Metabolic determinants of stemness in medulloblastoma

Martín-Rubio O et al. Metabolism of MB stem cells

Paula Martín-Rubio, Pilar Espiau-Romera, Alba Royo-Garcia, Laia Caja, Patricia Sancho

Abstract
Medulloblastomas (MB) are the most prevalent brain tumours in children. They are classified as grade IV, the highest in malignancy with about 30% metastatic tumours at the time of diagnosis. Cancer stem cells (CSCs) are a small subset of tumour cells that can initiate and support tumour growth. In MB, CSCs contribute to MB tumour initiation, metastasis and therapy resistance. Metabolic differences among the different MB groups have started to emerge: Sonic hedgehog tumours show enriched lipid, fatty acid and nucleic acid metabolism pathways; while Group 3-MB upregulate glycolysis, gluconeogenesis, glutamine anabolism and glutathione-mediated anti-oxidant pathways. Such differences impact the clinical behaviour of MB tumours and can be exploited therapeutically. In this review, we summarise the existing knowledge about metabolic rewiring in MB, with a particular focus on MB-CSCs. Finally, we highlight some of the emerging metabolism-based therapeutic strategies for MB.

Key Words: Medulloblastoma; Cancer stem cells; Stemness; Metabolism; Glycolysis; Lipids


Core Tip: Considering the profound cellular metabolism rewiring associated with the tumorigenesis process, metabolic targeting for cancer treatment has gained a lot of attention in the last years. Interestingly, increasing evidence indicates that cancer
stem cells (CSCs) show unique metabolic features within tumours. A deeper understanding of the metabolic vulnerabilities of medulloblastoma (MB) and MB-CSCs would accelerate the design of novel therapeutic strategies with the aim of improving the survival of paediatric patients suffering this disease.

INTRODUCTION

Medulloblastoma (MB) is the most common brain malignancy in young children. MB originates in the cerebellum and spreads via the cerebrospinal fluid to the brain and spine[1-3]. MB patients are classified in four different groups defined by the WHO, based on the developmental pathway involved in oncogenesis[2]: WNT, sonic hedgehog (SHH), Group 3 and Group 4 (Table 1).

The WNT group is the rarest, accounting for 10% of all MB. These tumours occur in children over 3 years or teenagers, and generally, they have very good prognosis. WNT tumours show activating somatic mutations in β-catenin (CTNNB1) or germline mutations in APC, both leading to constitutive WNT signalling[4,5].

SHH group represents 30% of MB patients, most of them either infants (≤ 3 years old) or adults (≥15 years old), with intermediate prognosis[4]. The molecular mechanism regulating this subtype implicates loss-of-function mutations in negative regulators or over-expression of different members of the SHH signalling pathway, such as PTCH1, SMO, GLI and SUFU[5].

Group 3 accounts for tumours with poor prognosis and high metastatic rate, occurring predominantly in males (2:1) and young adults (≥16 years old). These tumours display frequent amplification or overexpression of the MYCN gene, and other genetic events such as OTX2 amplification, SMARCA4 mutation and GFI enhancer activation[4,5].

Group 4 includes predominantly males (3:1), of all ages. These tumours show intermediate prognosis due to its metastatic potential: 30%-40% of Group 4 MB cases are already metastatic at diagnosis[3]. No underlying common cause has been described for Group 4 tumours, but they show chromosome 17 abnormalities and neuronal differentiation transcriptomic profile[4,5].
CANCER STEM CELLS IN MB

The cancer stem cell concept

Cancers are hierarchically organised structures with different levels of intratumoural heterogeneity. Indeed, distinct clone and cancer cell populations co-exist in a tumour. Among them, there is a subset of cancer cells, the so-called cancer stem cells (CSCs), which are at the origin of intratumoural heterogeneity. On the one hand, CSCs display the ability to self-renew, allowing them to preserve their identity as stem cells. On the other hand, these cells maintain intraclonal heterogeneity and give rise to all differentiated progenies within each cancer subclone in the primary tumour. These differentiated progenies have limited or no tumour-initiating and metastatic capacities, despite their high proliferation rate. Additionally, CSCs have an enhanced capacity to drive tumorigenesis and progression, resistance to conventional radio- and chemotherapy, and invasiveness. In fact, they are primarily responsible for tumour metastasis in secondary organs. Indeed, CSCs and tumour-differentiated cells can acquire mobility thanks to processes such as epithelial-to-mesenchymal transition (EMT). Both subpopulations can migrate to other tissues through the bloodstream, but only those with stem-like features will be able eventually to initiate secondary lesions causing metastasis.

Evidence of CSCs was first identified in acute myelogenous leukaemia and was verified subsequently in other diseases such as breast cancer, pancreatic cancer, prostate cancer, as well as in brain tumours like MB. Due to their malignant features, CSCs have become one of the main focuses for cancer therapy, with the aim of improving the understanding of cancer onset and progression, and finding new therapeutic strategies.

The origin of CSCs in the tumorigenesis process remains controversial nowadays, and it seems to be cancer type-specific. In general terms, two hypotheses have been formulated: The “Stem Cell hypothesis” and the “De-differentiation hypothesis”. The "Stem Cell hypothesis" accounts for the similarities observed between normal tissue SCs and CSCs, postulating that malignant transformation driven by frequent genetic mutations in SCs favours the aggressive behaviour of CSCs. The latter
consists of a de-differentiation of tumour cells caused by the acquisition of stem-like abilities and tumour-initiating potential[7,16].

**MB CSCs**

Although the “de-differentiation hypothesis” cannot be completely ruled out, it is clear nowadays that the stem or progenitor populations represent the cell-of-origin of MB tumours and their comprised CSCs (Table 1). Indeed, the main genes and pathways altered in MB (WNT, SHH, Notch, MYC, STAT3) are key controllers of cell cycle and stemness[17]. Early studies demonstrated that MB tumours originate from immature cerebellar granule neuron precursors[18,19] or neural stem cells[20-22] escaping self-renewal restriction upon activation of the SHH pathway. While this still holds true for the SHH subgroup, further studies have determined that the different subgroups arise from distinct developmental origins: Indeed, the WNT group originates from progenitors in the dorsal brainstem[23] and Group 3 tumours derive from cerebellar stem cells[24,25]. However, the origin of Group 4 MB is still under debate.

The original identification of brain CSCs in MB and other tumours involved the expression of the neural stem cell markers CD133 and Nestin[26] (Figure 1), enriching for a subpopulation of cells with enhanced neurosphere formation ability. These results were further developed in a subsequent study demonstrating that injecting as little as 100 CD133+ MB cells produced a tumour resembling the patient’s original tumour[27], definitely linking CD133 expression with CSCs in MB. In further studies, CD133+ MB cells have been characterised by their radiation[26] and chemotherapy[27] resistance, as well as their enhanced invasiveness[28,29]. Importantly, CD133 expression predicts poor prognosis in MB patients[30], associated with higher rates of recurrence and metastasis[27], especially in Group 3.

Besides the well-established role of CD133, the surface marker CD15 (SSEA-1) was found as marker of MB-CSCs in two independent studies using the Patched mutant mouse model of SHH MB (Figure 1)[31]. In both studies, CD15+ cells showed high tumorigenicity in transplantation experiments, giving rise to tumours histologically similar to the original ones. However, these studies contrasted in the definition of
CD15+ cells as progenitor or stem cell-like tumour cells, considering their CD133 expression and their ability to form neurospheres composed by different cellular subpopulations. Interestingly, Sox2+ cells have been shown to form a quiescent subpopulation within CD15+ cells with enhanced tumour-initiating abilities\cite{31,32} and both Sox2+ and CD15+ signatures associated with poor prognosis and lower survival rates in patients\cite{31,32}. Noteworthy, CD15+ signature was not restricted to any specific MB subgroup, suggesting that this marker predicts poor survival in MB patients, in general\cite{31}.

Interestingly, the specific expression pattern of CSC markers can distinguish the functional heterogeneity of MB-CSCs in the less aggressive subtypes such as SHH\cite{29}. Indeed, isolation of diverse clones from the well-studied SHH cell line DAOY, revealed that highly self-renewing cells expressed CD271 (p75 neurotrophin receptor (p75NTR)), while those with enhanced migratory capacity expressed CD133. However, CD271 expression is much lower in aggressive tumours from Groups 3 and 4, and does not allow for functional classification of their MB-CSCs.

Highly proliferative ki67+ CSCs have been also linked to worse prognosis, recurrence and metastasis in MB patients\cite{33}. Although the percentage of cells expressing CD133 was increased only slightly in relapsed tumours, authors found that the most significantly altered parameter in recurring tumours was the proliferative index of CSCs, calculated as the percentage of ki67 positive CSCs, especially in distant metastases.

Additionally, cells with CSC-like properties have been identified in MB patient samples using the side population (SP) technique, which entails the evaluation of the Hoechst 33342 dye exclusion by flow cytometry. Isolated MB-SP cells showed an increased neurosphere formation ability, as well as an overexpression of the stem cell markers Nestin, Notch1 and ABCG2\cite{34}.

**METABOLIC FEATURES OF MB CANCER (STEM) CELLS**

MB tumour cells must be provided not only with the essential metabolites to cover their energy requirements, but also with the macromolecules demanded for tumour growth. Consistent with this, increased lipogenesis and aerobic glycolysis have been
detected in MB\textsuperscript{35}. However, MB metabolic profile is not uniform among the different subtypes (Figure 1)\textsuperscript{36}.

**Lipid metabolism in MB**

SHH oncogenic signalling inhibits fatty acid oxidation while increasing fatty acid synthesis, an early critical step of lipogenesis, to promote tumour grow. Indeed, extensive lipid accumulation and elevated levels of the lipogenic enzyme fatty acid synthase (FASN) were detected in SHH-driven MBs from transgenic mice. In fact, the Rb/E2F tumour suppressor complex acted downstream of SHH, controlling FASN expression, and promoting lipogenesis and tumour growth\textsuperscript{37}. Furthermore, FASN and stearoyl-CoA desaturase (SCD), both with crucial roles in the fatty acid synthesis pathway, were identified as prognostic genes in SHH subgroup, Group 3 and Group 4 of MB (Figure 2)\textsuperscript{38}.

**Glucose metabolism in MB**

In many cancers, energy metabolism shifts from oxidative phosphorylation to aerobic glycolysis for ATP production, phenomenon known as the Warburg effect\textsuperscript{39}. Several studies indicate that this effect also occurs in MB\textsuperscript{40–42}.

High expression levels of the oncogene c-MYC, which is a downstream target of the WNT signalling pathway and it is overexpressed in Group 3 MB, has been related with increased glycolysis in MB\textsuperscript{42–44}. Indeed, MB from Groups 3 and WNT express significantly higher levels of the glycolytic enzyme LDHA downstream c-MYC than non-neoplastic cerebellum (Figure 2). Interestingly, LDHA levels have been linked with poor prognosis in Group 3 MBs\textsuperscript{44}. Besides LDHA, other glycolytic genes, such as hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2), are upregulated both in murine MYC-amplified and human Group 3 MBs. PKM2 upregulation was also associated with a subsequent downregulation of the PKM1 isoform, which promotes oxidative phosphorylation in MYC-amplified MB tumour cells\textsuperscript{43}.

Induction of glycolysis in SHH MBs requires activating both SHH and insulin/IGF/PI3K pathways\textsuperscript{41}. In addition, downstream activation of Myc-Max effector complex is also necessary for HK2 upregulation. Interestingly, rather than
LDHA upregulation, SHH subgroup showed higher levels of LDHB than the other groups\textsuperscript{[42]} suggesting that LDHB could be performing the expected role of LDHA\textsuperscript{[41]}. PPAR\gamma, a key regulator of fatty acid and glucose metabolism, is also upregulated in SHH-induced mouse MBs. This transcription factor, controlled by E2F1, induced the expression of key glycolytic enzymes, including HK2, PKM2, and glucose transporter type 4 (Glut4)\textsuperscript{[40]}.

Although an increased uptake of $^{18}$FDG, a readout of aerobic glycolysis, has been detected in human MB tumours\textsuperscript{[45]} and have been tested in clinical trials for MB detection and follow-up (i.e., NCT00381797), some evidence questions the universality of the Warburg phenotype in MB. On the one hand, an increased expression of genes associated with the mitochondrial respiratory chain and oxidative phosphorylation was detected in human SHH MBs, but not in mouse SHH-MB models. This suggests that murine SHH-MB models do not recapitulate the metabolic features of human MBs\textsuperscript{[48]} faithfully. On the other hand, a recent publication also questions the Warburg effect in MYC-amplified MBs: Higher glucose incorporation into the TCA cycle was detected in orthotopic tumours compared with normal brain, indicating that tumour cells use glycolysis and oxidative phosphorylation simultaneously. In addition, significant differences in glucose and glutamine metabolism were detected in high MYC-amplified MBs comparing in vitro, flank xenografts and orthotopic xenograft MB models, evidencing the importance of using orthotopic models for metabolic analysis in these tumours\textsuperscript{[47]}.

Other metabolic pathways altered in MB

Group 3 MB tumours not only display an enrichment of the glycolytic pathway, but also an upregulation of proteins involved in other metabolic pathways, such as gluconeogenesis (PCK2, PC), glutamine anabolism (GLUL), calcium signalling (Ryr3, CAMK2D), fatty acid synthesis (ACLY), glutathione-mediated antioxidant pathway (PTGDS, GSTZ1) and drug metabolism pathway (IMPDH1, GUSB)\textsuperscript{[48]}. In addition, the up-regulation of the TCA cycle, synthesis of nucleotides, hexosamines, amino acids and glutathione was also detected in MYC-amplified orthotopic
xenograft MBs\textsuperscript{[47]}. This highly metabolic profile could be responsible for the aggressiveness and chemoresistance of Group 3 and MYC-amplified tumours.

Importantly, several metabolic pathways with prognostic value have been identified in MB; this includes alanine, aspartate, and glutamate metabolism in SHH subgroup, pentose phosphate pathway and TCA cycle in Group 3, and one carbon pool by folate in Group 4\textsuperscript{[38]}.

**Metabolism in MB CSCs**

Thanks to recent advances, we know now that cellular metabolism and stemness are highly interconnected in normal development and cancer. Indeed, CSCs from different cancer types show distinct metabolic features when compared with their more differentiated progenies, though their dominant metabolic phenotype varies across tumour entities, patients and even subclones within a tumour\textsuperscript{[49]}. In fact, it has been shown that, depending on the type of cancer, CSCs may be either highly glycolytic or OXPHOS-dependent\textsuperscript{[50]}. Moreover, recent evidence from both pluripotent embryonic and adult stem cell studies suggests that reactive oxygen species (ROS) in synchrony with metabolism mediate the maintenance of CSC\textsuperscript{[51]}. A major role for the metabolic regulator mTOR in stemness maintenance has also been described in MB-CSCs\textsuperscript{[52,53]}. Surprisingly, although MB stem cells are thought to be responsible for chemoresistance and recurrence in this cancer\textsuperscript{[54]}, their metabolic properties have barely been studied.

A decreased oxidative phosphorylation, mitochondria mass and endogenous ROS production have been detected in established radioresistant MB stem-like clones (rMSLCS). These clones showed a higher pyruvate kinase activity and lactate production than parental cells. Mass spectrometry also detected an increased concentration of glycolysis intermediates (3-phosphoglyceric acid, 2-phosphoglyceric acid, phosphoenolpyruvic acid) and amino acids (arginine, glycine, histidine, leucine, lysine, serine) in rMSLCS, as well as decreased levels of NADH and ATP, and dysregulation of TCA cycle intermediates. Additionally, they showed an increased rate of conversion of pyruvic acid into lactic acid, and a lower rate of conversion of pyruvic acid into acetyl CoA, which indicated an increased glycolysis\textsuperscript{[55]}.
MB cells expressing high levels of BMI1 (a key regulator of neural stem cells) and low CHD7 (an ATP-dependent chromatin remodeller), showed a decreased mitochondrial function concomitant with higher glycolytic activity. Moreover, an enhanced expression of glycolytic genes [HK2, PFKP, ENO4, pyruvate dehydrogenase kinase 1 (PDK1) and LDHB] and increased levels of valine and leucine were detected in BMI1\textsuperscript{high} CHD7\textsuperscript{low} Group 3 MB tissues. Since both markers have been previously related to hypoxia, it was proposed that cells with this signature might present advantages in hypoxic conditions thanks to these metabolic adaptations (Figure 1)\textsuperscript{[56].}

Indeed, oxygen restriction in non-vascularised regions may support cell cycle entry and self-renewal \textit{via} Notch1 signalling, since hypoxia is one of the main expansion inducers of the CD133\textsuperscript{+} population\textsuperscript{[26,57].} In contrast, MB-CSCs associated to a more quiescent state have been reported to reside in a perivascular niche\textsuperscript{[58]}, in close interaction with endothelial cells that secrete factors to maintain their stemness.

Finally, it has been demonstrated that autophagy positively regulates stemness in MB cells. Indeed, the expression of the pro-autophagy factor AMBRA1 depends on c-MYC levels, and it is strongly upregulated in Group 3 MB CSCs\textsuperscript{[59].}

In general, all these reports suggest that the metabolic phenotype harboured by MB-CSCs is mainly glycolytic, which is favoured by hypoxia. Both glycolysis and hypoxia generate the anti-oxidant redox status needed to maintain their stemness. Moreover, the elevated availability of macromolecules needed to support their enhanced proliferation may be sustained by biosynthesis derived from glycolysis intermediates and an increased autophagy in these cells.

**TARGETED THERAPIES IN MB**

\textit{Standard therapies for MB}

Current standard treatments for MB include surgical resection of the tumour, craniospinal radiotherapy and systemic chemotherapy. Unfortunately, only 70% of patients survive. In addition, radiotherapy causes neurocognitive, neuroendocrine and psychosocial deficits, as well as the development of secondary malignancies\textsuperscript{[60,61].} Consequently, new treatment strategies for MB are needed, such as targeted
therapies that are less toxic and more effective in reducing the resulting long-term side effects[62].

**Molecular targeted therapies for MB**

Targeted therapies for WNT and SHH MB entail inhibiting the major pathways implicated in their tumourigenesis[62]. As an example, the use of SMOOTHE (SMO) inhibitors in the SHH MB group has been linked to tumour suppression in mouse models, and tumour regression in MB patients[63,64]. On the other hand, research on WNT MBs has focused on nuclear β-catenin inhibitors, leading to reduced cell migration and invasion[64,65].

In Groups 3 and 4 there are no major advances in targeted therapy as they have a low somatic mutation rate[62,64]. Group 3 MB is characterised by MYC oncogene amplification, so some epigenetic MYC inhibitors, such as histone deacetylases (HDACs) and bromodomain inhibitors, have been tested[66,67]. In addition, phosphatidylinositol-3 kinase (PI3K) inhibitors can act in synergy with HDAC modulators, preventing tumour proliferation and prolonging survival in Group 3 MB mice[68].

Apart from subgroup-specific therapies, other molecular approaches linked to the general population of MB patients are being explored nowadays. Selective inhibition of cell division protein kinase (CDK) 4/6 arrests the cell cycle potently and is therefore a favourable target for cancer treatment[69]. Another strategy is related to immunotherapy, as it may be effective against MB innate immunosuppressive properties and thus it may extend patient survival[70].

**Metabolic targeted therapies for MB**

Thoroughly investigated in the context of other cancer types, metabolic reprogramming might be also the source of putative targets for the design of new treatments against MB (Table 2). For example, specifically in lipid metabolism, direct inhibition of FASN by C75 reduced tumour proliferation in SHH tumours and increased survival in vivo, associated to reduced lipogenesis. In addition, C75 may act in synergy with the CDK inhibitor roscovitine[37].
Since glycolysis seems crucial for the metabolic requirements of MB, it may be important to target glycolytic enzymes. In that regard, LDH tetramers inhibition by oxamate attenuated glycolysis, proliferation, and motility significantly in MB cell lines\(^{[42]}\). In addition, LDHA antagonists GSK2837808a and FX11 reduced the growth of Group 3 MB cells\(^{[44]}\). Conditional suppression of HK2 altered both energy homeostasis and the balance between proliferation and differentiation markedly, thereby reducing tumour growth. Similarly, blockade of IL6 by bazedoxifene showed glycolysis downregulation, and impaired viability and proliferation of MB cells\(^{[71]}\). Finally, it is important to mention that pharmacological inhibition of PPARy by GW9662 increases SHH-driven MB cell death and mouse survival in vivo through glycolysis inhibition\(^{[40]}\).

Another interesting therapeutic strategy for MB is based on the inhibition of the metabolic regulator mTOR. Indeed, the TORC1/2 kinase inhibitor TAK228 improved the survival of orthotopic MYC-amplified Group 3 MB tumours through glutathione depletion\(^{[72]}\). In addition, the dual inhibition of PI3Kα and mTOR blocked the sphere-forming ability of SHH-driven MB cells, and repressed tumour growth in vivo\(^{[53]}\). In fact, several clinical trials are currently testing different conventional treatments in combination with mTOR inhibitors, such as Sirolimus (NCT02574728), Temsirolimus (NCT00784914) or Everolimus (NCT03387020) against MB and other brain tumours.

The elevated MYC expression characteristic of Group 3 MB is related to an increased glutamine transport and utilisation. For this reason, the glutamine antagonist 6-diazo-5-oxo-l-norleucine (DON) has been tested as therapy against Group 3 MB, although with little success in clinical trials due to its poor pharmacodynamic properties. Still, the prodrug isopropyl 6-diazo-5-oxo-2-(phenyl(pivoxyloxy) methoxy)-carbonyl) hexanoate (JHU395) has shown an improved cell penetration, enhanced growth reduction and increased apoptosis of Group 3 MB cells in vitro, as well as longer survival of mice in vivo\(^{[71]}\).

Different metabolic targeting approaches related to the toxic effect of ROS accumulation and redox imbalance have also been tested in clinical trials. On the one side, two clinical trials have studied the antitumoral properties of Photodynamic Therapy, which uses light and photosensitizing drugs to kill tumour cells via ROS
accumulation, in MB (NCT00002647, NCT01682746). Unfortunately, no results have been posted to date. On the other hand, the antiprotozoal compound Nifurtimox, with pro-oxidant properties, has also been approved by the Food and Drug Administration for its use in MB. In the Phase II trial NCT00601003, the combination of Nifurtimox with cyclophosphamide and topotecan is being tested as treatment for relapsed or refractory neuroblastoma and MB.

There are other alternative therapeutic approaches targeting MB metabolism currently being tested in clinical trials. On the one hand, the polyamine synthesis inhibitor Diuloromethylornithine (DFMO) is being tested in a phase II clinical trial as maintenance therapy for high molecular risk or very high risk and relapsed or refractory MB (NCT04696029). On the other hand, the combination of Vorinostat and Isotretinoin (13-cis-retinoic acid, vitamin A derivative) has been tested in a phase I clinical trial targeting young patients with recurrent or refractory lymphoma, leukemia or solid tumours such as MB (NCT00217412), although no results have been posted to this day.

**Targeted therapies for MB CSCs**

Though much less studied in the context of MB, metabolic pathways also play a key role in cancer resistance linked to CSCs. Treatment with the PDK inhibitor DCA suppressed CSC-like phenotypes, and increased intracellular ROS levels and radiosensitivity by inhibiting glycolysis and inducing mitochondrial aberrations\[^{55}\]. In addition, the polyphenolic compound curcumin, known to exert antitumor effects in many different cancer types, decreased anchorage-independent clonogenic growth and CD133\(^+\) stem-like population by inhibiting STAT3 and PI3K/AKT pathways\[^{74}\]. Furthermore, direct inhibition of the PI3K-AKT pathway with perifosine resensitised MB CSCs to radiotherapy by reducing CD133\(^+\) MB stem-like subpopulations\[^{75}\]. Inositol hexakisphosphate (IP6) treatment counteracted metabolic adaptation in BMII\(^{+}\) MB cells thus inhibiting their proliferation. Moreover, IP6 synergised with cisplatin, potentiating its cytotoxic activity *in vitro* and *in vivo*, and extending survival of the mice\[^{56}\] significantly. Finally, pharmacological inhibition of the pro-autophagy
factor AMBRA1 affected both the growth and invasion potential of MB group 3 CSCs profoundly, enhanced by the combination of AMBRA1 and STAT3 inhibition[59].

In addition to metabolic targeting, we can also find other checkpoints, such as the new SHH inhibitor chemotype 12 that overcomes the associated SMO receptor drug resistance. It reduced the ability to form spheres in number and size, associated with a reduced expression of stemness markers (NANOG, OCT4), and thus impaired stem-like cell growth \textit{in vitro} and \textit{in vivo}[70]. Moreover, CDK inhibitors, such as ribociclib, can also target CSCs[69]. Oncolytic engineered herpes simplex viruses (oHSV) may also have the potential to eliminate MB-CSCs specifically, as they express the primary HSV-1 entry molecule nectin-1 (CD111) and are shown to be highly sensitive to clinically relevant oHSV \textit{in vitro} and \textit{in vivo}[77].

CONCLUSION

Further research is urgently needed to elucidate the metabolic profile of MB tumours. Recent evidence suggests that metabolic features among MB groups and even among the different cell subpopulations within a tumour are highly heterogeneous. In addition, new data obtained in more clinically relevant models challenge most of the existing knowledge in the field, obtained from genetically modified mouse models. Moreover, the metabolic features of MB-CSCs remain absolutely unknown nowadays. The identification of metabolic vulnerabilities of these tumours and their highly aggressive CSCs could pave the way to the design of new therapeutic strategies improving the current treatment for this paediatric disease.

ACKNOWLEDGEMENTS

We want to thank Laura Sancho for proofreading the manuscript.

Figure Legends

\textbf{Figure 1 Metabolic features of medulloblastoma and medulloblastoma cancer stem cells.} Medulloblastoma (MB) tumours show activation of metabolic pathways such as glycolysis and fatty acid synthesis. MB cancer stem cells (MB-CSCs) display
several markers, including CD133, CD15 and nestin (NES). Increased autophagy and glycolysis have been detected in MB-CSCs compared to more differentiated cells. In addition, hypoxic conditions induce the expansion of MB-CSCs. On the other hand, MB-CSCs show decreased mitochondrial activity and reactive oxygen species. MB: Medulloblastoma; CSCs: Cancer stem cells; ROS: Reactive oxygen species.

Figure 2 Metabolic pathways upregulated in medulloblastoma. Enzymes from glycolysis or fatty acid synthesis pathways upregulated in medulloblastoma (MB) tumours are highlighted in different colours indicating the MB subgroup [Sonic hedgehog (SHH): Grey, WNT: Yellow, Group 3: Green, Group 4: Blue]. Statistically significant prognostic genes are marked with the asterisk (*). Glucose transporter type 4, hexokinase 2, pyruvate kinase M2, lactate dehydrogenase A, ATP Citrate Lyase, fatty acid synthase, stearoyl-CoA desaturase. SHH: Sonic hedgehog; GLUT4: Glucose transporter type 4; HK2: Hexokinase 2; PKM2: Pyruvate kinase M2; LDHA: Lactate dehydrogenase A; ACLY: ATP Citrate Lyase; FASN: Fatty acid synthase; SCD: Stearoyl-CoA desaturase.
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MB: Medulloblastoma; SHH: Sonic hedgehog.
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1 Compounds tested in clinical trials.

MB: Medulloblastoma; MB-CSCs: Medulloblastoma cancer stem cells.
### PRIMARY SOURCES

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<td>&quot;Oxamate, but Not Selective Targeting of LDH-A, Inhibits Medulloblastoma Cell Glycolysis, Growth and Motility&quot;, Brain Sciences, 2018</td>
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<td>&quot;The proteomic analysis shows enrichment of RNA surveillance pathways in adult SHH and extensive metabolic reprogramming in Group 3 medulloblastomas&quot;, Brain Tumor Pathology, 2021</td>
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"Reviews on New Drug Targets in Age-Related Disorders", Springer Science and Business Media LLC, 2021
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André A. Weber, Xiaojing Yang, Elvira Mennillo, Jeffrey Ding et al. "Lactational delivery of Triclosan promotes non-alcoholic fatty liver disease in newborn mice", Cold Spring Harbor Laboratory, 2022

Sara Badodi, Adrian Dubuc, Xinyu Zhang, Gabriel Rosser et al. "Convergence of BMI1 and CHD7 on ERK Signaling in Medulloblastoma", Cell Reports, 2017
