Molecular subgroups of medulloblastoma

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Abstract

Recent efforts at stratifying medulloblastomas based on their molecular features have revolutionized our understanding of this morbidity. Collective efforts by multiple independent groups have subdivided medulloblastoma from a single disease into four distinct molecular subgroups characterized by disparate transcriptional signatures, mutational spectra, copy number profiles and, most importantly, clinical features. We present a summary of recent studies that have contributed to our understanding of the core medulloblastoma subgroups, focusing largely on clinically relevant discoveries that have already, and will continue to, shape research.

Keywords

chromatin; genomics; medulloblastoma; molecular classification; pediatric brain tumors; subgroups

Medulloblastoma subgroup identification: a brief history of the confusion

During the past 5 or 6 years, multiple independent efforts at profiling the medulloblastoma transcriptome have led to the discovery and description of distinct molecular subgroups of this disease. However, bioinformatic analyses of gene-expression datasets generated from moderate (n = 46) to large (n = 194) cohorts of primary cases have revealed inconsistencies

in the number of molecular subgroups identified. To make things even less clear, each research group has assigned their own nomenclatures to these subgroups, causing an ever-growing degree of confusion among the pediatric brain tumor community.

Four principal expression array-based analyses have arguably had the greatest influence on our definition of the molecular subgroups of medulloblastoma during this relatively brief history [1–4]. In 2006, Thompson *et al.* were the first to introduce the modern concept of transcriptionally discrete subgroups of medulloblastoma, describing five distinct subgroups in a cohort of 46 cases [1]. These subgroups were designated A–E, and most notable were the 'B' and 'D' subgroups with activation of the WNT and Sonic hedgehog (SHH) pathways, respectively. Two years later, Kool *et al.* essentially recapitulated Thompson's finding of five medulloblastoma subgroups, this time in a series of 62 cases, once again assigned the letter designations A–E, but with no continuity with the nomenclature utilized in the Thompson study [2]. The WNT and SHH subgroups, respectively referred to as 'A' and 'B' by Kool *et al.*, were readily identified as standalone sample clusters, whereas the remaining 'C', 'D', and 'E' subgroups were shown to be more closely related. In 2010, independent studies led by our group in Toronto, Canada, and by Scott Pomeroy (Cho *et al.*) in Boston (MA, USA) published seemingly back-to-back medulloblastoma subgroup manuscripts [3,4]. Our expression analysis of 103 primary tumors demonstrated the greatest statistical support for four distinct variants of the disease (labeled as 'WNT', 'SHH', 'Group C', and 'Group D'), whereas the Boston study of 194 cases discriminated six molecular subtypes. In the latter report, subgroups were designated as 'C1–C6', with 'C6' representing the WNT cluster and 'C3' the SHH cluster. The remaining subgroups were more extensively characterized than in the previous publications, with 'C1'/'C5' and 'C2'/'C4' clusters representative of two unique subgroups with apparent substructure.

While it is evident from these findings that there are inconsistencies in the true nature/structure of the molecular subgroups of medulloblastoma and equal, if not greater, debate on the nomenclature of these subgroups, it has been firmly established that medulloblastoma comprises more than a single disease. There has been a general agreement in the community regarding the existence of WNT- and SHH-activated subgroups, fueled by their identification in all of the aforementioned studies and through extensive use of representative animal models that faithfully recapitulate the disease [5,6]. However, the true number of non-WNT/non-SHH subgroups has remained a divisive issue. Non-WNT/non-SHH medulloblastomas may represent one large subgroup [7,8] or, rather, four distinct subgroups as suggested in Cho *et al.*'s study [4]. Finally, in late 2010, a consensus was reached by leaders in the community at a closed meeting held in Boston, MA, USA. Based on the data presented in the recent Toronto and Boston series, as well as validation in subsequently published [9] and unpublished reports by independent groups, it was agreed that current evidence is most supportive of four core medulloblastoma subgroups, to be named WNT, SHH, Group 3 and Group 4 [10]. The somewhat generic names of Group 3 and Group 4 were thought to be most appropriate until the biology of these subgroups are better understood (Figure 1) [10].

Clinical significance

The clinical relevance associated with stratifying medulloblastoma based on their molecular and/or histological characteristics predates the publication of studies by Thompson, Kool, Northcott, and Cho that have collectively formulated much of our current understanding. In 2005, Ellison *et al.* provided early evidence that suggested that medulloblastomas with activated β-catenin belonged to a unique subgroup of the disease with a superior prognosis [11]. In this important study, 109 childhood medulloblastomas entered into the International Society of Pediatric Oncology/United Kingdom Children's Cancer Study Group Primitive Neuroectodermal Tumor 3 trial were examined for β-catenin nucleopositivity and in some cases mutation status. Patients exhibiting tumors with nucleopositive β-catenin had 5-year overall survival (OS) rates of 92%, compared with only 65% for nucleonegative cases. This exceptionally good prognosis among cases with activated β-catenin reported in the European study was subsequently recapitulated in the North American St Jude Medulloblastoma-96 trial reported by Gajjar *et al.* in 2006 [12]. In this study, 100% (10/10) of patients falling into this 'early' WNT subgroup were disease-free at 5 years, compared with only 68% (40/59) of non-WNT tumors. The association of an excellent prognosis with WNT medulloblastoma has since been validated in nearly all patient cohorts examined [3,4,7,9,13–16], thus making this important subgroup a candidate for possible de-escalated therapy in future clinical trials [17].

Molecular and pathological links to clinical outcomes for what is now accepted as the SHH subgroup were also evident prior to conventional subgrouping. In 2002, Pomeroy *et al.*'s seminal work published in *Nature* demonstrated that desmoplastic medulloblastomas are transcriptionally distinct from the more prevalent classic histology and exhibit activation of SHH target genes [18]. During the past decade, numerous clinical studies have investigated the role of histology in predicting medulloblastoma outcome, with desmoplastic/nodular histology confirmed to be a positive prognostic indicator in early childhood cases [19–22], although results have been conflicting. This connection between SHH activation, desmoplastic/nodular histology and an improved patient outcome has now been verified in the context of the four medulloblastoma subgroups. Specifically, nearly all reported desmoplastic medulloblastomas belong to the SHH subgroup. Furthermore, in the large cohorts examined on tissue microarrays (TMAs) and published by Northcott *et al.* [3] and Remke *et al.* [9,13], the 5-year OS probability for SHH cases was in excess of 80%. Among the 112 Children's Oncology Group cases (>3 years old) profiled in the Cho series for which outcome data were available, the SHH tumors exhibited a slightly diminished 6-year OS probability of approximately 65% [4]. This lower survival rate may be explained by the fact that children <3 years of age were excluded from this survival analysis, an age group in which the majority of patients belong to the SHH subgroup. The use of SHH tumors as a prognostic factor is largely based on pediatric cohorts. It is of particular importance to highlight that many prognostic markers are age-dependent in the SHH subgroup, including M-stage (adult only) and desmoplasia (pediatric only) [23]. The use of SHH tumors as a prognostic factor must be established using distinct cohorts of purely pediatric and purely adult cases to effectively establish the clinical significance of these distinct SHH variants.

Perhaps the most striking association between medulloblastoma subgroups and patient outcome came to light with the identification of the Group 3 subgroup [3,4]. Group 3 medulloblastomas are the most aggressive and malignant subgroup described to date, exhibiting 5-year OS probabilities ranging from approximately 20 to 30% [3,4,9]. This subgroup encompasses many molecular and pathological features that have been ascribed to medulloblastomas with a poor prognosis, including *MYC* amplification [15,16,24–27], large-cell anaplastic (LCA) histology [12,15,16,22,25,28] and metastatic disease [7,15,16,22,25,29]. In fact, the association between poor outcome and metastasis may be driven by the high prevalence of M+ disease in Group 3 tumors – we recently demonstrated that the power of M-stage as a predictor of patient outcome is lost when patients are stratified according to their molecular subgroup, with both M+ and M− Group 3 patients exhibiting a similarly dismal outcome [3].

A relevant clinical observation made by the Cho *et al.* study pertained to the separation of Group 3 medulloblastoma into two distinct subclasses, 'C1' and 'C5' [4]. Exposing this substructure revealed a pronounced difference in the 6-year OS, with rates of 21 versus 74% for 'C1' and 'C5', respectively. These trends suggest that the morbidity associated with Group 3 medulloblastomas is conferred by the 'C1' subclass, which harbors frequent *MYC* amplifications and is distinguished by the presence of metastasis.

Much like Group 3 medulloblastoma, our knowledge of the clinical behavior of Group 4 tumors is still rudimentary. Group 4 is the most common of all medulloblastoma subgroups, contributing to approximately 35% of all cases and thus accounting for a large percentage of what has often been referred to as non-WNT/non-SHH medulloblastoma in recent years. Group 4 tumors tend to have an 'intermediate' prognosis compared with the other subgroups, with reported OS rates of approximately 75–90% [3,4,9,13]. In a very recent study by Remke *et al.*, clinical heterogeneity was observed within the Group 4 subgroup, identifying *FSTL5* as a biomarker of high-risk Group 4 patients [9]. Future identification of similar biomarkers or, more likely, combinations of biomarkers will potentially lead to further dissection of high-risk patients within the core subgroups of medulloblastoma (Figure 2) [30].

Demographics

Age

Although medulloblastoma is the most common malignant pediatric brain tumor, the disease does occur in adults, albeit quite rarely compared with the more prevalent glioblastomas that represent the prototypical brain tumor of adulthood. Several original reports and epidemiological studies have shown that pediatric and adult medulloblastomas are genetically distinct and can behave quite differently from a clinical standpoint, suggesting that the two may represent distinct forms of the disease with potentially disparate origins [31–33]. Observations made in recent molecular subgrouping studies may account for some of these notable clinical differences that exist between younger versus older patients afflicted with medulloblastoma.

Medulloblastoma subgroups demonstrate a rather disparate distribution across distinct patient age groups. WNT tumors are found mainly in children and sometimes in adults, and are rarely seen in infants. SHH medulloblastomas are dominant in both infants and adults, accounting for more than approximately 60% of patients from either age group. By contrast, SHH tumors are underrepresented in childhood, constituting only approximately 10–15% of these cases. Group 3 medulloblastomas are found in pediatric patients but are exceptionally rare in older teenagers and almost never occur in adults. Group 4 cases are found in all age groups but appear to represent the bulk of childhood and adolescent medulloblastomas (Figure 3).

The uneven distribution of medulloblastoma subgroups across infants, children and adult patients has significant biological and clinical implications. Despite excellent survival rates repeatedly reported for WNT medulloblastomas, Remke *et al.* recently demonstrated that the improved survival of WNT patients is only applicable to pediatric cases, as adult WNT tumors exhibited a diminished OS rate of approximately 80% in the cohort examined [13]. While SHH medulloblastomas of different age groups do not display any significant clinical differences in their OS [13,23], our group has demonstrated that differences exist in the incidence of metastasis (higher in pediatric SHH), tumor localization (more frequently located in the vermis in pediatric SHH) and a trend towards a higher incidence in female patients in pediatric SHH versus adult SHH cases [23]. Furthermore, many prognostic markers demonstrate an age-dependent relationship, highlighting the differences between pediatric and adult SHH tumors [23].

Among all published cohorts with available subgroup associations, literally only a handful of patients over the age of 18 years have been assigned to Group 3, rendering this subgroup largely a pediatric disease. Indeed, in their paper characterizing the subgroups of adult medulloblastoma, Remke *et al.* concluded that adult medulloblastoma consists of just three distinct molecular variants: WNT, SHH and Group 4 [13]. Furthermore, in a very recent meta-analysis study carried out by Kool *et al.*, which included molecular and clinical data on 550 medulloblastoma cases, only four out of 177 (2.2%) adults were classified as Group 3, reaffirming the rarity of this subgroup in adults [34]. Although we are only beginning to uncover the molecular and cellular basis of this highly aggressive subgroup, it is of particular interest that Group 3 is confined to younger patients, further substantiating the hypothesis that pediatric and adult medulloblastomas are distinct diseases. Finally, while Group 4 medulloblastomas are found across all age groups, survival analyses performed in the adult study by Remke and colleagues indicate that not all Group 4 share the same prognosis, with adult Group 4 patients exhibiting a pronounced reduction in OS (~20%) compared with their pediatric counterparts [13].

Gender

It has long been reported that medulloblastoma is more commonly diagnosed in males, with an accepted male:female ratio of approximately 1.5:1 (Figure 1). Although this ratio has been repeatedly observed across patient cohorts, little insight has been gained in terms of possible mechanism(s) responsible for this gender bias. Recent integration of molecular subgrouping with gender information has shown that males and females exhibit distinct

frequencies with respect to their molecular subgroup makeup. Indeed, differences in subgroup distribution may explain the disparate responses to treatment that have been observed between males and females in the clinic [35].

Based on the combined cohorts of the recent studies published by Cho *et al.* [4] and our group [3], multiple relevant and significant observations related to subgroup distribution according to gender come to light. The male:female ratio for the combined cohort (n = 291) is 1.53:1, which is consistent with the accepted gender ratio for this malignancy. However, these trends change rather dramatically when examined in a subgroup-specific context, with male:female ratios of 0.5:1 and 2.8:1 for WNT and Group 3, respectively. These differences translate into a threefold increase in the prevalence of WNT tumors among females compared with males, occurring in 12 and 4% of females and males, respectively. Likewise, SHH tumors constitute a higher percentage of female medulloblastomas, accounting for 39% of cases versus only 23% of males. Moreover, Group 3 and Group 4 tumors account for 73% of male medulloblastomas versus only 48% of female cases. Collectively, data from these two cohorts show an uneven distribution of medulloblastoma subgroups according to patient gender. Future studies examining larger cohorts of patients will determine the validity of these reported distributions.

Histology

According to the 2007 WHO classification of tumors of the CNS [36], medulloblastoma consists of five histological variants, including (listed in order of prevalence) classic, desmoplastic/nodular, ana-plastic, large-cell and medulloblastoma with extensive nodularity (MBEN). The anaplastic and large-cell variants are typically amalgamated as a single LCA phenotype and regarded as a more clinically aggressive form of the disease compared with the other variants [37]. By contrast, the MBEN phenotype is enriched in very young infants and has recently been correlated with a very good prognosis [38–40]. The histological subtypes are not evenly distributed among the subgroups, the most notable of which are the desmoplastic variants that appear to be almost entirely restricted to SHH tumors [2–4,7]. Indeed, Ellison *et al.* reported that all desmoplastic tumors correlated with a SHH immunophenotype in a large study of 235 medulloblastomas investigated on a medulloblastoma TMA [7]. Likewise, MBENs have recently been shown to be strongly linked to Gorlin syndrome, and thus the SHH subgroup due to underlying *PTCH1* germline mutations [39]. Moreover, germline *SUFU* mutations have been recurrently catalogued in this rare subtype, further substantiating their association with SHH medulloblastoma [41]. WNT tumors are almost always of classic histology, although exceptionally rare LCA cases have also been reported. LCA tumors have been identified in all subgroups of medulloblastoma but have a significantly higher incidence in Group 3 tumors, in agreement with the poor outcome associated with this subgroup (Figure 1) [3,4]. Interestingly, the LCA histological subtype has been shown to correlate with *MYC* expression [42] and, reciprocally, over-expression (or amplification) of *MYC* leads to the development of an LCA phenotype, further linking these two markers of poor prognosis with the causal aggressiveness of Group 3 tumors [43].

Genomics

Transcriptomics

It could be argued that the 'molecular signature' of medulloblastoma subgroups is reflected in their underlying transcriptional profiles. Indeed, their discovery, much like the discovery of the major subtypes of GBM, can be credited to large-scale gene-expression profiling efforts that divulged the transcriptional heterogeneity of these diseases [44–46]. Despite considerable variation in the size of patient cohorts, gene-expression array platforms employed and unique combinations of bioinformatic tools, the consensus among the genes and pathways that are enriched among the subgroups has remained consistent.

WNT medulloblastomas express exorbitantly high levels of genes in the WNT pathway, including *WIF1*, *DKK1*, *DKK2*, *DKK4*, *LEF1*, *AXIN2*, *WNT11*, *WNT16*, *FZD6* and *FZD10* [1–4,47]. Several of these targets are known inhibitors of WNT signaling and are upregulated as part of a negative-feedback loop secondary to aberrant WNT activation. *MYC* is also expressed in WNT tumors and is a known target of the WNT cascade. Other transcriptional themes enriched in the WNT subgroup include TGF-β signaling, axonal guidance signaling and gene sets involved in the epithelial–mesenchymal transition.

SHH medulloblastomas, as implied by their nomenclature, are characterized by aberrant expression of a plethora of known SHH pathway genes, such as *HHIP*, *GLI1*, *GLI2*, *GLI3*, *PTCH2*, *BOC* and *ATOH1* [1–4]. WNT pathway genes *SFRP1*, *SFRP4* and *SFRP5* are also highly expressed in SHH tumors, as are members of the SOX gene family including *SOX2*, *SOX9* and *SOX13*. Comparing the transcriptomes of pediatric SHH tumors to their adult counterparts, we [23] and others [48] recently noted very distinct expression patterns between these age groups, with adult SHH tumors expressing elevated levels of HOX family genes (i.e., *HOXA5*, *HOXA9* and *HOXA2*) and genes involved in tissue development, whereas pediatric SHH tumors showed an enrichment of gene sets related to extracellular matrix function. The disparate transcriptional profiles observed between these two age groups of SHH medulloblastoma suggest they have distinct underlying biologies, and as a result may respond differently to current targeted therapies designed to abrogate SHH pathway activation [49,50].

Group 3 and Group 4 medulloblastomas remain quite poorly characterized compared with WNT and SHH tumors. Group 3 medulloblastomas tend to exhibit a retinal or phototransduction gene-expression signature, characterized by genes such as *GABRA5*, *NRL* and *CRX* [2–4], whereas Group 4 medulloblastomas are characterized by expression of neuronal factors including *GRM1* and *GRM8*, as well as potassium channel genes *KCNA1* and *KCNA5* [2–4]. In Cho *et al.*'s classification study, the Group 3 ('C1'/'C5') tumors were said to be GABAergic and Group 4 ('C2'/'C4') tumors to be glutamatergic with respect to their transcriptional profiles, in addition to the aforementioned phototransduction/neuronal signature distinction [4]. Perhaps the most striking difference between Group 3 and Group 4 at a gene expression level relates to *MYC*, which is expressed at very high levels in Group 3 and at only a modest baseline in Group 4 [2,3]. The aberrant expression of *MYC* reported in the poor-prognosis Group 3 subgroup is consistent with several previous reports that have linked elevated *MYC* activity with an inferior clinical outcome. Notably, in the Cho *et al.*'s

study, aberrant *MYC* levels were most prevalent in the poor-outcome 'C1' subclass when compared with 'C5' [4]. This important distinction further supports the concept of both biological and clinical heterogeneity within the core subgroups, providing rationale for dissecting them beyond the core hierarchy.

Cytogenetics

The molecular subgroups of medulloblastoma are easily distinguished as a result of their divergent transcriptional profiles. However, what is responsible for these unique transcriptional programs? The current body of evidence suggests that the medulloblastoma subgroups may originate from distinct cellular lineages [51–53]. However, the discriminatory karyotypes that characterize these subgroups also have strong influences on the observed gene-expression patterns. The most notable cytogenetic events that are enriched in the different subgroups are discussed below.

Monosomy 6 was the first cytogenetic aberration linked to a medulloblastoma subgroup [1,14], now confirmed in nearly all WNT tumors karyotyped to date. Interestingly, Thompson *et al.* associated the loss of chromosome 6 with WNT tumors somewhat indirectly, by virtue of the majority of genes on this chromosome exhibiting reduced expression in WNT tumors [1]. Since this discovery, monosomy 6 has become a defining feature of the WNT subgroup and is rarely (if ever) found in non-WNT tumors. As a result of the WNT-specific nature of this aberration, some have utilized chromosome 6 genomic status as a surrogate marker to identify or confirm WNT subgroup affiliation. Of equal importance, mutations in *CTNNB1* almost always coincide with monosomy 6, although rare exceptions of exclusivity for these events have been reported. Since WNT tumors have an exceptionally good prognosis, intensive efforts are currently being employed to determine an ideal 'test' for identifying these patients, including both *CTNNB1* mutational screening and chromosome 6q FISH. Outside of these genetic events, the WNT genome is extremely 'quiet' and essentially balanced, with very few gains and losses reported elsewhere.

Deletion of chromosome 9q, long suspected to be secondary to *PTCH1* mutation on 9q22, is found in approximately a third of SHH medulloblastomas. Loss of 9q appears to be highly restricted to SHH – found only in this subgroup in both the recent Cho *et al.* and Northcott *et al.* studies [3,4]. Additional over-represented copy number changes that have been reported in SHH tumors include gain of chromosomes 2 and 3, and losses of chromosomes 10q and 20p. Importantly, 9q and 10q deletions are generally mutually exclusive events, suggesting genetic heterogeneity within the SHH subgroup, some of which may be explained by underlying molecular differences between pediatric and adult SHH tumors [23,48], including a reduced frequency of 10q loss in adult cases [23]. These results, in conjunction with age-dependent prognostic markers, demonstrate that pediatric and adult cases of SHH medulloblastoma are biologically and clinically distinct [23].

From a karyotypic perspective, Group 3 and 4 share a number of common genomic features, including gain of chromosomes 7, 17q and 18, and loss of chromosomes 8, 11p and the X chromosome in females. Isochromosome 17q (i[17q]), the most frequently reported structural aberration in medulloblastoma [54,55], is prominent in both Group 3 and Group 4; however, it represents a defining feature of Group 4 tumors, as it is present in up to 80% of

this subgroup, whereas Group 3 medulloblastomas display this aberration less frequently. Despite their genomic similarities, discriminatory cytogenetic events do exist between these subgroups, including gain of chromosome 1q (Group 3), and chromosome 4 (Group 4), and losses of chromosomes 5q, 10q and 16q that are more prevalent in Group 3 tumors.

Oncogenes, tumor suppressors & medulloblastoma subgroups

CTNNB1

First published in a study by Zurawel *et al.* in 1998 [56], mutations affecting GSK3β phosphorylation sites in exon 3 of *CTNNB1* have since been reported in approximately 5–10% of medulloblastomas investigated [53–55]. By virtue of representing the apex of the WNT signaling pathway, it should have come as no surprise that *CTNNB1* mutations are confined to what is now known as the WNT subgroup. Indeed, the prevalence of *CTNNB1* mutations in subgroup 'B' reported by Thompson *et al.* [1] and later subgroup 'A' reported by Kool *et al.* [2] undoubtedly led to the definition of what is now accepted as the WNT subgroup.

TP53

Mutations in the *TP53* tumor-suppressor gene have historically been reported in approximately 10% of sporadic medulloblastomas [53–55], a frequency that is considerably lower than that observed in glioma, where mutations in the TP53 signaling pathway are believed to contribute to upwards of 85% of cases [46]. Li-Fraumeni patients who intrinsically harbor *TP53* germline mutations develop a wide range of cancers of different histologies, including medulloblastoma [53,57]. Although there have been conflicting reports regarding the prognostic significance of *TP53* mutation in medulloblastoma [58–61], recent studies have suggested an association between *TP53* mutation and the WNT subgroup. Pfaff *et al.* confirmed the mutational status of *TP53* in a series of 310 medulloblastomas, established a mutation frequency of 6.8% (21/310), and showed that *TP53* mutation frequently co-occurred with monosomy 6 and *CTNNB1* mutation (7/21 *TP53*-mutated tumors), both of which are defining characteristics of WNT medulloblastomas [59]. Using immunohistochemistry-based classification, the authors also showed that *TP53* mutations were found in WNT, SHH, and Group 4 tumors, but not in the prognostically inferior Group 3 subgroup. In a subsequent correspondence from Steve Clifford's group in the UK, a similar connection between *TP53* mutation and WNT subgroup affiliation was implicated, with three out of five *TP53*-mutated cases assigned to the WNT subgroup in a series of 50 tumors investigated [60].

PTCH1

Historically, *PTCH1* has been regarded as the 'prototypical' tumor-suppressor gene in medulloblastoma, reported to be mutated in approximately 8–10% of sporadic cases and known to be the underlying genetic basis of Gorlin syndrome, a hereditary cancer syndrome in which individuals harboring *PTCH1* germline mutation are predisposed to develop medulloblastoma [53,57]. Much like *CTNNB1* mutations typifying the WNT subgroup, mutations of *PTCH1* encoding the PATCHED transmembrane receptor (resulting in constitutive activation of the SHH signaling cascade) are intimately linked to the SHH

subgroup. *PTCH1* sequence analysis has ultimately influenced the definition of the SHH subgroup – collectively, *PTCH1* mutations were observed in nine out of 27 SHH tumors reported in the Thompson and Kool studies and not found in any of the non-SHH cases [1,2]. Other components of the SHH pathway, including *SUFU* and *SMO*, are known to be mutated in medulloblastoma and contribute to medulloblastoma pathogenesis [62,63]. Although mutations affecting these genes are presumably restricted to the SHH subgroup, this correlation has yet to be comprehensively evaluated.

MYC *family*

Proto-oncogenes belonging to the *MYC* family, including *MYCN*, *MYC* and *MYCL1*, comprise the most frequent targets of high-level amplification reported in medulloblastoma [15,64]. Importantly, patients harboring amplification of either *MYC* or *MYCN* have been repeatedly shown to exhibit significantly compromised progression-free survival and OS probabilities across multiple patient and clinical trial cohorts [15,16,65,66]. Although *MYC* and *MYCN* were among the first oncogenes reported as being amplified in medulloblastoma, the association of these oncogenes to a given subgroup has only recently come to light. To date, virtually all reported *MYC* amplifications have been linked to Group 3 tumors [3,4]. In contrast to the more restricted *MYC*-amplified cases, *MYCN*-amplified cases are found in either SHH or Group 4 medulloblastomas [3,4,65]. Amplifications affecting *MYCL1* are exceedingly rare in medulloblastoma and have yet to be connected to a particular subgroup(s). While initial reports demonstrated universal poor prognosis for both *MYC* and *MYCN* amplifications [15], recent studies have demonstrated that tumors with *MYCN* amplifications have a much greater genetic and clinical heterogeneity than originally believed [67].

MLL2 *& histone-modifying genes*

Numerous recent genomic profiling efforts have uncovered both recurrent copy number aberrations and DNA sequence variants that target genes encoding histone-modifying proteins (Figure 4). In 2009, we first reported recurrent homozygous deletions of *EHMT1*, a histone 3, lysine 9 methyltransferase on chromosome 9q34 [64]. In the same study, we reported recurrent amplification of *JMJD2C*, a jumonji family histone lysine demethylase on chromosome 9p24. Although patient subgroup information was not available at the time of this study, the observed somatic deletions of *EHMT1* occurred in the context of monosomy 9q, a genetic event confined to the SHH subgroup.

More recently, a landmark study by Parsons *et al.*, which involved whole-exome sequencing of 22 medulloblastomas, identified inactivating mutations of *MLL2* and *MLL3*, both histone lysine methyltransferases, in 16% of medulloblastomas [68]. Additional chromatin-modifying genes, particularly *SMARCA4*, *ARID1A* and *KDM6B*, were also found to be somatically mutated in this study, although at a much lower frequency. Although this study had limited information regarding molecular subgroup status, mutations in *MLL2* and *MLL3* appeared to be distributed across subgroups.

Other medulloblastoma oncogenes & tumor suppressors

OTX2 is among the most frequent targets of focal copy-number gain reported in medulloblastoma, accounting for approximately 10% of all cases [64]. Recently, Adamson *et al.* demonstrated that these aberrations are restricted to the Group 3 and Group 4 subgroups [69], thus potentially making *OTX2* an attractive candidate for molecularly targeted therapy in non-WNT/non-SHH tumors.

The *TSC1* tumor-suppressor gene on chromosome 9q34 is both mutated and recurrently focally deleted in medulloblastoma [64,68,70]. In collaboration with Bhatia *et al.*, we recently showed that these aberrations are highly over-represented in SHH medulloblastoma [64,70], implicating cooperation of aberrant SHH signaling and deregulation of the mTOR pathway in SHH-driven tumors. Since both of these pathways are actionable, therapeutic strategies combining inhibitors of both cascades may prove to be effective in future targeted therapies of this subgroup.

Technical aspects of medulloblastoma subgroup classification

Given what has recently been uncovered with respect to the clinical ramifications of medulloblastoma subgroups, an immense amount of interest is currently focused on the development and implementation of techniques for reliably assigning clinical samples to their appropriate subgroup. All of the key gene-expression array studies described throughout this review essentially used the same approach for defining the subgroups – profiling moderate-to-large cohorts on conventional oligonucleotide arrays using high-quality RNA extracted from fresh-frozen tissue. Unsupervised class discovery approaches such as hierarchical clustering and non-negative matrix factorization performed on the most variant genes among the cohorts has proven to be a robust method for discriminating medulloblastoma subgroups [1–4,9]. Furthermore, application of various class prediction algorithms and novel bioinformatic tools such as SubClass Mapping [71] and Metagene Projection [72] have allowed groups to define published series of array data in terms of their own concept of medulloblastoma subgroups [3,4,23]. Although these array-based methods have been informative, they are not practical for conditions typically encountered in a clinical setting where access to high-quality tumor RNA and expertise capable of performing the complex bioinformatics involved in microarray data processing are unlikely to be available.

To circumvent these limitations related to sample quality and available technologies, multiple groups have extracted information gained from the previous gene-expression array studies and chosen to focus on subsets of signature genes as molecular markers of the medulloblastoma subgroups [8,73]. Since virtually all clinical medulloblastoma samples are at some stage prepared as formalin-fixed paraffin-embedded (FFPE) material, IHC for top subgroup-specific signature genes has become both a convenient and effective method for medulloblastoma classification. To date, a variety of different markers have been tested for their ability to demarcate a particular subgroup using IHC, with some of these now effectively utilized by multiple independent laboratories. For instance, nuclear CTNNB1 staining is now widely used as more or less a definitive marker of WNT medulloblastoma [3,7,11–14,47,48,74], although antibodies against DKK1 [1,3,9] and DKK4 [1] have also

demonstrated efficacy in identifying WNT tumors. Similarly, SFRP1 [1,3,9,12,13,48], GLI1 [1,3,48] and, more recently, GAB1 [7] have all shown promise as molecular markers of SHH medulloblastoma. In collaboration with the team in Heidelberg, Germany, the authors have now published multiple reports whereby just four commercially available antibodies were used to establish subgroup status of several hundred FFPE medulloblastomas present on TMAs [3,9,23,65]. These markers include DKK1 (WNT), SFRP1 (SHH), NPR3 (Group 3) and KCNA1 (Group 4) and have proven to be extremely reliable markers of the four medulloblastoma subgroups. Despite these supportive published data, the transferability of our four antibody assay across the globe may be limited due to factors related to differences in sample preparation and fixation techniques used in different pathology laboratories, as well as potential lot-to-lot variability of the current antibody sources, most of which are rabbit polyclonal in origin. As such, novel molecular-based approaches for subgrouping medulloblastoma that are rapid, cost effective and compatible with FFPE-derived material routinely available in the clinical setting are warranted. Recently, we developed an assay based on NanoString Technology® that incorporated a 22-gene signature consisting of the most highly expressed subgroup-specific signature genes in order to predict medulloblastoma subgroup affiliation. This approach led to the accurate classification of medulloblastoma subgroups in approximately 98% (127/130) of fresh-frozen cases with known subgroup affiliation and, importantly, was highly compatible with FFPE-derived RNA from recent cases [73].

Preclinical models of medulloblastoma subgroups

Given the considerable progress that has been made in dissecting the molecular and clinical heterogeneity of medulloblastoma subgroups, the shift now moves towards the generation of rational and faithful preclinical models of the four subgroups. These models will be an essential tool for gaining further insight into the cellular origins of the subgroups and testing molecularly targeted therapies designed to inhibit the oncogenes and signaling pathways deregulated within them. Although multiple excellent reviews have recently summarized preclinical medulloblastoma models, particularly mouse models, we briefly discuss the current status of this topic here, highlighting the degree to which these published models match the subgroups described in the human disease. For more extensive reviews on the subject of medulloblastoma mouse models, the reader is encouraged to refer to other recent and more comprehensive publications [5,6,75].

Until only recently, most, if not all, mouse models of medulloblastoma relied on genetic perturbation of the SHH signaling pathway. The $Ptch1^{+/-}$ mouse is by far the most well characterized and has served as a workhorse for modeling the disease for >10 years [76–78]. Heterozygous loss of $Ptch1$ results in the generation of medulloblastomas within 3–6 months with a penetrance of approximately 15%. Breeding these mice with those on a $Tp53$-null background to generate $Ptch1^{+/-}$; $Tp53^{-/-}$ mice increases the incidence to nearly 100%. Sporadic mouse medulloblastomas have also been successfully produced through manipulation of other SHH signaling components, including transgenic over-expression of Smo (i.e., $ND2$-$SmoA1$) [79,80], deletion of $Sufu$ (i.e., $Sufu^{+/-}$; $Tp53^{-/-}$) [81], and through targeted deletion of $Ptch1$ in different cell types [82]. All of the above models, in addition to several others not mentioned here, faithfully represent the human SHH subgroup, which is

not surprising given their underlying genotypes. A multitude of studies strongly suggests that these tumors are derived from cerebellar granule neurons, are typically of classic histology and are characterized by elevated SHH signaling [53,83]. Furthermore, multiple recent publications have described a histological phenotype consistent with what is observed in the human MBEN subtype (which appear to be over-represented in the SHH subgroup) [39,41] when the SHH pathway is activated in combination with PI3K pathway perturbation in transgenic mice [84,85]. Given the relative paucity of MBEN among human medulloblastomas, the availability of a potentially reliable preclinical model of this subtype may provide alternative avenues for gaining an improved understanding of this rare subtype.

In 2010, Gibson *et al.* successfully introduced what was arguably the first *bona fide* model of non-SHH mouse medulloblastoma [52]. Targeted expression of activated β-catenin (*Blbp-Cre; Ctnnb1+/ lox[ex3]*) and concurrent loss of Tp53 to cells of the lower rhombic lip/ dorsal brainstem resulted in approximately 15% of mice developing medulloblastomas that were anatomically and molecularly distinct from the previous SHH models. Comparison of gene-expression data from these *CTNNB1*-driven mouse tumors with data derived from human cases demonstrated a significant correlation with the WNT subgroup, establishing this model as the first to accurately model a subgroup, other than SHH.

Another elegant model, also published in 2010, used an inducible system to bidirectionally overexpress both *Mycn* and luciferase (known as GTML) in the developing cerebellum [86]. Tumors derived from this genotype occurred in up to 75% of mice by 200 days, produced tumors of either classical or LCA pathologies, originated independently of cerebellar granule neuron progenitor cells, and only rarely (~5%) expressed SHH signature genes – strongly suggesting that GTML tumors are more often representative of non-SHH human medulloblastoma.

Very recently, two independent laboratories published back-to-back papers in *Cancer Cell* showing that aberrant expression of *Myc* combined with abrogation of Tp53 activity could faithfully recapitulate tumors resembling human Group 3 medulloblastoma [87–89]. These highly complimentary studies used an *ex vivo* approach to introduce *Myc* into either neural stem cells [88] or cerebellar progenitors [87] and transplanted the *Myc*-expressing cells intracranially into immunocompromised mice. When combined with loss of wild-type Tp53, either through the use of dominant negative Tp53 [88] or *Tp53* deletion [87], both of these strategies yielded highly penetrant, aggressive medulloblastomas with a short latency. Importantly, tumors derived from these animals were reminiscent of human LCA medulloblastoma, with a histology common to Group 3, and transcriptionally matched expression data derived from human Group 3 tumors. The requirement for Tp53 loss-of-function in both of these models as a mechanism to evade *Myc*-driven apoptosis is a topic of some debate, if these models are to be considered accurate representations of human Group 3 moving forward. Although *MYC* amplification and overexpression are highly specific to Group 3, direct inactivation of *TP53* via mutation or focal deletion is rarely seen in this subgroup, occurring much more frequently in WNT and SHH tumors, as described earlier [59,60]. Despite the general lack of *TP53* mutations in Group 3, chromosome 17p deletion (chromosome 17p being the genomic location of *TP53*) is quite prevalent, found in upwards of 50% of cases from this subgroup [3,4,34]. Moreover, the TP53 pathway may be affected

by mutations in genes other than *TP53* in Group 3, although this has yet to be thoroughly investigated. Nonetheless, these new *Myc*-driven models of medulloblastoma will undoubtedly serve as a valuable resource for future dissection of Group 3 biology and as a platform for preclinical testing in this poor-prognosis subgroup.

Using the sleeping beauty (SB) transposon system, Wu *et al.* recently performed 'saturation' mutagenesis of Math1-expressing CGNPs in an attempt to uncover novel medulloblastoma oncogenes and tumor suppressors [90]. Using the SB system combined with next-generation sequencing, Wu *et al.* were able to identify genes affected by common insertion of the SB transposon (also known as common insertion sites) and thus implicated in the maintenance, progression and metastasis of medulloblastoma. Indeed, using either $Ptch1^{+/-}$ or $Tp53^{-/-}$ sensitized backgrounds, the SB model system employed in this study induced medulloblastomas with nearly 100% incidence and a very high rate of leptomeningeal spread. The high incidence of metastasis allowed for a direct comparison of common insertion sites found in the primary tumors versus the metastases and resulted in the observation of an alarmingly low degree of overlap between the two compartments. The translational impact of these findings is of paramount interest to those treating the human disease, given that the majority of basic research to date has been focused on mutational events found in primary tumors, while metastases account for the bulk of the morbidity seen in medulloblastoma patients. In other words, if the development of future targeted therapies is based solely on information learned from studying the primary tumors, the likelihood of these treatments effectively curing the patients is speculative, given the apparent genetic differences between the primaries and the metastases.

With WNT, SHH and Group 3 medulloblastoma now successfully modeled in the mouse, the community is rapidly striving to generate an accurate model of Group 4 [89]. Elucidation of the cellular origin(s) of this subgroup and proper functional evaluation of candidate Group 4 oncogenes and tumor suppressors await the development of this highly desired reagent. These details are underscored by the fact that 35–40% of medulloblastomas belong to this subgroup and it continues to prevail as the subgroup we currently know the least about from both a genetic and cellular perspective.

Expert commentary

A genomic approach to large cohort studies has revolutionized our contemporary understanding of medulloblastoma. From original reports of small blue cells of the cerebella, medulloblastomas can and must now be recognized as four disparate molecular subgroups with specific biological and clinical properties. Throughout this review, we have highlighted some of the major cytogenetic, genomic and demographic characteristics associated with each molecular subgroup. WNT, a clinically less aggressive form of the disease, is largely characterized by monosomy 6, *CTNNB1* mutations, nuclear β-catenin and excellent survival outcomes (>90%). SHH medulloblastomas represent the most-characterized subgroup, with constitutive activation of SHH pathway and an approximately 65% OS, although recent evidence suggests that these trends are highly dependent on patient age. SHH tumors can present with 9q loss and *PTCH* mutation, and this subgroup is enriched for the desmoplastic histological subtype. Group 4 medulloblastomas remain a largely uncharacterized subgroup

with similar clinical characteristics to SHH tumors, often presenting with isochromosome 17q. Group 3 tumors, which largely occur in younger patients, are the most aggressive and most metastatic form of the disease, often presenting with histological (LCA) and genomic (*MYC* amplification) features associated with dismal outcome. With this new-found knowledge in hand, it will be critical to perform integrative clinical and genomic means of patient stratification, rapidly adapting our treatment to accommodate the ever-changing genomic landscape of medulloblastoma.

Five-year view

Given the present accepted subdivision of medulloblastoma – from a single entity into four distinct molecular subgroups, with disparate genetic, cytogenetic and transcriptional changes – and the advent of multiple, independent technologies to permit the rapid identification of subgroup association, the next generation of clinical trials will undoubtedly be focused on treating medulloblastoma according to its molecular subgroup. Initially, the disparate OS rates that have been retrospectively associated with each molecular variant will need to be evaluated in the context of prospective clinical trial settings. Important and 'hot' topics that are currently under debate among pediatric neuro-oncologists are centered on the de-escalation of therapy for WNT medulloblastomas and the intensification of treatment or targeted therapy for patients with Group 3 tumors [17,91]. The effectiveness of targeted therapies has already been demonstrated with the availability of orally bioactive SHH pathway inhibitors, such as GDC-0449 and NVP-LDE225, which repress SHH signaling by targeting the SMO receptor [92,93]. These inhibitors have demonstrated considerable effect on tumor growth, causing promising, albeit transient, remission of SHH medulloblastomas [92]. While targeted inhibition of SMO on its own has resulted in resistance and patient relapse, preliminary results suggest that combinatorial targeted therapies could provide an improved response, especially if the mechanisms of resistance are fully elucidated [50,93].

Over the next 5 years, the authors' understanding of medulloblastoma biology will continue to advance at an unprecedented rate. Improvements in sequencing technologies, and early efforts such as those reported by Parsons *et al.* [68], have led to a wave of whole-genome and whole-exome DNA sequencing and RNA sequencing studies. These efforts will undoubtedly describe the full mutational spectra associated with each molecular variant of medulloblastoma, probably cataloguing the true frequency of subgroup-specific mutations and identifying novel mechanisms of pathogenesis. Mutational data will be complimented by advances in our appreciation of the extent to which copy-number aberrations occur in medulloblastoma and between the medulloblastoma subgroups. We expect a major shift from discovery to validation as the first sequencing projects approach completion. This will require a concerted transformation in the field towards a more functional approach aimed at understanding the importance and relevance of the mutations and copy-number events identified during the current discovery era. Moreover, a major emphasis will be placed on identifying the cell of origin for Group 3 and Group 4 tumors and mimicking subtypes of these molecular subgroups in animal models. Furthermore, the overwhelming tumor-specific convergence on chromatin modifier genes advocates for the continued investigation, and possible use, of histone deacetylase inhibitors for treating medulloblastoma in the future [94–96].

Multiple, independent efforts led to the discovery of four core medulloblastoma subgroups. Now, with this molecular knowledge in hand, we have entered a new age in our understanding of this disease. The next 5 years of medulloblastoma research will continue on this trajectory, with refined tools and renewed interest, driving forward clinical advances in the management of this disease.

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Key issues

- M edulloblastoma is no longer considered as a single disease and is now generally accepted to comprise four distinct subgroups: WNT, Sonic hedgehog (SHH), Group 3 and Group 4.

- Group 3 medulloblastomas exhibit the worst prognosis of the four subgroups, while SHH and Group 4 represent intermediate-prognosis subgroups and WNT medulloblastomas currently have the best outlook in terms of patient survival.

- Demographic and clinical variables, including age, gender, and histology, are not equal among medulloblastoma subgroups, possibly accounting for clinical differences traditionally associated with these variables.

- Subgroups of medulloblastoma exhibit vastly different transcriptional and genomic profiles that imply distinct underlying biology and will be critical to consider in the future development of subgroup-specific targeted therapies.

- There is currently a great need for the further development of highly effective and accurate methods for discerning medulloblastoma subgroup affiliation from clinical samples, particularly those preserved as formalin-fixed paraffin-embedded material.

- The next 5 years of medulloblastoma research will involve two major waves:

  - A discovery phase, during which thousands of samples will be subjected to next-generation sequencing to comprehensively catalog all mutations found in medulloblastoma and especially those that are subgroup-restricted.

  - A validation phase, which will involve the generation of faithful preclinical models for each of the four subgroups, mimicking the genetic events observed in the human disease and subsequent utilization of these models for molecular cellular, and biochemical assays, especially those evaluating novel targeted therapies.
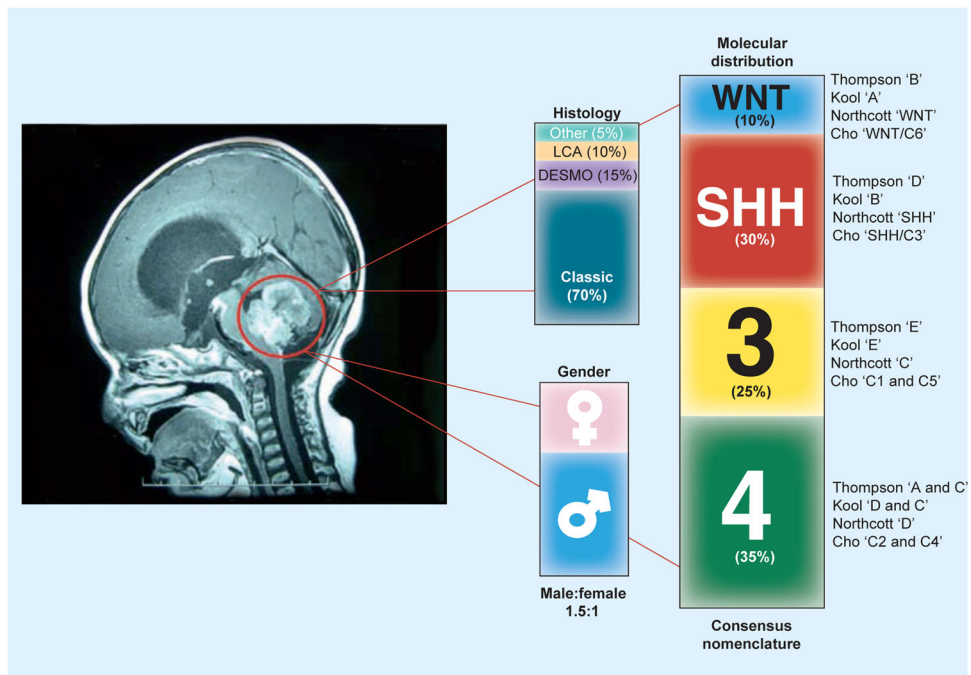
Figure 1.
Histological and gender distribution of medulloblastomas, as a single disease, as well as the prevalence of each of the consensus molecular subgroups: WNT (~10%), SHH (~30%), Group 3 (~25%) and Group 4 (~35%), across the four major studies that contributed to the discovery and definition of the molecular heterogeneity of medulloblastoma.
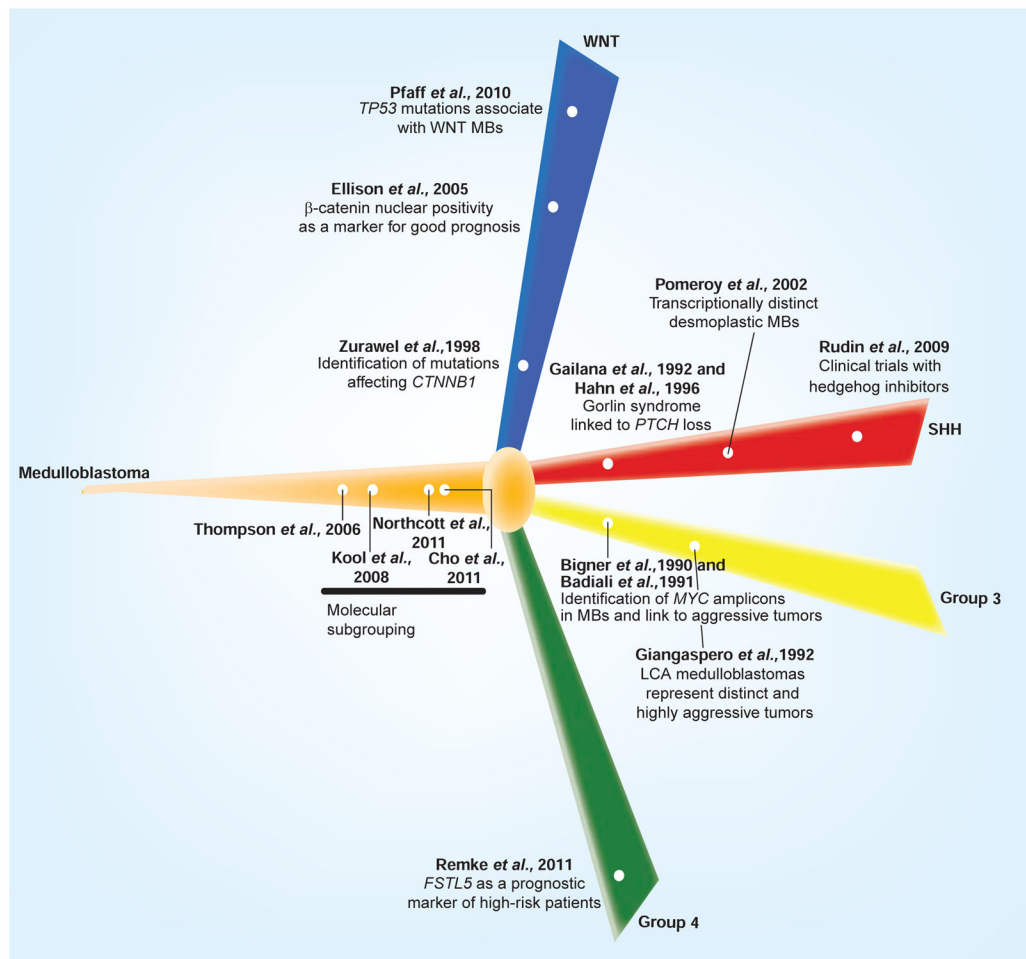DESMO: Desmoplastic; LCA: Large-cell anaplastic; SHH: Sonic hedgehog.

Figure 2.
Chronological reorganization of selected landmark papers that have significantly impacted our molecular or clinical understanding of the medulloblastoma subgroups consensus. LCA: Large-cell anaplastic; MB: Medulloblastoma; SHH: Sonic hedgehog.
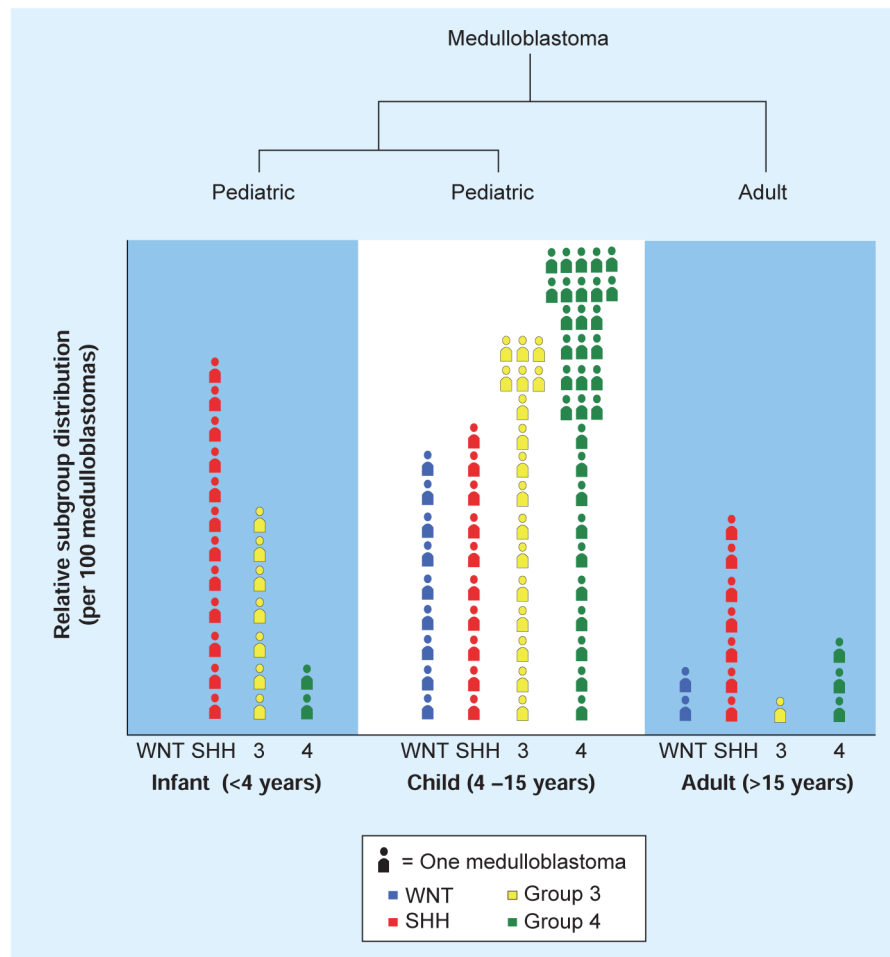
Figure 3.
Relative frequency of the four molecular subgroups of medulloblastoma across: infants (4
years of age), children (4–15 years of age) and adult patients (15 years of age) per 100
tumors, demonstrating both the prevalence (SHH: ~60% of infants) and rarity (Group 3: ~1–
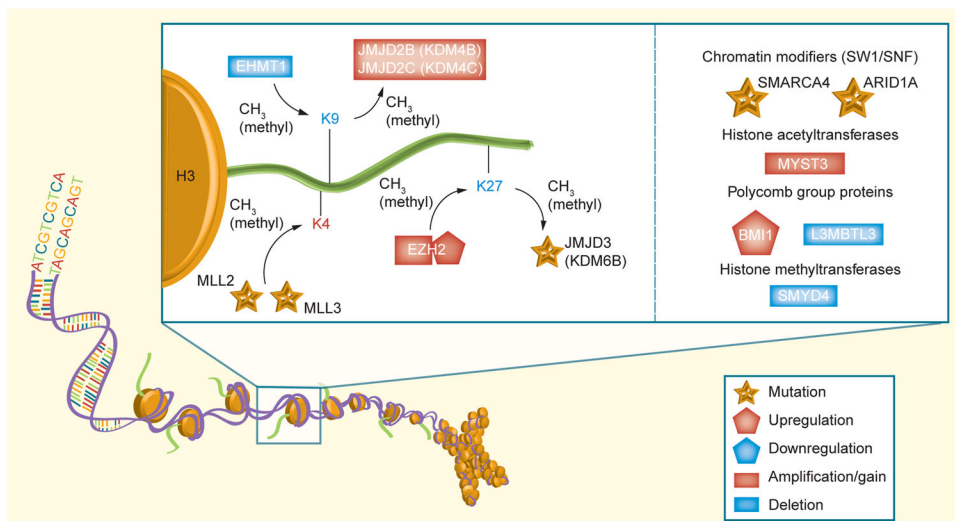2% of adults) of each molecular subgroup and their association with age.
SHH: Sonic hedgehog.

Figure 4.
Schematic representation of chromatin-modifying proteins identified as mutated, amplified/deleted, or over-/under-expressed in medulloblastoma
This important pathway affects a number of lysine residues (K4, K9 and K27), altering gene expression and inhibiting differentiation in a tumor-specific context.