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ZEB1 COMO MARCADOR DE SOBREVIDA SUBGRUPO-ESPECÍFICO EM MEDULOBLASTOMA E SUA INTERAÇÃO COM RNAS NÃO CODIFICANTES

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Orientador: Prof. Dr. Rafael Roesler

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LISTA DE ABREVIAÇÕES, SÍMBOLOS E UNIDADES

EMT – Transição epitélio-mesenquimal
GNPs – progenitores de neurônios granulares
lncRNA - RNA longo não codificante
MB - meduloblastoma
mRNA - RNA mensageiro
miR - microRNA
miRNA – microRNA
ng – nanograma
nM – nanomolar
NPCs - células progenitoras neuronais
pri-miRNA - microRNA primário
RT-qPCR - Reação em Cadeia da Polimerase associada a Transcriptase Reversa
SHH - Sonic Hedgehog
WNT - Wingless
ZEB1 - Zinc finger E-box Binding 1
μM – micromolar

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RESUMO

Meduloblastoma é o tumor maligno de Sistema Nervoso Central que mais acomete pacientes pediátricos. Há uma classificação molecular desse tumor em diferentes subgrupos, que apresentam incidência, prognóstico e marcadores distintos. O fator de transcrição ZEB1 promove a expressão de marcadores mesenquimais de indiferenciação e está intimamente relacionado à formação do cerebelo. Em outros tumores, a expressão de ZEB1 foi relacionada ao cenário metastático. Para investigar a ocorrência de ZEB1 em meduloblastoma, utilizamos como metodologia a análise da expressão de ZEB1 em bancos de dados disponibilizados na plataforma R2, por meio de scripts na linguagem R. Nesse trabalho, a expressão de ZEB1 em diferentes subgrupos moleculares foi acessada, e o subgrupo molecular SHH apresentou a maior expressão de ZEB1. A expressão de ZEB1 também foi relacionada à sobrevida de pacientes de meduloblastoma, com a maior expressão de ZEB1 sendo relacionada ao pior prognóstico de pacientes diagnosticados com o Grupo 3 e Grupo 4. Para investigar a relação de ZEB1 e RNAs não codificantes, foi realizada uma revisão da literatura. De forma inédita. Uma revisão compilou a relação de ZEB1 com o desenvolvimento de tecidos e desenvolvimento de tumores sólidos pediátricos, além da regulação com RNAs não codificantes. Em meduloblastoma, dois miRNAs diferencialmente expressos no cenário metastático e com relações inibitórias com ZEB1 foram analisados em um banco de dados de pacientes de meduloblastoma. Com base nos nossos resultados, ZEB1 é proposto como biomarcador subgrupo-específico e alvo terapêutico em meduloblastoma.

ABSTRACT

Medulloblastoma is the most common Central Nervous System malignant tumor in pediatric patients. Medulloblastoma is classified into subgroups presenting different incidences, prognosis and molecular markers. ZEB1 is a transcription factor involved in cerebellar development which promotes expression of mesenchymal markers. In other tumor types, ZEB1 expression has been associated with occurrence of metastasis. Aiming to investigate the occurence of ZEB1 in medulloblastoma, we applied R packages to data analysis in dataset from R2 Database as methodology. In this thesis, ZEB1 expression was accessed in different molecular subgroups of medulloblastoma, which SHH presenting the higher ZEB1 expression. ZEB1 levels were also related to the overall survival of medulloblastoma patients, where higher ZEB1 expression was found in patients with tumors of the molecular subgroups 3 and 4, which present the worst prognosis. To elucidate the relation between ZEB1 and non-coding RNAs, a literature review was performed. In an unprecedent way, the review compiled ZEB1 role in tissue development and pediatric solid tumors, and its interactions with non-coding RNAs. Finally, two miRNAs differentially expressed in metastasis and negatively regulating ZEB1 were analyzed in a dataset of human medulloblastoma tumors. In summary, on the basis of our findings we propose ZEB1 as regulator of non-coding RNAs with a possible role as subgroup-specific molecular marker and therapeutic target in medulloblastoma.

1. INTRODUÇÃO

1.1 MEDULOBLASTOMA

No Brasil, segundo estimativa publicada pelo INCA, cerca de 7.930 casos de câncer são esperados para cada ano do triênio 2023-2024-2025. Tumores infanto juvenis, diferentemente dos tumores em adultos, são frequentemente desenvolvidos a partir de tecidos embrionários, sendo leucemias, linfomas e tumores de Sistema Nervoso Central os diagnósticos mais comuns (INCA, 2022).

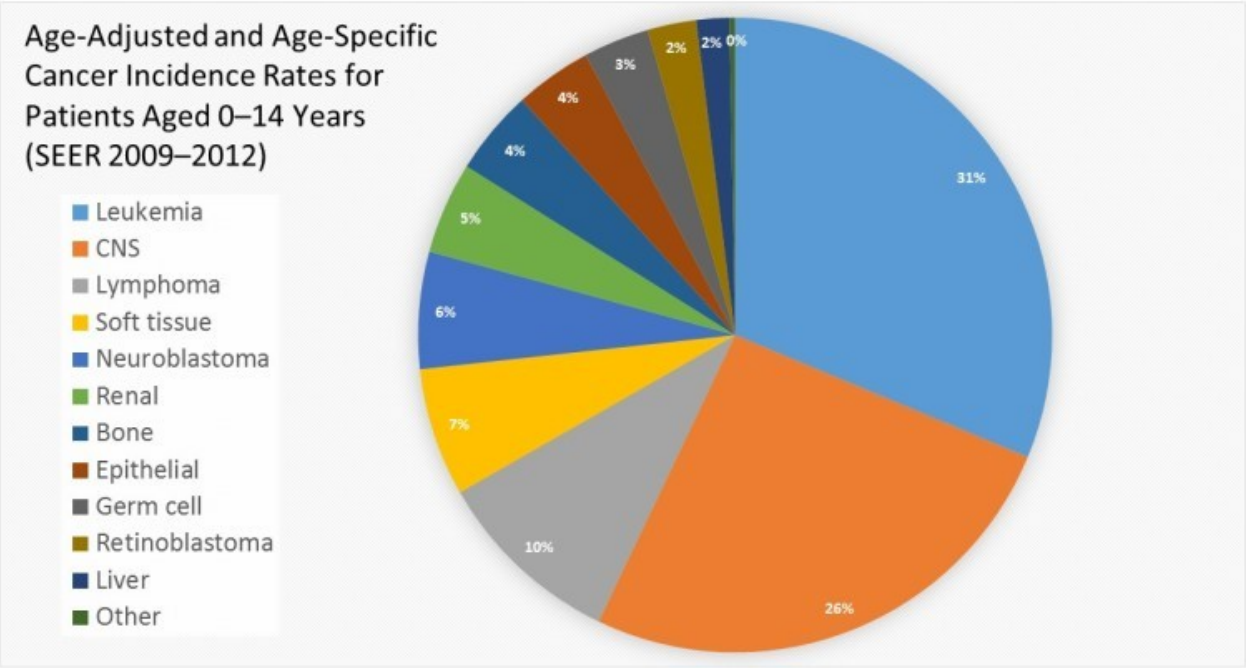

Figura 1. Incidência de tumores pediátricos em pacientes de 0 a 14 anos, de acordo com estimativa do programa de Vigilância, Epidemiologia e Resultados Finais (SEER) do *National Cancer Institute.* vPDQ Cancer Information Summaries [Internet]. Bethesda (MD): National Cancer Institute (US); 2018.

Uma vez que o câncer pediátrico não é prevenível, a principal ferramenta no combate ao câncer pediátrico, está o diagnóstico precoce. O conhecimento dos sinais e sintomas de tumores em crianças por familiares e profissionais de saúde é essencial para o curto período

de tempo entre a primeira consulta médica, realização de exames e diagnóstico da neoplasia. Entre os sintomas – que são inespecíficos e podem ser confundidos com outras doenças recorrentes – estão febre prolongada, vômitos, emagrecimento, sangramentos, dor óssea e palidez (MINISTÉRIO DA SAÚDE, BRASIL, 2017).

Meduloblastoma são tumores que se desenvolvem na fossa posterior, na região do cerebelo e é o tumor de Sistema Nervoso Central mais comum em crianças. Crianças de 4 a 9 anos tem a maior incidência (44%), seguidos por adolescentes de 10 a 16 anos 23% enquanto 12% da incidência ocorre em crianças na faixa de 0 a 3 anos de idade (Mahapatra & Amsbaugh, 2023). Em estudo brasileiro, foi visto que a idade média ao diagnóstico foi de 8.2 anos de idade (Oigman et al., 2022) .

Dor de cabeça, náusea e vômito e alterações na visão são os principais sintomas relatados pelos familiares de 114 pacientes diagnosticados com Meduloblastoma. Esses sintomas se devem ao crescimento da massa tumoral que obstrui o quarto ventrículo e ao aumento da pressão intracraniana. Junto aos sintomas, o diagnóstico requer exames por imagem, como a ressonância magnética (Oigman et al., 2022; Fang et al., 2022).

O tratamento adotado para meduloblastoma é a ressecção cirúrgica combinada a radioterapia e quimioterapia (Mahapatra & Amsbaugh, 2023). A quimioterapia é indicada principalmente para os casos em que há doença residual ou micrometástases, que não podem ser removidas cirurgicamente ou tratadas com radioterapia (Fang et al., 2022). Algumas abordagens envolvendo intensa quimioterapia adjuvante em detrimento da radioterapia estão sendo avaliadas para pacientes de meduloblastoma, mas ainda há carência de terapias disponíveis para meduloblastoma de alto risco (Lafay-Cousin et al., 2022). Um dos fatores que também influenciam no prognóstico de tumores infantojuvenis é o centro onde o paciente está sendo tratado. Pacientes tratados em centros especializados, com abordagem multidisciplinar e maior acesso a procedimentos neurocirúrgicos apresentam maior sobrevida do que pacientes tratados fora desses centros (Fischer et al., 2021)

Mesmo sendo bem tolerada, a radioterapia em pacientes pediátricos de meduloblastoma provoca dermatite, mucosite, alopecia, anorexia e fadiga. Náusea e vômitos também são observados mesmo com a administração de antieméticos. Além disso, sintomas hematológicos como diminuição de plaquetas e leucócitos também são observados (Ruggi et al, 2022).

Apesar de alterações em habilidades sociais felizmente não terem sido observadas com o tratamento (Ramjan et al., 2023), efeitos neuroendócrinos, como alterações em hormônios do crescimento e hipotireoidismo são encontradas nesses pacientes (Choi, 2023). Outro importante efeito de longo prazo do tratamento de meduloblastoma é o surgimento de tumores secundários como glioblastoma, indicando que a idade ao momento da primeira radioterapia poderia ser um fator de risco para o desenvolvimento de glioblastoma (Mesbahi et al., 2022).

Além da qualidade do centro onde os pacientes são tratados, a sobrevida de pacientes diagnosticados com meduloblastoma está estreitamente atrelado às características do tumor. O meduloblastoma era compreendido apenas histologicamente, sendo classificado como Clássico, Desmoplásico/nodular, ou Anaplásico/de células grandes (Borowska and Jóźwiak, 2016). Com a chegada de ferramentas de biologia molecular, o perfil transcricional pôde ser acessado e quatro grupos distintos foram identificados: Sonic Hedgehog (SHH), Wingless (WNT), Grupo 3 e Grupo 4, sendo reconhecidas pela Organização Mundial da Saúde em 2016 (Juraschka and Taylor, 2019).

	Subgroup	WNT	SHH	Group 3	Group 4
Clinical Characteristics	% of Cases	10	30	25	35
	Age at Diagnosis				
	Gender Ratio (M:F)	1:1	1:1	2:1	3:1
	Anatomic Location				
	Histology	Classic, Rarely LCA	Desmoplastic, Classic, LCA	Classic, LCA	Classic, LCA
	Metastasis at Diagnosis (%)	5-10	15-20	40-45	35-40
	Recurrence Pattern	Rare; Local or metastatic	Local	Metastatic	Metastatic
	Prognosis	Very good	Infants good, others intermediate	Poor	Intermediate
Molecular Characteristics	Proposed Cell of Origin	Progenitor cells in the lower rhombic lip	Granule precursors of the external granule layer	Neural stem cells	Unipolar brush cells
	Recurrent Gene Amplifications	-	*MYCN* *GLI1* or *GLI2*	*MYC* *MYCN* *OTX2*	*SNCAIP* *MYCN* *OTX2* *CDK6*
	Recurrent SNVs	*CTNNB1* *DDX3X* *SMARCA4* *TP53*	*PTCH1* *TERT* *SUFU* *SMO* *TP53*	*SMARCA4* *KBTBD4* *CTDNEP1* *KMT2D*	*KDM6A* *ZMYM3* *KTM2C* *KBTBD4*
	Cytogenetic Events ■ Gain ■ Loss	6	3q, 9p 9q, 10q, 17p	1q, 7, 18 8, 10q, 11, 16q i17q	7, 18q 8, 11p, X i17q
	Other Recurrent Genetic Events	-	-	*GFI1* and *GFI1B* enhancer hijacking	*PRDM6*, *GFI1*, and *GFI1B* enhancer hijacking

Age: Infant Child Adult

Figura 2. Subgrupos moleculares em Meduloblastoma. Características clínicas e moleculares de cada um dos grupos WNT, SHH, Grupo 3 e Grupo 4 são elencadas. Fonte: Juraschka K, Taylor MD. 2019.

O subgrupo molecular WNT representa cerca de 10% dos diagnósticos de meduloblastoma e é o subgrupo molecular com prognóstico mais favorável, raramente apresentam metástases (Skowron et al., 2015). Estima-se que meduloblastomas desse subgrupo molecular se desenvolvam a partir de células progenitoras do tronco cerebral (Gibson et al., 2010; Miranda Kuzan-Fischer et al., 2018). A alteração molecular mais frequente é no gene CTNNB1, que codifica a proteína β-catenina. A mutação nesse gene e o acúmulo nuclear da proteína são marcadores de diagnóstico do subgrupo WNT, caracterizando a ativação dessa via (Ramaswamy et al., 2016).

A ativação da via SHH com a mutação dos genes PTCH, SMO e SUFU caracteriza os tumores classificados como esse subgrupo molecular (Kool et al., 2014). Um terço dos casos de meduloblastoma são diagnosticados como SHH e o prognóstico é bastante variável. Os casos em que apresentam mutação no gene TP53 apresentam prognóstico desfavorável. Além disso, cerca de 20% dos meduloblastomas SHH apresentam metástases ao diagnóstico (Miranda Kuzan-Fischer et al., 2018). O surgimento desse subgrupo molecular ocorre a partir da proliferação desordenada de células granulares durante o desenvolvimento do cerebelo (Hatten & Roussel, 2011; Skowron et al., 2015).

Os demais tumores que não são categorizados como SHH ou WNT são compreendidos como Grupo 3 e Grupo 4. O grupo 3 contempla 25% dos diagnósticos de meduloblastoma e apresenta prognóstico desfavorável, com alta incidência de metástases, somadas à presença da amplificação do gene MYCN e à pouca idade ao diagnóstico (Cho et al., 2011; Menyhárt, & Győrffy, 2020). Já o Grupo 4 é o mais prevalente (cerca de 35%) e apresenta prognóstico intermediário, variando de acordo com a perda do cromossomo 11 , mas consideravelmente pior que o prognóstico de SHH e WNT (Menyhárt, & Győrffy, 2020)

Pacientes que apresentam doença localizada têm melhor prognóstico que pacientes diagnosticados com metástases (Oigman et al., 2022). Disseminação nas leptomeninges é o principal padrão de metástases de meduloblastoma (Fults et al., 2019). Características genéticas do tumor primário também foram encontradas nos focos metastáticos. Essa similaridade foi identificada principalmente em tumores do Grupo 3, enquanto no Grupo 4 foram encontradas divergências entre o sítio primário e as metástases (Richardson et al., 2022; Hill et al., 2020).

1.2 ZEB1

ZEB1 é um fator de transcrição que regula a transição epitélio-mesenquimal em processos fisiológicos, durante o desenvolvimento embrionário, e em processos patológicos, sendo amplamente descrito em mecanismos tumorais (Madany et al., 2018). A plasticidade celular regulada por ZEB1 é mediada pela inibição de miRNAs. Por outro lado, a depleção de ZEB1 reduz o fenótipo indiferenciado (“stemness”) e capacidade de colonização, limitando a plasticidade celular (Drápela et al., 2020; Krebs et al., 2017).

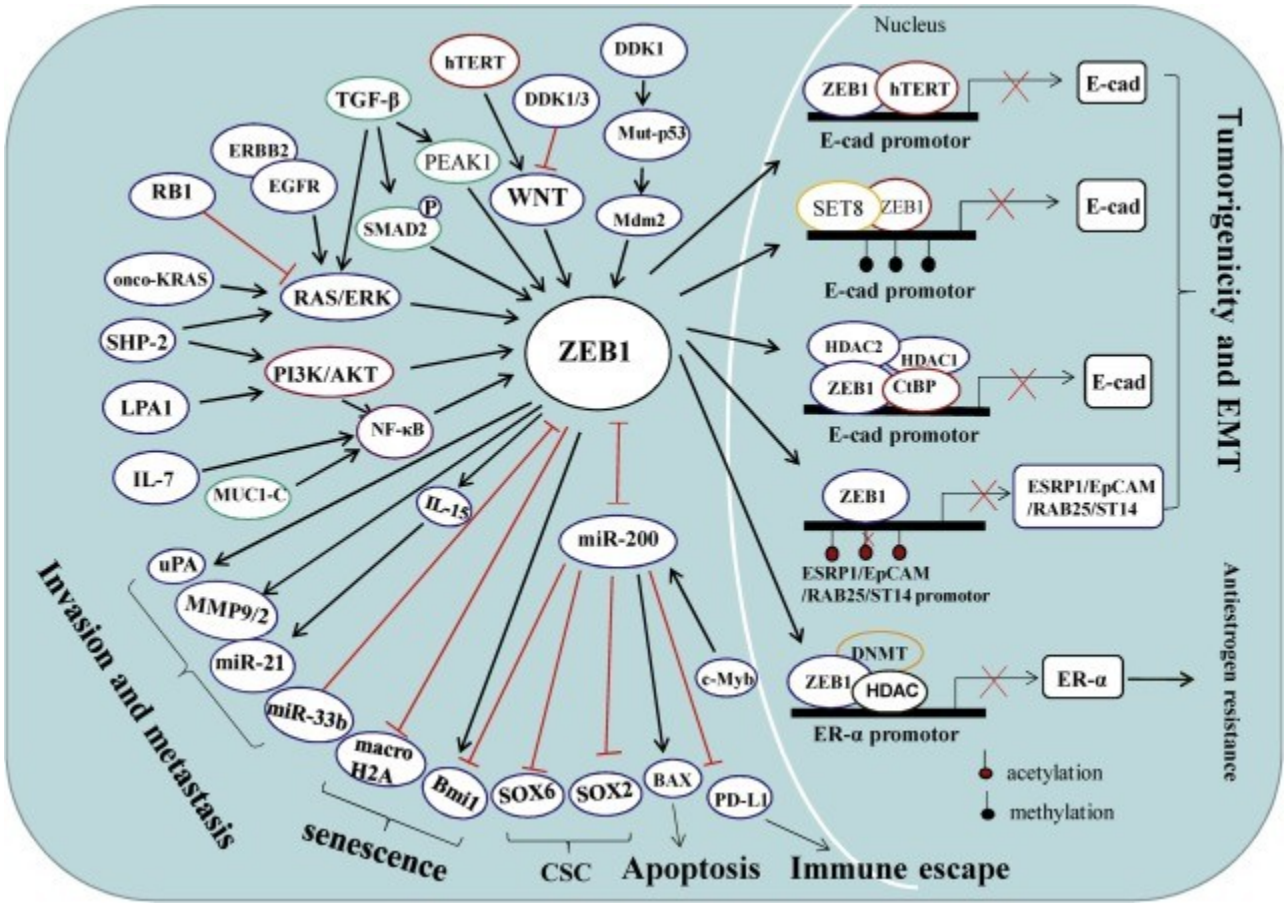

Figura 3. Rede de sinalização de ZEB1 em tumores. As relações de ativação e inibição com genes e miroRNAs são indicadas por meio das setas pretas e indicadores vermelhos, respectivamente. Os processos oncológicos modulados de invasão e metástase, senescência, células tronco tumorais, apoptose, escape tumoral e transição epitélio-mesenquimal são indicados. Fonte: Zhang Y, et al.. 2019.

ZEB1 contém dois clusters de dedos de zinco nas extremidades C-terminal e N-terminal que interagem com regiões E-box de genes alvo. ZEB1 também apresenta regiões que se ligam às proteínas SMAD e CtBP2. formando um complexo de regulação transcricional (Peinado et al., 2007; Wang et al., 2019). Junto à CtBP2, HDAC1 e HDAC2 também são recrutadas para o complexo de repressão de ZEB1, modulando a expressão de genes alvo (Aghdassi et al. 2012). ZEB1 também apresenta sítio de ligação para a proteína remodeladora de cromatina BRG1, que coopera na indução da transição epitélio-mesenquimal via repressão de E-caderina (Sanchez-Tilló et al. 2010).

Por outro lado, ZEB1 induz a expressão de N-caderina, vimentina, metaloproteinase 14 e CD44, contribuindo para fenótipo mesenquimal (Kim et al., 2020; Preca et al., 2015; Sánchez-Tilló et al., 2010; Suh et al., 2013). A ativação da expressão desses genes se dá via interação com SMAD, que recruta P/CAF-p300, formando um complexo de ativação da expressão gênica (Postigo et al., 2003).

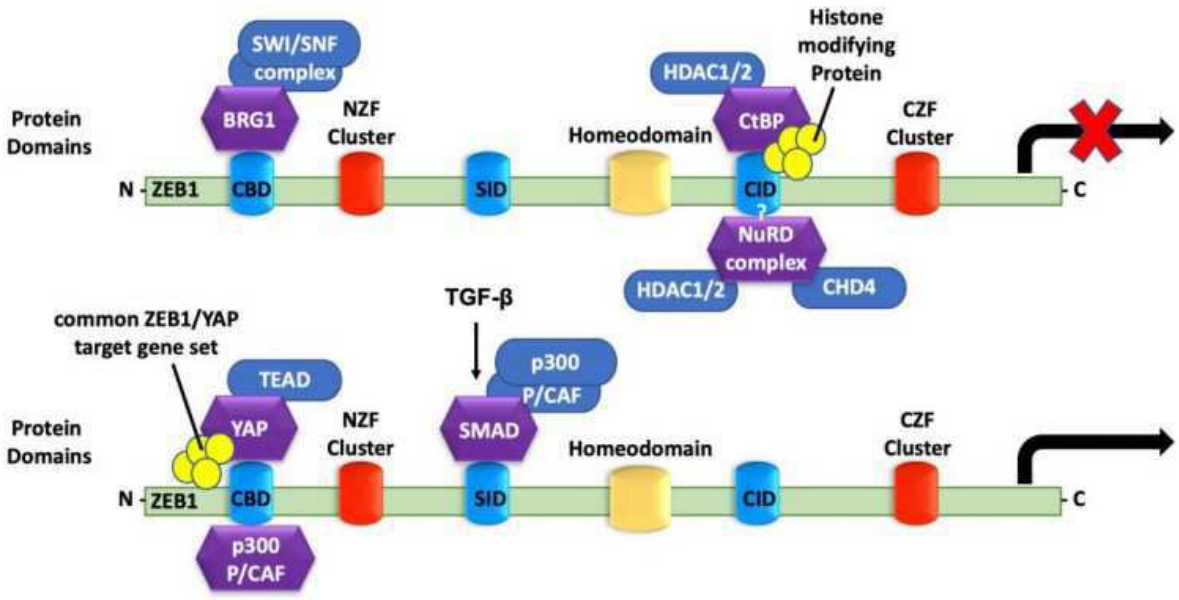

Figura 4. Estrutura da proteína de ZEB1. Sítios de ligação com DNA (NZR e CZF) são indicados em vermelho e sítios de ligação com outras proteínas, como HDACs, SMAD e p300 são ilustrdos. Fonte: Perez-Oquendo & Gibbons, 2022

Além da migração e invasão via transição-epitélio mesenquimal, alguns dos mecanismos regulados por ZEB1 são proliferação e plasticidade celular, resistência à terapias e reparo do DNA (Drápela et al., 2020; Soleymani et al., 2021). Por meio desses processos, ZEB1 atua no desenvolvimento da crista neural, do neocortex, cerebelo, retina e ossos (Vandewalle et al., 2009).

Durante o desenvolvimento do cerebelo, células progenitoras de neurônios granulares passam por uma etapa de proliferação e em seguida migram para as camadas mais internas do cerebelo, com aumento da expressão de genes de polaridade neuronal. Nesse cenário, ZEB1 desempenha papel essencial para a diferenciação de progenitores de neurônios granulares, reprimindo a expressão de genes de polaridade celular e maturidade neuronal, retendo os neurônios nas camadas mais externas do cerebelo, ao passo que a expressão de ZEB1 é extinta. Foi a primeira vez que ZEB1 foi descrito em meduloblastoma, em uma coorte de pacientes em que a maior expressão de ZEB1 foi identificada em tumores do subgrupo molecular SHH (Singh et al., 2016).

1.3 MicroRNAs

MiRNAs fazem parte do mecanismo conhecido como epigenética: alterações herdáveis no DNA que não correspondem a alterações na sequência nucleotídica. Alterações que impactam no remodelamento da cromatina e disposição de histonas como acetilação, metilação e lncRNA são mecanismos epigenéticos (Sharma et al., 2010; Roussel & Stripay, 2018).

MiRNAs são sequências curtas, compostas por cerca de 19-25 nucleotídeos. Essas moléculas atuam na região 3’ não codificante de mRNAs alvos, levando à sua degradação e consequente repressão da tradução (MacFarlane & Murphy, 2010; Roussel & Stripay, 2018). A biogênese de miRNAs inicia pela RNA polimerase II ou III , dando origem a um miRNA primário (pri-miRNA). No citoplasma, o pri-miRNA é clivado pela endonuclease Drosha em miRNA precursor (pre-miRNA) e subsequente uma molécula duplex de miRNA, novamente clivada por um complexo de enzimas envolvendo a endonuclease Dicer e então o miRNA maduro é liberado (Winter et al., 2009; MacFarlane & Murphy, 2010).

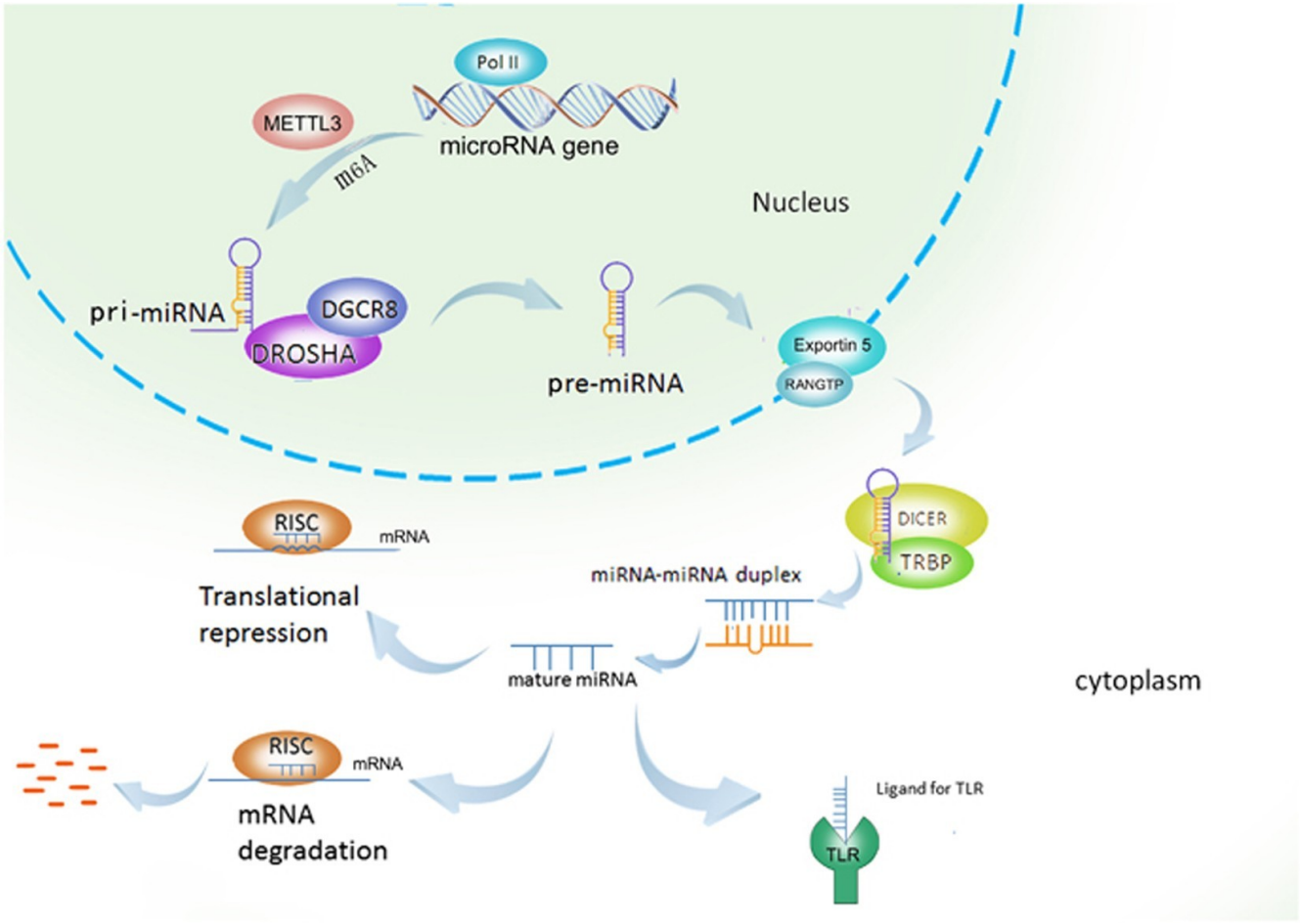

Figura 5. Biogênese de miRNAs. Ilustração do gene à molécula madura de miRNA, passando por suas fases intermediárias de pri-miRNA e pre-miRNA. As funções do miRNA maduro na repressão da tradução, degradação de mRNA e como ligante de receptores *toll like* são demonstradas.

Muitos miRNAs já foram identificados em meduloblastoma, tendo sido relacionados a diagnóstico e estratificação, progressão tumoral, regulação do ciclo celular, células tronco tumorais e metástases (Laneve & Caffarelli, 2020). A baixa expressão do miR-204 nos Grupos 3 e 4 foi relacionado a pior sobrevida em duas coortes analisadas (Bharambe et al., 2019). Com a identificação de mais miRNAs relacionados à estratificação de risco, um painel diagnóstico de miRNAs poderia ser construído como ferramenta auxiliar na decisão do plano de tratamento. Dado o papel de ZEB1 no desenvolvimento do cerebelo e de meduloblastoma, o melhor entendimento de miRNAs regulando ZEB1 configura mecanismo

chave para o desenvolvimento de novas estratégias de diagnóstico, monitoramento e alvo terapêutico em meduloblastoma.

O miR-101 já foi identificado em tumores de nasofaringe, de pulmão e câncer cervical, inibindo proliferação e resistência a quimioterápicos (Li et al., 2023; Han et al., 2022; Wang et al., 2021). Em meduloblastoma, o miR-101 foi encontrado superexpresso no plasma de pacientes, quando comparados com o plasma de indivíduos saudáveis. Experimentos in vitro e in vivo demonstraram que os genes FOXP4 e EZH2 são alvos de repressão de miR-101, ocasionando a inibição de proliferação, formação de colônias e migração de células tumorais (Xue et al., 2022).

Analisando a expressão do miR-148a entre subgrupos moleculares de meduloblastoma, foi observado que tumores do subgrupo WNT apresentavam a maior expressão (Kunder et al, 2013; Yogi et al., 2015). Além disso, a expressão induzida de miR-148a em linhagens celulares de meduloblastoma reduziu a proliferação, invasão e tumorigenicidade nessas células, via redução da expressão do gene NRP1 (Yogi et al., 2015).

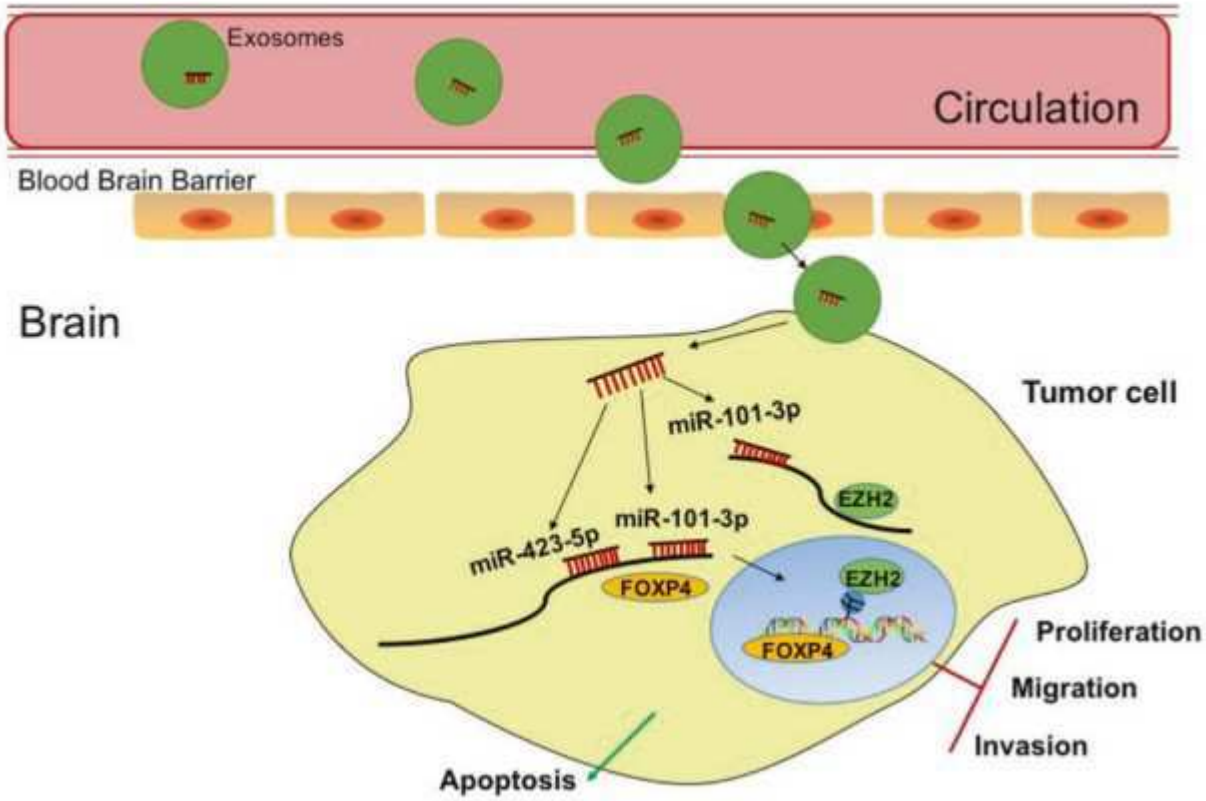

Figura 6. miR-101 inibe mecanismos tumorais em meduloblastoma. O miR-101 atua na inibição do mRNA de EZH2. Dessa forma, os processos de proliferação, migração e invasão mediados por essa proteína são modulados negativamente.

Fonte: Xue et al., 2022

2. HIPÓTESE

Dado o envolvimento de ZEB1 na formação do cerebelo e considerando o seu papel na indiferenciação e malignidade de tumores sólidos, é possível que ZEB1 esteja correlacionado com pior sobrevida em meduloblastoma no contexto in situ e metastático. Também estimamos que ZEB1 interaja com RNAs não codificantes e que essas moléculas também sejam preditores prognósticos em meduloblastoma. Dessa forma, a identificação de miRNAs que interajam com ZEB1 pode resultar em miRNAs alvos para investigações mais profundas de mecanismos celulares e correlações clínicas.

3. OBJETIVOS

3.1 Objetivo geral

Compreender o papel de ZEB1, sua expressão e sua regulação em meduloblastoma. Descrever a relação com RNAs não codificantes nos contextos fisiológico e oncológico.

3.2 Objetivos específicos

- Caracterizar a expressão de ZEB1 nos diferentes subgrupos moleculares de meduloblastoma;

- Avaliar a possível associação entre expressão de ZEB1 e sobrevida de pacientes de meduloblastoma;

- Analisar a relação entre ZEB1 e metástase em meduloblastoma;

- Revisar a relação entre ZEB1 e RNAs não codificantes no contexto oncológico.

4. CAPÍTULO I

Artigo “Oncogenic functions of ZEB1 in pediatric solid cancers: interplays with microRNAs and long noncoding RNAs”

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Oncogenic functions of ZEB1 in pediatric solid cancers: interplays with microRNAs and long noncoding RNAs

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Abstract

The transcription factor Zinc finger E-box binding 1 (ZEB1) displays a range of regulatory activities in cell function and embryonic development, including driving epithelial-mesenchymal transition. Several aspects of ZEB1 function can be regulated by its functional interactions with noncoding RNA types, namely microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). Increasing evidence indicates that ZEB1 importantly influences cancer initiation, tumor progression, metastasis, and resistance to treatment. Cancer is the main disease-related cause of death in children and adolescents. Although the role of ZEB1 in pediatric cancer is still poorly understood, emerging findings have shown that it is expressed and regulates childhood solid tumors including osteosarcoma, retinoblastoma, neuroblastoma, and central nervous system tumors. Here, we review the evidence supporting a role for ZEB1, and its interplays with miRNAs and lncRNAs, in pediatric cancers.

Keywords ZEB1 · MicroRNA · Long noncoding RNA · Noncoding RNA · Pediatric cancer

Introduction

Cancers of the childhood can be seen as developmental disorders possibly arising from defects in embryonic tissue formation. In pediatric solid tumors, these abnormalities often converge to alter epigenetic programs controlling development in neural crest cells and other developing cells [1, 2]. The striking biological parallels between cell plasticity during development and tumorigenesis are illustrated by epithelial-mesenchymal transition (EMT), a crucial process driving embryonic stem cell differentiation, pluripotency, neural crest formation, and progression of some cancers [3]. EMT is a transdifferentiation process that depends on reduced function of the epithelial marker E-cadherin and loss of cell–cell adhesion [4]. The transcription factor zinc finger E-box binding 1 (ZEB1) is importantly involved in EMT by, among other mechanisms, repressing expression of E-cadherin. ZEB1 is part of the complex transcriptional regulation system controlling development, supporting stemness, and repressing cellular differentiation and migration [5]. Accumulating evidence has described that ZEB1 influences cancer initiation, progression, and metastatic spreading [6]. However, its role in childhood tumors remains poorly understood. In this review, we present and discuss the current evidence indicating a role for ZEB1 in pediatric solid tumors.

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ZEB1 as a transcription factor: structure and function

ZEB1 was first described by two studies published in 1991. It was identified by Funahashi et al. [7] during chicken embryo development and named δ-crystallin enhancer factor 1 (δEF-1). A few months later, Williams et al. [8] described it as a zinc finger protein acting as a repressive element on interleukin 2 (IL-2) expression in a human T lymphocyte derived cell line (TCF8). It is currently recognized that ZEB1 (δEF-1, TCF8) and ZEB2 (SIP1) constitute the ZEB homeobox family of transcription factors. ZEB1 is encoded by the *ZEB1* gene located on Chr10p11.22, and its structure bears motifs characteristic of the third family of zinc fingers transcription factors, with two zinc finger clusters (namely N-terminal cluster, NZF, and C-terminal cluster, CZF) with a homeodomain between them, which is POU-like and does not bind DNA, so it might be mainly involved in protein–protein interactions. In contrast, ZEB1 zinc finger domains bind DNA with two hands to different CANNT sites, where N can be any nucleotide, thus regulating a set of genes presenting this nucleotide sequence on the promoters of target genes [9–14] (Fig. 1). Expression of ZEB1 is tightly regulated during development and coordinated with the expression of other transcription factors participating in tissue formation [6, 15]. Mechanisms regulating ZEB1 expression include the long non-coding mRNA LINC00152 acting through Zeste Homologue 2 (EZH2) histone methyltransferase [16].

Even though its main transcriptional function requires nuclear localization, ZEB1 can be translocated to the cytoplasm through phosphorylation by mitogen-activated protein kinase (MAPK)/extracellular-regulated kinase (ERK) downstream to insulin-like growth factor (IGF)-1 activation. Translocation to the cytoplasm might also be explained by the high amounts of ZEB1 in the nucleus upon induced expression [17, 18]. During mitosis, ZEB1 switches from being a chromatin-bound epithelial gene repressor to becoming a microtubule-associated protein, suggesting other roles for ZEB1 besides regulation of the transcription process [19].

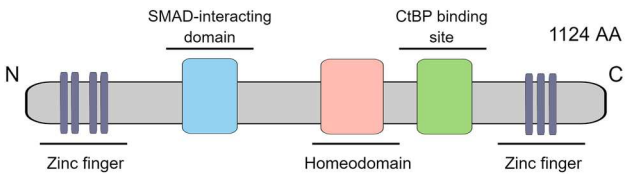

Fig. 1 Schematic ZEB1 protein representation. ZEB1 is composed of 1124 amino acids (AA) and characterized by two DNA-binding zinc fingers domains located near N-terminal and C-terminal regions, in addition to a central homeodomain. Binding sites for proteins SMAD and CtBP are found to the left and right sides of the homeodomain. *CtBP* C-terminal binding protein

ZEB1 regulates embryonic and nervous system development

Transcriptional targets of ZEB1 are typically involved in multiple pathways that regulate formation of tissues and organs including muscle, bone, cartilage, and the nervous system [20]. They include, for example, the vitamin D3 receptor, a steroid-thyroid receptor involved in cellular differentiation during development. Transcription of this receptor is likely directly activated by ZEB1 [21]. ZEB1 also represses type II collagen gene expression during chondrogenesis, influences type I collagen expression during osteoblast development, and regulates cell differentiation by repressing a specific vascular smooth muscle cell element within an enhancer region of the pro-Col1a2 gene [22]. In yet another example, ZEB1 negatively regulates CD4 gene expression, thus regulating T cell development [23].

Zeb1a and Zeb1b, the ZEB1 orthologs in zebrafish, regulate cell–cell adhesion during the gastrulation and segmentation stages of development [24]. ZEB1 modulates the generation of nephron cells in the mouse metanephric mesenchyme, so that knockdown of Zeb1 reduces cell proliferation and migration and promotes cell apoptosis, whereas Zeb1 overexpression results in opposite effects [25]. A functional interaction between Zeb1 and histone deacetylase (HDAC) 2 identifies early cardiovascular precursors and is crucial for mesendodermal differentiation of mouse embryonic stem cells (mESCs) [26]. MicroRNAs (miRNAs)-200C and -150 promote endothelial cell differentiation and vasculogenesis by inhibiting ZEB1 in experimental chick embryonic blood vessel formation [27]. In gonadal development that occurs during embryogenesis in chickens, ZEB1 expression increases between embryonic days 6 and 9 [28]. A role for ZEB1 in differentiation of smooth muscle and bone tissues has also been reported [22, 29].

Regarding nervous system development, ZEB1 exerts transcriptional regulation on a cohort of genes involved in cell differentiation and migration in cortical neural progenitor cells (NPCs), influencing the normal transition from proliferation to differentiation in the developing brain [5]. Human embryonic stem cells (hESCs) show high expression of ZEB1 upon differentiation into neuronal precursors. CRISPR/Cas9-mediated ZEB1 depletion prevents hESC-derived neural precursors from differentiating into neurons, indicating that ZEB1 is required for neuronal differentiation. In fact, ZEB1 overexpression stimulates neural differentiation and neuronal maturation and the generation of glutamatergic excitatory cortical neurons [30]. ZEB1 is found in human fetal periventricular stem and progenitor cells, and

its inhibition impairs migration of human neural stem cells (NSCs). In addition, the developing mouse brain displays high ZEB1 expression around the ventricles, in cerebellar progenitor cells and in the rostral migratory steam. Furthermore, analyses of fetal human brains from late first and early second trimester reveals moderate to strong expression of ZEB1 in the region around the ventricles and moderate expression in NPCs from the germinal matrix, whereas no expression is found in the adult human brain [31]. ZEB1 is directly involved in cerebellar development, repressing transcription of polarity genes and inhibiting polarization of granule neuron progenitors (GNPs) and retaining these cells in their germinal zone [32].

ZEB1 in tumor initiation, tumor progression, metastasis, and resistance to treatment: interactions with miRNAs and lncRNAs

In addition to regulating EMT, ZEB1 affects various other aspects of cellular function that impact on malignant transformation, proliferation, differentiation, stemness, and cell survival [33]. Early studies have focused mainly on adult solid tumors. For example, increased expression of ZEB1 is found in triple-negative and basal-like breast cancers [34]. ZEB1-influenced upregulation and downregulation of different sets of genes, through a direct interaction with the Hippo pathway effector YAP, may help predicting breast cancer patient survival, resistance to treatment, and risk for metastasis [13, 35]. These actions are mediated by multiple mechanisms. For example, ZEB1 inhibits oncosuppression by P53 and RB, unleashing enhanced cell cycle progression and hampering senescence and apoptosis [6, 36]. ZEB1 promotes malignant transformation of human bronchial epithelial cells and is required for tumor initiation in the $KRAS^{LA1}$ mouse model of lung adenocarcinoma [37, 38], as well as of melanocytes, whereas ZEB1 depletion delays tumorigenesis induced by $BRAF^{V600}$ in a mouse model of melanoma [39].

Interactions between ZEB1 and either miRNAs or long noncoding RNAs (lncRNAs) mediate several aspects of ZEB1 activities at the epigenetic level [6, 40, 41]. Both miRNAs and lncRNAs are types of noncoding RNAs that regulate embryogenesis as well as synaptic activity and other aspects of adult cellular function [42]. miRNAs constitute a class of small RNAs that fine-tune mRNA translation (thus protein synthesis) to regulate, for example, the definition of cellular identity and neural plasticity [43–46]. lncRNAs, in turn, influence gene expression through a variety of mechanisms, including serving as epigenetic regulators through lncRNA binding to chromatin-modifying proteins and recruiting them to act on specific sites in the genome, thereby modulating chromatin states [47, 48]. ZEB1 can promote tumorigenesis by repressing miRNAs that inhibit stemness [41], whereas, conversely, various miRNAs target and suppress ZEB1 inhibiting tumorigenesis [33, 49]. For example, ZEB1 is crucial for the tumor-initiating capacity of pancreatic and colorectal cancer cells, a function likely associated with ZEB1-induced repression of the stemness-inhibiting miR-203 [41], whereas tumor growth in cervical cancer is reduced by miRNA-211-induced ZEB1 inhibition [50].

Parallels between cell migration during embryogenesis and tumor biology have been exemplified by EMT during metastasis, and EMT-committed tumor cells more easily dissociate from primary tumors and disseminate to distant sites [4, 51]. EMT regulation by ZEB1 is directly linked to cell motility and invasiveness, which are crucially related to tumor metastatic capacity, and invasiveness in breast cancer cells can be inhibited by either miRNA-1236-3p or miRNA-409-3p targeting of ZEB1 [52, 53]. In addition, overexpression of ZEB1 in experimental breast and pancreatic cancers is associated with increased metastatic potential [13, 54]. ZEB1 may promote metastasis by repressing E-cadherin expression, via binding to its promoter and inhibiting transcription, translocating epithelial markers from the cell surface to the cytoplasm, in addition to increasing expression of mesenchymal markers N-cadherin and vimentin, and these activities can be inhibited by mi-200c [55]. In human colon, lung, and breast cancer cell lines, induced ZEB1 expression promotes invasiveness and migration and increases metastasis in in vivo models, and in a genetic mouse model of pancreatic cancer, ZEB1 was shown to be a key factor for invasion and metastasis [41, 54]. A transforming growth factor β (TGFβ)/ZEB/miR-200 network likely plays a major role in controlling metastasis, through TGFβ activation of ZEB1, ZEB2, and miR-200 [40]. Immunosuppression of tumor-infiltrating lymphocytes (TIL) is a common feature of cancer. miR-200 links CD8(+) TIL immunosuppression with EMT by repressing PD-L1, whereas ZEB1 counteracts this miR-200 action unleashing CD8(+) T-cell immunosuppression and metastasis in cancer cells. Evidence for this interplay is also supported by strong correlations between an EMT score based on a 76-gene EMT signature, miR-200 levels and PD-L1 expression in lung cancer tumors [56].

Expression of ZEB1 is epigenetically regulated by the lncRNA ZEB1 antisense 1 (AS1). The ZEB1 and ZEB1-AS1 genes are located physically next to one another, and ZEB1-AS1 increases ZEB1 expression. ZEB1-AS1 has been described as an oncogene promoting cell proliferation and migration as well as tumor progression. It is also increasingly characterized as a marker of poor prognosis, its expression predicting unfavorable survival outcome, across a variety of adult human solid tumors [57–59].

In addition to promoting tumor initiation, progression, and metastasis, ZEB1 contributes to resistance to anticancer treatment. EMT and stemness are associated with resistance

to radio- and chemotherapy, as well as targeted agents [60]. ZEB1 regulation of the DNA damage response leads to radioresistance in breast cancer cells [61]. miRNAs including miR-203, miR-429, and miR-200c, mediate either resistance or sensitivity to cytotoxic chemotherapy in several adult cancer cell types [60, 62, 63]. Overexpression of miR-574-3p in gastric carcinoma cells reduces resistance to cisplatin by suppressing ZEB1 [64]. Remarkably, chemoresistance associated with ZEB1 can be reversed by inhibition of class I HDACs, raising the proposal that HDAC inhibitors may be used to restore drug sensitivity by reversing the EMT/stemness phenotype [65]. ZEB1 can also promote resistance to targeted anticancer therapies, as illustrated by findings showing that it is associated to resistance to the epidermal growth factor receptor (EGFR) inhibitor erlotinib in both non-small cell lung cancer (NSCLC) [66] and head and neck squamous cell carcinoma [67] cells, as well as to MAPK and BRAF inhibitors in experimental melanoma, whereas ZEB1 depletion sensitizes cells to BRAF inhibitors [68].

ZEB1 and its interplays with miRNAs and lncRNAs in pediatric solid tumors

Osteosarcoma

Among pediatric cancer types, osteosarcoma is by far the one in which the role of ZEB1 has been most investigated. Osteosarcoma, arising from tissues of mesenchymal origin, is characterized by high local aggressiveness and metastasizing potential, resulting in poor survival outcome [69]. It should be noted that osteosarcoma afflicts children and adolescent as well as adult subjects, and several of the studies reviewed below describe results based on tumor samples, primary cells, and cell lines derived from both pediatric and adult patients. An early study showed that treatment with busulfan produces antitumor effects in experimental osteosarcoma, via upregulation of miR-200, which results in downregulation of ZEB1 and ZEB2 [70]. Transfection of ZEB1 siRNA results in inhibition of cell proliferation and invasion accompanied by lower mRNA and protein expression of ZEB1 in MG-63 osteosarcoma cells [71]. A positive feedback involving ZEB1 interplay with sirtuin 1 (SIRT1) enhances EMT and stimulates metastasis in experimental osteosarcoma [72]. The zinc finger transcription factor Ovo-like zinc finger 2 (Ovol2) suppresses ZEB1 expression by binding to the ZEB1 promoter and is associated with reduced expression of ZEB1 in osteosarcoma tumors [73]. Silencing of ZEB1 by siRNA in osteosarcoma cells leads to downregulation of Cyclin D1, matrix metalloproteinase-2 (MMP-2), and bcl-2, whereas ZEB1 overexpression increases proliferation, migration, and levels of Cyclin D1, MMP2, and bcl-2 [74].

ZEB1 expression is enhanced in human osteosarcoma cells showing resistance to doxorubicin. Silencing ZEB1 reduces metastatic potential and restores doxorubicin sensitivity. ZEB1 upregulation increases expression of interlukin-6 (IL-6) and is counteracted by an E3 ubiquitin-protein ligase (SIAH1) [75]. Induction of resistance to cisplatin in osteosarcoma cells is associated with parallel increases in ZEB1, the adipocyte hormone visfatin, and the zinc-finger transcription factor Snail. Knockdown of visfatin restores cisplatin sensitivity. Visfatin increases ZEB1 protein stability and mediates the increased ZEB1 expression by upregulating the serine/threonine kinase ATM, which can in turn phosphorylate and stabilize ZEB1 [76]. In CD166 + osteosarcoma stem cells, EMT induced by TGFβ promotes resistance against the EGFR inhibitor erlotinib, by decreasing miR-499a expression through the direct binding of Snail1/Zeb1 to the miR-499a promoter, and miR-499a overexpression inhibits TGFβ-induced erlotinib-resistance [77].

Several other miRNAs and lncRNAs play a role in osteosarcoma by interacting with ZEB1. Significant downregulation of miR-200b has been observed in osteosarcoma tissues and cell lines, and reduced miR-200b levels are associated with advanced clinical stage and distant metastasis. ZEB1 is a target gene of miR-200b, its expression being negatively regulated by miR-200b in osteosarcoma cells and negatively correlated with miR-200b expression in osteosarcoma tumors. ZEB1 is significantly upregulated in osteosarcoma cells and tumors, and inhibition of ZEB1 expression reduces cell proliferation, migration, and invasion [78]. Osteosarcoma cells show an inverse correlation between levels of miR-141 and miR-146b-5p and those of the mRNA-binding protein AUF1. These miRNAs suppress the promotion of mesenchymal features by AUF1 in osteosarcoma cells, through a mechanism involving repression of ZEB1 and targeting AUF1, which binds the 3′-UTR of ZEB1 mRNA and reduces its turnover [79]. ZEB1 is the main target gene of miR-429, and expression of ZEB1 is negatively related to miR-429 expression in osteosarcoma. Higher levels of miR-429 in osteosarcoma cells significantly suppressed the migration, invasion and proliferation of cells and induced apoptosis; thus, miR-429 may suppress growth and metastasis of osteosarcoma by downregulating ZEB1 [80].

As with miR-429, miRNA 130a also inhibits growth and metastasis of osteosarcoma cells by directly targeting ZEB1. This miRNA is downregulated in osteosarcoma tumors and cell lines compared with normal bone tissue or a normal osteoblast cell line. Expression levels of miR-130a is negatively correlated with clinical stage and metastasis in osteosarcoma patients. Overexpression of miR-130a inhibits osteosarcoma cell proliferation, migration and invasion. Downregulating ZEB1 with a small interfering RNA mimics the effects of transfection with an miR-130a analog [81]. Similar patterns of findings are obtained with miR150,

miR-708, and miR-409-3p, which target and negatively regulate ZEB1 to function as tumor suppressors in osteosarcoma [82–84]. Knockdown of ZEB1 inhibits, whereas ZEB1 overexpression enhances, metastasis in MG63 and SaOS-2 osteosarcoma cells, effects shown to be due to ZEB1 acting as a target of miR-708-5p [85]. Transfection of miR-643 inhibits proliferation and invasion in osteosarcoma cells, and ectopic expression of ZEB1 counteracts the effects of miR-643 transfection. There is a significant inverse correlation between expression of miR-643 and ZEB1, and either low expression of miR-643 or high expression of ZEB1 is associated with poor patient survival outcomes [86]. Finally, miR-340 is downregulated in both osteosarcoma tumors and chemoresistant cells, and a negative correlation is found between miR-340 and ZEB1 expression. Forced expression of miR-340 in resistant osteosarcoma cells enhances sensitivity to cisplatin by reducing viability and promoting apoptosis. ZEB1 was identified as a direct target of miR-340, and miR-340 negatively regulates ZEB1 expression. Ectopic expression of ZEB1 reverses the effects of miR-340 on cell viability and apoptosis, thus miR-340 can restore sensitivity to chemotherapy by targeting ZEB1 [87].

The lncRNA ZEB1-AS1 is upregulated in osteosarcoma tumors and cells. Increased expression of ZEB1-AS1 is correlated with larger tumor size, metastasis, and poorer recurrence-free and overall survival outcomes. In addition, there is a significant correlation between expression of ZEB1-AS1 and ZEB1 in osteosarcoma tumors. Enhanced expression of ZEB1-AS1 promotes, whereas ZEB1-AS1 knockdown inhibits, osteosarcoma cell proliferation and migration. Experimental findings indicate that ZEB1-AS1 directly binds and recruits p300 to the ZEB1 promoter region, induces an open chromatin structure, and activates ZEB1-related transcription in osteosarcoma cells. ZEB1 depletion abrogates the roles of ZEB1-AS1 on cell proliferation and migration. Together, these findings indicate that ZEB1-AS1 acts as an oncogene in osteosarcoma via epigenetic activation of ZEB1 [88]. These actions of ZEB1-AS1 on ZEB1 and osteosarcoma cell function involve an interplay with miR-200s [89].

In addition to ZEB1-AS1, other lncRNAs interact with ZEB1 and miRNAs to influence osteosarcoma (Table 1). For instance, lncRNA activated by transforming growth factor-β (lncRNA-ATB), which is increased in tumors and serum of osteosarcoma patients and is positively associated with recurrence, metastasis and poorer survival outcomes, inhibits miR-200s and upregulates ZEB1 and ZEB2 [90]. High expression of the lncRNA maternally expressed gene 3 (MEG3) is found in osteosarcoma cells, and MEG3 silencing reduces cell viability, migration and invasion, while promoting apoptosis. MEG3 binds to miR-127, which targets ZEB1, and the actions of MEG3 are prevented by miR-127 knockdown. Impairments in cell growth and metastasis induced by miR-127 overexpression are attenuated by ZEB1. Conversely, miR-127 suppression activates the c-Jun N-terminal kinase (JNK) and Wnt pathways, and these activities are prevented by silencing ZEB1 [91].

Table 1 Long non-coding RNAs targeting microRNAs that modulate ZEB1 in solid pediatric cancers

Cancer type	miRNA	lncRNA	References
Osteosarcoma	miR-200s	ZEB1-AS1	[89]
Osteosarcoma	miR-200s	ATB	[90]
Osteosarcoma	miR-205	SNHG16	[92]
Osteosarcoma	miR-217	HOTAIR	[93]
Osteosarcoma	miR-150-5p	MIAT	[94]
Osteosarcoma	miR-101	SPRY4-IT1	[95]
Osteosarcoma	miR-143-3p	PCAT6	[96]
Osteosarcoma	miR-214-5p	LINC00612	[97]
Osteosarcoma	miR-8081	NR_136400	[98]
Retinoblastoma	miR-101	XIST	[103]

Downregulation of the lncRNA small nucleolar RNA host gene 16 (SNHG16) inhibits osteosarcoma cell proliferation and upregulates ZEB1 expression by binding miR-205 [92]. Another lncRNA, HOTAIR, which represses osteosarcoma cell growth, migration and invasion and promotes apoptosis, acts by upregulating ZEB1 expression through acting as a competitive endogenous RNA (ceRNA) targeted by miR-217 [93]. Proliferation and spreading of osteosarcoma cells are hindered by knockdown of the lncRNA myocardial infarction associated transcript (MIAT), which is upregulated in osteosarcoma tumors. MIAT likely functions by competing with critical RNAs to target miR-150-5p and activate ZEB1 [94]. Knockdown of the lncRNA SPRY4-IT1 is accompanied by increased expression of miR-101 and decreased expression of ZEB1 and ZEB2 in osteosarcoma cells and xenografts. SPRY4-IT1 may reduce ZEB1 and ZEB2 expression by binding miR-101 [95]. High levels of the lncRNA prostate cancer associated transcript 6 (PCAT6) potentiate the malignant phenotype of osteosarcoma cells by binding miR-143-3p, resulting in upregulation of ZEB1 [96]. LINC00612 is a lncRNA that acts as a ceRNA to target miR-214-5p in osteosarcoma cells. It is upregulated in osteosarcoma cells and metastatic osteosarcoma, and its overexpression enhances proliferation, invasion, and in vivo growth of osteosarcoma. The mechanisms mediating LINC00612 actions include EMT resulting from increased ZEB1 expression [97]. Downregulation of the lncRNA NR_136400 induces osteosarcoma cell proliferation, apoptosis, and invasion, and stimulates EMT partially by binding miR-8081 and enhancing ZEB1 expression [98].

Retinoblastoma

Retinoblastoma, the most common primary intraocular pediatric cancer, is initiated by biallelic inactivation of the retinoblastoma 1 (*RB1*) gene [99]. In a model of retinoblastoma formation based on inactivation of *RB1* in hESCs using CRISPR/Cas9, analysis of the resulting teratomas revealed ZEB1 as a transcription factor involved in RB1-mediated ectoderm differentiation [100]. ZEB1 constitutes a bidirectional inhibitory negative feedback loop with miR-23a to regulate EMT in retinoblastoma cells [101]. Transcripts of the type I receptor of the TGF-β family, activin A receptor (ACVR1C or ALK7) and its ligands Nodal, activin A and B, and growth differentiation factor-3 (GDF3) are expressed in retinoblastoma cells. Pharmacological inhibition, or genetic downregulation using shRNA, of ACVR1C hinders cell invasion, growth, survival, and these effects are accompanied by decreased protein levels of ZEB1 and Snail [102]. Expression of the lncRNA X inactive specific transcript (XIST), ZEB1 and ZEB2 is increased, whereas miR-101 expression is reduced, in retinoblastoma tumors and cells. Knockdown of XIST inhibits proliferation, migration, invasion and EMT while promoting apoptosis and caspase-3 activity. XIST acts as a ceRNA for miR-101 to derepress ZEB1 and ZEB2 in retinoblastoma [103] (Table 1).

Neuroblastoma

Neuroblastoma is the most common extracranial solid childhood tumor, likely deriving from the abnormal development of embryonal neural crest cells that later give rise to the sympathetic nervous system. Biological modifications leading to neuroblastoma tumorigenesis may involve epigenetic alterations [104, 105]. At least three miRNAs, namely miR152, miR200B, and miR338, are dysregulated in neuroblastoma and influence differentiation and apoptosis. ZEB1 is among the targets repressed by these miRNAs, and ZEB1 expression is reduced upon treatment with pre-miR-200b [106]. Nodal can increase the malignancy of neuroblastoma cells by enhancing ZEB1 activity. Knockdown of ZEB1 attenuates Nodal-induced malignancy, and Nodal increases the protein stability of ZEB1 via phosphorylation mediated by Ataxia telangiectasia mutated kinase (ATM), rather than affecting its mRNA expression [107]. In human SH-SY5Y cells, miR-205 is positively regulated by Angelica polysaccharide and targets ZEB1 [108].

Central nervous system tumors

In children, brain cancers as a group of diagnoses are the most common solid tumors and the leading cause of cancer-related death. Medulloblastoma is the most common type of malignant pediatric brain tumor. This cancer type is currently classified into molecular subgroups with distinct genomic, epigenetic, and clinical features: WNT, Sonic Hedgehog (SHH, Tp53 wild-type or Tp53 mutant), Group 3, and Group 4. Medulloblastoma occurs in the cerebellum and likely originates from abnormal changes in epigenetic and signaling mechanisms involved in embryonic development [109, 110]. ZEB1 controls neuronal differentiation by transcriptionally repressing polarity genes in neuronal progenitors, retaining polarization in GNPs and maintaining them in their germinal zone. Expression of ZEB1 is enhanced in the SHH medulloblastoma subgroup, which originates from GNPs and displays persistent SHH activation [32].

In ependymoma, evaluation of tumor samples shows increases in ZEB1 mRNA and protein content. Enhanced ZEB1 expression is significantly associated with shorter progression-free survival [111]. Immunohistochemical assessment of 295 surgical samples of various types of pediatric and adult brain cancers reveals higher ZEB1 expression in infiltrative lesions compared to less invasive tumor. ZEB1 is also present in human fetal periventricular stem and progenitor cells and ZEB1 inhibition impairs migration of human NSCs [31]. Figure 2 illustrates some of the interactions of ZEB1 with miRNAs, lncRNAs, and receptor signaling.

Conclusion

It is becoming increasingly clear that ZEB1 plays multiple roles in cancer beyond regulating EMT. Cellular functions crucially influenced by ZEB1 include the maintenance of a stem-cell phenotype, senescence, and apoptosis. As

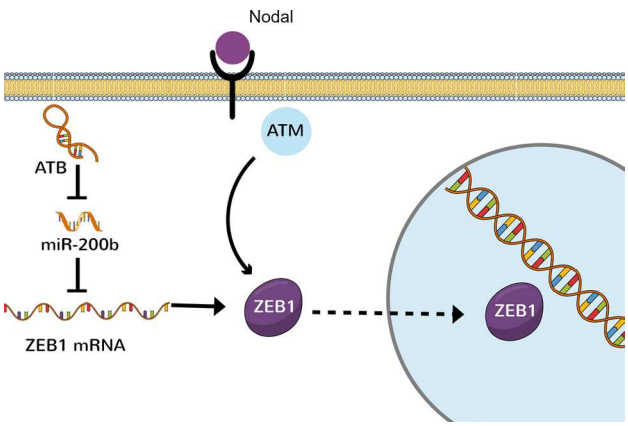

Fig. 2 ATM can be stimulated by receptors activated by ligands including Nodal, resulting in phosphorylation and stabilization of ZEB1. lncRNAs such as ATB inhibit miR-200b, leading to upregulation of ZEB1. Although the function of ZEB1 as a transcription factor requires nuclear localization, ZEB1 can be translocated to cytoplasm through phosphorylation to regulate mitosis and migration. *ATM* Ataxia Telangiectasia Mutated, *lncRNA-ATB* ATB: long noncoding RNA Activated by TGF-Beta, *miR-200b* microRNA-200b

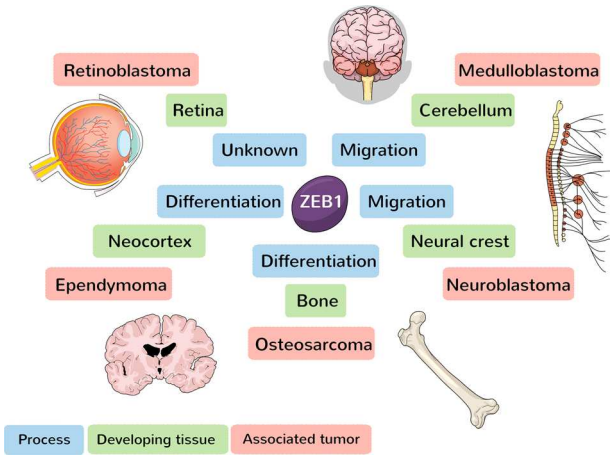

Fig. 3 ZEB1 integrates cellular signals and epigenetic effects regulating various aspects of cellular function in developing tissues, enabling it to influence pediatric cancer progression and metastasis. Blue boxes illustrate cellular processes involving ZEB1 during normal development of tissues (shown in green boxes). Pink boxes represent solid cancers associated with each developing tissue. (Color figure online)

discussed above, functional interplays with epigenetic processes, including those involving miRNAs and lncRNAs, are key in mediating ZEB1 activities. As a focus of integration of dynamic intracellular signals coordinating gene expression patterns, ZEB1 can be seen as a key regulator of cellular plasticity, resulting in enabling tumor adaptability, resistance, and spreading through metastasis (Fig. 3). A newer development of ZEB1 research is the description of its involvement in different solid cancers of childhood. As with adult tumors, the early evidence available so far indicates the possibility of ZEB1 expression being investigated and characterized as a biomarker of clinical outcomes in pediatric patients. Also, targeting ZEB1 function, for example by inhibiting associated miRNAs with antisense oligonucleotides [112], may lead to novel avenues for the development of therapeutic strategies.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lawlor ER, Thiele CJ (2012) Epigenetic changes in pediatric solid tumors: promising new targets. Clin Cancer Res 18:2768–2779. https://doi.org/10.1158/1078-0432.CCR-11-1921
2. Marshall GM, Carter DR, Cheung BB et al (2014) The prenatal origins of cancer. Nat Rev Cancer 14(4):277–289. https://doi.org/10.1038/nrc3679
3. Nieto MA, Huang RY, Jackson RA, Thiery JP (2016) EMT: 2016. Cell 166:21–45. https://doi.org/10.1016/j.cell.2016.06.028
4. Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139:871–890. https://doi.org/10.1016/j.cell.2009.11.007
5. Wang H, Xiao Z, Zheng J et al (2019) ZEB1 represses neural differentiation and cooperates with CTBP2 to dynamically regulate cell migration during neocortex development. Cell Rep 27:2335-2353.e6. https://doi.org/10.1016/j.celrep.2019.04.081
6. Caramel J, Ligier M, Puisieux A (2018) Pleiotropic roles for ZEB1 in cancer. Cancer Res 78:30–35. https://doi.org/10.1158/0008-5472.CAN-17-2476
7. Funahashi J, Kamachi Y, Goto K, Kondoh H (1991) Identification of nuclear factor δEF1 and its binding site essential for lens-specific activity of the δ1-crystallin enhancer. Nucleic Acids Res 19:3543–3547. https://doi.org/10.1093/nar/19.13.3543
8. Williams TM, Moolten D, Burlein J et al (1991) Identification of a zinc finger protein that inhibits IL-2 gene expression. Science 254:1791–1794. https://doi.org/10.1126/science.1840704
9. Lai Z, Fortini ME, Rubin GM (1991) The embryonic expression patterns of zfh-1 and zfh-2, two *Drosophila* genes encoding novel zinc-finger homeodomain proteins. Mech Dev 34:2–3. https://doi.org/10.1016/0925-4773(91)90049-C
10. Funahashi J, Sekido R, Murai K, Kamachi Y, Kondoh H (1993) Delta-crystallin enhancer binding protein delta EF1 is a zinc finger-homeodomain protein implicated in postgastrulation embryogenesis. Development 119:433–446
11. Takagi T, Moribe H, Kondoh H, Higashi Y (1998) DeltaEF1, a zinc finger and homeodomain transcription factor, is required for skeleton patterning in multiple lineages. Development 125:21–31
12. Remacle JE, Kraft H, Lerchner W et al (1999) New mode of DNA binding of multi-zinc finger transcription factors: δEF1 family members bind with two hands to two target sites. EMBO J 18:5073–5084. https://doi.org/10.1093/emboj/18.18.5073
13. Wu HT, Zhong HT, Li GW et al (2020) Oncogenic functions of the EMT-related transcription factor ZEB1 in breast cancer. J Transl Med 18:51. https://doi.org/10.1186/s12967-020-02240-z
14. Fortini ME, Lai ZC, Rubin GM (1991) The *Drosophila* zfh-1 and zfh-2 genes encode novel proteins containing both zinc-finger and homeodomain motifs. Mech Dev 34(2–3):113–122. https://doi.org/10.1016/0925-4773(91)90048-b
15. Gheldof A, Hulpiau P, van Roy F, De Craene B, Berx G (2012) Evolutionary functional analysis and molecular regulation of the ZEB transcription factors. Cell Mol Life Sci 69(15):2527–2541. https://doi.org/10.1007/s00018-012-0935-3
16. Zhang S, Liao W, Wu Q, Huang X, Pan Z, Chen W, Gu S, Huang Z, Wang Y, Tang X, Liang S, Zhang X, Chen Y, Chen S, Chen W, Jiang Y, Chen C, Qiu G (2020) LINC00152 upregulates ZEB1 expression and enhances epithelial-mesenchymal transition and oxaliplatin resistance in esophageal cancer by interacting

with EZH2. Cancer Cell Int 20(1):569. https://doi.org/10.1186/s12935-020-01620-1
17. Llorens MC, Lorenzatti G, Cavallo NL et al (2016) Phosphorylation regulates functions of ZEB1 transcription factor. J Cell Physiol 231:2205–2217. https://doi.org/10.1002/jcp.25338
18. Siles L, Ninfali C, Cortés M, Darling DS, Postigo A (2019) ZEB1 protects skeletal muscle from damage and is required for its regeneration. Nat Commun 10:1364. https://doi.org/10.1038/s41467-019-08983-8
19. Fouani L, Huang MLH, Cole L, Jansson PJ, Kovacevic Z, Richardson DR (2020) During mitosis ZEB1 "switches" from being a chromatin-bound epithelial gene repressor, to become a microtubule-associated protein. Biochim Biophys Acta Mol Cell Res 1867:118673. https://doi.org/10.1016/j.bbamcr.2020.118673
20. Vandewalle C, Van Roy F, Berx G (2009) The role of the ZEB family of transcription factors in development and disease. Cell Mol Life Sci 66(5):773–787. https://doi.org/10.1007/s00018-008-8465-8
21. Lazarova DL, Bordonaro M, Sartorelli AC (2001) Transcriptional regulation of the vitamin D(3) receptor gene by ZEB. Cell Growth Differ 12:319–326
22. Ponticos M, Partridge T, Black CM, Abraham DJ, Bou-Gharios G (2004) Regulation of collagen type I in vascular smooth muscle cells by competition between Nkx2.5 and deltaEF1/ZEB1. Mol Cell Biol 24:6151–6161. https://doi.org/10.1128/MCB.24.14.6151-6161.2004
23. Yasui DH, Genetta T, Kadesch T, Williams TM, Swain SL, Tsui LV, Huber BT (1998) Transcriptional repression of the IL-2 gene in Th cells by ZEB. J Immunol 160:4433–4440
24. Vannier C, Mock K, Brabletz T, Driever W (2013) Zeb1 regulates E-cadherin and Epcam (epithelial cell adhesion molecule) expression to control cell behavior in early zebrafish development. J Biol Chem 288:18643–18659. https://doi.org/10.1074/jbc.M113.467787
25. Gu Y, Zhao Y, Zhou Y, Xie Y, Ju P, Long Y et al (2016) Zeb1 is a potential regulator of Six2 in the proliferation, apoptosis and migration of metanephric mesenchyme cells. Int J Mol Sci 17:1283. https://doi.org/10.3390/ijms17081283
26. Cencioni C, Spallotta F, Savoia M et al (2018) Zeb1-Hdac2-eNOS circuitry identifies early cardiovascular precursors in naive mouse embryonic stem cells. Nat Commun 9:1281. https://doi.org/10.1038/s41467-018-03668-0
27. Luo Z, Wen G, Wang G et al (2013) MicroRNA-200C and -150 play an important role in endothelial cell differentiation and vasculogenesis by targeting transcription repressor ZEB1. Stem Cells 31:1749–1762. https://doi.org/10.1002/stem.1448
28. Lim W, Song G (2015) Novel genes and hormonal regulation for gonadal development during embryogenesis in chickens. Gen Comp Endocrinol 211:20–27. https://doi.org/10.1016/j.ygcen.2014.11.009
29. Yang S, Zhao L, Yang J (2007) deltaEF1 represses BMP-2-induced differentiation of C2C12 myoblasts into the osteoblast lineage. J Biomed Sci 14:663–679. https://doi.org/10.1007/s11373-007-9155-5
30. Jiang Y, Yan L, Xia L (2018) Zinc finger E-box-binding homeobox 1 (ZEB1) is required for neural differentiation of human embryonic stem cells. J Biol Chem 293:19317–19329. https://doi.org/10.1074/jbc.RA118.005498
31. Kahlert UD, Suwala AK, Raabe EH et al (2015) ZEB1 promotes invasion in human fetal neural stem cells and hypoxic glioma neurospheres. Brain Pathol 25:724–732. https://doi.org/10.1111/bpa.12240
32. Singh S, Howell D, Trivedi N et al (2016) Zeb1 controls neuron differentiation and germinal zone exit by a mesenchymal-epithelial-like transition. Elife 5:e12717. https://doi.org/10.7554/eLife.12717
33. Cheng L, Zhou MY, Gu YJ, Chen L, Wang Y (2021) ZEB1: new advances in fibrosis and cancer. Mol Cell Biochem 476(4):1643–1650. https://doi.org/10.1007/s11010-020-04036-7
34. Karihtala P, Auvinen P, Kauppila S, Haapasaari KM, Jukkola-Vuorinen A, Soini Y (2013) Vimentin, zeb1 and Sip1 are up-regulated in triple-negative and basal-like breast cancers: association with an aggressive tumour phenotype. Breast Cancer Res Treat 138:81–90. https://doi.org/10.1007/s10549-013-2442-0
35. Lehmann W, Mossmann D, Kleemann J (2016) ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types. Nat Commun 7:10498. https://doi.org/10.1038/ncomms10498
36. Liu Y, El-Naggar S, Darling DS, Higashi Y, Dean DC (2008) Zeb1 links epithelial-mesenchymal transition and cellular senescence. Development 135:579–588. https://doi.org/10.1242/dev.007047
37. Liu Y, Lu X, Huang L et al (2014) Different thresholds of ZEB1 are required for Ras-mediated tumour initiation and metastasis. Nat Commun 5:5660. https://doi.org/10.1038/ncomms6660
38. Larsen JE, Nathan V, Osborne JK et al (2016) ZEB1 drives epithelial-to-mesenchymal transition in lung cancer. J Clin Invest 126:3219–3235. https://doi.org/10.1172/JCI76725
39. Caramel J, Papadogeorgakis E, Hill L (2013) A switch in the expression of embryonic EMT-inducers drives the development of malignant melanoma. Cancer Cell 24:466–480. https://doi.org/10.1016/j.ccr.2013.08.018
40. Gregory PA, Bert AG, Paterson EL et al (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 10:593–601. https://doi.org/10.1038/ncb1722
41. Wellner U, Schubert J, Burk UC et al (2009) The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 11:1487–1495. https://doi.org/10.1038/ncb1998
42. Pauli A, Rinn JL, Schier AF (2011) Non-coding RNAs as regulators of embryogenesis. Nat Rev Genet 12:136–149. https://doi.org/10.1038/nrg2904
43. Bicker S, Schratt G (2008) MicroRNAs: tiny regulators of synapse function in development and disease. J Cell Mol Med 12(5A):1466–1476. https://doi.org/10.1111/j.1582-4934.2008.00400.x
44. Fiore R, Siegel G, Schratt G (2008) MicroRNA function in neuronal development, plasticity and disease. Biochim Biophys Acta 1779:471–478. https://doi.org/10.1016/j.bbagrm.2007.12.006
45. Schratt G (2009) Fine-tuning neural gene expression with microRNAs. Curr Opin Neurobiol 19:213–219. https://doi.org/10.1016/j.conb.2009.05.015
46. Bredy TW, Lin Q, Wei W, Baker-Andresen D, Mattick JS (2011) MicroRNA regulation of neural plasticity and memory. Neurobiol Learn Mem 96:89–94. https://doi.org/10.1016/j.nlm.2011.04.004
47. Mercer TR, Mattick JS (2013) Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 20:300–307. https://doi.org/10.1038/nsmb.2480
48. Briggs JA, Wolvetang EJ, Mattick JS, Rinn JL, Barry G (2015) Mechanisms of long non-coding RNAs in mammalian nervous system development, plasticity, disease, and evolution. Neuron 88:861–877. https://doi.org/10.1016/j.neuron.2015.09.045
49. Ashrafizadeh M, Ang HL, Moghadam ER (2020) MicroRNAs and their influence on the ZEB family: mechanistic aspects and therapeutic applications in cancer therapy. Biomolecules 10:1040. https://doi.org/10.3390/biom10071040
50. Chen G, Huang P, Xie J, Li R (2018) MicroRNA-211 suppresses the growth and metastasis of cervical cancer by directly targeting ZEB1. Mol Med Rep 17:1275–1282. https://doi.org/10.3892/mmr.2017.8006

51. Lamouille S, Xu J, Derynck R (2014) Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 15:178–196. https://doi.org/10.1038/nrm3758
52. Ma Z, Li Y, Xu J, Ren Q, Yao J, Tian X (2016) MicroRNA-409-3p regulates cell invasion and metastasis by targeting ZEB1 in breast cancer. IUBMB Life 68:394–402. https://doi.org/10.1002/iub.1494
53. Liang TC, Fu WG, Zhong YS (2019) MicroRNA-1236-3p inhibits proliferation and invasion of breast cancer cells by targeting ZEB1. Eur Rev Med Pharmacol Sci 23:9988–9995. https://doi.org/10.26355/eurrev_201911_19565
54. Krebs AM, Mitschke J, Lasierra Losada M et al (2017) The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nat Cell Biol 19:518–529. https://doi.org/10.1038/ncb3513
55. Hur K, Toiyama Y, Takahashi M et al (2013) MicroRNA-200c modulates epithelial-to-mesenchymal transition (EMT) in human colorectal cancer metastasis. Gut 62:1315–1326. https://doi.org/10.1136/gutjnl-2011-301846
56. Chen L, Gibbons DL, Goswami S (2014) Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun 5:5241. https://doi.org/10.1038/ncomms6241
57. Li T, Xie J, Shen C et al (2016) Upregulation of long noncoding RNA ZEB1-AS1 promotes tumor metastasis and predicts poor prognosis in hepatocellular carcinoma. Oncogene 35:1575–1584. https://doi.org/10.1038/onc.2015.223
58. Su W, Xu M, Chen X et al (2017) Long noncoding RNA ZEB1-AS1 epigenetically regulates the expressions of ZEB1 and downstream molecules in prostate cancer. Mol Cancer 16:142. https://doi.org/10.1186/s12943-017-0711-y
59. Zuo XL, Cai J, Chen ZQ et al (2018) The utility of long non-coding RNA ZEB1-AS1 as a prognostic biomarker in human solid tumors: a meta-analysis. Clin Chim Acta 485:14–20. https://doi.org/10.1016/j.cca.2018.06.018
60. Shibue T, Weinberg RA (2017) EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol 14:611–629. https://doi.org/10.1038/nrclinonc.2017.44
61. Zhang P, Wei Y, Wang L (2014) ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. Nat Cell Biol 16:864–875. https://doi.org/10.1038/ncb3013
62. Siebzehnrubl FA, Silver DJ, Tugertimur B (2013) The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance. EMBO Mol Med 5:1196–1212. https://doi.org/10.1002/emmm.201302827
63. Zou J, Liu L, Wang Q (2017) Downregulation of miR-429 contributes to the development of drug resistance in epithelial ovarian cancer by targeting ZEB1. Am J Transl Res 9:1357–1368
64. Wang M, Zhang R, Zhang S, Xu R, Yang Q (2019) MicroRNA-574-3p regulates epithelial mesenchymal transition and cisplatin resistance via targeting ZEB1 in human gastric carcinoma cells. Gene 700:110–119. https://doi.org/10.1016/j.gene.2019.03.043
65. Meidhof S, Brabletz S, Lehmann W et al (2015) ZEB1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. EMBO Mol Med 7:831–847. https://doi.org/10.15252/emmm.201404396
66. Zhang Z, Lee JC, Lin L et al (2012) Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet 44:852–860. https://doi.org/10.1038/ng.2330
67. Haddad Y, Choi W, McConkey DJ (2009) Delta-crystallin enhancer binding factor 1 controls the epithelial to mesenchymal transition phenotype and resistance to the epidermal growth factor receptor inhibitor erlotinib in human head and neck squamous cell carcinoma lines. Clin Cancer Res 15:532–542. https://doi.org/10.1158/1078-0432.CCR-08-1733
68. Richard G, Dalle S, Monet MA et al (2016) ZEB1-mediated melanoma cell plasticity enhances resistance to MAPK inhibitors. EMBO Mol Med 8:1143–1161. https://doi.org/10.15252/emmm.201505971
69. Yang J, Zhang W (2013) New molecular insights into osteosarcoma targeted therapy. Curr Opin Oncol 25(2013):398–406. https://doi.org/10.1097/CCO.0b013e3283622c1b
70. Mei Q, Li F, Quan H, Liu Y, Xu H (2014) Busulfan inhibits growth of human osteosarcoma through miR-200 family microRNAs in vitro and in vivo. Cancer Sci 105:755–762. https://doi.org/10.1111/cas.12436
71. Xu XM, Liu W, Cao ZH, Liu MX (2017) Effects of ZEB1 on regulating osteosarcoma cells via NF-κB/iNOS. Eur Rev Med Pharmacol Sci 21:1184–1190
72. Yu XJ, Guo XZ, Li C et al (2019) SIRT1-ZEB1-positive feedback promotes epithelial-mesenchymal transition process and metastasis of osteosarcoma. J Cell Biochem 120:3727–3735. https://doi.org/10.1002/jcb.27653
73. Liu J, Wu Q, Wang Y et al (2018) Ovol2 induces mesenchymal-epithelial transition via targeting ZEB1 in osteosarcoma. Onco Targets Ther 11:2963–2973. https://doi.org/10.2147/OTT.S157119
74. Ming H, Chuang Q, Jiashi W et al (2018) Naringin targets Zeb1 to suppress osteosarcoma cell proliferation and metastasis. Aging (Albany NY) 10:4141–4151. https://doi.org/10.18632/aging.101710
75. Han X, Liu F, Zhang C, Ren Z, Li L, Wang G (2019) SIAH1/ZEB1/IL-6 axis is involved in doxorubicin (Dox) resistance of osteosarcoma cells. Biol Chem 400:545–553. https://doi.org/10.1515/hsz-2018-0292
76. Wang D, Qian G, Wang J (2019) Visfatin is involved in the cisplatin resistance of osteosarcoma cells via upregulation of Snail and Zeb1. Cancer Biol Ther 20:999–1006. https://doi.org/10.1080/15384047.2019.1591675
77. Wang T, Wang D, Zhang L et al (2019) The TGFβ-miR-499a-SHKBP1 pathway induces resistance to EGFR inhibitors in osteosarcoma cancer stem cell-like cells. J Exp Clin Cancer Res 38:226. https://doi.org/10.1186/s13046-019-1195-y
78. Li Y, Zeng C, Tu M et al (2016) MicroRNA-200b acts as a tumor suppressor in osteosarcoma via targeting ZEB1. Onco Targets Ther 9:3101–3111. https://doi.org/10.2147/OTT.S96561
79. Al-Khalaf HH, Aboussekhra A (2014) MicroRNA-141 and microRNA-146b-5p inhibit the prometastatic mesenchymal characteristics through the RNA-binding protein AUF1 targeting the transcription factor ZEB1 and the protein kinase AKT. J Biol Chem 289:31433–31447. https://doi.org/10.1074/jbc.M114.593004
80. Deng Y, Luan F, Zeng L, Zhang Y, Ma K (2017) MiR-429 suppresses the progression and metastasis of osteosarcoma by targeting ZEB1. EXCLI J 16:618–627. https://doi.org/10.17179/excli2017-258
81. Yi L, Liu M, Tang Z (2017) MicroRNA-130a inhibits growth and metastasis of osteosarcoma cells by directly targeting ZEB1. Mol Med Rep 16:3606–3612. https://doi.org/10.3892/mmr.2017.6968
82. Xu J, Wang Z, Liao Z, Dai D, Ma X (2017) MicroRNA-150 functions as an antioncogenic regulator in osteosarcoma. Oncol Lett 14:2483–2490. https://doi.org/10.3892/ol.2017.6393
83. He J, Xiang D, Lin Y (2019) MicroRNA-708 inhibits the proliferation and invasion of osteosarcoma cells by directly targeting ZEB1. Mol Med Rep 19:3948–3954. https://doi.org/10.3892/mmr.2019.10013
84. Wu L, Zhang Y, Huang Z et al (2019) MiR-409-3p inhibits cell proliferation and invasion of osteosarcoma by targeting zinc-finger e-box-binding homeobox-1. Front Pharmacol 10:137. https://doi.org/10.3389/fphar.2019.00137

85. Feng T, Zhu Z, Jin Y et al (2020) The microRNA-708-5p/ZEB1/EMT axis mediates the metastatic potential of osteosarcoma. Oncol Rep 43:491–502. https://doi.org/10.3892/or.2019.7452
86. Wang H, Xing D, Ren D et al (2017) MicroRNA-643 regulates the expression of ZEB1 and inhibits tumorigenesis in osteosarcoma. Mol Med Rep 16:5157–5164. https://doi.org/10.3892/mmr.2017.7273
87. Yan H, Zhang B, Fang C, Chen L (2018) miR-340 alleviates chemoresistance of osteosarcoma cells by targeting ZEB1. Anticancer Drugs 29:440–448. https://doi.org/10.1097/CAD.0000000000000614
88. Liu C, Lin J (2016) Long noncoding RNA ZEB1-AS1 acts as an oncogene in osteosarcoma by epigenetically activating ZEB1. Am J Transl Res 8:4095–4105
89. Liu C, Pan C, Cai Y, Wang H (2017) Interplay between long noncoding RNA ZEB1-AS1 and miR-200s regulates osteosarcoma cell proliferation and migration. J Cell Biochem 118:2250–2260. https://doi.org/10.1002/jcb.25879
90. Han F, Wang C, Wang Y, Zhang L (2017) Long noncoding RNA ATB promotes osteosarcoma cell proliferation, migration and invasion by suppressing miR-200s. Am J Cancer Res 7:770–783
91. Wang Y, Kong D (2018) Knockdown of lncRNA MEG3 inhibits viability, migration, and invasion and promotes apoptosis by sponging miR-127 in osteosarcoma cell. J Cell Biochem 119:669–679. https://doi.org/10.1002/jcb.26230
92. Zhu C, Cheng D, Qiu X, Zhuang M, Liu Z (2018) Long noncoding RNA SNHG16 promotes cell proliferation by sponging microRNA-205 and upregulating ZEB1 expression in osteosarcoma. Cell Physiol Biochem 51:429–440. https://doi.org/10.1159/000495239
93. Wang B, Qu XL, Liu J, Lu J, Zhou ZY (2019) HOTAIR promotes osteosarcoma development by sponging miR-217 and targeting ZEB1. J Cell Physiol 234:6173–6181. https://doi.org/10.1002/jcp.27394
94. Jin H, Jin X, Chai W et al (2019) Long non-coding RNA MIAT competitively binds miR-150-5p to regulate ZEB1 expression in osteosarcoma. Oncol Lett 17:1229–1236. https://doi.org/10.3892/ol.2018.9671
95. Yao H, Hou G, Wang QY et al (2020) LncRNA SPRY4-IT1 promotes progression of osteosarcoma by regulating ZEB1 and ZEB2 expression through sponging of miR-101 activity. Int J Oncol 56:85–100. https://doi.org/10.3892/ijo.2019.4910
96. Wu K, Feng Q, Li L et al (2020) Long-noncoding RNA PCAT6 aggravates osteosarcoma tumourigenesis via the MiR-143-3p/ZEB1 axis. Onco Targets Ther 13:8705–8714. https://doi.org/10.2147/OTT.S258415
97. Zhou Y, Li X, Yang H (2020) LINC00612 functions as a ceRNA for miR-214-5p to promote the proliferation and invasion of osteosarcoma in vitro and in vivo. Exp Cell Res 392:112012. https://doi.org/10.1016/j.yexcr.2020.112012
98. Liu L, Zheng M, Wang X, Gao Y, Gu Q (2020) LncRNA NR_136400 suppresses cell proliferation and invasion by acting as a ceRNA of TUSC5 that is modulated by miR-8081 in osteosarcoma. Front Pharmacol 11:641. https://doi.org/10.3389/fphar.2020.00641
99. Kivelä T (2009) The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. Br J Ophthalmol 93:1129–1131. https://doi.org/10.1136/bjo.2008.150292
100. Avior Y, Lezmi E, Yanuka D, Benvenisty N (2017) Modeling developmental and tumorigenic aspects of trilateral retinoblastoma via human embryonic stem cells. Stem Cell Reports 8:1354–1365. https://doi.org/10.1016/j.stemcr.2017.03.005
101. Wang Y, Luo Y, Guan W, Zhao H (2018) Role of miR-23a/Zeb1 negative feedback loop in regulating epithelial-mesenchymal transition and tumorigenicity of intraocular tumors. Oncol Lett 16:2462–2470. https://doi.org/10.3892/ol.2018.8940
102. Asnaghi L, White DT, Key N (2019) ACVR1C/SMAD2 signaling promotes invasion and growth in retinoblastoma. Oncogene 38:2056–2075. https://doi.org/10.1038/s41388-018-0543-2
103. Cheng Y, Chang Q, Zheng B et al (2019) LncRNA XIST promotes the epithelial to mesenchymal transition of retinoblastoma via sponging miR-101. Eur J Pharmacol 843:210–216. https://doi.org/10.1016/j.ejphar.2018.11.028
104. Fetahu IS, Taschner-Mandl S (2021) Neuroblastoma and the epigenome. Cancer Metastasis Rev 40:173–189. https://doi.org/10.1007/s10555-020-09946-y
105. Almeida VR, Vieira IA, Buendia M et al (2017) Combined treatments with a retinoid receptor agonist and epigenetic modulators in human neuroblastoma cells. Mol Neurobiol 54:7610–7619. https://doi.org/10.1007/s12035-016-0250-3
106. Ragusa M, Majorana A, Banelli B et al (2010) MIR152, MIR200B, and MIR338, human positional and functional neuroblastoma candidates, are involved in neuroblast differentiation and apoptosis. J Mol Med (Berl) 88:1041–1053. https://doi.org/10.1007/s00109-010-0643-0
107. Wu J, Cheng P, Huang Z, Tan Q, Qu Y (2019) Nodal increases the malignancy of childhood neuroblastoma cells via regulation of Zeb1. BioFactors 45:355–363. https://doi.org/10.1002/biof.1505
108. Yang J, Shao X, Wang L et al (2019) Angelica polysaccharide exhibits antitumor effect in neuroblastoma cell line SH-SY5Y by up-regulation of miR-205. BioFactors. https://doi.org/10.1002/biof.1586
109. Northcott PA, Robinson GW, Kratz CP et al (2019) Medulloblastoma. Nat Rev Dis Primers 5:11. https://doi.org/10.1038/s41572-019-0063-6
110. Thomaz A, Jaeger M, Brunetto AL et al (2020) Neurotrophin signaling in medulloblastoma. Cancers (Basel) 12:2542. https://doi.org/10.3390/cancers12092542
111. Malgulwar PB, Nambirajan A, Pathak P et al (2018) Epithelial-to-mesenchymal transition-related transcription factors are up-regulated in ependymomas and correlate with a poor prognosis. Hum Pathol 82:149–157. https://doi.org/10.1016/j.humpath.2018.07.018
112. Simonson B, Das S (2015) MicroRNA therapeutics: the next magic bullet? Mini Rev Med Chem 15:467–474. https://doi.org/10.2174/1389557515666150324123208

5. CAPÍTULO II

Artigo “ZEB1 is a Subgroup-Specific Marker of Prognosis and Potential Drug Target in Medulloblastoma”

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ORIGINAL ARTICLE

ZEB1 is a Subgroup-Specific Marker of Prognosis and Potential Drug Target in Medulloblastoma

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Abstract

Medulloblastoma (MB) is a malignant brain tumor that afflicts mostly children and adolescents and presents four distinct molecular subgroups, known as WNT, SHH, Group 3, and Group 4. ZEB1 is a transcription factor that promotes the expression of mesenchymal markers while restraining expression of epithelial and polarity genes. Because of ZEB1 involvement in cerebellum development, here we investigated the role of ZEB1 in MB. We found increased expression of ZEB1 in MB tumor samples compared to normal cerebellar tissue. Expression was higher in the SHH subgroup when compared to all other MB molecular subgroups. High ZEB1 expression was associated with poor prognosis in Group 3 and Group 4, whereas in patients with WNT tumors poorer prognosis were related to lower ZEB1 expression. There was a moderate correlation between ZEB1 and MYC expression in Group 3 and Group 4 MB. Treatment with the immunomodulator and histone deacetylase (HDAC) inhibitor fingolimod (FTY720) reduced ZEB1 expression specifically in D283 cells, which are representative of Group 3 and Group 4 MB. These findings reveal novel subgroup-specific associations of ZEB1 expression with survival in patients with MB and suggest that ZEB1 expression can be reduced by pharmacological agents that target HDAC activity.

Keywords ZEB1 · Transcription factor · Fingolimod · Histone deacetylase · Medulloblastoma · Brain tumor

Introduction

Medulloblastoma (MB) comprises a set of brain tumors arising from the posterior fossa, responsible for around 20% of brain tumors in pediatric patients and entailing mutism, ataxia, and psychosocial and psychiatric consequences (Lanier & Abrams, 2017). Progenitor cells, granule cell precursors, neural stem cells, and unipolar brush cells are undifferentiated cells present in embryonic stages proposed as possible cells of origin for MB (Juraschka & Taylor, 2019; Vladoiu et al., 2019). These different cell types may give rise to the distinct molecular subgroups of MB tumors classified into Wingless-activated (WNT), Sonic Hedgehog-activated (SHH), Group 3, and Group 4, according to active signaling pathways and epigenetic features, presenting distinct age incidence, anatomic location, metastasis risk, and prognosis (Juraschka & Taylor, 2019; Taylor et al., 2012).

Mortality associated with MB tumor often results from metastatic dissemination, mostly as a recurrence site, despite the presence of metastatic foci at diagnosis being only seen in about 20–30% of pediatric patients. Leptomeningeal

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metastasis in MB patients was described early in reports of posterior fossa tumors by Harvey Cushing (Kunschner, 2002). In contrast, metastasis is rarely found in extra neural sites such as lymph nodes, bones, and lungs. Within distinct molecular subgroups, Group 3 tumors show the highest rates of metastasis at diagnosis (40–45%), followed by Group 4 (35–40%) and SHH (15–20%) (Van Ommeren et al., 2020).

Zinc finger E-box binding 1 (ZEB1) is a transcriptional factor that modulates differentiation, migration, and adhesion in tissue development but also in tumor development, in both pediatric and adult tumors (Fratini et al., 2021). During cerebellum formation, ZEB1 is highly expressed and prevents neuronal progenitor differentiation by repressing epithelial and polarization genes (Singh et al., 2016a, 2016b). This repression can be mediated through formation of a complex where ZEB1 cooperates with CtBP2, histone deacetylase (HDAC) 1 (HDAC1) and HDAC2 at the promoter site (Wang et al., 2009, 2019), whereas mesenchymal genes such as CDH2 and VIM can be increased via ZEB1 association to chromatin modelers p300 and PCAF to maintain an undifferentiated cell phenotype (Mizuguchi et al., 2012; Zhang et al., 2019). HDAC inhibitors (HDACis) have been shown to reduce migration and revert chemotherapy resistance through a reduction of ZEB1 in solid cancers (Meidhof et al., 2015; Singh et al., 2016a, 2016b).

The immunosuppressant agent fingolimod (FTY720), which is currently used in the treatment of multiple sclerosis, acts also as an HDACi and displays anticancer activities. FTY720 impairs viability and increases histone H3 acetylation in MB cells (Perla et al., 2020a, 2020b) and reduces tumor growth in Group 3 MB-derived xenografts (Garner et al., 2018). It has anticancer actions and can act as an HDACiFTY720 decreases tumorigenesis in group 3 medulloblastoma patient-derived xenografts. Here, we reveal a potential role for ZEB1 as a subgroup-specific biomarker in MB patients, report that FTY720 can reduce ZEB1 expression in MB cells.

Materials and Methods

Gene Expression Analysis

Datasets of MB tumor samples from patients GSE85217 ($n = 763$, described by Cavalli et al., 2017), GSE28192 ($n = 92$) and "CCLE Cancer Cell Line Encyclopedia—21q4—Broad" ($n = 1389$) were acquired from "R2: Genomics Analysis and Visualization Platform" (http://r2.amc.nl) normalized and transformed (log2). In order to identify ZEB1 targets and related genes, a heatmap was built in the "heatmaply" package in R (version 4.0.4). Overall survival data were available for 625 patients from GSE85217.

Cell Culture and Treatment

Human Daoy (HTB186™) and D283 (HTB185™) MB cells were obtained from the American Type Culture Collection (ATCC, Rockville, USA) and tested to confirm line identity and rule out contamination. Cells were cultured in tissue culture flasks at 37 °C with humidified atmosphere and 5% CO_2. Cells were maintained with DMEM low glucose (Gibco, Grand Island, USA), 1% penicillin and streptomycin and 0.1% amphotericin B, supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Bedford, USA). FTY720 (Sigma-Aldrich, St Louis, Missouri, USA) was diluted in injection water and stored at – 20 °C. Cells were plated and treated after 24 h with FTY720 at IC50 doses, as previously determined (Perla et al., 2020b). After 48 h under treatment, cells were detached using trypsin/EDTA (Gibco; Thermo Fisher Scientific) and centrifuged.

RNA Extraction and cDNA Synthesis

RNA was extracted using Invitrogen™ PureLink™ RNA Mini Kit (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's protocol. After RNA extraction, samples were treated with DNAse (Promega; Madison, USA) according to the manufacturer's protocol. cDNA was synthesized using the reverse transcription (RT) PCR kit SuperScriptTM III First-Strand Synthesis SuperMix (Invitrogen, Thermo Fisher Scientific).

Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

Expression of ZEB1 mRNA was measured with RT-qPCR performed using the QuantStudio 3 Real Time PCR System (Thermo Fisher Scientific), with negative controls. Reactions were prepared with GoTaq® qPCR Master Mix (Promega). Every reaction contained 10 ng sample input cDNA and 500 nM each primer. The standard cycle program

Table 1 Primers used for measurement of ZEB1 mRNA by RT qPCR

Primer	Sequence
ZEB1 forward	5′-ACCCTTGAAAGTGATCCAGC-3′
ZEB1 reverse	5′-CATTCCATTTTCTGTCTTCCGC-3′
β-actin forward	5′AAACTGGAACGGTGAAGGTG-3′
β-actin reverse	5′-AGAGAAGTGGGGTGGCTTTT-3′

indicated by the manufacturer was followed. ZEB1 expression levels were evaluated using the $2^{-\Delta\Delta CT}$ method, with β-actin used as the housekeeping gene. Table 1 shows the primers used for mRNA expression measurement.

Statistical Analysis

Significant differences were evaluated using the "PMCMR plus" package on R (version 4.0.4). Experiments in cell lines were independently conducted three times, each time with triplicates, and analyzed using an independent Student T-test. Gene expression was analyzed using the Wilcoxon test for comparisons between two groups and Kruskal–Wallis followed by Dunn's test when comparing more than two groups. Survival analysis was performed using a log-rank test. The "survival" and "survminer" R packages (version 4.0.4) were used to determine a cutoff for ZEB1 expression in each molecular subgroup and for plotting Kaplan–Meier graphs. Correlational analyses were performed using Spearman correlation. The differences were considered statistically significant when P-values were < 0.05.

Results

ZEB1 Expression is Increased in Human MB Tumors

ZEB1 mRNA levels were evaluated in data from MB tumors and adult non-tumoral cerebellar samples. ZEB1 expression was increased in MB samples when compared to normal adult cerebellum (Fig. 1A). Given that MB subgroups show clinical and molecular distinct features, ZEB1 expression was also analyzed in the larger dataset generated by Cavalli et al. (2017). ZEB1 level was significantly higher in the SHH-activated subgroup (Fig. 1B). There was no significant difference among subgroups WNT, Group 3, and Group 4. There were also no differences between female and male patients ($P = 0.58$, data not shown). We then analyzed ZEB1 mRNA expression in MB cell lines. D283 cells, which originate from a metastatic site and are representative of Group 3 and Group 4 MB, displayed higher ZEB1 levels than Daoy cells, which represent the SHH molecular subgroup of MB (Ivanov et al., 2016) (Fig. 1C).

High ZEB1 Expression is a Potential Marker Poor Prognostic in Group 3 and Group 4 MB and Good Prognosis in WNT MB

Considering that the MB molecular subgroups presented different ZEB1 levels, we investigated if these differences could have an impact on clinical prognosis. Thus, a cutoff point was set for each molecular subgroup, dividing the samples into either high ZEB1 or low ZEB1 expression profile. Kaplan–Meier curves for the SHH subgroup (Fig. 2A) showed no statistical difference between high- and low-expressing tumors ($P = 0.16$). However, Group 3 (Fig. 2B) and Group 4 (Fig. 2C) presented a significant association between high ZEB1 expression and poor prognosis ($P = 0.016$ and $P = 0.009$, respectively). The opposite was observed in the WNT molecular subgroup, where poorer prognosis was related to lower ZEB1 expression ($P = 0.007$; Fig. 2D).

ZEB1 Expression Correlations with MYC Expression in Group 3 and Group 4 MB

MYC is a proto-oncogene involved in driving MB and associated with poor prognosis in high-risk patients (Pei et al., 2012; Qin et al., 2022). Analysis of the whole dataset of tumors using Spearman correlation showed no important correlation between ZEB1 and MYC expression ($R = -0.09$, $P = 0.015$). However, we found moderate correlations between ZEB1 and MYC specifically in both MB subgroups that show poorer prognosis, Group 3 and Group 4 MB ($R = 0.56$, $P = 3.4e-13$ and $R = 0.31$, $P = 7e-9$, respectively; Fig. 3).

Gene Targets and Corepressors of ZEB1 are Independent of Metastasis Status

We then went on to evaluate whether ZEB1 expression could be related to metastasis, given that genes associated to cell adhesion (CDH1, CHL1, and CNTN2), differentiation (SORL1, BHLHE40, LIMK2, FAT2, and RBFOX3), and polarity (PARD3, PARD6A, LIN7A, DDIT4, and DLG2) are repressed by ZEB1 (Ma et al., 2015; Singh

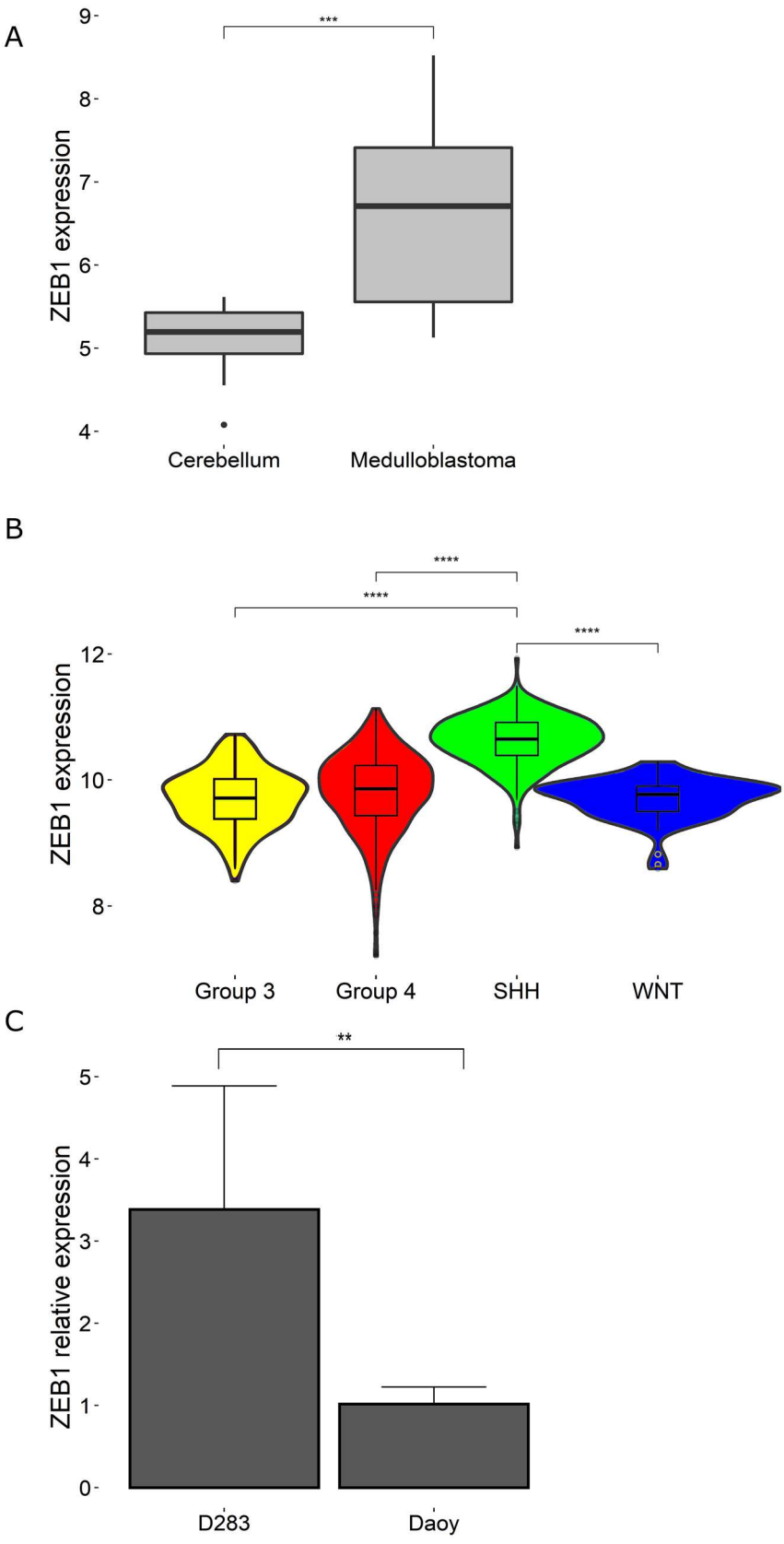


Fig. 1 ZEB1 transcript levels are increased in MB. **A** Expression of ZEB1 in MB ($n = 18$) and normal adult cerebellum ($n = 12$) samples reveal a significant increase in MB (Wilcoxon test, $^{***}P = 0.00024$). **B** ZEB1 levels are higher in the SHH molecular subgroup of MB ($n = 223$) compared to WNT ($n = 70$), Group 3 ($n = 144$) and Group 4 ($n = 326$) MB (Kruskal–Wallis followed by Dunn's all-pairs test, $^{****}P < 0.0001$). **C** ZEB1 levels are higher in D283 cells (metastatic site, Group 3, and Group 4 MB) compared to Daoy cells (primary tumor, SHH MB) ($n = 3$, independent *T* test, $^{*}P < 0.012$)

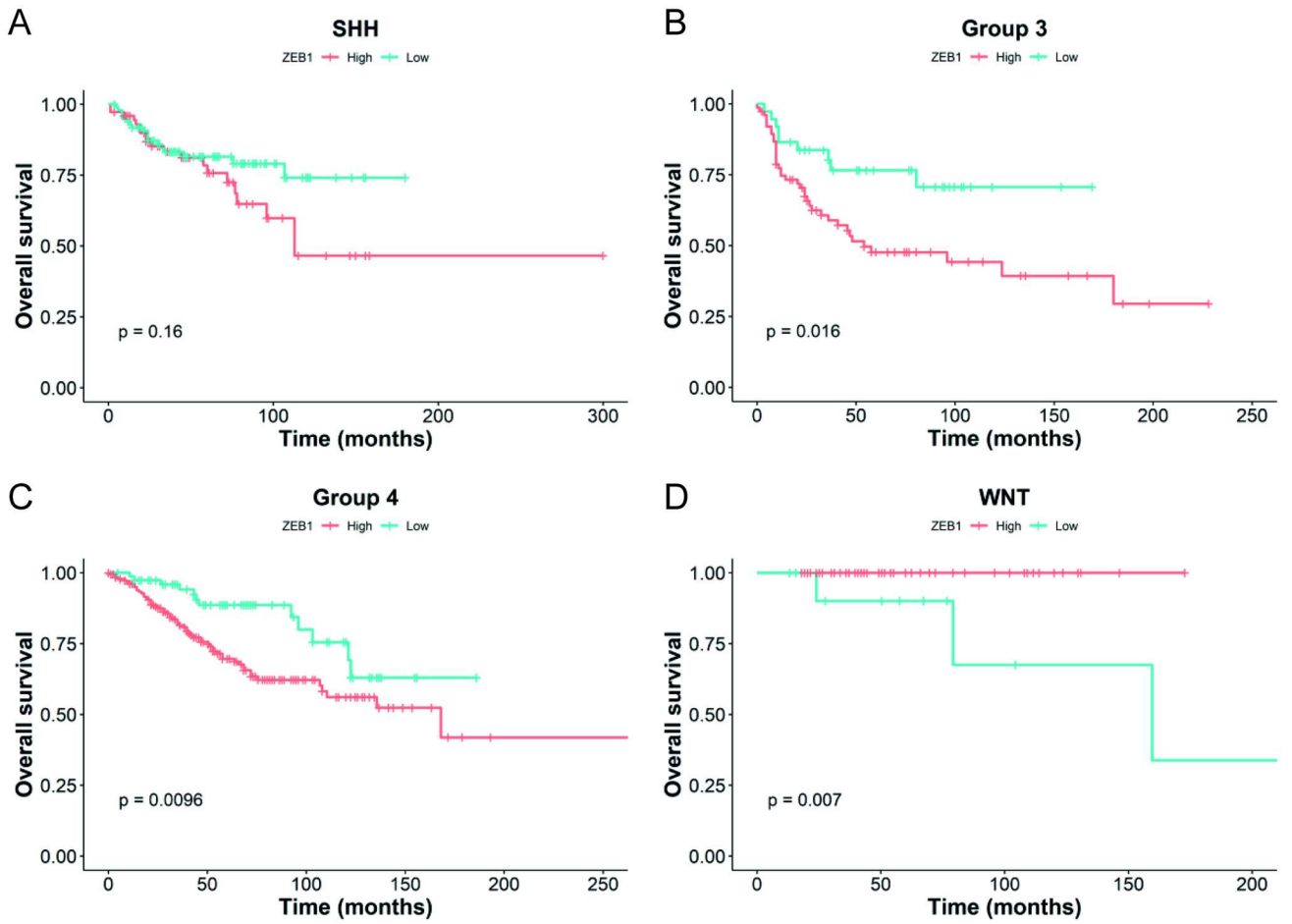


Fig. 2 ZEB1 is a prognosis marker in MB in a subgroup-specific manner. Overall survival and ZEB1 expression of patients with MB tumors belonging to molecular subgroups **A** SHH ($n = 172$, no significant association, log-rank test, $P = 0.16$), **B** Group 3 ($n = 113$, high ZEB1 expression was associated with poor prognosis, log-rank test, $P = 0.016$), **C** Group 4 ($n = 264$, high ZEB1 expression was associated with poor prognosis, log-rank test, $P = 0.0096$), **D** WNT ($n = 63$, high ZEB1 expression associated with better outcome, log-rank test, $P = 0.007$)

et al., 2016a, 2016b), whereas mesenchymal markers such as CDH2, VIM, and MMP14 and the stemness gene CD44 are positively regulated (Kim et al., 2020; Preca et al., 2015; Sánchez-Tilló et al., 2010; Suh et al., 2013). Genes belonging to the repressor complex, such as CTBP2, HDAC1, and HDAC2 (Aghdassi et al., 2012; Papadopoulou et al., 2010; Wang et al., 2009) were evaluated as well. Based on gene expression similarity, samples from patients (Fig. 4A) and MB cell lines (Fig. 4B) were grouped according to molecular subgroups. No cluster formation according to metastasis status could be observed. Moreover, a reduced ZEB1 expression in MB samples from patients who had metastasis was observed (Fig. 4C).

FTY720 Selectively Decreases ZEB1 Expression in MB Cells that Show MYC Amplification and are Representative of Group 3 and Group 4 Tumors

We treated D283 and Daoy cells with FTY720 at the IC50 dose for each cell line (6.2 µM and 8.9 µM, respectively). D283 cells show MYC amplification and display features of Group 3 and Group 4 MB, whereas Daoy cells represent the SHH MB subgroup (Ivanov et al., 2016). ZEB1 expression

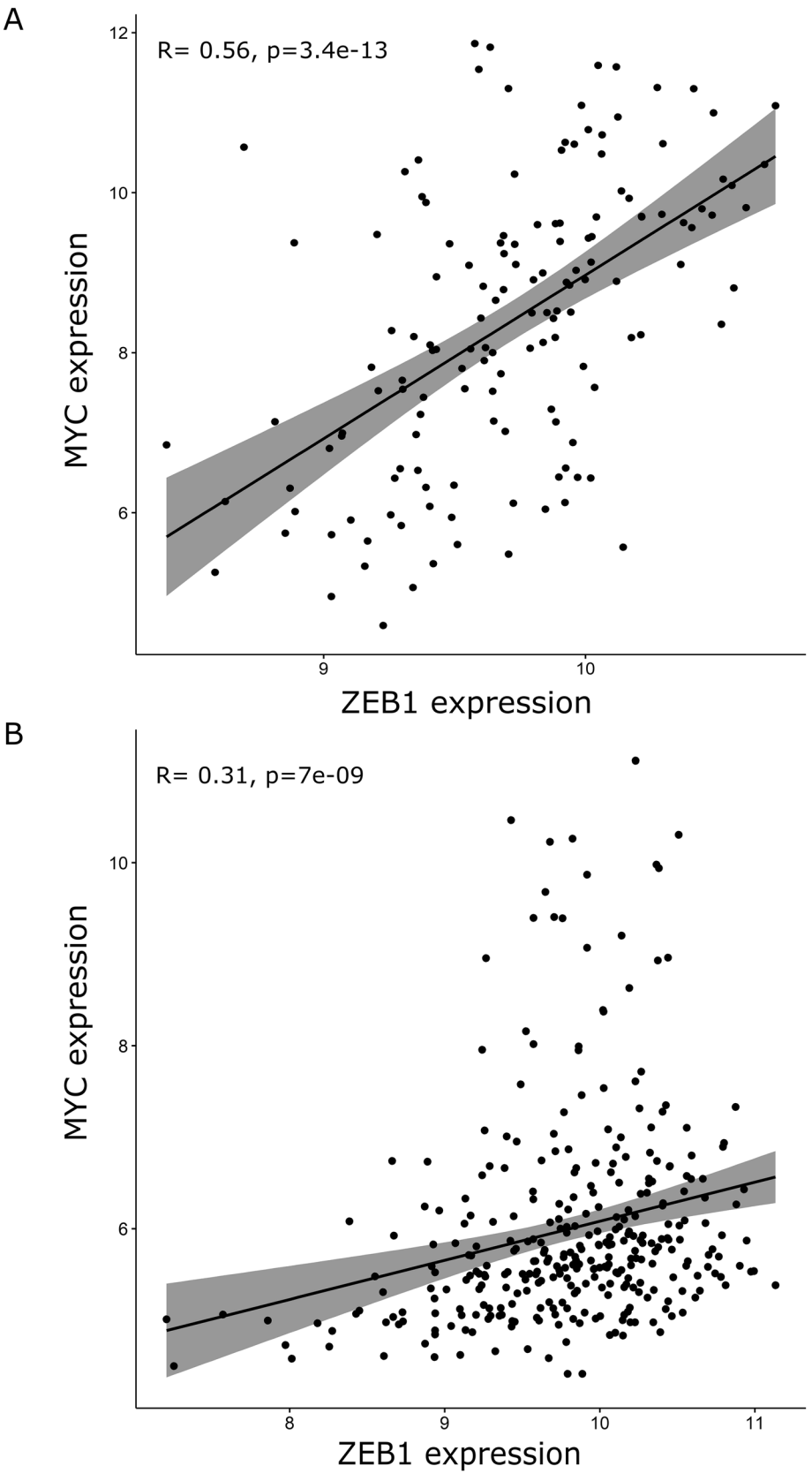


Fig. 3 Correlational analysis of ZEB1 and MYC expression in **A** Group 3 ($n = 144$) and **B** Group 4 ($n = 326$) MB (Spearman correlation analysis, $R = 0.56$, $P = 3.4e-13$ and $R = 0.31$, $P = 7e-9$, respectively)

was significantly decreased in D283 cells, after FTY720 treatment (Fig. 5), whereas no changes were observed in Daoy cells.

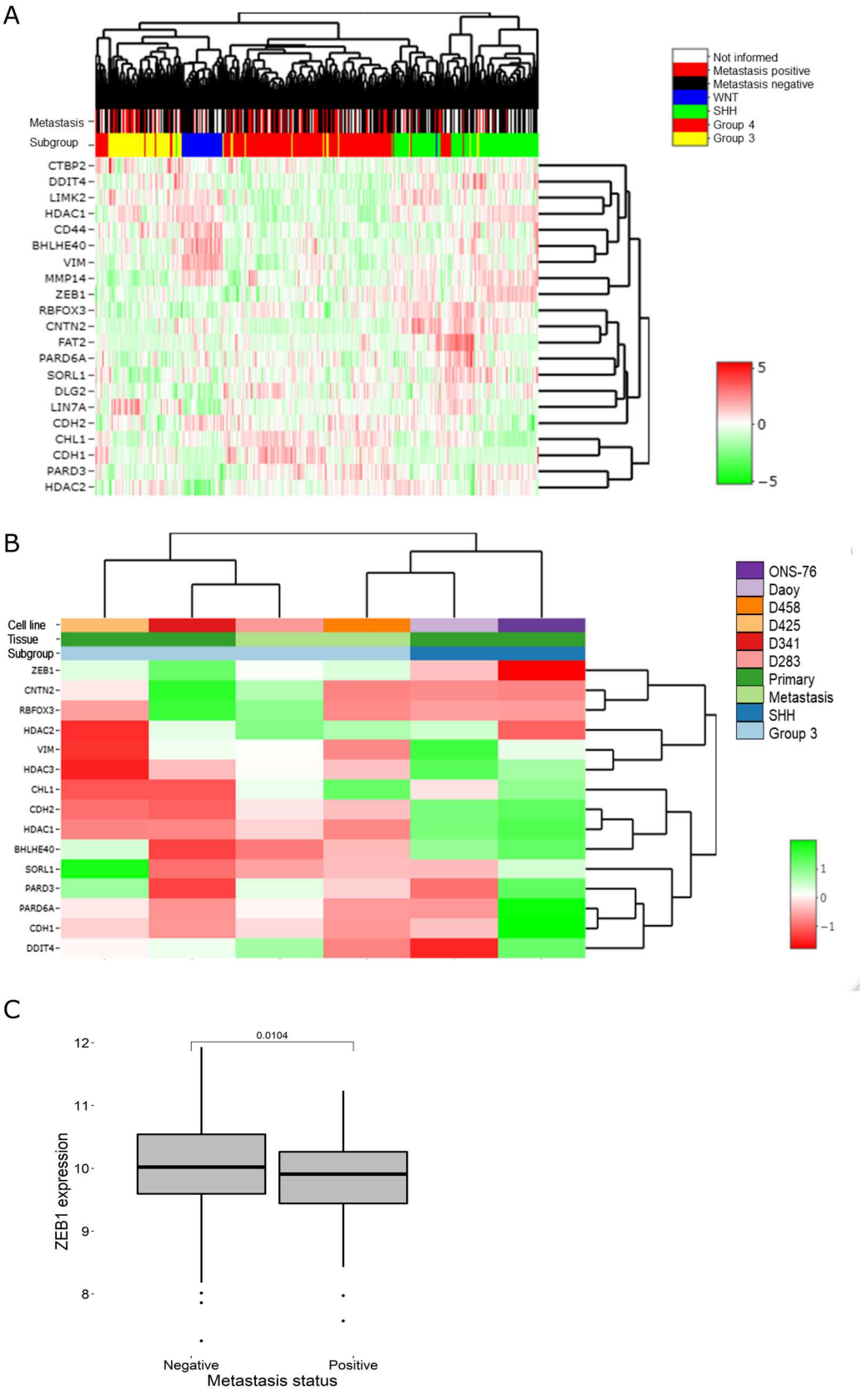
Discussion

In the present report, we describe higher ZEB1 mRNA levels in human MB tumors compared to non-tumoral cerebellar tissue. These findings are consistent with the increased ZEB1 expression previously described in in a dataset of 74 human MB samples as well as in a mouse model of SHH MB, where ZEB1 expression level was higher than the peak level found in the cerebellum during normal development (Singh et al., 2016a, 2016b). Our results significantly expand those previous data to an analysis of a large dataset comprising 763 tumor samples. Interestingly, ZEB1 protein expression is increased in high-grade brain and infiltrative tumors, including MB, when compared to low-grade and non-infiltrative brain tumor types, such as pilocytic astrocytoma and ependymoma (Kahlert et al., 2015).

Our findings also provide the first evidence for associations of ZEB1 gene expression with overall survival in patients with MB. These associations are dependent on the molecular subgroup, so that higher ZEB1 indicates poorer outcome in Group 3 and Group 4 MB but better outcome in WNT MB and moderately correlates with MYC expression in Group 3 and Group 4 MB. Tumors in these molecular subgroups occur in the majority of patients diagnosed with MB and are associated with high-risk features that include MYC amplification and metastasis at diagnosis (Juraschka & Taylor, 2019), but even patients with tumors that lack these features show relapsed disease (Pizer & Clifford, 2009). The finding of better prognosis in patients with WNT MB is somewhat surprising, given that ZEB1 both regulates WNT expression and is a target of the WNT pathway (Sánchez-Tilló et al., 2015), playing a role in metastasis in colorectal (Zhang et al., 2018), lung (Yang et al., 2015) and hepatocellular cancer (Li et al., 2021) through mechanisms related to WNT signaling. SHH tumors, which present the highest ZEB1 expression among molecular subgroups of MB, showed any association with prognosis, although ZEB1 is part of the SHH signaling system (Singh et al., 2016a, 2016b).

Metastasis is a strong feature in clinical prognosis of MB. We found a reduced ZEB1 expression in patients who presented metastasis. Other factors such as resistance to treatment (Khatua et al., 2018), cancer stem cell population (Casciati et al., 2020) and genetic features such as TP53 mutation status (Ramaswamy et al., 2016) must be taken into account as factor influencing outcome. Our analysis was carried on data from primary tumor samples. Our finding that the D283 cell line, originally isolated from a metastatic site, has a higher ZEB1 expression than Daoy cells might be a preliminary finding raising the possibility that metastatic sites could present higher ZEB1 levels compared to primary tumors. Previous findings indicate that only about 10% of cells from metastatic lesions in brain metastasis present ZEB1 expression, but a higher expression was detected in stromal cells from metastatic lesions (Nagaishi et al., 2017). Single-cell analysis in MB samples as well as stromal cell expression of ZEB1 may be required to a better understanding of ZEB1 in metastasis context.

We have previously evaluated HDAC inhibitors as anticancer agents in MB cells, showing that they are able to modulate cellular phenotype and induce cell death (Jaeger et al., 2020; Nör et al., 2013; Perla et al., 2020b). FTY720 is a clinically used immunomodulator for the treatment of relapsed multiple sclerosis (Brinkmann et al., 2010) that acts as a HDAC inhibitor in its active form (Hait et al., 2014) and can readily enter the brain due to its lipophilic nature (Brinkmann et al., 2010). In MB cells, FTY720 impairs viability and survival and increased histone H3 acetylation (Perla et al., 2020b), and also reduces migration, invasion and tumor growth in patient-derived xenografts representing Group 3 MB. In this study, FTY720 was able to reduce ZEB1 expression in the metastatic site-derived D283, which displays features of Group 3 and Group 4 MB, but not in the Daoy cell line, which is derived from a primary tumor and represents the SHH subgroup.


A
Not informed
Metastasis positive
Metastasis negative
WNT
SHH
Group 4
Group 3
Metastasis
Subgroup
CTBP2
DDIT4
LIMK2
HDAC1
CD44
BHLHE40
VIM
MMP14
ZEB1
RBFOX3
CNTN2
FAT2
PARD6A
SORL1
DLG2
LIN7A
CDH2
CHL1
CDH1
PARD3
HDAC2
5
0
−5
B
ONS-76
Daoy
D458
D425
D341
D283
Primary
Metastasis
SHH
Group 3
Cell line
Tissue
Subgroup
ZEB1
CNTN2
RBFOX3
HDAC2
VIM
HDAC3
CHL1
CDH2
HDAC1
BHLHE40
SORL1
PARD3
PARD6A
CDH1
DDIT4
1
0
−1
C
0.0104
12
11
10
9
8
ZEB1 expression
Negative
Positive
Metastasis status

◂**Fig. 4** ZEB1 expression is independent of metastatic status. Heatmap with ZEB1 targets and corepressors shows a clustering according to molecular subgroups in **A** MB patients (n = 763) and **B** 6 MB cell lines. **C** ZEB1 expression is higher in primary samples from patients who did not present metastasis (Wilcoxon test, P = 0.010)

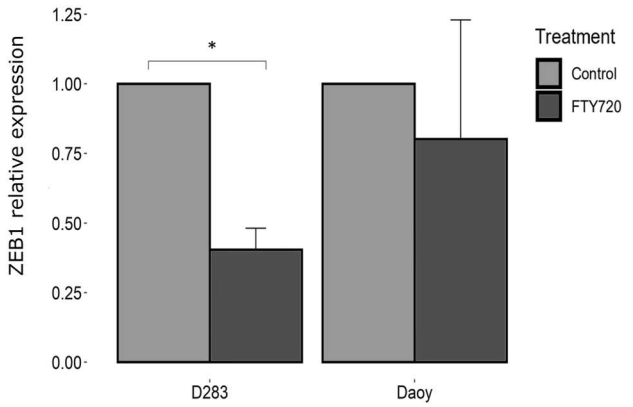


Fig. 5 FTY720 reduces ZEB1 expression in D283, but not Daoy human MB cells. Cells were cultured and treated for 48 h as described in "Materials and Methods" section. Expression of ZEB1 mRNA was measured with RT-qPCR. Results are shown as mean + standard error of mean relative expression (n = 3, independent T test, * P = 0.039)

Conclusion

Taken together, the present findings provide early evidence highlighting a role of ZEB1 as a potential subgroup-specific prognosis biomarker and drug target in MB.

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Author Contributions LF conceived and carried out experiments and gene expression analyses, analyzed data, and wrote the first draft of the manuscript. MGSD carried out experiments, analyzed data, and wrote the manuscript. MS, ASP, CBF, ALB, ATB, MCJ, and RR contributed to the experimental design, study conception, securing and managing funds for the study, data analysis, data interpretation, writing, and revision of this article.

Data Availability Data sharing: No new datasets were generated in this study. Datasets analyzed are available and described in the Methods section.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Aghdassi, A., Sendler, M., Guenther, A., Mayerle, J., Behn, C. O., Heidecke, C. D., Friess, H., Büchler, M., Evert, M., Lerch, M. M., & Weiss, F. U. (2012). Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer. *Gut, 61*, 439–448. https://doi.org/10.1136/gutjnl-2011-300060

Brinkmann, V., Billich, A., Baumruker, T., Heining, P., Schmouder, R., Francis, G., Aradhye, S., & Burtin, P. (2010). Fingolimod (FTY720): Discovery and development of an oral drug to treat multiple sclerosis. *Nature Reviews Drug Discovery, 9*, 883–897. https://doi.org/10.1038/nrd3248

Casciati, A., Tanori, M., Manczak, R., Saada, S., Tanno, B., Giardullo, P., Porcù, E., Rampazzo, E., Persano, L., Viola, G., Dalmay, C., Lalloué, F., Pothier, A., Merla, C., & Mancuso, M. (2020). Human medulloblastoma cell lines: Investigating on cancer stem cell-like phenotype. *Cancers, 12*, 226. https://doi.org/10.3390/cancers12010226

Cavalli, F.M.G., Remke, M., Rampasek, L., Peacock, J., Shih, D.J.H., Luu, B., Garzia, L., Torchia, J., Nor, C., Morrissy, A. S., Agnihotri, S., Thompson, Y. Y., Kuzan-Fischer, C. M., Farooq, H., Isaev, K., Daniels, C., Cho, B.-K., Kim, S.-K., Wang, K.-C., … Taylor, M. D. (2017). Intertumoral heterogeneity within medulloblastoma subgroups. *Cancer Cell*, *31*, 737–754.e6. https://doi.org/10.1016/j.ccell.2017.05.005

Fratini, L., Jaeger, M., de Farias, C. B., Brunetto, A. T., Brunetto, A. L., Shaw, L., & Roesler, R. (2021). Oncogenic functions of ZEB1 in pediatric solid cancers: Interplays with microRNAs and long noncoding RNAs. *Molecular and Cellular Biochemistry, 476*, 4107–4116. https://doi.org/10.1007/s11010-021-04226-x

Garner, E. F., Williams, A. P., Stafman, L. L., Aye, J. M., Mroczek-Musulman, E., Moore, B. P., Stewart, J. E., Friedman, G. K., & Beierle, E. A. (2018). FTY720 decreases tumorigenesis in Group 3 medulloblastoma patient-derived xenografts. *Scientific Reports, 8*, 6913. https://doi.org/10.1038/s41598-018-25263-5

Hait, N. C., Wise, L. E., Allegood, J. C., O'Brien, M., Avni, D., Reeves, T. M., Knapp, P. E., Lu, J., Luo, C., Miles, M. F., Milstien, S., Lichtman, A. H., & Spiegel, S. (2014). Active, phosphorylated fingolimod inhibits histone deacetylases and facilitates fear extinction memory. *Nature Neuroscience, 17*, 971–980. https://doi.org/10.1038/nn.3728

Ivanov, D. P., Coyle, B., Walker, D. A., & Grabowska, A. M. (2016). In vitro models of medulloblastoma: Choosing the right tool for the job. *Journal of Biotechnology, 236*, 10–25. https://doi.org/10.1016/j.jbiotec.2016.07.028

Jaeger, M. C., Ghisleni, E. C., Cardoso, P. S., Siniglaglia, M., Falcon, T., Brunetto, A. T., Brunetto, A. L., de Farias, C. B., Taylor, M. D., Nör, C., Ramaswamy, V., & Roesler, R. (2020). HDAC and MAPK/ERK inhibitors cooperate to reduce viability and stemness in medulloblastoma. *Journal of Molecular Neuroscience, 70*, 981–992. https://doi.org/10.1007/s12031-020-01505-y

Juraschka, K., & Taylor, M. D. (2019). Medulloblastoma in the age of molecular subgroups: A review: JNSPG 75th Anniversary invited review article. *Journal of Neurosurgery: Pediatrics, 24*, 353–363. https://doi.org/10.3171/2019.5.PEDS18381

Kahlert, U. D., Suwala, A. K., Raabe, E. H., Siebzehnrubl, F. A., Suarez, M. J., Orr, B. A., Bar, E. E., Maciaczyk, J., & Eberhart, C. G. (2015). ZEB1 promotes invasion in human fetal neural stem cells and hypoxic glioma neurospheres. *Brain Pathology, 25*, 724–732. https://doi.org/10.1111/bpa.12240

Khatua, S., Song, A., Sridhar, D. C., & Mack, S. C. (2018). Childhood medulloblastoma: Current therapies, emerging molecular landscape and newer therapeutic insights. *Current*

Neuropharmacology, 16, 1045–1058. https://doi.org/10.2174/1570159X15666171129111324

Kim, J. Y., Cho, K. H., Jeong, B. Y., Park, C. G., & Lee, H. Y. (2020). Zeb1 for RCP-induced oral cancer cell invasion and its suppression by resveratrol. *Experimental & Molecular Medicine, 52*, 1152–1163. https://doi.org/10.1038/s12276-020-0474-1

Kunschner, L. J. (2002). Harvey Cushing and medulloblastoma. *Archives of Neurology, 59*, 642–645. https://doi.org/10.1001/archneur.59.4.642

Lanier, J. C., & Abrams, A. N. (2017). Posterior fossa syndrome: Review of the behavioral and emotional aspects in pediatric cancer patients. *Cancer, 123*, 551–559. https://doi.org/10.1002/cncr.30238

Li, L., Yang, J., Rong, F., Luo, Z., Hu, S., Fang, H., Wu, Y., Yao, R., Kong, W., Feng, X., Chen, B., Li, J., & Xu, T. (2021). ZEB1 serves an oncogenic role in the tumourigenesis of HCC by promoting cell proliferation, migration, and inhibiting apoptosis via Wnt/β-catenin signaling pathway. *Acta Pharmacologica Sinica, 42*, 1676–1689. https://doi.org/10.1038/s41401-020-00575-3

Ma, Y., Zheng, X., Zhou, J., Zhang, Y., & Chen, K. (2015). ZEB1 promotes the progression and metastasis of cervical squamous cell carcinoma via the promotion of epithelial-mesenchymal transition. *International Journal of Clinical and Experimental Pathology, 8*, 11258–11267.

Meidhof, S., Brabletz, S., Lehmann, W., Preca, B., Mock, K., Ruh, M., Schüler, J., Berthold, M., Weber, A., Burk, U., Lübbert, M., Puhr, M., Culig, Z., Wellner, U., Keck, T., Bronsert, P., Küsters, S., Hopt, U. T., Stemmler, M. P., & Brabletz, T. (2015). ZEB 1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. *EMBO Molecular Medicine, 7*, 831–847. https://doi.org/10.15252/emmm.201404396

Mizuguchi, Y., Specht, S., Lunz, J. G., Isse, K., Corbitt, N., Takizawa, T., & Demetris, A. J. (2012). Cooperation of p300 and PCAF in the control of microRNA 200c/141 transcription and epithelial characteristics. *PLoS ONE, 7*, e32449. https://doi.org/10.1371/journal.pone.0032449

Nagaishi, M., Nakata, S., Ono, Y., Hirata, K., Tanaka, Y., Suzuki, K., Yokoo, H., & Hyodo, A. (2017). Tumoral and stromal expression of Slug, ZEB1, and ZEB2 in brain metastasis. *Journal of Clinical Neuroscience, 46*, 124–128. https://doi.org/10.1016/j.jocn.2017.08.050

Nör, C., Sassi, F. A., de Farias, C. B., Schwartsmann, G., Abujamra, A. L., Lenz, G., Brunetto, A. L., & Roesler, R. (2013). The histone deacetylase inhibitor sodium butyrate promotes cell death and differentiation and reduces neurosphere formation in human medulloblastoma cells. *Molecular Neurobiology, 48*, 533–543. https://doi.org/10.1007/s12035-013-8441-7

Papadopoulou, V., Postigo, A., Sánchez-Tilló, E., Porter, A. C. G., & Wagner, S. D. (2010). ZEB1 and CtBP form a repressive complex at a distal promoter element of the BCL6 locus. *Biochemical Journal, 427*, 541–550. https://doi.org/10.1042/BJ20091578

Pei, Y., Moore, C. E., Wang, J., Tewari, A. K., Eroshkin, A., Cho, Y. J., Witt, H., Korshunov, A., Read, T. A., Sun, J. L., Schmitt, E. M., Miller, C. R., Buckley, A. F., McLendon, R. E., Westbrook, T. F., Northcott, P. A., Taylor, M. D., Pfister, S. M., Febbo, P. G., & Wechsler-Reya, R. J. (2012). An animal model of MYC-driven medulloblastoma. *Cancer Cell, 21*, 155–167. https://doi.org/10.1016/j.ccr.2011.12.021

Perla, A. S., Fratini, L., Cardoso, P. S., de Farias, C. B., Jaeger, M. C., & Roesler, R. (2020b). Fingolimod (FTY720) reduces viability and survival and increases histone H3 acetylation in medulloblastoma cells. *Pediatric Hematology and Oncology, 37*, 170–175. https://doi.org/10.1080/08880018.2019.1699213

Perla, A., Fratini, L., Cardoso, P. S., Nör, C., Brunetto, A. T., Brunetto, A. L., de Farias, C. B., Jaeger, M., & Roesler, R. (2020a). Histone deacetylase inhibitors in pediatric brain cancers: Biological activities and therapeutic potential. *Frontiers in Cell and Developmental Biology, 8*, 1–14. https://doi.org/10.3389/fcell.2020.00546

Pizer, B. L., & Clifford, S. C. (2009). The potential impact of tumour biology on improved clinical practice for medulloblastoma: Progress towards biologically driven clinical trials. *British Journal of Neurosurgery, 23*, 364–375. https://doi.org/10.1080/02688690903121807

Preca, B. T., Bajdak, K., Mock, K., Sundararajan, V., Pfannstiel, J., Maurer, J., Wellner, U., Hopt, U. T., Brummer, T., Brabletz, S., Brabletz, T., & Stemmler, M. P. (2015). A self-enforcing CD44s/ZEB1 feedback loop maintains EMT and stemness properties in cancer cells. *International Journal of Cancer, 137*, 2566–2577. https://doi.org/10.1002/ijc.29642

Qin, N., Paisana, E., Langini, M., Picard, D., Malzkorn, B., Custódia, C., Cascão, R., Meyer, F. D., Blümel, L., Göbbels, S., Taban, K., Bartl, J., Bechmann, N., Conrad, C., Gravemeyer, J., Becker, J. C., Stefanski, A., Puget, S., Barata, J. T., … Remke, M. (2022). Intratumoral heterogeneity of MYC drives medulloblastoma metastasis and angiogenesis. *Neuro Oncology*. https://doi.org/10.1093/neuonc/noac068

Ramaswamy, V., Nör, C., & Taylor, M. D. (2016). p53 and Medulloblastoma. *Cold Spring Harbor Perspectives in Medicine, 6*, a026278. https://doi.org/10.1101/cshperspect.a026278

Sánchez-Tilló, E., de Barrios, O., Valls, E., Darling, D. S., Castells, A., & Postigo, A. (2015). ZEB1 and TCF4 reciprocally modulate their transcriptional activities to regulate Wnt target gene expression. *Oncogene, 34*, 5760–5770. https://doi.org/10.1038/onc.2015.352

Sánchez-Tilló, E., Lázaro, A., Torrent, R., Cuatrecasas, M., Vaquero, E. C., Castells, A., Engel, P., & Postigo, A. (2010). ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. *Oncogene, 29*, 3490–3500. https://doi.org/10.1038/onc.2010.102

Singh, S., Howell, D., Trivedi, N., Kessler, K., Ong, T., Rosmaninho, P., Raposo, A. A. S. F., Robinson, G., Roussel, M. F., Castro, D. S., & Solecki, D. J. (2016a). Zeb1 controls neuron differentiation and germinal zone exit by a mesenchymal-epithelial-like transition. *eLife, 5*, e12717. https://doi.org/10.7554/eLife.12717

Singh, T., Prasad, R., & Katiyar, S. K. (2016b). Therapeutic intervention of silymarin on the migration of non-small cell lung cancer cells is associated with the axis of multiple molecular targets including class 1 HDACs, ZEB1 expression, and restoration of miR-203 and E-cadherin expression. *American Journal of Cancer Research, 6*, 1287–1301.

Suh, Y., Yoon, C.-H., Kim, R.-K., Lim, E.-J., Oh, Y. S., Hwang, S.-G., An, S., Yoon, G., Gye, M. C., Yi, J.-M., Kim, M.-J., & Lee, S.-J. (2013). Claudin-1 induces epithelial–mesenchymal transition through activation of the c-Abl-ERK signaling pathway in human liver cells. *Oncogene, 32*(41), 4873–4882. https://doi.org/10.1038/onc.2012.505

Taylor, M. D., Northcott, P. A., Korshunov, A., Remke, M., Cho, Y. J., Clifford, S. C., Eberhart, C. G., Parsons, D. W., Rutkowski, S., Gajjar, A., Ellison, D. W., Lichter, P., Gilbertson, R. J., Pomeroy, S. L., Kool, M., & Pfister, S. M. (2012). Molecular subgroups of medulloblastoma: The current consensus. *Acta Neuropathologica, 123*, 465–472. https://doi.org/10.1007/s00401-011-0922-z

Van Ommeren, R., Garzia, L., Holgado, B. L., Ramaswamy, V., & Taylor, M. D. (2020). The molecular biology of medulloblastoma metastasis. *Brain Pathology, 30*, 691–702. https://doi.org/10.1111/bpa.12811

Vladoiu, M.C., El-Hamamy, I., Donovan, L.K., Farooq, H., Holgado, B.L., Sundaravadanam, Y., Ramaswamy, V., Hendrikse, L.D., Kumar, S., Mack, S.C., Lee, J.J.Y., Fong, V., Juraschka, K., Przelicki, D., Michealraj, A., Skowron, P., Luu, B., Suzuki, H., Morrissy, A. S., … Taylor, M.D. (2019). Childhood cerebellar tumours mirror conserved fetal transcriptional programs. *Nature, 572*, 67–73. https://doi.org/10.1038/s41586-019-1158-7

Wang, H., Xiao, Z., Zheng, J., Wu, J., Hu, X. L., Yang, X., & Shen, Q. (2019). ZEB1 represses neural differentiation and cooperates with CTBP2 to dynamically regulate cell migration during neocortex development. *Cell Reports, 27*, 2335-2353.e6. https://doi.org/10.1016/j.celrep.2019.04.081

Wang, J., Lee, S., Teh, C. E. Y., Bunting, K., Ma, L., & Shannon, M. F. (2009). The transcription repressor, ZEB1, cooperates with CtBP2 and HDAC1 to suppress IL-2 gene activation in T cells. *International Immunology, 21*, 227–235. https://doi.org/10.1093/intimm/dxn143

Yang, X., Li, L., Huang, Q., Xu, W., Cai, X., Zhang, J., Yan, W., Song, D., Liu, T., Zhou, W., Li, Z., Yang, C., Dang, Y., & Xiao, J. (2015). Wnt signaling through Snail1 and Zeb1 regulates bone metastasis in lung cancer. *American Journal of Cancer Research, 5*, 748–755.

Zhang, M., Miao, F., Huang, R., Liu, W., Zhao, Y., Jiao, T., Lu, Y., Wu, F., Wang, X., Wang, H., Zhao, H., Ju, H., Miao, S., Wang, L., & Song, W. (2018). RHBDD1 promotes colorectal cancer metastasis through the Wnt signaling pathway and its downstream target ZEB1. *Journal of Experimental & Clinical Cancer Research, 37*, 22. https://doi.org/10.1186/s13046-018-0687-5

Zhang, Y., Xu, L., Li, A., & Han, X. (2019). The roles of ZEB1 in tumorigenic progression and epigenetic modifications. *Biomedicine and Pharmacotherapy, 110*, 400–408. https://doi.org/10.1016/j.biopha.2018.11.112

6. CAPÍTULO III

Resultados não publicados: miR-101 é relacionado à melhor sobrevida no Grupo 4 de Meduloblastoma

6. 1 Metodologia

Buscando compreender a relação de ZEB1 e miRNAs, utilizamos os diferencialmente expressos descritos por Yang et al. (2015). Dentre os miRNAs diferencialmente expressos entre pacientes metastáticos e não metastáticos descritos pelo artigo, a ferramenta TransmiR v2.0 (https://www.cuilab.cn/transmir) foi utilizada para identificar os miRNAs relacionados a ZEB1. Dessa forma, dois miRNAs foram selecionados para mais análises: miR-101-3p e miR-148a-3p. Utilizando a mesma metodologia aplicada no artigo do Capítulo 2 desta tese, a expressão dos dois miRNAs foi acessada entre os subgrupos moleculares de meduloblastoma e analisados pelo teste estatístico não paramétrico de Wilcoxon. Para a análise de sobrevida, foram utilizados os pacotes “survival” e “survminer” no software R (versão 4.0.4)

6.2 Resultados

Na Tabela I podemos conferir a relação do miR-101 e do miR-148a em meduloblastoma, bem como a relação descrita com ZEB1 em outros tumores. A expressão de ambos miRNAs foi descrita por Yang et al (2015) como reduzida em amostras de MB metastático quando comparadas a amostras de MB i*n situ*. Tanto miR-101 quanto o miR-148a inibem processos oncológicos em meduloblastoma. O miR-101 inibe ZEB1 nas linhagens celulares tumorais HeLa, SCC-9 e OvCa. Já o miR-148a é alvo de ZEB1, sendo inibido por ele na linhagem celular 373P.

Analisando a expressão de miR-148a entre os subgrupos de meduloblastoma, a maior expressão foi encontrada no subgrupo WNT, que apresenta melhor prognóstico dentre os subgrupos moleculares de meduloblastoma. Esse resultado vai de encontro ao resultado descrito na Tabela 1, que mostra relação inibitória de processos celulares pró-tumorais,

processos esses que são menos ativos em WNT quando comparados aos demais grupos moleculares de meduloblastoma, uma vez que esse é o grupo molecular de melhor prognóstico. Já para o miR-101, a maior expressão foi encontrada no Grupo 4, quando comparado aos demais grupos. Esse resultado foi inesperado e deve ser mais bem investigado em outros modelos de análise.

Para melhor entender o perfil dos miRNAs em meduloblastoma, a análise de sobrevida dos miRNAs foi realizada entre os subgrupos moleculares. Somente o miR-101 no Grupo 4 mostrou resultado significativo, em que maior expressão foi relacionada a melhor prognóstico. A relação com sobrevida em pacientes de meduloblastoma de miR-101 nos demais subgrupos e de miR-148a não geraram resultados estatisticamente significativos no teste de log-rank.

Tabela 1. miRNAs diferencialmente expressos no cenário metastático de meduloblastoma e relacionados a ZEB1

MicroRNA		Expression in metastatic MB	miRNA action in MB	Modulation by ZEB1 (cell line)	Method	References (PMID)
miRBase ID	miRBase acession					
hsa-miR-101-3p	MIMAT0000099	Decreased	Inhibits tumorigenesis	Inhibits ZEB1 expression (HeLa, SCC-9 and OvCa)	Luciferase assay	26506238, 24677166, 27429852, 30098599, 34294888
hsa-miR-148a-3p	MIMAT0000243	Decreased	Inhibits proliferation, invasion and tumorigenicity	Inhibited by ZEB1 (373P)	Luciferase assay	18973228, 26097868, 30195786, 26506238, 28440450, 27869652

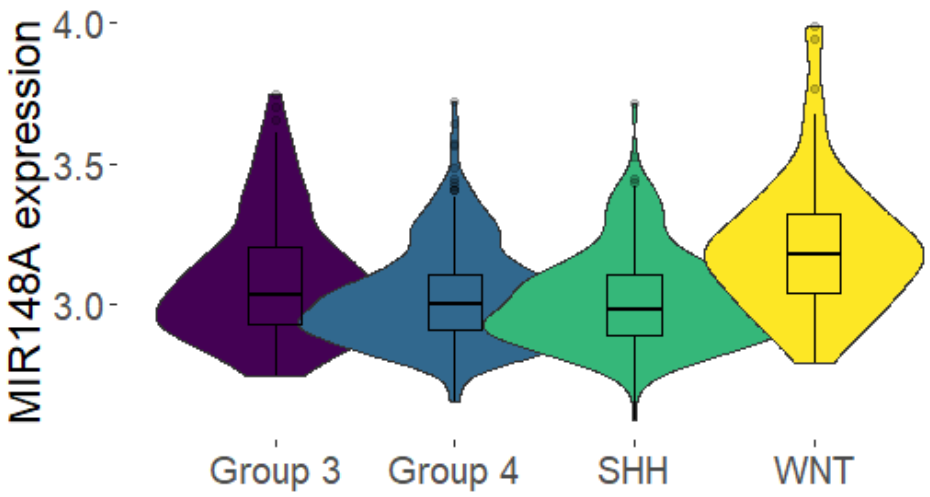

Figura 7. Expressão de miRNA-148a é maior no subgrupo molecular WNT de meduloblastoma,quando comparada aos demais grupos 3, 4 e WNT.

Tabela 2. Expressão de miR-148a nos subgrupos moleculares de meduloblastoma

Grupos comparados	Valor p
Group 4 - Group 3 == 0	0.03520719 *
SHH - Group 3 == 0	0.03158793 *
WNT - Group 3 == 0	0.00060911 ***
SHH - Group 4 == 0	1.00000000
WNT - Group 4 == 0	9.8275e-10 ***
WNT - SHH == 0	1.6615e-09 ***

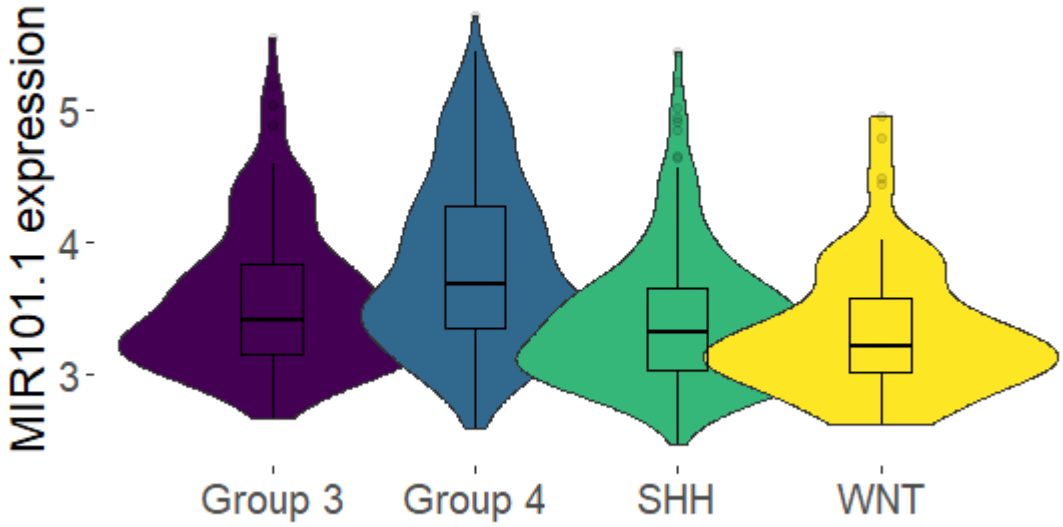

Figura 8. Expressão de miRNA-101 é maior no subgrupo molecular Grupo 4 de meduloblastoma, quando comparada aos demais grupos 3, SHH e WNT.

Tabela 3. Expressão de miRNA101 nos subgrupos moleculares de meduloblastoma

Grupos comparados	Valor p
Group 4 - Group 3 == 0	0.00015945 ***
SHH - Group 3 == 0	0.04006117 *
WNT - Group 3 == 0	0.01494683 *
SHH - Group 4 == 0	1.7889e-15 ***
WNT - Group 4 == 0	3.7859e-10 ***
WNT - SHH == 0	1.00000000

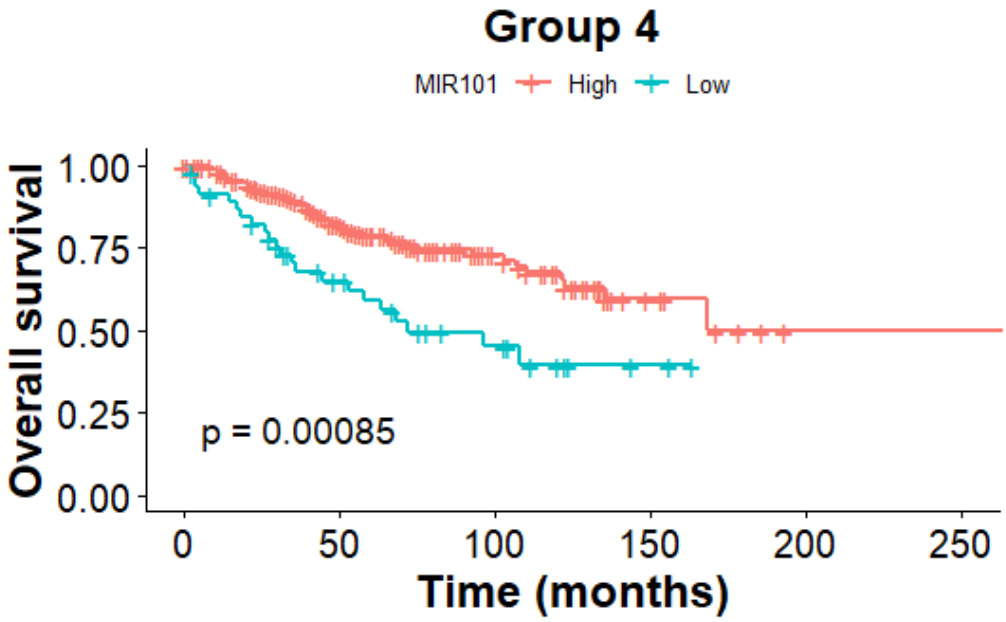

Figura 9. A maior expressão de miRNA-101 está relacionada ao melhor prognóstico no Grupo 4 de meduloblastoma.

7. DISCUSSÃO

Ao longo dos anos, a compreensão de meduloblastoma do ponto de vista molecular permitiu estratificação de risco mais adequada e novas abordagens terapêuticas, aumentando a sobrevida global para 80% (Gottardo et al., 2014). Da mesma forma, foi visto que a expressão de ZEB1 se distribui diferentemente entre os subgrupos moleculares de meduloblastoma. A análise da expressão de ZEB1 foi realizada em uma coorte maior do que a analisada em Singh et al 2016, mas o resultado foi replicado. Nesse trabalho, assim como Singh et al (2016) já havia visto em amostras de camundongos, foi observado que a expressão de ZEB1 em meduloblastoma era maior que a expressão em amostras de cerebelo de tecidos humanos.

De forma inédita, a expressão de ZEB1 foi associada à sobrevida em pacientes de meduloblastoma. A maior expressão de ZEB1 foi encontrada em pacientes com pior prognóstico dos grupos moleculares 3 e 4. Em contrapartida, pacientes do subgrupo molecular WNT com maior expressão de ZEB1 tiveram melhor sobrevida. Não foi observada relação com a sobrevida de pacientes de SHH MB. Esse achado foi inesperado, uma vez que, em modelo in vivo de SHH MB, a expressão de ZEB1 se mostrou elevada quando a vida SHH estava ativada e em culturas celulares tratadas com agonistas dessa via, ZEB1 estava altamente expresso, sugerindo uma relação entre ZEB1 e SHH (Singh et al, 2016).

Quando a expressão de ZEB1 foi analisada junto a seus alvos moleculares e correpressores, as amostras foram clusterizadas de acordo com o subgrupo molecular. Amostras de linhagens celulares imortalizadas dos subgrupos WNT e Grupo 3 também foram clusterizadas diferencialmente. Dessa forma, podemos entender a expressão de ZEB1 como um marcador prognóstico em diferentes subgrupos moleculares de meduloblastoma.

Diferentemente de outros estudos que correlacionam a maior expressão de ZEB1 à metástase em tumores sólidos (Horny et al. 2023; Mohammadpour et al., 2023), foi encontrada uma maior expressão de ZEB1 em amostras de pacientes negativos para metástases, mas não foi observada formação de clusters em relação ao status metastático

das amostras. É possível que a relação de ZEB1 em metástases seja vista quando analisada entre amostras de sítio primário em comparação com amostras de sítio metastático dos mesmos pacientes.

Um maior entendimento da função de ZEB1 em meduloblastoma poderia ser alcançado a partir da análise da expressão da proteína em amostras de meduloblastoma. É sabido que ZEB1 pode sofrer alterações pós-transcricionais como fosforilação, sumoilação, ubiquitinação e deubiquitinação, que podem alterar a função de ZEB1 na progressão do câncer (Park et al., 2022) , por isso tais análises poderiam elucidar a relação de ZEB1 em metástases de meduloblastoma. Em contrapartida, para tais análises utilizando amostras biológicas de tumores, enfrentaríamos a limitação do tamanho amostral, já que a incidência de meduloblastoma é baixa e seria necessário um longo período de tempo para reunir tamanho amostral significativo para análises estatísticas mais robustas. Nesse sentido, a análise de banco de dados abordadas neste trabalho foi adequada ao tumor estudado.

Durante as análises deste trabalho, nenhum resultado estatisticamente significativo de sobrevida foi observado no subgrupo molecular SHH, apesar de um de apresentar a maior expressão de ZEB1 dentre os subgrupos moleculares. Um dos marcadores que conferem agressividade aos tumores desse subgrupo é a presença do gene TP53 mutado (Sursal et al., 2022). Da forma que os dados foram adquiridos, através do portal R2 (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi?open_page=login), não foi disponibilizado o dado da mutação de TP53. Especulamos que uma análise de SHH com estratificação do status de TP53 possa revelar alguma relação com a sobrevida e o status de TP53.

A relação de ZEB1 e miRNAs tem sido investigada em células tronco tumorais - células que podem ser responsáveis pela resistência tumoral e recidiva. A expressão de ZEB1 a nível de proteína foi inibida por miR-101, entre outros miRNAs testados. É importante destacar que esse resultado não foi visualizado quando a expressão de ZEB1 foi acessada a nível de mRNA, ressaltando a importância de avaliar a expressão de ZEB1 nesses dois níveis, se tratando de regulação via miRNAs (Ferreira et al., 2023).

No artigo de revisão publicado, foi sintetizada a relação de ZEB1 com miRNAs e RNAs longos não codificantes, tanto em cenários fisiológicos - durante o desenvolvimento - quanto nos cenários oncológicos. Os dois miRNAs identificados apresentaram expressão diferenciada dentre os subgrupos de meduloblastoma. Apenas o miRNA-101 apresentou relação com a sobrevida no Grupo 4. Como esperado, de acordo com a relação inibitória com ZEB1, a maior expressão desse miRNA foi encontrada em pacientes com melhor prognóstico. A relação oposta foi encontrada em ZEB1, onde pacientes com maior expressão de ZEB1 apresentaram pior prognóstico. Experimentos inibitórios da expressão de miR-101 e miR-148a e avaliação da expressão de ZEB1 são necessários para validar essas relações em meduloblastoma.

A epigenética está intimamente ligada a hábitos de vida e fatores externos como estresse, dieta e exercícios, consumo de álcool e tabaco que regulam histona deacetilases,DNA transferases e DNA-metiltransferases e alteram a expressão gênica (Galkin et al., 2023; Daniel & Tollefsbol, 2015). Fármacos que agem em mecanismos epigenéticos atuam em múltiplos alvos e têm sido empregados no tratamento de doenças como malária, depressão, ansiedade, doenças cardiovasculares e tumores (Gladkova et al., 2023). Entre os fármacos, estão os inibidores de histona deacetilases como o mocetinostat, que já demonstrou reverter o fenótipo resistente em células tumorais, via indução da expressão de miRNAs da família miR-200 e repressão de ZEB1 (Meidhof et al., 2015).

Em trabalhos anteriores do nosso grupo, já foi visto que o fingolimod, utilizado no tratamento de esclerose múltipla refratária, também desempenha o papel de inibidor de histona deacetilase em meduloblastoma, além de reduzir a viabilidade de células deste tumor (Perla et al., 2020). Em pacientes de esclerose múltipla, foi visto que o fingolimod aumenta a expressão de miRNAs neuroprotetores (Vargas-Medrano et al., 2019). No artigo “ZEB1 is a Subgroup-Specific Marker of Prognosis and Potential Drug Target in Medulloblastoma”, fruto dessa tese, foi visto que o fingolimod reduz a expressão de ZEB1 em linhagem celular do subgrupo molecular Grupo 3, resultado esse já publicado em 2018 (Garner et al., 2018). São necessárias novas análises para verificar se o fingolimod é capaz de modular a expressão de miR-101 e de miR-148a.

O ensaio clínico de fase I com análogo de miR-34a, em pacientes oncológicos, teve encerramento precoce devido a eventos adversos sérios de ordem imunológica (Hong et al., 2020). Para o tratamento de hepatite C, o ensaio clínico com o análogo de miR-122 está em fase II. Além disso, há análogos de miRNAs em ensaios pré-clínicos e de fase I para nefropatias, doenças vasculares e linfomas (Chakraborty et al., 2021) . O tratamento com análogos de miRNAs que inibam ZEB1 pode representar uma opção viável para futuras terapias em meduloblastoma.

8. CONCLUSÃO

De forma inédita, esse trabalho mostrou que ZEB1 é mais expresso em amostras de meduloblastoma do que em amostras de cerebelo humano Também pela primeira fez, foi demonstrado que ZEB1 tem relação com a sobrevida de diferentes subgrupos moleculares de meduloblastoma. A relação foi significativa nos grupos moleculares 3 e 4, que apresentam pior prognóstico clínico quando comparados aos demais grupos WNT e SHH. Ademais, a expressão de ZEB1 foi acessada de modo mais robusto em um banco de dados diferente do que já existia na literatura, corroborando com os resultados já publicados.

No cenário metastático, apesar de amostras positivas para metástases apresentarem expressão menor de ZEB1 quando comparadas a amostras de meduloblastoma sem metástases, quando avaliados genes alvo e co-repressores, não foi observado a formação de clusters. Essa relação em cenário metastático precisa ser mais bem avaliada a nível de proteína pois, dado o papel de RNAs não codificantes em ZEB1, pode haver discrepâncias entre os níveis de expressão de ZEB1 em bancos de dados de RNA seq e dados de bancos de proteína. Sobre os microRNAs identificados no banco de dados de amostras metastáticas e primárias, experimentos inibitórios com miR-101, miR-148a e ZEB1 são necessários para entender se há relação causal em meduloblastoma.

A revisão de literatura demonstrando o importante papel de ZEB1 e RNAs não codificantes durante o desenvolvimento de diversos tecidos e órgãos evidencia ZEB1 como possível ator em oncologia pediátrica, uma vez que tumores pediátricos muitas vezes emergem de alguma alteração no desenvolvimento normal de tecidos.

9. REFERÊNCIAS

Aghdassi, A., Sendler, M., Guenther, A., Mayerle, J., Behn, C.-O., Heidecke, C.-D., Friess, H., Büchler, M., Evert, M., Lerch, M. M., & Weiss, F. U. (2012). Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer. *Gut*, *61*(3), 439–448. https://doi.org/10.1136/gutjnl-2011-300060

Bharambe HS, Paul R, Panwalkar P, Jalali R, Sridhar E, Gupta T, Moiyadi A, Shetty P, Kazi S, Deogharkar A, Masurkar S, Yogi K, Kunder R, Gadewal N, Goel A, Goel N, Chinnaswamy G, Ramaswamy V, Shirsat NV.(2019). Downregulation of miR-204 expression defines a highly aggressive subset of Group 3/Group 4 medulloblastomas. Acta Neuropathol Commun. Apr 3;7(1):52. doi: 10.1186/s40478-019-0697-3.

Borowska, A., & Jóźwiak, J. (2016). Medulloblastoma: molecular pathways and histopathological classification. *Archives of Medical Science*, *3*, 659–666. https://doi.org/10.5114/aoms.2016.59939

Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Especializada e Temáticas. Protocolo de diagnóstico precoce do câncer pediátrico [recurso eletrônico] / Ministério da Saúde, Secretaria de Atenção à Saúde, Departamento de Atenção Especializada e Temáticas. – Brasília : Ministério da Saúde, 2017. 29 p. https://bvsms.saude.gov.br/bvs/publicacoes/protocolo_diagnostico_precoce_cancer_pediatrico.pdf Acessado em 04 de fevereiro de 2024.

Chakraborty, C., Sharma, A. R., Sharma, G., & Lee, S.-S. (2021). Therapeutic advances of miRNAs: A preclinical and clinical update. *Journal of Advanced Research*, *28*, 127–138. https://doi.org/10.1016/j.jare.2020.08.012

Cho, Y.-J., Tsherniak, A., Tamayo, P., Santagata, S., Ligon, A., Greulich, H., Berhoukim, R., Amani, V., Goumnerova, L., Eberhart, C. G., Lau, C. C., Olson, J. M., Gilbertson, R. J., Gajjar, A., Delattre, O., Kool, M., Ligon, K., Meyerson, M., Mesirov, J. P., & Pomeroy, S. L. (2011).

Integrative Genomic Analysis of Medulloblastoma Identifies a Molecular Subgroup That Drives Poor Clinical Outcome. *Journal of Clinical Oncology*, *29*(11), 1424–1430. https://doi.org/10.1200/JCO.2010.28.5148

Choi, J. Y. (2023). Medulloblastoma: Current Perspectives and Recent Advances. *Brain Tumor Research and Treatment*, *11*(1), 28. https://doi.org/10.14791/btrt.2022.0046

Daniel, M., & Tollefsbol, T. O. (2015). Epigenetic linkage of aging, cancer and nutrition. *Journal of Experimental Biology*, *218*(1), 59–70. https://doi.org/10.1242/jeb.107110

Dutra, L. F (2018). Modulação de ZEB1 em células de meduloblastoma humano. Dissertação de mestrado, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre.

Drápela, S., Bouchal, J., Jolly, M. K., Culig, Z., & Souček, K. (2020). ZEB1: A Critical Regulator of Cell Plasticity, DNA Damage Response, and Therapy Resistance. *Frontiers in Molecular Biosciences*, *7*. https://doi.org/10.3389/fmolb.2020.00036

Fang, F. Y., Rosenblum, J. S., Ho, W. S., & Heiss, J. D. (2022). New Developments in the Pathogenesis, Therapeutic Targeting, and Treatment of Pediatric Medulloblastoma. *Cancers*, *14*(9), 2285. https://doi.org/10.3390/cancers14092285

Ferreira, L. A. M., Bezerra, M. A. dos S., Kawasaki-Oyama, R. S., Fernandes, G. M. de M., Castanhole-Nunes, M. M. U., Serafim Junior, V., Castilho, R. M., Pavarino, É. C., Maniglia, J. V., & Goloni-Bertollo, E. M. (2023). Effect of ZEB1 Associated with microRNAs on Tumor Stem Cells in Head and Neck Cancer. *International Journal of Molecular Sciences*, *24*(6), 5916. https://doi.org/10.3390/ijms24065916

Fischer, A. N., Roecker, R., Saba da Silva, N., Cavalheiro, S., Finlay, J. L., Cappellano, A., & Osorio, D. S. (2021). Validated quantitative needs assessment differences in the management of children with central nervous system cancer between Brazil, an upper

middle-income country, and the United States of America, a high income country. *Pediatric Blood & Cancer*, *68*(6). https://doi.org/10.1002/pbc.28958

Fratini, L., Jaeger, M., de Farias, C. B., Brunetto, A. T., Brunetto, A. L., Shaw, L., & Roesler, R. (2021). Oncogenic functions of ZEB1 in pediatric solid cancers: interplays with microRNAs and long noncoding RNAs. *Molecular and Cellular Biochemistry*, *476*(11), 4107–4116. https://doi.org/10.1007/s11010-021-04226-x

Fults, D. W., Taylor, M. D., & Garzia, L. (2019). Leptomeningeal dissemination: a sinister pattern of medulloblastoma growth. *Journal of Neurosurgery: Pediatrics*, *23*(5), 613–621. https://doi.org/10.3171/2018.11.PEDS18506

Galkin, F., Kovalchuk, O., Koldasbayeva, D., Zhavoronkov, A., & Bischof, E. (2023). Stress, diet, exercise: Common environmental factors and their impact on epigenetic age. *Ageing Research Reviews*, *88*, 101956. https://doi.org/10.1016/j.arr.2023.101956

Garner EF, Williams AP, Stafman LL, Aye JM, Mroczek-Musulman E, Moore BP, Stewart JE, Friedman GK, Beierle EA. (2018). FTY720 Decreases Tumorigenesis in Group 3 Medulloblastoma Patient-Derived Xenografts. Sci Rep; 8(1):6913. doi: 10.1038/s41598-018-25263-5.

Gibson, P., Tong, Y., Robinson, G., Thompson, M. C., Currle, D. S., Eden, C., Kranenburg, T. A., Hogg, T., Poppleton, H., Martin, J., Finkelstein, D., Pounds, S., Weiss, A., Patay, Z., Scoggins, M., Ogg, R., Pei, Y., Yang, Z.-J., Brun, S., … Gilbertson, R. J. (2010). Subtypes of medulloblastoma have distinct developmental origins. *Nature*, *468*(7327), 1095–1099. https://doi.org/10.1038/nature09587

Gladkova, M. G., Leidmaa, E., & Anderzhanova, E. A. (2023). Epidrugs in the Therapy of Central Nervous System Disorders: A Way to Drive on? *Cells*, *12*(11), 1464. https://doi.org/10.3390/cells12111464

Gottardo, N. G., Hansford, J. R., McGlade, J. P., Alvaro, F., Ashley, D. M., Bailey, S., Baker, D. L., Bourdeaut, F., Cho, Y.-J., Clay, M., Clifford, S. C., Cohn, R. J., Cole, C. H., Dallas, P. B., Downie, P., Doz, F., Ellison, D. W., Endersby, R., Fisher, P. G., … Gajjar, A. (2014). Medulloblastoma Down Under 2013: a report from the third annual meeting of the International Medulloblastoma Working Group. *Acta Neuropathologica*, *127*(2), 189–201. https://doi.org/10.1007/s00401-013-1213-7

Han, L., Zhang, Y., Zhao, B., Yue, J., Chen, Z., Lei, G., Huang, C., & Chen, W. (2022). MicroRNA 101 Attenuated NSCLC Proliferation through IDH2/HIFα Axis Suppression in the Warburg Effect. *Oxidative Medicine and Cellular Longevity*, *2022*, 1–12. https://doi.org/10.1155/2022/4938811

Hatten, M. E., & Roussel, M. F. (2011). Development and cancer of the cerebellum. *Trends in Neurosciences*, *34*(3), 134–142. https://doi.org/10.1016/j.tins.2011.01.002

Hill, R. M., Richardson, S., Schwalbe, E. C., Hicks, D., Lindsey, J. C., Crosier, S., Rafiee, G., Grabovska, Y., Wharton, S. B., Jacques, T. S., Michalski, A., Joshi, A., Pizer, B., Williamson, D., Bailey, S., & Clifford, S. C. (2020). Time, pattern, and outcome of medulloblastoma relapse and their association with tumour biology at diagnosis and therapy: a multicentre cohort study. *The Lancet Child & Adolescent Health*, *4*(12), 865–874. https://doi.org/10.1016/S2352-4642(20)30246-7

Hong, D. S., Kang, Y.-K., Borad, M., Sachdev, J., Ejadi, S., Lim, H. Y., Brenner, A. J., Park, K., Lee, J.-L., Kim, T.-Y., Shin, S., Becerra, C. R., Falchook, G., Stoudemire, J., Martin, D., Kelnar, K., Peltier, H., Bonato, V., Bader, A. G., … Beg, M. S. (2020). Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *British Journal of Cancer*, *122*(11), 1630–1637. https://doi.org/10.1038/s41416-020-0802-1

Horny, K., Sproll, C., Peiffer, L., Furtmann, F., Gerhardt, P., Gravemeyer, J., Stoecklein, N. H., Spassova, I., & Becker, J. C. (2023). Mesenchymal–epithelial transition in lymph node

metastases of oral squamous cell carcinoma is accompanied by ZEB1 expression. *Journal of Translational Medicine*, *21*(1), 267. https://doi.org/10.1186/s12967-023-04102-w

INCA, 2022. Instituto Nacional de Câncer (Brasil). Estimativa 2023 : incidência de câncer no Brasil / Instituto Nacional de Câncer. – Rio de Janeiro. Disponível em https://www.inca.gov.br/sites/ufu.sti.inca.local/files/media/document/estimativa-2023.pdf

Juraschka, K., & Taylor, M. D. (2019). Medulloblastoma in the age of molecular subgroups: a review. *Journal of Neurosurgery: Pediatrics*, *24*(4), 353–363. https://doi.org/10.3171/2019.5.PEDS18381

Kim, J. Y., Cho, K. H., Jeong, B. Y., Park, C. G., & Lee, H. Y. (2020). Zeb1 for RCP-induced oral cancer cell invasion and its suppression by resveratrol. *Experimental & Molecular Medicine*, *52*(7), 1152–1163. https://doi.org/10.1038/s12276-020-0474-1

Kool, M., Jones, D. T. W., Jäger, N., Northcott, P. A., Pugh, T. J., Hovestadt, V., Piro, R. M., Esparza, L. A., Markant, S. L., Remke, M., Milde, T., Bourdeaut, F., Ryzhova, M., Sturm, D., Pfaff, E., Stark, S., Hutter, S., Şeker-Cin, H., Johann, P., … Pfister, S. M. (2014). Genome Sequencing of SHH Medulloblastoma Predicts Genotype-Related Response to Smoothened Inhibition. *Cancer Cell*, *25*(3), 393–405. https://doi.org/10.1016/j.ccr.2014.02.004

Krebs, A. M., Mitschke, J., Lasierra Losada, M., Schmalhofer, O., Boerries, M., Busch, H., Boettcher, M., Mougiakakos, D., Reichardt, W., Bronsert, P., Brunton, V. G., Pilarsky, C., Winkler, T. H., Brabletz, S., Stemmler, M. P., & Brabletz, T. (2017). The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. *Nature Cell Biology*, *19*(5), 518–529. https://doi.org/10.1038/ncb3513

Kunder, R., Jalali, R., Sridhar, E., Moiyadi, A., Goel, N., Goel, A., Gupta, T., Krishnatry, R., Kannan, S., Kurkure, P., Deopujari, C., Shetty, P., Biyani, N., Korshunov, A., Pfister, S. M., Northcott, P. A., & Shirsat, N. V. (2013). Real-time PCR assay based on the differential expression of microRNAs and protein-coding genes for molecular classification of formalin-

fixed paraffin embedded medulloblastomas. *Neuro-Oncology*, *15*(12), 1644–1651. https://doi.org/10.1093/neuonc/not123

Lafay-Cousin, L., Baroni, L., Ramaswamy, V., & Bouffet, E. (2022). How do we approach the management of medulloblastoma in young children? *Pediatric Blood & Cancer*, *69*(10). https://doi.org/10.1002/pbc.29838

Laneve, P., & Caffarelli, E. (2020). The Non-coding Side of Medulloblastoma. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.00275

Li, T., Zhang, G., Li, W., Xiao, J., Zhou, Z., Tan, G., & Ai, J. (2023). MicroRNA-101-3p inhibits nasopharyngeal carcinoma cell proliferation and cisplatin resistance through ZIC5 down-regulation by targeting SOX2. *Biological Chemistry*, *0*(0). https://doi.org/10.1515/hsz-2022-0329

MacFarlane, L.-A., & R. Murphy, P. (2010). MicroRNA: Biogenesis, Function and Role in Cancer. *Current Genomics*, *11*(7), 537–561. https://doi.org/10.2174/138920210793175895

Madany, M., Thoma, T., & Edwards, L. A. (2018). The Curious Case of ZEB1. *Discoveries*, *6*(4), e86. https://doi.org/10.15190/d.2018.7

Mahapatra S, Amsbaugh MJ. Medulloblastoma. 2023 Feb 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 28613723.

Meidhof, S., Brabletz, S., Lehmann, W., Preca, B., Mock, K., Ruh, M., Schüler, J., Berthold, M., Weber, A., Burk, U., Lübbert, M., Puhr, M., Culig, Z., Wellner, U., Keck, T., Bronsert, P., Küsters, S., Hopt, U. T., Stemmler, M. P., & Brabletz, T. (2015). ZEB1-associated drug resistance in cancer cells is reversed by the class I HDAC inhibitor mocetinostat. *EMBO Molecular Medicine*, *7*(6), 831–847. https://doi.org/10.15252/emmm.201404396

Menyhárt, O., & Győrffy, B. (2020). Molecular stratifications, biomarker candidates and new therapeutic options in current medulloblastoma treatment approaches. *Cancer and Metastasis Reviews*, *39*(1), 211–233. https://doi.org/10.1007/s10555-020-09854-1

Mesbahi, T., Zaine, H., Mahazou Abdou, I., Chekrine, T., Sahraoui, S., Karkouri, M., & Lakhdar, A. (2022). Glioblastoma Following Treated Medulloblastoma After 29 Years in the Posterior Fossa: Case Report and Review of Literature. *Frontiers in Oncology*, *12*. https://doi.org/10.3389/fonc.2022.760011

Miranda Kuzan-Fischer, C., Juraschka, K., & Taylor, M. D. (2018). Medulloblastoma in the Molecular Era. *Journal of Korean Neurosurgical Society*, *61*(3), 292–301. https://doi.org/10.3340/jkns.2018.0028

Mohammadpour, S., Esfahani, A., Khorasaniasl, S., Karimpour, R., Bakhshian, F., Moradi, A., & Nazemalhosseini-Mojarad, E. (2022). High expression of ZEB1 is associated with EMAST & metastasis in colorectal cancer patients. *Indian Journal of Medical Research*, *156*(1), 64. https://doi.org/10.4103/ijmr.IJMR_1062_20

Oigman, G., Osorio, D. S., Ferman, S., Stanek, J. R., Aversa do Souto, A., Christiani, M. M. C., Magalhaes, D. M. A., Finlay, J. L., & Vianna, D. A. (2022). Epidemiological characteristics and survival outcomes of children with medulloblastoma treated at the National Cancer Institute (INCA) in Rio de Janeiro, Brazil. *Pediatric Blood & Cancer*, *69*(1). https://doi.org/10.1002/pbc.29274

Park, M. K., Lee, H., & Lee, C. H. (2022). Post-Translational Modification of ZEB Family Members in Cancer Progression. *International Journal of Molecular Sciences*, *23*(23), 15127. https://doi.org/10.3390/ijms232315127

Peinado, H., Olmeda, D., & Cano, A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nature Reviews Cancer*, *7*(6), 415–428. https://doi.org/10.1038/nrc2131

Perez-Oquendo, M., & Gibbons, D. L. (2022). Regulation of ZEB1 Function and Molecular Associations in Tumor Progression and Metastasis. *Cancers*, *14*(8), 1864. https://doi.org/10.3390/cancers14081864

Perla, A. S., Fratini, L., Cardoso, P. S., de Farias, C. B., da Cunha Jaeger, M., & Roesler, R. (2020). Fingolimod (FTY720) reduces viability and survival and increases histone H3 acetylation in medulloblastoma cells. *Pediatric Hematology and Oncology*, *37*(2), 170–175. https://doi.org/10.1080/08880018.2019.1699213

Postigo, A. A., Depp, J. L., Taylor, J. J., & Kroll, K. L. (2003). Regulation of Smad signaling through a differential recruitment of coactivators and corepressors by ZEB proteins. *The EMBO Journal*, *22*(10), 2453–2462. https://doi.org/10.1093/emboj/cdg226

Preca, B.-T., Bajdak, K., Mock, K., Sundararajan, V., Pfannstiel, J., Maurer, J., Wellner, U., Hopt, U. T., Brummer, T., Brabletz, S., Brabletz, T., & Stemmler, M. P. (2015). A self-enforcing CD44s/ZEB1 feedback loop maintains EMT and stemness properties in cancer cells. *International Journal of Cancer*, *137*(11), 2566–2577. https://doi.org/10.1002/ijc.29642

Ramaswamy, V., Remke, M., Bouffet, E., Bailey, S., Clifford, S. C., Doz, F., Kool, M., Dufour, C., Vassal, G., Milde, T., Witt, O., von Hoff, K., Pietsch, T., Northcott, P. A., Gajjar, A., Robinson, G. W., Padovani, L., André, N., Massimino, M., … Pomeroy, S. L. (2016). Risk stratification of childhood medulloblastoma in the molecular era: the current consensus. *Acta Neuropathologica*, *131*(6), 821–831. https://doi.org/10.1007/s00401-016-1569-6

Ramjan, S., Levitch, C., Sands, S., Kim, S. Y., Barnett, M., Bledsoe, J., & Holland, A. A. (2023). Executive and social functioning in pediatric posterior fossa tumor survivors and healthy controls. *Neuro-Oncology Practice*, *10*(2), 152–161. https://doi.org/10.1093/nop/npac090

Richardson, S., Hill, R. M., Kui, C., Lindsey, J. C., Grabovksa, Y., Keeling, C., Pease, L., Bashton, M., Crosier, S., Vinci, M., André, N., Figarella-Branger, D., Hansford, J. R., Lastowska, M., Zakrzewski, K., Jorgensen, M., Pickles, J. C., Taylor, M. D., Pfister, S. M., … Clifford, S. C. (2022). Emergence and maintenance of actionable genetic drivers at medulloblastoma relapse. *Neuro-Oncology*, *24*(1), 153–165. https://doi.org/10.1093/neuonc/noab178

Roussel, M. F., & Stripay, J. L. (2018). Epigenetic Drivers in Pediatric Medulloblastoma. *The Cerebellum*, *17*(1), 28–36. https://doi.org/10.1007/s12311-017-0899-9

Ruggi, A., Melchionda, F., Sardi, I., Pavone, R., Meneghello, L., Kitanovski, L., Zaletel, L. Z., Farace, P., Zucchelli, M., Scagnet, M., Toni, F., Righetto, R., Cianchetti, M., Prete, A., Greto, D., Cammelli, S., Morganti, A. G., & Rombi, B. (2022). Toxicity and Clinical Results after Proton Therapy for Pediatric Medulloblastoma: A Multi-Centric Retrospective Study. *Cancers*, *14*(11), 2747. https://doi.org/10.3390/cancers14112747

Sánchez-Tilló, E., Lázaro, A., Torrent, R., Cuatrecasas, M., Vaquero, E. C., Castells, A., Engel, P., & Postigo, A. (2010). ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. *Oncogene*, *29*(24), 3490–3500. https://doi.org/10.1038/onc.2010.102

Sharma, S., Kelly, T. K., & Jones, P. A. (2010). Epigenetics in cancer. *Carcinogenesis*, *31*(1), 27–36. https://doi.org/10.1093/carcin/bgp220

Singh, S., Howell, D., Trivedi, N., Kessler, K., Ong, T., Rosmaninho, P., Raposo, A. A., Robinson, G., Roussel, M. F., Castro, D. S., & Solecki, D. J. (2016). Zeb1 controls neuron differentiation and germinal zone exit by a mesenchymal-epithelial-like transition. *ELife*, *5*. https://doi.org/10.7554/eLife.12717

Skowron, P., Ramaswamy, V., & Taylor, M. D. (2015). Genetic and molecular alterations across medulloblastoma subgroups. *Journal of Molecular Medicine*, *93*(10), 1075–1084. https://doi.org/10.1007/s00109-015-1333-8

Soleymani, L., Zarrabi, A., Hashemi, F., Hashemi, F., Zabolian, A., Banihashemi, S. M., Moghadam, S. S., Hushmandi, K., Samarghandian, S., Ashrafizadeh, M., & Khan, H. (2021). Role of ZEB Family Members in Proliferation, Metastasis, and Chemoresistance of Prostate Cancer Cells: Revealing Signaling Networks. *Current Cancer Drug Targets*, *21*(9), 749–767. https://doi.org/10.2174/1568009621666210601114631

Suh, Y., Yoon, C.-H., Kim, R.-K., Lim, E.-J., Oh, Y. S., Hwang, S.-G., An, S., Yoon, G., Gye, M. C., Yi, J.-M., Kim, M.-J., & Lee, S.-J. (2013). Claudin-1 induces epithelial–mesenchymal transition through activation of the c-Abl-ERK signaling pathway in human liver cells. *Oncogene*, *32*(41), 4873–4882. https://doi.org/10.1038/onc.2012.505

Sursal, T., Ronecker, J. S., DicpinigaitisI, A. J., Mohan, A. L., Tobias, M. E., Gandhi, C. D., & Jhanwar-Unyal, M. (2022). Molecular Stratification of Medulloblastoma: Clinical Outcomes and Therapeutic Interventions. *Anticancer Research*, *42*(5), 2225–2239. https://doi.org/10.21873/anticanres.15703

Vandewalle, C., van Roy, F., & Berx, G. (2009). The role of the ZEB family of transcription factors in development and disease. *Cellular and Molecular Life Sciences*, *66*(5), 773–787. https://doi.org/10.1007/s00018-008-8465-8

Vargas-Medrano, J., Yang, B., Garza, N. T., Segura-Ulate, I., & Perez, R. G. (2019). Up-regulation of protective neuronal MicroRNAs by FTY720 and novel FTY720-derivatives. *Neuroscience Letters*, *690*, 178–180. https://doi.org/10.1016/j.neulet.2018.10.040

Wang, H., Xiao, R., & Yang, B. (2021). MiR-101-3p Suppresses Progression of Cervical Squamous Cell Carcinoma by Targeting and Down-Regulating KPNA2. *Technology in*

Cancer Research & Treatment, *20*, 153303382110559. https://doi.org/10.1177/15330338211055948

Wang, H., Xiao, Z., Zheng, J., Wu, J., Hu, X.-L., Yang, X., & Shen, Q. (2019). ZEB1 Represses Neural Differentiation and Cooperates with CTBP2 to Dynamically Regulate Cell Migration during Neocortex Development. *Cell Reports*, *27*(8), 2335-2353.e6. https://doi.org/10.1016/j.celrep.2019.04.081

Winter, J., Jung, S., Keller, S., Gregory, R. I., & Diederichs, S. (2009). Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nature Cell Biology*, *11*(3), 228–234. https://doi.org/10.1038/ncb0309-228

Xue, P., Huang, S., Han, X., Zhang, C., Yang, L., Xiao, W., Fu, J., Li, H., & Zhou, Y. (2022). Exosomal miR-101-3p and miR-423-5p inhibit medulloblastoma tumorigenesis through targeting FOXP4 and EZH2. *Cell Death & Differentiation*, *29*(1), 82–95. https://doi.org/10.1038/s41418-021-00838-4

Yang, S. Y., Choi, S. A., Lee, J. Y., Park, A.-K., Wang, K.-C., Phi, J. H., Koh, E. J., Park, W.-Y., Park, S.-H., Hwang, D. W., Jung, H. W., & Kim, S.-K. (2015). miR-192 suppresses leptomeningeal dissemination of medulloblastoma by modulating cell proliferation and anchoring through the regulation of *DHFR* , integrins, and *CD47*. *Oncotarget*, *6*(41), 43712–43730. https://doi.org/10.18632/oncotarget.6227

Yogi, K., Sridhar, E., Goel, N., Jalali, R., Goel, A., Moiyadi, A., Thorat, R., Panwalkar, P., Khire, A., Dasgupta, A., Shetty, P., & Shirsat, N. V. (2015). MiR-148a, a microRNA upregulated in the WNT subgroup tumors, inhibits invasion and tumorigenic potential of medulloblastoma cells by targeting Neuropilin 1. *Oncoscience*, *2*(4), 334–348. https://doi.org/10.18632/oncoscience.137

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4. **Experiência profissional ou didática anterior**

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5. **Artigos completos publicados**

JAEGER, MARIANE ; GHISLENI, EDUARDA C. ; **FRATINI, LÍVIA** ; BRUNETTO, ALGEMIR L. ; GREGIANIN, LAURO JOSÉ ; BRUNETTO, ANDRÉ T. ; SCHWARTSMANN, GILBERTO ; DE FARIAS, CAROLINE B. ; ROESLER, RAFAEL .

Viability of D283 medulloblastoma cells treated with a histone deacetylase inhibitor combined with bombesin receptor antagonists. Child's Nervous System (Online), v. 32, p. 61-64, 2015.

PERLA, ALEXANDRE S. ; **FRATINI, LÍVIA** ; CARDOSO, PAULA S. ; DE FARIAS, CAROLINE BRUNETTO ; DA CUNHA JAEGER, MARIANE ; ROESLER, RAFAEL . Fingolimod (FTY720) reduces viability and survival and increases histone H3 acetylation in medulloblastoma cells. Pediatric Hematology-Oncology, v. 37, p. 170-175, 2020.

PERLA, ALEXANDRE ; **FRATINI, LÍVIA** ; CARDOSO, PAULA S. ; NÖR, CAROLINA ; BRUNETTO, ANDRÉ T. ; BRUNETTO, ALGEMIR L. ; DE FARIAS, CAROLINE BRUNETTO ; JAEGER, MARIANE ; ROESLER, RAFAEL . Histone Deacetylase Inhibitors in Pediatric Brain Cancers: Biological Activities and Therapeutic Potential. FRONTIERS IN CELL AND DEVELOPMENTAL BIOLOGY, v. 8, p. 546, 2020

FRATINI, LÍVIA; JAEGER, MARIANE ; DE FARIAS, CAROLINE BRUNETTO ; BRUNETTO, ANDRÉ T. ; BRUNETTO, ALGEMIR L. ; SHAW, LISA ; ROESLER, RAFAEL . Oncogenic functions of ZEB1 in pediatric solid cancers: interplays with microRNAs and long noncoding RNAs. MOLECULAR AND CELLULAR BIOCHEMISTRY, v. 476, p. 4107-4116, 2021.

FRATINI, LIVIA; DALMOLIN, MATHEUS GIBEKE SIQUEIRA ; SINIGAGLIA, MARIALVA ; DA SILVEIRA PERLA, ALEXANDRE ; DE FARIAS, CAROLINE BRUNETTO ; BRUNETTO, ALGEMIR L. ; BRUNETTO, ANDRÉ T. ; DA CUNHA JAEGER, MARIANE ; ROESLER, RAFAEL . ZEB1 is a Subgroup-Specific Marker of Prognosis and Potential Drug Target in Medulloblastoma. NEUROMOLECULAR MEDICINE, v. 1, p. 1, 2022

6. Resumos e trabalhos apresentados em congressos

FRATINI, LÍVIA; Dalmolin, M. ; Sinigaglia, M. ; Jaeger, M. ; de Farias, C. B. ; Roesler, R. . ZEB1 expression in medulloblastoma: in silico analysis. 2021. (Apresentação de Trabalho/Simpósio).

DUTRA, L. F.; Miyamoto, K. N. ; Gregianin, L. J. ; de Farias, C. B. ; Jaeger, M. ; ROESLER, RAFAEL . Eixo ZEB1-microRNAs e metástases: uma análise in silico em meduloblastoma. 2020. (Apresentação de Trabalho/Congresso).

DUTRA, L. F.; PERLA, ALEXANDRE ; SOUZA, B. K. ; Jaeger, M. ; BRUNETTO, ALGEMIR L. ; de Farias, C. B. ; Roesler, R. . Investigação da expressão de ZEB1 em linhagens celulares de meduloblastoma. 2018. (Apresentação de Trabalho/Congresso).

FRATINI, LÍVIA; RIES, S. ; Jaeger, M. ; Portich, J P ; Meneses, C. F. ; Loss, J. ; GREGIANIN, LAURO JOSÉ ; BRUNETTO, ALGEMIR L. ; ROESLER, RAFAEL ; DE FARIAS, CAROLINE B. . Avaliação da Expressão do Receptor P75NTR em pacientes pediátricos de leucemia linfocítica aguda. 2017. (Apresentação de Trabalho/Congresso).

DUTRA, L. F.; Perla, A. S. ; Jaeger, M. ; Thomaz, A. C. G. ; Ghisleni, E. C. ; Brunetto, A. T. ; Gregianin, L. J. ; Brunetto, A. L. ; de Farias, C. B. ; Roesler, R. . Efeitos do fingolimod (FTY720) sobre células de meduloblastoma humano. 2016. (Apresentação de Trabalho/Outra).

DUTRA, L. F.; Gil, M. S. ; Santos, R. P. ; Casagrande, P. R. ; Meneses, C. F. ; Loss, J. ; Brunetto, A. L. ; Gregianin, L. J. ; Roesler, R. ; de Farias, C. B. . O Papel de Agonistas e Antagonistas de Neurotrofinas em Leucemias Pediátricas Agudas. 2015. (Apresentação de Trabalho/Outra).

DUTRA, L. F.; Santana, D.B. ; BECKENKAMP, A. ; NASCIMENTO, J. ; BRUNO, A. N. ; PILGER, D. A. ; BUFFON, A. . AVALIAÇÃO DA ECTO-ADENOSINA DEAMINASE

E EFEITO DA ADENOSINA EM LINHAGENS DE CÂNCER CERVICAL. 2013. (Apresentação de Trabalho/Outra).

DUTRA, L. F.; Santana, D.B. ; BECKENKAMP, A. ; BRUNO, A. N. ; Paccez, J. ; Zerbini, L. F. ; WINK, M. ; NASCIMENTO, J. ; BUFFON, A. . Expression profile and biochemical characterization of an ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) in cervical carcinoma cells. 2013. (Apresentação de Trabalho/Congresso).

DUTRA, L. F.. AVALIAÇÃO DA EXPRESSÃO DA DPPIV/CD26 E MECANISMOS TUMORAIS EM CULTURA DE CÉLULAS DE CARCINOMA CERVICAL HUMANO. 2013. (Apresentação de Trabalho/Outra).

7. **Participação em bancas**

FRATINI, LÍVIA. Participação em banca de Tatiane Madeira Reis.Avaliação da influência do estado nutricional em modificações do comprimento telomérico. 2019. Trabalho de Conclusão de Curso (Graduação em Biomedicina) - Universidade Federal do Rio Grande do Sul.