Molecular Determinants of Medulloblastoma Metastasis and Leptomeningeal Dissemination
Min Li, Yuhao Deng, and Wangming Zhang

ABSTRACT

Medulloblastoma is the most common malignant brain cancer in pediatrics consisting of four molecular subgroups, namely wingless (WNT), sonic hedgehog (SHH), Group 3, and Group 4. One of the biggest challenges in the clinical management of this disease is the leptomeningeal dissemination (LMD) of tumor cells with high morbidity and mortality. Many molecular regulators to date have been identified to participate in medulloblastoma metastasis. In the SHH subgroup, the co-upregulation of CXCR4 and PDGFR, as well as the activation of c-MET, show significant promigratory effects on medulloblastoma cells. Amplification or overexpression of genes on the long arm of chromosome 17, such as LASP1 and WIP1, facilitates tumor invasion in both Group 3 and Group 4 medulloblastomas. PRUNE1, NOTCH1, and MYC interactor JPO2 are more specific genetic drivers of metastatic Group 3 tumors. The RAS/MAPK and PI3K/AKT pathways are two crucial signal transduction pathways that may work as the convergent downstream mechanism of various metastatic drivers. Extracellular signals and cellular components in the tumor microenvironment also play a vital role in promoting the spread and colonization of medulloblastoma cells. For instance, the stromal granule cells and astrocytes support tumor growth and dissemination by secreting PlGF and CCL2, respectively. Importantly, the genetic divergence has been determined between the matched primary and metastatic medulloblastoma samples. However, the difficulty of obtaining metastatic medulloblastoma tissue hinders more profound studies of LMD. Therefore, identifying and analyzing the subclone with the metastatic propensity in the primary tumor is essential for future investigation.

Introduction

Medulloblastoma, the most frequent malignant brain tumor in children, is now considered a highly heterogeneous disease (1). The classification of medulloblastoma has evolved from only relying on histopathological features to its combination with molecular characteristics (2). A consensus was reached in the early 2010s when four major medulloblastoma subgroups designated wingless (WNT), sonic hedgehog (SHH), Group 3, and Group 4 were proposed on the basis of genomic and transcriptomic studies (3, 4). These subgroups are thought to have different cellular origins and also show demographic and clinical differences (5). Generally, the prognosis is best for WNT medulloblastomas, followed by SHH and Group 4 subgroups and worst for Group 3 medulloblastomas. The standard treatment for medulloblastoma includes the maximum safe surgical debulking, craniospinal radiotherapy, and cytotoxic chemotherapy (1, 6). Risk stratification plays a vital role in treating patients with medulloblastoma, which determines the intensity of the treatment. Molecular risk-adapted management also emerged in recent years as the classiﬁcation developed into the molecular era (1, 7).

Owing to multimodal and risk-adapted therapies, the survival of patients with medulloblastoma has signiﬁcantly improved over the past decades. However, the prognosis for about 30% of patients remains poor, and many of the survivors suffer from severe side effects because of intensive therapeutic regimens (6). One of the leading causes of treatment failure and death is metastases of tumor cells from the primary site (posterior fossa) to intracranial or spinal leptomeninges (the most common metastatic deposits; ref. 8). Metastasis occurs in 20% to 40% of newly diagnosed patients and approximately 70% of recurrent cases (9–11). Group 3 tumors with the worst prognosis have the highest incidence of metastasis at initial diagnosis and recurrence, whereas WNT medulloblastomas show the lowest (11). Importantly, to prevent or treat metastases, patients have to receive therapies with high-toxicity rates such as irradiation for the entire craniospinal axis, which often results in long-term sequelae. Furthermore, craniospinal irradiation is unacceptable for children under 3 years of age due to adverse effects on the developing central nervous system, rendering more dismal outcomes for these patients (1). Therefore, a deeper understanding of medulloblastoma metastasis and more targeted therapeutic strategies are desperately needed.

Over the last two decades, studies have revealed that a number of molecular players and associated signaling pathways are involved in medulloblastoma metastasis. Some are upregulated genes in medulloblastoma (or medulloblastoma with metastasis), which show pro-metastatic effects in vitro or in vivo and are considered to be genetic drivers of medulloblastoma metastasis. Also, extracellular signals that promote the process of anchoring or colonization of tumor cells and enhance cell interactions in the tumor microenvironment (TME) have been reported by researchers. In this review, we summarize and classify these molecular players and essential signal transduction pathways, and also analyze current challenges and possible strategies for improving future research.
Table 1. Summary of molecular players of metastasis associated with MB subgroups.

<table>
<thead>
<tr>
<th>Name</th>
<th>Downstream/upstream/manipulation</th>
<th>Effects on metastasis</th>
<th>Subgroup specificity</th>
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<tr>
<td>GRK6 (12)</td>
<td>PDGFR/Src, CXCR4/CXCL12</td>
<td>Inhibits cell migration</td>
<td>SHH</td>
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<tr>
<td>LSD1 (15)</td>
<td>REST</td>
<td>Promotes cell migration</td>
<td></td>
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<tr>
<td>USP7 (14)</td>
<td>Shh pathway</td>
<td>Promotes cell migration</td>
<td></td>
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<tr>
<td>MAP4K4 (16, 17)</td>
<td>c-MET</td>
<td>Promotes cell invasion in vitro and brain tissue infiltration ex vivo</td>
<td></td>
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<tr>
<td>OGR1 (18)</td>
<td>TRPC4</td>
<td>Promotes cell migration</td>
<td></td>
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<tr>
<td>ATOH1 (19)</td>
<td>Transgene expression</td>
<td>Promotes LMD in mouse models</td>
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<tr>
<td>PIK3CA (20)</td>
<td>Mutations</td>
<td>Promotes LMD in mouse models</td>
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<tr>
<td>ERAS (21)</td>
<td>Ectopic expression in Nestin-expressing neural progenitors</td>
<td>Promotes LMD in mouse models</td>
<td></td>
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<tr>
<td>LHX1 (21)</td>
<td>OCT4 and mTOR</td>
<td>Promotes LMD in mouse models</td>
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<tr>
<td>CCRK (21)</td>
<td>Chromosomal aberrations and non-coding RNA expression</td>
<td>Promotes cell motility in vitro and LMD in vivo</td>
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<tr>
<td>AKT (21)</td>
<td>miR-206</td>
<td>Promotes cell migration</td>
<td>Group 3 and Group 4</td>
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<td>ARNT (22)</td>
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<td>GD12 (22)</td>
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<td>MYCN (23)</td>
<td>OCT4A (24)</td>
<td>Promotes cell motility in vitro and LMD in vivo</td>
<td></td>
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<tr>
<td>LSD1 (13)</td>
<td>REST</td>
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<td>GRK6 (12)</td>
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<td>SETD8 (36)</td>
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<td>JPO2 (37)</td>
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<td>LEDGF/p75 (39)</td>
<td>JPO2, PIK3/AKT</td>
<td>Promotes cell migration in vitro and LMD in vivo</td>
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<tr>
<td>ID3 (40)</td>
<td>/</td>
<td>Promotes cell migration in vitro and LMD in vivo</td>
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<td>SNCA (41)</td>
<td>/</td>
<td>Inhibits cell invasion</td>
<td>Group 4</td>
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<td>DDX3 (42)</td>
<td>Rac1 and β-Catenin</td>
<td>Modulates cell motility and invasion</td>
<td></td>
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<tr>
<td>MiR-148a (44)</td>
<td>NRPI</td>
<td>Inhibits cell invasion</td>
<td>WNT</td>
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SHH

The co-upregulation of a group of genes implies the potential collaboration of them in regulating a biological process. Yuan and colleagues (12) observed the co-overexpression of CXCR4, CXCL12, and PDGFR in primary medulloblastoma specimens of the SHH subgroup in comparison with non-SHH medulloblastomas. And a pro-migration signaling axis consisting of PDGFR, SRC, GRK6, and CXCR4 was found in SHH medulloblastoma cells where PDGFR-SRC negatively regulated GRK6 expression via the proteasomal pathway, consequently enhanced CXCR4 signaling and promoted medulloblastoma cell migration (Fig. 1A; ref. 12). Co-elevation of lysine-specific demethylase 1 (LSD1) with its deubiquitylases ubiquitin–specific peptidase 7 (USP7) and REI-silencing transcription factor (REST) was also demonstrated in a subset of SHH medulloblastoma tumors, and REST–LSD1 interaction positively regulated tumor cell migration (13). In addition, in another study, USP7 promoted cell migration by activating the Shh pathway (14). Several studies have revealed c-MET as a marker of SHH medulloblastomas and a promoter of tumor cell dissemination (15, 16). Targeting c-MET by its inhibitor (foretinib) suppresses HGF-mediated migration and invasion in vitro and decreases metastases in xenograft and transgenic mouse models of SHH medulloblastoma (15). Furthermore, MAP4K4 is identified as a key downstream mediator of c-MET-induced invasiveness by controlling F-actin dynamics, integrin β1 activation, and c-MET endocytosis (Fig. 1A, Table 1; refs. 16, 17).

The SHH subgroup of medulloblastoma is believed to originate from cerebellar granule neuron progenitors (GNP; ref. 5). Therefore, manipulating Shh signaling in GNP provides the possibility for creating cell models or mouse models of this subgroup. In granule precursor-derived medulloblastoma cells, ovarian cancer G protein–coupled receptor 1 (OGRI) can enhance the expression of short transient receptor potential channel 4 (TRPC4). Activation of these channels leads to a large Ca2+ influx and promotes migration (18). Moreover, researchers have investigated genetic drivers of metastatic dissemination using the transgenic mouse models of SHH medulloblastoma. Both ATOH1 transgene expression and PIK3CA mutations accelerate tumor growth and promote leptomeningeal dissemination (LMD) in mouse models with mutated PTCH1 or SMO (19, 20). ERAS, LHX1, CCRK, AKT, ARNT, and GD12 are also deemed to be genetic drivers of metastasis because ectopic
expression of any of these genes in shh-induced medulloblastoma can facilitate LMD in mice (21, 22). More recently, Cancer and colleagues (23) used two types of human neuroepithelial stem (NES) cells, primary human hindbrain-derived neuroepithelial stem (hhNES) cells and induced pluripotent stem cell (iPSC)-derived NES cells, to generate humanized models of SHH medulloblastoma by overexpressing MYCN (23). Both of cell models recapitulated hallmarks of infant SHH medulloblastoma and developed LMD in mice through OCT4 upregulation and mTOR hyperactivation (Fig. 1A). Targeting mTOR significantly prolonged survival and reduced spinal cord metastases of NES tumor-grafted mice. High OCT4A (an isoform of OCT4) levels were also found to drive the metastatic potential of medulloblastoma cells. Overexpression of OCT4A in medulloblastoma cell lines significantly increased cell motility in vitro and LMD in vivo (Table 1; ref. 24).

Although blocking the hyperactive Shh signaling by SMO inhibitors appeared to be a promising therapy for SHH medulloblastoma, resistance mutations such as SUFU mutations developed rapidly in tumors after treatment. Hence, targeting the downstream effectors of SMO/SUFU like GLI1/2 may be a possible strategy to overcome the resistance. A novel casein kinase 1a (CK1a) agonist, SSTC3, with good blood–brain barrier permeability remarkably attenuates tumor growth and blocks metastases by targeting GLI1/2 in vismodegib (SMO inhibitor)-resistant metastatic SHH medulloblastoma mouse models.
An alternative cause of the resistance to SMO inhibition is the activation of the RAS/MAPK pathway, which drives tumor growth independent of the Shh pathway and enhances metastatic behavior (26). In addition, the crossstalk between FGFR and Shh signaling pathways has been demonstrated in controlling medulloblastoma tumor invasion. Specifically, although sustained and low-level activation of SMO promotes proliferation and tissue invasion, acute and pronounced SMO activation represses FGFR-driven invasiveness (27).

**Group 3 and 4**
Isochromosome 17 or gain of 17q that frequently occurs in Group 3 and 4 tumors is a hallmark cytogenetic event of these subgroups (28). LiM and SH3 protein 1 (LASP1), a gene on chromosome 17q, is significantly upregulated in medulloblastoma tumors with 17q gain. High expression of this gene is strongly correlated with metastatic dissemination and inferior survival. And LASP1 knockdown dramatically reduces the migration of medulloblastoma cells in vitro (29). LASP1 is also identified as a target of miR-206 that is a significantly downregulated miRNA in medulloblastoma associated with tumor seeding (Fig. 1B, Table 1). Overexpression of this miRNA in D341 cells, a Group 3 cell line, notably reduces cell migration (30). WIP1 is another oncogene on chromosome 17q and also overexpressed in Group 3 and 4 medulloblastomas. In high WIP1-expressing cells, WIP1 represses GRK3-induced CXCXR4 phosphorylation and internalization and then enhances the cell surface localization of CXCXR4 (31). Stimulation of CXCXR4 on these cells activates PI3K/AKT signaling and promotes proliferation and invasion (Fig. 1B).

With regard to more specific regulators in Group 3 tumors, a highly expressed gene PRUNE1 has been identified as a molecular driver of metastasis. PRUNE1 activates canonical TGF-β signaling by binding to NME1 and then enhances OXT2 expression and PTEN inhibition, which together constitute a signaling axis to promote metastasis (Fig. 1B, Table 1). In orthotopic xenograft mouse models of Group 3 medulloblastoma, small molecules that enhance PRUNE1 degradation or impair PRUNE1/NME1 complex formation inhibit tumor growth and metastatic dissemination (32). NOTCH1 is also a pivotal driver of metastasis initiation in Group 3 medulloblastomas with WNT subtypes. In high WNT expression medulloblastoma cells, a Group 3 cell line, notably reduces cell migration (30). And BMI1 has been identified as a molecular driver of metastasis in various medulloblastoma subtypes may also be different, which warrants further investigation.

**Molecular players of medulloblastoma metastasis in non-subgroup-specific studies**
PDGFRα is one of the earliest identified driver genes that is upregulated in metastatic primary medulloblastoma specimens compared with non-metastatic primary specimens. Stimulating the receptor with PDGFA enhances medulloblastoma cell migration in vitro and activates the RAS/MAPK signaling pathway (Fig. 2A). In contrast, blocking PDGFRα with neutralizing antibodies inhibits the phosphorylation of RAS/MAPK signaling components (MEK1/2 and ERK1/2) and counteracts PDGFA-stimulated migration (46). Gilbertson and Clifford (47) pointed out that the oligonucleotide probe of PDGFRα in that study actually detects PDGFRB. They also confirmed the overexpression of PDGFRB by analyzing 14 metastatic and 13 non-metastatic primary medulloblastoma tumors. Hence, it is possible that both PDGFRs have a role in medulloblastoma metastasis. Subsequently, a second receptor tyrosine kinase, ERBB2, was identified as a molecular driver of medulloblastoma metastasis (48, 49). ERBB2 promotes cell invasion and migration by upregulating expression of S100A4 (a known pro-metastatic gene) via the MAPK pathway (Fig. 2A). Also, the high expression of EphB2 is observed in both medulloblastoma samples and cell lines (50, 51). Stimulation of medulloblastoma cells with its ligand ephrin-B1 increases cell invasion.
capacity with p38, ERK, and mTOR as the downstream mediators (Fig. 2A, Table 2; ref. 50).

Netrin-1 is another promoter of medulloblastoma cell invasiveness, which activates the MAPK pathway through its receptors UNC5B and Neogenin and stimulates ERK phosphorylation (Fig. 2A, Table 2; ref. 52). Interestingly, the urinary level of netrin-1 in patients with invasive medulloblastoma is also significantly higher than in patients with noninvasive medulloblastoma. And this high level of netrin-1 in urine decreases after surgical resection of medulloblastoma, implying that netrin-1 is a potential biomarker of a disseminated phenotype. Real-time sensing of MAPK signaling in medulloblastoma cells reveals that the nuclear activation of ERK1/2 correlates with the speed of cell migration (53). Exosomal miRNAs isolated from Group 3 medulloblastoma cells promotes in vitro migration and invasion of a less invasive SHH medulloblastoma cell line (Daoy) by activating ERK1/2 in RAS/MAPK signaling (54). Several other regulators, such as folate receptor 1 (Folr1) and long noncoding RNA (lncRNA) CCAT1, also mediate medulloblastoma invasiveness via the MAPK pathway (55, 56). Furthermore, treating medulloblastoma mouse models with reovirus significantly decreases LMD, and the oncolytic effect of reovirus depends on the high level of activated RAS in tumor cells (57).

Altogether, these findings suggest that the MAPK signaling plays an essential role in medulloblastoma tumor dissemination, which may function as the convergent downstream mechanism of various metastatic drivers.

In addition to the MAPK pathway, the PI3K/AKT signaling pathway also serves as the key downstream effector or upstream modulator of many molecular players in medulloblastoma metastasis. MiR-183 cluster highly upregulated in non-SHH medulloblastomas shows positive regulation of medulloblastoma cell migration through...
activating the PI3K/AKT/mTOR pathway (Fig. 2A, refs. 58, 59). Conversely, pharmacological inhibition of mTOR with rapamycin diminishes the pro-metastatic effects of miR-183 cluster (59). Like miR-183 cluster, tripartite motif-containing 9 (TRIM9) and IRX1 LIMXL1-AS1 also promote cell migration via the PI3K/AKT pathway in medulloblastoma (60, 61). FBW7 is an SCF(SKP1/CUL1/F-box)-type ubiquitin ligase and a tumor suppressor that can degrade SOX9. Mutation or down-regulation of FBW7 in medulloblastoma results in the stabilization of SOX9, which in turn promotes tumor metastasis and treatment resistance. The phosphorylation of threonine 236 (T236) in SOX9 by GSK3 kinase is necessary for FBW7 to degrade SOX9, and this process is inhibited by the PI3K/AKT/mTOR pathway (62). Thus, the PI3K/AKT/mTOR pathway as the upstream modulator enhances the stabilization of SOX9 (Fig. 2A, Table 2).

Both radiotherapy and hypoxia can lead to increased invasiveness of medulloblastoma cells. The urokinase-type plasminogen activator receptor (uPAR) works as a crucial regulator in these processes. uPAR downregulation retards invasion/migration and EMT induced by radiotherapy and intermittent hypoxia, respectively (63, 64). The suppressive effect in the former is mediated by the inhibition of FAK/Rac1 signaling (64). Similarly, the calcium/calmodulin-activated kinase kinase (CaMKK) pathway regulates the migration of medulloblastoma cells via Rac1 (65). Some studies also reported that Rac1 can stimulate medulloblastoma cell migration in vitro (66, 67). Moreover, the p21-activated kinase-1 (Pak1) as the essential downstream effector of Rac1 can be activated by PDGF through PDGFR/ERK/Rac1 signaling (68). Consequently, Rac1 may be a pivot of the signal transduction for some pro-metastatic factors (Table 2).

Filopodia formation is a critical process of cell motility and migration. In medulloblastoma cells, ezrin interacts with CD44 to promote the formation of filopodia, in vitro cell invasion, and in vivo leptomeningeal metastasis (69). A potassium channel Ether-a-go-go 2 (EAG2) concentrated in the trailing edge of migrating medulloblastoma cells plays an important role in regulating local cell volume dynamics and facilitating cell motility (70). Besides the promoters of metastasis mentioned above, there are some negative regulators of invasiveness in medulloblastoma. Growth arrest and DNA-damage-inducible 45 alpha (Gadd45a) sensitizes medulloblastoma cells to ionizing radiation (IR), downregulates MMP-9, and suppresses EMT (71). MiR-199b-5p is another negative regulator and dramatically repressed in metastatic medulloblastoma resulting from the binding of Hes1 to its promoter and an epigenetic mechanism with the methylation of CpG island upstream of its promoter. Therefore, miR-199b-5p cannot suppress the pro-metastatic ERK and AKT pathways to impair tumor cell migration (72). Programmed cell death 4 (PDCD4) is also a metastasis suppressor, and the overexpressed miR-21 in medulloblastoma downregulates PDCD4, which leads to the disinhibition of invasion mediators MAP4K1 and JNK (Table 2; ref. 73).

Modulators in the process of chemotaxis, anchoring, and colonization

Apart from the function of upregulated genes in medulloblastoma cells, the chemotaxis to extracellular signals is also important for tumor cell dissemination. Conditioned media (CM) from a meningothelial cell line (BMEN1) enhances medulloblastoma cell migration and adhesion. Although VEGFA shows an elevated level in BMEN1 CM compared with Daoy CM, exogenous recombinant VEGFA fails to increase tumor cell migration, and the VEGFA monoclonal antibody bevacizumab does not reduce the promigratory effects of BMEN1 CM. These results suggest that paracrine chemokines from BMEN1, but not VEGFA, are crucial for the spreading of medulloblastoma cells (74). Besides, EGF has been identified as potent external signaling that increases the cell motility and induces chemotactic responses of medulloblastoma cells (75, 76).

The adhesion or anchoring of medulloblastoma cell to the leptomeninges is a prerequisite for the survival and colonization of tumor cells at a metastatic site. In a Group 3/4 medulloblastoma cell line D283, integrins (α9 and β1 subunits) are vital mediators for cell adhesion by binding to the extracellular matrix protein tenascin and activating ERK1/2 (77). IHC of medulloblastoma patient specimens also demonstrates the increased expression of these integrins and tenasin in leptomeningeal tumor implants. Another study reported that miR-192 is downregulated in medulloblastoma with cerebrospinal fluid (CSF) seeding compared with medulloblastoma without CSF seeding. And this miRNA suppresses cellular anchoring via the inhibition of integrins (ITGAV, ITGB1, and ITGB3) and CD47 (78).
The activation of IGF1R on medulloblastoma cells by IGF1 in CSF is also involved in tumor cell adhesion and leptomeningeal metastasis. Meningothelial cells appear to secrete abundant proteases to liberate IGF1 from the IGF1–IGFBP3 complex in CSF. Thus, free IGF1 at the leptomeningeal surface can bind to IGF1R on tumor cells and promote metastasis establishment (Table 2; ref. 79).

### Tumor microenvironmental factors in medulloblastoma metastasis

The stromal cells and their secreted factors in the TME have been discovered to support medulloblastoma growth and dissemination. Snuderl and colleagues (80) found that both tumor cells and stromal granule cells can secrete the placental growth factor (PIGF) that binds to its receptor NRPI on tumor cells to activate ERK1/2. The initial tumor growth relies on autocrine PIGF from medulloblastoma cells. As the tumor grows, shh secreted by medulloblastoma cells can stimulate stromal granule cells to produce more abundant PIGF. Then this paracrine signaling accelerates tumor growth and metastatic spread (Table 2, Fig. 2B; ref. 80). Basic fibroblast growth factor (bFGF) in the TME also promotes medulloblastoma cell invasion by activating FGF receptor and the downstream mediators, FGR substrate 2 (FRS2) and ERK1/2 (Fig. 2C). More importantly, this promigratory effect of bFGF/FGFR signaling is regulated by TGF-β in a context-dependent manner (Table 2). When bFGF abundance is low in the TME, TGF-β activates the Rho-associated kinase (ROCK) and ERK1/2, which represses the bFGF/FGFR/FRS2 signaling and promotes a non-motile phenotype (Fig. 2D). Inversely, when the FRS2 pathway is inhibited by negative-feedback regulation of high bFGF-induced ERK1/2, TGF-β can abrogate this negative regulation and revitalize the promigratory signaling (Fig. 2E; ref. 81).

Most recently, Liu and colleagues (82) investigated the interplay between tumor-associated astrocytes (TAA) and medulloblastoma stem-like cells (MBSC) in disseminated medulloblastoma. MBSCs recruit astrocytes via the secretion of IL-6, and then TAAAs produce CCL2 to maintain the stemness properties of MBSCs by activating JAK2/STAT3-mediated Notch signaling. Furthermore, the production of CCL2 depends on the necroptosis of TAAs via RIP1/RIP3/MLKL-mediated salvage of TAAs. Notch signaling further promotes the production of CCL2 via the RIP1/RIP3/MLKL-mediated salvage of TAAs. This results corroborate the findings of other studies in which highly expressed CCL2 is found in medulloblastoma leptomeningeal metastasis as well as in the CSF of metastatic medulloblastomas and overexpression of CCL2 in medulloblastoma drives LMD in mouse models (83, 84). In addition, the astrocyte conditioned media can also accelerate the self-proliferation and metastasizing capability of MBSCs (82). These results also support the findings of other studies in which highly expressed CCL2 is found in medulloblastoma leptomeningeal metastasis as well as in the CSF of metastatic medulloblastomas and overexpression of CCL2 in medulloblastoma drives LMD in mouse models (83, 84). In addition, the astrocyte conditioned media can also accelerate the self-proliferation and metastasizing capability of MBSCs (82).

### Conclusion and outlook

Currently, risk stratification plays a significant role in the management of patients with medulloblastoma on account of the tumor suppression and progression. However, the suppression of TME also promotes medulloblastoma cell invasion by activating FGF receptor and the downstream mediators, FGR substrate 2 (FRS2) and ERK1/2 (Fig. 2C). More importantly, this promigratory effect of bFGF/FGFR signaling is regulated by TGF-β in a context-dependent manner (Table 2). When bFGF abundance is low in the TME, TGF-β activates the Rho-associated kinase (ROCK) and ERK1/2, which represses the bFGF/FGFR/FRS2 signaling and promotes a non-motile phenotype (Fig. 2D). Inversely, when the FRS2 pathway is inhibited by negative-feedback regulation of high bFGF-induced ERK1/2, TGF-β can abrogate this negative regulation and revitalize the promigratory signaling (Fig. 2E; ref. 81).
heterogeneity and the lack of targeted therapies for metastasis. Multimics data analysis and molecular classification by using tumor biopsies or specimens can facilitate the prediction of metastatic probability and the selection of optimal or more individualized treatments. Of note, circulating tumor DNA (ctDNA) has been reported to be abundant in CSF but barely found in plasma (87). Except for tumor samples, the analysis of CSF ctDNA also contributes to the molecular characterization, subgrouping, and risk stratification of medulloblastoma. More importantly, this relatively noninvasive method allows the study of intratumor genomic heterogeneity and genomic evolution, facilitating the longitudinal analysis of tumor progression such as metastasis and recurrence. By means of this kind of monitoring, therapeutic strategies could be adjusted to molecular features of tumors throughout the course of the disease (87). In addition, higher levels of some CSF cytokines (CCL2, CXCL1, IL6, and IL8) have been identified in metastatic Group 3 and 4 medulloblastomas compared with non-metastatic controls (84), further underscoring the importance of CSF for studying medulloblastoma metastasis.

Although medulloblastoma subgroups remain unchanged in the metastatic or recurrent tumors (9, 88), the genetic divergence has been determined in these tumor cells compared with the primary tumor (89, 90). Hence, dissecting the peculiarities of disseminated tumor cells guarantees a better understanding of medulloblastoma metastasis. Unfortunately, the difficulty of routinely obtaining patient specimens from a metastatic site immensely limits the investigation of the characteristics and mechanisms of medulloblastoma dissemination. Most of the studies summarized in this review originate from either comparing primary specimens of patients with metastasis with those without metastasis or examining the differences between medulloblastoma specimens and normal controls. Only a few research groups have analyzed the genomic and transcriptomic features of the metastatic medulloblastoma tissue (88, 89). One of these studies has demonstrated that clonal genetic events in the medulloblastoma metastases can be detected in a minor subclone of the primary tumor, implicating that only a small number of tumor cells within the primary lesion have the ability to metastasize (89). In view of this, identifying the subclones with the highly invasive and metastatic propensity in the primary tumor and developing targeted therapies for these small subsets of tumor cells is an essential aspect of future investigations. The emerging methods, such as single-cell RNA sequencing, may provide more possibilities to identify those metastatic subclones in the primary tumors. Using genetically engineered mouse models (GEMM) to manipulate genes and investigate the genetic drivers of metastasis have also been performed by some researchers (21, 22). It is noticeable that this approach is, in most cases, only suitable for the SHH subgroup due to the lack of GEMMs in other subgroups. And results from these mouse models need to be verified by further human data.

In consideration of the heterogeneity of medulloblastoma, profound investigations of LMD according to specific molecular subgroups are also important, especially for Group 3 and 4 medulloblastomas, which have a higher incidence of dissemination but less experimental models. With the in-depth research on their nature and cell-of-origin (5), good models may be established in the future for facilitating studies on the regulatory mechanism of metastasis in these two subgroups. As metastatic nodules are almost invariably found on the leptomeninges of the brain or spinal cord, medulloblastoma metastases are presumably caused by the spread of tumor cells through CSF. A recent study, however, provided evidence of a hematogenous route for LMD (83), further indicating the complexity of medulloblastoma metastasis. The critical molecular regulators of the invasation and extravasation of medulloblastoma cells in this process warrant further investigation. Besides, more efforts should also be made to examine the role of TME on medulloblastoma dissemination, including its influences on enhancing the metastasizing capability of medulloblastoma cells in primary tumors and supporting the colonization/survival of disseminated medulloblastoma cells at metastatic sites. On the basis of a more precise understanding of the molecular programs for medulloblastoma metastasis, the development of new treatments targeting not only primary but also metastatic compartments holds the promise of improving clinical efficacy for this refractory disease.

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