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Advances in Molecular Targets for the Treatment of Medulloblastomas

Abstract

Purpose: To present an assortment of molecular targets evident from a variety of signal transduction pathways and downstream effectors, which may have clinical relevance for the treatment of medulloblastomas.

Source: Data were archived from MEDLINE, using Boolean-formatted queries on the keywords including: medulloblastoma, pathology, prognosis, classification, tumor regression, inhibition, therapy, clinical trial, therapeutic agent, drug, molecular inhibitor, and signalling pathway. Only the most reputable articles were selected for critical analyses based on the qualitative assessment of the citation index, novelty of the findings and relevance to prospective novel ways of targeted therapies for medulloblastomas.

Principal findings: Medulloblastomas are highly aggressive embryonal tumors of the cerebellum, akin to primitive neuroectodermal tumors elsewhere in the brain. Current treatments for medulloblastomas which include a combination of surgery, chemotherapy and radiation, remain challenging especially, for younger patients; however, advances in understanding regulatory pathways in medulloblastomas are crucial to develop more effective therapeutic targets. Evidence showing several molecular and pharmacological targets within key signalling pathways, such as HEDGEHOG, WNT, NOTCH, Receptor Tyrosine Kinase (ERB, IGF-IR, c-MET, PDGF, Estrogen, p75NTR), their downstream effectors like PI3K/AKT, c-MYC and STAT3, and as well as other targets such as telomerase and cytoskeletal elements, is summarized. All molecular and pharmacological targets have pivotal roles in the pathogenesis of medulloblastomas. Most importantly, these pathways can be effectively pharmacologically targeted to regress the growth of medulloblastomas. Pre-clinical studies were routinely undertaken with a variety of human and murine cell lines and as well as murine models of medulloblastomas. Thus far, two drugs which target the NOTCH and HEDGEHOG signalling have completed Phase I clinical trials, but with evidence of low efficacies; hence, reinforcing the importance of continuing investigations in search of new therapeutic agents and targets.

Conclusion: Novel therapies, based on better understanding key biological pathways in medulloblastomas, hold promise for improved treatments in due course among patients with medulloblastomas.

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Medulloblastomas are most common among children, representing up to 30% of diagnosed pediatric primary brain tumor cases [1]. About 40% of these cases are diagnosed when children are younger than five years old. These younger cases are usually therapeutically challenging and correlate with the poorest prognosis. Furthermore, about 30% of cases are diagnosed between the ages of five and nine, while a majority of the remaining cases are diagnosed between 10 and 19 years of age. Medulloblastomas account for less than 2% of primary brain tumors in adults. The main neurological symptoms among affected children are associated with hydrocephalus due to fourth ventricle obstruction, but progressive worsening of symptoms occurs when an increase in tumor size begins to affect other major cranial nerve functions. The incidence tends to be higher in males when compared with females (~1.6:1 ratio). [1]

The historical definition of medulloblastoma is a distinct cerebellar tumor; while histologically similar, tumors that reside elsewhere are classified as primitive neuroectodermal tumors of the central nervous system [1]. In 2007, the World Health Organization (WHO) defined medulloblastomas as “a malignant, invasive embryonal tumour of the cerebellum with preferential manifestation in children, predominantly neuronal differentiation, and an inherent tendency to metastasize via cerebrospinal pathways [1].” These tumors, like many other cancers, are genetically and epigenetically heterogeneous, and ongoing world-wide research is aimed at precisely defining the inherent cellular and molecular mechanisms regulating tumor growth and recurrence. The 2007 WHO classification recognizes five histologic variants of medulloblastomas: classic medulloblastomas, nodular/desmoplastic medulloblastomas, medulloblastomas with extensive nodularity, large cell medulloblastomas; and, anaplastic medulloblastomas, although the latter two entities are often grouped together as large cell/anaplastic medulloblastomas [1]. All variants correspond histologically to WHO grade IV. Among these pathological variants, younger patients with nodular/desmoplastic medulloblastomas have a better survival with current therapies [2]. Similar histology in older patients may be a favorable prognostic sign according to some studies [3]. Medulloblastomas can also be classified into two groups according to risk-adapted treatments based on the patient's age, extent of surgical resection and evidence of tumor infiltration: the high-risk and average risk groups, [4]. Recent studies describe about 80% of patients with average risk medulloblastomas and 70% of patients with high risk medulloblastomas respond favorably to current treatments [5].

Ongoing molecular studies have identified multiple subtypes of medulloblastomas with limited concordance to previously defined histologic subtypes. Current consensus recognizes four molecular subgroups: WNT/Wingless (WNT), characterized by alterations in the Wnt signaling pathway, Sonic Hedgehog (SHH), characterized by alterations in the Hedgehog signaling pathway, and Groups 3 and 4 [6]. Thus far, Group 3 medulloblastomas, which are associated with a particularly poor prognosis, can be categorized as MYC-driven [7, 8]. The nodular/desmoplastic medulloblastomas and most medulloblastomas with extensive nodularity fall into the SHH group, but classic and large cell/anaplastic medulloblastomas can be found in any molecular subgroup, while the large cell/anaplastic medulloblastomas remain a poor prognosis subgroup regardless of molecular status [9].

The treatments for medulloblastomas in large pediatric neuro-oncology centres follow guidelines developed over the last 30 years from multi-centre trials in North America and Europe. In fact, current protocols for infants older than 36 months include craniospinal radiotherapy, chemotherapy (like Cisplatin, Cyclophosphamide, Vinblastine), and in some cases autologous stem cell transplantation [10, 11]. The five-year event-free-survival for patients having high-risk medulloblastomas was 70% in a recent US study using 36 Gy of radiation administered to the craniospinal axis, and adjuvant Cyclophosphamide-based dose-intensive chemotherapy combined with hematopoietic stem cell support [12]. In contrast, infants younger than 36 months do not receive radiotherapy due to the risk of acquiring severe side effects including mental retardation, growth failure and leukoencephalopathy [12]. Instead, these infants are treated with chemotherapy alone (like Cyclophosphamide and Vincristine). Studies from the Pediatric Oncology Group reported that 74% of infants less than 24 months and 91% of those aged 24-36 months had no disease progression after one year following chemotherapy and total surgical resection of a solitary tumor [13]. Unfortunately, despite current treatment modalities (surgery, radiation and chemotherapy), poor outcomes are still frequent among children having infiltrative unresectable medulloblastomas. This justifies the need to explore alternative therapeutic agents and targets; a topic reviewed in this article.

Methods

Data were archived from MEDLINE, using Boolean-formatted queries on keywords including: medulloblastoma, pathology, prognosis, classification, tumor regression, inhibition, therapy, clinical trial, therapeutic agent, drug, molecular inhibitor and signalling pathway. From over 4000 articles on

these topics, the focus was restricted for analyses only to those articles describing novel strategies and relevant new insights into the biology and/or therapeutic targeting of medulloblastomas within a pre-clinical and/or clinical context; hence representing less than 2% of all articles. Emphasis was also placed on those articles published by leading researchers within the field of therapeutic research in medulloblastomas and deciphering signal transduction pathways. Furthermore, the data was analysed to determine whether any novel therapeutic target or agent is in use with current or proposed clinical trials as archived from the website: clinicaltrials.gov.

Results

Considerable progress has been made towards understanding the biology of medulloblastomas, which is advantageous in discovering alternative therapeutic targets that have the potential to offer more effective treatments for medulloblastomas. Indeed, numerous targeted therapies aimed at modulating signaling pathways mediating growth, survival, differentiation, proliferation, migration, invasion and angiogenesis were developed. A wide multitude of new targets from signalling pathways in medulloblastomas that are crucial to drive tumor progression are summarized in Figure 1.

Hedgehog signalling

Initially discovered as a regulator of segment polarity in *Drosophila*, Hedgehog signalling has emerged as a crucial player in both vertebrate and invertebrate development [14]. Aberrant Hedgehog signalling is eminent in about 30% of medulloblastomas [15]. Hedgehog signalling is controlled by both upstream activators and repressors. For instance, in the absence of the Hedgehog ligand, the Patched transmembrane receptor (PTCH1) attenuates the function of the neighbouring Smoothened receptor (SMO); however, binding of the Hedgehog ligand to the Patched receptor relinquishes inhibition of the Smoothened receptor, leading to the downstream activation of the GLI1 and GLI2 proteins in the cytoplasm. These activated GLI proteins then translocate to the nucleus and induce the expression of genes, including *PTCH1*, *HHIP* (Hedgehog interacting protein) and *SUFU* (Suppressor of Fused homolog), the latter being a repressor of Hedgehog signalling [14]. In fact, a wide spectrum of mutations including in the *SMO*, *PTCH1* and *SUFU* genes was identified to contribute to the pathogenesis of medulloblastomas [19]. Moreover, about 5% of patients with Gorlin syndrome who have loss of function mutations in the *PTCH1* gene, develop medulloblastomas [14].

Much progress in finding suitable pharmaceutical agents for the treatment of medulloblastomas has emerged from the use of the *Ptch1* mouse model, as well as medulloblastoma cell lines established from mice and from human tumor specimens. For example, the PTCH1 protein was capable of being restored in the cerebellum of a *Ptch1* conditional knock-out mouse model with the drug Bortezomib, a 26S proteasome inhibitor [16], and this treatment could ultimately significantly repress the growth of medulloblastomas. Similarly, treating human medulloblastoma cell lines, DAOY, D283 and D341, with Bortezomib profoundly repressed the growth of these tumor cell lines [16]. Vismodegib (GDC-0449), an inhibitor of the Smoothened receptor, has completed Phase I clinical trials involving patient with medulloblastomas [17]. Vismodegib is very effective *in vitro* and in studies with mouse model [18], as well as being capable of inducing tumor regression in a patient with refractory metastatic medulloblastoma [19]. These initial findings have generated a lot of excitement prompting the need for additional clinical trials with Vismodegib, including an ongoing Phase I study and a planned Phase II study in patients with recurrent or relapse with medulloblastomas. One pitfall is the initial observation of acquired resistance with Vismodegib, which challenges the efficacy of this drug within a clinical context. Following drug treatments, *de novo* substitution mutation commonly occurs within a conserved aspartate residue of the Smoothened receptor to prevent the binding of Vismodegib; hence, causing relapse [20]. Such phenomenon is similarly noted with another Smoothened inhibitor, NVP-LDE225 [21]. Tumor regression is initially observed; however, acquired resistance shortly occurs with *de novo* activation mutations within the Smoothened receptor, as well as gene amplification mutations of the *GLI2* gene; and therefore relapse with tumor re-growth is seen as a consequence of re-activated constitutive Hedgehog signalling.

Other classes of Smoothened receptor inhibitors are being investigated in cell culture and murine models of medulloblastomas, and may offer an alternative for the treatment of resistant medulloblastomas. For instance, Saridegib (IPI-926) can still attenuate Hedgehog signalling in medulloblastoma cells by maintaining strong binding to Smoothened receptor mutants, which are incapable of being bound by Vismodegib [22]. In this manner, Saridegib can prolong the survival of *Ptch1* mice by inducing tumor regression [22]. Similarly, other smoothened inhibitors, including PF-5274857, Cyclopamine, HhAntag, and even using a polymeric nanoparticle encapsulated with NanoHHI, a small-molecule inhibitor of Hedgehog signalling, can efficiently induce tumor regression in several murine models of medulloblastomas [23, 24, 25]. Cyclopamine, an alkaloid

FIGURE 1. Schematic of signalling pathways and downstream effectors that have pivotal roles in driving the progression of medulloblastomas. These include a variety of transmembrane receptors such as ERB2, ER, PDGF, c-MET, Smoothed, Patched, NOTCH and p75NTR; and intracellular effectors like γ -secretase, MYC, STAT3, PI3K, AKT, β -catenin. A summary of relevant pharmacological agents and their respective molecular targets are also shown. ----| represents inhibition of a molecular target by a pharmacological agent such as from drugs, miRNA genes or other proteins.

of corn lily, was reported to regress tumor growth in allograft models, as well as in a *Ptc1*^{+/−}*p53*^{−/−} murine double mutant [24, 25]; however, Cyclopamine can also activate the neutral sphingomyelin phosphodiesterase 3 protein in the human DAOY medulloblastoma cell line to increase the production of neuronal-nitric oxide synthase (n-NOS), leading to nitric oxide mediated cell death [26].

Abrogation of intracellular signalling mediated by the GLI proteins is another prospective therapeutic strategy for the treatment of medulloblastomas, since the GLI proteins are

crucial to regulate the expression of downstream genes in Hedgehog signalling. HhAntag and SANT-1, potent antagonists of the Smoothed receptor [27], not only significantly induce tumor regression to prolong survival in the *Ptc1*^{+/−}*p53*^{−/−} mouse model of medulloblastomas, but significantly decreases the expression of the *GLI1* gene, and other downstream genes such as *SFRP1* (Secreted frizzled-related protein 1), *MATH1* and *PTCH2* [27]. Even though it was not investigated, this effect is expected to also occur with the use of other drugs such as Cyclopamine, Saridegib and Vismodegib. Simi-

larly, Forskolin and H89, agonists for protein kinase A (PKA), a downstream mediator of Hedgehog signalling, can result in the maintenance of the GLI protein complex in a repressor state [28]; and hence, repressing Hedgehog signalling which is crucial for the progression of a subset of medulloblastomas.

WNT signalling

WNT signalling, an important mediator of cell growth and differentiation is activated following the co-binding of WNT ligands to a family of seven transmembrane frizzled (Fz) receptors, which form heterodimers with the low-density lipoprotein receptor-related protein (LRP) [29]. This induces the phosphorylation of Dishevelled and β -catenin, and the inhibition of glycogen synthase kinase 3-beta (GSK-3beta). The phosphorylated β -catenin then translocates to the nucleus and interacts with other transcription factors of the T cell factor/lymphoid enhancer factor (Tcf/Lef) family, which subsequently induces the expression of several genes including *c-MYC* and *CCND1*. Elevated WNT signalling is prevalent in ~25% of medulloblastomas with mutations most common in the *APC*, *AXIN1* and *CTNNB1* genes [30]. In fact, among patients with familial adenomatous polyposis, germline mutations in the *APC* gene are associated with a high risk of developing medulloblastomas and colorectal adenomas [30]. Furthermore, frequent epigenetic mutations are prevalent in the promoters of the Secreted Frizzled-related protein 1, 2 and 3 (*SFRP1*, *SFRP2* and *SFRP3*) genes [30]. Many studies have investigated the possibility of therapeutically targeting WNT signalling in medulloblastomas. For instance, elevating the expression of the *SFRP1* gene in the D283 and ONS76 medulloblastoma cell lines leads to a significant reduction in the extent of phosphorylated or activated DVL2 (Dishevelled homolog 2) protein, a downstream effector of WNT signalling, and a subsequent decrease in tumor growth from an orthotopic intracerebellar xenograft model [31]. Similarly, increasing the expression of the *DICKOPF1* (*DKK1*) gene, a Wnt antagonist, in D283 medulloblastoma cell line augments apoptosis and hinders tumor growth [32]. In addition, the pharmacological inhibition of WNT signalling with the drug Norcantharidin, in the medulloblastoma DAOY cell line, significantly decreases the cellular levels of β -catenin and its translocation to the nucleus, and further regresses intracranial xenograft tumor growth in immunodeficient mice [33].

Notch signalling

Notch is a transmembrane heterodimeric receptor, comprising four family members, which mediates cell fate and pattern

formation during development [34]. Binding of the notch ligands, namely Delta and Jagged, which are reciprocally expressed in adjacent cells, triggers the proteolytic cleavage of the Notch ligands by α - and γ -secretases causing the release of the Notch intracellular domain. The intracellular domain then translocates to the nucleus and binds to CSL protein (CBFI/Suppressor of hairless/LAG-1) to recruit transcription factors that regulate the expression of genes such as *c-MYC*, *NF-kB2*, *p21*, *HES* and *HEY* family members. Elevated Notch signalling, mechanistically caused by factors such as increased expression of Notch receptors, specifically NOTCH2, has pivotal roles in driving the progression of medulloblastomas [35]. Hence, the Notch pathway possesses attractive therapeutic targets that warrant pre-clinical investigations. In fact, studies using the medulloblastoma DAOY cell line showed that inhibition of Notch signalling via the γ -secretase inhibitors, MK-0752, DFK-167 and GSI-18, can reduce the expression of Notch downstream effector genes like *HES1*, the overall tumor growth and the cancer stem cell populations within the xenograft tumor masses [35, 36, 37]. MK-0752 has completed Phase I clinical trials but with dismally low responses [37]; however, further clinical studies may still be pursued.

Re-expression of tumour suppressors into tumour cells is also another effective therapeutic strategy to regress tumor growth. The *NEURL1* gene functions in the ubiquitination of Notch Ligands to facilitate ligand degradation [38]. *NEURL1* is also a tumor suppressor gene in medulloblastomas and its expression negatively correlates with the *GLI1* and *GLI2* genes. Therapeutically, re-expression of *NEURL1* in the DAOY medulloblastoma cell line regresses tumor growth, and mechanistically, it acts by blocking the expression of Notch signalling effector genes like the *HES1* and *HEY1* family of transcription factors, leading to an increase in apoptosis [38].

Notch signalling is also regulated by several micro-RNA genes, which can be exploited for therapeutic applications. Micro RNA genes encode single stranded non-coding RNA molecules that repress translation via binding to the 5' or 3' untranslated regions of mRNA transcripts. Apart from having important roles in the biology of medulloblastomas, miRNA genes can be used informatively as clinical biomarkers in tumor classification and predicting patient prognosis [39]. Several examples of therapeutic applications with miRNA genes were previously reported [39]. For instance, the *miR-199b-5p* gene negatively regulates the *HES1* gene of the Notch pathway, the *CD15* cancer stem cell marker, as well as other genes including *AKT* and *ERK*; while the *miR-34a* gene negatively regulates the Notch ligand – Delta-like 1. Since both of these miRNA genes are important modulators of cancer stem cell mainte-

nance and tumor progression [39], delivering high copies of these miRNA genes into tumor cells with methods such as nucleic-acid-lipid encapsulations or adenoviruses, can significantly reduce the growth of medulloblastomas from an orthotopic DAOY cell line model [39].

SIGNALING MEDIATED BY RECEPTOR TYROSINE KINASES AND DOWNSTREAM EFFECTORS

Receptor tyrosine kinases (RTK) belong to a family of transmembrane receptors that transduce biological signals from a wide variety of ligands such as FGF, EGF, PDGF, steroids, HGF, IGF-I neuregulins and neurotrophins to modulate many cancer progression pathways in medulloblastomas, including cell proliferation, death, differentiation and metastasis [40]. Like other signalling pathways, RTK signalling is stringently regulated in normal cells. In medulloblastomas, aberrancies in signalling that result in driving tumorigenesis, are common. Below, advances in pre-clinical research on several molecular targets from signalling pathways that have therapeutic relevance are outlined.

RECEPTORS

ERB receptor family

The ERB proteins belong to the type I family of growth factor receptors comprising of c-ErbB-1 (EGFR), c-ErbB-2 (HER-2/neu), c-ErbB-3 (HER-3) and c-ErbB-4 (HER-4) [40]. In fact, ErbB-2 has elevated expression in medulloblastomas as a consequence of gene amplification, and correlates with poor overall survival [41]. Overexpression of ErbB-2 promotes the infiltration of medulloblastoma cells in the brain parenchyma by increasing the expression of the pro-metastatic genes such as *S100A4* [42]. This phenomenon can be abrogated with the ErbB tyrosine kinase inhibitor, OSI-774 [42]. Further pharmacological studies using the D341 and DAOY medulloblastoma cell lines indicate the EGFR inhibitor, Gefitinib, can significantly induce tumor regression in xenograft models. Interestingly, overexpressing the *HER2* genes in DAOY cell lines can further induce sensitization to Gefitinib treatments by causing up to 78% reduction in xenograft tumour explant volumes [43].

Insulin-like growth factor I receptor

Crucial in cell proliferation, differentiation, DNA repair and resistance to apoptosis is the Insulin-like growth factor I receptor (IGF-IR) [44]. IGF-IR becomes activated upon the binding of ligands such as IGF1, IGF2 or insulin, leading to the

phosphorylation of several downstream effectors like phosphatidylinositol 3-kinase (PI3K), and the C-terminal SRC kinase (CSK) [45], to modulate a variety of cellular processes. An increase in IGF-IR signaling in medulloblastomas is evident from experiments involving cancer cell lines, animal models and surgical specimens [45-49]. For instance, by using the human (DAOY, TE-671, D283 Med) and murine (BsB8, BsB13, Bs-1b, Bs-1c) medulloblastoma cell lines, it was discovered that IGF-I induces the rapid phosphorylation of IGF-IR to promote cell proliferation. This effect can be inhibited by using an IGF-IR protein with a dominant negative mutation or by silencing its expression with antisense oligonucleotides [45]. Pre-clinical studies with pyrrole[2,3-d]pyrimidine derivatives (NVP-AEW541 and NVP-ADW742) have shown some promise of anti-neoplastic properties for the treatment of medulloblastomas and as well as in other cancers [46]. The drug NVP-AEW541 functions as a competitive inhibitor of ATP in the binding of IGF-IR to abrogate downstream signaling and growth of the murine (BsB8) and human (DAOY and D384) medulloblastoma cell lines. Further treatments of these cell lines with sodium nitroprusside, which inhibits the phosphorylation of the GSK-3 β protein, can synergize with NVP-AEW541 to decrease the survival of medulloblastoma cells. Interestingly, the drug NVP-ADW742 has similar effects as NVP-AEW541, and can further chemosensitize the DAOY medulloblastoma cell line to Temozolomide [47]. Other pre-clinical studies using medulloblastoma cell lines (D343 and DAOY) and the Ptch1 medulloblastoma mouse model, have demonstrated additional small molecule inhibitors like Fenofibrate and Picropodophyllin, can block IGF-IR signalling in medulloblastoma cells and decrease cell survival by augmenting apoptosis [48, 49].

c-MET receptor

c-MET is a transmembrane protein of the tyrosine kinase receptor family, which has elevated expression in human medulloblastomas [50]. Binding of ligands, such as Scatter factor or Hepatocyte growth factor, to the c-MET receptor leads to the activation of c-MET and recruitment of adaptor proteins in the intracellular space, followed by the activation of several downstream signaling pathways such as RAS and phosphoinositide 3-kinase (PI3K); pathways important to mediate cell migration, growth and survival [50]. Attenuation of the growth of medulloblastomas can be accomplished with gene therapy or with the use of pharmacological agents. For instance, the tumor suppressor gene, Serine protease inhibitor kunitz-type 2 (SPINT2), is epigenetically silenced in 34.3% of primary medulloblastoma specimens [51]. Re-expression of the

SPINT2 gene in medulloblastoma cell lines can attenuate the growth of these cell lines in vitro and in vivo [51]. Furthermore, the pharmacological targeting of c-MET signaling, using the small molecule inhibitor SGX523, can effectively decrease the growth and migration of the human medulloblastoma cell line, DAOY [52]. Interestingly, natural flavonoid compounds like Quercetin, Kaempferol and Myricetin, found abundantly in vegetables and fruits, can inhibit the phosphorylation of c-MET and AKT, leading to decreased growth and migratory properties of the DAOY cell line [53]. These pre-clinical findings collectively suggest targeting c-MET signaling could be an effective alternative in the treatment of medulloblastomas.

Platelet-derived growth factor receptor

Platelet-derived growth factor receptor (PDGFR) is another tyrosine kinase transmembrane receptor with two major isoforms, PDGFR α and PDGFR β , which mediate their signalling upon ligand binding in a manner similar to the generic receptor, tyrosine kinase. In fact, PDGFR α gene amplification mutations are present in approximately 4% of medulloblastomas, while 13% of these tumors also have elevated protein expression [54]. Elevated PDGFR β protein expression is commonly observed among metastatic medulloblastomas [55]. Of therapeutic relevance, treating medulloblastoma cells with the PDGFR inhibitors, such as Gleevec or Sunitinib, can inhibit the in vitro and in vivo growth and invasiveness of human medulloblastoma DAOY and D556 cell lines [56]. Both drugs can efficiently cross the blood-brain barrier [56]; however, further studies are required to determine the combined efficacies of these drugs in cell line models and as well as in mouse models for medulloblastomas.

Estrogen receptor

The synthesis of steroids such as 17 β -estradiol occurs in several regions of the body, including the brain, to mediate a wide spectrum of biological functions [57]. Among malignancies involving steroidogenic tissues such as the breast, the binding of estradiol to the estrogen receptor and other non-steroid receptors like the epidermal growth factor receptor, activates downstream signalling events via pathways regulated by proteins such as RAS and Phosphatidylinositol 3-kinase (PI3K) [58]. Estradiol can also increase the viability of developing rat neonatal cerebellar neurons in primary culturing experiments [58]. Most remarkably, the estrogen receptor- β has elevated expression in medulloblastoma surgical specimens and in human medulloblastoma cell lines such as D283Med and DAOY [59]. In fact, estradiol and an agonist of the estrogen receptor-

beta (2, 3-bis(4-hydroxyphenyl)-propionitrile), can accelerate the growth of human medulloblastoma cell lines [59]. Evidence from xenograft tumors grown with the human D283 medulloblastoma cell line, indicates that this phenomenon can be reversed by treating the tumors with Faslodex (ICI182,780), a drug which functions as an antagonist to the estrogen receptor [59]. Estradiol also influences the infiltrative properties of medulloblastomas via downstream signalling with the calcium/calmodulin activated kinase kinase (CaMKK) pathway [60] through the activation RAC1. In fact, treating the human medulloblastoma DAOY cell line with a CaMKK inhibitor, STO-609, attenuates the migration of these cells [60]; suggesting that CaMKK could be another therapeutic target for metastatic medulloblastomas. These findings should be examined cautiously since ovariectomized *Ptch1*^{+/-} female mice are naturally susceptible to a higher incidence of developing medulloblastomas [61], but this phenomenon can be reversed following the administration of the estrogen receptor-beta agonist, 2, 3-bis (4-hydroxyphenyl)-propionitrile [62]. The direct influence of estradiol on tumor progression in the *Ptch1* mouse model for medulloblastomas has not yet been established.

p75 neurotrophin receptor

p75 neurotrophin receptor (p75^{NTR}) is a multifunctional type I transmembrane protein belonging to the tumor necrosis factor superfamily, which is expressed in a subset of medulloblastomas [63]. The binding of the neurotrophin ligand leads to receptor activation and subsequently the proteolysis of the α - and γ -secretases of the intramembrane cause the release of the extracellular and intracellular domains [64]. These events are important to regulate tumor invasion. Most importantly, recent evidence suggests that the pharmacological targeting of γ -secretase in medulloblastoma animal models can effectively abrogate p75^{NTR} function by significantly diminishing tumor metastasis along the spinal column [65]. Hence, targeting p75^{NTR} signalling is another potential strategy for the treatment of medulloblastomas.

DOWNSTREAM SIGNALLING PROTEINS PI3K/AKT

The phosphatidylinositol 3-kinase (PI3K) and AKT (Protein Kinase B) proteins function to bridge many signalling pathways, such as those mediated by the IGF-1R and Neurotrophin-3 (TRKC) receptors in medulloblastomas, to regulate crucial processes in cancer progression, including cell proliferation, migration, apoptosis and survival [45, 66]. PI3K/AKT can also be aberrantly activated by the loss of function of PTEN, a tumor suppressor, either by gene deletions or pro-

motor methylation mutations, as seen among 16% of medulloblastomas [67]. Furthermore, activating mutations in the *PIK3CA* gene is prevalent among a wide assortment of human cancers, including medulloblastomas [68]. Prior pre-clinical studies have investigated whether targeting PI3K and AKT could potentially treat medulloblastomas. For instance, the catalytic subunit of PI3K, p110 α , when competitively bound by the drug, YM024, could impair Doxorubicin-induced phosphorylation of the AKT and S6 proteins leading to significant decreases in the survival and migratory properties of the human DAOY medulloblastoma cell line and inhibition of anchorage independent growth [69]. In another example, the third generation Colecobix inhibitor (OSU03012 or 2-amino-*N*(4-5-(2-phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]phenyl)acetamide), acts very potently in the human D283 medulloblastoma cell line to decrease the phosphorylation of selected serine and threonine residues on the PDK1 and AKT proteins and likewise downstream targets of the mTOR pathway (S6K1 and 4E-BP1), resulting in significant *in vitro* and *in vivo* growth arrest [70]. Furthermore, the inhibitory effect of OSU03012 on PDK1 and AKT, causes GSK-3 β to become activated, leading to the phosphorylation of amino terminus of β -catenin, and subsequently retention in the cytoplasm rather than in the nucleus [70]. Nevertheless, TCL/LEF transactivation declines and results in comparable decreases in the expression of the *CYCLIN D* and *c-MYC* genes in the D283 medulloblastoma cell line [70].

Other drugs, such as the Rapamycin analog CCI-779, also demonstrate potent anti-neoplastic properties with the human DAOY cell line by inhibiting *in vitro* and *in vivo* growth [71]. Despite the potent cytotoxic effect of CCI-779 on medulloblastoma cells, most mTOR inhibitors can induce AKT activation; however, this problem can be circumvented by the combined use of CCI-779 and OSU03012, which synergistically block the phosphorylation of AKT, leading to a more profound decrease in the *in vitro* and *in vivo* growth of the D283 medulloblastoma cell line [70]. Recently, some studies have demonstrated that targeting the PI3K/AKT pathway can also be beneficial in the treatment of medulloblastomas. This provides, evidence of therapeutic resistance having emerged from aberrancies in Hedgehog signaling. In this manner, NVP-BKM120 (an inhibitor of PI3K) and NVP-BEZ235 (an inhibitor of both PI3K and mTOR) can very effectively reduce the survival of resistant medulloblastoma cells treated with Smoothened receptor antagonist NVP-LDE225 [21]. It is important to note that PI3K also has crucial roles in modulating the survival of medulloblastoma stem cells following irradiation [72] and in tumor migration via the activation of

RhoGTPases [73]. Hence, it is anticipated that agents that target PI3K and its downstream signalling are expected to be beneficial for use as chemotherapy agents - alone or as adjuvant therapies with radiation treatments.

c-MYC

The protein levels of the transcription factor *c-MYC* are frequently elevated in up to 72% of human medulloblastomas specimens, and with about 10% of these tumors having gene amplification mutations amongst the most aggressive large cell/anaplastic variants [74]. *c-MYC* expression is modulated by several signaling pathways including Hedgehog, PI3K/AKT, WNT/ β -catenin and *c-MET* [68, 75] and appears to be an attractive candidate for therapeutic targeting. For instance, in medulloblastomas, upon ligand activation of *c-MET* signaling, downstream PI3K/MAPK signaling becomes active, which causes the inhibition of GSK3- β , the translocation of β -catenin to the nucleus, and eventually induces expression of the *c-MYC* gene [75]. Several studies have investigated the therapeutic potential of targeting the *c-MYC* gene in medulloblastomas. One example of this targeting is the Telomestatin derivative, S2T1-6OTD, which binds to the NHEIII1 sequence in the promoter of *c-MYC* impairing transcriptional activation and thereby decreasing the viability of medulloblastoma cells [76]. S2T1-6OTD also inhibits the expression of the human *hTERT* gene required for telomere maintenance [76]. Other agents, such as the natural-occurring plant derived polyphenol, Resveratrol (3, 5, 4'-trihydroxy-*trans*-stilbene), the anticonvulsant and histone acetylase inhibitor, Valproic acid, the Quassinoid analogue, NBT-272, and all-*trans*-retinoic acid, can all effectively inhibit the *c-MYC* gene expression and subsequently the *in vitro* and *in vivo* growth of medulloblastoma cells [74, 77-79].

STAT3

The signal transducer and activator of transcription 3 (STAT3) protein, a crucial member of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling cascade, is constitutively activated in medulloblastomas [80]. STAT3 can be therapeutically targeted with pharmacological agents or via the use of shRNA technology. For instance, Sunitinib, Resveratrol and the JAK₂-specific inhibitor AG490, can all potently inhibit the phosphorylation of the STAT3 protein, thereby disrupting the expression of downstream genes including *c-MYC*, *SURVIVIN*, *COX2*, and *CYCLIN D1*, and leading to a diminished growth of the human UW228-3 or DAOY medulloblastoma cell lines [81,82]. Sunitinib (SU11248), a

FDA-approved agent for the treatment of renal carcinoma and Imatinib-refractory gastrointestinal tumours [83], can effectively inhibit in medulloblastomas the phosphorylation of AKT and the activation of GSK3 β and mTOR, leading to diminished survival of tumor cells. This phenomenon can be mechanistically correlated with the decreased expression of the *CYCLIN E/D2/D3* genes, and conversely, increased expression of the *BAK* and *BIM* genes [82]. Other STAT3 inhibitors such as Sorafenib (BAY43-9006, Nexavar), LLL12, Curcumin and Cucurbitacin have similar effects to inhibit the *in vitro* and *in vivo* growth of medulloblastoma cells [84-87]. In fact, Sorafenib targets the RAF and receptor tyrosine kinases (RTK), to abrogate STAT3 phosphorylation and to cause drastic decreases in the levels of the CYCLIN D/E and MCL-1 proteins [84]; important regulators of cell cycle and apoptosis.

OTHER MOLECULAR TARGETS

Telomerase

In normal cells, progressive shortening of the chromosome telomeres occurs after each cell division until a threshold is met, then the cell enters senescence and eventually apoptosis [88]. In cancerous cells, such as medulloblastomas, elevated activity of telomerase caused by over-expression and amplification mutations of the *hTERT* gene, and via other mechanisms such as alternate lengthening of telomeres, can cause sustained telomere lengths [89]. Telomerase is an attractive candidate for therapeutic targeting based on findings from several studies. For instance, the use of compounds such as all-trans retinoic acids, and Abacavir (an anti-retroviral reverse transcriptase inhibitor) can perturb the growth of medulloblastoma cell lines, and is correlated with decreased telomerase expression and activity [90,91]. Furthermore, photodynamic therapy [92], which uses light to activate photosensitizers to produces reactive oxygen species, is also effective in pre-clinical therapeutic studies on medulloblastomas. In fact, by administering 5-aminolevulinic acid (5-ALA) and ALA hexylester in conjunction with irradiation treatments of the TE-671 medulloblastoma cell line can significantly inhibit *in vivo* growth in immuno-deficient mice and is accompanied by a decline in hTERT expression in the tumor cells [92].

Cytoskeletal elements

Microtubules binding agents, including Paclitaxel, Docetaxel, Vinorelbine, have demonstrated variable efficacies in clinical use among patients with medulloblastomas as well as other cancers [93-95]; hence, the need to investigate other suitable pharmacological agents. Recent studies suggest that Curcumin

has potent anti-neoplastic properties. For instance, research undertaken with the human DAOY medulloblastoma cell line showed that in addition to STAT3, Curcumin functions by inhibiting the activity of histone deacetylase 4 (HDAC4), thereby causing alterations in the microtubule dynamics by increasing tubulin acetylations, and resulting in mitotic arrest [96]. In medulloblastoma clinical specimens, a wide assortment of other microtubule-associated proteins, such as Polo-like kinase 1 (PLK1) and Aurora Kinase A (AURKA), which regulate processes during mitosis such as chromosomal segregation and cytokinesis, are over-expressed in medulloblastomas [97] and therefore represent promising alternative therapeutic targets. Indeed, the pharmacological inhibition of PLK1 and AURKA, with BI 2536 and C1368 inhibitors, demonstrated evidence of a significant decrease in tumor growth by mechanistically arresting tumors cells in the G2M phase of the cell cycle [97]. Inhibition of PLK1 activity can further radio sensitize medulloblastoma cells [98], while inhibiting AURKA activity seems to improve chemotherapy sensitization with adjuvant treatments of medulloblastoma cells with Etoposide and Cisplatin [97]. Similar therapeutic outcomes are evident from the pharmacological inhibition of the DNA repair protein, Poly (ADP-ribose) polymerase-1 (PARP1), by drugs such as INO-1001 and Olaparib [99,100]. PARP1 has crucial roles in DNA repair in response to DNA damage and, by inhibiting its activity in medulloblastoma cells, can confer increase in chemotherapy sensitization to Temozolomide [99] and to radiation sensitization [100]. The pharmacological targeting of PARP1 may also offer improved efficacies in adjuvant treatments for medulloblastomas.

Conclusion and Perspectives

Over the years, tremendous progress has been made to better understand the biology of medulloblastomas, in conjunction with modern advances in the development of surgical, radiotherapy and chemotherapy techniques. Indeed, this research has improved the overall survival and quality of life of children with medulloblastomas. A subset of patients still relapse; hence, the need to explore new therapeutic agents. To assist with a better understanding of these tumors, an assortment of whole genome analyses have been undertaken and have uncovered a great wealth of genetic information concerning chromosomal imbalances, gene mutations and gene expressions. This information was beneficial in modernizing pathological diagnoses of medulloblastomas, and is being used to develop better biomarkers for predicting patient prognosis and deciding the best treatment modalities. Moreover, a wide variety of disciplines, such as molecular biology, biochemistry, pharmacology,

oncology and cell biology, have provided invaluable knowledge concerning signal transduction pathways and their role in driving the progression of medulloblastomas. Indeed, as discussed in this article, rigorous experimental therapeutic research by researchers world-wide has revealed novel pharmacological and molecular targets originating from several signalling pathways and their downstream effectors, as well as other targets such as telomerase and cytoskeletal elements; all of which have pivotal roles in driving the progression of medulloblastomas. In fact, two of the drugs from these studies were investigated in Phase I clinical trials, namely MK-0752 (a target of γ -secretase in NOTCH signalling) and Vismodegib (a target of the Smoothed receptor in Hedgehog signalling). Unfortunately, these drugs exhibited only low efficacies but the search continues for better drugs for the treatment of medulloblastomas. In this manner, it is still imperative for the continuation of research on targeted therapy strategies, including the identification of new molecular and pharmacological agents. This is a field of research that will require the use of a wider spectrum of pre-clinical models like cancer stem cells (tumorspheres), tumor cell lines, murine mouse models and tumor cells from surgical resections that are freshly maintained in artificial cerebrospinal fluid, prior to administering human clinical trials. Discordance in results from “bench to bedside” and concomitant toxicity issues are expected to occur with a subset of the drugs, but this research will eventually lead to a narrower selection of more effective therapeutic agents and strategies.

Many patients relapse due to acquired drug resistance that can be relieved by a change in the treatment modality, such as by using another chemotherapy agent that is intolerant to the resistance conferred by the tumor. The extensive “cross-talk” eminent among signalling pathways such as NOTCH, Hedgehog and WNT, is beneficial for the design of more effective drugs that can be optimized for treating resistant tumors. The ultimate drug is one that has multiple targets within tumor cells, strong efficacies among a wide spectrum of tumor cells, and low toxicity to neighbouring normal cells. Furthermore, combined treatment modalities still remain the most robust method to eradicate the tumor or extend the overall survival and quality of life among the patients. From the advances in the development of novel pharmacological agents and the understanding of molecular targets, as summarized in this article, future clinical assessments are needed to determine the efficacy of novel agents to either replace existing treatments or for use adjuvantly, to treat newly diagnosed patients and as well as patients who are recurrent or relapsed.

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