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MINI-SYMPOSIUM: When Genetics Meets Epigenetics—a New Option for Therapeutic Intervention in Brain Tumors?

The Role of Chromatin Remodeling in Medulloblastoma

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Abstract

The unexpectedly high frequency and universality of alterations to the chromatin machinery is one of the most striking themes emerging from the current deluge of cancer genomics data. Medulloblastoma (MB), a malignant pediatric brain tumor, is no exception to this trend, with a wealth of recent studies indicating multiple alterations at all levels of chromatin processing. MB is typically now regarded as being composed of four major molecular entities (WNT, SHH, Group 3 and Group 4), which vary in their clinical and biological characteristics. Similarities and differences across these subgroups are also reflected in the specific chromatin modifiers that are found to be altered in each group, and each new cancer genome sequence or microarray profile is adding to this important knowledge base. These data are fundamentally changing our understanding of tumor developmental pathways, not just for MB but also for cancer as a whole. They also provide a new class of targets for the development of rational, personalized therapeutic approaches. The mechanisms by which these chromatin remodelers are dysregulated in MB, and the consequences both for future basic research and for translation to the clinic, will be examined here.

INTRODUCTION

Brain tumors currently account for more than a quarter of all deaths from pediatric cancer (3). Medulloblastoma (MB), the most common embryonal brain tumor, is a major contributor to this unfortunate figure. Improvements in patient outcomes over the past few decades have largely been achieved through advances in the application of chemotherapy and craniospinal radiation, as opposed to the biology-driven risk stratification that has been so successful in treating leukemia, for example (58). A significant fraction of patients will still die from their disease, and those who survive often experience long-term side effects (19, 42, 63). Thus, there is clearly scope for a shift toward more rational patient stratification and for the application of personalized targeted therapy based on sound tumor-biological data (55).

In the recent era of high-throughput genomics, several major advances have been made in terms of our understanding of the biology behind MB tumorigenesis (47). Arguably, the most important paradigm shift has been the recognition that MB is not a single entity, but rather is composed of multiple molecular subgroups (WNT, SHH, Group 3 and Group 4) that differ in their age and gender distribution, clinical outcome and molecular genetic features (10, 34, 35, 48, 75, 76). Despite these differences, some common threads have also emerged. One such feature that is found across subgroups is the recurrent alteration of the chromatin remodeling machinery, as discussed in the following paragraphs. Even here, however, the importance of molecular classification is highlighted by the fact that certain types of chromatin modifier alterations are restricted to specific subgroups.

The packaging and organization of chromatin is the principal mechanism by which cell-specific transcriptional programs are established and maintained during organismal development (8, 39, 41), including the specification of neural cell types (28, 73). This organization is mediated in large part by an array of post-translational modifications (PTMs) at key residues on histone tails—the so-called histone code (29, 36). Genes involved in this process of chromatin regulation are typically grouped into four main categories. First, there are "writers" of covalent histone marks such as histone methyltransferases (HMTs) and histone acetyltransferases (HATs) that establish patterns of PTMs on histone tails. Next, there are "erasers," histone demethylases (HDMs) and histone deacetylases (HDACs), which can remove these PTMs. An additional class of genes, the "readers" of PTMs, binds to specific histone tail motifs in order to coordinate the downstream consequences of these marks. Finally, there are also large-scale remodelers of nucleosome structure and chromatin architecture such as the SWI/SNF, INO80 or CHD/NuRD complexes (11, 25, 38, 61, 78), or CTCF (52).

Disruptions of these normal regulatory processes, including in nervous system formation, have been linked to multiple disorders (23, 37, 64). Indeed, it has become increasingly clear in recent years that alterations in how chromatin structure controls cellular processes are one of the most important features of human cancer, irrespective of tumor site or histology (9, 12, 84). Intriguingly, mutations at key regulatory residues in histone proteins themselves (histone 3.1 and the variant 3.3), as well as the histone chaperones *ATRX* and *DAXX*, have recently been identified in a subset of another malignant pediatric brain tumor—glioblastoma (33, 68, 81) (see also

Fontebasso *et al* in this issue). Importantly, these alterations in chromatin modifying genes also provide new classes of candidates for targeted therapeutic intervention, with several compounds already showing promise in preclinical or clinical settings (13, 56).

The purpose of this article is to highlight what is currently known about the role of dysregulation of chromatin remodeling in MB and to provide an outlook as to how this knowledge may be expanded upon in the future, as focus shifts toward translating these findings into benefit for patients.

COPY-NUMBER CHANGES AND ALTERED CHROMATIN IN MB

The first strong evidence for a role of altered chromatin in MB development emerged from genome-wide investigation of DNA copy-number alterations (49). A study by Northcott *et al* identified multiple changes affecting chromatin modifying genes, including focal gain or amplification of the histone acetyltransferase *KAT6A* (formerly *MYST3*) and the lysine demethylases *JMJD2B* and *JMJD2C*, together with deletions of polycomb group genes (*L3MBTL2*, *L3MBTL3* and *SCML2*) and histone methyltransferases (*EHMT1*, *SMYD4*). Many of these genes converge on regulation of H3K9 methylation status, and hypomethylation at H3K9 was revealed by immunohistochemical analysis to occur in 41% of tumor samples investigated (49). The same study demonstrated that re-expression of *L3MBTL3* in DAOY cells, in which the gene is deleted, could restore H3K9 methylation and block cellular proliferation.

This work was recently expanded upon in a comprehensive copy-number profiling analysis of more than 1000 MBs by an international consortium led by the same Toronto group (50), which confirmed the importance of copy-number alterations at chromatin modifying genes in a subset of cases. These alterations were particularly enriched in Group 4 MBs, and novel homozygous deletions on chromosome X affecting the H3K27 demethylase *KDM6A* (also called *UTX*) were identified in this subgroup. Interestingly, this gene was also found to be recurrently mutated in the same subgroup, as described in the following paragraphs.

It is also interesting to note that alterations of chromatin architecture may be a cause of certain structural changes in a cell's DNA rather than simply a consequence. For example, a process of catastrophic rearrangement occurring at a single time point [termed chromothripsis (70)] has recently been linked to *TP53*-mutated SHH-subgroup MB (SHH-MB) (60). One hypothesis as to how these dramatic rearrangements might arise is through a critical loss of specialized chromatin structures at the telomeres, resulting in chromosome end-to-end fusions and subsequent mechanical shearing during mitosis (77). A link between germline *TP53* mutation [as was frequently observed in SHH-MB with chromothripsis (60)], accelerated telomere shortening and age of tumor onset has previously been established (72). It will therefore be of interest to further investigate the role of telomeric chromatin alterations in the generation of these complex DNA copy-number changes.

MUTATIONS OF CHROMATIN MODIFYING GENES—INSIGHTS FROM LARGE-SCALE SEQUENCING STUDIES

The first large-scale, unbiased sequencing effort in MB, published by Parsons *et al* in 2011, provided a number of further insights into the role of chromatin modifying genes in this tumor type (53). Several truncating mutations in the histone methyltransferases *MLL2* and *MLL3* were reported, suggesting a tumor suppressor function of these two genes in MB. Alterations in chromatin modifiers in general were clearly a recurring theme, with rarer mutations being found in the histone demethylase *KDM6B*, as well as the SWI/SNF chromatin remodeling complex members *SMARCA4* and *ARID1A*, among others. The subgroup specificity of these changes, however, was not clear in this study.

The theme of chromatin modification was also an obvious feature of three next-generation sequencing studies published in the summer of 2012 (30, 57, 62), and was summarized in a recent review and meta-analysis (47). One-third of all tumors, across all subgroups, were reported as having a mutation in a gene mapping to the Gene Ontology (GO) term "Chromatin modification" (GO:0015168). A summary of recurrently mutated chromatin modifiers, and their frequency of mutation, is shown in Figure 1.

Mutations of *SMARCA4*, as identified in the Parsons *et al* study, were found to be largely restricted to WNT and Group 3 MB, where they were identified in 25% and 11%, respectively, of tumors in

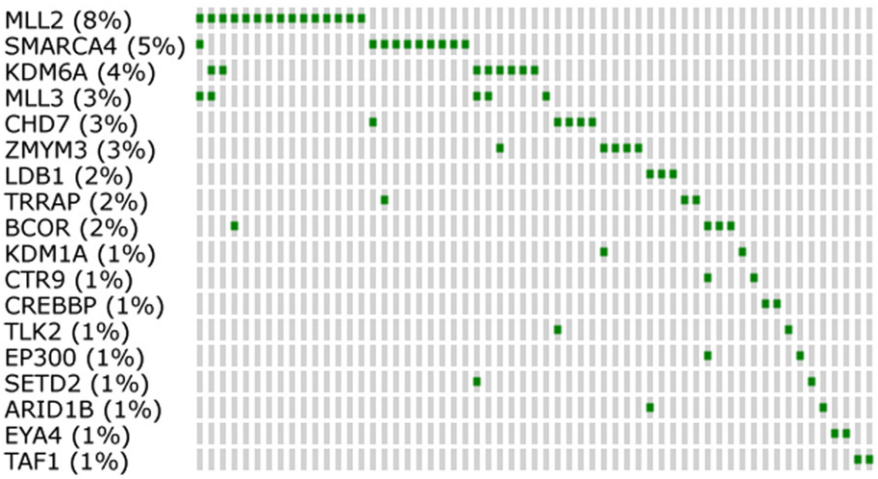

Figure 1. *Chromatin modifier genes recurrently altered in medulloblastoma (MB).* This figure indicates those genes related to the GO term "Chromatin Modification" (GO:0015168), which are mutated at least twice in the discovery cohorts (whole-exome or whole-genome sequencing, n = 189) of the three next-generation sequencing studies described herein (30, 57, 62). Percentages by gene names indicate the overall frequency of mutation in this gene across the three studies. NB—cases with no mutation in the listed genes are not shown.

these subgroups (47). Rarer mutations in *ARID1B* and *ARID2* were also observed, again implicating the SWI/SNF complex as an important component of MB development. The question of whether there may be a link between hyperactivation of c-Myc oncogene signaling (common to WNT and Group 3 tumors) and alterations in this complex will be an interesting avenue for further investigation.

SHH-MBs showed a strong enrichment for alterations in several members of the nuclear receptor co-repressor (N-CoR) complex, which is associated with histone deacetylation and is thought to repress target genes by inducing chromatin condensation (51). Genes of this complex mutated in SHH-MB include *BCL6 co-repressor* (*BCOR*), *LIM-domain binding 1* (*LDB1*) and *G-protein pathway suppressor 2* (*GPS2*). In addition, *LIM-domain only 4* (*LMO4*), a binding partner of LDB1 (14), has been identified as a recurrently amplified gene in SHH-MB (50), further supporting a key role for this complex in the pathogenesis of this subgroup.

One of the most striking single gene changes was the recurrent inactivating mutation of *KDM6A* in 12% of Group 4 tumors (47). This gene acts in concert with the most commonly mutated chromatin modifier in MB as a whole, *MLL2*, to elevate H3K4me3 and remove H3K27me3 marks in order to activate target genes (65, 66). Robinson *et al* further speculated that this loss of KDM6A function, as well as additional mutations in *ZMYM3* and *CHD7* (regulators of H3K4me3-mediated patterns) and overexpression of the H3K27 methyltransferase *EZH2*, may lead to an imbalance between trimethylated H3K4 and H3K27 and altered differentiation signaling in this subgroup (62) (see Figure 2).

In a follow-up study of these sequencing efforts, Dubuc *et al* screened a large series of MBs specifically for mutations in *MLL2* and *KDM6A* (17). *MLL2* mutations were seen in approximately 8% of MBs, with roughly half of those predicted to be truncating alterations and no clear enrichment in a particular MB subgroup. Alterations of *KDM6A*, in contrast, were largely restricted to Group 4 tumors (in keeping with the above sequencing and copy-number studies) and were mostly truncating (17). The two alterations were found to be mutually exclusive, as might be expected from the above-noted similarities in the predicted outcome of these changes. When further considering copy-number and transcriptional changes of *EZH2*, *KDM6A* and *KDM6B*, the authors found further support for the concept postulated by Robinson *et al* (62) that a subset of Group 3 and Group 4 MBs may show an enrichment of H3K27me3 marks ("K27+"). This was further supported by immunohistochemical staining for various histone marks, as well as an overrepresentation of differential expression of polycomb repressive complex 2 (PRC2) targets (which are typically linked to H3K27me3 levels) in Group 3 and Group 4 tumors with or without this K27+ phenotype (17).

As with copy-number changes, evidence has recently emerged that the relationship between these DNA mutations and chromatin architecture may not be solely a one-way process. For example, it seems that certain marks of heterochromatin, particularly H3K9 trimethylation, are highly enriched in regions of the genome that show a higher rate of mutation (67), indicating perhaps that DNA repair processes are not as efficient in these repressive chromatin domains. It is unclear as yet whether a similar phenomenon may be shaping the mutational landscape of somatic changes in MB.

OTHER LINKS BETWEEN CHROMATIN ALTERATIONS AND MB

As noted earlier, several of the reported alterations in chromatin modifying genes seem to converge on methylation of lysine 27 of

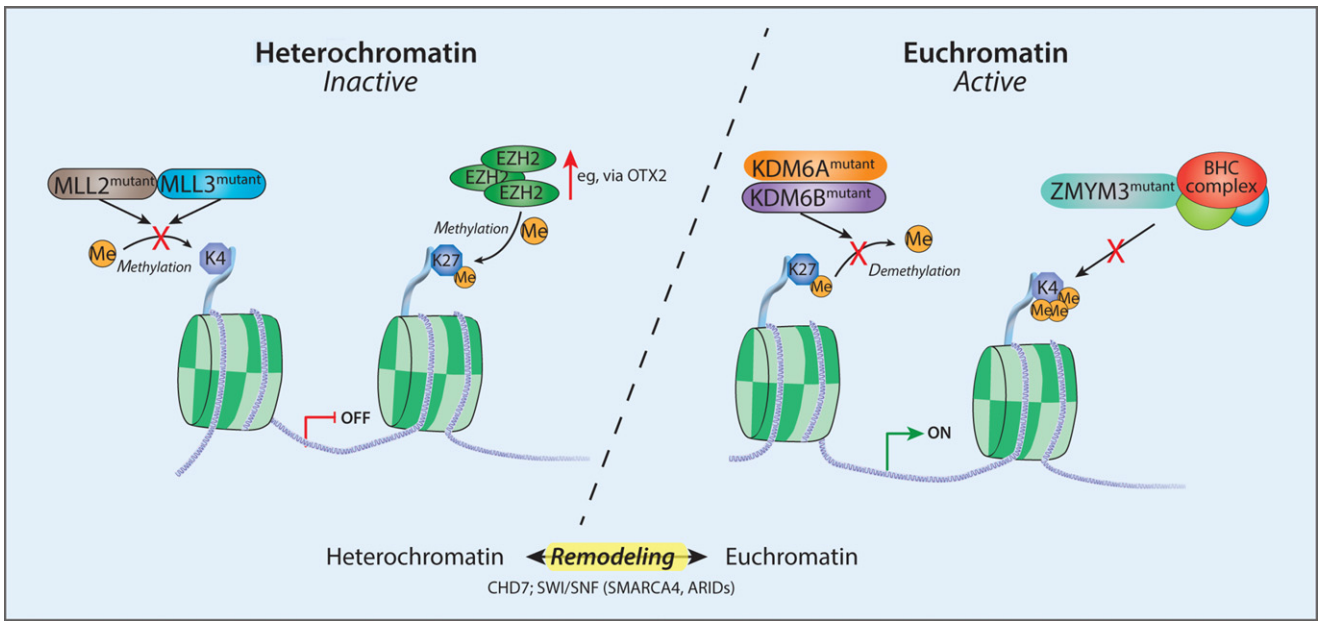

Figure 2. *Schematic of key chromatin-related mutations in medulloblastoma (MB).* This schematic view indicates some of the recurrently mutated chromatin modifier genes in medulloblastoma, and their possible effects on disrupting chromatin marks, with a particular focus on the disrupted H3K4/H3K27 methylation frequently observed in Group 3 and Group 4 tumors.

histone 3, particularly elevated H3K27me3 in Group 3 and Group 4 tumors (17, 62). In a similar vein, one recent report investigated the link between polycomb group genes (required for induction of H3K27 methylation) and the transcription factor *OTX2* (7), which is occasionally amplified and almost universally overexpressed in Group 3 and Group 4 tumors (1, 6, 50). The authors found upregulation of multiple genes belonging to PRC1 and PRC2, including *EZH2*, *EED* and *SUZ12*, across MB subgroups, but particularly in Group 3 and Group 4 MBs (7). Upon silencing of *OTX2* in the MB cell line D425, Bunt *et al* saw downregulation of these polycomb genes in conjunction with induction of H3K27 demethylases (e.g. *KDM6A*, *KDM6B* and *KDM7A*), resulting in reduced levels of H3K27me3 (7). Thus, further investigation of the role of *OTX2* as a regulator of key chromatin modifiers in certain MB subgroups may be warranted.

In addition to these *OTX2*-mediated changes, other examples of polycomb deregulation have also been reported. In particular, the PRC1 regulator and stem cell self-renewal factor *BMI1* has been linked to SHH-MB [the only MB subgroup in which *OTX2* is essentially not expressed (1)]. Using Bmi1-null mice, Leung *et al* first showed in 2004 that Bmi1 is essential for normal cerebellar development (40). The same study identified a subset of human MB characterized by concordant overexpression of *PTCH1* and *BMI1*. This was built upon by Michael *et al* in 2008, in a study which used a SmoA1 mouse model to demonstrate that *Bmi1* is essential for SHH-MB tumorigenesis (43). A recent report also identified a feedback loop between hedgehog pathway activation and *BMI1*, whereby Shh ligand can induce expression of *BMI1* in MB tumor-initiating cells, which then further upregulates hedgehog target genes (79).

OUTLOOK

The current era of MB research is an exciting one. Significant advances have been made over the past few years, with the development of important subgroup classification schema and the identification of multiple prognostic markers. As large-scale genomics projects have come to fruition, chromatin remodeling defects have taken center stage in multiple studies. There is still much to be done, however, in translating this increased knowledge into true clinical benefit. While the genetic alterations affecting chromatin remodelers are becoming better understood, the downstream epigenetic consequences of these alterations remain relatively understudied. In addition, it is not clear whether these changes in chromatin architecture are really seen across the whole genome, or whether they affect only a subset of specific targets. The transcriptional consequences of mutations in chromatin modifiers are also still somewhat unclear. More detailed characterization of the MB epigenome will therefore be an important area of focus in the coming years. Several such studies are under way, for example, under the auspices of International Cancer Genome Consortium (ICGC) projects in Germany and Canada or the Pediatric Cancer Genome Project in the USA, to look at global DNA methylation (Illumina Infinium 450k arrays and whole-genome bisulfite sequencing), non-coding RNAs (RNA-seq, miRNA-seq) and patterns of critical histone modifications (ChIP-seq).

The repertoire of potential therapeutic targets has also expanded rapidly in the genomics era. Many novel therapeutic agents targeting chromatin modifiers are currently in development or in early stage trials, although not many have achieved Food and Drug Administration (FDA) approval as yet (5). The best known class of approved drugs are histone deacetylase inhibitors such as vorinostat (31) and romidepsin (26), which are both in phase I/II clinical trials for pediatric brain tumors (see http://www.clinicaltrials.gov). Vorinostat has shown some promising results in preclinical MB models (44, 46, 69, 74), and knockdown of specific HDACs in MB cells results in decreased proliferation and cell viability (45). More recently, inhibition of bromodomain-containing proteins, which interact with acetylated histones and recruit transcriptional regulators (59), has been suggested as one way of indirectly targeting Myc-driven tumorigenesis (15). This is of particular interest given the strong transcriptional upregulation and/or amplification of c-Myc in WNT and Group 3 MBs. Targeting the aberrant regulation of H3K27 methylation observed in MB has also been suggested as a therapeutic avenue (17, 62). As further support for this concept, DZNep (3-deazaneplanocin A), an inhibitor of the H3K27 methyltransferase *EZH2*, has recently been shown to suppress MB cell growth *in vitro* (2). Phase II clinical trials of *EZH2* inhibitors are now part of the Children's Oncology Group (COG) blueprint for MB research (20). Particularly for those tumors with elevated *OTX2* expression, the combination of polycomb inhibition with a differentiating agent such as all-trans retinoic acid (ATRA) has also been suggested (4, 7, 16). As it seems, however, that the global process of histone methylation is affected, rather than a single gene or pathway, therapeutic targeting of these alterations may prove challenging.

Finally, the development of tumor models that faithfully recapitulate these epigenetic alterations will also be crucial, both for further characterizing the biological changes resulting from epigenetic dysregulation and for the development and testing of novel therapeutics. The majority of the established MB cell lines are thought to most closely resemble Group 3 MB, with no WNT models and only poor representation of the SHH and Group 4 subgroups. As with any long-term culture model, there are also concerns as to how well these lines truly reflect the primary tumor. As such, a lot of effort is currently being put into developing suitable *in vivo* models. There are already genetically engineered mouse models (GEMMs) of three of the four molecular subgroups of MB (18): WNT (21), SHH (22, 24, 27, 80, 82, 83) and Group 3 (32, 54). A MYCN-driven model that may resemble Group 4 tumors in certain contexts has also been established (71). None of these GEMMs, however, are driven by alterations in chromatin modifiers, and it will therefore be necessary to build a larger repertoire to model the variety of changes identified through high-throughput genomics. A number of research groups are also building collections of xenografted tumor material from the various molecular subgroups, which will help recapitulate the wide spectrum of alterations observed in primary tumors and provide a useful additional tool for preclinical testing of targeted agents (85).

Thus, while it is right to acknowledge the multiple advances that have recently been made in the field, building on these insights into the role of chromatin remodeling in MB in order to transfer this knowledge to the bedside will be one of the major challenges in the emerging post-genomics era.

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