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Neuropeptídeos GRP e BDNF como Alvos Moleculares em Neoplasias Femininas

Daniela Baumann Cornelio

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Orientador: Prof. Dr. Rafael Roesler

Co-orientador: Prof. Dr. Gilberto Schwartsmann

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SUMÁRIO

LISTA DE ABREVIATURAS

ASCUS	células escamosas atípicas de significado indeterminado (do inglês, atypical squamous cells of undetermined significance)
BB3R	receptor órfão da bombesina subtipo 3
BB4R	receptor de bombesina subtipo 4
BDNF	fator neurotrófico derivado de cérebro (do inglês, brain-derived neurotrophic factor)
bFGF	fator de crescimento básico de fibroblasto (do inglês, basic fibroblast growth factor)
BLP	peptídeo semelhante à bombesina (do inglês, bombesin-like peptide)
CSC	células-tronco tumorais (do inglês, cancer stem cell)
COX2	cicloxigenase 2
DNA	ácido desoxirribonucleico
EGF	fator de crescimento epidérmico (do inglês, epidermal growth factor)
EGFR	receptor do fator de crescimento epidérmico
ERK	do ingles, extracellular signal-regulated protein kinase
FAK	quinase de adesão focal
GRP	peptídeo liberador da gastrina
GRPR	receptor do peptídeo liberador da gastrina

GTPase	enzima que hidrolisa guanosina trifosfato
HER	receptor do fator de crescimento epidérmico (do inglês, human epidermal growth factor receptor)
HPV	papiloma vírus humano
HSIL	neoplasia intraepitelial escamosa de alto grau (do inglês, high-grade squamous intraepithelial lesion)
IGF	fator de crescimento insulínico (do inglês, insulin growth factor)
IL-8	interleucina 8
JEC	junção escamo-colunar
KRAS	do inglês, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LH-RH	hormônio liberador de hormônio luteinizante (do inglês lutein hormone releasing hormone)
LSIL	neoplasia intraepitelial escamosa de baixo grau (do inglês, low-grade squamous intraepithelial lesion)
MAPK	proteína quinase mitógeno-ativada
MTT	3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NTs	neurotrofinas
NIC	neoplasia intraepitelial cervical
NGF	fator de crescimento de nervo (do inglês, nerve growth factor)
NMBR	receptor de neuromedina B

p16INK4a inibidor da proteína quinase dependente de ciclina

pAkt proteína quinase B fosforilada (do inglês, phosphorylated protein kinase B)

PARP poli(ADP-ribose) polimerase

PGE2 prostaglandina E2

PI3K fosfatidil inositol 3 quinase

PKC proteína quinase C

PLA2 fosfolipase A2

PLC fosfolipase C

RNAm ácido ribonucléico mensageiro

Rt-PCR reação em cadeia da polimerase em tempo real

TNF-α fator de necrose tumoral alfa (do inglês, tumor necrosis factor alfa)

VEGF fator de crescimento vascular endotelial (do inglês, vascular endothelial growth factor)

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RESUMO

Receptores de neuropeptídeos e neurotrofinas constituem importantes alvos moleculares no câncer. Fatores de crescimento como o peptídeo liberador da gastrina (GRP) e fator neurotrófico derivado do cérebro (BDNF) estão envolvidos na proliferação celular e progressão do câncer, influenciando na invasão local, angiogênese, metastatização e apoptose.

O receptor de GRP (GRPR) tem sido identificado em muitos tumores humanos, mas até o presente trabalho não havia nenhuma informação na literatura quanto à sua expressão em câncer cervical. Nosso estudo inicial demonstrou pela primeira vez a expressão aberrante em GRPR em displasias e câncer do colo uterino, levantando a hipótese de que este receptor poderia estar implicado no processo carcinogênico destes tumores. Para explorar o papel de GRPR como um biomarcador de lesões de colo uterino, em nosso segundo estudo objetivamos avaliar o potencial diagnóstico da detecção de GRPR por imunocitoquímica, técnica que também não havia sido previamente descrita. Verificamos que este receptor foi fortemente associado com displasia e neoplasia cervical invasora. Além disso, o exame demonstrou elevada acurácia para lesões classificadas como células escamosas atípicas de significado indeterminado (ASCUS). Com base nestes resultados, concluímos que a expressão de GRPR por imunocitoquímica pode ser considerada como um método adicional para a detecção de lesões cervicais.

Estudos prévios indicam que o bloqueio de GRPR ou do receptor tropomiosina quinase B (TrkB) pode ter efeito antiproliferativo em células de câncer. Neste trabalho mostramos que a ativação do GRPR pode reduzir, ao passo que o bloqueio pode aumentar a viabilidade de células de câncer de ovário, mama e colo uterino. Além disso, demonstramos que a inibição TrkB reduz a viabilidade destas células, sendo que o tratamento com BDNF aumentou a viabilidade de células de ovário. Os resultados obtidos reforçam o conhecimento de que as sinalizações GRP/GRPR e BDNF/TrkB regulam a viabilidade de células de câncer. Ainda mais importante, fornecem a primeira evidência de que, sob certas condições, a ativação de GRPR pode inibir, em vez de estimular, células neoplásicas de mama, ovário e colo uterino.

ABSTRACT

Neuropeptide and neurotrophin receptors are increasingly important molecular targets in cancer. Growth factors as the gastrin-releasing peptide (GRP) and brain-derived neurotrophic factor (BDNF) are involved in cell proliferation and cancer progression, enhancing local invasion, angiogenesis, distant metastasis and apoptosis.

The GRP receptor has been identified in many human malignancies, but no information regarding its expression in cervical cancer was found in the literature. Considering that cervical cancer is a very important cause of morbidity and mortality worldwide, we aimed to evaluate the GRPR expression profile in preinvasive and invasive cervical lesions. Our initial study demonstrated for the first time the aberrant GRPR expression in human cervical dysplasia and cancer, raising the hypothesis that GRPR could be implicated in the carcinogenic process of cervical tumors. To further exploit GRPR as a biomarker, in our second study we aimed to evaluate the diagnostic potential of GRPR immunocytochemistry in detecting cervical dysplasia and invasive cancer. This was the first immunocytochemical evaluation of GRPR expression in cervical epithelial cells. This receptor was strongly associated with cervical dysplasia and invasive cancers. Additionally, GRPR immunosignaling showed high accuracy in detecting dysplasias in cells classified as atypical squamous cells of undetermined significance (ASCUS). Based on these results, we concluded that immunocytochemistry for GRPR may be regarded as a valuable method for early detection of cervical intraepithelial neoplasia.

Previous studies have indicated that compounds that act by blocking gastrin-releasing peptide receptors (GRPR) or tropomyosin receptor kinase B (TrkB) receptors can display antiproliferative activities against cancer cells. Here we show that GRPR activation can reduce, whereas its blockade can increase, the viability of breast, ovarian, and cervical cancer cell lines. In addition, we demonstrate that TrkB inhibition reduces the viability of these cells and BDNF increases the viability of ovarian cells. The results support the view that GRPR and BDNF/TrkB signaling regulate cancer cell viability. Most importantly, the findings provide the first evidence that, under certain conditions, GRPR activation can inhibit, rather than stimulate, breast, ovarian and cervical cancer cells.

I – INTRODUÇÃO

1.1 Peptídeo Liberador da Gastrina (GRP)

1.1.1 Descoberta do GRP

O tetradecapeptídeo bombesina foi primeiramente isolado e caracterizado a partir da pele do anfíbio *Bombina bombina* (ANASTASI *et al*., 1971). Posteriormente, peptídeos semelhantes à bombesina (BLPs) foram identificados em mamíferos, sendo os maiores níveis observados em células pulmonares neuroendócrinas (JOHNSON *et al*., 1982). O principal BLP foi chamado de peptídeo liberador da gastrina devido à sua primeira atividade conhecida de indução da secreção de gastrina a partir das células G do antro gástrico. O GRP possui 27 aminoácidos e compartilha com a bombesina uma sequência altamente conservada de 7 aminoácidos C-terminal, o que é essencial para a imunogenicidade e para uma ligação de alta afinidade ao receptor preferencial do GRP (SUNDAY *et al*., 1988) (Figura 1). Assim, GRP e bombesina apresentam essencialmente efeitos fisiológicos idênticos.

Bombesina

Pyr-Gln-Arg-Leu-Gly-Asn-Gln-**Trp-Ala-Val-Gly-His-Leu-Met-NH2**

GRP

Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-

Tyr-Pro-Arg-Gly-Asn-His-**Trp-Ala-Val-Gly-His-Leu-Met-NH2**

Figura 1: Sequências de bombesina e GRP. As porções C-terminais de bombesina e GRP, marcadas em negrito, são idênticas.

Já foram identificados três RNAs mensageiros (mRNA) GRP em humanos, os quais codificam proteínas contendo 138, 141 e 148 aminoácidos. Estes mRNAs são originados de uma fita simples de RNAm nascente por *splicing* alternativo. Eles irão codificar igualmente o peptídeo GRP 1-27, porém sua sequencia que codifica a extensão C-terminal varia (SAUSVILLE, *et al.*, 1986; SPINDEL, *et al.*, 1986).

1.1.2 Receptor Preferencial do GRP, GRPR

Os receptores de GRP pertencem ao grupo de receptores acoplados à proteína G, considerada a maior família de moléculas de superfície celular envolvida na transmissão de sinais e que contribui com mais de 2% dos genes codificados pelo genoma humano (DORSAM & GUTKIND, 2007). Uma característica central deste grupo é a estrutura comum de sete domínios α-hélices transmembrana, sendo que a ligação à proteína G se dá através do domínio intracelular (Figura 2). De uma maneira geral, a natureza da cascata de sinalização gerada depende da especificidade de ligação de cada receptor à proteína G.

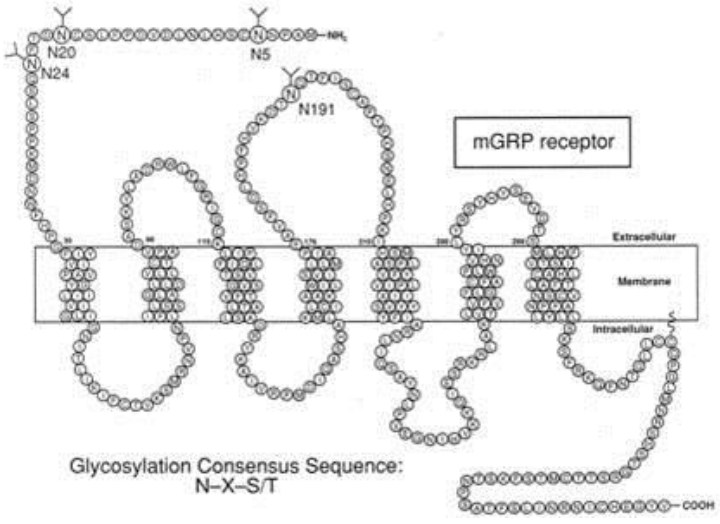

Figura 2: Estrutura molecular do GRPR (BENYA *et al.*, 2000).

Até o presente momento já foram descritos quatro subtipos de receptores para a família dos BLPs; o receptor preferencial do GRP, GRPR (SPINDEL *et al.*, 1990; BATTEY *et al*., 1991), receptor de neuromedina B, NMBR (WADA *et al.*, 1991), receptor órfão da bombesina subtipo 3, BB3R (FATHI *et al*., 1993) e receptor de bombesina subtipo 4, BB4R (NAGALLA *et al*., 1995), este último exclusivo dos anfíbios. Estes receptores podem ser distinguidos com base na sua afinidade pelos agonistas e antagonistas. O GRPR possui alta afinidade por bombesina e GRP, enquanto que praticamente não se liga à neuromedina B, outro conhecido membro dos BLPs (GILADI *et al*., 1993). Existe uma marcada diferença na ligação dos agonistas aos receptores de GRP entre humanos e ratos, o que não se observa em receptores NMB (UEHARA *et al.*, 2011).

O GRPR humano é composto por 384 aminoácidos e possui elevada homologia ao GRPR de ratos, com 90% de identidade (OHKI-HAMAZAKI, *et al*., 2005; JENSEN, *et al*., 2008). Este receptor já foi clonado parcial ou totalmente em vinte e uma espécies, sendo que as regiões mais conservadas são o terceiro domínio extracelular e os domínios transmembranas (BALDWIN *et al*., 2007).

1.1.3 Sinalização Celular Mediada por GRPR

Quando um agonista se liga ao GRPR, ocorre ativação da fosfolipase C e aumento das concentrações de inositol trifosfato, diacilglicerol e cálcio (HELLMICH *et al.*, 1999). A liberação de cálcio se dá inicialmente por uma liberação dos estoques intracelulares e é posteriormente sustentada pelo influxo deste cátion através da membrana celular (Figura 3).

Entre as vias de sinalização celular ativadas pelos GRPRs já foram caracterizadas a da proteína quinase mitógeno-ativada (MAPK), proteína quinase C (PKC) e quinase de adesão focal (FAK) (APRIKIAN *et al*., 1997; CHEN & KRUG, 2010), responsáveis por promover crescimento e proliferação. Em células de câncer hepatocelular, GRP demonstrou promover o crescimento, acelerar a progressão do ciclo celular e reduzir a apoptose, ativando a via da MAPK por mecanismos independentes do receptor do fator de crescimento epidérmico (EGFR) (LI *et al*., 2010). Também em linhagem celular de câncer de mama, GRPR provou independência de EGFR na proliferação celular, enquanto que houve sinergismo com EGFR na regulação da migração celular e na expressão de interleucina 8 (IL-8) (CHAO *et al*., 2009).

Outros pesquisadores encontraram efeito sinérgico no bloqueio de GRPR e EGFR na inibição da proliferação celular e na apoptose em células de câncer de cabeça e pescoço (ZHANG *et al*., 2007). De Farias e colaboradores (2009a) evidenciaram que GRP combinado com agentes que estimulam a via do monofosfato cíclico de adenosina (cAMP) / proteína quinase A (PKA) levou à proliferação de células de glioma humanas. Nenhum dos compostos utilizados demonstrou ação isoladamente, levando os autores a sugerir que possa haver uma interação de GRP/GRPR na via de sinalização de cAMP/PKA. Também em células de gliomas, GRPR parece regular a proliferação celular através de um mecanismo mediado pela sinalização de fosfatidilinositol-3-hidroxiquinase (PI3K) (FLORES *et al*., 2008).

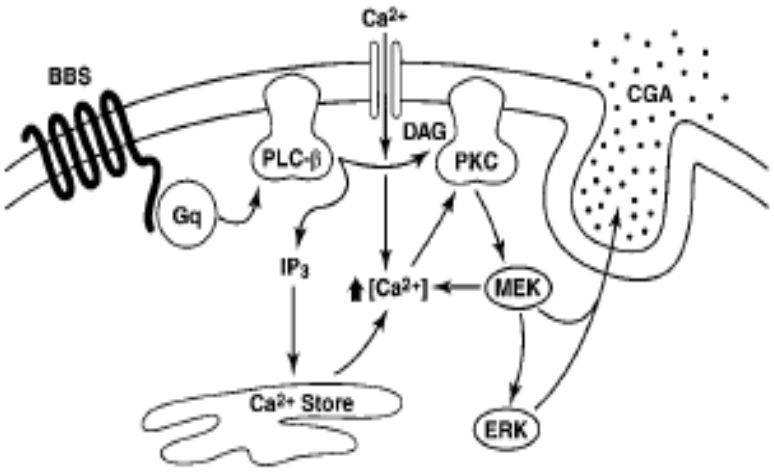

Figura 3: modelo de secreção mediada por GRPR (HELLMICH *et al.*, 1999).

Os receptores de GRP também estão envolvidos na migração celular e angiogênese. GRP e GRPR atuam como morfogenes quando expressos em câncer colo-retal, promovendo uma melhor diferenciação fenotípica através da regulação da mobilidade celular. Dados evidenciam que GRP super-regula a proteína de adesão intracelular 1 (ICAM-1) via FAK, levando à mobilidade celular e adesão à matriz extracelular (TAGLIA *et al*., 2007). GRPRs foram frequentemente encontrados na vasculatura de uma grande variedade de tumores. Além disso, muitas vezes eles estão associados à presença de receptores de fator de crescimento vascular (VEGF) (REUBI *et al*., 2011). Em modelo animal com enxertos de neuroblastomas, o tratamento com bombesina levou a um aumento do volume dos tumores e a uma maior expressão de marcadores de angiogênese (KANG *et al*., 2007).

Na cascata de sinalização de GRPR ocorre também ativação da GTPase Rho, que tem um papel central na migração celular através da ativação da ROCK (MARINISSEN & GUTKIND, 2001), e de fosfolipase A2 (PLA2) e cicloxigenase 2 (COX2) (WEN *et al*., 2011), aumentando a produção de prostaglandina E2 (PGE2)

(ROZENGURT *et al.*, 2002). A figura 4 mostra as principais vias de sinalização ativadas por GRPR.

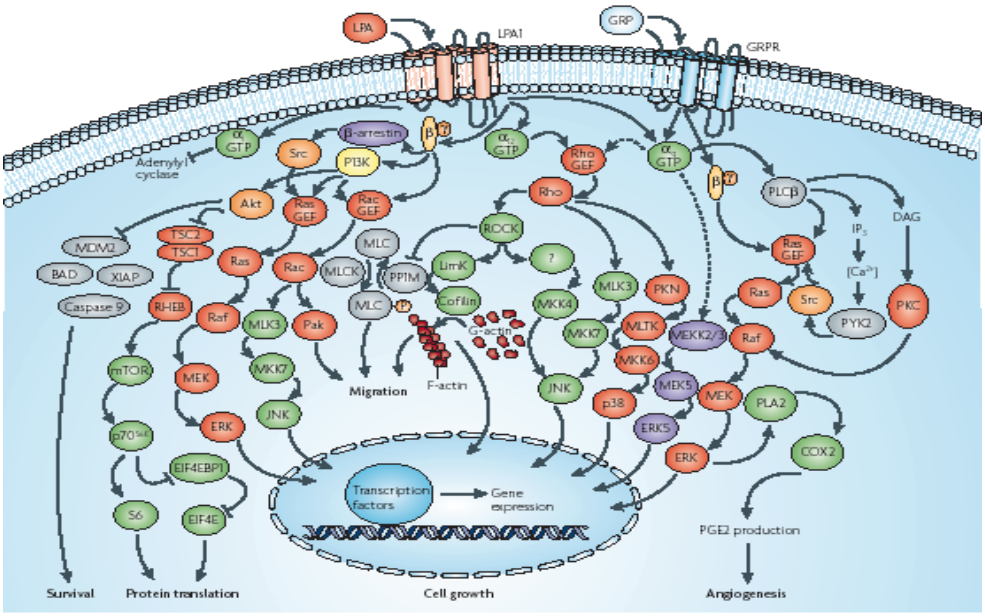

Figura 4: Vias de sinalização ativadas pelo GRPR (DORSAM & GUTKIND, 2007).

1.1.4 Funções do GRP

O GRP desempenha diversos papéis fisiológicos além do estímulo à secreção ácida gástrica. Os BLPs são responsáveis por promover a liberação de vários hormônios no trato gastrointestinal, incluindo gastrina, somatostatina e colecistoquinina (SCHUBERT, *et al.*, 2002), e por estimular a secreção de enzimas pancreáticas (NIEBERGALL-ROTH & SINGER, 2001). Além disso, participam da contração da musculatura lisa em diversos tecidos e atuam como neurotransmissores no sistema nervoso central (BUNNET, 1994).

Entre os efeitos neurológicos dos BLPs já foram descritas alterações comportamentais, anorexia e melhora da consolidação da memória em modelos animais

(ROESLER *et al*., 2006a, ROESLER *et al*., 2009). Recentemente foi demonstrado que a sinalização de GRP/GRPR pode influenciar a memória aversiva através da modulação da fisiologia da amídala lateral (CHAPERON *et a.l*, 2012) e o medo de extinção através da amídala lateral e do córtex frontal (MARTEL *et al.*, 2012). O bloqueio de GRPR no período neonatal de ratos levou à diminuição da interação social e à deficiência na memória de reconhecimento de objetos (PRESTI-TORRES *et al*., 2012).

Estes peptídeos podem estar envolvidos na patogênese de diversas desordens do sistema nervoso central, como a doença de Parkinson, a esquizofrenia e a doença de Alzheimer (ROESLER *et al*., 2006b; ROESLER *et al*., 2007), bem como em transtornos depressivos (MONJE *et al.*, 2011). Já foi sugerido que o transtorno bipolar possa estar associado ao estresse oxidativo. Em um estudo que avaliou um modelo animal com mania, o bloqueio de GRPR com um antagonista demonstrou propriedades antioxidantes no cérebro (VALVASSORI *et al.*, 2010). Ainda, o gene de GRP parece estar associado à etiologia da síndrome do pânico, conforme estudo genético que avaliou 120 casos com este transtorno (HODGES *et al*., 2009).

Os BLPs também demonstram participar de processos imunológicos e inflamatórios (PETRONILHO *et al*., 2007). O GRP modula, estimulando ou inibindo, a função de linfócitos (MEDINA *et al*., 1999), fagócitos (DE LA FUENTE *et al*., 1991), neutrófilos (CZEPIELEWSKI *et al.*, 2012) e mastócitos, estando implicado na patologia de displasia bronco-pulmonar (SUBRAMANIAM *et al*., 2003), asma (ZHOU *et al*., 2011) e artrite reumatóide (GRIMSHOLM *et al*., 2005; OLIVEIRA *et al*., 2011). O GRPR igualmente parece estar envolvido no processo de prurido (JEFFRY *et al*., 2011; SU & CO, 2011;). Receptores de GRP já foram identificados em neurônios espinais que estão crucialmente envolvidos no prurido, mas não no processo de dor (STÄNDER *et*

al., 2011). Além disso, através do bloqueio da cascata desencadeada por GRPR observou-se uma diminuição no prurido induzido por opióides (LIU *et al.*, 2011).

Outra doença inflamatória em que GRPRs demonstraram estar envolvidos é a uveíte. Utilizando um antagonista de GRPR em um modelo animal de uveíte, Pereira e coautores (2009) evidenciaram ações anti-inflamatórias através da redução da atividade da mieloperoxidase e do decréscimo dos níveis de fator de necrose tumoral alfa (TNF-α) e de proteína-1 quimioatrativa de monócitos. Também em sepse, o uso de um antagonista seletivo de GRPR em um modelo animal provou aumentar a sobrevida de roedores, demonstrando um efeito protetor aos danos teciduais e atenuando a liberação de citoquinas inflamatórias (DAL-PIZZOL *et al*., 2006).

Receptores de bombesina em mamíferos ainda atuam na regulação da homeostase energética e podem representar um alvo atraente para o tratamento farmacológico da obesidade e certos transtornos alimentares (MAJUMDAR & WEBER, 2011). Evidências experimentais apontam que o BB3-R é um importante regulador do peso corporal, do desgaste energético e da homeostase da glicose (MAJUMDAR & WEBER, 2012).

Finalmente, evidências preliminares sugerem que os GRPRs podem estar envolvidos com a formação de células-tronco tumorais (CSCs). Flores e colaboradores (2009) avaliaram o papel das CSCs em gliomas e meduloblastomas e demonstraram, através da técnica de imuno-histoquímica, que o GRPR pode estar implicado na expansão de CSCs cerebrais in vitro.

1.1.5 GRP, GRPR e Câncer

O GRP e a bombesina possuem efeitos mitogênicos já bem estabelecidos. Estes peptídeos estimulam o crescimento de tecidos normais, como pâncreas (PAREKH *et al*., 1994), mucosa gastrointestinal (CHU *et al*., 1995) e epitélio brônquico (WILLEY *et al*., 1984). O primeiro estudo em neoplasias mostrou que o emprego de um anticorpo monoclonal dirigido ao GRP, impedindo a ligação ao seu receptor, inibiu o crescimento de câncer de pulmão de pequenas células *in vitro* e *in vivo* (CUTTITTA *et al*., 1985). Desde então, muitos grupos de pesquisa passaram a investigar o papel do GRP no desenvolvimento e progressão do câncer. Através de estudos com linhagens celulares e modelos animais, o tratamento com bombesina/GRP promoveu proliferação em diversas neoplasias, incluindo próstata (BOLOGNA *et al*., 1989), cólon (NARAYAN *et al*., 1990), estômago (KIM *et al*., 1996) e mama (BURNS *et al*., 1999).

A detecção simultânea de GRP e seu receptor nos tecidos, bem como os efeitos antiproliferativos dos anticorpos anti-GRP, levaram ao reconhecimento deste peptídeo como um fator de crescimento autócrino no câncer. Posteriormente, demonstrou-se que o GRP também é capaz de estimular os receptores de forma parácrina (HEASLEY, 2001).

A ampla expressão de receptores de fatores de crescimento na superfície das células malignas reconhecidamente confere maior agressividade biológica aos tumores. Além de promover proliferação celular, os fatores de crescimento estão envolvidos em processos de invasão local, metastatização, angiogênese e apoptose (PERONA, 2006). Estes parâmetros também vêm sendo investigados nos estudos com GRPRs. Analisando neoplasias de cólon, alguns autores relacionaram invasão linfática e perda de diferenciação celular à maior expressão de GRPRs (SAURIN *et al*., 1999).

Recentemente foi identificada uma via de sinalização de GRPR através da proteína heterocromatina 1HSβ, que foi relacionada com maior invasividade do câncer de cólon (TELL *et al*., 2011). Outros pesquisadores foram capazes de associar níveis superiores de GRPRs a tumores de ovário mais indiferenciados (SUN *et al.*, 2000a), bem como a neuroblastomas mais agressivos (KIM *et al*., 2002). GUGGER & REUBI (1999) avaliaram tumores de mama metastáticos para linfonodos axilares e relataram 100% de expressão de GRPR nas metástases de tumores primariamente positivos para este receptor. Em estudo com carcinomas renais implantados em ratos, receptores GRP foram encontrados na microcirculação tumoral, enquanto que a neoangiogênese foi significativamente inibida com o emprego de um antagonista GRPR (HEUSER *et al*., 2005).

1.1.6 Análogos de GRPR

A estratégia de tratamentos dirigidos a receptores de fatores de crescimento progrediu significativamente na última década. Os resultados obtidos na prática clínica com terapias-alvo, a exemplo das terapias anti-EGFR (VIVANCO & MELLINGHOFF, 2010), vêm encorajando pesquisadores a desenvolver compostos capazes de interagir com estes receptores, através de biomarcadores para detecção e estadiamento de neoplasias, ou de análogos e antagonistas para tratamento das mesmas. Muitos estudos têm explorado o GRPR como alvo diagnóstico e terapêutico.

Na área de diagnóstico por imagem já foram desenvolvidos diversos conjugados com análogos de GRP (POOL *et al*., 2010). Nos últimos vinte anos já foram realizados mais de 200 estudos a esse respeito (SANCHO *et al*., 2011). Análogos de GRP ou bombesina radiomarcados podem ser usados como ferramentas não invasivas para

diagnosticar, monitorar e tratar potencialmente tumores, como o câncer de próstata, já tendo sido testados em pacientes, sem efeitos colaterais importantes (LEARS *et al.*, 2011). O composto conjugado de bombesina e 177Lu-Amba já foi avaliado em estudo clínico fase I como método diagnóstico e radioterapia sistêmica, após excelente desempenho em estudos pré-clínicos (LANTRY *et al*., 2006).

Com finalidades terapêuticas, análogos de bombesina têm sido combinados a diversos agentes citotóxicos, atuando como veículos carreadores de drogas. Um análogo citotóxico de bombesina / GRP, AN-215, contendo 2-pyrrolino-Dox, demonstrou inibir o crescimento de várias neoplasias humanas que expressam receptores de bombesina / GRP. A toxicidade, farmacocinética e as doses máximas toleradas de AN-152 foram avaliadas em mulheres com câncer de ovário ou endométrio em um ensaio clínico fase I, que mostrou estabilização da doença e respostas objetivas. O análogo AN-152 encontra-se atualmente em ensaios clínicos de fase II (SCHALLY *et al*., 2011). A abordagem com anticorpos monoclonais também já foi testada em estudos clínicos. O anticorpo 2A11, que se liga com alta afinidade ao GRP, demonstrou perfil de toxicidade aceitável para o tratamento de pacientes com câncer de pulmão (CHAUDHRY *et al*., 1999).

1.1.7 Antagonistas de GRPR, RC-3095 e RC-3940-II

Uma classe importante de drogas utilizadas em terapias-alvo são os antagonistas. Idealmente, estes compostos devem possuir elevada afinidade pelos receptores para bloquear a cascata de sinalização celular que seria desencadeada pelo acoplamento do ligante. Na última década foram desenvolvidos diversos antagonistas GRPR com capacidade de inibir o crescimento de tumores (HOHLA & SCHALLY, 2010).

Um composto que tem demonstrado notável atividade antitumoral é o antagonista RC-3095, já testado em diversas neoplasias, cuja estrutura molecular pode ser vista na figura 5.

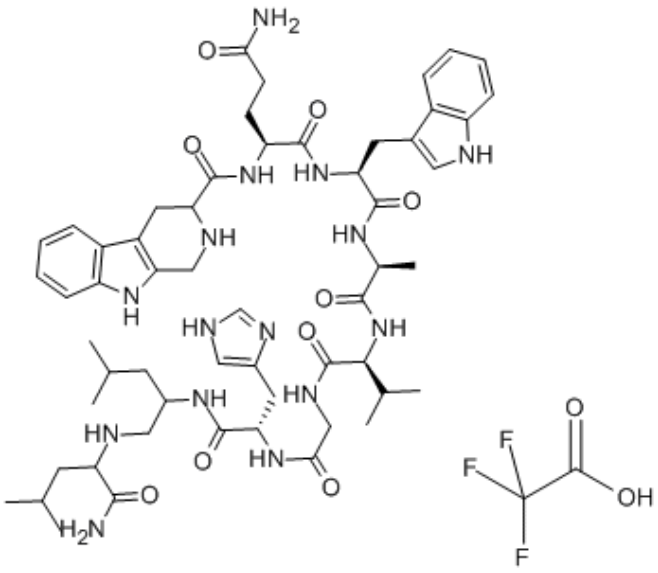

Figura 5: Estrutura molecular do composto RC-3095.

Este agente já provou ser ativo contra câncer de mama (SZEPESHAZI *et al*., 1997; MIYAZAKI *et al*., 1998), pulmão (KOPPAN *et al*., 1998), ovário (CHATZISTAMOU *et al*., 2000; CHATZISTAMOU *et al*., 2001), próstata (STANGELBERGER *et al*., 2005a), glioblastomas (KIARIS *et al*., 1999), entre outros. Seu efeito parece interferir com importantes vias de sinalização como a do fator de crescimento epidérmico (EGF) e fator de crescimento de insulina (IGF) (PLONOWSKI *et al*., 2000; LIU *et al*., 2007; ZHANG *et al*., 2007). Em animais com enxertos de câncer de mama, a administração de RC-3095 foi associada com substancial redução da expressão de fator de crescimento básico de fibroblasto (bFGF), IGF-II e VEGF, além de promover a inibição do crescimento tumoral (BAJO *et al*., 2004). O RC-3095 também demonstrou interação com a expressão de HER2 em linhagens celulares de câncer de mama (BAJO *et al*., 2002) e próstata (SOTOMAYOR *et al*., 2010). De Farias

e coautores (2009b) encontraram uma diminuição na secreção de fator de crescimento neural (NGF) com o uso deste composto em células de câncer de cólon, sugerindo que este possa ser um mecanismo antiproliferativo de antagonistas de GRPR.

O efeito antiproliferativo do RC-3095 já foi testado com outros agentes em sinergismo. A administração simultânea de análogo do hormônio liberador de hormônio luteinizante (LH-RH) e de RC-3095 a animais com tumores de próstata levou a uma importante redução do crescimento tumoral (PINSKI *et al*., 1993). Já em modelo animal de câncer de pâncreas, o sinergismo de RC-3095 com análogo de LH-RH não se mostrou superior aos agentes utilizados isoladamente (SZEPESHAZI *et al*., 1994). Em modelo experimental de glioblastoma, a combinação de RC-3095 com temozolamida produziu importante inibição do crescimento celular de gliomas in vitro, bem como redução do volume tumoral em ratos (DE OLIVEIRA *et al*., 2009).

Em nosso centro, o RC-3095 já foi avaliado em linhagens celulares de tumores e modelos animais, bem como em estudos clínicos. Foi realizado um estudo fase I com vinte e cinco pacientes portadores de neoplasias sólidas avançadas, em que este antagonista GRPR demonstrou perfil de toxicidade favorável, após aplicações subcutâneas diárias. Neste mesmo ensaio, uma única dose de RC-3095 administrada a um paciente hipergastrinêmico, portador da Síndrome de Zolliger-Ellison, produziu decréscimo superior a 50% nos níveis plasmáticos de gastrina (SCHWARTSMANN *et al*., 2006).

O RC-3940-II é outro composto desenvolvido como antagonista de GRPR, cuja estrutura molecular pode ser visualizada na figura 6 abaixo.

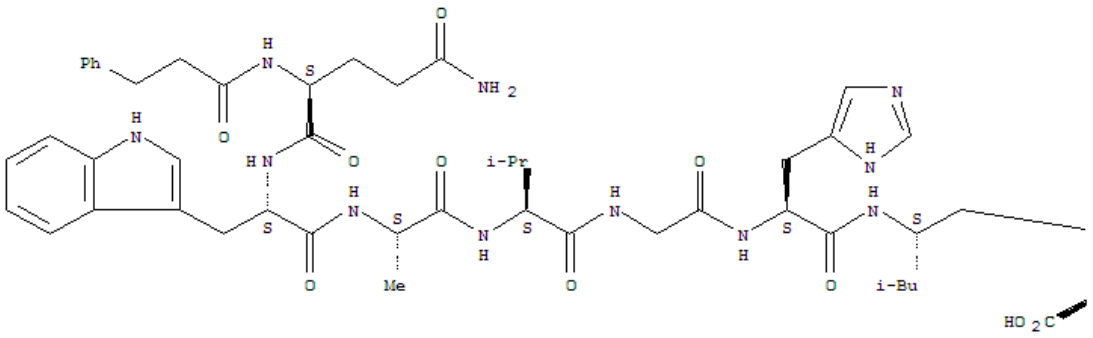

Figura 6: Estrutura molecular do composto RC-3940-II.

Da mesma forma que o RC-3095, o RC-3940-II também já demonstrou importante atividade antiproliferativa em tumores, incluindo neoplasias de pâncreas (QIN *et al*., 1995), rim (JUNGWIRTH *et al*., 1998), ovário (CHATZISTAMOU, *et al*., 2000), mama (MIYAZAKI *et al*., 1998; BAJO *et al*., 2002) e pulmão (KOPPAN *et al*., 1998). Em camundongos com enxertos de câncer de pulmão, o RC-3940-II inibiu o crescimento dos enxertos em até 50%, além de reduzir a expressão de K-Ras, COX-2 e da proteína quinase B fosforilada (pAkt) (HOHLA *et al*., 2007). Em outro estudo com modelo animal de câncer de pulmão, além de redução tumoral, a administração de RC-3094-II foi associada com a diminuição da expressão de EGFR (KANASHIRO *et al*., 2007). Estudando linhagem celular de câncer de cólon, pesquisadores verificaram atividade antiproliferativa do RC-3940-II, bem como redução da expressão de COX-2 e consequente menor liberação de prostaglandina (CORRAL *et al*., 2007). Além disso, este antagonista também demonstrou influenciar a expressão de fatores de crescimento tumoral como VEGF e bFGF, bem como receptores da família de EGF (STANGELBERGER *et al*., 2005b).

1.1.8 Expressão de GRPR em Tumores

A caracterização do perfil de expressão dos receptores de fatores de crescimento nos processos neoplásicos é fundamental para que se identifiquem aqueles tumores passíveis de serem diagnosticados ou tratados com abordagens mais seletivas. A expressão aberrante de GRPRs já está documentada em diversas neoplasias humanas através de diferentes técnicas de ligação ao receptor, imunohistoquímica, ou rt-PCR, provando ser mais ampla no câncer em comparação aos tecidos normais (CORNELIO *et al*., 2007). A tabela 1 sumariza os tipos tumorais já estudados até a data da publicação do artigo "Gastrin-releasing peptide receptor as a molecular target in experimental anticancer therapy", fruto da dissertação de mestrado da autora.

Type of cancer	No. cases	No. positive	%	Method
Prostate	12	12	100	PCR
	30	30	100	Binding
	80	50	63	Binding
	22	20	91	PCR
	12	12	100	Binding
Gastrinoma	5	5	100	Binding
Breast	100	33	33	Binding
	71	44	62	Binding
	57	41	72	Binding
Ovarian	22	17	77	PCR
Pancreatic	12	2	17	Binding
	26	2	8	PCR
	29	0	0	Binding
Colon	21	5	24	Binding
	29	27	93	PCR
	50	38	76	IH
	23	23	100	PCR
Renal	4	4	100	PCR
	16	6	35	Binding
	18	13	72	PCR
Lung (SCLC)	7	2	29	PCR
	9	3	33	Binding
Head and Neck	25	25	100	PCR
Neuroblastoma	33	24	73	IH
	19	19	100	PCR
Esophageal	12	10	83	PCR
GI carcinoid	26	22	85	IH
Gastric	23	12	50	Binding
	20	8	40	PCR
Uterine	29	11	38	Binding

Tabela 1: Expressão de GRPR em tecidos neoplásicos humanos

(CORNELIO *et al*., 2007).

Em certos tumores, já se verificou taxa de expressão do GRPR de 100%, incluindo próstata (MARKWALDER & REUBI, 1999; SCHROEDER *et al*., 2010; NAGASKI *et al*., 2012), glioma (FLORES et al., 2010), cólon (CHAVE *et al*., 2000), rim (PANSKI *et al*., 2000), neuroblastoma (SEBESTA *et al*., 2001), cabeça e pescoço (LANGO *et al*., 2002) e gastrinoma (REUBI *et al*., 2002). A superexpressão de GRPR já havia sido descrita em neoplasias de pulmão de pequenas células. Recentemente, a presença de GRPR em epitélio brônquico não neoplásico foi significativamente associada com a presença de câncer de pulmão do tipo adenocarcinoma e epidermóide, tanto em fumantes, como em não fumantes, sem diferença entre homens e mulheres (EGLOFF *et al*., 2012).

Entre as neoplasias ginecológicas, já foram detectados GRPRs em carcinomas de ovário (SUN *et al*., 2000a) e de corpo uterino (FLEISHMANN *et al*., 2005), porém não existiam dados referentes à expressão deste receptor em neoplasias de colo uterino até o estudo da presente autora.

1.2 Neurotrofinas

1.2.1 Caracterização e Função das Neurotrofinas

As neurotrofinas (NTs) constituem uma família conhecida de fatores de crescimento do sistema nervoso central, descobertas há mais de 50 anos (LEVI-MONTALCINI, 1998). Enquanto diversas moléculas de sinalização podem regular funções e estruturas neuronais, e, portanto serem consideradas fatores neurotróficos, classicamente a família das NTs é formada por apenas quatro polipeptídeos: fator de crescimento nervoso (NGF), fator neurotrófico derivado de cérebro (BDNF), NT3 e NT4 (REICHARDT, 2006; SKAPER, 2008). As formas maduras de NTs em mamíferos

são polipeptídeos de 13-15 kDa, todos com similaridades estruturais e funcionais (MC DONALD & CHAO, 1995) (Figura 7). A homologia entre as espécies chega a 90-100%, o que facilitou muito o uso de modelos animais com administração exógena de NTs ou o uso de animais transgênicos com ausência de aspectos específicos da sinalização de NTs.

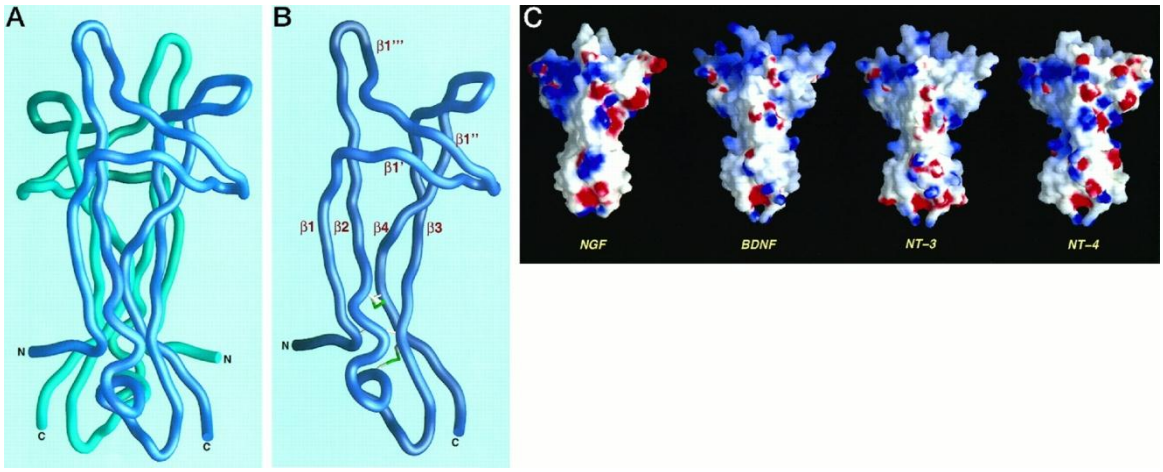

Figura 7: Representação tridimensional de NGF (A e B) e dos 4 subtipos de neurotrofinas (C) (Mc Donald & Chao, 1995).

Desde sua descrição inicial, as NTs eram consideradas como reguladoras da neurogênese, da diferenciação e sobrevivência dos neurônios, bem como de sua condução e plasticidade (KALB, 2005; CHAO *et al*., 2006). Atualmente se sabe que as NTs também estão envolvidas em desordens degenerativas como a doença de Alzheimer (SCHULTE-HERBRUGGEN *et al*., 2008; SARAGOVI *et al*., 2009), tumores cerebrais (THIELE *et al*., 2009), reparos a injúrias na medula espinal (BLESCH *et al*., 2002) e outras condições clínicas relevantes (DWIVEDI, 2009).

1.2.2 Receptores e Sinalização das Neurotrofinas

As NTs exercem seus efeitos através da ligação com receptores da superfície celular. Existem dois tipos diferentes de receptores que estão envolvidos na sinalização. Todas as NTs se ligam ao receptor p75NTR, um pan receptor de baixa afinidade de NT com 75 kDa (BARKER, 2004; CHEN *et al*., 2009). A família de receptores de alta afinidade tropomiosina quinase relacionados (Trk) consiste do TrkA, que se liga preferencialmente ao NGF, TrkB, que se liga preferencialmente ao BDNF e NT4, e TrkC, sítio preferencial de ligação do NT3 (TENG & HEMSTEAD, 2004) (Figura 8).

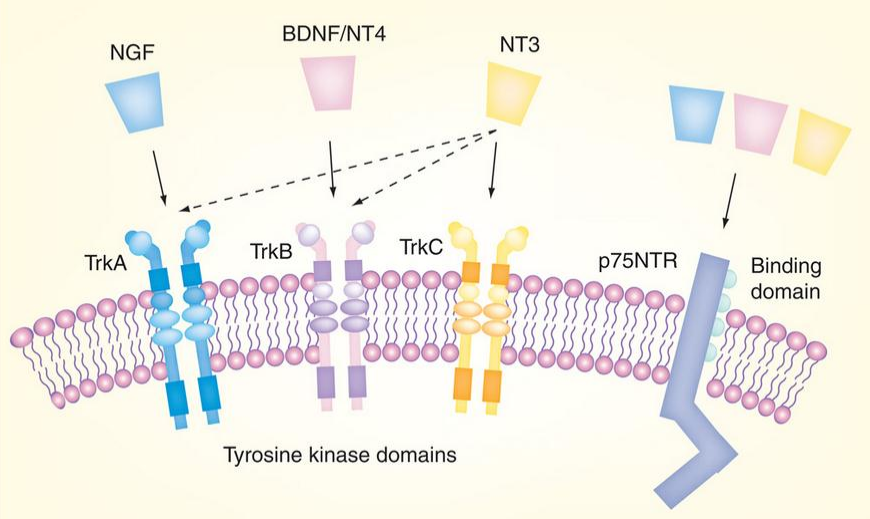

Figura 8: Receptores de neurotrofinas (PRAKASH *et al*., 2010).

Ambos os receptores Trk e p75NTR ativam múltiplas e distintas vias de sinalização. Os Trks são receptores de tirosina quinase que dimerizam e são autofosforilados em resíduos tirosina intracelulares após a ligação das NTs, resultando em uma rápida cascata de sinalização, que inclui a ativação de Ras, PI3K/Akt (proteína quinase B), e fosfolipase C gama (SKAPER, 2008). Estas cascatas importantes podem ativar fatores de transcrição celulares específicos envolvidos em diferenciação, sobrevida, apoptose e crescimento. De uma maneira geral, as NTs podem alterar o

balanço entre sobrevida / crescimento e morte celular (LU *et al*., 2005). Um esquema representativo da sinalização celular de NTs e seus receptores pode ser visualizado na figura 9.

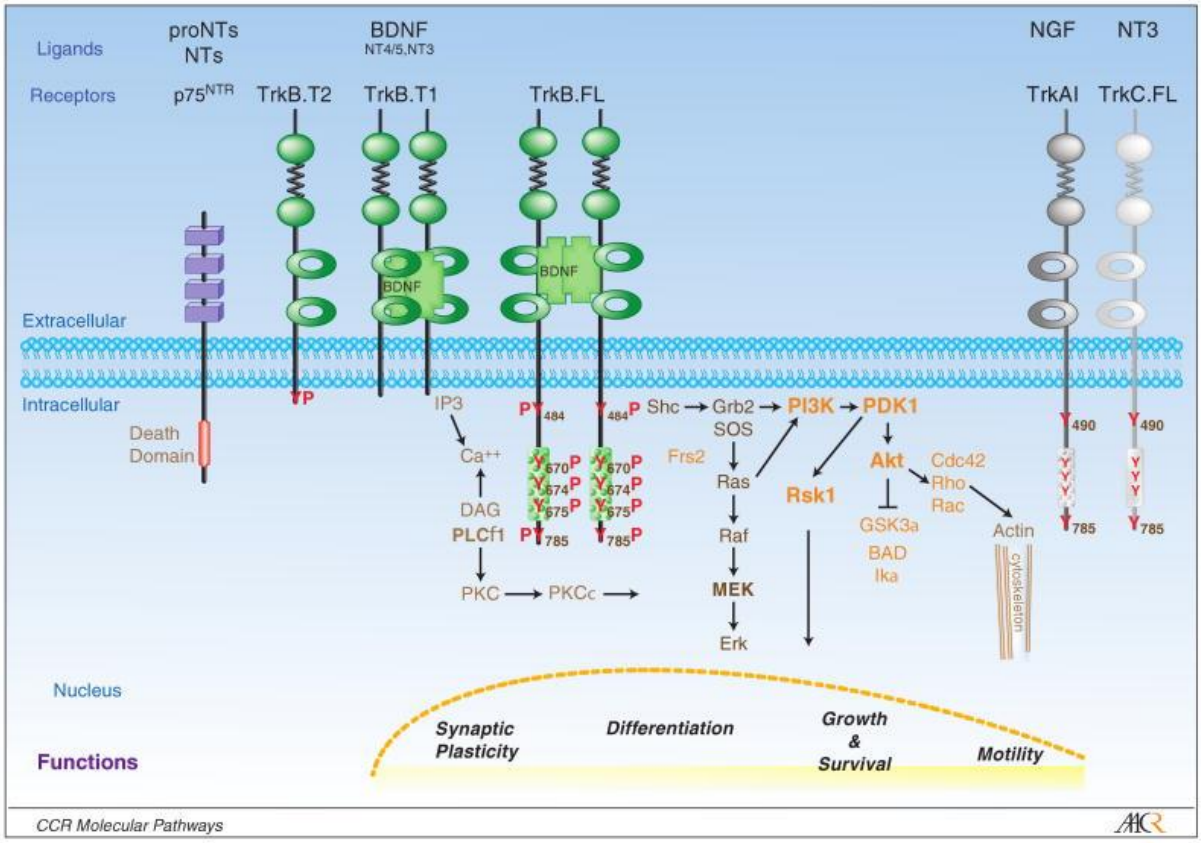

Figura 9: Esquema representativo dos receptores Trk e principais vias de sinalização (THIELE *et al*., 2009).

1.2.3 Expressão de Neurotrofinas em Tecidos Humanos

Existem evidências crescentes que ambos os receptores de baixa e alta afinidade de NTs são amplamente distribuídos em tecidos não neuronais que não estão necessariamente associados a inervação. Tal fato suscita a hipótese de que as NTs

liberadas por tecidos periféricos poderiam ter efeitos autócrinos ou parácrinos sobre estruturas vizinhas além dos nervos. Neste sentido, parece haver uma grande heterogeneidade na expressão dos receptores de NTs em tecidos específicos, variando conforme a idade, patologias e outros fatores. Os receptores de NTs já foram descritos em pulmão, mucosa nasal, pele, sistemas gastrointestinal e geniturinário, músculo cardíaco, músculos esqueléticos e lisos (PRAKASH *et al*., 2010). Além disso, as NTs parecem estar envolvidas com diversas condições clínicas relevantes, como alergia de pele (RAAP & KAPP, 2005), constipação crônica (SCHILLER, 2004), doença arterial coronariana (CAPORALI & EMANUELI, 2009) e diversos tumores (THIELE *et al*., 2009).

1.2.4 BDNF / TrkB e Câncer

A primeira associação clínica entre Trks e câncer surgiu através de achados de mutações ativadoras causadas por rearranjos cromossômicos ou mutações em TrkA em carcinomas de tireoide papilar e medular, respectivamente (PIEROTTI & GRECO, 2006). Nos últimos anos, diversos estudos detalharam a expressão de Trks em diferentes tipos de tumores, correlacionando sua expressão com prognóstico ou estádio tumoral (DESMET & PEEPER, 2006; ROESLER *et al*., 2011). Entretanto, até o momento não existe um padrão claro de associação entre uma isoforma de Trk e prognóstico. Por exemplo, em neuroblastomas, pacientes com níveis elevados de TrkA (NAKAGAWARA *et al*., 1993) ou TrkC (YAMASHIRO *et al*., 1996) possuem prognóstico melhor, enquanto que aqueles que demonstram elevada expressão de TrkB e BDNF possuem prognóstico pobre (NAKAGAWARA *et al*., 1994; AZGHARZADEH *et al*., 2006).

Nos casos em que os Trks não estão mutados ou translocados, questiona-se se eles poderiam ser apenas marcadores tumorais ou desempenhar um papel na fisiopatologia do câncer. Considerando-se o racional de que a ativação de Trks por NTs media sinais de sobrevida e estimula a neurogênese e a migração celular de neurônios normais (KNÜSEL *et al*., 1994), pode-se pensar que o mesmo processo possa ser explorado pela células tumorais para sobreviver a insultos citotóxicos e metastatizar. Células de neuroblastoma que expressam TrkB tratadas com BDNF são menos sensíveis a drogas citotóxicas (SCALA *et al*., 1996; JABOIN *et al*., 2002), exibem maior invasividade (MATSUMOTO *et al*., 1995), sobrevivem em condições limitadas de fatores de crescimento (KIM *et al*., 1999) e apresentam produção aumentada de VEGF (NAKAMURA *et al*., 2006). Além disso, células de neuroblastoma que sobrevivem a repetidas exposições de agentes citotóxicos expressam níveis aumentados de BDNF, sugerindo que a via de sinalização NT-Trk contribui para um fenótipo multi resistente a drogas (MATSUMOTO *et al*., 1995). BDNF e TrkB já foram detectados em tumores e linhagens celulares de câncer colo-retal (DE FARIAS *et al*., 2010). Outros pesquisadores verificaram que em células de câncer de colo retal, o tratamento com BDNF produziu um efeito anti-apoptótico via TrkB, e que em pacientes portadores desta neoplasia, havia maior expressão de transcritos de BDNF e TrkB nos tumores em comparação com os tecidos normais adjacentes, especialmente nos casos avançados (AKIL *et al*., 2011). Da mesma forma, em células de câncer de pulmão observou-se expressão de BDNF e TrkB, sendo que a estimulação das células com BDNF ativou a proteína apoptótica Akt. Inversamente, o tratamento destas células com um antagonista de Trk inibiu a apoptose e diminuiu a proliferação (PEREZ-PINERA *et al*., 2007).

Em neoplasias de mama, a expressão de BDNF mostrou ser mais elevada nas amostras tumorais do que em tecidos mamários não neoplásicos, sendo que os níveis de

transcritos de BDNF foram associados com parâmetros patológicos desfavoráveis e desfechos clínicos adversos (PATANI *et al*., 2011). Além disso, BDNF também induziu resistência a apoptose em células de câncer de mama, enquanto que a injeção de um anticorpo anti-BDNF reduziu o crescimento de tumores enxertados em camundongos (VANHECKE *et al*., 2011). Com relação a outras neoplasias femininas, BDNF igualmente demonstrou estar expresso em tumores de colo uterino (MOON *et al.*, 2011) e ovário (QUIU *et al*., 2006; YU *et al*., 2008; AU *et al*., 2009), tendo sido associado com promoção de metástases e pior sobrevida nestes últimos.

1.3 Câncer de Colo Uterino

1.3.1 Incidência

O câncer de colo uterino é a segunda principal causa de morte em mulheres no mundo, constituindo um importante problema de saúde pública, especialmente nos países em desenvolvimento (FOROUZANFAR, *et al*, 2011). No Brasil, o câncer de colo de útero é considerado o segundo mais comum entre as mulheres, principalmente aquelas com idades entre 40 e 60 anos, sendo superado apenas pelo câncer de mama. Com relação à mortalidade, é a quarta neoplasia mais letal, tendo sido responsável por 5063 mortes em 2009. As estimativas de incidência para 2012 são de 17.540 novos casos (INCA, 2012). Entre os estados brasileiros, considera-se que o Rio Grande do Sul contribua com uma parcela significativa deste número, com 14,51 a 18,88 casos estimados para cada 100.000 mulheres (Figura 10).

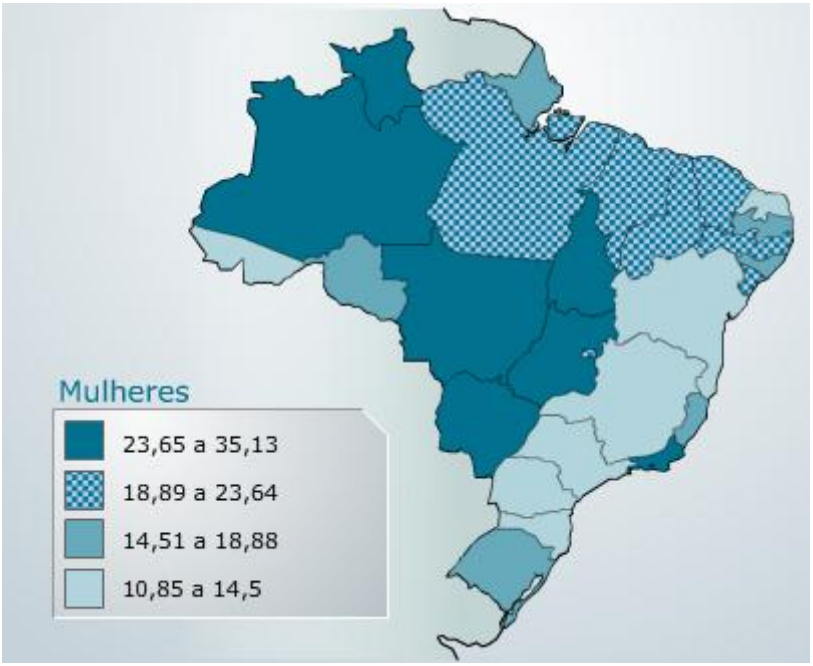

Figura 10: Representação espacial das taxas brutas de incidência de câncer de colo uterino por 100.000 mulheres estimada para o ano de 2012, segundo a Unidade da Federação (INCA, 2012).

Quando diagnosticado nas fases iniciais, o câncer de colo de útero pode ter 100% de cura. Nas últimas décadas houve uma sensível redução na mortalidade e incidência nos países com programas bem estabelecidos de rastreamento, especialmente através do exame Papanicolau (SCARINSI, *et al*, 2010). O impacto do rastreamento na progressão à doença invasiva pode ser verificado pelas diferenças nas incidências idade-específicas entre países desenvolvidos e em desenvolvimento. Comparando-se dados do Reino Unido e Brasil, por exemplo, observa-se que as taxas de incidência entre mulheres jovens são semelhantes, sugerindo níveis similares de exposição aos fatores de risco, enquanto que divergem rapidamente nas mulheres mais velhas, provavelmente

refletindo as diferenças na disponibilidade de rastreamento de massa entre os dois países (BOSH & SANJOSE, 2003) (Figura 11).

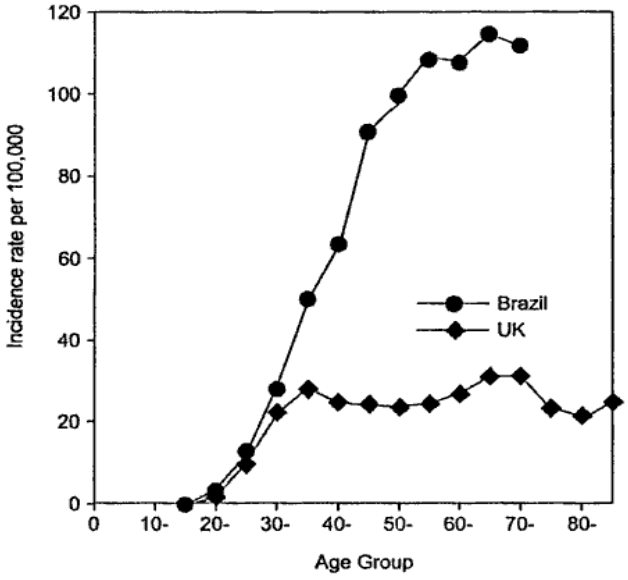

Figura 11: Incidências idade-específicas de câncer de colo de útero invasor no Brasil e Reino Unido (BOSH & SANJOSE, 2003).

1.3.2 Patologia, História Natural e Prognóstico

Cerca de 80% das neoplasias cervicais são carcinomas epidermóides, 20% são adenocarcinomas, enquanto que os sarcomas são raros (SMITH *et al*., 2000). Existem dois tipos celulares embriologicamente distintos que compõem o epitélio cervical. A ectocérvice é a porção do colo que se estende até a vagina, e é constituída de epitélio estratificado escamoso não queratinizado, semelhante ao revestimento da vagina. Já a endocérvice é a parte interna do colo que leva até o útero, sendo constituída de epitélio colunar secretor de muco (SCHIFFMANN *et al*., 1996). A junção dos epitélios colunar e escamoso é chamada de junção escamo-celular (JEC). Com a idade, a junção escamo-

celular acaba migrando para a ectocérvice, ocorrendo a substituição das células colunares por epitélio escamoso estratificado. Este processo é chamado de metaplasia escamosa, e leva à formação da zona de transformação, ou seja, aquela entre a JEC original e a atual. Devida à rápida multiplicação celular, a zona de transformação é altamente suscetível a carcinógenos, carcinogênese e infecções por HPV. A maioria das neoplasias cervicais e suas lesões precursoras são originadas na zona de transformação (Figura 12).

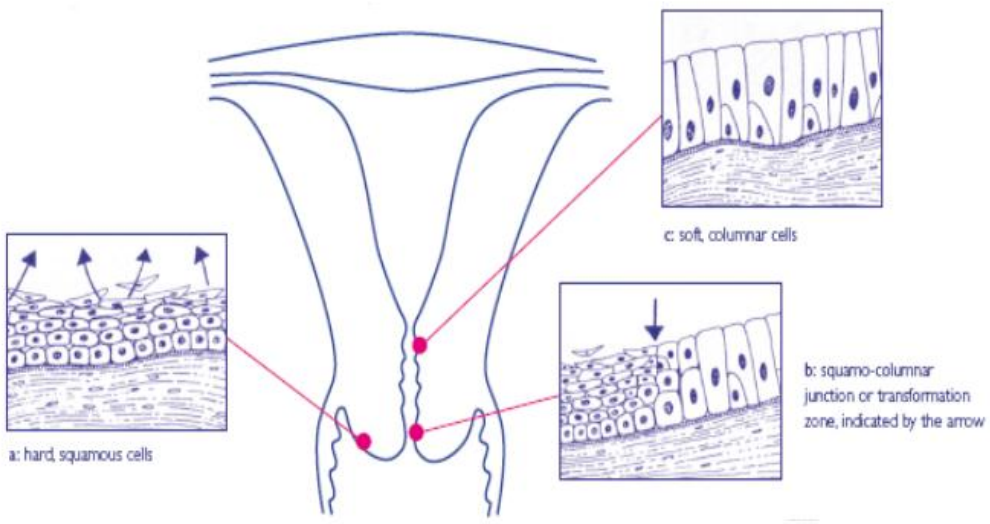

Figura 12: Epitélios constituintes do colo uterino: a) epitélio escamoso, b) junção escamo-celular c) epitélio colunar.

A história natural do câncer de colo uterino já está bem entendida. De uma maneira geral, as lesões iniciam na JEC e progridem lentamente de displasia a carcinoma in situ a câncer invasor. Uma pequena percentagem de lesões evolui em um período de tempo substancialmente mais curto.

As lesões pré-malignas são denominadas neoplasia intraepitelial cervical (NIC) I, II e III conforme a proporção de espessura epitelial acometida por células displásicas (Figura 13). De acordo com a classificação de Bethesda criada inicialmente em 1992,

NIC I corresponde a neoplasia intraepitelial escamosa de baixo grau (LSIL), e NIC II e III a neoplasia intraepitelial escamosa de alto grau (HSIL) (TABBARA et al, 1992). Enquanto na NIC I as alterações são limitadas à camada basal, na NIC III a população neoplásica chega a ocupar até o terço superior do epitélio, já constituindo um carcinoma in situ. Nesta fase a maioria das lesões é assintomática.

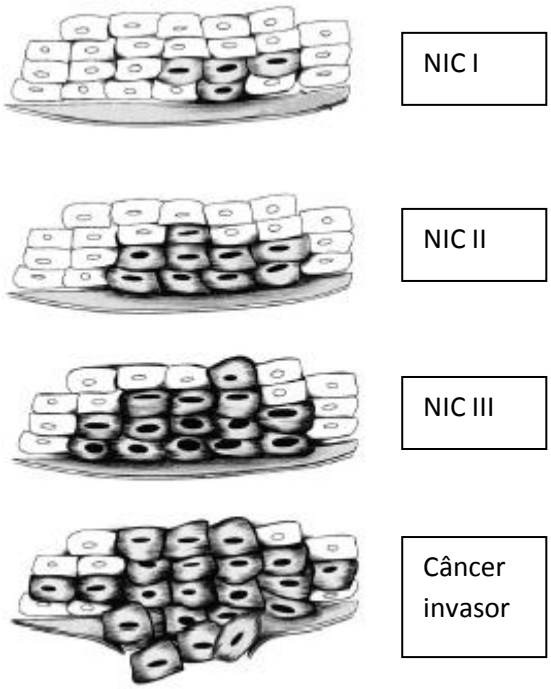

Figura 13: Progressão do câncer de colo uterino.

Após o rompimento da camada basal o tumor se dissemina primariamente por extensão local ao corpo do útero, vagina e outras estruturas pélvicas e sequencialmente às cadeias de linfonodos. Nos pacientes com câncer localmente avançado as metástases hematológicas são pouco comuns (ARENDS *et al*, 1998). Estima-se que 60% das mulheres com NIC I vão apresentar regressão espontânea, 30% podem apresentar persistência da lesão como tal e menos de 10% vão evoluir para NIC III, sendo a chance de progressão a câncer invasor cerca de 1% (SYRJANEN, 1996). O prognóstico desta

neoplasia é marcadamente afetado pela extensão da doença no momento do diagnóstico, sendo influenciado pelo volume e grau do tumor, tipo histológico, disseminação linfática e invasão vascular (HAIE-MEDER, *et al*. 2009).

O tratamento para o câncer de colo uterino inclui cirurgia ou radioterapia, podendo ser adicionada terapia sistêmica com agentes quimioterápicos ou terapias-alvo, conforme o estádio da doença.

1.3.3 Papel do HPV

Estudos epidemiológicos convincentemente demonstram que o principal fator de risco para o desenvolvimento de lesões neoplásicas pré-invasivas e invasivas no colo do útero é a infecção pelo papiloma vírus humano (HPV) (figura 14), superando outros fatores como elevada paridade, maior número de parceiros sexuais, início precoce da atividade sexual, baixo nível socioeconômico e tabagismo (SCHIFFMAN *et al*, 2011). Já foi demonstrado que o DNA do HPV está presente em mais de 95% dos carcinomas cervicais (MUNOZ *et al*., 2003). Supostamente a integração do DNA do HPV ao genoma humano leva à transcrição persistente dos genes E6 e E7, que rompem mecanismos de controle do ciclo celular através da inativação dos oncogenes p53 e Rb, respectivamente (SCHEFFNER *et al*., 1990; TRINGLER *et al*., 2004). Apesar de ser causa necessária para o desenvolvimento de câncer invasor, a especificidade e o valor preditivo positivo da detecção do HPV de alto risco são baixos. Diversos estudos demonstraram que grande parte das lesões intraepiteliais escamosas de baixo grau são positivas para HPVs de alto risco (cerca de 80%) e, mesmo assim, regridem espontaneamente sem tratamento (ALTS GROUP, 2000; EVANS *et al*., 2002).

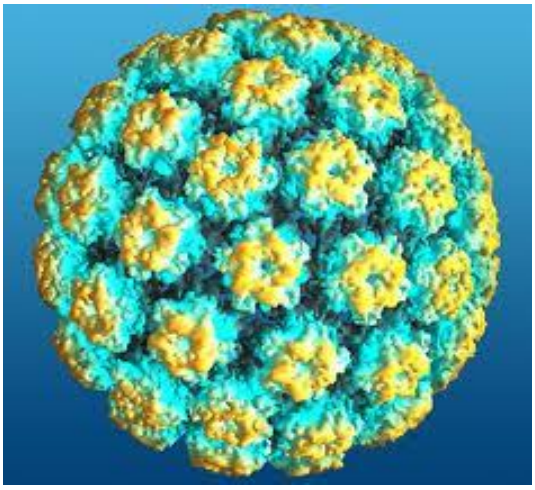

Figura 14: Papilomavírus humano, HPV (virologyspring, 2011).

1.3.4 Exame Papanicolau e Perspectivas de Novos Biomarcadores

A avaliação citológica de esfregaços cervicais foi introduzida na década de 1940 por George Papanicolaou. Este exame é o mais amplamente utilizado no mundo todo para rastreamento do câncer de colo uterino e suas lesões precursoras. O teste baseia-se na coleta de células descamadas da superfície das ecto- e endocérvices uterinas, seguida da observação microscópica das células fixadas e coradas, na busca por anormalidades morfológicas.

Apesar do enorme sucesso do teste na redução das taxas de incidência e mortalidade por câncer de colo uterino, o exame Papanicolau tem muitas limitações, particularmente com relação a resultados falso-negativos no rastreamento (SAFAEIAN *et al.*, 2007). Estudos demonstram que um único teste é apenas 50% sensível para detectar lesões de alto grau ou carcinoma invasivo (FRASER *et al.*, 2005). Melhorias na técnica do Papanicolau, como a citologia em base líquida, não demonstraram melhorar a sensibilidade e especificidade para a detecção de NIC em comparação com a citologia

convencional (ARBYN *et al*., 2008). Além disso, até 10% dos exames Papanicolau são classificados como ASCUS (células escamosas atípicas de significado indeterminado), ou seja, não é possível fazer uma categorização clara de lesão normal, moderada ou grave, ou tumor. No entanto, a experiência mostra que até 10% desta população ASCUS tem lesões de alto grau, que podem passar despercebidas (MANOS *et al.*, 1999). Devido a isso, pesquisar tecnologias alternativas para triagem e diagnóstico para o câncer cervical é essencial. Muitos métodos moleculares foram avaliados para essa finalidade nos últimos anos, entre eles o teste do HPV, MIB-1 e inibidor da proteína quinase dependente de ciclina (p16INK4a) (NAUCLER *et al*., 2007; PINTO *et al*., 2012).

O p16INK4a é considerado um marcador para as atividades oncogênicas do HPV em células cervicais e sua hiperexpressão está bem estabelecida em NIC e câncer invasivo através de muitos estudos (KALOF *et al*., 2006; BENEVOLO *et al*., 2008). Apesar de demonstrar boas correlações com a severidade das alterações celulares, não existem evidências suficientes para o uso rotineiro do p16INK4a na prática clínica. As limitações para este biomarcador incluem a falta de critérios padronizados para avaliar a coloração imunocitoquímica, a falta de consenso quanto aos critérios de positividade e presença de imunorreatividade esporádica em células escamosas normais, atróficas e metaplásicas, bem como em células endocervicais, inflamatórias e em bactérias (TSOMPOU *et al*., 2009).

O Ki-67 é um antígeno que identifica células em proliferação e é expresso em todas as fases do ciclo celular. MIB-1 é um anticorpo monoclonal que detecta este antígeno no núcleo de células fixadas ou em tecidos embebidos em parafina. A infecção por HPV ativa a progressão do ciclo celular do hospedeiro com aumento da cinética do ciclo celular, e este fenômeno é refletido em um aumento da coloração por MIB-1. Os primeiros estudos com este marcador mostraram resultados promissores (DUNTON *et*

al., 1997), porém o MIB-1 nunca foi testado em uma grande coorte. Quando comparado com outros marcadores, como o p16INK4a, geralmente apresenta menor sensibilidade e especificidade para detecção de HSIL (LONGATTO *et al.*, 2005; HALLOUSH *et al.*, 2008).

Apesar das pesquisas promissoras com MIB-1 e p16INK4a, entre outros, até o presente momento ainda não foi encontrado um biomarcador ideal para lesões de colo uterino. Portanto, são necessários outros métodos para detectar o potencial de infecção persistente e de progressão a formas invasivas nas neoplasias cervicais. Além do importante papel na determinação de prognóstico e comportamento biológico das neoplasias, a identificação de novos marcadores biológicos pode contribuir para o melhor rastreamento e tratamento do câncer de colo de útero.

1.4 Câncer de Mama

O câncer de mama é o tumor maligno mais comum entre as mulheres ocidentais e representa um importante problema de saúde pública. Segundo o Instituto Nacional do Câncer, para o ano de 2012 são estimados 52.680 novos casos e cerca de 12.000 mortes no Brasil. O câncer de mama é responsável por um terço das mortes por câncer em mulheres com idade entre 35-55 anos (INCA, 2012). De uma maneira geral, a incidência do câncer de mama vem aumentando nas últimas décadas, tanto em países desenvolvidos, quanto em países em desenvolvimento, como pode ser visualizado na figura 15.

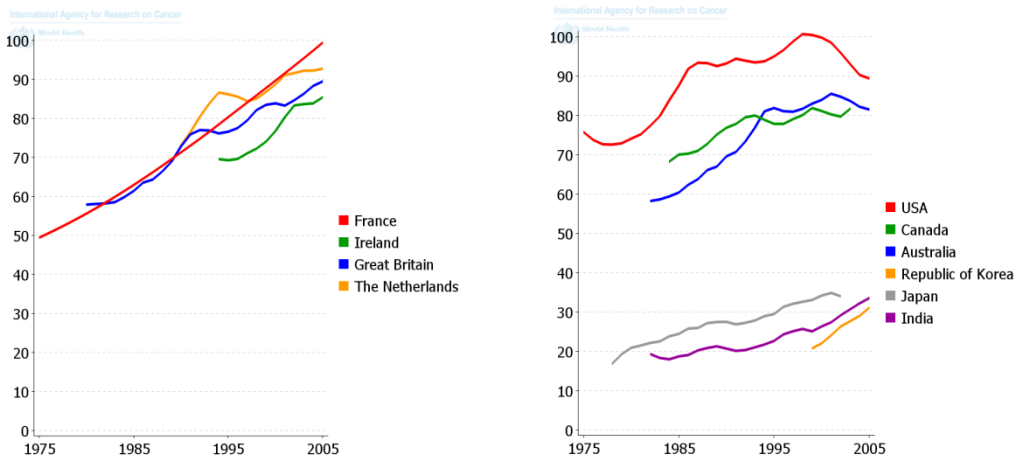

Figura 15: Incidências de câncer de mama em diferentes países do mundo (GLOBALCAN, 2008).

São fatores de risco a idade avançada, a exposição prolongada aos hormônios femininos, o excesso de peso, a história familiar, não ter tido filhos ou ter tido o primeiro filho após os 35 anos, menarca precoce e menopausa tardia (DAVIES et al., 2011; MACCIÒ & MADEDDU, 2011). No entanto, há casos de mulheres que desenvolvem a doença sem apresentar fatores de risco identificáveis.

As neoplasias malignas mamárias se dividem em tumores epiteliais (carcinomas) que podem ser de origem ductal ou lobular, e sarcomas que se originam no tecido conjuntivo. Os carcinomas são a maioria das neoplasias malignas da mama, sendo o carcinoma ductal invasor o tipo mais comum, presente em 90% dos casos. Os carcinomas ainda podem ser in situ, quando confinados aos ductos ou lóbulos, ou invasores, quando invadem o estroma.

O rastreamento do câncer de mama tem como objetivo primordial detectar tumores insipientes, quando ainda não há comprometimento linfonodal e sistêmico. A detecção do câncer de mama em suas fases iniciais tem impacto significativo na redução da mortalidade e no aumento da expectativa de vida (MICHAELSON *et al.*, 2003).

Portanto, muitos esforços da oncologia moderna estão focados em alcançar diagnósticos mais precoces, quando a doença ainda é limitada e os tumores são ressecáveis, possibilitando tratamentos com intenção curativa (HOWARD & BLAND, 2012).

O tratamento para o câncer de mama é baseado em cirurgia, radioterapia e terapia sistêmica, esta última podendo incluir hormônio ou quimioterapia, além de tratamentos com drogas alvo. Estudos emergentes têm revelado mecanismos genéticos e moleculares de transformação neoplásica que estão levando a perspectivas terapêuticas mais individualizadas, potencialmente com maior efetividade e com redução de toxicidade e custos. Diversas características biomoleculares específicas já foram identificadas no câncer de mama, como mutações nos genes BRCA1 e BRCA 2 (GAGE *et al*., 2012; HARTMAN *et al*., 2012), superexpressão do receptor do EGFR-2 (HER-2) (ARTEAGA *et al*, 2011; TSANG & FINN, 2012) e ativação do VEGF (ALVAREZ *et al*., 2011; MONTERO *et al*., 2012). Além desses, muitos outros biomarcadores vêm sendo estudados, e os resultados obtidos até agora encorajam a busca e o desenvolvimento de novos compostos para o tratamento mais efetivo do câncer de mama.

1.5 Câncer de Ovário

O câncer de ovário é o tumor ginecológico mais difícil de ser diagnosticado e o que apresenta as menores chances de cura. No ano de 2010 ocorreram 2.979 mortes no Brasil, e para o ano de 2012 são esperados 6.190 casos novos (INCA, 2012) (Figura 16).

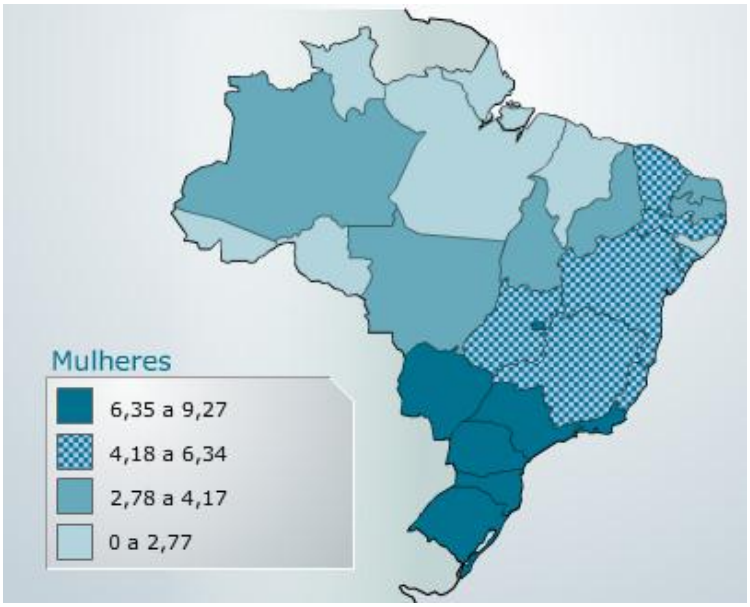

Figura 16: Representação espacial das taxas brutas de incidência de câncer de ovário por 100.000 mulheres estimada para o ano de 2012, segundo a Unidade da Federação (INCA, 2012).

Devido a sintomas inespecíficos, ao diagnóstico em fases avançadas, à falta de biomarcadores confiáveis e à presença de tipos histológicos resistentes a drogas, o prognóstico e as taxas de cura em longo prazo são limitados, tornando o câncer de ovário a mais letal das neoplasias ginecológicas. Aproximadamente 70% das mulheres se apresentam com doença avançada ao diagnóstico, e 65% morrem dentro dos primeiros cinco anos (LUTZ. *et al.*, 2012).

Apesar de controvérsias quanto à histogênese dos tumores ovarianos, acredita-se que a maioria se origina do epitélio da superfície ovariana, sendo o carcinoma epitelial o subtipo mais comum. (FEELEY & WELLS, 2001). No passado, os carcinomas epiteliais ovarianos eram considerados uma única doença, tanto de uma perspectiva biológica como terapêutica. Atualmente, com os avanços em estudos genéticos e patológicos, são reconhecidos diversos subtipos histológicos, os quais possuem diferentes fatores de risco, anormalidades genéticas e vias de sinalização oncogênicas,

que determinam seu comportamento biológico, resposta à quimioterapia e prognóstico (LALWANI *et al*., 2011).

Os tumores ovarianos geralmente se disseminam localmente para a cavidade peritoneal, seguido por implantes no peritônio, invasão de intestino e bexiga. Nos estádios iniciais, o tratamento baseia-se em histerectomia total, salpingo-ooforectomia bilateral e omentectomia (YOUNG *et al*., 1983; ELATTAR *et al*., 2011). Já em tumores grau III, densamente aderentes ou estádio IV, a chance de recidiva e morte é elevada, podendo atingir 30% (MONGA *et al*., 1991; KOLOMAINEN *et al*., 2003). Nesses casos, podem ser indicadas radioterapia (HEINZELMANN-SCHWARTZ, 2011), quimioterapia intraperitoneal (JAABACK *et al*., 2011) ou quimioterapia sistêmica baseada em platina isolada ou em combinação com agentes alquilantes ou taxanos (MEI *et al*., 2010; PIGNATA *et al*., 2011).

As terapias-alvo também estão sendo introduzidas para o tratamento câncer de ovário. O anticorpo monoclonal anti-VEGF, bevacizumab, é o mais estudado até o momento, tanto como monoterapia, quanto em associação com outras drogas, demonstrando aumentar o intervalo livre de doença e até mesmo a sobrevida global (MONK *et al*., 2005; BRAGHIROLI *et al*., 2012). Terapias dirigidas a membros da família do EGFR também estão sendo igualmente avaliadas com os anticorpos monoclonais anti-HER2, trastuzumab e pertuzumab (BOOK *et al*., 2003; SIMS *et al*., 2012) e com o anticorpo monoclonal anti-HER1 cetuximab (SECORD *et al*., 2008). Inibidores de poly(ADP-ribose) polymerase (PARP), inicialmente preconizados para tumores com mutações germinativas do gene BRCA, têm demonstrado atividade em neoplasias epiteliais ovarianas em estudos de fase I e II. As respostas têm sido observadas tanto em tumores deficientes em BRCA ou esporádicos (ZORN, 2012).

2 – OBJETIVOS

2.1. OBJETIVOS GERAIS

2.1.1 Avaliar a expressão de GRPR em amostras cervicais não neoplásicas, lesões pré-invasivas e invasivas.

2.1.2 Avaliar a influência de GRP/GRPR e BDNF/Trk na viabilidade de células de câncer de colo uterino, ovário e mama.

2.2 OBJETIVOS ESPECÍFICOS

2.2.1 Avaliar a expressão de GRPR em tecidos cervicais sadios, em neoplasias pré-invasivas e invasivas através da técnica de imuno-histoquímica.

2.2.2 Avaliar por imuno-histoquímica se existe diferença de expressão do GRPR entre as formas pré-invasivas (NIC I, II e III) e invasivas (carcinomas epidermóides e adenocarcinomas) de colo uterino.

2.2.3 Avaliar por imuno-histoquímica se existe diferença de expressão do GRPR entre as lesões neoplásicas e os tecidos cervicais adjacentes.

2.2.4 Avaliar a expressão de GRPR e p16 em esfregaços cervicais não neoplásicos e neoplásicos através da técnica de imunocitoquímica e em seus tecidos correspondentes através da técnica de imuno-histoquímica.

2.2.5 Realizar o exame de Papanicolau em esfregaços cervicais não neoplásicos e neoplásicos.

2.2.6 Comparar a expressão de GRPR nos esfregaços cervicais com as amostras teciduais correspondentes.

2.2.7 Comparar o potencial diagnóstico da análise da expressão de GRPR por imunocitoquímica com o exame Papanicolau.

2.2.8 Avaliar a expressão de GRPR e BDNF em linhagens celulares de câncer de mama (MCF-7), ovário (OVCAR-3) e colo uterino (HeLa) através da técnica de Rt-PCR.

2.2.9 Avaliar a viabilidade celular nas linhagens MCF-7, OVCAR-3 e HeLa após os tratamentos com GRP, antagonistas de GRPR, RC-3095, RC-3094-II, BDNF e antagonista de TrKB, K252a, através da técnica de MTT.

Capítulo I

Gastrin-Releasing Peptide Receptor Expression in Cervical Cancer

Artigo publicado em Oncology em 2008

Parte dos resultados apresentados neste capítulo foram previamente apresentados em formato preliminar na Dissertação de Mestrado da candidata (Cornelio, D.B., "Expressão do receptor do peptídeo liberador da gastrina em câncer decolo de útero", Dissertação, Programa de Pós-Graduação em Biologia Celular e Molecular, Universidade Federal do Rio Grande do Sul, 2007).

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Gastrin-Releasing Peptide Receptor Expression in Cervical Cancer

Daniela Baumann Cornelio[a, b] Luise Meurer[c] Rafael Roesler[a, b, e]
Gilberto Schwartsmann[a, d]

[a]Cancer Research Laboratory, Academic Research Center, [b]Graduate Program in Cellular and Molecular Biology, Center for Biotechnology, Departments of [c]Pathology and [d]Internal Medicine, Faculty of Medicine, and [e]Cellular and Molecular Neuropharmacology Research Group, Department of Pharmacology, Institute for Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Key Words
Cervical cancer · Gastrin-releasing peptide · Gastrin-releasing peptide receptor · Bombesin · Immunohistochemistry

Abstract
Objectives: It was the aim of this study to evaluate whether cervical neoplasms and adjacent tissues express the gastrin-releasing peptide receptor (GRPR) and whether there is a significant difference in its distribution among preinvasive and invasive cancers. ***Methods:*** Sections of paraffin-embedded cervical tumors (n = 88) and non-neoplastic control cervical tissues (n = 14) obtained from women registered in the Pathology Department, Academic Hospital, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, were investigated by immunohistochemistry for GRPR. ***Results:*** GRPR was detected in 99% of tumor specimens, mostly exhibiting a diffuse strong staining. The receptors were seldom detected in the endocervices, while ectocervices expressed GRPRs only when adjacent to neoplastic lesions. No correlation between GRPR expression and preinvasive and invasive neoplasms was found. ***Conclusions:*** To the best of our knowledge, this is the first demonstration of the widespread GRPR expression in human cervical cancer. The presence of receptors both in tumors and surrounding tissues may suggest that GRPR can play a role in the cervical carcinogenic process. These data provide a molecular basis for exploiting GRPR as a target for diagnostic and therapeutic purposes in cervical neoplasms.

Introduction

Cervical cancer is the second largest cause of cancer deaths in woman worldwide, with higher rates in developing countries [1]. Epidemiologic studies convincingly demonstrate that the major risk factor for development of preinvasive or invasive carcinoma of the cervix is human papillomavirus infection, which far outweighs other known risk factors [2]. Cervical cancer progresses from dysplasia to carcinoma in situ to cancer, and the prognosis is markedly affected by the extent of the disease at the time of diagnosis. There has been a reduction in incidence and mortality in countries where there is an established screening program, especially through Papanicolaou smear [3]. Although this is an effective screening tool, it lacks high sensitivity. In recent years, there has been much effort to develop newer molecular methods for diagnostic and therapeutic purposes [4].

Gastrin-releasing peptide (GRP), the mammalian equivalent of bombesin, is a neuroendocrine peptide shown to have growth-stimulatory effect on many types

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Daniela Baumann Cornelio, MD, MSc
Hospital de Clínicas de Porto Alegre, 3° andar Leste
Rua Ramiro Barcelos, 2350
90035-003, Porto Alegre, RS (Brazil)
Tel. +55 51 2101 8012, Fax +55 51 3388 2877, E-Mail danicornelio@terra.com.br

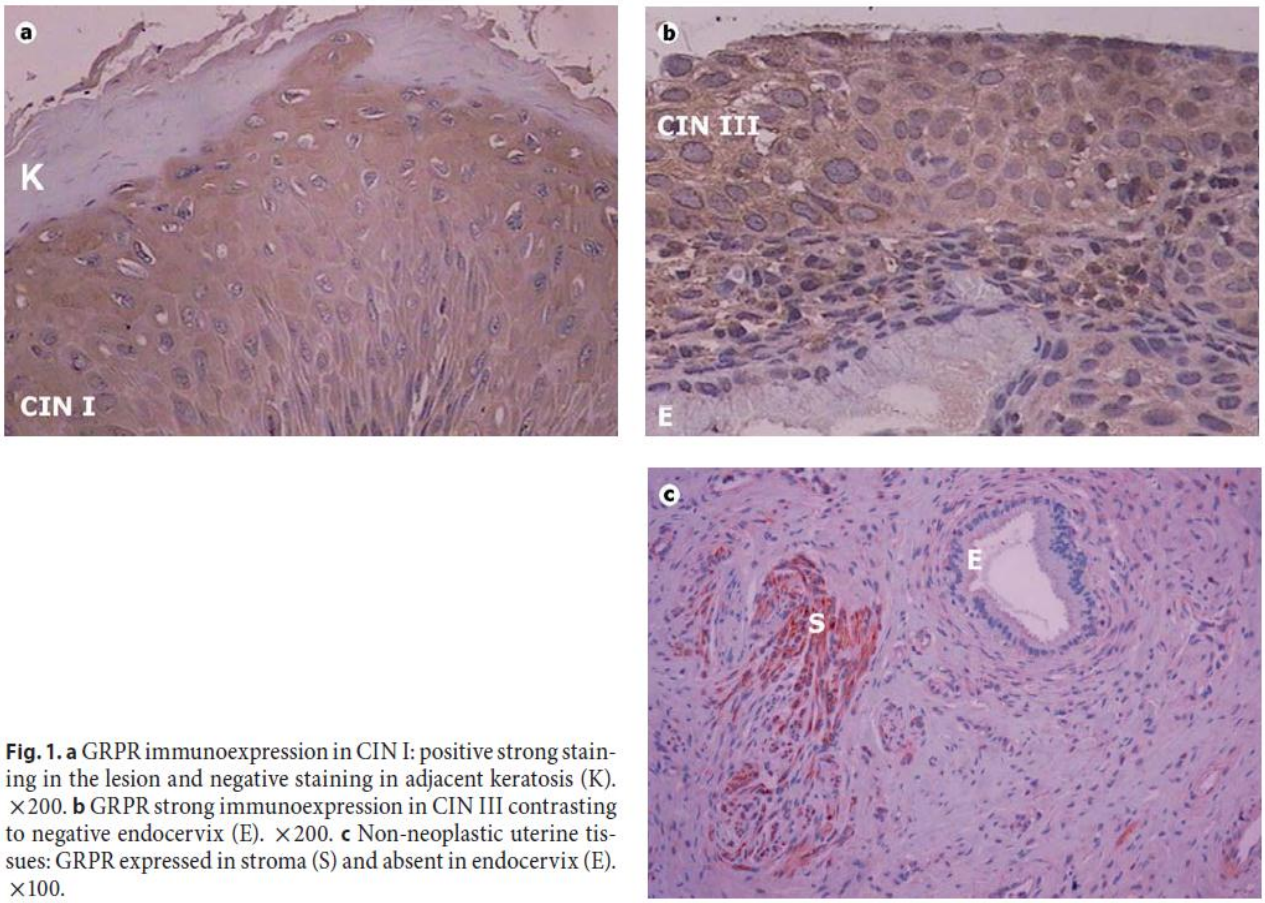

Fig. 1. a GRPR immunoexpression in CIN I: positive strong staining in the lesion and negative staining in adjacent keratosis (K). ×200. **b** GRPR strong immunoexpression in CIN III contrasting to negative endocervix (E). ×200. **c** Non-neoplastic uterine tissues: GRPR expressed in stroma (S) and absent in endocervix (E). ×100.

of cancer [5–7]. The GRP-preferring receptor (GRPR) belongs to the G-protein receptor superfamily and activates multiple signal transduction pathways, resulting in cell proliferation and growth [8]. For that reason, many GRPR antagonists have been developed as anticancer candidate compounds, exhibiting impressive antitumor activity both in vitro and in vivo in various murine and human tumors [9–11]. GRP analogues are also currently being investigated for their ability to bind to GRPRs, acting as carriers for cytotoxins, immunotoxins or radioactive compounds. Similar to somatostatin analogues, which are already being applied in the clinical practice, GRP analogues are showing promising results for tumor detection through scintigraphy [12, 13].

GRPR is overexpressed in a wide variety of human malignancies, including prostate [14–16], breast [17–19], lung [17, 20], head and neck [21], gastric [22, 23], colon [24–27], esophageal [28] and renal cancer [29, 30]. Among gynecological tumors, GRPRs were detected in high density in ovarian [31] and uterine carcinomas [32]. To our knowledge, the status of GRPR expression has not yet been systematically examined in cervical cancer. Due to the relevance of this tumor type in terms of morbidity and mortality, especially in developing countries, we aimed to examine whether GRPR is present in cervical cancer and adjacent tissues and if there is a significant difference in its expression among the preinvasive and invasive neoplasms.

Materials and Methods

Eighty-eight cervical tumors were identified from the pathology records of the Pathology Department, Academic Hospital, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, between January and December 2004. The samples consisted of 22 cervical intraepithelial neoplasias (CIN) I, 19 CIN II, 26 CIN III, 13 squamous cancers and 8 adenocarcinomas. An additional 14 samples of non-neoplastic uterine cervices were also selected as

Table 1. GRPR incidence, distribution and density in cervical cancer and adjacent ectocervix and endocervix

Pathological grade	GRPR immunoexpression				
	negative	weak focal	weak diffuse	moderate diffuse	strong diffuse
CIN I	0	0	3 (13.6)	10 (45.5)	9 (40.9)
Ectocervix	2 (14.3)	1 (7.1)	8 (57.1)	3 (21.4)	0
Endocervix	11 (84.6)	2 (15.4)	0	0	0
CIN II	1 (5.3)	0	2 (10.5)	12 (63.2)	4 (21.1)
Ectocervix	2 (15.4)	3 (23.1)	5 (38.5)	3 (23.1)	0
Endocervix	14 (100)	0	0	0	0
CIN III	0	2 (7.7)	7 (26.9)	10 (38.5)	7 (26.9)
Ectocervix	4 (26.7)	2 (13.3)	7 (46.7)	2 (13.3)	0
Endocervix	15 (71.4)	5 (23.8)	0	1 (4.8)	0
Squamous cancer	0	0	3 (23.1)	3 (23.1)	7 (53.8)
Ectocervix	2 (100)	0	0	0	0
Endocervix	2 (100)	0	0	0	0
Adenocarcinoma	0	1 (12.5)	1 (12.5)	4 (50.0)	2 (25.0)
Ectocervix	0	1 (33.3)	2 (66.7)	0	0
Endocervix	NA	NA	NA	NA	NA
Total lesions	1 (1.1)	3 (3.4)	16 (18.2%)	39 (44.3)	29 (32.9)
Total ectocervices	10 (21.3)	7 (14.9)	22 (46.8)	8 (17)	0 (0)
Total endocervices	42 (84)	7 (14)	0 (0)	1 (2)	0 (0)

Figures in parentheses are percentages. NA = Not accessed.

controls. A representative paraffin wax block was chosen for each case. Samples were processed by immunohistochemical technique. The primary antibody used was a rabbit polyclonal antibody anti-GRPR (Affinity Bioreagents, Golden, Colo., USA). After dewaxing, inactivating endogenous peroxidase activity and blocking cross-reaction with normal serum, 4-μm sections were incubated overnight at 4°C with a diluted solution of the primary antibody (1:50). Identification of primary antibody location was achieved by subsequent application of biotinylated antibody, streptavidin horseradish peroxidase conjugate (LSAB, Dako) and diaminobenzidine tetrahydrochloride/H_2O_2 (Kit DAB, Dako). A pancreatic cancer known as positive was used as positive control, and negative control was obtained by omitting the primary antibody. Staining was interpreted by 2 blinded pathologists, with a percent of agreement >90%.

Immunohistochemical staining was scored semiquantitatively according to intensity and distribution, similar as described by Scott et al. [33], in the following way: for intensity, 0 = no staining; 1 = weak staining; 2 = moderate staining, and 4 = strong staining; for distribution, 1 = less than 10% cells stained (focal), and 3 = more than 10% cells stained (diffuse). For the purposes of data presentation, tumors were considered negative if the sum score of intensity and distribution was ≤1, weak positive if the sum score was between 2 and 4 (weak diffuse, moderate or strong focal) and strong positive if the sum score was ≥5 (moderate or strong diffuse). Comparisons among lesions and surrounding tissues, as well as comparisons among the distinct pathological grades of cervical cancer were performed using Kruskal-Wallis analysis of variance followed by Mann-Whitney tests.

Results

GRPR immunoexpression demonstrated a diffuse cytoplasmic staining pattern among the neoplastic lesions (fig. 1a). The marker was expressed in 99% of the tumoral tissues, mostly ranging from moderate to strong staining. In contrast, the receptors were seldom detected in endocervices surrounding the lesions; 15% of the samples were classified as weak focal and therefore considered negative, and only 1 sample showed positivity (fig. 1b). GRPR was expressed in 63.8% of ectocervices adjacent to neoplastic processes, mostly with a weak diffuse signal. None of these ectocervices exhibited a strong diffuse staining. Kruskal-Wallis analysis of variance showed a significant difference in GRPR expression among the malignant processes and the adjacent tissues (d.f. = 2; H = 118.28; $p < 0.0001$). Further analysis using Mann-Whitney tests confirmed that GRPR expression was significantly higher in the lesions when compared with both endocervices and ectocervices ($p < 0.0001$). The results between endocervices and ectocervices were also significantly different ($p < 0.0001$). When control non-neoplastic uterine samples were analyzed, 85.7% of the endocervices had no GRPR expression (fig. 1c), and Mann-Whitney tests showed no significant differences in the

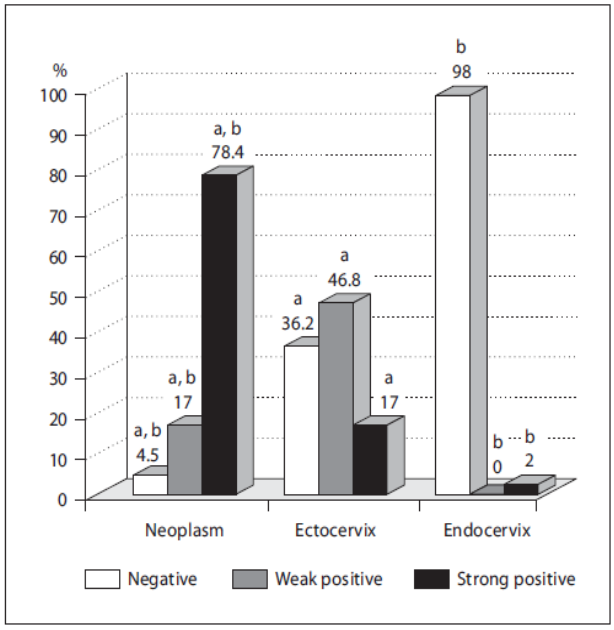

Fig. 2. Overall expression of GRPR in cervical cancer and adjacent tissues. [a] $p < 0.0001$ compared with endocervix; [b] $p < 0.0001$ compared with ectocervix.

endocervices among neoplastic and control tissues ($p = 0.11$). On the other hand, 100% of the ectocervices were negative for GRPR, contrasting with the positive expression found in ectocervices adjacent to malignant lesions. This difference was also of statistical significance ($p < 0001$). Table 1 describes the results of GRPR immunostaining for the different pathological grades.

The receptor distribution was homogeneous among the tumoral specimens; from the preinvasive form CIN I to the invasive cancers, no statistical differences were noted (d.f. = 4; H = 4.82; $p = 0.3$). Also, the ectocervices and endocervices surrounding the lesions did not differ significantly regarding GRPR status among the groups (d.f. = 4; H = 4.18; $p = 0.4$, and d.f. = 3; H = 1.38; $p = 0.7$, respectively). Figure 2 shows the overall GRPR expression in malignant and normal tissues.

Discussion

In this study, we demonstrated the presence of abundant expression of GRPRs in human cervical cancer, while the adjacent ectocervices had a less pronounced receptor density, and the endocervices had virtually no detectable receptors. To the best of our knowledge, this detailed evaluation of the status of GRPR expression in cervical cancer has not been previously reported in the literature.

Cancer cells are known to proliferate excessively in response to stimuli, such as growth factors. Over the last decades, several lines of experimental evidence have suggested that GRP and other bombesin-like peptides (BLPs) may play a role in cancer development [5–7]. GRP has been recognized as an autocrine mitogen based on the detection of the growth factor and its cognate receptor in the same tissue, resulting in proliferation [5]. Recent data show that GRP also has paracrine and endocrine effects and functions as a morphogen and a proangiogenic agent [9]. Additionally, the inhibition of GRPR was demonstrated to interfere with other relevant growth factor pathways such as the epidermal growth factor- and vascular endothelial growth factor-dependent signaling pathways [34, 35].

We recently reviewed the GRPR expression status for human cancers [36]. These receptors have been localized in many human malignancies through different methods, most commonly binding assays or mRNA detection with RT-PCR. The use of distinct techniques lead to variable results in receptor distribution. Even when the same methods were employed, the quantification of results was not uniform between authors. We have opted for immunohistochemistry for our analysis, because it is considered a simple and affordable technology that is already part of the pathologist's routine. Moreover, immunohistochemistry preserves both tissue architecture and cellular morphology, so the immunoreactions can be attributed to specific subpopulations.

Our results demonstrated a diffuse immunopositivity in 99% of the neoplastic lesions, with the great majority exhibiting a strong staining pattern. Other types of cancer showed the same widespread GRPR expression. Prostate cancer specimens were found to be 100% GRPR positive by distinct authors and methods [14, 15, 17]. When matched normal prostatic tissues were analyzed, they either rarely expressed measurable amounts of GRPR or displayed a low message staining [14, 15]. Based on these findings, it was postulated that these receptors could be useful in differentiating hyperplasia from neoplasia. Chave et al. [25] evaluated normal and neoplastic colorectal tissues and detected GRPRs in all samples, with overexpression in the tumoral ones, supporting the possibility of GRP acting as an autocrine growth factor. Pansky and colleagues [29] also demonstrated GRPR expression in renal cell carcinoma, but not in normal kidney tissues. Other researchers reinforced the role of GRP in cancer

development by their results in patients with neuroblastomas [37], as well as in patients with squamous cell carcinoma of the head and neck [21], where the receptor was found in all tumoral tissues analyzed. In summary, the expression of GRPR is much more commonly found in malignancies than in normal human tissues.

BLPs have been extensively demonstrated in normal tissues. They play many physiological roles including the regulation of smooth muscle contraction, the release of hormones and the secretion of enzymes [38]. During fetal development, GRPR expression has been established at various locations in the respiratory, nervous, urogenital and gastrointestinal systems [39]. In adults, GRPR has been shown to occur in peripheral blood cells from healthy subjects [40] and in several organs, such as the gastric antrum [41] and the pancreas [42]. Gugger and Reubi [19] described a ubiquitous GRPR expression in non-neoplastic breast lobules and ductules, suggesting a role of GRP in breast physiology. Regarding the female genital tract, physiological functions such as contractions of the rat uterine smooth muscles [43] and growth of human endometrial stroma cells [44] have been reported. High concentrations of BLPs were found in vaginas of rat females [45]. In humans, various uterine tissues, namely myometrium, endometrial glands and vessels, are able to express GRPRs under physiological conditions. Fleischmann and colleagues [32] recently evaluated normal and neoplastic uteri and described overexpression of GRPRs in normal myometrium in all phases of the cycle and after menopause, as well as in functional endometrium. In the present study, we confirm these previously reported findings, as our non-neoplastic specimens exhibited GRPRs in uterine stroma cells and myometrium.

To date, no data have been reported for normal cervical tissues. Histologically, the ectocervix is covered with stratified squamous epithelium that is essentially identical to the epithelium of the vagina. Since BLPs have been described in normal vagina [45], we were not surprised to find GRPRs in most ectocervices of our neoplastic specimens; GRPRs could be promoting physiological trophic effects in these tissues. However, when non-neoplastic cervical samples were analyzed, no receptors were found in the ectocervices. Based on these findings, we could assume that the presence of GRPRs in tumor-adjacent ectocervices could represent an early molecular event in cervical carcinogenesis. Interestingly, GRPR immunosignaling in the endocervices adjacent to neoplastic lesions was significantly lower as compared with ectocervices. Further evaluation is required to elucidate the heterogeneous GRPR distribution in cervical nonmalignant tissues.

It remains unclear whether GRPR expression in neoplasms represents only a tumor characteristic or signals the aggressive biologic behavior of these cancers. Trying to address this question, we attempted to correlate the level of GRPR expression with the invasiveness of cervical cancer. Some authors were able to associate a higher incidence of GRPRs with poorly differentiated or more aggressive carcinomas [26, 31, 46]. In our study, we found no significant differences among the neoplastic specimens; GRPRs were quantitatively present in all of the lesions, from preinvasive CIN I to invasive carcinoma. As shown in previous studies, GRPR expression could not be associated with disease progression [16, 37].

New emerging technologies are having a great impact on cancer screening, detection, treatment and prevention. Like epidermal and other growth factor receptors, GRPRs are being exploited for diagnostic and therapeutic interventions [9, 10, 36]. GRP analogues can either be labeled with radionuclides for tumor imaging and detection or conjugated to toxins for drug delivery. Furthermore, monoclonal antibodies are already in phase II clinical trials achieving interesting results, and GRPR antagonists are demonstrating remarkable antitumor activity for many cancer types.

Since cervical cancer contributes significantly to cancer-related morbidity and mortality worldwide, and given the aberrant GRPR expression found in this study, we propose that these neoplasms should also be considered for potential clinical applications of GRP analogues and GRPR antagonists.

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Capítulo II

The Gastrin-Releasing Peptide Receptor as a Marker of Dysplastic Alterations in Cervical Epithelial Cells

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The Gastrin-Releasing Peptide Receptor as a Marker of Dysplastic Alterations in Cervical Epithelial Cells

Daniela Baumann Cornelio[a, e] Luise Meurer[b] Gilberto Schwartsmann[a, c, e]
Rafael Roesler[a, d, e]

[a]Cancer Research Laboratory, University Hospital Research Center (CPE-HCPA), Departments of [b]Pathology and [c]Internal Medicine, Faculty of Medicine, and [d]Laboratory of Neuropharmacology and Neural Tumor Biology, Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, and [e]National Institute for Translational Medicine, Porto Alegre, Brazil

Key Words
Gastrin-releasing peptide · Gastrin-releasing peptide receptor · Cervical dysplasia · Cervical cancer

Abstract
Background: Cervical cancer is a leading cancer in women worldwide. The Papanicolaou test (Pap test) remains the main screening tool; however, it produces high rates of false-negative and false-positive results. Gastrin-releasing peptide is a growth factor that has been implicated in many cancers, and its main receptor, the gastrin-releasing peptide receptor (GRPR), is nearly always expressed in cervical dysplasias and invasive carcinomas. The aim of this study was to evaluate the diagnostic potential of GRPR immunocytochemistry in detecting cervical dysplasia and invasive cancer. ***Methods:*** Cervical smears were collected from 66 women in Brazil and subjected to GRPR immunocytochemistry and the Pap test. GRPR and p16 immunohistochemistry were performed in biopsies if abnormalities were detected. ***Results:*** GRPR immunostaining sensitivity in detecting cervical lesions was 87.5% and its specificity was 76.7%. GRPR immunostaining showed 80% accuracy in identifying atypical squamous cells of undetermined significance (ASCUS), with 88% sensitivity and 71% specificity. ***Conclusion:*** This is the first immunocytochemical evaluation of GRPR expression in cervical epithelial cells. This biomarker was strongly associated with cervical dysplasia and invasive cancers. GRPR immunosignaling showed high accuracy in detecting dysplasias in cells classified as ASCUS by Pap tests. Based on these results, immunocytochemistry for GRPR may be regarded as a valuable method for early detection of cervical intraepithelial neoplasia.

Introduction

Cervical cancer is the second most common cancer in women worldwide, with about 500,000 new cases each year. The incidences are highest in developing countries, where carcinoma of the cervix is the leading cause of female cancer mortality [1]. Since the introduction of the Papanicolaou cytological screening (Pap test) there has been a significant reduction in the incidence and mortality from cervical cancer. However, many women are still

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Daniela Baumann Cornelio
Cancer Research Laboratory, University Hospital Research Center (CPE-HCPA)
Federal University of Rio Grande do Sul
90035-003 Porto Alegre, RS (Brazil)
E-Mail danicornelio@terra.com.br

not properly screened, either because of low coverage of Pap testing or because of the limitations of this method.

The efficacy of the Pap test, a single test only 50% sensitive in detecting high-grade lesions or invasive carcinomas, is hampered by high rates of false-negative and false-positive results [2]. Technical improvements of the Pap test, such as liquid-based cytology, have not improved its sensitivity or specificity in detecting cervical intraepithelial neoplasia (CIN) compared to conventional cytology [3]. Furthermore, up to 10% of Pap tests are classified as atypical squamous cells of undetermined significance (ASCUS), i.e. specimens that cannot be clearly categorized as normal, or as displaying moderate or severe lesions, or tumors. However, experience shows that up to 10% of patients with an ASCUS classification have high-grade lesions, which are thus overlooked [4]. Therefore, it is essential to search for alternative screening and diagnostic technologies in cervical cancer. Many molecular methods have been evaluated for this purpose in recent years, among them human papillomavirus (HPV) testing, Ki-67 and p16INK4a (p16) detection [5]. The cyclin-dependent kinase inhibitor protein p16 is considered a surrogate marker of the oncogenic activities of HPV in cervical cells, and its overexpression has been well established in CIN and invasive cancer by many studies [6–9].

Gastrin-releasing peptide (GRP) is a neuroendocrine peptide shown to have growth-stimulatory effects on many types of cancer [10]. The GRP-preferring receptor (GRPR, BB2 receptor) belongs to the G-protein receptor super family and activates multiple signal transduction pathways, resulting in cell proliferation and growth [11]. GRPR is overexpressed in a wide variety of human malignancies, including prostate, breast, ovary, lung, head and neck, gastric, colon, esophageal and renal cancers, and glioma [10, 12]. In addition, we have recently described the aberrant expression of GRPR in both cervical dysplasias and invasive carcinomas. Based on the presence of this receptor in 99% of the evaluated samples, but not in non-malignant cervices, it has been suggested that GRPR may play a role in cervical carcinogenesis [13]. These data provided the molecular basis for exploiting GRPR as a target for cervical cancer diagnosis. GRPR is already being studied for diagnostic purposes in other types of cancer, like breast and prostate cancer, with promising results [14–17]. However, to date no studies have evaluated GRPR expression in cancer using immunocytochemistry.

In this study, we aimed to investigate the sensitivity and specificity of GRPR immunocytoexpression in detecting cervical dysplasia and invasive cancer in comparison to conventional cytology. We also intended to verify whether immunocytochemical staining in cervical smears correlates to immunohistochemical signaling in the corresponding tissue specimens.

Materials and Methods

Data and Specimen Collection

Between 2009 and 2010, 66 cervical samples were randomly collected from women who attended the Gynecological Clinic at the University Hospital of Irmandade Santa Casa de Misericórdia in Porto Alegre, Brazil. The sample population consisted of 36 selected women who had been referred for colposcopy because of abnormal Pap tests (16 ASCUS, 5 CIN I, 15 CIN II–III), and 30 women undergoing routine cervical cancer screening. The study was approved by the local Research Ethics Committee (3025/09); all subjects invited to take part in the study agreed to do so, and informed consent was obtained. Patients included were aged 21–64, with a median age of 37 years. Two samples were collected from each individual by one specialist gynecologist using a spatula and cytobrush. The first smear was used for GRPR immunocytochemistry and the second for the conventional Pap test, which was performed at the hospital's pathology laboratory and interpreted by a certified pathologist. All specimens were classified according to the criteria of the World Health Organization as either normal cervix, ASCUS, mild dysplasia (CIN I), moderate dysplasia (CIN II), severe dysplasia or carcinoma in situ (CIN III), invasive squamous or adenocarcinoma. According to the Bethesda criteria, CIN I is classified as a low-grade squamous intraepithelial lesion (LSIL) and CIN II–III as a high-grade squamous intraepithelial lesion (HSIL). Cases were managed clinically according to test results. Biopsies were taken if abnormalities suggestive of cervical premalignancy or malignancy were visualized either at the time of initial specialist examination or during any subsequent colposcopy. The biopsies were evaluated by immunohistochemistry for GRPR and p16 expression.

Immunohistochemistry and Immunocytochemistry

The GRPR expression analysis was performed using a rabbit anti-GRPR polyclonal antibody (Affinity Bioreagents, Golden, Colo., USA) as primary antibody. For p16 expression, we used a p16-INK4A-specific monoclonal antibody, clone E6H4 (Dako AS, Glostrup, Denmark), primary antibody. The immunohistochemical methods have been described in our previous study [13]. Briefly, after dewaxing, inactivating endogenous peroxidase activity and blocking cross-reactions with normal serum, 4-μm sections were incubated overnight at 4°C with a diluted solution of the primary antibody (1:50). Identification of primary antibody location was achieved by subsequent application of biotinylated antibody, streptavidin horseradish peroxidase conjugate (LSAB, Dako, and diaminobenzidine tetrahydrochloride/H_2O_2; DAB detection Kit, Dako). The procedure for the immunocytochemical analysis was identical to that described above, except that the dewaxing step in xylene and the antigen retrieval steps were omitted. A known pancreatic cancer was used as positive control and the negative control was obtained by omitting the primary antibody.

In the immunohistochemical analysis, lesions were considered positive for both GRPR and p16 if more than 10% of the cells stained moderatly or strongly. Inversely, lesions were considered

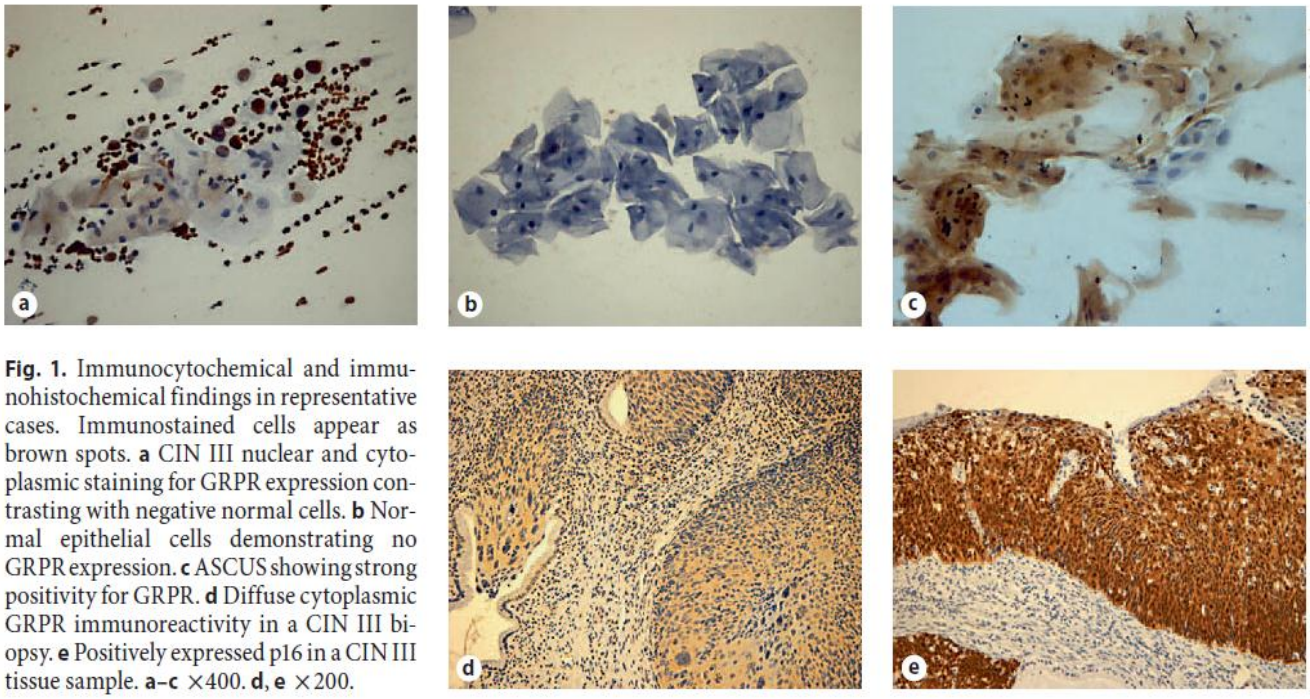

Fig. 1. Immunocytochemical and immunohistochemical findings in representative cases. Immunostained cells appear as brown spots. **a** CIN III nuclear and cytoplasmic staining for GRPR expression contrasting with negative normal cells. **b** Normal epithelial cells demonstrating no GRPR expression. **c** ASCUS showing strong positivity for GRPR. **d** Diffuse cytoplasmic GRPR immunoreactivity in a CIN III biopsy. **e** Positively expressed p16 in a CIN III tissue sample. **a–c** ×400. **d**, **e** ×200.

Table 1. Evaluation of the diagnostic performance of immunocytochemical analysis (IC) of GRPR expression in detecting cervical dysplastic and neoplastic lesions

IC GRPR	Biopsy/colposcopy		Total
	lesion	control	
+ (≥5)	28	7	35
– (<5)	4	23	27
Total	32	30	62

Sensitivity: 87.5% (95% CI: 71.0–96.5); specificity: 76.7% (95% CI: 57.7–90.1); positive likelihood ratio: 3.75 (95% CI: 1.93–7.27); negative likelihood ratio: 0.16 (95% CI: 0.06–0.42).

negative if less than 10% of the cells stained weakly. Positive cytoplasmic or nuclear staining reactions will appear as brown spots. GRPR expression was considered positive if immunostaining was positive in at least 5 epithelial cells in every slide, based on the classification proposed by Guo et al. [18]. All samples were reviewed independently by two pathologists blinded to the previous diagnosis. In case of discrepancies, a consensus was reached with the involvement of a third pathologist.

Statistical Analysis

The sensitivity and specificity of the Pap test and GRPR overexpression in cervical smears were estimated according to the histological findings in the corresponding biopsies, considered as the gold standard in the diagnosis of cervical disease. In the control group, however, biopsies were not performed for ethical reasons. Thus a normal colposcopy was defined as absence of neoplastic lesions. Sensitivity and specificity were calculated using PEPI version 4.0; the 95% confidence intervals (95% CIs) were calculated using a binomial distribution. Likelihood ratios were obtained using Stat Calc/epi-info, and for CIs, Taylor series were used.

Results

Immunocytochemical Staining for GRPR

Histologically confirmed biopsies consisted of 6 CIN I, 23 CIN II–III and 3 squamous carcinomas. The immunostaining analysis of the corresponding cervical smears showed GRPR positivity in the vast majority of cervical lesions. The receptor was identified in 83% of CIN I, 86% of CIN II–III and 100% of invasive carcinomas samples, mostly in dysplastic cells, but sometimes in normal epithelial cells in the same slide (fig. 1a). Most of the cells exhibited cytoplasmic staining or a combination of cytoplasmic and nuclear staining. None of the cases showed a nuclear signal only. The greater part of normal smears was negative for GRPR expression (fig. 1b), while a small number of normal smears showed immunopositivity in epithelial and inflammatory cells. The main results are summarized in table 1. GRPR immunostaining sensitivity in detecting cervical dysplas-

tic lesions and invasive cancer was 87.5% (95% CI: 71.0–96.5) and specificity was 76.7% (95% CI: 57.7–90.1). The positive likelihood ratio was 3.75 (95% CI: 1.93–7.27), while the negative likelihood ratio was 0.16 (95% CI: 0.06–0.42) (fig. 2).

Pap Test Results

The Pap test indicated altered cells in 24 smears of 31 cervical lesions. However, Pap tests matched the histological diagnosis in only 5 of 16 CIN II–III and 2 of 5 CIN I, corresponding to 32.2% of the neoplastic samples analyzed. It is important to emphasize that the Pap test failed to recognize invasive cancer cells, since 1 sample of invasive carcinoma was classified as CIN III and the other 2 were classified as ASCUS. In 32 controls with normal colposcopy, 21 of the Pap tests were normal while 11 cases were classified as abnormal (6 ASCUS, 4 CIN II–III and 1 CIN I). The results are summarized in table 2. The sensitivity of the Pap test in detecting cervical dysplastic lesions and invasive cancer was 77.4% (95% CI: 60.4–90.0) and specificity was 65.6% (95% CI: 48.1–80.4). The positive likelihood ratio was 2.25 (95% CI: 1.35–3.77), while the negative likelihood ratio was 0.34 (0.17–0.69) (fig. 2).

ASCUS Lesions

Among 63 patients analyzed, the Pap test classified 16 lesions as ASCUS. Of those, 7 related to normal colposcopies in the control group. The other 9 proved to be 1 CIN I, 6 CIN II–III and 1 invasive squamous carcinoma by histological analysis. GRPR immunostaining was performed in 15 of those patients classified as ASCUS. The marker was positive in 7 of 8 abnormal samples (fig. 1c) and negative in 2 of 5 nonmalignant lesions, which led to a test accuracy of 80%. The sensitivity was 88% and the specificity 71%, while the positive and negative predictive values were 77 and 83%, respectively. Of note, 2 lesions classified as ASCUS turned out to be invasive carcinomas, and GRPR showed strong immunostaining in those samples.

Immunohistochemical Staining for GRPR and p16

In 25 patients with confirmed dysplasias or invasive cervical carcinomas, immunohistochemical staining for GRPR was performed. In 88% of the samples, the expression of GRPR was positive, generally with a strong signal in dysplastic and neoplastic epithelia, clearly distinct from adjacent normal epithelia or stroma cells (fig. 1d). Most of the samples exhibited a predominantly cytoplasmic staining pattern.

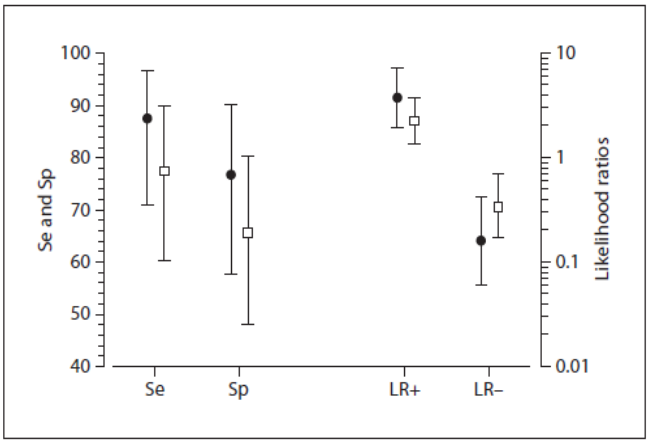

Fig. 2. Graphic representation of proportions and error bars representative of the sensitivity (Se) and specificity (Sp) of the immunocytochemical analysis of GRPR expression (black circle) and the Pap test (white square) in detecting cervical lesions, and positive and negative likelihood ratios (LR+, LR–), with the respective 95% CIs.

Table 2. Evaluation of the diagnostic performance of the Pap test in detecting cervical dysplastic and neoplastic lesions

Pap test	Biopsy/colposcopy		Total
	lesion	control	
+	24	11	35
–	7	21	28
Total	31	32	63

Sensitivity: 77.4% (95% CI: 60.4–90.0); specificity: 65.6% (95% CI: 48.1–80.4); positive likelihood ratio: 2.25 (95% CI: 1.35–3.77); negative likelihood ratio: 0.34 (95% CI: 0.17–0.69).

p16 immunohistochemical staining was performed in 26 neoplastic tissues. Intense positive staining was found in 88.4% of the samples. Most dysplastic and neoplastic cells showed cytoplasmic staining (fig. 1e). In both GRPR and p16, the markers were expressed in CIN I, CIN II–III and invasive cancer. We did not observe higher signaling in more advanced lesions. The degree of agreement between GRPR immunostaining in cervical smears and the corresponding cervical lesions was 88%. Only in 3 CIN II–III did we find no GRPR expression, whereas immunocytochemical analysis yielded positive tests.

Discussion

The Pap test is currently the method of choice for detecting cervical cancer. The Pap test is a subjective method that has remained substantially unchanged for many decades. There are several concerns, however, regarding its performance. The reported sensitivity of a single Pap test is low and shows wide variation (30–87%), and the specificity of a single Pap test might be as low as 86% in a screening population [2]. In recent years, technological advances in sample collection and processing have been achieved in cervical screening programs, with a positive impact on the detection of cervical lesions. However, the morphological interpretation of cytological tests still has limitations. It has been demonstrated that misinterpretation and interobserver discrepancies are common, specifically within the ASCUS and LSIL cytology categories [19]. A significant percentage of Pap smears characterized as ASCUS or LSIL are actually high-grade lesions [3]. Thus, a reliable method for diagnosing cervical disease that is independent of, or works in conjunction with, the conventional Pap test is needed. A wide array of potential biomarkers has been tested to test their diagnostic usefulness in the evaluation of cervical cancer and its precursors.

The GRPR is emerging as a very promising target for both cancer diagnosis and treatment. Over the last decades, several lines of experimental evidence have suggested that GRP may play a role in cancer development [20–22]. GRP has been recognized as an autocrine mitogen based on the detection of the growth factor and its cognate receptor in the same tissue, resulting in proliferation [23]. Recent data show that GRP also has paracrine and endocrine effects and functions as a morphogen and a proangiogenic agent [24]. Additionally, inhibition of GRPR was demonstrated to interfere with other relevant growth factor pathways such as the epidermal growth factor- and vascular endothelial growth factor-dependent signaling pathways [25, 26]. We recently reviewed the GRPR expression status in human cancers. These receptors have been identified in many malignancies, including prostate, breast, ovary, lung, head and neck, gastric, colon, esophageal and renal cancers, but no data have been reported for cervical carcinomas [10]. In an earlier immunohistochemical study, we found aberrant GRPR expression in dysplastic squamous lesions and invasive squamous carcinomas of the cervix, in contrast to low expression in normal epithelia and endocervices. Based on those findings, we postulated that GRPR could be involved in cervical carcinogenesis [13]. These results prompted us to continue investigating GRPR immunosignaling in cervical smears.

The first objective of this study was to evaluate the feasibility of GRPR immunocytochemistry in cervical smears since this technique had not been previously described for any tumors. We believe immunocytochemistry is a highly interesting approach since it is a simple and affordable method that can be performed in a nonsophisticated environment. Additionally, it offers the advantage of enabling a patient to avoid a surgical intervention. We were able to perform GRPR immunocytochemical staining successfully in cervical smears using the same fixative method as that required for the Pap test. Of the 66 samples analyzed in this study, only 3 could not be interpreted due to failures in the staining technique.

In this study, the staining intensity was not graded to avoid subjective findings. We only counted the number of immunopositive cells in a medium-power microscopic field as described by Guo et al. [18] for p16 expression. Since this is the first description of GRPR immunocytochemistry in cervical smears, we had no previous parameters with GRPR to compare with. Therefore, we based our research on extensive data from p16 immunocytochemical studies. There is no consensus, so far, on a standard methodology, especially regarding the interpretation of cervical smear results. For the time being there are no clear-cut arguments for establishing threshold values above which a sample becomes 'positive'. In the systematic review and meta-analysis by Klaes et al. [27], cut-off for p16 positivity ranged from 1 cell to more than 30% of cells.

GRPR immunocytochemical staining was found in the great majority of cervical lesions; 83% of CIN I, 86% of CIN II–III and 100% of invasive carcinomas samples. Compared to p16, the most studied biomarker of cervical dysplasia, this expression was higher in CIN I and equivalent in CIN II–III, according to data reported in a meta-analysis of p16 expression. Although the positivity cut-off varied, the average p16 positivity for CIN I and CIN II–III was 45% (37–57%) and 89% (84–95%), respectively [25]. Most GRPR-positive cells predominantly exhibited cytoplasmic staining. This finding is similar to our previous results with GRPR immunohistochemistry. However, the features of nuclear versus cytoplasmic staining have not yet been analyzed systematically, and are currently not considered to be relevant by the majority of authors [27]. Interestingly, in abnormal smears, GRPR immunosignaling was not limited to dysplastic cells, but could also be seen in some normal epithelial cells. On the other hand, GRPR expression was seldom detected in normal smears.

This confirms our previous findings, i.e. normal cells adjacent to lesions exhibited GRPR immunosignaling, contrasting to no signaling in nonmalignant tissues. We concluded that the presence of GRPRs in those cases could represent an early molecular event in cervical carcinogenesis.

In patients with confirmed dysplasia or invasive cervical carcinomas, immunohistochemical staining for GRPR showed 88% positivity, generally with a strong signal in dysplastic and neoplastic epithelia, clearly distinct from adjacent normal epithelia or stroma. This result was superior to that of our previous study, in which 78.4% of the tissues exhibited such moderate or strong diffuse GRPR staining. p16 immunohistochemistry was also performed in the present study and yielded virtually the same results as GRPR expression, i.e. 88.4% positive results.

GRPR was homogeneously expressed through the different grades of CIN and squamous carcinomas in both smears and tissue specimens. We did not find a larger number of positive cells to be associated with increased severity of the lesions. This reproduced our earlier results, as we found no significant differences in GRPR expression among the dysplastic and neoplastic specimens. Data in the literature are controversial: some authors were able to associate a higher incidence of GRPRs with poorly differentiated or more aggressive lesions [28–30], while others did not find GRPR expression to be associated with disease progression [31, 32].

In our previous work on cervical tissues, we demonstrated that GRPR is aberrantly expressed in cervical squamous dysplasia and neoplasia, but is absent in nonmalignant ectocervices [13]. The use of biopsies has the advantage of preserving tissue architecture and cellular morphology, enabling immunoreactions to be attributed to specific subpopulations; however, they require invasive procedures. Thus, another important goal of this study was to verify whether immunocytochemical staining in cervical smears correlated to immunohistochemical signaling in the corresponding tissue specimens, so that the GRPR status could be evaluated in a noninvasive manner. The degree of agreement between GRPR immunostaining in cervical smears and the corresponding cervical lesions was 88%. GRPR expression was absent in only 3 CIN II–III samples, contrasting with a positive immunocytochemical test.

Despite several limitations, the Pap test remains the most widely used method for cervical cancer screening. In the current study, it was compared with the histologic diagnosis in biopsy samples taken from the same patients. The Pap test indicated altered cells in 24 smears of 31 cervical lesions. Its sensitivity was 77.4% and its specificity was 65.6%. A systematic review of the sensitivity and specificity of conventional cervical cytological tests found biases in many studies and wide variation in their results. Evaluating the studies with the best methodology and valid controls, the sensitivity of a conventional Pap test for the diagnosis of CIN I or worse ranged from 30 to 87% with 86–100% specificity [33]. According to the current literature, there is clearly some correlation between abnormal cytological results and subsequent histological findings in biopsies, but a direct correspondence is found in only about half of the patients [34]. Consistent with this information, our Pap test results matched the histological diagnosis in only 32.2% of the neoplastic samples analyzed. Of note, 3 invasive squamous carcinomas were underdiagnosed by the Pap test.

A major objective of this study was to compare the diagnostic utility of GRPR immunocytochemistry with that of Pap test. Figure 2 shows the sensitivity, specificity, and the positive and negative likelihood ratios of both tests. There is a clear diagnostic advantage for GRPR expression in all measures. The ability of GRPR immunostaining to detect cervical lesions was 10.1% higher than that of the Pap test, with a gain of 11.1% in specificity. The likelihood ratios are used for assessing the value of performing a diagnostic test. They use the sensitivity and specificity of the test to determine whether a test result usefully changes the probability that a disease exists. As expected, the positive likelihood ratio of GRPR immunocytochemistry was superior to that of the Pap test while the negative likelihood ratio was inferior. GRPR immunostaining showed higher diagnostic utility compared with the Pap test, although the sample size was too small to produce statistically significant results. We believe that further studies including a larger number of patients are needed.

Finally, another important issue was to evaluate lesions classified as ASCUS with respect to GRPR expression. We had a biased higher proportion of ASCUS compared to the normal population since we are a reference colposcopy unit. Seven of 16 samples classified as ASCUS had been diagnosed as normal colposcopies in the control group, and 9 had been identified as dysplastic or invasive lesions proven by histological analysis. It is well recognized that the Pap test is less sensitive when changes consistent with ASCUS are seen [35]. Additionally, the diagnosis of ASCUS has considerable interobserver variability, even among expert pathologists. In the ASCUS/LSIL study, the diagnostic agreement was of only 55% of the

submitted ASCUS cases [34]. Unfortunately, the low sensitivity of the Pap test in this regard affects millions of women because up to 5% of all Pap tests will be classified as ASCUS, and only a small subset of those patients (5–17%) will have a biopsy proving CIN II–III lesions, which definitely require treatment [36]. For this reason, methods for identifying those patients erroneously classified as ASCUS following Pap tests but more likely to have dysplasia are needed. GRPR immunocytochemistry showed high accuracy (80%) in the ASCUS diagnosis, either in detecting the presence or absence of lesions. The sensitivity was 88% and the specificity 71%, while the positive and negative predictive values were 77% and 83%, respectively. In the present work, 2 patients referred for colposcopy because of an ASCUS diagnosis had invasive carcinomas. GRPR showed strong immunostaining in those samples.

This study had limitations, mainly related to sample size, although the major objective was to assess whether GRPR expression could be used as a potential diagnostic tool. We believe that a cohort study that includes at least 1,000 patients is necessary to demonstrate a significant role of GRPR expression as a marker of dysplastic cervical lesions.

Conclusion

To the best of our knowledge, this is the first immunochemical study evaluating GRPR expression in cervical epithelial cells. The biomarker was strongly associated with cervical dysplasia and invasive cancers. GRPR immunosignaling showed high accuracy in detecting precancerous lesions among Pap tests classified as ASCUS. Based on these results, immunocytochemistry for GRPR expression may be regarded as a valuable test for the early detection of CIN.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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Capítulo III

Influence of GRPR and BDNF/Trk on the Viability of Breast and Gynecologic Cancer Cells

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Influence of GRPR and BDNF/TrkB signaling on the viability of breast and gynecologic cancer cells

DANIELA B. CORNELIO[1-3*], CAROLINE B. DE FARIAS[1-4*], DÉBORA S. PRUSCH[1-3], TIAGO E. HEINEN[1-3], RAFAEL P. DOS SANTOS[1-3], ANA L. ABUJAMRA[1-4], GILBERTO SCHWARTSMANN[1,3,5] and RAFAEL ROESLER[1-3]

[1]Cancer Research Laboratory, University Hospital Research Center (CPE-HCPA);
[2]Laboratory of Neuropharmacology and Neural Tumor Biology, Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul;
[3]National Institute for Translational Medicine (INCT-TM);
[4]Children's Cancer Institute; [5]Department of Internal Medicine, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

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Abstract. Neuropeptide and neurotrophin receptors are increasingly important molecular targets in cancer. Scientific findings indicate that compounds blocking gastrin-releasing peptide receptors (GRPR) or tropomyosin receptor kinase (Trk) receptors are likely to have antiproliferative activities against cancer cells. The present study aimed to demonstrate that, in contrast to previous findings, GRPR activation reduces, whereas its blockade increases the viability of breast, ovarian and cervical cancer cell lines. However, consistent with previous studies, Trk inhibition was demonstrated to reduce the viability of these cells. MCF-7 (breast), OVCAR-3 (ovarian) and HeLa (cervical) human cancer cell lines were treated with GRP, the GRPR antagonists RC-3095 and RC-3940-II, brain-derived neurotrophic factor (BDNF) and the Trk antagonist K252α. Cell viability was measured by the MTT assay. Expression of GRPR and BDNF was confirmed with reverse transcription-polymerase chain reaction (RT-PCR). GRP reduced, whereas RC-3940-II enhanced the viability of the three cell lines. Treatment with K252α inhibited the viability of the cell lines, while BDNF increased the viability of OVCAR-3 cells. The results supported the hypothesis that GRPR and BDNF/TrkB signaling regulates cancer cell viability. Most importantly, these findings are the first to demonstrate that GRPR blockade can stimulate, rather than inhibits the viability of breast and gynecologic cancer cell lines.

Correspondence to: Dr Rafael Roesler, Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, Rua Sarmento Leite, 500 (ICBS, Campus Centro/UFRGS), 90050-170 Porto Alegre, RS, Brazil
E-mail: rafael.roesler@pq.cnpq.br

*Contributed equally

Key words: gastrin-releasing peptide receptor, brain-derived neurotrophic factor, tropomyosin receptor kinase B, gynecologic cancer, cancer cell

Introduction

Increasing evidence indicates that neuropeptide and neurotrophin receptors and their ligands may be overexpressed in cancer cells and are involved in cell survival and growth. Neuropeptide receptors aberrantly expressed in several human cancers include the gastrin-releasing peptide receptor (GRPR), activated in mammals by the bombesin-like neuropeptide gastrin-releasing peptide (GRP). GRPR activation has been shown to stimulate cancer cell proliferation, whereas GRPR antagonists to reduce tumor growth in a range of experimental cancer models (1,2). In gynecologic cancers, GRPR is likely to be highly expressed in breast, ovarian and cervical tumors, as well as in breast cancer cell lines, whereas its expression is low or absent in non-neoplastic tissue and healthy cells (1,3-8). The pharmacological blockade of GRPR by synthetic peptides acting as selective antagonists (RC-3940-II, RC-3095) has been shown to reduce human breast and ovarian tumor growth xenografted into nude mice (9-11).

Additional growth factor receptors increasingly implicated in tumor progression include tropomyosin receptor kinase (Trk) receptors. Trks are receptor tyrosine kinases activated endogenously by neurotrophins. TrkA, TrkB and TrkC are the preferred receptors for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), respectively (12). Increased BDNF and TrkB expressions have recently been found in several human tumors (13-15). BDNF/TrkB promote cancer cell survival and resistance to chemotherapy, while small-molecule inhibitors of Trk, such as K252α, inhibit cell growth and induce apoptotic death in cancer cells (16-19). TrkB expression has been described in ovarian and cervical cancers (20,21), while being associated with a shorter survival and the promotion of metastasis in ovarian cancer patients (20). In addition, studies using ovarian cancer cells indicated that TrkB is involved

in cell proliferation, migration and suppression of anoikis (22,23). In breast cancer, BDNF expression has been shown to be higher in tumor samples compared to non-neoplastic tissues, while BDNF transcript levels have been associated with unfavorable pathological parameters and adverse clinical outcomes (24). Moreover, BDNF has been shown to induce resistance to apoptosis in breast cancer cells, while injection of an anti-BDNF antibody has been proven to reduce the growth of breast tumors xenografted in mice (25).

The present study aimed to explore the effects of GRPR and TrkB ligands on the viability of human breast, ovarian and cervical cancer cells *in vitro*. Notably, under the experimental conditions used in this study, GRP produced a small, but statistically significant reduction of cell viability, whereas the RC-3940-II-induced GRPR blockade led to a statistically significant increase in cell viability. In addition, K252α-induced Trk inhibition was found to have reduced cell viability.

Materials and methods

Cell culture and treatments. MCF-7, OVCAR-3 and HeLa human cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were plated in 96-well plates (TPP) at a density of 4, 7 and $3x10^3$ cells/well in sextuplets, respectively, and then cultured and maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Carlsbad, CA, USA) (MCF-7 and HeLa cells) and RPMI-1640 (Gibco-BRL) (OVCAR-3 cells), containing 2% (w/v) LH-glutamine and 10% (v/v) fetal bovine serum (FBS; Sorali, Campo Grande, Brazil). For the experiments GRP treatment was used, whereby cells were starved for 24 h in medium supplemented with 0.5% serum medium and then treated with human recombinant GRP (0.001, 0.01, 0.1, 1 or 10 μM; Sigma-Aldrich, St. Louis, MO, USA). For other treatments, 24 h after medium and serum addition, the cells were treated with the GRPR antagonist [D-Tpi^6, Leu^{13} psi(CH_2NH)-Leu^{14}] bombesin (RC-3095; 0.001, 0.01, 0.1, 1 or 10 μM; Zentaris GmbH, Frankfurt, Germany), the GRPR antagonist [Hca^6, Leu^{13} psi(CH_2N)-Tac14-bombesin$^{6-14}$] (RC-3940-II; 0.01, 0.1, 0.5, 1 or 5 μM; Zentaris GmbH) (10), human recombinant BDNF (1, 10 or 100 ng/ml; Sigma-Aldrich) or K252α (0.01, 0.1 or 1 μM, Sigma-Aldrich). The cells were kept at a temperature of 37˚C, in a minimum relative humidity of 95% and an atmosphere of 5% CO_2 in air.

MTT assay. The cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich) 48 h subsequent to treatment. Eleven microliters of MTT 5 mg/ml solution were added to each well of the plate, followed by incubation for 4 h at 37˚C. The plate was left at room temperature until completely dry. Dimethyl sulfoxide was added and the absorbance was measured at 492 nm in a multiplate reader. The experiments were performed at least in triplicate.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from MCF-7, OVCAR-3 and HeLa cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer's instructions, and reverse transcribed with SuperScript® III First-Strand Synthesis SuperMix® (Invitrogen). The human BDNF and GRPR primers were designed according to the corresponding GenBank sequence. The forward and reverse primers used for RT-PCR amplification are shown in Table I. The PCR experiments were carried out with 1.5 mM $MgCl_2$, 0.1 μM for each primer, 0.2 mM dNTPs, 0.5 M betain (only to BDNF primers), 1 unit Taq Platinum® (Invitrogen) and 2 μl cDNA template. The expression of β-actin was measured as an internal control using the primers shown in Table I. The PCR reaction was performed in a total volume of 20 μl using a concentration of 0.04 mM dNTPs, 0.2 units Taq polymerase in the supplied reaction buffer, 0.3 mM $MgCl_2$ and 10 pmol of each primer. Amplification conditions consisted of 1 min at 95˚C followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 59˚C for 30 sec, extension of primers at 72˚C for 45 sec, followed by a final extension at 72˚C for 10 min. The products of BDNF (362 bp), GRPR (190 bp) and β-actin (190 bp) were electrophoresed through 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet illumination (17,26,27). Each experiment was performed twice using RNA isolated from two independent cell cultures.

Statistical analysis. Data were shown as the mean ± SEM. Differences between the mean values were evaluated by one-way analysis of variance followed by Tukey *post hoc* tests, when appropriate. In the comparisons, $P<0.05$ was considered to indicate a statistically significant difference.

Results

GRPR activation reduced, whereas GRPR blockade increased the viability of MCF-7, OVCAR-3 and HeLa cells. Treatment with recombinant GRP induced a small (range, 11.3-36.0%), yet statistically significant reduction of cell viability in the three cell lines studied (Fig. 1A). Viability was reduced by GRP at all the doses used in MCF-7 cells, with the exception of 0.001 μM in OVCAR-3 cells and only at 1 μM in HeLa cells (Fig. 1B). The GRPR antagonist RC-3940-II led to increases ranging from 200.1 to 476.8% in the viability of all three cell lines, at all doses used, with the exception of the 0.01 μM in OVCAR-3 and the 5 μM in HeLa cells. The less potent (10) GRPR antagonist RC-3095 demonstrated an increase of ~24% in the viability of OVCAR-3 cells, however, no statistically significant effect was observed in OVCAR-3 or HeLa cells (Fig. 1C). Given these negative findings, RC-3095 in MCF-7 cells was not tested. These results indicated that GRPR activation reduced, whereas GRPR blockade increased the viability of MCF-7, OVCAR-3 and HeLa cells.

Trk inhibition reduced the viability of MCF-7, OVCAR-3 and HeLa cells. Treatment with BDNF had no significant effect on cell viability, except for a small effect at a dose of 1 ng/ml in OVCAR-3 cells (Fig. 2A). The Trk inhibitor K252α demonstrated a notable inhibitory effect on cell viability (ranging from 13.5 to 44.6%), at a dose of 1 μM in the three cell lines, and at a dose of 0.01 μM in MCF-7 cells (Fig. 2B). These results indicated that Trk inhibition reduced the viability of MCF-7, OVCAR-3 and HeLa cells in a dose-dependent manner.

GRPR and BDNF expression in MCF-7, OVCAR-3 and HeLa cells. RT-PCR analyses demonstrated that MCF-7,

Table I. Forward and reverse primers used for RT-PCR amplification.

Gene	Primer sequences	PCR product size (bp)
GRPR	Forward: 5'-CAAGATCTTCTGCACGGTCA-3' Reverse: 5'-TCAGTTTGCAGCCAATTCTG-3'	190
BDNF	Forward: 5'-GCGTGAATGGGCCCAAGGCAGG-3' Reverse: 5'-TGTGACCGTCCCGCCCGACATG-3'	362
β-actin	Forward: 5'-AAACTGGAACGGTGAAGGTG-3' Reverse: 5'-AGAGAAGTGGGGTGGCTTTT-3'	190

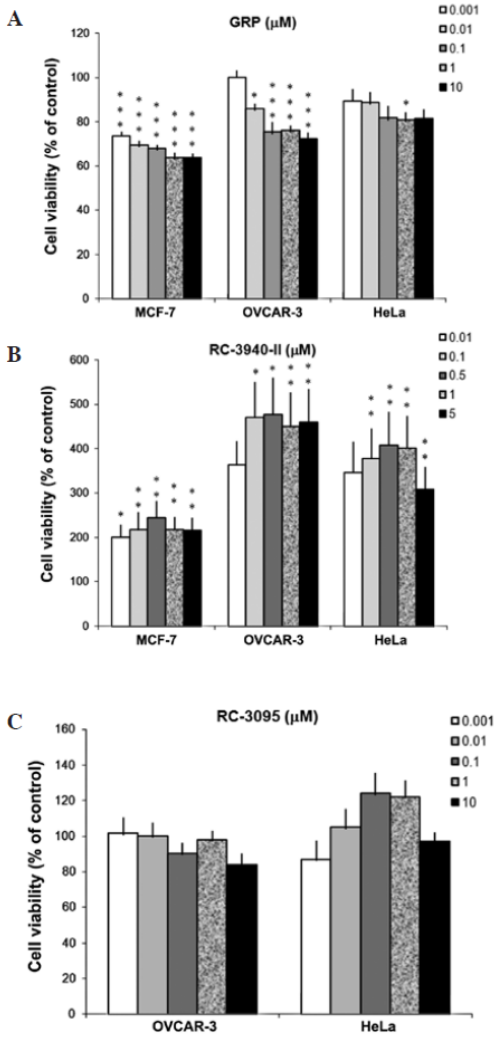

Figure 1. A GRPR agonist reduces, whereas a GRPR antagonist increases the viability of human breast, ovarian and cervical cancer cell lines *in vitro*. MCF-7, OVCAR-3 and HeLa cells were treated with (A) human recombinant GRP (0.001, 0.01, 0.1, 1 or 10 μM); (B) RC-3940-II (0.01, 0.1, 0.5, 1 or 5 μM) and (C) RC-3095 (0.001, 0.01, 0.1, 1 or 10 μM). RC-3095 was tested in OVCAR-3 and HeLa cells only. Cell viability was measured using an MTT assay, as described in Materials and methods. Data are the mean ± SEM of 3-5 different experiments performed in 4-6 wells each. The mean value for the control cells was taken as 100%; *P<0.05, **P<0.01 and ***P<0.001 compared to control cells.

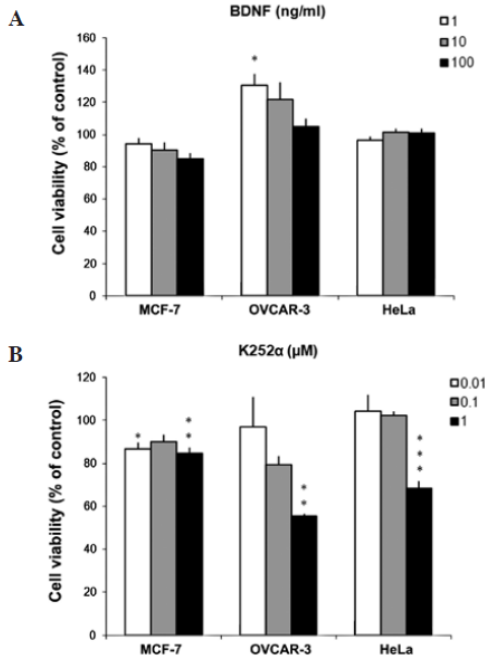

Figure 2. Trk inhibition reduces the viability of human breast, ovarian and cervical cancer cell lines *in vitro*. MCF-7, OVCAR-3 and HeLa cells were treated with (A) human recombinant BDNF (1, 10 or 100 ng/ml) or (B) K252α RC-3940-II (0.01, 0.1 or 1 μM). Cell viability was measured using an MTT assay, as described in Materials and methods. Data are the mean ± SEM of 3-4 different experiments performed in 4-6 wells each. The mean value for the control cells was taken as 100%; *P<0.05 and ***P<0.001 compared to the control cells.

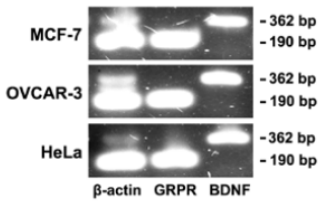

Figure 3. RT-PCR analysis of GRPR and BDNF mRNA expression in MCF-7, OVCAR-3 and HeLa human cancer cells is shown. RNA was extracted from the cells and RT-PCR analysis was performed, as described in Materials and methods. Transcript sizes of 190 and 362 bp were identified, representing fragments of GRPR and BDNF, respectively.

VCAR-3 and HeLa cells showed an mRNA expression for both GRPR and BDNF. Transcript sizes of 190 and 362 bp, representing fragments of GRPR and BDNF, respectively, were identified in the cells (Fig. 3).

Discussion

The most important finding of the present study is that, in contrast to findings of previous studies (1,2,9-11), pharmacological blockade of the GRPR exhibited an enhancing, rather than an inhibitory action on cancer cells, whereas GRP decreased cell viability. In addition, Trk inhibition was found to reduce the viability of the breast, ovarian and cervical cancer cell lines used, although treatment with BDNF did not alter cell viability.

GRPR antagonists have been previously shown to reduce experimental breast and ovarian tumor growth *in vivo* (9-11). However, the effects of the GRPR agonists and antagonists on cancer cell growth depends markedly on specific cell culture and experimental conditions. Consistent with these hypotheses, Yano *et al* (28) found that bombesin (showing a GRPR agonistic action comparable to that of GRP) at doses ranging from 0.001 to 1 μM stimulated, while RC-3095 at doses between 0.01 and 10 μM inhibited the proliferation of human breast cancer cell lines only when cells were cultured in heat-inactivated and dextran-coated charcoal-treated FBS (DCC-FBS), but not in the presence of untreated FBS. Additionally, MCF-7 cells failed to respond to bombesin or RC-3095 in the presence of either FBS or DCC-FBS. The authors of that study suggested that bombesin-like peptides or other growth factors present in the culture medium may compete with GRPR ligands, thus altering the cell response to the treatments. Authors of other studies (29) found that bombesin failed to affect MCF-7 cell proliferation, although the cells expressed GRP binding sites, while bombesin stimulated calcium mobilization and inositol lipid hydrolysis. Discrepancies among different studies might be due to differences in cell clones, cell culture conditions and methods used to assess proliferation and viability (e.g., trypan blue dye exclusion vs. MTT) in different laboratories.

In a previous study using Neuro2A mouse neuroblastoma cells, a lower dose of RC-3095 was found to reduce, while a higher dose to increase cell viability (30). Notably, in experiments examining cancer cell growth (27,30,31), as well as in other experimental models (32-34), GRPR agonists and antagonists often show drug-response patterns, in which intermediate doses have more pronounced biological, whereas higher doses have no or even contrary effects. Moreover, the lack of a significant effect of RC-3095 in the present study might be owed to its lower potency compared to RC-3940-II. The latter has been shown to inhibit cancer cell proliferation at lower dose ranges, and was more effective compared to RC-3095, in inhibiting experimental breast cancer cell growth (10). Taken together, these data raise the possibility that GRPR agonists and antagonists have highly varying effects depending on the dose and the presence of endogenous GRP, as well as other factors in the tissue microenvironment, with potential implications of their effects *in vivo* in both experimental animals and patients undergoing clinical studies.

Although several molecular mechanisms downstream of GRPR activation have been described and proposed to mediate GRPR-induced cancer cell growth regulation, the mechanisms underlying the stimulatory effects of GRPR blockade on cancer cells observed in the present study and previous experiments (30) remain unknown and have yet to be investigated in future studies. Cell responses to GRPR activation are mediated by multiple protein kinase pathways, including phospholipase C (PLC)/protein kinase C (PKC), mitogen-activated protein kinase (MAPK)/extracellular signal-regulated protein kinase (ERK) and phosphatidylinositol 3-kinase (PI3K) cascades (35). Studies focusing on experimental breast and gynecologic cancers have demonstrated that GRPR is associated with cell migration and interleukin-8 expression in breast tumors (36), whereas GRPR antagonists reduce ErbB-2/HER-2 expression in breast cancer cells and epidermal growth factor receptor (EGFR), as well as c-jun and c-fos oncogenes in experimental breast and ovarian tumors (9,11,38).

BDNF/TrkB signaling has been suggested to promote cancer cell survival and resistance to chemotherapy (12-14). Previous studies on breast and ovarian cancer cells have suggested that BDNF/TrkB stimulates cell survival and migration (20,22,23,25). BDNF is likely not to enhance viability since the BDNF/TrkB pathway is already activated at its optimal level by BDNF secreted from the cells as an autocrine factor. The possibility that BDNF is secreted as an autocrine factor from cultured cells would be consistent with our finding that the three cell lines expressed mRNA for BDNF. Results of this study demonstrating that K252α decreased cell viability are consistent with the hypothesis that TrkB needs to be further examined as a potential anticancer target in breast and gynecologic cancers. Since TrkB has the potential to crosstalk with GRPR and other growth factor receptors, including EGFR, in regulating cancer cell survival and proliferation (17,22), combining compounds acting on different receptors might prove to be the most effective strategy to inhibit tumor growth by targeting neuropeptide and neurotrophin signaling.

In conclusion, the present study is the first to demonstrate that, at least under certain experimental conditions, GRPR activation negatively regulates the viability of breast, ovarian and cervical cancer cells *in vitro*. In addition, these findings are consistent with the hypothesis that Trk signaling regulates the viability of breast and gynecologic cancer cells. Experimental findings suggesting that there are conditions under which GRPR blockade stimulates cell viability and proliferation are likely to have implications for the clinical testing of GRPR antagonists as potential anticancer medications. Based on the *in vitro* findings reported in this study, additional studies using *in vivo* models and tumor samples from patients are required in order to examine the potential inhibitory role of GRPR activation in breast and gynecologic cancer development.

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Capítulo IV

Emerging Therapeutic Agents for Cervical Cancer

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Emerging Therapeutic Agents for Cervical Cancer

Daniela B. Cornelio[1,2,3,4]*, Rafael Roesler[1,2,4,5] and Gilberto Schwartsmann[1,4,6]

[1]*Cancer Research Laboratory, Academic Hospital Research Center, Federal University of Rio Grande do Sul, 90035-003 Porto Alegre, RS, Brazil,* [2]*Graduate Program in Cellular and Molecular Biology, Center for Biotechnology, Federal University of Rio Grande do Sul, 91501-070 Porto Alegre, RS, Brazil,* [3]*Department of Gynecology and Obstetrics, Federal University of Health Sciences, 90020-090, Porto Alegre, RS, Brazil,* [4]*National Institute for Translational Medicine (INCT-TM), 90035-003 Porto Alegre, RS, Brazil,* [5]*Cellular and Molecular Neuropharmacology Research Group, Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, 90046-900 Porto Alegre, RS, Brazil,* [6]*Department of Internal Medicine, Faculty of Medicine, Federal University of Rio Grande do Sul, 90035-003 Porto Alegre, RS, Brazil*

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Abstract: Cervical cancer is the second most frequent malignancy affecting women worldwide. The highest incidences occur in the developing world, where, in most countries, cervical cancer is the leading cause of cancer mortality in women. Although surgery and chemoradiotherapy can cure 80-95% of women with early stage cancer and 60% of locoregionally advanced cancer, the recurrent and metastatic disease remains a major cause of cancer death. The current cytotoxic treatment options for advanced and metastatic cancer demonstrate modest results, with response rates of maximum 30% and overall survival of less than 10 months. Given this limited degree of success with conventional therapies, interest has increased in other therapeutic alternatives. In this way, targeted agents are emerging as potential candidates for improving survival in cervical cancer patients. In this review we highlight the main current therapeutic strategies for cervical cancer and summarize the most relevant patents from the latest five years. Special attention was given to patents with potential applications in the clinical practice.

Keywords: Targeted therapy, cervical cancer, growth factors.

INTRODUCTION

Cervical cancer is the second most frequent malignancy affecting women worldwide, with approximately 500,000 new cases diagnosed and 280,000 deaths each year [1]. The highest incidences occur in the developing world, where, in most countries, cervical cancer is the leading cause of cancer mortality in women [2]. Although surgery and chemoradiotherapy can cure 80-95% of women with early stage cancer and 60% of locoregionally advanced cancer, the recurrent and metastatic disease remains a major cause of cancer death [3]. The current cytotoxic treatment options for advanced and metastatic cancer demonstrate modest results, with response rates of maximum 30% and overall survival of less than 10 months [4]. Given this limited degree of success with conventional therapies, interest has increased in other therapeutic alternatives. In this way, targeted agents are emerging as potential candidates for improving survival in cervical cancer patients. This review article will highlight the current therapeutic strategies and the most recent patents for cervical cancer treatment.

EARLY-STAGE DISEASE

In developed countries, where screening for cervical cancer is effective, approximately half of the patients present with stage I disease at the time of diagnosis. For these patients there are a number of acceptable treatment options that are based on surgery and/or radiation therapy. Most retrospective studies suggest that radical hysterectomy and pelvic radiation therapy are equally effective for the treatment of stage IB1. As tumor size increases, there is a higher risk for treatment failure. In this way, additional therapeutic modalities are being included for patients with stage IB2 disease. For patients stage IB2 who undergo radiotherapy, concurrent cisplatin-based chemotherapy has proven to improve overall and progression-free survival. Based on these results, chemotherapy associated to radiation is being considered the standard treatment for early-stage bulky tumors [5-8]. Neoadjuvant chemotherapy is also being employed for some groups of patients, including stage IB2 [9]. Randomized trials have suggested advantage in survival with the use of chemotherapy prior to radical pelvic surgery [10, 11]. Results from meta-analysis, however, are conflicting. While some authors found no survival advantage using neoadjuvant chemotherapy [12], other encountered a significant decrease in the risk of death from cervical cancer with this approach [13]. Additional controlled studies are needed to incorporate the neoadjuvant chemotherapy in the therapeutic arsenal for early-stage cervical cancer.

LOCALLY ADVANCED DISEASE

The patients who present with advanced lesions at diagnosis are at greater risk of recurrence and account for the majority of cervical cancer deaths. The treatment of choice for locally advanced tumors (stages IIA-IVA) has usually been based on pelvic external-beam radiation or intracavitary

*Address correspondence to this author at the Hospital de Clínicas de Porto Alegre, Laboratório de Pesquisas em Câncer, Rua Ramiro Barcelos, 2350, 90035-003, Porto Alegre, RS, Brazil; Tel: +55 51 2101 7616; Fax: +55 51 3388 2877; E-mail: danicornelio@terra.com.br

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brachitherapy. However, radiotherapy alone fails to control progression of cervical cancer in 35% to 90% of women with locally advanced disease. As in many other solid malignancies, the addition of concurrent chemotherapy has been employed in attempt to minimize the risk of recurrence and distant metastasis. In cervical carcinomas, several randomized phase III trials have shown overall survival advantage for cisplatin-based therapy given concomitant with radiation therapy. The risk of death from cervical cancer decreased by 30% to 50% with the use combined chemoradiotherapy. Based on these results, there is a strong recommendation for adjuvant cisplatin-based chemotherapy for patients who undergo radiotherapy [5-8, 14, 15].

Whether cisplatin is more effective as a single agent or in combination with other drugs is currently under investigation [15, 16]. The GOG Trial of pelvic radiotherapy plus concurrent single-agent cisplatin versus cisplatin plus FU plus hydroxyurea versus hydroxyurea alone showed significant improvements in progression-free and overall survival in patients randomly assigned for either cisplatin-containing arm. In this study, cisplatin alone was equally effective and less toxic than the three-drug regimen [15]. A second GOG trial tested cisplatin and hydroxyurea as single agents and cisplatin followed by FU concomitant to radiotherapy for patients with stages IIB to IVA. Again, survival rates were higher in both cisplatin-containing regimens [16]. Carboplatin, a platin derivative which is associated with less toxicity than cisplatin, has been investigated as a radiation-sensitizing agent in advanced cervical cancer. In phase I and II studies, carboplatin at a weekly schedule demonstrated to be effective and safe [17, 18]. Although this is a promising drug for cervical cancer treatment, phase III studies should be conducted comparing carboplatin with cisplatin during radiotherapy.

Other chemotherapeutic agents have been studied for advanced disease as well. In a general way, their performance as single agents is poor, but in combination with cisplatin the effectiveness is increased. The Taiwanese trial randomly assigned women with bulky IIB or IIIB cervical cancer to radiotherapy with or without concurrent multiagent chemotherapy (cisplatin, vinblastine and bleomycin). In this study, chemotherapy did not improve overall survival or disease-free survival after a median 47 month follow-up [19]. Paclitaxel in association to carboplatin was tested in a phase II trial that included women stages IB to IVA cervical cancer who undergone radiotherapy. The 3-year overall survival rates were 91%, 88% and 50% for stages IIB, III and IV, respectively [20]. Gemcitabine alone concomitant to radiotherapy showed elevated response rates at very low toxicity in a phase I study among patients with advanced cervical carcinoma [21]. Gemcitabine was further investigated as a radiosensitizer in a phase III trial in comparison to cisplatin. Even though the toxicity and overall response rates were similar among both agents, the complete responses were higher in the gemcitabine group [22]. The addition of topotecan to cisplatin during pelvic irradiation for locally advanced cervical cancer led to a complete response rate of 92% in a recent phase I trial [23]. Recent data suggest that topotecan, when used concurrently with cisplatin, may be the new standard of care for the management of advanced cervical cancer. Ongoing phase III studies will compare this combination with other cisplatin-containing and cisplatin-free combinations [24]. Although these and other combinations are promising regimens for advanced carcinoma of the cervix, weekly cisplatin in association to radiotherapy remains the standard of care.

RECURRENT AND DISSEMINATED DISEASE

Recurrent and disseminated cervical cancers are associated with poor survival rates. The prognosis in recurrent disease depends on the site of recurrence and the ability to pursue potentially curative therapy, among other factors. In locally recurrent tumors, pelvic exenteration can lead to a 5-year survival rate of 32% to 62% in selected patients [25]. Salvage radiotherapy may be an option for some patients if it has not been administered before.

Once the disease is spread beyond the confines of a radiation or surgical field, no standard treatment is available. In these cases, the main objective is palliation of symptoms. Radiotherapy may be useful to relieve pelvic pain or bleeding from advanced lesions. In metastatic disease, radiation can control pain from skeletal metastases or symptoms related to brain lesions [26].

Patients under palliative treatment should be candidates for single agent or combined chemotherapy. In a general way, the single agent approach is reserved for women with poor health, who would not tolerate combination chemotherapy. Several single chemotherapy agents and combination regimens have demonstrated activity in metastatic disease or in recurrences not amenable to local therapy. Cisplatin is to date the most active drug against cervical cancer, with response rates that range from 18% to 38% [27]. As renal and gastrointestinal toxicities increase proportionately to higher doses of cisplatin, other platinum derivatives have been studied with the intent of obtaining lower toxicity levels. Carboplatin at a dose of 400mg/m^2 every 4 weeks produced response rates as high as 28% and median survival of 6 to 7 months [28]. Iproplatin was studied in a randomized phase III trial of advanced cervical carcinomas, showing poorer response rates (10,8% versus 15,4%) in comparison to carboplatin [29]. Other classes of chemotherapeutic agents have shown activity in recurrent and metastatic cervical cancer, among them paclitaxel, topotecan, vinorelbine and ifosfamide [30]. Nevertheless, no single agent proved to be superior to cisplatin. In this way, research is being directed to combining agents with cisplatin or comparing combination therapy to single-agent cisplatin in randomized controlled trials.

The addition of active agents to cisplatin generally results in higher response rates, although the benefit in prolonged survival is small. The patients who have better response rates are those naïve of prior treatments. Many agents have been investigated in phase III trials in combination to cisplatin, including gemcitabine, vinorelbine, topotecan, ifosfamide, paclitaxel, fluoracil and bleomycin. The great majority demonstrated a response advantage over single-agent cisplatin [30]. Ifosfamide [31], paclitaxel [32] and topotecan [33] showed progression-free survival advantages compared to cisplatin alone. Regarding overall survival, topotecan is the only agent in combination to cisplatin that demonstrated advantage in a controlled trial [33].

In attempt to achieve even better response rates, multidrug chemotherapy has been tested for recurrent and metastatic cervical cancer. Several triplets combining active agents with cisplatin have demonstrated response rates of more than 50% and survival rates slightly superior to single-agent or doublet regimens in phase II studies. However, this benefit has not been confirmed in phase III trials; no triplet has proven greater activity than cisplatin as single-agent or in a doublet regimen [34-38].

TARGETED THERAPIES

In the last decade, several molecular events in cervical carcinogenesis have been elucidated, leading to the development of targeted agents for both diagnostic and therapeutic purposes. Noteworthy, the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) have been widely studied in many solid malignancies. In cervical cancer, anti-EGFR and anti-VEGF therapies have been evaluated, in one effort to improve the limited results from current cytotoxic therapies.

Anti-EGFR Therapies

The EGF family of tyrosine kinases receptors is divided is four members: EGFR (HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) [39]. EGFR is highly expressed in primary and recurrent cervical tumors, and this expression has been correlated to poor prognosis and to more advanced stages of disease [40-42]. Additionally, EGFR has been shown to modulate chemosensitivity and radiosensitivity in pre-clinical trials [43, 44]. The EGFR has been successfully targeted either through monoclonal antibodies (cetuximab) or through small molecules inhibitors of tyrosine kinase (erlotinib and gefitinib).

Cetuximab, a chimerized antibody of the immunoglobulin G1 subclass, is highly specific for EGFR and has proven activity as monotherapy or in adjuvance to chemotherapy in head and neck, colorectal and lung cancers [45]. Based on these results, cetuximab has been tested as single agent or combined to radiotherapy and chemotherapy for cervical cancer treatment. In experimental studies, cetuximab demonstrated notable cellular toxicity and tumor growth inhibition [46]. Results from ongoing clinical trials with cetuximab are awaited, among them a phase II trial of cetuximab plus cisplatin as first-line chemotherapy for persistent or recurrent cervical carcinoma (GOG-0076DD), a study of cetuximab as monotherapy for recurrent or persistent cervical carcinoma (GOG-0227E) and a study of cetuximab in adjuvance to radiotherapy in early-stage disease (GOG-9918). Cetuximab might be a novel and attractive therapeutic strategy in patients harboring chemotherapy-resistant, recurrent, or metastatic cervical cancer.

EGFR tyrosine kinase inhibitors are also being studied for cervical malignancies. Gefitinib was investigated in a phase II clinical trial as second- and third-line single agent for recurrent squamous or adenocarcinoma of the cervix. No objective responses were found, though 20% of the patients had disease stabilized, with a median duration of stable disease of approximately 100 days [47]. Erlotinib, another specific EGFR tyrosine kinase inhibitor, is currently being tested in combination with radiotherapy and chemotherapy for locally advanced cervical cancer (NCT00428194) and as a single agent for persistent or recurrent disease (GOG-0227D). In a phase I trial, erlotinib in combination to cisplatin and pelvic radiotherapy was well tolerated in patients with locally advanced cervical tumors [48]. Dai and colleagues found that acquired resistance to cytotoxic therapy in cervical cancer cell lines was associated with enhanced sensitivity to erlotinib, which correlated with increased EGFR expression [49]. The authors suggested that EGFR tyrosine kinase inhibitors might be more effective as second- or third-line treatment for certain patients with tumors that were previously treated with multiple chemotherapy regimens. Lapatinib, another tyrosine kinase inhibitor which targets both EGFR and HER2, is being evaluated as monotherapy in a phase II clinical trial for locally advanced (IVB), persistent or recurrent cervical cancer (VEG105281).

Anti-VEGF Therapies

VEGF signaling is an attractive target for cancer therapy given its role in tumor angiogenesis and in endothelial cancer cell proliferation, differentiation, survival and migration. The overexpression of VEGF is usually related to poor prognosis and progression of cervical carcinomas [50]. Additionally, increased VEGF expression and tumor vascularization can be independent predictors of poor disease-free and overall survival [51]. Currently, the most studied anti-VEGF agent is bevacizumab, a humanized immunoglobulin G1 monoclonal antibody that binds to VEGF. In experimental studies, bevacizumab inhibited VEGF-induced proliferation of endothelial cells and decreased microvessel density in tumor xenografts [50]. Bevacizumab in addition to chemotherapy significantly improved overall survival in patients with metastatic colorectal and non-small lung cancer. These favorable results provided a rationale for testing this agent for cervical cancer treatment. The use of bevacizumab in heavily pretreated women with recurrent cervical carcinoma demonstrated clinical benefit in 67% of the patients, which included 1 (17%) complete response, 1 (17%) partial response and two (33%) patients with stable disease. The median time to progression for the women who demonstrated clinical benefit was 4.3 months [52].

Other Potential Targets

Beyond EGFR and VEGF, many other targets have been investigated for cervical cancer treatment. The phosphatidylinositol 3-kinase (PI3K) signaling pathway has been implicated in cervical carcinogenesis. PI3K was shown to be over expressed in cervical tumors, but not in normal tissues. Through cell lines experiments, a PI3K inhibitor, LY29400, significantly inhibited Hela cells growth and induced apoptosis [53]. LY29400 has also been tested as a radiosensitizer in cervical cancer cells. Although LY294002 alone did not produce cytotoxic effects, PI3K inhibition with this antagonist produced significant radiosensitization, showed significant time-dependent effects, increased apoptosis, and altered gene expression [54].

The heat shock protein 90 (Hsp90) is a conserved chaperone involved in crucial signaling events in normal and malignant cells. It is believed that tumor cells are particularly

dependent on Hsp90 for survival as well as for malignant progression. Hsp90 inhibitors, which are derivates of the natural compound geldanamycin, such as the orally bioavailable 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG), are currently being tested in clinical trials and small molecule inhibitors are in development. Through *in vitro* experiments, some researchers evaluated the effect of 17-DMAG in a panel of cervical carcinoma cell lines and demonstrated that Hsp90 inhibition effectively induced apoptosis and growth arrest [55]. HSP90 has also been identified as a molecular target for ionizing radiation. The treatment of two human cervical carcinoma cell lines (Hela and SiHa) with geldanamycin and its 17-allylamino-17-demethoxy analog (17-AAG) resulted in cytotoxicity and, when combined with ionizing radiation, enhanced the radiation response [56].

Cyclin-dependent kinases (CDK) play a crucial role in the control of the cell cycle. Recently, inhibition of CDKs by pharmacological inhibitors became a promising therapeutic option. Roscovitine, a selective CDK inhibitor, is known to efficiently target human malignant cells and induce cell cycle arrest and apoptosis through activation of p53 tumor suppressor protein. This effect was demonstrated in cervical cancer as well. Through *in vitro* studies with Hela cells, Roscovitine induced site-specific phosphorylation of p53 protein and apoptosis [57]. Another CDK inhibitor, NU6140, was tested in Hela cells, alone or in association with paclitaxel, with respect to apoptosis, inhibition of cell proliferation and cell cycle progression. Results from this study indicated that NU6140 significantly potentiated the apoptotic effect of paclitaxel, with inhibition of survivin expression/phosphorylation as the potential mechanism [58].

Finally, another interesting target is the Notch signal transduction pathway. In the latest years, it has been established that the Notch pathway mediates cell differentiation and proliferation. Intracellular forms of Notch1 have been detected in human cervical cancers, and its signaling pathway seems to complement the function of papillomavirus oncogenes. The activation of PI3K/Akt pathway and the up regulation of c-Myc have been proposed by some researchers as possible pro-oncogenic effector mechanisms [59]. Wang and colleagues showed that the overexpression of active Notch1 inhibited cervical carcinomas cells growth through induction of cell cycle arrest. Increased Notch1 signaling induced a downmodulation of human papillomavirus transcription through suppression of activator protein (AP)-1 activity by upregulation of c-Jun and the decreased expression of c-Fos [60]. In another study that used RNA interfering vectors to construct a course recombination enzyme-dependent short hairpin RNA expression plasmid targeting Notch1 in Hela cells, Notch1 expression was inhibited, intracellular Notch1 signal level was decreased and the cellular proliferation was suppressed [61].

Table **1** summarizes the current therapeutic options and the new perspectives for cervical cancer treatment according to FIGO stage.

RECENT PATENTS FOR CERVICAL CANCER TREATMENT

US20080171051 - Cancer Treatment

In this invention the combined treatment of a death receptor ligand, such as an anti FAS antibody, with a chemotherapeutic agent like 5-FU or an antifolate drug, produces a synergistic effect in killing cancer cells. However, the synergistic effect achieved is abrogated in cancer cells which overexpress c-FLIP. In cell lines which demonstrate overexpression of c-FLIP and associated resistance to chemotherapy induced apoptosis, inhibition of FLIP expression reversed the resistance to chemotherapy-induced apoptosis.

Table 1. Current Therapeutic Options and New Perspectives for Cervical Cancer Treatment

FIGO Stage	Current Therapeutic Options	New Perspectives
IA - IB1 IIA (<4cm)	S or RT	
IB2 IIA (> 4cm)	S ± adjuvant RT CT (cisplatin) + RT ± adjuvant S	Neoadjuvant CT + S ± RT Combination CT + RT Other CT agents* Biologic agents**
IIB - IVA	CT (cisplatin) + RT	Neoadjuvant CT Combination CT + RT Other CT agents* Biologic agents**
IVB	Palliative CT (cisplatin)	Combination CT Other CT agents* Biologic agents**

CT: Chemotherapy; RT: Radiotherapy; S: Surgery.

*including carboplatin, nedaplatin, paclitaxel, gemcitabine, capecitabine, vinorelbine, ifosfamide and topotecan.

** including cetuximab, bevacizumab, erlotinib, gefitinib, lapatinib, sorafenib and celecoxib.

On further investigating this effect, the inventors tested a number of cell lines having a p53 mutation or p53 null genotype, and observed that down-regulation of c-FLIP markedly enhanced apoptosis in response to certain chemotherapeutic agents. This observation led to the invention of c-FLIP inhibi-tors combined to chemotherapeutic agents for the treatment of malignancies associated with p53 mutations, among them cervical cancer.

The c-FLIP inhibitor and the chemotherapeutic agent (thymidylate synthase inhibitor, platinum cytotoxic agent or topoisomerase inhibitor) may be provided and administered in the absence of other active agents. However, in a preferred embodiment of theses aspects of the invention, there is provided a death receptor binding member, or a nucleic acid encoding said binding member. Any suitable death receptor binding member may be used. Death receptors include Fas, TNFR, DR-3, DR-4 and DR-5. Preferably, the c-FLIP inhibitor and the chemotherapeutic agent are administered in a potentiating ratio, such that the cytotoxic activity of the combination is greater than that of either component alone or of the additive activity that would be predicted for the combinations based on the activities of the individual components. Thus in a potentiating ratio, the individual components act synergistically. The c-FLIP inhibitor can be an RNAi agent, which modulates expression of the c-FLIP gene, or a siRNA, a shRNA, a ddRNAi construct or a transcription template thereof, e.g., a DNA encoding a shRNA [62].

US20080113340 - Diagnosis and Treatment of Cervical Cancer

In US20080113340 the invention relates to methods of diagnosing cervical diseases or conditions, including cervical cancer, cervical precancerous lesions, or immortalization of cervical cells, by using a panel of biomarkers. The invention also relates to methods of treating cervical diseases by targeting one or more of these biomarkers.

The diagnosing method comprises analyzing the status of at least two of the following biomarkers: human telomerase reverse transcriptase (hTERT), insulin-like growth factor binding protein 3 (IGFBP-3), transferrin receptor, beta-catenin, Myc-human papilloma virus (HPV) E6 interaction, HPV E7, and telomere length, in cervical cells of the female. If the biomarker is hTERT, IGFBP-3, transferrin receptor or HPV E7, the status to be assessed is the expression level of the biomarker. Preferably, the expression level of HPV E7 is analyzed by flow cytometry. Increased expression level of the biomarker relative to an appropriate control level (e.g., obtained from a healthy female) indicates that the female has cervical cancer or is at increased risk of developing this tumor. If the biomarker is beta-catenin, the status to be assessed is the level and localization of beta-catenin in the cytoplasm and/or nucleus. In case the biomarker is Myc-HPV E6 interaction, the association between Myc and HPV E6 is analyzed. Further on, if the biomarker is telomere length, an increased telomere length relative to control indicates that the female has cervical carcinoma or has a higher risk of developing it.

The invention also provides a method of classifying the grade of a cervical lesion for diagnostic and/or prognostic purposes. Such method aims to determinate the status of one or more biomarkers (including hTERT, IGFBP-3, transferrin receptor, beta-catenin, Myc-HPV E6 interaction, HPV E7, and telomere length, and combinations) in a cervical cell of a female to provide an individual biomarker diagnostic for cervical lesions. The status of the individual biomarker can be combined with a biomarker reference panel and the cervical cancer lesion can be classified according this comparison. Preferably, the biomarker reference panel of the method comprises a constituent panel developed using cervical cancer, high grade cervical lesion, low grade cervical lesion, and control group populations.

Moreover, the invention provides a method of treating cervical cancer or preventing the onset of cervical cancer and reducing the extent to which it occurs. Such method comprises administering to the female a therapeutically effective amount of an agent which targets and blocks or decreases the function of one or more of the biomarkers. In one case, the agent blocks interaction between Myc and HPV E6. In other cases, the agent blocks or reduces the expression level of hTERT, IGFBP-3, transferrin receptor, beta-catenin, HPV E6, or HPV E7. In a particular case, the agent blocks signaling through the beta-catenin pathway. Exemplary therapeutic agents in such methods include, but are not limited to, small molecules, polypeptides, antibodies, and nucleic acids. In specific embodiments, the present invention contemplates the use of antisense nucleic acids or RNA interference (RNAi) nucleic acids to block or reduce gene expression of one or more of the above biomarkers.

The methods can be used alone or in combination with other anti-viral or anti-cancer therapeutic approaches (e.g., administration of an anti-viral or anti-cancer agent, radiation therapy, phototherapy or immunotherapy) directed to treatment or prevention of cervical cancer or virus infections. Thus, the methods of the invention may further include as optional ingredients one or more agents already known for their use in the inhibition of cervical cancer, for added clinical efficacy. These agents include interleukin-2,5′-fluorouracil, nedaplatin, methotrexate, vinblastine, doxorubicin, carboplatin, paclitaxel (Taxol), cisplatin, 13-cis retinoic acid, pyrazoloacridine, vinorelbine, artemisinin, and artemisinin analogs. Appropriate amounts in each case will vary with the particular agent, and will be either readily known to those skilled in the art or readily determinable by routine experimentation. In other cases, the subject methods of the invention may further include as optional ingredients one or more agents already known for their anti-viral effects, including 5′-fluorouracil, interferon alpha, imiquimod, lamivudine, arsenic trioxide, capsaicin, nucleoside analogues (e.g., acyclovir), and antiviral vaccines [63].

US20080187513 - Treatment of Solid Cancers

US20080187513 patent contemplates a method for treating or preventing cancer growth and metastasis by administering angeloyl substituted ingenanes or derivatives directly or proximally to the tumor, in order to induce primary necrosis in the cancer cells and to stimulate the generation of cancer-specific T-cells. The cancer-specific T-cells include $CD8^+$ T-cells and $CD4^+$ T-cells or their precursors. The angeloyl substituted ingenanes can be either

combined with genetic, immunological or cytological agents which enhance, co-operate or otherwise synergize the induced cancer-specific T-cells, or with other anti-cancer regimens including radiotherapy and chemotherapy. Angeloyl substituted ingenanes can be co-administered with a cancer vaccine such as a dendritic cell vaccine or a vaccine based on virus vector or recombinant protein or cancer cell lysate, which is capable of presenting a cancer antigen or epitope to the immune system.

The method of the present invention assists in the treatment of primary tumors and prevents or reduces the growth of secondary tumors. Thus, this immunostimulatory chemoablation therapy not only debulks the tumor burden but in so doing also induces cancer-specific T-cells such as $CD8^+$ T-cells and $CD4^+$ T-cells. The angeloyl substituted ingenanes or derivatives may be synthetically produced or may be derived from extracts of a plant of the Euphorbiaceae family, particularly *Euphorbia peplus* [64].

US20080286781 - Compositions, Kits, and Methods for Identification, Assessment, Prevention, and Therapy of Cervical Cancer

In US20080286781 the invention relates to cancer markers that can be exploited for cervical cancer diagnosis, staging, prognosis and treatment, including carcinomas (carcinoma *in situ*, invasive carcinoma, metastatic carcinoma) and pre-malignant conditions (dysplasia, including CIN or SIL). The invention provides a diagnostic method of assessing whether a patient has cervical cancer or has higher than normal risk for its development, by comparing the level of expression of a marker of the invention in a patient sample and the normal level of expression of the marker in a control, e.g., a sample from a patient without cervical cancer. The markers are selected such that the positive predictive value is at least about 10%, preferably about 25%, more preferably about 50% and most preferably about 90%. The methods of the invention may be used to measure response to therapy, for example, to verify the reduction in tumor burden, evaluating the expression of the marker in a patient before, during and after treatment.

The invention further provides a method of inhibiting cervical cancer in a patient, which consists in obtaining a sample comprising cancer cells from the patient, separately maintaining aliquots of the sample in the presence of a plurality of compositions, comparing expression of a marker of the invention in each of the aliquots and administering to the patient at least one of the compositions which significantly lowers the level of expression of the marker The cells may be found in a cervical smear or body fluid ncluding blood, lymph, ascitic fluids, gynecological fluids, urine, and fluids collected by vaginal rinsing.

Additionally, the invention includes the use of untibodies which bind specifically with a marker protein or a fragment of the protein. The invention also provides methods for making such antibody, antibody derivative, and antibody fragment, which may comprise immunizing a mammal with a protein or peptide containing the entirety of a marker protein, wherein the protein or peptide may be obtained from a cell or by chemical synthesis. The methods of the invention also encompass producing monoclonal and single-chain antibodies, which would further comprise isolating splenocytes from the immunized mammal, fusing the isolated splenocytes with an immortalized cell line to form hybridomas, and screening individual hybridomas for those that produce an antibody that binds specifically with a marker protein or a fragment of the protein [65].

US20080213220 - Cancer-Targeted Viral Vectors

US20080213220 patent relates to viral vectors that are targeted to cancer cells, including cervical cancer. The viral vectors of the invention are adenoviruses having a PEG-3 promoter driving the expression of the viral genes E1A and E1B. The PEG-3 promoter exhibits increased activity in malignant cells. Adenoviruses of the invention show increased replication in malignant cells, thereby producing a cytopathic effect. The viral vectors of the invention further comprise additional genes of interest, and may have altered capsid proteins that may enhance infection of and target infection to cancer cells. Additional cell types derived from diseased states in which the PEG-3 promoter is selectively active are also therapeutic target of the viral vectors of the instant invention including those generating allergic, autoimmune and inflammatory responses [66].

WO2008104804 - Proteins

WO2008104804 patent relates to the identification of membrane proteins associated with cervical cancer, among other malignancies. These proteins can be useful as tumor markers and as targets against which antibodies or other pharmaceutical agents can be made. The first aspect of the invention provides methods of treating cervical cancer that consists in administering to a cervical cancer patient a therapeutically effective amount of a compound that modulates (up regulates or down regulates) or complements the expression or the biological activity of one or more proteins of the invention.

The second aspect of the invention consists on a method for cervical cancer detection, diagnosis and/or screening and disease progression monitoring. It comprises detecting the presence or level of the proteins of the invention, and also the fragments or nucleic acid that encodes these proteins. This may include the step of obtaining a biological sample (serum or tissue) from the patient. The analyses can be made either through imaging technologies or through immunohistochemistry on tissue sections. Immunohistochemistry is a technique which detects the localization of an antigen by the use of specific labeled antibodies. Antigen-antibody interactions can be visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.

In a further aspect, this invention provides the use of the proteins as vaccine compositions, for either prophylactic or therapeutic purpose. The vaccine compositions can include one or more immuno stimulants [67].

US20080260729 - Method of Treating Cancer Comprising a VEGF-B Agonist

US20080260729 patent provides a method of inhibiting the growth of cancer including tumor tissue and pre-cancerous tissue using an antagonist of VEGF-B. Com-

positions are also provided comprising one or more VEGF-B antagonists alone or in combination with other anti-cancer agents or other angiogenesis inhibiting agents. An antagonist contemplated by the present invention may be an antibody which inhibits interaction between VEGF-B and VEGFR-1, an antisense compound which reduces VEGF-B expression, or an interfering nucleic acid which reduces VEGF-B expression. The preferred antibodies bind to VEGF-B and interfere with VEGF-B interaction with its receptor. The antibody and other antagonists are proposed for use in treating certain conditions mediated in whole or in part, or directly or indirectly, by VEGF-B. Preferably, the antibodies are monoclonal antibodies or antigen-binding fragments thereof. Even more preferably, the antibodies are humanized antibodies including deimmunized or chimeric antibodies or human antibodies suitable for administration to humans. Antibodies in accordance with this invention include the murine monoclonal antibodies 1C6, 2F5, 2H10 and 4E12, and humanized, deimmunized or chimeric forms of mAbs 1C6, 2F5, 2H10 and 4E12 [68].

US20070269407 - Use of Interleukin-19 to Treat Cervical Cancer

US20070269407 patent is based on treating a mammalian having HPV infection, cervical dysplasia, cervical intra-epithelial neoplasia and carcinoma of the cervix with interleukin-19 (IL-19). The invention also provides a method for inhibiting the growth of cervical cancer cells by bringing IL-19 or fragments comprising helices A-D of IL-19, into contact with these cells. The quantities of IL-19 for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medications administered. Methods for administration include intravenous, peritoneal, intramuscular, transdermal or administration into the lung or trachea in spray form by means or a nebulizer or atomizer. Dosage ranges would ordinarily be expected from 1μg to 1000μg per kilogram of body weight per day. However, the doses may be higher or lower as can be determined by a medical doctor with ordinary skill in the art.

For cervical cancer diagnosis, suitable detectable molecules (radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles) may be directly or indirectly attached to IL-19. For treatment purposes, IL-19 can be administered in conjunction to radiation and chemotherapic agents, such as bleomycin, chlorambucil, epirubicin, 5-fluorouracil, ifosfamide, mitomycin, methotrexate, vincristine, cisplatin and vinblastine. Cytotoxic molecules may be directly or indirectly attached to IL-19, and include bacterial or plant toxins, as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90. In addition, IL-19 polypeptide-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation. IL-19 cytokine fusion proteins can be used for *in vivo* killing of cervical cancer, where IL-19 receptors are expressed. The described fusion proteins enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. IL-19 polypeptides target an undesirable cancerous cell or tissue, and the fused cytokine mediated improves target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor [69].

US20070166328 - Genetic Immunization Against Cervical Carcinoma

US20070166328 patent is an alphavirus vector system comprising nucleic acid derived from a HPV. Alphariuses include a nucleocapsid with one copy of a single-stranded RNA molecule surrounded by envelope containing spike proteins. Alphavirus RNA has a positive polarity, thus enabling the genomic RNA to initiate an infection when introduced into the cytoplasm of a cell. Further, the RNA is self-replicating since it encodes its own replicase, wherein replication results in high-level expression of the viral proteins in host cells.

The nucleic acids are derived from a HPV type 16 or type 18, and can be a gene, a functional part of a gene, a precursor of a gene, a transcribed gene on any nucleic acid level or a gene product derived therefrom that can overcome cell cycle suppression. The cell cycle suppression may be overcome by inactivating major tumor suppressor proteins, such as P53 and pRB gene products, respectively, leading to loss of normal cellular differentiation and the development of a carcinoma.

The alphavirus viral system is suited to safely induce cellular immune responses against oncoproteins such as HPV 16/18 E6 and E7. The invention further discloses an alphavirus vector system or other viral vector systems wherein the nucleic acid further encodes a cytokine gene or functional fragment thereof. Cytokines are primarily involved in signaling between cells of the immune system. It is provided to use Granulocyte-Macrophage Colony-Stimulating-Factor (GM-CSF) and/or Interleukin 12 (IL-12). However, the cytokines IL-2, IL-6, IL-18, and others are also contemplated.

The invention also provides the incorporation of an alphavirus vector system and/or a cell infected with an alphavirus vector system for the preparation of a vaccine for cervical cancer treatment. The method includes providing an alphaviral vector system with a broad host range comprising nucleic acid encoding tumor antigens devoid of capacity to bind to the cellular tumor suppressor products pRB and P53 and capable of inducing an HPV-specific cytotoxic T lymphocyte (CTL) response against HPV-transformed tumor cells expressing tumor antigens. CTLs can destroy cells expressing foreign antigens through recognition of foreign peptides generated within the cell, transported to the cell surface and presented by histocompatibility complex (MHC) class I antigens. The CTLs are, thus, potentially powerful agents of tumor cell destruction [70].

US20060029613 - Biological Compositions and Methods for Treatment of Cervical Cancer

US20060029613 patent relates to biological or oral compositions useful for subjects with cervical cancer. The methods of the invention comprise culturing yeast cells in the presence of a series of electromagnetic fields, such that the yeast cells become metabolically active. The electromagnetic fields used are each defined by one of five

frequency ranges and a broad range of field strength. The starting yeast cells are commercially available and accessible to the public as *Saccharomyces*. The methods for making the biological compositions of the invention further comprise conditioning the activated yeast cells in plant extracts and the gastric juice of animals, while in the presence of another series of electromagnetic fields.

The methods of manufacturing also comprise expanding the number of activated or activated and conditioned yeast cells in large scale cultures in the presence of yet another series of electromagnetic fields, performing quality control measures, and packaging. Pharmaceutical compositions of the invention comprise activated and conditioned yeast cells and one or more pharmaceutically acceptable excipients or carriers. Additional ingredients, such as vitamins and/or flavors may be added to the biological compositions to form the oral compositions of the invention. Such additional carriers and ingredients can improve the healthful benefits, pharmacological properties, and organoleptic characteristics of the oral compositions. During the manufacturing process, the activated or activated and conditioned yeast cells may be dried and stored for a period of time. The biological or oral compositions of the invention are ingested by the subject or used as an additive to be incorporated into food to be consumed by the subject. Dietary supplement and nutritional compositions comprising activated and conditioned yeast cells are encompassed by the invention.

The biological composition of the invention can retard the growth of cervical cancer cells and prolong the time of survival of an animal with cervical cancer which received the composition orally [71].

US20060039919 - Fusion Protein for Inhibiting Cervical Cancer

US20060039919 patent refers to a fusion protein for inducing immune response in cervical cancers. The fusion protein of the present invention can effectively inhibit the proliferation of carcinoma cells, induce cytotoxic T lymphocytes (CTL) and antibody protection *in vivo* and destroy the infected cells by presenting the antigen. The pharmaceutical composition of the present invention also comprises a medical compound such as a fusion protein for preventing or inhibiting cancer induced by HPV type 16, wherein the compound is able to control the proliferation or the increase of carcinoma cells. The invention also discloses an antibody composition, which targets the antigen of E7 peptide *in vivo* [72].

US6825226 - Apoptosis Inducing Adamanthyl Derivatives and their Usage as Anti-Cancer Agents, Especially for Cervical Cancers and Dysplasias

US6825226 patent relates to the discovery that specific adamantyl or adamantyl group derivatives containing retinoid-related compounds induce apoptosis of cancer cells and therefore may be used for the treatment of cancer, including advanced malignancies. Also, the present invention relates to novel adamantyl or adamantyl group derivatives containing retinoid compounds and their usage for prevention of cancer, keratinization disorders and dermatological conditions. More specifically, it has been shown that such adamantyl compounds, e.g., 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoicacid, 2-[3-(1-adamantyl)-4-methoxyphenyl]-5-benzimidazole carboxylic acid, and 6-[3-(1-adamantyl)-4,5-methylenedioxyphenyl]-2-naphthoic acid, can be used to treat or prevent cervical cancers and precancers such as cervical dysplasias, including high grade and low grade dysplasias [73].

US20030161811 - Method for Treating Cervical Cancer

Molecular and epidemiologic studies have demonstrated a strong relationship between HPV, cervical intraepithelial neoplasia, (CIN), and invasive carcinoma of the cervix. This invention refers to administering interleukin-20 (IL-20) to a mammalian having cervical cancer or HPV infection. The invention also provides a method for inhibiting the growth of cervical cancer cells by bringing IL-20 into contact with these cells. Interleukin-20 (formally called Zcyto10) can be produced according to the method described in International Patent Application US98/25228 filed on Nov. 25, 1998. The human IL-20 polypeptide is comprised of a sequence of 176 amino acids.

IL-20 can be administered intralesionally, or intramuscularly for localized disease. For metastatic disease, IL-20 can also be administered by intraperitoneal administration including intravenous administration. IL-20 can be administered alone or in conjunction with standard therapies such as surgery, radiation or other chemotherapeutic agents such as bleomycin, chlorambucil, epirubicin, 5-fluorouracil, ifosfamide, mitomycin, methotrexate, vincristine, cisplatin and vinblastine.

Cells infected with HPV can be treated with IL-20 to inhibit the proliferation of the virus. Anogenital warts caused by HPV type 6, 11, 16, 18, 31, 33 and 35 are transmitted sexually and have an incubation period of 1 to 6 months. Endocervical wart infections caused by type 16 or 18 have been implicated as a cause of cervical intraepithelial neoplasia and cervical cancer. HPV types 16 and 18 generally do no not cause external genital warts, which are usually caused by types 6 and 1. IL-20 can be administered directly into lesions containing cells infected with HPV alone or with standard therapies such as interferon alpha or interferon beta both of which are commercially available. IL-20 can also be administered with other standard therapies for treating HPV including antimitotics such as podophyllotoxin, podophyllin, or 5-fluorouracil; caustics such as trichloroacetic acid; or interferon inducers such as imiquimod. The quantities of IL-20 for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medications administered [74].

The above described patents are summarized in Table **2**.

CURRENT & FUTURE DEVELOPMENTS

Despite significant advances in the understanding and management of cervical cancer, the mortality rates for this malignancy remain exceedingly high. It is believed that the greatest impact in cervical cancer burden will be achieved by primary prevention, either through better screening programmes or by restricting the spread of its viral cause.

Table 2. Overview of New Patents for Cervical Cancer Treatment

Patent Number	Invention	Mechanism of Action	Reference
US20080171051	c-FLIP inhibitor	Enhances cytotoxicity of CT	[62]
US20080113340	Biomarkers	Target therapy	[63]
US20080187513	Angeloyl substituted ingenanes	Induce primary necrosis; activate the immune system	[64]
US20080286781	Biomarkers	Target therapy	[65]
US20080213220	Viral vectors	Target cancer cells	[66]
WO2008104804	Biomarkers	Target therapy	[67]
US20080260729	VEGF-B antagonist	Target therapy	[68]
US20070269407	Interleukin-19	Activate the immune system	[69]
US20070166328	Alphavirus vectors	Activate the immune system	[70]
US20060029613	Metabolically active yeast cells	Retard tumor growth; prolong survival	[71]
US20060039919	Fusion protein	Activate the immune system	[72]
US20046825226	Adamantly derivates	Induce apoptosis of cancer cells	[73]
US20030161811	IL-20	Activate the immune system	[74]

CT: chemotherapy.

There is hope that the widespread HPV vaccination might benefit many women in the future, although these results are not expected for the next decades. Meanwhile, the main challenge is to improve the current therapeutic options, especially in respect to advanced lesions. Although no agent has shown superiority to cisplatin, much effort is being concentrated in the search for new drugs and optimal regimens to combine with radiotherapy. Additionally, various promising biologic agents are being developed and tested, and their results are enthusiastically awaited.

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CONFLICT OF INTEREST

Dr Daniela B. Cornelio, Rafael Roesler and Gilberto Schwartsmann do not have any financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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7. DISCUSSÃO GERAL

O envolvimento dos neuropeptídeos GRP e BDNF com câncer vem sendo muito estudado nos últimos anos em diversos tipos e modelos tumorais. Porém, antes deste trabalho não havia dados na literatura quanto ao papel de GRP/GRPR em lesões displásicas e neoplásicas de colo uterino. Também havia consenso de que GRP teria um papel mitogênico em células tumorais, enquanto que o bloqueio de GRPR deveria inibir a proliferação celular. O presente estudo descreveu pela primeira vez os seguintes pontos: a expressão de GRPR em tecidos neoplásicos e sadios de colo uterino (capítulo I); a técnica de imunocitoquímica para GRPR, a expressão de GRPR em esfregaços cervicais e o potencial diagnóstico desta técnica para o diagnóstico de lesões de colo uterino (capítulo II); o efeito antiproliferativo do tratamento de células de câncer de colo uterino, mama e ovário com GRP e o inverso aumento da viabilidade celular com o bloqueio de GRPR com o antagonista RC-3095 (capítulo III).

Os resultados do estudo do capítulo I evidenciaram superexpressão de receptores de GRPR em neoplasias cervicais humanas. Estes receptores também foram encontrados, em menor densidade, nas ectocervices adjacentes às lesões neoplásicas, enquanto que praticamente não foram mensuráveis nas endocérvices. De acordo com o nosso conhecimento, esta avaliação da expressão do GRPR em câncer de colo de útero não havia sido previamente descrita na literatura.

Os peptídeos semelhantes ao GRP podem ser encontrados em tecidos normais, desempenhando funções fisiológicas. Durante o desenvolvimento fetal, a presença de GRPRs já foi estabelecida em diversas localizações nos sistemas respiratório, nervoso, urogenital e gastrintestinal (BATTEY *et al*., 1994). Em adultos, já foram demonstrados GRPRs em células sanguíneas periféricas de indivíduos sadios (SHINGYOJI *et al*., 2003) e em vários órgãos, como o estômago (FERRIS *et al*., 1997) e o pâncreas (XIAO *et al*., 2001). Alguns autores demonstraram a expressão ubíqua destes receptores em

lóbulos e dutos mamários sadios, sugerindo papel do GRP na fisiologia da mama (GUGGER & REUBI, 1999).

Quanto ao trato genital feminino, já foram encontradas elevadas concentrações de BLPs em tecidos vaginais de ratas (GHATEI *et al*., 1985). Adicionalmente, já foram descritas funções fisiológicas do GRP em contrações da musculatura lisa uterina (STJERNQUIST *et al*., 1986) e no crescimento de células do estroma endometrial humano (ENDO *et al*., 1991). Também em humanos, diversas estruturas uterinas como miométrio, glândulas endometriais e vasos foram capazes de expressar GRPRs em condições fisiológicas. Fleishmann e colaboradores (2005) avaliaram tecidos uterinos sadios e neoplásicos e descreveram superexpressão de GRPRs no miométrio normal, em todas as fases do ciclo menstrual e na pós-menopausa, bem como no endométrio funcional.

Histologicamente, a ectocérvice é recoberta por epitélio estratificado escamoso, que é essencialmente idêntico ao epitélio que recobre a vagina. Na medida em que GRPRs já foram descritos em tecidos vaginas sadios (GHATEI *et al*., 1985), encontrar estes receptores em nossas amostras já era esperado, salientando que a imunosinalização foi sempre menos intensa quando comparada às respectivas lesões neoplásicas. Uma possibilidade a considerar é que os GRPRs poderiam estar envolvidos na promoção de efeitos tróficos nas ectocérvices. Entretanto, quando avaliamos amostras cervicais não neoplásicas, não encontramos GRPRs nas ectocérvices. Com base nesses achados, hipotetizamos que a presença de GRPRs nas ectocérvices adjacentes a tumores ou displasias poderia representar um evento molecular precoce na carcinogênese cervical. Em contraste, as endocérvices praticamente não exibiram GRPRs. Uma explicação possível é que este tecido apresenta uma taxa de *"turnover"* menor comparada à

ectocérvice, a qual está constantemente exposta às injúrias do meio. Esta questão ainda necessita de estudos adicionais para ser elucidada.

No câncer, o GRP já está estabelecido como fator de crescimento, com efeitos mitogênicos autócrinos e parácrinos, atuando também como morfogene e como agente proangiogênico (PATEL *et al*., 2006). Os GRPRs já foram localizados em muitas neoplasias humanas através de diferentes métodos, mais comumente ensaios de ligação ao receptor, detecção de RNAm com rt-PCR, ou imuno-histoquímica (CORNELIO *et al*., 2007). Com o emprego de técnicas tão distintas, os resultados na distribuição do receptor são variáveis entre os estudos. Mesmo quando utilizados métodos iguais, a quantificação dos resultados não é uniforme entre os autores. Neste estudo optamos pelo método da imuno-histoquímica, por ser uma tecnologia simples e acessível, sendo amplamente empregada em rotinas de patologia. Além disso, a imuno-histoquímica preserva a arquitetura dos tecidos e a morfologia celular, de maneira que as reações imunológicas podem ser atribuídas a subpopulações específicas.

Os resultados encontrados neste estudo demonstraram reação imunológica positiva difusa em 99% das lesões neoplásicas, com a grande maioria exibindo um forte padrão de coloração. Estes achados são concordantes com a expressão aberrante exibida em outros tipos de câncer já estudados. No câncer de próstata, as amostras se mostraram 100% positivas para expressão de GRPR por diferentes autores e diferentes métodos (BARTHOLDI *et al*., 1998; MARKWALDER & REUBI, 1999; REUBI *et al*., 2002). Quando foram avaliados os respectivos tecidos sadios adjacentes às lesões, eles raramente expressaram quantidades mensuráveis de GRPRs ou exibiram sinais fracos de coloração (BARTHOLDI *et al*., 1998; MARKWALDER & REUBI, 1999). Baseado nestes achados foi proposto que estes receptores poderiam ser úteis na diferenciação entre hiperplasia e neoplasia. Chave e colaboradores (2000) avaliaram tecidos colo

retais normais e neoplásicos e detectaram GRPRs em todas as amostras, mas com superexpressão nas tumorais, reforçando o papel do GRP como fator de crescimento autócrino no processo carcinogênico. Outros pesquisadores também demonstraram expressão de GRPR no carcinoma de rim, mas não nos tecidos renais normais (PANSKY *et al*., 2000). Resultados semelhantes foram obtidos analisando pacientes com neuroblastomas (SEBESTA *et al*., 2001) e carcinomas escamosos de cabeça e pescoço (LANGO *et al*., 2001), em que os receptores foram encontrados em todas as amostras avaliadas. Como podemos observar, a expressão de GRPRs é muito mais comumente encontrada em malignidades do que em tecidos humanos sadios. O que permanece incerto é se a expressão de GRPR é apenas um marcador tumoral ou se sinaliza um comportamento biológico agressivo destas neoplasias.

Na tentativa de responder esta questão, objetivou-se neste primeiro estudo correlacionar os níveis de expressão de GRPR com os diferentes graus do câncer de colo uterino, para verificar se haveria uma maior densidade de receptores em lesões mais invasivas. Alguns autores foram capazes de associar maior incidência de GRPRs em carcinomas mais agressivos ou pobremente diferenciados (SAURIN *et al*., 1999; SUN *et al*., 2000a; KIM *et al*., 2002). Em nossa análise, entretanto, não foram encontradas diferenças significativas entre os diferentes subtipos patológicos; os receptores estavam quantitativamente presentes em todas as amostras, de NIC I a câncer invasor. Estes resultados são consistentes com outros estudos, onde a expressão de GRPR não pôde ser associada a fatores patológicos ou prognósticos (SUN *et al.*, 2000b; SEBESTA *et al.*, 2001).

Os resultados obtidos nesta primeira pesquisa nos motivaram a seguir avaliando GRPR em colo uterino como um potencial biomarcador. No estudo do capítulo II, procuramos avaliar a presença de GRPRs em esfregaços cervicais através da técnica de

imunocitoquímica, no intuito de detectar lesões displásicas e neoplásicas, dadas as limitações no rastreamento com o teste citopatológico convencional.

O teste citopatológico de Papanicolau consiste em um método subjetivo de avaliação morfológica das células cervicais e permanece até hoje como o exame de escolha para a detecção de câncer cervical. Embora seja a principal ferramenta no rastreamento do câncer de colo de útero, não é um teste ideal por não possuir elevada sensibilidade (SHARMA & MENON, 2006). Nos últimos anos, avanços tecnológicos foram instituídos na coleta e processamento das amostras, com impacto positivo na detecção de lesões cervicais. No entanto, a interpretação morfológica dos testes citológicos ainda apresenta limitações. Tem sido demonstrado que as interpretações errôneas e as discrepâncias interobservadores são comuns, especialmente dentro das categorias de ASCUS e LSIL (STOLER & SCHIFFMANN, 2001). De fato, muitas das lesões caracterizadas como ASCUS ou LSIL na verdade consistem em lesões de alto grau (ARBYN *et al*., 2008). Assim, é importante buscar um método confiável para o diagnóstico de lesões cervicais, para ser utilizado independentemente ou em conjunto com o teste de Papanicolau convencional.

O primeiro objetivo do estudo do capítulo II foi avaliar a possibilidade de realizar a técnica de imunocitoquímica com GRPR, já que esta não havia sido descrita previamente para nenhum tipo de tumor. Fomos capazes de realizar a técnica com sucesso, utilizando o mesmo fixador empregado para o exame de Papanicolau. Das 66 amostras analisadas, apenas três não puderam ser interpretadas devido a falhas na técnica de coloração.

Para a interpretação dos resultados, a intensidade da coloração não foi graduada como na técnica de imuno-histoquímica, com o intuito de evitar conclusões subjetivas.

Conforme descrito por Guo *et al.* (2008) para a expressão de p16, nós avaliamos quantitativamente a presença de células imunopositivas em um campo microscópico de média potência. Uma vez que esta foi a primeira descrição da expressão de GRPR por imunocitoquímica em esfregaços do colo do útero, não tínhamos parâmetros anteriores com GRPR para comparar. Portanto, baseamos nossa pesquisa em dados de estudos de imunocitoquímica com p16. Verificamos que não há consenso até agora, de um método padrão para a interpretação dos resultados de imunocitoquímica, particularmente em esfregaços cervicais. Ainda não existem argumentos claros para o estabelecimento de um ponto de corte, a partir do qual uma amostra é considerada positiva. Na revisão sistemática e meta-análise de Klaes e colaboradores (2001), o ponto de corte para positividade de p16 variou de uma célula até mais de 30% de células coradas. Neste trabalho, consideramos expressão positiva de GRPR a presença de mais de cinco células coradas.

A expressão de GRPR foi positiva na grande maioria das lesões cervicais; em 83% de NIC I, 86% de NIC II-III e em 100% das amostras de carcinomas invasivos. Comparado ao p16, o marcador mais estudado para displasia de colo de útero, essa expressão de GRPR foi maior do que a de p16 em NIC I e equivalente em CIN II-III, de acordo com os dados reportados em uma meta-análise da expressão de p16. Embora com ponto de corte variável, a positividade média de p16 para NIC I e NIC II-III foi de 45% (37-57%) e 89% (84-95%), respectivamente (KLAES *et al*., 2001). A maioria das células com expressão de GRPR exibiu predominantemente coloração citoplasmática. Este achado é semelhante aos nossos resultados prévios com imuno-histoquímica de GRPR.

Nos esfregaços alterados de acordo com o teste de Papanicolau, a imunosinalização não foi limitada apenas a células displásicas, mas também pôde ser

visualizada em algumas células epiteliais normais. Por outro lado, a expressão de GRPR foi raramente detectada em esfregaços normais. Isso confirma nossos achados anteriores, ou seja, as células normais adjacentes a lesões exibiram receptores de GRP, contrastando com a pouca ou ausente sinalização em tecidos não malignos. Concluímos que a presença de GRPRs nesses casos pode representar um evento molecular precoce na carcinogênese cervical.

Naquelas pacientes com esfregaços alterados foram realizadas biópsias ou cirurgias. A análise imuno-histoquímica de GRPR mostrou positividade de 88%, geralmente com um sinal forte nos epitélios displásicos e neoplásicas, claramente diferente dos epitélios normais adjacentes ou do estroma. Esta expressão foi ainda maior do que a encontrada no estudo do capítulo I, em que 78,4% dos tecidos exibiram coloração difusa moderada ou forte. Também foi realizada análise imuno-histoquímica de p16 nestas amostras, e evidenciou praticamente os mesmos resultados do que a expressão de GRPR, ou seja, 88,4% de positividade.

GRPR foi homogeneamente expresso nos diferentes graus de NIC e carcinomas de células escamosas, tanto nos esfregaços, quanto nos tecidos. Não encontramos associação entre um maior número de células positivas com um aumento da severidade das lesões, achado também congruente com o primeiro estudo. O grau de concordância entre a expressão de GRPR em esfregaços do colo do útero e dos tecidos correspondentes foi de 88%. Este resultado é bastante interessante, já que através de uma técnica não invasiva como a coleta do raspado cervical, podemos obter uma informação bastante fidedigna do status de expressão tecidual de GRPR.

No presente estudo o exame de Papanicolau apresentou sensibilidade de 77,4% e especificidade de 65,6%. Uma revisão sistemática da sensibilidade e especificidade dos

testes citológicos convencionais encontrou vieses em muitos estudos e uma grande variação nos seus resultados. Avaliando aqueles com melhor metodologia e controles válidos, a sensibilidade de um exame Papanicolau para o diagnóstico de NIC I ou lesão mais avançada variou de 30 a 87%, com especificidade 86-100% (NANDA *et al*., 2000). De acordo com a literatura atual, existe claramente uma correlação entre os resultados citológicos anormais e os respectivos exames anátomo-patológicos, mas uma correspondência direta é encontrada em apenas metade dos pacientes (ALTS GROUP, 2003). Consistente com esta informação, nossos resultados dos exames Papanicolaou coincidiram com o diagnóstico histológico em apenas 32,2% das amostras neoplásicas analisadas. É importante salientar que três carcinomas invasores foram subdiagnosticados pelo teste de Papanicolau em nosso estudo.

Outra questão importante foi avaliar lesões classificados como ASCUS com respeito à expressão de GRPR. Está bem estabelecido na literatura que o exame Papanicolau é menos sensível quando as alterações citológicas são classificadas como ASCUS (ALTS GROUP, 2000). Adicionalmente, o diagnóstico de ASCUS tem considerável variabilidade interobservadores, mesmo entre patologistas especializados. No estudo ASCUS/LSIL, a concordância diagnóstica foi de apenas 55% entre os casos de ASCUS (ALTS GROUP, 2003). Infelizmente, a baixa sensibilidade do teste de Papanicolau nesta questão afeta milhões de mulheres, porque até 5% de todos os exames Papanicolau são classificados como ASCUS, e apenas uma pequena parcela das pacientes (5 - 17%) efetivamente terá lesões NIC II-III que necessitam de tratamento. Inversamente, existem casos em que exames ASCUS podem corresponder a lesões mais avançadas, incluindo câncer invasor, e não podem ser subtratados (NYGARD *et al*., 2003). Nós obtivemos uma proporção maior de resultados ASCUS em relação à população normal, pois o estudo foi realizado em um centro de referência de patologia

cervical. Sete de 16 amostras classificadas como ASCUS correspondiam a colposcopias normais no grupo controle e 9 correspondiam a lesões displásicas ou neoplásicas comprovadas por análise histológica. A imunocitoquímica de GRPR mostrou alta acurácia (80%) no diagnóstico de lesões classificadas como ASCUS, quer em detectar a presença ou ausência de lesões. A sensibilidade foi de 88% e a especificidade de 71%, enquanto que os valores preditivos positivos e negativos foram de 77% e 83%, respectivamente. No presente trabalho, duas pacientes encaminhadas para colposcopia devido a um diagnóstico ASCUS apresentavam carcinomas invasivos. Nestas amostras, GRPR estava fortemente expresso.

Finalmente, um dos principais objetivos deste segundo estudo foi comparar a utilidade diagnóstica da expressão de GRPR por imunocitoquímica com o exame Papanicolau. A sensibilidade da expressão de GRPR em detectar lesões foi de 87,5%, contrastando com 77,4% do exame Papanicolau, ou seja, 10,1% maior. Também na especificidade houve um ganho de 11,1%, comparando valores de 76,7% da expressão de GRPR com 65,6% do exame Papanicolau. Estes resultados não atingiram significância estatística devido ao limitado tamanho da amostra. Todavia, o principal objetivo deste trabalho era avaliar se a expressão de GRPR poderia ser utilizada como uma potencial ferramenta diagnóstica. A condução de um estudo incluindo um número maior de mulheres é necessária para poder comprovar o benefício da técnica de imunocitoquímica de GRPR na detecção de lesões cervicais.

No capítulo III avaliamos o efeito dos neuropeptídeos GRP e BDNF e de antagonistas de seus respectivos receptores, GRPR e Trk, na viabilidade celular de linhagens de câncer de mama (MCF-7), colo uterino (HeLa) e ovário (OVCAR-3). O principal achado deste estudo é que o bloqueio farmacológico de GRPR promoveu proliferação celular nestas linhagens, enquanto que o tratamento com GRP

inversamente diminuiu a viabilidade celular. Tais resultados são contrastantes ao reconhecido papel mitogênico de GRP descrito em várias evidências anteriores (PATEL *et al*., 2006; CORNELIO *et al*., 2007). Além disso, a inibição de TRK reduziu a viabilidade das linhagens testadas de ovário, colo de útero e mama, porém o tratamento com BDNF não produziu alterações na viabilidade celular.

Antagonistas de GRPR já demonstraram reduzir o crescimento de tumores experimentais de mama e ovário in vivo (YANO *et al*., 1994; MIYAZAKI *et al*., 1998; CHATZISTAMOU *et al*., 2001). Entretanto, o efeito de agonistas e antagonistas de GRPR no crescimento das células tumorais parece depender criticamente de culturas celulares específicas e das condições experimentais. Alguns pesquisadores descreveram que bombesina em doses entre 0.001 a 1 µM estimulou, enquanto que o RC-3095 em doses entre 0.01 a 10 µM inibiu a proliferação celular de linhagens celulares de câncer de mama, apenas quando as células foram cultivadas em soro bovino fetal (FBS) inativado pelo calor ou tratado com carvão coberto com dextran, mas não com FBS não tratado (YANO *et al*., 1994). Os autores sugeriram que BLPs ou outros fatores de crescimento presentes no meio de cultura poderiam competir com os ligantes de GRPR, alterando, assim, a resposta celular aos tratamentos. Outros pesquisadores relataram que bombesina não alterou a proliferação celular de células MCF-7, apesar de as células expressarem sítios de ligação para GRP e de bombesina estimular a mobilização de cálcio e a hidrólise de inositol (PATEL & SCHREY, 1990). As disparidades encontradas entre os estudos podem ser atribuídas a diferenças nos clones celulares, nas condições das culturas ou nos métodos utilizados para avaliar a proliferação e viabilidade (por exemplo, exclusão por trypan blue ou MTT) entre os diversos laboratórios. Em um estudo prévio em nosso laboratório utilizando células de neuroblastoma Neuro 2A, verificamos que uma dose menor de RC-3095 reduziu,

enquanto que uma dose maior aumentou a viabilidade celular (ABUJAMRA *et al*., 2009). Achados semelhantes já foram descritos por diversos autores. Tanto em experimentos com linhagens celulares de câncer (PINSKI *et al*., 1994; FLORES *et al*., 2008), como em outros modelos experimentais (ROESLER *et al*., 2003, ROESLER *et al*., 2006a; CZEPIELEWSKI *et al*., 2012), agonistas e antagonistas de GRPR frequentemente demonstram padrões de dose-resposta nos quais doses intermediárias apresentam efeitos biológicos mais pronunciados, enquanto doses mais elevadas produzem efeitos opostos ou nenhum efeito.

Tomados em conjunto, estes dados permitem inferir que os efeitos de agonistas e antagonistas de GRPR podem variar consideravelmente na dependência das dosagens das drogas e na presença de GRP endógeno e outros fatores no microambiente tecidual, que podem ter implicações nos efeitos *in vivo* tanto em animais de experimentação como em pacientes de estudos clínicos.

Apesar de diversos mecanismos moleculares da sinalização de GRPR já terem sido descritos e implicados na regulação do crescimento das células neoplásicas, os mecanismos subjacentes aos efeitos estimulatórios do bloqueio de GRPR observado no presente estudo e experimentos prévios (ABUJAMRA *et al*., 2009) permanece incerto e deve ser investigado em estudos subsequentes. Conforme descrito anteriormente, as respostas celulares à ativação de GRPR são mediadas por múltiplas vias de sinalização de proteínas quinases, incluindo PKC, PLC, MAPK, ERK e PI3K (revisado por JENSEN *et al*., 2008). Estudos direcionados especificamente a tumores experimentais de mama indicaram que GRPR também estava associado com migração celular e expressão de interleucina-8, enquanto que antagonistas de GRPR levaram à redução da expressão de HER2 em células de câncer de mama (CHAO *et al*., 2009). O bloqueio de GRPR com antagonistas também reduziu a expressão de EGFR e dos oncogenes c-jun e

c-fos em tumores de mama e ovário experimentais (YANO *et al*., 1999; CHATZISTAMOU *et al*., 2000; CHATZISTAMOU *et al*., 2001).

A sinalização de BDNF/TrkB tem sido implicada em promover sobrevida nas células de câncer, bem como resistência à quimioterapia (HUANG & REICHARDT, 2003; DESMET & PEEPER, 2006; ROESLER *et al.*, 2011). Estudos prévios com linhagens celulares de câncer de mama e ovário sugeriram que BDNF/TrkB estimulam a sobrevida celular e migração (QIU *et al*., 2006; YU *et al*., 2008; AU *et al*., 2009; VANHECKE *et al.*, 2011). Nossos achados de que o inibidor K252a diminuiu a viabilidade celular são consistentes com a visão de que TrkB deve continuar a ser explorado como potencial alvo antitumoral em neoplasias de mama e ginecológicas. Como TrkB pode interagir com GRPR e outros receptores de fatores de crescimento, incluindo EGFR, na regulação da sobrevida e proliferação (QIU *et al*., 2006; DE FARIAS *et al*., 2010), compostos combinados dirigidos a ambas as vias de sinalização de neuropeptídios e neurotrofinas podem ser uma estratégia eficaz para inibir o crescimento de tumores.

8. CONCLUSÕES

- ✓ GRPR é amplamente expresso em neoplasias cervicais pré-invasivas e invasivas;
- ✓ GRPR é igualmente expresso em neoplasias pré-invasivas e invasivas de colo uterino, não sendo verificada associação entre expressão e agressividade;
- ✓ GRPR é mais fortemente expresso nas lesões cervicais do que nos tecidos não neoplásicos adjacentes;
- ✓ Em amostras cervicais não neoplásicas, GRPR não demonstra expressão em ectocérvices;
- ✓ GRPR pode sinalizar um evento molecular precoce na carcinogênese cervical;
- ✓ A expressão de GRPR pode ser avaliada através da técnica de imunocitoquímica em células de esfregaços cervicais;
- ✓ GRPR e p16 são amplamente expressos em esfregaços de neoplasias cervicais pré-invasivas e invasivas;
- ✓ Existe forte concordância entre a expressão de GRPR e p16 nos esfregaços cervicais e seus tecidos correspondentes, inferindo que os achados nos esfregaços são representativos dos eventos teciduais subjacentes;
- ✓ A expressão de GRPR demonstrou potencial diagnóstico superior ao exame Papanicolau convencional para detecção de lesões de colo uterino;

- ✓ A expressão de GRPR demonstrou elevada acurácia para o diagnóstico de lesões ASCUS;
- ✓ GRPR e BDNF são expressos em linhagens celulares de câncer de mama (MCF-7), ovário (OVCAR-3) e colo uterino (HeLa) através de Rt-PCR;
- ✓ O bloqueio farmacológico de GRPR promoveu proliferação celular em linhagens de câncer de mama, ovário e colo uterino, enquanto que o tratamento com GRP inversamente diminuiu a viabilidade celular.
- ✓ A inibição de TRK reduziu a viabilidade celular das linhagens de ovário, colo de útero e mama.

9. PERSPECTIVAS

O presente trabalho deixa importantes perspectivas no sentido de aplicar os achados científicos na prática clínica. Através de nossos resultados fomos capazes de submeter três pedidos de patente ao INPI. A autora, juntamente com outras duas sócias, fundou a empresa Ziel Biosciences, atualmente incubada na Incubadora Empresarial do Centro de Biotecnologia da Universidade Federal do Rio Grande do Sul.

A patente "Uso de Anticorpos Anti-GRPR, Método de Detecção e Kit de Diagnóstico de Lesões Displásicas Neoplásicas" já se encontra em processo de licenciamento para a Ziel Biosciences e em breve daremos início ao desenvolvimento de um anticorpo próprio. Acreditamos que o GRPR pode ser utilizado como um marcador, em conjunto ou isoladamente ao exame Papanicolau, para o rastreamento e diagnóstico de lesões de colo uterino.

As outras patentes "Composição Compreendendo Agentes Moduladores Antitumorais, Método de Modulação de Tumores Utilizando Agentes Moduladores e Uso de Agentes Moduladores de BDNF/TrkB Para Modulação da Resistência a Agentes Antitumorais" e "Uso de Agentes Moduladores (GRP) para Preparação de Medicamentos Antitumorais, Composição Compreendendo Agentes Moduladores Antitumorais, Método de Modulação de Tumores Utilizando Agentes Moduladores" relacionam-se a dados mais embrionários, e necessitam de estudos mais aprofundados, tanto *in vitro* como *in vivo*, para um potencial desenvolvimento de produtos.

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Anexo I

HER2 as a cancer stem-cell target

Artigo publicado em Lancet Oncology em 2010

1126 patients with head and neck cancer, we found that 3% had upfront neck dissection.[2] This proportion is likely to be higher in other small retrospective studies, including series of patients with early T-stage hypopharyngeal cancer and resectable advanced nodal disease. Indeed, some clinicians advocate that a pretreatment neck dissection in a chemoradiation regimen for hypopharyngeal cancer does not delay radiation therapy, has low complication rates, and high nodal-disease control.[3] However, a strategy of neck dissection for residual disease after chemoradiation could avoid half of unnecessary dissections.[2]

To clarify the role of neck dissection in the management of patients with head and neck cancer, we need prospective assessment in a randomised trial that stratifies patients according to T and N stage, resectability of nodal disease, and primary site. We also clearly need data on toxicity, quality of life, and outcomes by biomarkers and human papillomavirus status, of which the latter seems to differ by tumour site and possibly by geographical area (seemingly more frequent in the USA than in Europe).

Juliette Thariat, Pierre-Yves Marcy*
Department of Radiation Oncology (JT) and Department of Radiology (PYM), CRLC Antoine Lacassagne, University Nice Sophia-Antipolis, Nice, France
jthariat@hotmail.com

The authors declared no conflicts of interest.

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HER2 as a cancer stem-cell target

The recent review by Jones and Buzdar[1] comprehensively examines the role of the human epidermal growth-factor receptor-2 (HER2) in breast cancer initiation and progression, and the role of agents targeting HER2 (trastuzumab, lapatinib, and others) in the treatment of breast cancer. However, the authors did not mention recent evidence that HER2-mediated carcinogenesis and tumorigenesis might be due to the protein's action on tumour-initiating cells or cancer stem cells in HER2-positive breast cancers, and that the clinical efficacy of trastuzumab might be related to the drug's ability to target cancer stem cells.

The cancer stem-cell hypothesis posits that tumours may be initiated and maintained by a subset of cells that maintain or acquire stem-cell properties. Cancer stem cells were identified in breast cancers several years ago,[2] and increasing evidence suggests that, in HER2-positive breast cancers, HER2 promotes carcinogenesis, invasion, and metastasis at least partly by maintaining and increasing cancer stem cells.[3-5] For instance, Korkaya and colleagues[3] showed that HER2 overexpression in breast cancer cell lines increases the number of cancer stem cells, leading to increased invasion in vitro and increased tumorigenesis in mice. Moreover, Magnifico and colleagues[5] have noted that, in breast cell lines, cells displaying stem-cell characteristics, such as mammosphere formation, also have increased HER2 expression. Most importantly, HER2-overexpressing cancer stem cells have preferential sensitivity to trastuzumab and lapatinib.[4,5]

As we recently pointed out when reviewing the biology of cancer stem cells in brain tumours,[6] growth-factor receptors are likely to become increasingly important molecular targets in the search for new anticancer therapies aimed at cancer stem-cell signalling. The findings reviewed above strongly suggest that the role of anti-HER2 therapeutic strategies in cancer treatment should be investigated, taking into account the specific effects of HER2 inhibition on the cancer stem-cell subpopulation of tumour cells.

Rafael Roesler, Daniela Baumann Cornelio, Ana Lucia Abujamra, Gilberto Schwartsmann*
Cancer Research Laboratory, University Hospital Research Center (CPE-HCPA), and Departments of Pharmacology and Internal Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
rroesler@terra.com.br

Reflection and Reaction

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Errata

Saito M, Aogi K, Sekine I, et al. Palonosetron plus dexamethasone versus granisetron plus dexamethasone for prevention of nausea and vomiting during chemotherapy: a double-blind, double-dummy, randomised, comparative phase III trial. Lancet Oncol 2009; **10:** 115–24—The Role of the Funding Source for this Article should read "Under agreement with the study sponsor, the medical adviser, medical statistical adviser, and coordinating investigators of the study worked on the design and conduct of this study, and in the analysis and interpretation of the data collaboratively with the principal investigators at the trial sites. The study sponsor approved the study protocol, the statistical analysis plan, and the clinical study report. Study data were collected by investigators and the conduct of the study was monitored by the sponsor. All authors had access to all the data. The corresponding author had final responsibility for the decision to submit for publication." The Trial Members should read "Mamoru Tsukuda (medical adviser; Yokahama City University School Of Medicine, Yokahama, Japan), Chikuma Hamada (medical statistical adviser; Tokyo University of Science, Tokyo, Japan), Yutaka Ariyoshi (coordinating investigator; Aichi Cancer Center, Aichi Hospital, Okazaki, Japan), Tomohide Tamura (coordinating investigator, National Cancer Center, Tokyo, Japan), Toshiaki Saeki (coordinating investigator, Saitama Medical University, Hidaka, Japan)." The Conflicts of Interest statement should read: "Mamoru Tsukuda, Chikuma Hamada, Yutaka Ariyoshi, Tomohide Tamura, and Toshiaki Saeki have received consulting fees or honoraria from Taiho Pharmaceutical. Chikuma Hamada has also received grants from Taiho Pharmaceutical. Toshiaki Saeki has received grants from Chugai Pharmaceutical and Pfizer, and has received honoraria from Bayer and Bristol-Myers Squibb. The study authors declared no conflicts of interest."

Wolchok JD, Neyns B, Linette G, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol 2010; **11:** 155–64—In this Article, the third sentence of the methods section in the Summary should read "217 patients with previously treated stage III (unresectable) or stage IV melanoma were randomly assigned a fixed dose of ipilimumab of either 10 mg/kg (n=72), 3 mg/kg (n=72), or 0·3 mg/kg (n=73) every 3 weeks for four cycles (induction) followed by maintenance therapy every 3 months." Additionally, the last sentence of the second paragraph of the Results section should read "Median follow-up was for 10·7 (IQR 3·6–23·3), 8·7 (4·0–22·3), and 8·3 (3·5–15·3) months for 10 mg/kg, 3 mg/kg, and 0·3 mg/kg groups, respectively."

Anexo II

Depósito de Pedido de Patente "Uso de Anticorpos Anti-GRPR, Método de Detecção e Kit de Diagnóstico de Lesões Displásicas Neoplásicas"

Inventores: Daniela Baumann Cornelio, Rafael Roesler, Gilberto Schwartsmann

Número de protocolo PI 1102458-5, 2011.

< Uso exclusivo do INPI >

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Ao Instituto Nacional da Propriedade Industrial:
O requerente solicita a concessão de um privilégio na natureza e nas condições abaixo indicadas

1. Depositante (71):

1.1 Nome: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

1.2 Qualificação:

1.3 CNPJ/CPF: 92969856000198

1.4 Endereço Completo: AV. PAULO GAMA, 110. CENTRO, PORTO ALEGRE - RS BR

1.5 CEP: 90040-060 1.6 Telefone: 51 3308 4236 1.7 Fax: 51 3308 4237

1.8 E-mail: SEDETEC@UFRGS.BR

☐ continua em folha anexa

2. Natureza: ◉ Invenção ○ Modelo de Utilidade ○ Certificado de Adição

Escreva, obrigatoriamente, e por extenso, a Natureza desejada: PATENTE DE INVENÇÃO

3. Título da Invenção ou Modelo de Utilidade ou Certificado de Adição(54):

USO DE ANTICORPOS ANTI-GRPR, MÉTODO DE DETECÇÃO E KIT DE DIAGNÓSTICO DE LESÕES DISPLÁSTICAS E NEOPLÁSTICAS

☐ continua em folha anexa

4. Pedido de Divisão: do pedido Nº Data de Depósito:

5. Prioridade: ☐ interna ☐ unionista

O depositante reivindica a(s) seguinte(s):

País ou organização de origem	Número de depósito	Data do depósito

6. Inventor (72):

☐ Assinale aqui se o(s) mesmo(s) requer(em) a não divulgação de seu(s) nome(s)

6.1 Nome: DANIELA BAUMANN CORNÉLIO

6.2 Qualificação: MÉDICA 6.3 CPF: 65414845034

6.4 Endereço completo: CEL LUCAS DE OLIVEIRA, 1585/402 BELA VISTA PORTO ALEGRE RS BR

6.5 CEP: 6.6 Telefone: 6.7 Fax:

6.8 E-Mail: DANICORNELIO@TERRA.COM.BR

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7. Declaração na forma do item 3.2 do Ato Normativo nº 127/97:

☐ 7.1 Declaro que os dados fornecidos no presente formulário são idênticos ao da certidão de depósito ou documento equivalente do pedido cuja prioridade está sendo reivindicada.

☐ em anexo

8. Declaração de divulgação anterior não prejudicial: (Período de Graça):
(art. 12 da LPI e item 2 do AN nº 127/97) ☐ em anexo

9. Procurador (74)

9.1 Nome:

9.2 CNPJ/CPF: 9.3 API/OAB:

9.4 Endereço completo:

9.5 CEP: 9.6 Telefone: 9.7 Fax:

9.8 E-Mail:

10. Listagem de sequências Biológicas (documentos anexados) (se houver):

☐ Listagem de sequências em arquivo eletrônico: nº de CDs ou DVDs (original e cópia).

☐ Código de controle alfanumérico no formato de código de barras: fl.

☐ Listagem de sequências em formato impresso: fls.

☐ Declaração de acordo com o artigo da Resolução INPI nº 228/09: fls.

11. Documentos anexados (assinale e indique também o número de folhas):
(Deverá ser indicado o nº total de somente uma das vias de cada documento)

☒	11.1 Guia de Recolhimento	1 fls.	☒	11.5 Relatório descritivo	18 fls.
☐	11.2 Procuração	fls.	☒	11.6 Reivindicações	1 fls.
☐	11.3 Documentos de Prioridade	fls.	☐	11.7 Desenhos	fls.
☐	11.4 Doc. de contrato de trabalho	fls.	☒	11.8 Resumo	1 fls.
☒	11.9 Outros que não aqueles definidos no campo 11 (especificar) TERMO DE CESSÃO, COPIA DOU, PORTARIA DE COMPETENCIA, ANEXOS				10 fls.

12. Total de folhas anexadas (referentes aos campos 10 e 11): 31 fls.

13. Declaro, sob penas da Lei, que todas as informações acima prestadas são completas e verdadeiras.

06/05/2011, Porto Alegre
Local e Data

Assinatura e Carimbo
Profª Raquel S. Mauler
Secretária de Desenvolvimento Tecnológico
UFRGS

ANEXO DE INVENTORES

Título: USO DE ANTICORPOS ANTI-GRPR, MÉTODO DE DETECÇÃO E KIT DE

Nome: GILBERTO SCHWARTSMANN
Qualificação: DOCENTE
CPF: 28994647015
Endereço Completo: SANTO INACIO, 525/1201 MOINHOS DE VENTO PORTO ALEGRE RS BR
CEP:
Telefone:
FAX:
E-mail:

Nome: RAFAEL ROESLER
Qualificação: DOCENTE
CPF: 73711276091
Endereço Completo: DONA LEONOR, 194/708 RIO BRANCO PORTO ALEGRE RS BR
CEP:
Telefone:
FAX:
E-mail:

Anexo III

Depósito de Pedido de Patente "Composição Compreendendo Agentes Moduladores Antitumorais, Método de Modulação de Tumores Utilizando Agentes Moduladores e Uso de Agentes Moduladores de BDNF/TrkB Para Modulação da Resistência a Agentes Antitumorais"

Inventores: Ana Lucia Abujamra, Caroline Brunetto de Farias, Daniela Baumann Cornelio, Gilberto Schwartsmann, Rafael Roesler

Número de protocolo PI BR 10 2012 010889-5, 2012.

Esta patente será anexada às teses das alunas de doutorado Caroline Brunetto de Farias e Daniela Baumann Cornelio.

< Uso exclusivo do INPI >

INPI INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL PROTOCOLO GERAL
Toqazzi
08/05/2012 16:23 DERS
0161200002259
BR 10 2012 010889 5

Espaço reservado ao protocolo

Espaço para etiqueta

DEPÓSITO DE PEDIDO DE PATENTE OU DE CERTIFICADO DE ADIÇÃO

Ao Instituto Nacional da Propriedade Industrial:
O requerente solicita a concessão de um privilégio na natureza e nas condições abaixo indicadas

1. Depositante (71):

1.1 Nome: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

1.2 Qualificação: INST. PÚBLICA DE ENSINO SUP

1.3 CNPJ/CPF: 92969856000198

1.4 Endereço Completo: AV. PAULO GAMA, 110. CENTRO, PORTO ALEGRE - RS Brasil

1.5 CEP: 90040-060 1.6 Telefone: 51 3308 3800 1.7 Fax: 51 3308 4237

1.8 E-mail: sedetec@ufrgs.br

☒ continua em folha anexa

2. Natureza: ◉ Invenção ○ Modelo de Utilidade ○ Certificado de Adição

Escreva, obrigatoriamente, e por extenso, a Natureza desejada: Patente de Invenção

3. Título da Invenção ou Modelo de Utilidade ou Certificado de Adição(54):

COMPOSIÇÃO COMPREENDENDO AGENTES MODULADORES ANTITUMORAIS, MÉTODO DE MODULAÇÃO DE TUMORES UTILIZANDO AGENTES MODULADORES E USO DE AGENTES MODULADORES DE BDNF/TRKB PARA MODULAÇÃO DA RESISTÊNCIA A

☒ continua em folha anexa

4. Pedido de Divisão: do pedido Nº Data de Depósito:

5. Prioridade: ☐ interna ☐ unionista

O depositante reivindica a(s) seguinte(s):

País ou organização de origem	Número de depósito	Data do depósito

6. Inventor (72):

☐ Assinale aqui se o(s) mesmo(s) requer(em) a não divulgação de seu(s) nome(s)

6.1 Nome: RAFAEL ROESLER

6.2 Qualificação: DOCENTE 6.3 CPF: 737.112.760-91

6.4 Endereço completo: DONA LEONOR, 194 APTO 708 RIO BRANCO PORTO ALEGRE RS BR

6.5 CEP: 90420180 6.6 Telefone: 33083183 6.7 Fax: 33083121

6.8 E-Mail: RROESLER@TERRA.COM.BR

☒ continua em folha anexa

7. Declaração na forma do item 3.2 do Ato Normativo nº 127/97:

☐ 7.1 Declaro que os dados fornecidos no presente formulário são idênticos ao da certidão de depósito ou documento equivalente do pedido cuja prioridade está sendo reivindicada.

☐ em anexo

8. Declaração de divulgação anterior não prejudicial: (Período de Graça):
(art. 12 da LPI e item 2 do AN nº 127/97) ☐ em anexo

9. Procurador (74)

9.1 Nome:

9.2 CNPJ/CPF: 9.3 API/OAB:

9.4 Endereço completo:

9.5 CEP: 9.6 Telefone: 9.7 Fax:

9.8 E-Mail:

10. Listagem de sequências Biológicas (documentos anexados) (se houver):

☐ Listagem de sequências em arquivo eletrônico: nº de CDs ou DVDs (original e cópia).

☐ Código de controle alfanumérico no formato de código de barras: fl.

☐ Listagem de sequências em formato impresso: fls.

☐ Declaração de acordo com o artigo da Resolução INPI nº 228/09: fls.

11. Documentos anexados (assinale e indique também o número de folhas):
(Deverá ser indicado o nº total de somente uma das vias de cada documento)

☒	11.1 Guia de Recolhimento	1 fls.	☒	11.5 Relatório descritivo	18 fls.
☒	11.2 Procuração	1 fls.	☒	11.6 Reivindicações	2 fls.
☐	11.3 Documentos de Prioridade	fls.	☒	11.7 Desenhos	9 fls.
☐	11.4 Doc. de contrato de trabalho	fls.	☒	11.8 Resumo	1 fls.
☒	11.9 Outros que não aqueles definidos no campo 11 (especificar) Autorizações de cessão de invenção; Portaria de Competência; cópia Diário Oficial da União.				9 fls.

12. Total de folhas anexadas (referentes aos campos 10 e 11): 41 fls.

13. Declaro, sob penas da Lei, que todas as informações acima prestadas são completas e verdadeiras.

Porto Alegre 08/MAIO/2012
Local e Data

Raquel S. Mauler
Assinatura e Carimbo

Profª Raquel S. Mauler
Secretária de Desenvolvimento Tecnológico
UFRGS

INPI

ANEXO DE INVENTORES

Título: COMPOSIÇÃO COMPREENDENDO AGENTES MODULADORES ANTITUMORAIS, MÉTODO DE MODULAÇÃO DE TUMORES UTILIZANDO AGENTES MODULADORES E...

Nome: CAROLINE BRUNETTO DE FARIAS

Qualificação: Estudante

CPF: 003.146.320-77

Endereço Completo: Rua Sarmento Leite, 781, Apto. 304 Centro Porto Alegre RS BR

CEP: 90050-170

Telefone: 51-30234824

FAX:

E-mail: carolbfarias@gmail.com

Nome: DANIELA BAUMANN CORNÉLIO

Qualificação: MÉDICA

CPF: 65414845034

Endereço Completo: CEL LUCAS DE OLIVEIRA, 1585/402 BELA VISTA PORTO ALEGRE RS BR

CEP:

Telefone:

FAX:

E-mail: DANICORNELIO@TERRA.COM.BR

Nome: ANA LUCIA ABUJAMRA

Qualificação: Biomédica

CPF: 271.634.108-75

Endereço Completo: Rua Artur Rocha, 1121, Apto. 801 Rio Branco Porto Alegre RS BR

CEP: 90620-110

Telefone: 51-33597616

FAX:

E-mail: aabujamra@hcpa.ufrgs.br

Nome: GILBERTO SCHWARTSMANN

Qualificação: DOCENTE

CPF: 289.946.470-15

Endereço Completo: SANTO INACIO, 525/1201 MOINHOS DE VENTO PORTO ALEGRE RS BR

CEP: 90450-170

Telefone: 51-33954912

FAX:

E-mail: gilberto.ez@terra.com.br

Anexo IV

Depósito de Pedido de Patente "Uso de Agentes Moduladores (GRP) para Preparação de Medicamentos Antitumorais, Composição Compreendendo Agentes Moduladores Antitumorais, Método de Modulação de Tumores Utilizando Agentes Moduladores"

Inventores: Ana Lucia Abujamra, Caroline Brunetto de Farias, Daniela Baumann Cornelio, Gilberto Schwartsmann, Rafael Roesler

Número de protocolo BR 102012012574-9, 2012.

Esta patente será anexada às teses das alunas de doutorado Caroline Brunetto de Farias e Daniela Baumann Cornelio.

< Uso exclusivo do INPI >

INPI INSTITUTO NACIONAL DA PROPRIEDADE IN... PROTOCOLO GERAL
Fogazzi
25/05/2012 01612000581 15:35 DI RS
BR 10 2012 012574 9

Espaço reservado ao protocolo

Espaço para etiqueta

DEPÓSITO DE PEDIDO DE PATENTE OU DE CERTIFICADO DE ADIÇÃO

Ao Instituto Nacional da Propriedade Industrial:
O requerente solicita a concessão de um privilégio na natureza e nas condições abaixo indicadas

1. Depositante (71):

1.1 Nome: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

1.2 Qualificação: INST. PÚBLICA DE ENSINO SUP

1.3 CNPJ/CPF: 92969856000198

1.4 Endereço Completo: AV. PAULO GAMA, 110 - CENTRO, PORTO ALEGRE - RS, Brasil

1.5 CEP: 90040-060 1.6 Telefone: 51 3308 3800 1.7 Fax: 51 3308 4237

1.8 E-mail: sedetec@ufrgs.br

☒ continua em folha anexa

2. Natureza: ◉ Invenção ○ Modelo de Utilidade ○ Certificado de Adição

Escreva, obrigatoriamente, e por extenso, a Natureza desejada: Patente de Invenção

3. Título da Invenção ou Modelo de Utilidade ou Certificado de Adição(54):

USO DE AGENTES MODULADORES (GRP) PARA PREPARAÇÃO DE MEDICAMENTOS ANTITUMORAIS, COMPOSIÇÃO COMPREENDENDO AGENTES MODULADORES ANTITUMORAIS, MÉTODO DE MODULAÇÃO DE TUMORES UTILIZANDO AGENTES

☒ continua em folha anexa

4. Pedido de Divisão: do pedido Nº Data de Depósito:

5. Prioridade: ☐ interna ☐ unionista

O depositante reivindica a(s) seguinte(s):

País ou organização de origem	Número de depósito	Data do depósito

6. Inventor (72):

☐ Assinale aqui se o(s) mesmo(s) requer(em) a não divulgação de seu(s) nome(s)

6.1 Nome: RAFAEL ROESLER

6.2 Qualificação: DOCENTE 6.3 CPF: 73711276091

6.4 Endereço completo: DONA LEONOR, 194/708 - RIO BRANCO, PORTO ALEGRE - RS, BR

6.5 CEP: 6.6 Telefone: 6.7 Fax:

6.8 E-Mail:

☒ continua em folha anexa

7. Declaração na forma do item 3.2 do Ato Normativo nº 127/97:

☐ 7.1 Declaro que os dados fornecidos no presente formulário são idênticos ao da certidão de depósito ou documento equivalente do pedido cuja prioridade está sendo reivindicada.

☐ em anexo

8. Declaração de divulgação anterior não prejudicial: (Período de Graça):
(art. 12 da LPI e item 2 do AN nº 127/97) ☐ em anexo

9. Procurador (74)

9.1 Nome:

9.2 CNPJ/CPF: 9.3 API/OAB:

9.4 Endereço completo:

9.5 CEP: 9.6 Telefone: 9.7 Fax:

9.8 E-Mail:

10. Listagem de sequências Biológicas (documentos anexados) (se houver):

☐ Listagem de sequências em arquivo eletrônico: nº de CDs ou DVDs (original e cópia).

☐ Código de controle alfanumérico no formato de código de barras: fl.

☐ Listagem de sequências em formato impresso: fls.

☐ Declaração de acordo com o artigo da Resolução INPI nº 228/09: fls.

11. Documentos anexados (assinale e indique também o número de folhas):
(Deverá ser indicado o nº total de somente uma das vias de cada documento)

☒	11.1 Guia de Recolhimento	1 fls.	☒	11.5 Relatório descritivo	12 fls.
☒	11.2 Procuração	1 fls.	☒	11.6 Reivindicações	1 fls.
☐	11.3 Documentos de Prioridade	fls.	☒	11.7 Desenhos	4 fls.
☐	11.4 Doc. de contrato de trabalho	fls.	☒	11.8 Resumo	1 fls.
☒	11.9 Outros que não aqueles definidos no campo 11 (especificar) Autorizações de cessão; Portaria de Competência; cópia Diário Oficial da União.				9 fls.

12. Total de folhas anexadas (referentes aos campos 10 e 11): 29 fls.

13. Declaro, sob penas da Lei, que todas as informações acima prestadas são completas e verdadeiras.

Porto Alegre, 25/05/2012
Local e Data

Assinatura e Carimbo

Profª Raquel S. Mauler
Secretária de Desenvolvimento Tecnológico
UFRGS

ANEXO DE INVENTORES

Título: USO DE AGENTES MODULADORES (GRP) PARA PREPARAÇÃO DE MEDICAMENTOS ANTITUMORAIS, COMPOSIÇÃO COMPREENDENDO...

Nome: CAROLINE BRUNETTO DE FARIAS

Qualificação: Estudante

CPF: 003.146.320-77

Endereço Completo: Rua Sarmento Leite, 781, Apto. 304 - Centro, Porto Alegre - RS, BR

CEP: 90050-170

Telefone: 51-30234824

FAX:

E-mail: carolbfarias@gmail.com

Nome: DANIELA BAUMANN CORNÉLIO

Qualificação: MÉDICA

CPF: 65414845034

Endereço Completo: CEL LUCAS DE OLIVEIRA, 1585/402 - BELA VISTA, PORTO ALEGRE - RS, BR

CEP:

Telefone:

FAX:

E-mail: DANICORNELIO@TERRA.COM.BR

Nome: GILBERTO SCHWARTSMANN

Qualificação: DOCENTE

CPF: 289.946.470-15

Endereço Completo: SANTO INACIO, 525/1201 - MOINHOS DE VENTO, PORTO ALEGRE - RS, BR

CEP: 90450-170

Telefone: 51-33954912

FAX:

E-mail: gilberto.ez@terra.com.br

Nome: ANA LUCIA ABUJAMRA

Qualificação: Biomédica

CPF: 271.634.108-75

Endereço Completo: Rua Artur Rocha, 1121, Apto. 801 - Rio Branco, Porto Alegre - RS, BR

CEP: 90620-110

Telefone: 51-33597616

FAX:

E-mail: aabujamra@hcpa.ufrgs.br

---

12. CURRICULUM VITAE

Daniela Baumann Cornelio
Curriculum Vitae

Dados Pessoais

Nome Daniela Baumann Cornelio
Filiação Ricardo Edi Cornélio e Annemarie Baumann Cornelio
Nascimento 29/09/1973 - Porto Alegre/RS - Brasil
Carteira de Identidade 3030301992 SSP - RS - 16/05/1994
CPF 65414845034

Endereço residencial Rua Pedro Ivo, 224/402
Mon't Serrat - Porto Alegre
90450-210, RS - Brasil
Telefone: 51 91138123

Endereço profissional Laboratório de Pesquisas em Câncer, Hospital de Clínicas de Porto Alegre
Rua Ramiro Barcelos, 2350
- Porto Alegre
90035-003, RS - Brasil
Telefone: 51 33597616

Endereço eletrônico
e-mail para contato : danicornelio@terra.com.br
e-mail alternativo : dbcornelio@gmail.com

Formação Acadêmica/Titulação

2011 Especialização - Residência médica.
Santa Casa de Misericórdia de Porto Alegre, SCM/PORTO ALEGRE, Porto Alegre, Brasil
Título: Breast Surgery
Bolsista do(a): MEC

2008 Doutorado em Biologia Celular e Molecular.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil
Título: Análise do potencial Diagnóstico e Terapêutico do Receptor do Peptídeo Liberador da Gastrina em Neoplasias Femininas
Orientador: Rafael Roesler

2008 - 2011 Especialização - Residência médica.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil
Título: Ginecologia e Obstetrícia
Bolsista do(a): Governo Federal

2006 - 2007 Mestrado em Biologia Celular e Molecular.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil
Título: Análise da expressão dos receptors GRP/bombesina em neoplasias humanas, Ano de obtenção: 2007
Orientador: Rafael Roesler

2001 - 2006 Graduação em Medicina.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil

1992 - 1996 Graduação em Odontologia.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

Formação complementar

2010 - 2010 Extensão universitária em Clinical Attachment at Breast Unit.
Royal Marsden Hospital, Marsden, Inglaterra

2010 - 2010 Curso de curta duração em Curso de Atualização em Mastologia.
Centro de Mama Hospital Santa Rita, HSR, Brasil

2010 - 2010 Curso de curta duração em Temas em Ginecologia.
Congresso Integrado de Ginecologia e Obstetrícia - CIGO 2010, CIGO, Brasil

2010 - 2010 Curso de curta duração em Oncoplastic Core Skills Course.
Royal College Of Surgeons Of England, London, Inglaterra

2009 - 2009 Curso de curta duração em III Curso de Atualização em Ginecologia da Infânci.
Irmadade da Santa Casa de Misercórdia de Porto Alegre, ISCMPA, Brasil

2008 - 2008 Extensão universitária em Estresse Oxidativo em Eucariotos e Procariotos.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2008 - 2008 Curso de curta duração em Colposcopia e Patologias do Colo Uterino, Vagina e.
Associação Médica do Rio Grande do Sul, AMRIGS, Brasil

2008 - 2008 Extensão universitária em oncologia molecular.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2007 - 2007 Curso de curta duração em Curso Prático de Laser.
Sociedade Brasileira de Laser em Medicina e Cirurgia, SBLMC, Brasil

2007 - 2007 Proficiência em Língua Inglesa.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2007 - 2007 Proficiência em Língua Espanhola.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2006 - 2006 Neurobiologia Celular e Molecular.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2006 - 2006 Terapia Gênica e Celular de Doenças.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2006 - 2006 Biologia Molecular e Celular do Câncer.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2006 - 2006 Extensão universitária em Curso de Estresse Oxidativo.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2006 - 2006 Introdução à Gestão da Inovação e Produção.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2005 - 2005 Extensão universitária em Curso de Ecg.

Instituto de Cardiologia do Rio Grande do Sul, IC-FUC, Porto Alegre, Brasil

2005 - 2005 Tópicos de Pesquisa Em Clínica Médica II.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2004 - 2004 Extensão universitária em Simpósio Inaugural Liga de Hipertensão da FFFCMPA.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil

2004 - 2004 Curso de curta duração em Curso de Eletrocardiograma.
Hospital de Clínicas de Porto Alegre, HCPA, Porto Alegre, Brasil

2003 - 2003 Extensão universitária em Curso de Fisiopatologia.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil

Atuação profissional

1. Hospital de Clínicas de Porto Alegre - HCPA

Vínculo institucional

2005 - Atual Vínculo: pesquisador , Carga horária: 0 Regime: Parcial

Atividades

2008 - Atual Projetos de pesquisa, Laboratório de Pesquisas em Câncer
Participação em projetos:
ESTUDOS COM O ANTAGONISTA DO RECEPTOR DO PEPTÍDEO LIBERADOR DA GASTRINA RC-3095 EM NEOPLASIAS DE MAMA E COLO UTERINO: ANÁLISE DO EFEITO ANTIPROLIFERATIVO E DO SINERGISMO COM OUTROS AGENTES TERAPÊUTICOS

06/2005 - Atual Projetos de pesquisa, Serviço de Patologia
Participação em projetos:
Análise da Expressão de Receptores GRP/bombesina em Neoplasias Humanas

06/2005 - Atual Pesquisa e Desenvolvimento, Serviço de Patologia
Linhas de Pesquisa:
análise de receptores-alvo em neoplasias

2. Ziel Biosciences

Vínculo institucional

2011 - Atual Vínculo: sócia fundadora , Enquadramento funcional: diretora de pesquisa e desenvolvimento, Regime: Parcial

3. Hospital do Pronto Socorro Municipal de Porto Alegre - HPSPA*

Vínculo institucional

2004 - 2004 Vínculo: Estágiario , Enquadramento funcional: Estágio Curricular , Carga horária: 36, Regime: Parcial

Atividades

07/2004 - 12/2004 Estágio, Emergência
Estágio:
Estágio de emergência em: Traumatologia, Cirurgia, Cirurgia Ambulatorial, Politraumatizados, Clínica Médica e Ambulância (SAMU)

4. Irmadade da Santa Casa de Misercórdia de Porto Alegre - ISCMPA

Vínculo institucional

2004 - 2004 Vínculo: Estagiário , Enquadramento funcional: Estágio , Carga horária: 40, Regime: Integral

2006 - 2007 Vínculo: pesquisador , Enquadramento funcional: pesquisador , Carga horária: 0, Regime: Parcial

Atividades

10/2005 - 2008 Pesquisa e Desenvolvimento, Serviço de Gastroenterologia
Linhas de Pesquisa:
análise genética de neoplasias

12/2006 - 2008 Projetos de pesquisa, Serviço de Gastroenterologia
Participação em projetos:
Análise da Diferença de Expressão Gênica entre Mucosa Gástrica Normal, Metaplasia e Câncer Gástrico por cDNA Microarray , Análise da Expressão Gênica e sua Relação com a Patogênese do Carcinoma Epidermóide de Esôfago.

2012 - Atual Projetos de pesquisa, Laboratório de Medicina Nuclear
Participação em projetos:
AVALIAÇÃO DA ACURÁCIA DA MAMOCINTILOGRAFIA NO DIAGNÓSTICO COMPLEMENTAR DE LESÕES MAMÁRIAS

2011 - Atual Projetos de pesquisa, Centro de Mama do Hospital Santa Rita
Participação em projetos:
Quimioterapia Neoadjuvante em Câncer de Mama

2009 - Atual Projetos de pesquisa, Departamento de Ginecologia e Obstetrícia
Participação em projetos:
ANÁLISE DO RECEPTOR DO PEPTÍDEO LIBERADOR DA GASTRINA COMO MARCADOR MOLECULAR DIAGNÓSTICO EM NEOPLASIAS DE COLO UTERINO

01/2004 - 01/2004 Estágio, Cirúrgia Cardiovascular
Estágio:
Período de aprendizagem

5. Fundação Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA

Vínculo institucional

2003 - 2003 Vínculo: Estagiário , Enquadramento funcional: Estágio , Carga horária: 0, Regime: Parcial

Atividades

01/2003 - 02/2003 Estágio, Departamento de Medicina Interna, Disciplina de Nefrologia
Estágio:
Estágio extracurricular de férias

6. Royal Marsden Hospital

Vínculo institucional

2010 - 2010 Vínculo: estágio , Enquadramento funcional: estágio , Carga horária: 50, Regime: Integral

Linhas de pesquisa

1. análise de receptores-alvo em neoplasias

Objetivos:Identificar receptores de fatores de crescimento em neoplasias e testar eficácia de drogas-alvo contra estes receptores.

2. análise genética de neoplasias

Objetivos:Avaliar alterações genéticas relacionadas a neoplasias gastrointestinais por tecnologia de microarrays

Projetos

2012 - Atual AVALIAÇÃO DA ACURÁCIA DA MAMOCINTILOGRAFIA NO DIAGNÓSTICO COMPLEMENTAR DE LESÕES MAMÁRIAS
Situação: Em Andamento Natureza: Pesquisa
Alunos envolvidos: Especialização (1);
Integrantes: Daniela Baumann Cornelio (Responsável); ; Rogério Grossmann; Cássio F. Paganini; Neivo da Silva Junior
Financiador(es):

2011 - Atual Quimioterapia Neoadjuvante em Câncer de Mama
Descrição: Projeto que visa avaliar linfonodo sentinela e diversos parâmetros cirúrgicos, anátomo-patológicos e imuno-histoquímicos em pacientes portadoras de câncer de mama submetidas a quimioterapia neoadjuvante
Situação: Em Andamento Natureza: Pesquisa
Integrantes: Daniela Baumann Cornelio; Sandra W. Hohgraefe; Rogério Grossmann; Simão Grossmann; Gabriela R. Santos (Responsável); Sérgio Lago
Financiador(es):

2009 - Atual ANÁLISE DO RECEPTOR DO PEPTÍDEO LIBERADOR DA GASTRINA COMO MARCADOR MOLECULAR DIAGNÓSTICO EM NEOPLASIAS DE COLO UTERINO

Situação: Em Andamento Natureza: Pesquisa
Alunos envolvidos: Doutorado (1);
Integrantes: Daniela Baumann Cornelio (Responsável); ; Suzana Arenhart Pessini; Gustavo Py Gomes da Silveira
Financiador(es): FIPE - HCPA-FIPE - HCPA, South American Office for Anticancer Drug Discovery-SOAD

2008 - Atual ESTUDOS COM O ANTAGONISTA DO RECEPTOR DO PEPTÍDEO LIBERADOR DA GASTRINA RC-3095 EM NEOPLASIAS DE MAMA E COLO UTERINO: ANÁLISE DO EFEITO ANTIPROLIFERATIVO E DO SINERGISMO COM OUTROS AGENTES TERAPÊUTICOS

Situação: Em Andamento Natureza: Pesquisa
Alunos envolvidos: Graduação (1); Doutorado (1);
Integrantes: Daniela Baumann Cornelio (Responsável); ; Gilberto Schwartsmann; Rafael Roesler; Caroline Brunetto de Farias; Ana Lúcia Abujamra; Rodrigo Cruz Lima
Financiador(es): Hospital de Clínicas de Porto Alegre-HCPA

2006 - 2008 Análise da Expressão Gênica e sua Relação com a Patogênese do Carcinoma Epidermóide de Esôfago.

Descrição: Estudo que pretende comparar geneticamente casos de carcinoma epidermóide de esôfago de pacientes de duas regiões distintas do Brasil: RS e SP
Situação: Desativado Natureza: Pesquisa
Alunos envolvidos: Especialização (0); Mestrado acadêmico (0); Mestrado profissionalizante (0); Doutorado (0);
Integrantes: Daniela Baumann Cornelio; Gilberto Schwartsmann; Julio Carlos Pereira Lima (Responsável)
Financiador(es): Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP, Instituto Ludwig de Pesquisa sobre o Câncer-ILPC

2006 - 2008 Análise da Diferença de Expressão Gênica entre Mucosa Gástrica Normal, Metaplasia e Câncer Gástrico por cDNA Microarray

Descrição: Este projeto visa comparar tecidos sadios e neoplásicos de pacientes com câncer gástrico através de um painel de microarranjos de DNA
Situação: Desativado Natureza: Pesquisa
Alunos envolvidos: Graduação (0); Especialização (0); Mestrado acadêmico (0); Mestrado profissionalizante (0); Doutorado (0);
Integrantes: Daniela Baumann Cornelio; Gilberto Schwartsmann; Julio Carlos PereiraLima; Luis Fernando Reis (Responsável); Ricardo Brentani
Financiador(es): Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq

2005 - Atual Análise da Expressão de Receptores GRP/bombesina em Neoplasias Humanas

Descrição: Este projeto tem como objetivo avaliar a expressão dos receptores de GRP/bombesina em tecidos neoplásicos humanos, para potencial uso de terapia-alvo com anticorpo anti-GRPR
Situação: Em Andamento Natureza: Pesquisa
Alunos envolvidos: Graduação (0); Especialização (0); Mestrado acadêmico (1); Mestrado profissionalizante (0); Doutorado (0);
Integrantes: Daniela Baumann Cornelio; Gilberto Schwartsmann (Responsável); Rafael Roesler; Luise Meurer
Financiador(es): Fundação Soad-SOAD, Hospital de Clínicas de Porto Alegre-HCPA

Revisor de periódico

1. Respiratory Research (Cessou em 2002. Cont. ISSN 1465-993X Respiratory Rese -

Vínculo

2011 - Atual Regime: Parcial

2. European Neurology -

Vínculo

2010 - Atual Regime: Parcial

3. Oncology (Basel) -

Vínculo

2009 - Atual Regime: Parcial

4. Cancer Therapy -

Vínculo

2009 - Atual Regime: Parcial

5. Current Stem Cell Research & Therapy -

Vínculo

2009 - Atual Regime: Parcial

6. Cancer Letters (Print)

Vínculo

2009 - Atual Regime: Parcial

Prêmios e títulos

2012 Poster premiado no Workshop de Mama e Oncogenética do Hospital Santa Rita, ISCMPA

2011 Menção Honrosa por Melhor Trabalho de Conclusão de Residência Médica, Universidade Federal de Ciências da Saúde de Porto Alegre

2011 Poster premiado no Workshop de Mama do Hospital Santa Rita, ISCMPA

2010 finalista, Prêmio Santander de Empreendedorismo

2007 1º lugar no exame AMRIGS para ginecologia e obstetrícia, AMRIGS

1997 1º lugar em concurso público para cirurgião-dentista, Prefeitura Municipal de Igrejinha

Produção em C, T& A

Produção bibliográfica

Artigos completos publicados em periódicos

1. CORNELIO, Daniela Baumann, MEURER, Luise, SCHWARTSMANN, Gilberto, ROESLER, Rafael
The Gastrin-Releasing Peptide Receptor as a Marker of Dysplastic Alterations in Cervical Epithelial Cells. Oncology. , v.82, p.90 - 97, 2012.

2. ROESLER, Rafael, CORNELIO, Daniela Baumann, Abujamra, A.L., SCHWARTSMANN, Gilberto
HER2 as a cancer stem cell target. Lancet Oncology. , v.11, p.225 - 226, 2010.

3. CORNELIO, Daniela Baumann, BRAGA, R. P., ROSA, M. W., AYUB, A.C.
Devic's neuromyelitis optica and pregnancy: distinction from multiple sclerosis is essential.. Archives of Gynecology and Obstetrics (Print). , v.280, p.475 - 477, 2009.

4. CORNELIO, Daniela Baumann, ROESLER, Rafael, SCHWARTSMANN, Gilberto
Emerging Therapeutic Agents for Cervical Cancer. Recent Patents on Anti-Cancer Drug Discovery. , v.4, p.196 - 206, 2009.

5. CORNELIO, Daniela Baumann, GLIETSCH, M. L., MOREIRA, Luís Fernando, SCHWARTSMANN, Gilberto
Câncer Gastrointestinal. Âmbito Hospitalar. , v.187, p.14 - 28, 2007.

6. CORNELIO, Daniela Baumann, ROESLER, Rafael, SCHWARTSMANN, Gilberto
Gastrin-releasing peptide receptor as a molecular target in experimental anticancer therapy.. Annals of Oncology. , v.18, p.1457 - 1466, 2007.

7. CORNELIO, Daniela Baumann, MEURER, Luise, ROESLER, Rafael, SCHWARTSMANN, Gilberto
Gastrin-releasing peptide receptor expression in cervical cancer. Oncology (Basel). , v.73, p.340 - 345, 2007.

8. CORNELIO, Daniela Baumann, DAL-PIZZOL, F., ROESLER, Rafael, SCHWARTSMANN, Gilberto
Targeting the Bombesin/Gastrin-Releasing Peptide Receptor to treat sepsis. Recent Patent Reviews on Anti-Infective Drug Discovery. , v.2, p.131 - 139, 2007.

9. CORNELIO, Daniela Baumann, MOREIRA, Luís Fernando, SCHWARTSMANN, Gilberto
Neoplasias Gastrintestinais. Ambito Hopitalar. , v.181, p.13 - 28, 2006.

Capítulos de livros publicados

1. Lago, S, CORNELIO, Daniela Baumann

Biologia Molecular das Metástases In: TRATADO DE MASTOLOGIA DA SBM ed.São Paulo : Revinter, 2010, p. 692-700.

2. LAZARETTI, N.S., CORNELIO, Daniela Baumann, POZZI, B., SCHWARTSMANN, Gilberto
IMUNOTERAPIA E CÂNCER In: MANUAL DE ONCOLOGIA 3ª EDIÇÃO, 2008, v.2

3. CORNELIO, Daniela Baumann, POHLMANN, Paula Raffin, SCHWARTSMANN, Gilberto
BASES DA QUIMIOTERAPIA In: Tratado de Clínica Médica.2 ed.São Paulo : Roca, 2007

4. CORNELIO, Daniela Baumann, SCHWARTSMANN, Gilberto
CIGARETTE SMOKING AND SMOKING CESSATION In: ESMO HANDBOOK ON CANCER PREVENTION ed.Londres : Informa Healthcare, 2007, p. 67-72.

5. CORNELIO, Daniela Baumann, SCHWARTSMANN, Gilberto
IMUNOTERAPIA E CÂNCER In: Manual de Oncologia.2 ed.São Paulo : BBS Editora, 2006, v.1, p. 1187-1194.

6. CORNELIO, Daniela Baumann, LEAL, Fábio, SCHWARTSMANN, Gilberto
NOVAS DROGAS EM TUMORES DE CABEÇA E PESCOÇO In: CÂNCER DE CABEÇA E PESCOÇO: DIAGNÓSTICO E TRATAMENTO.1ª ed.São Paulo : Âmbito, 2006, p. 44-48.

Trabalhos publicados em anais de eventos (completo)

1. CORNELIO, Daniela Baumann
The Gastrin-Releasing Peptide Receptor as a Target for Cervical Cancer Diagnosis In: 2nd Annual Congress of Antibodies, 2010, Beijing.
BIT Life Sciences 2nd Annual Congress Of Antibodies. Beijing: , 2010. v.1. p.257 - 257

Trabalhos publicados em anais de eventos (resumo)

1. MOREIRA, A.P., CORNELIO, Daniela Baumann, TURRA, S. E., MALDOTTI, V., PETRACCO, R., BORGES, B., PAGANINI, C. F., GROSSMANN, R., HOHGRAEFE, S. W., GROSSMANN, S.
Carcinoma de Mama Localmente Avançado: Abordagem Multidisciplinar In: III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul, 2009, Porto Alegre.
III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul. , 2009. v.1. p.45 - 45

2. CORNELIO, Daniela Baumann, MEURER, Luise, ROESLER, Rafael, SCHWARTSMANN, Gilberto
Expressão do Peptídeo Liberador da Gastrina no Câncer de Colo Uterino In: III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul, 2009, Porto Alegre.
III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul. , 2009. v.1. p.errata -

3. CORNELIO, Daniela Baumann, ROSA, M. W.
Leiomiomatose Peritoneal Disseminada: Relato de Caso In: III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul, 2009, Porto Alegre.
III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul. , 2009. v.1. p.39 - 40

4. CORNELIO, Daniela Baumann, TURRA, S. E., MOREIRA, A.P., HOHGRAEFE, S. W., GROSSMANN, R., PAGANINI, C. F., BATISTA, L., GROSSMANN, S.
Tratamento Cirúrgico de Metástases Pulmonares do Câncer de Mama In: III Congresso de Oncologia do Hospital Santa Rita - I Congresso de Oncologia do Mercosul, 2009, Porto Alegre.
III Congresso de Oncologia do Hospital Santa Rita - I Congresso de Oncologia do Mercosul. , 2009. v.1. p.errata -

5. MOREIRA, A.P., CORNELIO, Daniela Baumann, TURRA, S. E., MALDOTTI, V., PETRACCO, R., BORGES, B., PAGANINI, C. F., MACHADO, L., GROSSMANN, R., BORGHETTI, K. M., SANTOS, G. R., HOHGRAEFE, S. W., GROSSMANN, S.
Tumor Filoides em Mulher Jovem: Relato de Caso e Revisão da Literatura In: III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul, 2009, Porto Alegre.
III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul. , 2009. v.1. p.47 - 48

6. TURRA, S. E., CORNELIO, Daniela Baumann, MOREIRA, A.P., HOHGRAEFE, S. W., SANTOS, G. R., MACHADO, L., BORGHETTI, K. M., GROSSMANN, S.
Tumor Filóides Maligno em Homem: Relato de Caso e Revisão da Literatura In: III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul, 2009, Porto Alegre.
III Congresso de Oncologia do Hospital Santa Rita – I Congresso de Oncologia do Mercosul. , 2009. v.1. p.48 - 48

Apresentação de Trabalho

1. CORNELIO, Daniela Baumann
Breast Cancer with Solitary Brain Metastasis, 2012. (Comunicação,Apresentação de Trabalho)

2. Dini, I, CORNELIO, Daniela Baumann
Mastectomia higiênica seguida de cirurgia reconstrutiva de mama como tratamento paliativo de carcinoma lobular avançado com lesão ulcerada, 2012. (Comunicação,Apresentação de Trabalho)

3. CORNELIO, Daniela Baumann, GROSSMANN, R., PAGANINI, C. F., BATISTA, L., BORGHETTI, K. M., SANTOS, G. R., MACHADO, L.
CÂNCER DE MAMA COM METÁSTASES PULMONARES: QUAL O PAPEL DA RESSECÇÃO CIRÚRGICA?, 2011. (Outra,Apresentação de Trabalho)

4. CORNELIO, Daniela Baumann
Câncer de Mama em Homens, 2011. (Conferência ou palestra,Apresentação de Trabalho)

5. SANTOS, G. R., BORGHETTI, K. M., CORNELIO, Daniela Baumann, MACHADO, L., PAGANINI, C. F., BATISTA, L., GROSSMANN, R., GROSSMANN, S.
CÂNCER DE MAMA EM PACIENTES JOVENS: EXPERIÊNCIA DO CENTRO DE MAMA DO HOSPITAL SANTA RITA, 2011. (Outra,Apresentação de Trabalho)

6. CORNELIO, Daniela Baumann
Câncer de Mama Triplo Negativo, 2011. (Conferência ou palestra,Apresentação de Trabalho)

7. BORGHETTI, K. M., CORNELIO, Daniela Baumann, GROSSMANN, R., GROSSMANN, S., MACHADO, L., BATISTA, L., SANTOS, G. R., PAGANINI, C. F.
CARACTERÍSTICAS CLÍNICAS E ANÁTOMO-PATOLÓGICAS DE 557 CASOS DO CENTRO DE MAMA DO HOSPITAL SANTA RITA, 2011. (Outra,Apresentação de Trabalho)

8. CORNELIO, Daniela Baumann
Cirurgia no Câncer de Mama recorrente ou Metastático, 2011. (Conferência ou palestra,Apresentação de Trabalho)

9. CORNELIO, Daniela Baumann
Classificação TNM - Atualizações e Proposta IEO, 2011. (Conferência ou palestra,Apresentação de Trabalho)

10. CORNELIO, Daniela Baumann, Coelho, M, GROSSMANN, R., GROSSMANN, S., BORGHETTI, K. M., BATISTA, L., MACHADO, L., PAGANINI, C. F., SANTOS, G. R.

DIAGNÓSTICO DIFERENCIAL DE MASSAS AXILARES: LINFOMA, 2011. (Outra,Apresentação de Trabalho)

11. CORNELIO, Daniela Baumann, Coelho, M, BORGHETTI, K. M., GROSSMANN, R., GROSSMANN, S., MACHADO, L., PAGANINI, C. F., SANTOS, G. R., BATISTA, L.
DIAGNÓSTICO DIFERENCIAL DE MASSAS AXILARES: MELANOMA, 2011. (Outra,Apresentação de Trabalho)

12. CORNELIO, Daniela Baumann
Discussão de artigo: Axilary vs No Axillary Dissection in Women with Invasive Breast Cancer and Sentinel Node Metastasis, 2011. (Conferência ou palestra,Apresentação de Trabalho)

13. CORNELIO, Daniela Baumann
Discussão de Artigo: Dedicated Dual-Head Gamma Imaging for Breast Cancer Screening in Women with Mammographically Dense Breasts, 2011. (Conferência ou palestra,Apresentação de Trabalho)

14. CORNELIO, Daniela Baumann
Exames para Diagnóstico e Seguimento do Câncer de Mama, 2011. (Conferência ou palestra,Apresentação de Trabalho)

15. CORNELIO, Daniela Baumann
Ginecomastia, 2011. (Conferência ou palestra,Apresentação de Trabalho)

16. CORNELIO, Daniela Baumann
Linfonodo Sentinela: Discussão de caso, 2011. (Conferência ou palestra,Apresentação de Trabalho)

17. CORNELIO, Daniela Baumann
Massa Axilar: Discussão de Caso, 2011. (Conferência ou palestra,Apresentação de Trabalho)

18. CORNELIO, Daniela Baumann, Abujamra, A.L., Farias, C.B., ROESLER, Rafael
Relato de Caso: Empresa Incubada na UFRGS - case Ziel Biosciences, 2011. (Simpósio,Apresentação de Trabalho)

19. CORNELIO, Daniela Baumann, HOHGRAEFE, S. W., SANTOS, G. R., Dal Pizzol Jr, A, Lago, S, MACHADO, L., BORGHETTI, K. M., PAGANINI, C. F., GROSSMANN, R., GROSSMANN, S.
RESPOSTA À QUIMIOTERAPIA NEOADJUVANTE NO TRATAMENTO DO CÂNCER DE MAMA, 2011. (Outra,Apresentação de Trabalho)

20. Abujamra, A.L., CORNELIO, Daniela Baumann, Farias, C.B.
Ziel Biosciences - apresentação da empresa, 2011. (Simpósio,Apresentação de Trabalho)

21. CORNELIO, Daniela Baumann, Farias, C.B., Abujamra, A.L.
Ziel Biosciences - case, 2011. (Outra,Apresentação de Trabalho)

22. CORNELIO, Daniela Baumann
Câncer de Mama em Idosas, 2010. (Conferência ou palestra,Apresentação de Trabalho)

23. CORNELIO, Daniela Baumann
Leiomiossarcoma: Discussão de Caso, 2010. (Conferência ou palestra,Apresentação de Trabalho)

24. CORNELIO, Daniela Baumann
Primary outcome results of NSABP-32, a randomized phase III clinical trial to compare sentinel node resection to conventional axillary dissection in clinicaly node-negative breast cancer patients, 2010. (Conferência ou palestra,Apresentação de Trabalho)

25. CORNELIO, Daniela Baumann
Selected presentations ASCO 2010 - Primary outcome results of NSABP-32, a randomized phase III clinical trial to compare sentinel node resection to conventional axillary dissection in clinicaly node-negative breast cancer patients, 2010. (Conferência ou palestra,Apresentação de Trabalho)

26. CORNELIO, Daniela Baumann, MEURER, Luise, ROESLER, Rafael, SCHWARTSMANN, Gilberto
The Gastrin-Releasing Peptide Receptor as a Target for Cervical Cancer Diagnosis, 2010. (Congresso,Apresentação de Trabalho)

27. CORNELIO, Daniela Baumann
The Gastrin-Releasing Peptide Receptor as a Target for Cervical Cancer Diagnosis, 2010. (Conferência ou palestra,Apresentação de Trabalho)

28. CORNELIO, Daniela Baumann
Trabalho de Conclusão de Curso no Programa de Ginecologia e Obstetrícia, 2010. (Outra,Apresentação de Trabalho)

29. MOREIRA, A.P., CORNELIO, Daniela Baumann, TURRA, S. E., MALDOTTI, V., PETRACCO, R., BORGES, B., PAGANINI, C. F., GROSSMANN, R., HOHGRAEFE, S. W., GROSSMANN, S.
Carcinoma de Mama Localmente Avançado: Abordagem Multidisciplinar, 2009. (Congresso,Apresentação de Trabalho)

30. CORNELIO, Daniela Baumann, MEURER, Luise, ROESLER, Rafael, SCHWARTSMANN, Gilberto
Expressão do Peptídeo Liberador da Gastrina no Câncer de Colo Uterino, 2009. (Congresso,Apresentação de Trabalho)

31. CORNELIO, Daniela Baumann
Factors Associated with Bilateral vs. Unilateral Mastectomy in a Diverse, Population-based Sample of Breast Cancer Patients, 2009. (Congresso,Apresentação de Trabalho)

32. CORNELIO, Daniela Baumann, ROSA, M. W.
Leiomiomatose Peritoneal Disseminada: Relato de Caso, 2009. (Congresso,Apresentação de Trabalho)

33. CORNELIO, Daniela Baumann
Manejo e prevenção de complicações pós-operatórias em cirurgias ginecológicas, 2009. (Conferência ou palestra,Apresentação de Trabalho)

34. CORNELIO, Daniela Baumann
Papel de Marcadores Tumorais no Seguimento do Câncer de Mama, 2009. (Conferência ou palestra,Apresentação de Trabalho)

35. CORNELIO, Daniela Baumann, TURRA, S. E., MOREIRA, A.P., HOHGRAEFE, S. W., GROSSMANN, R., PAGANINI, C. F., BATISTA, L., GROSSMANN, S.
Tratamento Cirúrgico de Metástases Pulmonares do Câncer de Mama, 2009. (Congresso,Apresentação de Trabalho)

36. CORNELIO, Daniela Baumann, TURRA, S. E., MOREIRA, A.P., HOHGRAEFE, S. W., PAGANINI, C. F., GROSSMANN, R., GROSSMANN, S.
Tratamento Cirúrgico de Metástases Pulmonares do Câncer de Mama, 2009. (Outra,Apresentação de Trabalho)

37. MOREIRA, A.P., CORNELIO, Daniela Baumann, TURRA, S. E., MALDOTTI, V., PETRACCO, R., BORGES, B., PAGANINI, C. F., MACHADO, L., GROSSMANN, R., BORGHETTI, K. M., SANTOS, G. R., HOHGRAEFE, S. W., GROSSMANN, S.

Tumor Filoides em Mulher Jovem: Relato de Caso e Revisão da Literatura, 2009. (Congresso,Apresentação de Trabalho)

38. TURRA, S. E., CORNELIO, Daniela Baumann, MOREIRA, A.P., HOHGRAEFE, S. W., SANTOS, G. R., MACHADO, L., BORGHETTI, K. M., GROSSMANN, R.
Tumor Filoides Maligno em Homem: Relato de Caso e Revisão da Literatura, 2009. (Congresso,Apresentação de Trabalho)

39. CORNELIO, Daniela Baumann
Attom: Randomized Trial of 10 versus 5 years of adjuvant tamoxifen among 3694 women with estrogen receptor+ (ER+) or ER untested breast cancer: preliminary results., 2008. (Conferência ou palestra,Apresentação de Trabalho)

40. CORNELIO, Daniela Baumann
Câncer de Mama Associado à Gestação, 2008. (Conferência ou palestra,Apresentação de Trabalho)

41. CORNELIO, Daniela Baumann, ROSA, M. W.
LEIOMIOMATOSE PERITONEAL DISSEMINADA: RELATO DE CASO, 2008. (Congresso,Apresentação de Trabalho)

42. CORNELIO, Daniela Baumann, ROSA, M. W., AYUB, A.C.
NEUROMIELITE ÓPTICA DE DEVIC DURANTE A GESTAÇÃO: RELATO DE CASO, 2008. (Congresso,Apresentação de Trabalho)

Demais produções bibliográficas

1. CORNELIO, Daniela Baumann, PAGLIOLLI, Ricardo
Odontologia: Consulta Rápida. Porto Alegre:Editora Artes Médicas, 1997. (Livro, Tradução)

2. CORNELIO, Daniela Baumann, Abujamra, A.L., ROESLER, Rafael
Nature Molecule Page - Bb2 Receptor. Nature Molecule Page. :UCSD Nature Signaling Gateway, 2009. (Outra produção bibliográfica)

Produção Técnica

Produtos tecnológicos com registro ou patente

1. CORNELIO, Daniela Baumann, Abujamra, A.L., Farias, C.B., ROESLER, Rafael, SCHWARTSMANN, Gilberto
Composição Compreendendo Agentes Moduladores Anti-Tumorais, Método de Modulação de Tumores Utilizando Agentes Moduladores e Uso de Agentes Moduladores de BDNF/TrkB para Modulação da Resistência a Agentes Antitumorais, 2012

2. CORNELIO, Daniela Baumann, Abujamra, A.L., Farias, C.B., ROESLER, Rafael, SCHWARTSMANN, Gilberto
Uso de Agentes Moduladores (GRP) para Preparação de Medicamentos Antitumorais, Composição Compreendendo Agentes Moduladores Antitumorais, Método de Modulação de Tumores Utilizando Agentes Moduladores, 2012

3. CORNELIO, Daniela Baumann, ROESLER, Rafael, SCHWARTSMANN, Gilberto
USO DE ANTICORPOS ANTI-GRPR, MÉTODO DE DETECÇÃO E KIT DE DIAGNÓSTICO DE LESÕES DISPLÁSICAS E NEOPLÁSICAS, 2011

Trabalhos técnicos

1. Abujamra, A.L., Farias, C.B., CORNELIO, Daniela Baumann, ROESLER, Rafael
Oncoterapia Individualizada, 2010

2. POHLMANN, Paula Raffin, DIAS, Eduardo Coelho, CORNELIO, Daniela Baumann, GRAUDENZ, Márcia, BEDIN JR, Ademar José
ELABORAÇÃO DO BANCO DE TECIDOS DO HOSPITAL MOINHOS DE VENTO, 2005

Demais Trabalhos

1. CORNELIO, Daniela Baumann
Monitora Bolsista da Disciplina de Nefrologia do Departamento de Medicina Interna do Curso de Medicina, 2004.

2. CORNELIO, Daniela Baumann
Monitora da Disciplina de Cirurgia Geral do Departamento de Cirurgia Geral, 2004.

3. CORNELIO, Daniela Baumann
Monitora Bolsista da Disciplina de Nefrologia, 2003.

4. CORNELIO, Daniela Baumann
Monitora da Disciplina de Histologia Geral, 1992.

Eventos

Participação em eventos

1. **Workshop de Mama e Oncogenética do Hospital Santa Rita**, 2012. (Oficina)
Breast Cancer with Solitary Brain Metastasis.

2. Apresentação Oral no(a) **Workshop de Inovação Farmacêutica**, 2011. (Oficina)
Relato de Caso: Empresa Incubada na UFRGS - case Ziel Biosciences.

3. Apresentação de Poster / Painel no(a) **Workshop de Mama Hospital Santa Rita**, 2011. (Oficina)
RESPOSTA À QUIMIOTERAPIA NEOADJUVANTE NO TRATAMENTO DO CÂNCER DE MAMA.

4. Apresentação Oral no(a) **13º Laboratório de Aprendizagem Brasil - EUA**, 2011. (Oficina)
Ziel Biosciences - apresentação da empresa.

5. Apresentação Oral no(a) **Aula Final da Disciplina de Informática da UFRGS**, 2011. (Outra)
Ziel Biosciences - case.

6. **2nd Annual Meeting of the Global Federation of Competitiveness Councils**, 2011. (Encontro)
.

7. **54 Congresso Brasileiro de Ginecologia e Obstetrícia**, 2011. (Congresso)
.

8. Moderador no(a) **VI Congresso Franco-Brasileiro de Oncologia**, 2010. (Congresso)
Câncer Ginecológico Parte I.

9. Moderador no(a) **VI Congresso Franco-Brasileiro de Oncologia**, 2010. (Congresso)
Câncer Ginecológico Parte II.

10. Apresentação (Outras Formas) no(a)**I Forum Intersetorial de Controle de Câncer de Mama no Estado do Rio Grande do Sul**, 2010. (Simpósio)
I Forum Intersetorial de Controle de Câncer de Mama no Estado do Rio Grande do Sul.

11. Conferencista no(a) **The Best of Asco**, 2010. (Congresso)
Primary outcome results of NSABP-32, a randomized phase III clinical trial to compare sentinel node resection to conventional axillary dissection in clinicaly node-negative breast cancer patients.

12. Conferencista no(a) **VI Congresso Franco-Brasileiro de Oncologia**, 2010. (Congresso)
Primary outcome results of NSABP-32, a randomized phase III clinical trial to compare sentinel node resection to conventional axillary dissection in clinicaly node-negative breast cancer patients.

13. Conferencista no(a) **2nd Annual International Congress of Antibodies**, 2010. (Congresso)
The Gastrin-Releasing Peptide Receptor as a Target for Cervical Cancer Diagnosis.

14. **Congresso Integrado de Ginecologia e Obstetrícia**, 2010. (Congresso)
.

15. **Câncer de Mama Gramado 2010**, 2010. (Congresso)
.

16. **Workshop de Mama**, 2010. (Oficina)
.

17. Apresentação de Poster / Painel no(a) **III CONGRESSO DE ONCOLOGIA DO HOSPITAL SANTA RITA - I CONGRESSO DE ONCOLOGIA DO MERCOSUL**, 2009. (Congresso)
EXPRESSÃO DO PEPTÍDEO LIBERADOR DA GASTRINA NO CÂNCER DE COLO UTERINO.

18. Conferencista no(a) **The Best of ASCO**, 2009. (Congresso)
Factors Associated with Bilateral vs. Unilateral Mastectomy in a Diverse, Population-based Sample of Breast Cancer Patients.

19. Apresentação de Poster / Painel no(a) **Workshop de Mama Hospital Santa Rita / Hospital Albert Einstein**, 2009. (Oficina)
Tratamento Cirúrgico de Metástases Pulmonares em Câncer de Mama.

20. Apresentação (Outras Formas) no(a)**Ciclo de Palestras para Profissionais da Saúde em Homenagem ao Dia Internacional da Mulher**, 2008. (Oficina)
Anticoncepção - Atualização.

21. Conferencista no(a) **The Best of ASCO**, 2008. (Simpósio)
BREAST CANCER: Adjuvant Therapy.

22. Apresentação de Poster / Painel no(a) **II CONGRESSO DE GINECOLOGIA E OBSTETRICIA DA SANTA CASA - UFCSPA**, 2008. (Congresso)
LEIOMIOMATOSE PERITONEAL DISSEMINADA: RELATO DE CASO e NEUROMIELITE ÓPTICA DE DEVIC DURANTE A GESTAÇÃO: RELATO DE CASO.

23. **XIV Congresso Sulbrasileiro de Ginecologia e Obstetrícia**, 2008. (Congresso)
.

24. **SIMPÓSIO SULBRASILEIRO DE ENDOMETRIOSE**, 2008. (Simpósio)
.

25. **Curso de Aleitamento Materno**, 2008. (Outra)
.

26. **II Simpósio Internacional de Laser**, 2007. (Simpósio)
.

27. **Simpósio Gaúcho sobre Terapia Gênica e Celular**, 2007. (Simpósio)
.

28. **The Best of ASCO 2007**, 2007. (Congresso)
.

29. **Workshop Elaborando um Projeto de Pesquisa**, 2007. (Oficina)
.

30. **II Annual Meeting of Aesthetic Procedures**, 2006. (Encontro)
.

31. **VII simpósio internacional de cirurgia plástica**, 2006. (Simpósio)
.

32. **Novos Paradigmas em TNE e GIST**, 2006. (Encontro)
.

33. **III Encontro Científico Anual do Instituto de Cardiologia do RS -**, 2005. (Encontro)
.

34. **III Encontro Científico Anual do Instituto de Cardiologia do RS**, 2005. (Encontro)
.

35. **I Congresso Internacional de Mastologia do Hospital Moinhos de Vento**, 2005. (Congresso)
.

36. **Seminário RC-3095**, 2005. (Seminário)
.

37. **Workshop em oncologia torácica**, 2005. (Oficina)
.

38. **III Workshop de Tumores Neuroendócrinos**, 2005. (Oficina)
.

39. **Cancer Today: from molecular biology to treatment**, 2005. (Congresso)
.

40. **Simpósio Inaugural da Liga de Hipertensão da FFFCMPA**, 2004. (Simpósio)
.

41. **IV Encontro do Serviço de Endocrinologia e Centro de Diabetes da Santa Casa**, 2004. (Encontro)
.

42. **I Workshop em Melanoma**, 2004. (Oficina)
.

43. **Patologia Renal e EQU**, 2003. (Outra)
.

44. **IX Jornada Gaúcha de Nefrologia e Enfermagem em Nefrologia**, 2003. (Outra)
.

45. **Simpósio 15 anos do Serviço de Cardiologia do Hospital São Francisco - 200 Anos da Santa Casa de Porto Alegre**, 2003. (Simpósio)
.

46. **XVIII Semana Acadêmica da FFFCMPA**, 2002. (Outra)
.

Organização de evento

1. CORNELIO, Daniela Baumann
Curso de Férias Temas de Urgências Cirúrgicas, 2005. (Outro, Organização de evento)

Totais de produção

Produção bibliográfica

Artigos completos publicados em periódico	9
Capítulos de livros publicados	6
Trabalhos publicados em anais de eventos	7
Apresentações de Trabalhos (Comunicação)	2
Apresentações de Trabalhos (Conferência ou palestra)	19
Apresentações de Trabalhos (Congresso)	10
Apresentações de Trabalhos (Simpósio)	2
Apresentações de Trabalhos (Outra)	9
Traduções (Livro)	1
Demais produções bibliográficas	1

Produção Técnica

Produtos tecnológicos (outro)	3
Trabalhos técnicos (elaboração de projeto)	2

Eventos

Participações em eventos (congresso)	15
Participações em eventos (seminário)	1
Participações em eventos (simpósio)	8
Participações em eventos (oficina)	11
Participações em eventos (encontro)	6
Participações em eventos (outra)	5
Organização de evento	

Demais trabalhos relevantes