

Review Article

Medulloblastoma stem cells: Promising targets in medulloblastoma therapy

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Medulloblastoma is a primitive neuroepithelial tumor originating from the hindbrain and represents one of the leading causes of pediatric tumor-related death.^(1,2) The 2007 WHO classification defines four histological variants of MB: classic, LCA, desmoplastic/nodular, and MB with extensive nodularity.⁽³⁾ In 2010, several large-scale transcriptional profiling studies led to a consensus that MB contains four distinct major subgroups at the molecular level: Wnt, Shh, Group 3, and Group 4.⁽⁴⁾ Each differs with respect to demographics, histological phenotypes, somatic mutations, and clinical outcomes.⁽⁴⁾ Current clinical practice stratifies a high risk group as follows: infants <3 years old, patients with a post-surgical residual tumor >1.5 cm², LMD at diagnosis, or LCA histology.^(5,6) Indeed, relapsed disease and LMD are still major causes of poor outcomes.⁽⁶⁾ With gradually increasing understanding of MB at the pathological and molecular levels, along with the continuous refinements in clinical risk stratification, therapeutic regimens have immensely improved, and the overall survival rate of MB patients has increased from 20% to approximately 80% in the last 35 years.⁽⁶⁾

Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Despite great improvements in the therapeutic regimen, relapse and leptomeningeal dissemination still pose great challenges to the long-term survival of MB patients. Developing more effective strategies has become extremely urgent. In recent years, a number of malignancies, including MB, have been found to contain a subpopulation of cancer cells known as cancer stem cells (CSCs), or tumor initiating/propagating cells. The CSCs are thought to be largely responsible for tumor initiation, maintenance, dissemination, and relapse; therefore, their pivotal roles have revealed them to be promising targets in MB therapy. Our growing understanding of the major medulloblastoma molecular subgroups and the derivation of some of these groups from specific stem or progenitor cells adds additional layers to the CSC knowledge base. Herein we review the current knowledge of MB stem cells, highlight the molecular mechanisms relating to MB relapse and leptomeningeal dissemination, and incorporate these with the need to develop more effective and accurate therapies for MB patients.

The molecular classification has provided key guidance in the development of new targeted therapies. For example, vismodegib (GDC-0449), an Shh pathway inhibitor targeting Smo, triggers the rapid regression of an MB patient's metastatic tumors, but it is soon followed by relapse due to mutations in Smo causing therapeutic resistance.^(7,8) Molecular classification can also guide the reduced treatment of medulloblastoma patients with less aggressive disease. While aggressive treatment strategies usually result in improved survival, it is unfortunately at the cost of severely affecting the quality of life of MB survivors. The devastating side-effects include cognitive deficits, endocrine disorders, and increased incidence of secondary cancers later in life.⁽²⁾

Recently, the intratumor heterogeneity revealed in malignancies such as breast cancer⁽⁹⁾ and glioblastoma⁽¹⁰⁾ has offered new insights into MB relapse. The spatial and temporal patterns of genetic, phenotypic, and functional diversity enable cancer cells to respond differently to targeted agents, with less sensitive or even insensitive populations surviving and leading to tumor relapse.⁽¹¹⁾ Among multiple theories proposed to interpret the intratumor heterogeneity,⁽¹²⁾ the CSC model established by

studies of malignancies such as leukemia,⁽¹³⁾ breast cancer,⁽¹⁴⁾ and brain tumors⁽¹⁵⁾ is of intense interest. Cancer stem cells show unlimited self-renewal and differentiation capacity, leading to phenotypically and functionally heterogeneous progenies and fueling tumor initiation, maintenance, and progression.⁽¹²⁾ Additional layers of heterogeneity may come from clonal evolution and environmental differences within tumors, giving rise to distinct subpopulations (Fig. 1).⁽¹⁶⁾

Cancer stem cells survive chemoradiotherapy and further contribute to treatment failure through multiple drug resistance mechanisms (i.e., the activation of pro-survival/anti-apoptosis pathways).⁽¹⁷⁾ Cancer stem cells have also been found to be tightly associated with tumor dissemination through molecular networks such as EMT, which increases the invasion and motility of cancer cells, and confers them a stem-like property essential for distant colonization.⁽¹⁸⁾ Elucidating the molecular mechanisms of MBSCs will definitely contribute to the discovery of more efficient agents that will notably improve the survival rate and quality of life of MB patients.

Medulloblastoma Cell-of-Origin versus MBSCs

The CSCs required for long-term neoplastic growth and xenograft initiation are sometimes confused with the “cell-of-ori-

gin” from within specific tumors. The term “CSC” refers to stem-like tumor cells containing multiple mutations, whereas the “cell-of-origin” refers to a normal cell that undergoes an initial transforming event.⁽¹⁹⁾

The cells of origin for human MBs are difficult to identify, as the earliest steps of the neoplastic process occur in a “black box.” However, the establishment of a series of GEM models of MB provided important insights into the cellular origins of these tumors (Fig. 2).⁽²⁰⁾ Granule neuron progenitors in the external germinal layer on the surface of the developing cerebellum have long been proposed as cells of origin for MB, and it is now clear that Shh-driven MB arises from this group.^(21,22) In contrast, the Wnt group is now thought to originate from progenitors in the dorsal brainstem.⁽²³⁾ Group 3, which is the most aggressive subgroup of MB and is characterized by high levels of MYC amplification or overexpression, appears to derive from cerebellar stem cells.^(24,25) The origins of Group 4, the most prevalent MB subgroup, remain unknown.^(26,27)

Sorting and Identifying MBSCs

The last decade has produced great advances in sorting and identifying MBSCs using markers, such as CD133 and CD15,

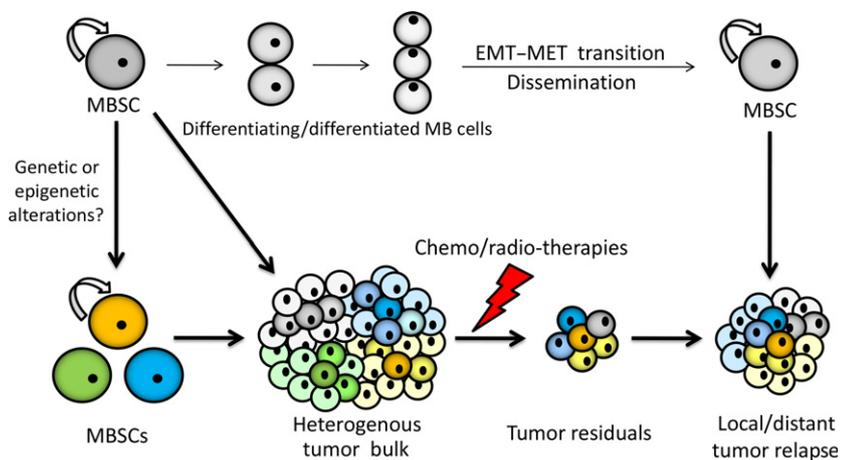
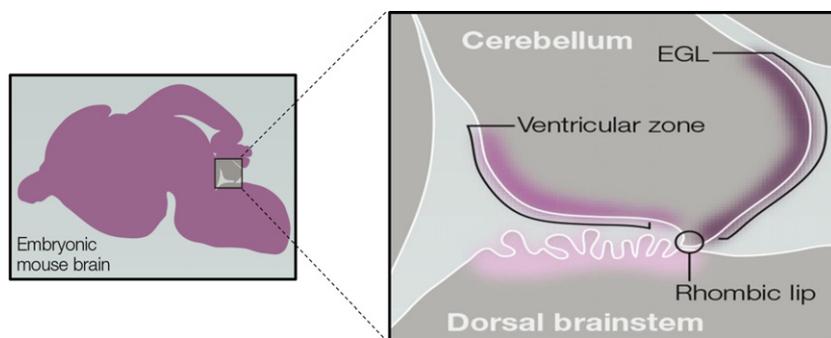


Fig. 1. Role of medulloblastoma stem cells (MBSCs) in medulloblastoma (MB) heterogeneity and relapse. MBSCs possess the ability to self-renew while differentiating into phenotypical and functional heterogeneous progenies. The possible existence of MBSC subpopulations further contributes to MB heterogeneity. When treated with chemoradiotherapy, insensitive MB cells will survive and lead to MB relapse. Meanwhile, the transition between epithelial and mesenchymal properties of MB cells through the epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) programs also cause MB relapse by endowing MB cells with stemness and multidrug resistance features.



Symbols	MB type	GEM models	Cells of origin	Ref.
●	WNT	Ctnnb1 ^{lox(ex3)} , Trp53 ^{flx/flx}	Dorsal brainstem neuronal progenitors	24
●	SHH	Ptch loss or ND2-SmoM2	EGL and RL GNPs	22, 23
●●	Group 3	MycT58A, Trp53+/-	VZ neural progenitors or EGL and RL GNPs	25, 26
?	Group 4	Glt1-MYCIN	Cerebellum progenitors or stem cells?	27, 28

Fig. 2. Cells of origin of medulloblastomas (MBs) and a summary of the related genetically engineered mouse (GEM) models. The top panels are adapted from the review by Rusert *et al.*⁽²⁾ EGL, external granular layer; GNP, granular neuroprogenitor; RL, rhombic lip; SHH, Sonic Hedgehog; VZ, ventricular zone; WNT, Wingless.

and varying methods, such as flow cytometry, xenograft models, and lineage tracing.

In 2003, a CD133⁺/Nestin⁺ cell from human MB tissue was first reported, which shows a marked capacity for proliferation, self-renewal, and differentiation *in vitro*.⁽¹⁵⁾ Further exploration showed that, in an immunodeficient mouse, 100 CD133⁺ cells were sufficient to produce a tumor resembling the original human tumor, whereas 100 000 CD133⁻ cells could not,⁽²⁸⁾ indicating that CD133 serves as a marker of MBSCs. However, these findings were called into question by later reports showing that tumor-initiating properties were also possessed by CD133⁻ MB cells.^(29,30) These discrepancies suggest more efforts need to be made to effectively single out MBSCs.

The GEM models established based on the molecular classification of MB facilitated the identification of MBSCs. In 2009, a tumor-propagating CD15⁺ cell was isolated from a *Patched* haploinsufficient (*Ptch*^{+/-}) MB model.⁽²⁹⁾ These cells were suggested to represent progenitor-like cells as they did not display multilineage differentiation or form neurospheres in stem-cell growth conditions.⁽²⁹⁾ However, this was challenged by a study showing that CD15⁺ MB cells from *Ptch*^{+/-} mice can be propagated long term in similar conditions while showing stem-like and tumor-initiating capacity.⁽³¹⁾ Recently, quiescent Sox2⁺ MB cells from post-irradiated *Ptch*^{+/-} mice were found to be tumorigenic.⁽³²⁾ Interestingly, more than 80% of Sox2⁺ cells were also CD15⁺, while Sox2⁺ cells formed a minority (<10%) of the CD15⁺ population; these results indicated that MB relapse may actually be propagated by a subset of CD15⁺ cells that are also Sox2⁺.⁽³²⁾

Medulloblastoma stem cells identified in the more aggressive Group 3 MB have rarely been reported. Recently, tumor-derived neurosphere cell lines from a MYCN-driven Group 3 MB were established.⁽³³⁾ These highly tumorigenic cells displayed features of partially committed neural stem and progenitor cells (i.e., upregulation of CD133, Nestin, and Musashi).⁽³³⁾ As MB patients harboring stem-like tumor cells that display stemness signatures are often characterized by poor prognoses,^(15,28,34) more research needs to be focused on identifying MBSCs in the more aggressive MB subtypes, Group 3 and Group 4.

Additionally, MBSCs can also be sorted out by markers such as CD271⁽³⁵⁾ and ABCG2 (marker of the SPs),⁽³⁶⁾ each representing a subpopulation efficient in tumor initiation or propagation.

Regulation of MBSC Key Properties on MB Relapse and LMD

Accumulating research on MBSCs indicates that the key properties related to MB relapse and LMD are stemness, therapeutic resistance, invasion, and motility. The stemness property is extremely vital, as it allows MBSCs to reproduce tumors in both post-surgical residual disease and LMD.

Critical regulator candidates of MBSC stemness. *Shh signaling pathway.* The Shh pathway is the major mitogenic regulator that promotes granule neuron progenitor proliferation.⁽³⁷⁾ Excessive activation of Shh pathways had been reported to cause MB tumorigenesis.⁽³⁷⁾

Unlike the CSCs in human leukemia, which are more quiescent than the “blasts” that make up the majority of the tumor, brain CSCs seem to show higher proliferation than do non-CSCs from the same tumor.^(15,28) Consistent with this, when the aforementioned CD15⁺ MBSCs were compared with those of CD15⁻ cells in *Ptch*^{+/-} MB, CD15⁺ cells expressed increased levels of Shh target genes *Gli1* and *CyclinD1*, and showed higher incorporation levels of thymidine *in vitro* and

BrdU *in vivo*.^(29,32) This evidence suggests that the increased proliferative capacity of CD15⁺ MBSCs is closely related with increased Shh pathway activation.

The transcription factors Gli1/2 the main effectors of the Shh pathway, have been reported to interact with stem-related factors, such as Nanog,⁽³⁸⁾ MYCN,⁽³⁹⁾ and Bmi-1, in the self-renewal regulation of MBSCs. The reprogramming factor Nanog was found highly expressed in stem cells from postnatal cerebellum and MB, and its specific cis-regulatory sequences were direct targets of *Gli1/2*.⁽³⁸⁾ When the Shh pathway was blocked by Smo-antagonist KAAD-cyclopamine, the expression of Nanog was reduced, and the self-renewal of MB neurospheres was also significantly inhibited⁽³⁸⁾ (Fig. 3).

A member of the polycomb protein family, Bmi-1, is a key regulator of stemness.⁽⁴⁰⁾ A direct feedback mechanism between downregulators of *bmi-1* and Shh signaling was found in CD133⁺ MBSCs, indicating that *bmi-1* and Shh are mutually indispensable in MBSC maintenance.⁽⁴¹⁾ In addition, *bmi-1* was also found to promote the self-renewal and tumorigenicity of CD15⁺ MBSCs *in vivo* through reciprocal promoter occupancy with FoxG1.⁽⁴²⁾ Together, these findings suggest that the Shh pathway plays a key role in regulating the self-renewal and maintenance of MBSCs (Fig. 3).

MYC family. Group 3 MB is characterized by elevated expression of MYC and LCA histopathology. Medulloblastoma patients with MYC amplification have a particularly high risk of relapse and the poorest prognosis of all MB patients.⁽²⁾

Early in 2006, when co-overexpressed with a neuronal differentiation repressor gene *REST/NRSF*, MYC was shown to block the differentiation of NSCs derived from the external germinal layer and cause MB formation.⁽⁴³⁾ Recently, JQ1, a small molecule inhibitor uniquely targets BRD4 (a key mediator of MYC-driven transcription programs), could strongly repress the expression of critical stem-related factors Sox2, Nestin, and Nanog in MB cells.⁽⁴⁴⁾ Moreover, JQ1 strongly suppressed the ability of these cells to form neurospheres *in vitro* and tumors *in vivo*.⁽⁴⁴⁾ These results indicate that MYC is tightly associated with MBSC stemness.

Another MYC family member, MYCN, was shown to be aberrantly amplified across MB subtypes.⁽⁴⁵⁾ Notably, Ahmad *et al.* recently established a MYCN-driven MB displaying the large-cell histopathology and expression profiles resembling Group 3 MB. Furthermore, CD133⁺ neurosphere cell lines were established, the self-renewal and growth of which were highly dependent on MYCN.⁽³³⁾ Specifically, the neurospheres showed restricted growth and reduced Ki-67, Nestin, and CD133 after 48 h of MYCN withdrawal,⁽³³⁾ indicating that MYCN is critical in the development and maintenance of aggressive MBSCs.

Notch signaling pathway. The Notch pathway is known for its regulation of stem cells in both normal and cancerous brain tissue. Hes1, the main effector of the Notch pathway, was found upregulated exponentially in CD133⁺ MB cells. When it was downregulated by Notch pathway γ -secretase inhibitors, the CD133⁺ MB cells and SP were largely reduced,⁽⁴⁶⁾ indicating that the Notch pathway plays an important role in MBSC self-renewal. Furthermore, Notch signaling was shown to interact with hypoxia-inducible factor-1 α to maintain NSCs and MBSCs in an undifferentiated status.⁽⁴⁷⁾ Particularly, stimulating Notch1 with Dll4 ligand under hypoxic conditions drives the expansion of MB-derived CD133⁺/Nestin⁺ precursors, while treating with γ -secretase inhibitors promoted the neuronal differentiation of these cells (Fig. 4).⁽⁴⁷⁾

MicroRNA. MicroRNA, an endogenous, 19–25-nt long, non-coding RNA, has been broadly shown to be involved in MB

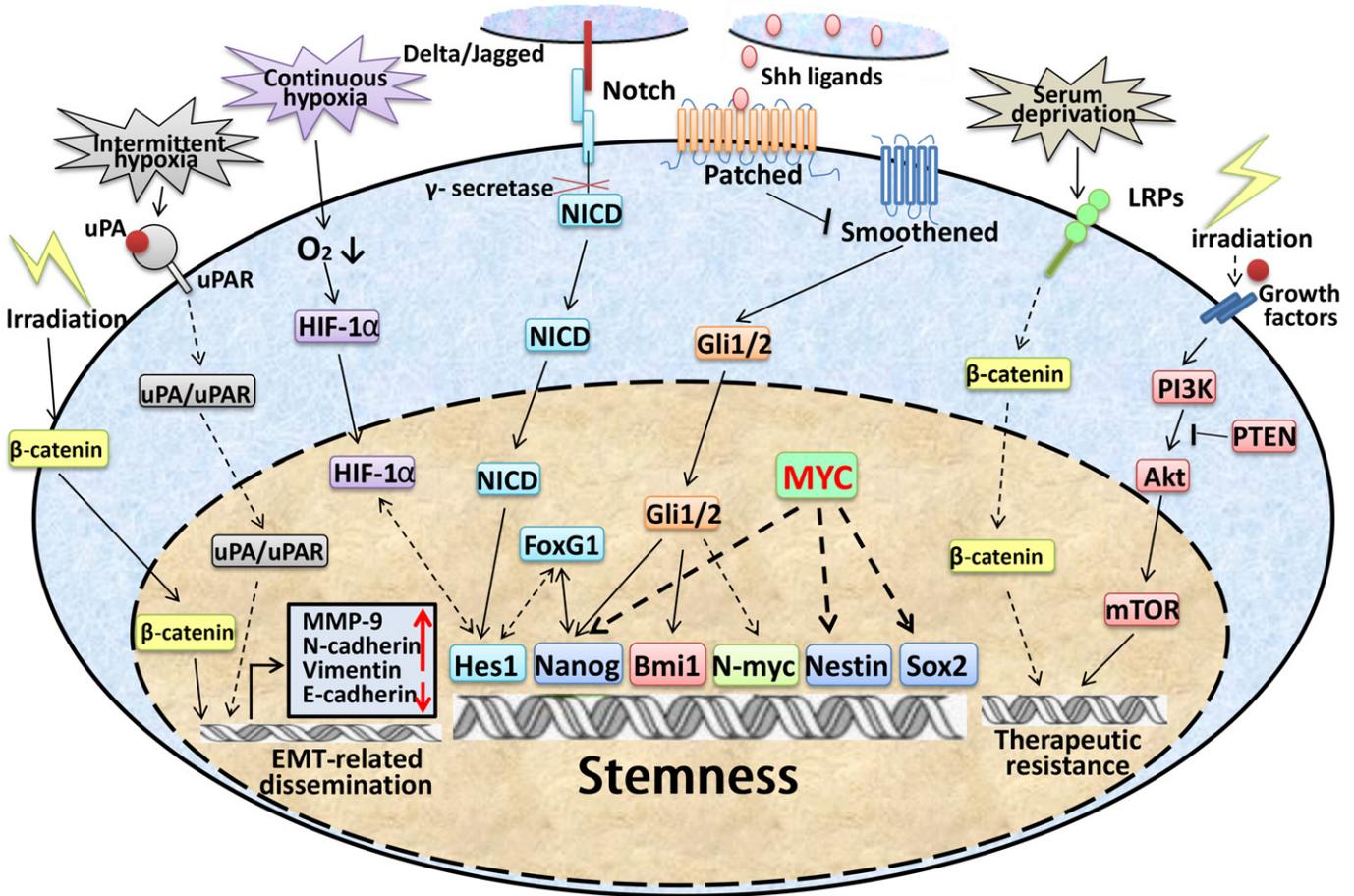


Fig. 3. Regulation mechanisms of medulloblastoma stem cell key properties in relation to medulloblastoma relapse and leptomeningeal dissemination. Akt, protein kinase B; EMT, epithelial–mesenchymal transition; FoxG1, Forkhead box 1; HIF-1 α , hypoxia-inducible factor 1 α ; LRP, low-density lipoprotein receptor-related protein; mTOR, mammalian target of rapamycin; MYC, v-Myc avian myelocytomatosis viral oncogene homolog; NICD, Notch1 intracellular domain; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Shh, Sonic Hedgehog; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor.

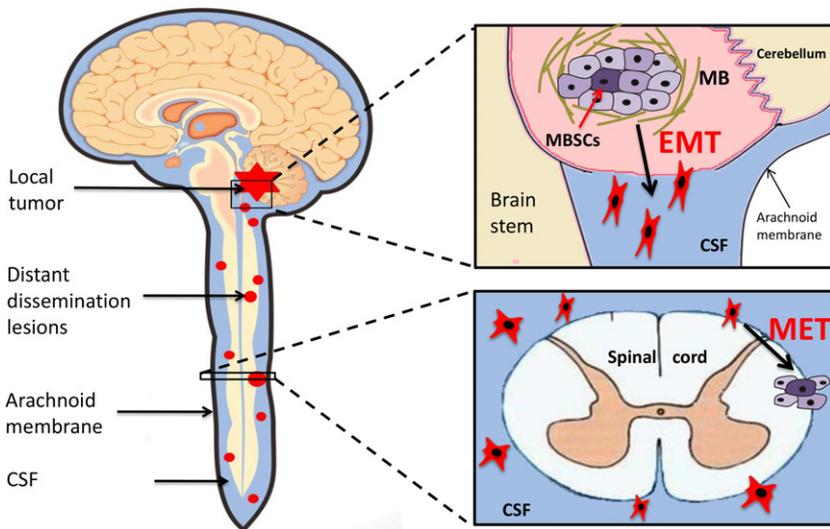


Fig. 4. Relationship between medulloblastoma (MB) leptomeningeal dissemination, MB stem cells (MBSCs), and epithelial–mesenchymal transition. The epithelial–mesenchymal transition program allows the epithelial MB cells to acquire a mesenchymal phenotype with increased motility and invasiveness, facilitating the initiation of MB dissemination. Inversely, the mesenchymal–epithelial transition (MET) program enables mesenchymal MB cells to settle and self-renew, leading to medulloblastoma leptomeningeal dissemination and relapse. CSF, cerebrospinal fluid.

tumorigenesis and progression,⁽⁴⁸⁾ and its roles in MBSCs are becoming gradually illuminated. For example, miR-199b-5p was found to regulate the self-renewal of CD15⁺ and CD133⁺ MBSCs by interacting with Hes1 through reciprocal binding

on the promoter regions.^(49,50) The famous stem-related miR-34a also regulates MBSC maintenance by inhibiting the PI3K/Akt/PTEN pathway.⁽⁵¹⁾ When miR-34a was overexpressed, the proportion of active Akt decreased, and the subpopulations

of CD15⁺ and CD133⁺ cells were significantly reduced.⁽⁵¹⁾ Moreover, the tumorigenic properties of MBSCs can also be strongly inhibited by miR-135a.⁽⁵²⁾ Together, these data suggest that microRNAs may serve as powerful tools in eradicating MBSCs.

Therapeutic resistance regulation mechanisms of MBSCs. The resistance characteristics possessed by CSCs, such as quiescence, activation of pro-survival/anti-apoptosis pathways, and interaction with microenvironmental factors (i.e., hypoxia),⁽¹⁷⁾ constitute an additional level of mechanisms that lead to treatment failure.

Targeting the Shh pathway seemed to be an effective way to eradicate MBSCs. However, the results were disappointing. Although vismodegib was found to be effective in eliminating most proliferating MB cells, it also significantly increased the frequency of quiescent Sox2⁺ MB cells in Ptch^{+/-} MB mouse models, strongly indicating that the quiescence feature can serve as an efficacious defense mechanism for MBSCs in resisting chemoradiotherapy.⁽³²⁾

Another example is the activation of the PI3K/Akt signaling pathway, which promotes tumor cell survival by regulating multiple apoptosis-related proteins (i.e., the Bcl-2 family).⁽⁵³⁾ Hambarzumyan *et al.* discovered a Nestin⁺ MBSC in the perivascular niche in different post-irradiated Shh-MB mouse models that could re-enter the cell cycle (72 h after radiation) through radiation-induced PTEN loss and the subsequent activation of the PI3K/Akt pathway.⁽⁵⁴⁾ When pretreated with the PI3K/Akt inhibitor perifosine, Nestin⁺ MBSCs displayed increased sensitivity to radiation-induced apoptosis,⁽⁵⁴⁾ indicating that activation of the PI3K/Akt pathway helps Nestin⁺ MBSCs to survive radiotherapy.

The Wnt pathway may also play a role in the therapeutic resistance of MBSCs. The LRP5/8 are membrane co-receptors of the Wnt/ β -catenin pathways⁽⁵⁵⁾ and have been shown to mediate the resistance phenotypes of many malignancies.⁽⁵⁶⁾ Annabi *et al.*⁽⁵⁶⁾ found that DAOY CD133⁺ MB cells mainly express LRP5/8, whereas under nutrient derivation, these cells acquire an increase of CD133⁺ cells expressing LRP1/1b/5, indicating that the LRP expression pattern mediates MBSC adaptation to nutrient derivation.

Leptomeningeal dissemination of MB, EMT, and MBSCs. Leptomeningeal dissemination, the metastatic spread of tumor cells through the cerebrospinal fluid to the brain and spinal cord, is a defining characteristic of MB and is associated with short survival time for MB patients.⁽⁵⁷⁾ Recently, the characteristics shared by CSCs and cancer cells in the EMT in many malignancies provided new insights into the elusive mechanisms of LMD.⁽⁵⁸⁾ The EMT program is a process characterized by the repression of epithelial markers (i.e., E-cadherin), the acquisition of mesenchymal makers (i.e., vimentin, N-cadherin) and the loss of cell adhesion. Together with its reverse process, mesenchymal-epithelial transition, the EMT has been found to play vital roles in tumor metastasis (Fig. 4).⁽⁵⁹⁾

The nuclear translocation of β -catenin (Wnt pathway key effector) was found to be induced by irradiation therapy in MB cells.⁽⁶⁰⁾ Interestingly, in the nucleus, β -catenin was then found to activate the EMT effector MMPs, such as MMP-9, and to promote MB invasion and migration.⁽⁶⁰⁾ The uPA/uPAR complex, which is a major regulator of ECM degradation,⁽⁶¹⁾ has been found to be activated by intermittent hypoxia and can induce the activation of the EMT programs of MB cells.⁽⁶¹⁾ It has been reported that uPAR⁺ cells in small-cell lung cancer are multidrug resistant and highly clonogenic and co-express CSC markers CD44 and multidrug resistance protein 1 (MDR1).⁽⁶²⁾ Thus,

uPA/uPAR may also be responsible for MB cell acquisition of therapeutic resistance and stem-like properties.

In spite of these findings, it is critical to define the role of MYC or MYCN in the more malignant MB subtypes, Group 3 and Group 4, the metastasis rates of which are up to 45% and 40%, respectively.⁽²⁾

Approaches for Eradicating MBSCs

Cancer stem cells can be eliminated through a plethora of methods, such as blocking pro-survival/anti-apoptosis pathways, targeting the mechanisms maintaining quiescence, or disrupting CSC interaction with the microenvironment.^(63,64) The molecular classification of MB has undoubtedly helped develop therapeutic strategies for targeting unique signaling pathways, specific oncogenes, or tumor suppressor genes aberrantly regulated in specific MB subtypes. However, many of these targeted therapies poorly eliminate MBSCs.^(32,33)

To date, most studies aiming to eradicate MBSCs have focused on targeting specific signaling pathways. The preceding sections have discussed that when the Notch pathway was blocked by γ -secretase inhibitors, miR-199b-5p, or exposure to 20% oxygen concentration, the fractions of stem-like CD133⁺, CD15⁺ MB cells and SPs were significantly reduced. Inhibiting the PI3K/Akt pathway also seems to be effective in removing MBSCs in some contexts. Apart from the aforementioned perifosine, GDC-0941, which is another highly specific PI3K inhibitor, could significantly reduce the stem-like CD133⁺ MB subpopulations as well as their clonogenicity.⁽⁶⁵⁾ More importantly, it strongly delayed tumor growth of a mouse that orthotopically received the most aggressive Group 3 MB xenograft.⁽⁶⁵⁾

In addition, the STAT3 pathway was found to be enhanced in CD133⁺ MB cells, and the STAT3 inhibitors, cucurbitacin I or celecoxib, can strongly inhibit the stem-like properties of CD133⁺ MBSCs, as well as improve the chemoradiosensitivity of, and survival time, with CD133⁺ MBSCs xenografts.^(66,67) Taken together, these data showed that developing agents targeting these pathways is promising for the elimination of MBSCs.

Conclusion and Perspectives

Given the pivotal role of stem-like tumor cells in MB relapse and LMD, improved treatment strategies combining traditional and MBSC-targeted therapies may be required to significantly improve the survival time and quality of life of MB patients. However, despite great efforts and multiple encouraging discoveries, the current knowledge of MBSCs is still not sufficient and the optimal clinical application of MBSC-targeted agents awaits future groundbreaking work. Goals to achieve include: (i) finding more reliable MBSC markers and gaining a comprehensive knowledge of the discrepancies between MBSCs and NSCs/neural progenitor cells to develop agents that uniquely target MBSCs; (ii) investigating MBSC subclones and the essential molecular mechanisms they share, or with which they interact; and (iii) understanding further CSC plasticity and the possibility that stemness can reflect a transient state rather than a metastable state, although the flux of non-CSCs into the CSC compartment appears relatively low.⁽⁶⁸⁾

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Disclosure Statement

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Abbreviations

Akt	protein kinase B
CSC	cancer stem cell
EMT	epithelial–mesenchymal transition
GEM	genetically engineered mouse
LCA	large cell/anaplastic
LMD	leptomeningeal dissemination

LRP	low-density lipoprotein receptor-related protein
MB	medulloblastoma
MBSC	medulloblastoma stem cell
miR	microRNA
MYC	v-Myc avian myelocytomatosis viral oncogene homolog
MYCN	v-Myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog
NSC	neural stem cell
PI3K	phosphatidylinositol 3-kinase
PTEN	phosphatase and tensin homolog
Shh	Sonic Hedgehog
Smo	Smoothen
SP	side population
STAT3	signal transducer and activator of transcription 3
uPA	urokinase-type plasminogen activator
uPAR	urokinase-type plasminogen activator receptor
Wnt	Wingless

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