Role of MYC in Medulloblastoma

Martine F. Roussel[1] and Giles W. Robinson[2]

[1]Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

[2]Division of Neuro-Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

Correspondence: martine.roussel@stjude.org

Since its discovery as an oncogene carried by the avian acute leukemia virus MC29 in myelocytomatosis (Roussel et al. 1979) and its cloning (Vennstrom et al. 1982), *c-MYC* (*MYC*), as well as its paralogs *MYCN* and *MYCL1*, has been shown to play essential roles in cycling progenitor cells born from proliferating zones during embryonic development, and in all proliferating cells after birth. *MYC* deletion induces cell-cycle exit or cell death, depending on the cell type and milieu, whereas *MYC* and *MYCN* amplification or overexpression promotes cell proliferation and occurs in many cancers. Here, we review the relationship of MYC family proteins to the four molecularly distinct medulloblastoma subgroups, discuss the possible roles MYC plays in each of these subgroups and in the developing cells of the posterior fossa, and speculate on possible therapeutic strategies targeting MYC.

Medulloblastoma (MB), the most common malignant brain tumor of childhood, is a diverse and heterogeneous disease. Most frequent in children between the ages 0 and 9, this aggressive tumor can occur at any age (Louis et al. 2007). Histopathological variants also exist and are associated with different outcomes (Rutkowski et al. 2005; Ellison et al. 2011b). Most recently, transcriptional profiling identified four major MB subgroups: two associated with pathogenic abnormalities in the Wingless (WNT) and Sonic Hedgehog (SHH) developmental signaling pathways (the WNT and SHH subgroups), and two that are less well molecularly characterized and referred to as group 3 and group 4 tumors. These transcriptional subgroups relate to differences in age at diagnosis, sex, histopathology, incidence of metastatic dis-ease, somatic variations, and provide an improved prediction of clinical outcome (Fig. 1A) (Thompson et al. 2006; Kool et al. 2008; Cho et al. 2011; Northcott et al. 2011; Taylor et al. 2012). Despite this heterogeneity, medulloblastomas are still treated uniformly. Patients receive surgery, radiation, and adjuvant chemotherapy, and although curative at about 70%, this regimen leaves survivors with debilitating side effects and fails to cure all comers (Mulhern et al. 2005; Gajjar et al. 2006; Packer et al. 2006). Before this suboptimal but broadly effective treatment can be changed, an improved understanding of the pathogenesis of the subtypes is needed. If therapy is to become truly tailored to each molecular subgroup, bona fide tumorigenic targets for each subgroup must be identified and drugs developed to hit those targets.
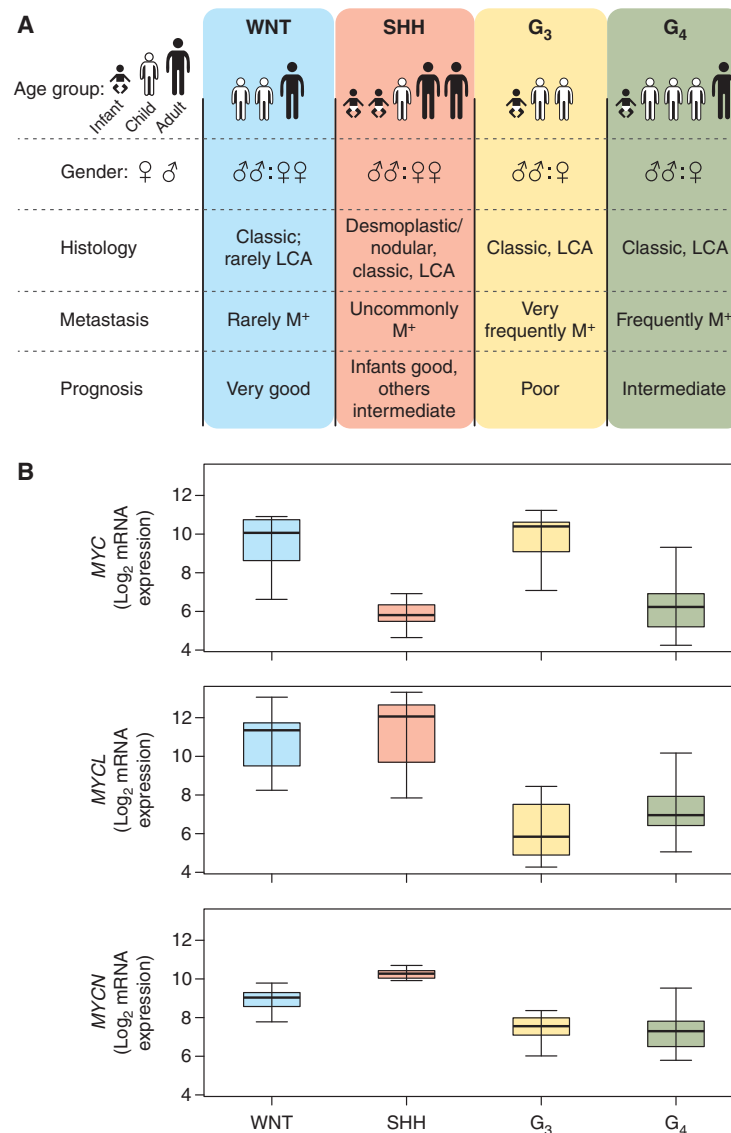
Figure 1. Features of the human medulloblastoma subgroups and variable expression of *MYC* genes. (*A*) Clinical and demographic features of medulloblastoma subgroups (LCA, large cell anaplastic; M^+, positive for metastatic disease). Number of human figures in age group row and symbols in gender row represent the relative distribution in the four subgroups (for example, Group 4 [G_4] medulloblastoma is most common in children and in males) (based on data from Taylor et al. 2012). (*B*) *MYC*, *MYCL1*, and *MYCN* Log_2 mRNA expression across the four subgroups of medulloblastoma (expression data from Robinson et al. 2012 [$n = 74$]). Group 3, G_3.

One such target is the MYC family of proteins (MYC, MYCN, and to a lesser degree MYCL1). These proteins are of interest because they appear to play different roles in each of the medulloblastoma subgroups. This relationship to the different subtypes underscores the diver-

sity of these proteins and promises to reveal clues about medulloblastoma tumorigenesis, especially if we can gain a better understanding of the relationship of MYC protein function to normal and transformed cells of the posterior fossa, the intracranial cavity that houses the cer-

ebellum and the brain stem within which medulloblastomas arise.

MYC AND THE MEDULLOBLASTOMA SUBGROUPS

MYC proteins are associated with many cancers and medulloblastoma is no exception. *MYC*, *MYCN*, and *MYCL1* amplifications have all been described in medulloblastomas (Northcott et al. 2012b). *MYC* and *MYCN* amplification and expression have been intensely scrutinized in medulloblastoma because highly aggressive tumors frequently harbor *MYC* or *MYCN* amplification and/or overexpression (McManamy et al. 2007; Pfister et al. 2009; Cho et al. 2011). Classification schemes correlating expression and amplification of these proteins to poor outcome have been proposed (de Haas et al. 2008; Park et al. 2012). However inconsistencies, such as high expression in a subset of good responders, have made these criteria difficult to apply. These discrepancies arise because MYC proteins relate differently to each subgroup. When viewed in this context, the relationship of MYC to prognosis becomes clearer.

MYC in the WNT Subgroup

The WNT subgroup of medulloblastoma is the most curable with >90% of patients surviving on current therapy (Ellison et al. 2005; Clifford et al. 2006; Gajjar et al. 2006; Ellison et al. 2011a). It is also the least common medulloblastoma subtype, accounting for only 10%. Patients are generally older, with an average age of about 10 years. There is an even distribution of females to males. The histology is overwhelmingly classic, and the tumors are very infrequently metastatic (Fig. 1A) (Kool et al. 2012; Taylor et al. 2012). Transcriptional profiling reveals a high expression of WNT pathway genes in these tumors compared with the other subgroups (Thompson et al. 2006; Kool et al. 2008; Northcott et al. 2011). Next-generation sequencing studies show that ~90% of these tumors harbor activating mutations in *β-CATENIN* (*CTNNB1*), the central orchestrator of the canonical WNT pathway (Jones et al. 2012; Northcott et al.

2012a; Pugh et al. 2012; Robinson et al. 2012). The only mouse model of this disease requires an activating mutation in *Ctnnb1* and loss of *Trp53* (Gibson et al. 2010). Partners of CTNNB1 and members of the canonical WNT pathway are also frequently mutated in this subgroup (Robinson et al. 2012). These data strongly support the constitutive activation of the WNT pathway as causal to this medulloblastoma subgroup.

In the WNT subgroup, although *MYCN* and *MYCL1* have a moderately high overall expression level in comparison to group 3 and group 4, *MYC* expression is very high and comparable only to that of group 3 (Fig. 1B). This is interesting because, as previously noted, *MYC* overexpression has been associated with a worse prognosis (de Haas et al. 2008; Park et al. 2012). High expression in the subgroup with the best overall survival refutes this idea and is the primary reason that *MYC* expression alone is a poor prognosticator. Moreover, unlike the other subgroups, *MYC*, *MYCN*, and *MYCL1* amplifications almost never occur in the WNT subgroup (Fig. 2A–E) (Jones et al. 2012; Northcott et al. 2012b; Pugh et al. 2012; Robinson et al. 2012).

MYC is a downstream target of WNT signaling and its expression is increased on the formation of a transcription factor complex through the interaction of CTNNB1 with TCF/LEF family proteins (He et al. 1998). It is not, therefore, surprising that *MYC* expression is elevated in tumors driven by constitutive WNT signaling. Furthermore, many mouse models of medulloblastoma have been developed through enforced *MYC* and *MYCN* expression in various cell types throughout the posterior fossa and none of these results in the formation of WNT subgroup (Zindy et al. 2007; Swartling et al. 2010; Kawauchi et al. 2012; Swartling et al. 2012). These data suggest that *MYC*'s relationship to the WNT subgroup is not tumorigenic or progressive, but rather a marker of enhanced WNT pathway activity.

MYC in the SHH Subgroup

The SHH subgroup of medulloblastoma has an intermediate prognosis and makes up ~25%–
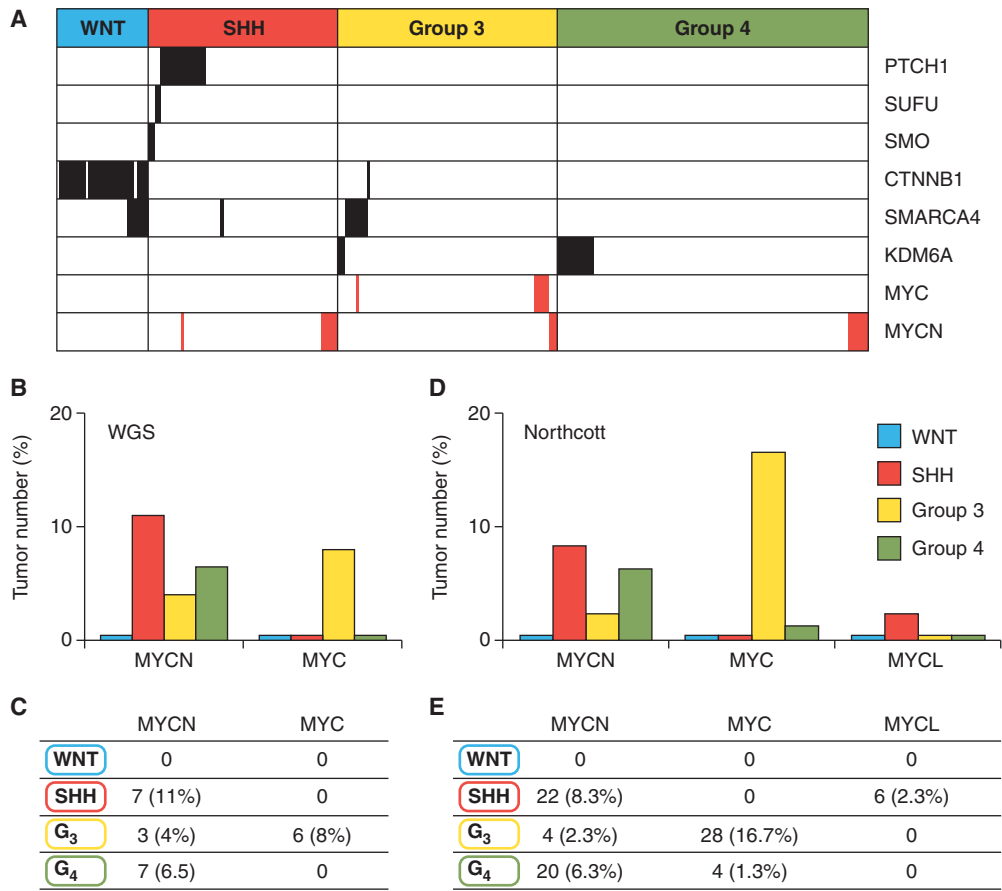
Figure 2. Amplifications of *MYC* genes in human medulloblastoma. (*A*) *MYC* and *MYCN* amplifications, shown in red, and the most common recurrent subgroup mutations (missense, indels, frameshift mutations), shown in black, relative to the four medulloblastoma subgroups. Results are reported across three genomic studies (Jones et al. 2012; Pugh et al. 2012; Robinson et al. 2012). (*B,C*) *MYCN* and *MYC* amplifications relative to four medulloblastoma subgroups across three whole genome sequencing (WGS) studies (Jones et al. 2012; Pugh et al. 2012; Robinson et al. 2012). (*D,E*) *MYCN* and *MYC* amplifications relative to four medulloblastoma subgroups in 827 medulloblastomas (Northcott et al. 2012b).

30% of all medulloblastomas. SHH subgroup patients tend to be very young (<5 years old) or older (>16 years old). Both males and females are equally likely to be diagnosed with SHH medulloblastoma. All three major histologic variants (nodular desmoplastic, classic, and large cell/anaplastic [LCA]) are described within this group (Northcott et al. 2011; Kool et al. 2012; Taylor et al. 2012). Outcome correlates with histology, and nodular desmoplastic SHH tumors have a good overall survival, whereas LCA tumors show a worse outcome

(McManamy et al. 2007; Ellison et al. 2011a). Metastatic disease is uncommon but associates with other poor prognostic features such as LCA histology and older age at diagnosis (Fig. 1A) (Ellison et al. 2011a). Transcriptional profiling shows significant overexpression of SHH signaling genes in this group relative to the other subgroups (Thompson et al. 2006; Kool et al. 2008; Cho et al. 2011; Northcott et al. 2011). WGS and other studies have identified numerous mutations in genes that regulate the SHH pathway, all of which result in its constitutive activation

(Jones et al. 2012; Northcott et al. 2012a; Pugh et al. 2012; Robinson et al. 2012). Numerous mouse models of this disease have been developed by engineering mutations that activate the SHH pathway (Goodrich et al. 1997; Hallahan et al. 2004; Uziel et al. 2005; Zindy et al. 2007; Hatton et al. 2008; Ayrault et al. 2010).

MYCN and *MYCL1* are highly expressed in the SHH subgroup relative to the other subgroups (Fig. 1B). Moreover *MYCN* and *MYCL1* amplification both occur in SHH medulloblastoma (Fig. 2A–E) (Northcott et al. 2012b). *MYCN* amplification in this subgroup is associated with poor prognosis (Ellison et al. 2011a). Additionally, combined data from WGS studies show a strong association between *MYCN* amplification, LCA histology, and *TP53* mutational status; each are markers of poor prognosis (Jones et al. 2012; Pugh et al. 2012; Rausch et al. 2012; Robinson et al. 2012). These data suggest that although *MYCN* expression is uniformly high in this subgroup, *MYCN* amplification occurs in more aggressive, drug-resistant tumors.

Parallels are seen in mouse models of SHH medulloblastoma. Activation of the SHH pathway promotes the expression and stabilization of MYCN (Oliver et al. 2003; Thomas et al. 2009). Enforced expression of MYCN in granule neural precursors (GNPs) collaborates with the loss of one copy of the *Ptch1* mutant and *Cdkn2c* ($p18^{Ink4c}$), or with the loss of *Trp53* and *Cdkn2c* to accelerate SHH MB development in mice from 9 to 3 months after birth and to increase penetrance from 14% to 60% (Uziel et al. 2005; Zindy et al. 2007; Ayrault et al. 2009; Kawauchi et al. 2012). Moreover, enforced MYCN expression produces more invasive, drug-resistant tumors (Kessler et al. 2009).

To the SHH subgroup, therefore, MYCN is both a marker of enhanced SHH activity and a harbinger of aggressivity. These activities can be distinguished by copy number but not expression. Analogous to *MYC* in the WNT subgroup, *MYCN* expression increases on SHH pathway activation, meaning that all SHH tumors show high *MYCN* expression including highly curable nodular desmoplastic tumors. However, more predictive of prognosis, is the copy number because, when amplified, these tumors associate

with higher risk features and respond poorly to therapy. These data suggest that *MYCN*, especially if amplified and intrinsically deregulated, will induce and maintain SHH tumor progression.

MYC in the Group 3 Subgroup

Group 3 medulloblastomas have the worst overall survival at <50% survival on many protocols (Cho et al. 2011; Northcott et al. 2011; Taylor et al. 2012). These tumors account for ~25%–30% of medulloblastoma. They occur in infants (<3 years old) and children but not adults. Patients are twice as likely to be male as female. Metastases are more prevalent in this subgroup than any other, exceeding 40% in some studies, and LCA histology—another high-risk feature—is also prevalent (Fig. 1A) (Kool et al. 2012; Northcott et al. 2012a; Taylor et al. 2012). Transcriptional profiling analysis did not reveal appreciable signaling pathways but enrichment for genes involved in ribosomal biosynthesis, nucleotide metabolism, photoreceptor differentiation, and GABAergic function (Kool et al. 2008; Cho et al. 2011). Next-generation sequencing of 76 group 3 primary tumors in three independent studies yielded few recurrent mutations in this group (Jones et al. 2012; Northcott et al. 2012a; Pugh et al. 2012; Robinson et al. 2012). However, numerous mutations have been identified in genes involved in the reading, writing, and erasing of epigenetic marks. These mutations overlapped with those found in group 4 suggesting a common transforming pathway (Fig. 3A,B) (Robinson et al. 2012).

MYC is more intricately linked to this subgroup than to any other. In group 3 tumors, *MYC* is significantly more highly expressed compared with SHH and group 4, but not the WNT subgroup (Fig. 1B). *MYC* amplifications occur at a much higher frequency (10%–17%) than in any other subgroup (Fig. 2A–E) (Northcott et al. 2012b). *MYCN* amplifications also occur in this group albeit much more infrequently (2%–4%) (Fig. 2A–E) (Northcott et al. 2012b). These *MYC* and *MYCN* amplifications occur in a mutually exclusive fashion suggesting that they
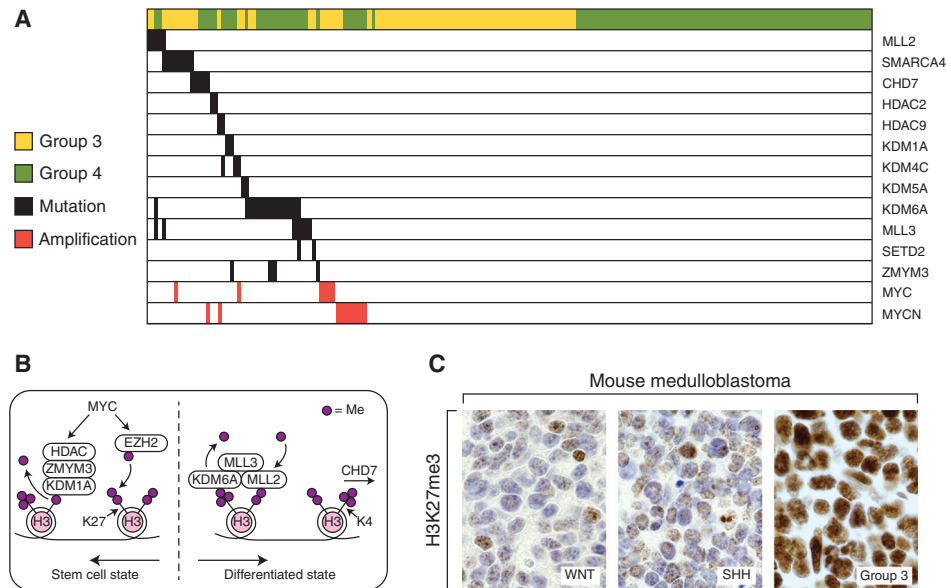
Figure 3. *MYC* genes and the epigenetic aberrations seen in human group 3 and group 4 medulloblastoma. (*A*) *MYC* and *MYCN* amplifications, shown in red, relative to the most common recurrent epigenetic mutations (missense, indels, frameshift mutations) in group 3 and group 4 reported across three genomic studies (Jones et al. 2012; Pugh et al. 2012; Robinson et al. 2012). (*B*) A diagram of epigenetic regulators altered in group 3 and group 4 medulloblastoma that influence the methylation of H3K27 and H3K4 histone marks. Somatic aberrations found in group 3 and group 4 medulloblastoma drive cell toward a stem-cell-like epigenetic state by favoring H3K27 trimethylation. (*C*) H3K27me3 expression in WNT, SHH, and group 3 mouse models (From Robinson et al. 2012; adapted, with permission, from the author.)

may have overlapping functions in this setting (Fig. 2A) (Northcott et al. 2012a).

Two *MYC*-driven mouse models of group 3 MBs have been generated. Our model was generated by enforced expression of the wild-type *MYC* gene in GNPs purified from *Trp53*-null mice (Kawauchi et al. 2012); the other relied on enforced expression of a partially stabilized mutant of *MYC* (MYC^{T58A}) with a dominant-negative form of *Trp53* in CD133/prominin 1-positive neural stem cells (NSCs) (Pei et al. 2012). The resultant tumors showed that the LCA phenotypes were highly aggressive, and transcriptionally resembled group 3 MB. *MYC* withdrawal caused complete tumor regression (Pei et al. 2012). These data suggest that *MYC* is a significant contributor to the initiation, maintenance, and progression of this disease (see Gabay et al. 2013).

Of note, many mouse models of MB, including these *MYC*-driven group 3 models, re-

quire the loss of *Trp53* function; yet somatic mutations of *TP53* occur mostly in WNT and SHH medulloblastoma and are associated with *MYCN* rather than *MYC* amplification (Pfaff et al. 2010; Rausch et al. 2012). The absence of these mutations in group 3 tumors means that *TP53* loss is not required for human tumor initiation, that another cooperative genetic event assists tumor initiation, or that the TP53 pathway is in some other way compromised. To clarify this question, an analysis of TP53 protein expression and function and its regulation in human primary medulloblastoma samples grown as neurospheres or xenografts is warranted (Milde et al. 2012; Zhao et al. 2012).

MYC in the Group 4 Subgroup

Group 4 medulloblastomas have a similar prognosis to SHH tumors. They account for ~40%–50% of medulloblastoma, occur mainly in chil-

dren but can occur in adults, and, similar to group 3, predominantly occur in males. Most group 4 MBs have a classic histology (Fig. 1A) (Kool et al. 2012; Northcott et al. 2012a; Taylor et al. 2012). Transcriptional profiling revealed enrichment for genes of neuronal differentiation and glutamatergic receptors (Kool et al. 2008; Cho et al. 2011). Next-generation sequencing identified recurrent mutations in the molecular machinery that reads, writes, and erases epigenetic marks (described below), some overlapping with those found in group 3 tumors (Fig. 3A,B) (Jones et al. 2012; Northcott et al. 2012a; Pugh et al. 2012; Robinson et al. 2012).

Group 4 tumors generally have low *MYC* and *MYCN* expression compared with the other subgroups (Fig. 1B). However, mean *MYCN* expression levels are still comparatively high when compared with mature cerebellum and exist at comparable levels to fetal cerebella (Swartling et al. 2010). This implies that a certain level of *MYCN* may be required for tumor maintenance. In addition, *MYCN* amplifications occur in some group 4 tumors and these, like group 3, appear to be mutually exclusive with epigenetic mutations (Fig. 3A) and to other amplifications (Northcott et al. 2012a; Robinson et al. 2012). This suggests that, although rare, *MYCN* amplification may initiate group 4 medulloblastoma.

Mouse cerebellar NSCs from postnatal day 0, but not from embryonic day 16, transduced with a partially stabilized form of MYCN (MYCN^{T58A}) produce SHH-independent medulloblastoma (Swartling et al. 2012). Similarly, when conditional *MYCN*^{T58A} expression is targeted to neural cells from postnatal day 1 through adulthood, mice develop group 4-like MBs (Swartling et al. 2010, 2012). These tumors enter senescence on removal of stabilized MYCN^{T58A} (Swartling et al. 2010). These experiments show the influence of the timing of the oncogenic stimulus and its requirement for tumorigenesis (see Gabay et al. 2013).

This temporal association draws parallels to the peak age of this disease occurring in older children rather than infants and implies that there may be a finite period during which certain cell pools are particularly vulnerable to tu-

morigenesis. These data suggest that *MYCN* plays a role in initiation and maintenance of group 4 medulloblastoma.

MYC IN THE DEVELOPING POSTERIOR FOSSA

The cerebellum is a unique brain structure because, unlike the rest of the brain, granule neural progenitors actively proliferate after birth (Roussel and Hatten 2011). Cerebellar progenitor neurons arise during embryogenesis from the ventricular zone and the upper rhombic lip (URL) to form the cerebellar anlage (Fig. 4). During this time, neural progenitors are also born in the lower rhombic lip (LRL) on the floor of the fourth ventricle and express the *Blbp* gene (Gibson et al. 2010). The neural progenitors within the ventricular zone of the cerebellar anlage migrate radially outward and give rise to Bergman glia and Purkinje cells, which are postmitotic by birth. Conversely neural progenitors from the upper rhombic lip give rise to GNPs that rapidly proliferate after birth in the external granule layer (EGL) of the developing cerebellum, exit cycle, and migrate inward. Once the GNPs cross the Purkinje cell layer they come to rest and form the internal granule layer (IGL) as postmitotic neurons (Roussel and Hatten 2011). The white matter within the core of the mature cerebellum contains neuronal stem cells expressing CD133/prominin that are capable of generating new neurons during the life of the organism.

MYC, *MYCN*, and *MYCL* expression in the developing posterior fossa appears to be cell context dependent (Fig. 4). GNPs express *MYCN*, but not *MYC*, in their proliferative phase in response to SHH. Neuronal progenitors in the Purkinje cell layer and in the ventricular zone express *MYCL* but not *MYCN* or *MYC* (www.cdtdb.neuroinf.jp/CDT/Top.jsp). *MYC* is expressed in brain lipid-binding protein (BLBP)-positive glial and LRL neural progenitors and possibly in CD133/prominin NSCs that reside in the white matter (Wey et al. 2010). This differential expression of *MYC* and *MYCN* by distinct cerebellar progenitors lends credence to the argument that the different me-
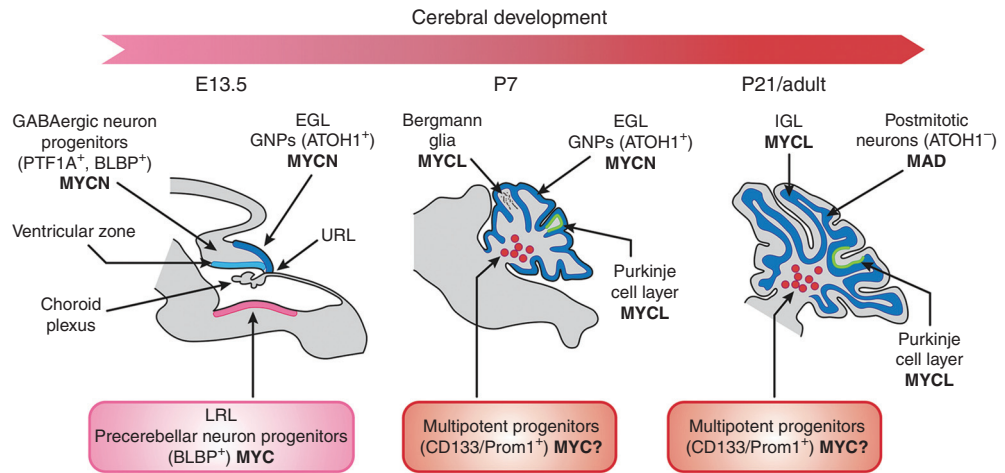
Figure 4. Expression of *MYC* genes during mouse cerebellar development. At embryonic day E13.5, GNPs that migrate from the upper rhombic lip (URL) and form the external granule layer (EGL) and GABAergic neural progenitors in the cerebellar ventricular zone (VZ) express *MYCN*. *MYC* is found expressed in precerebellar neuron progenitors in the lower rhombic lip. At P7, whereas the GNPs in the EGL continue to express *MYCN*, the Purkinje cell layer, composed of Bergman glia and Purkinje cells, expresses *MYCL* and CD133/prominin-positive multipotent progenitors. At P21, all GNPs have exited cycle, no longer express *MYCN* but do express MXD (see Eisenman 2013), and have migrated through the Purkinje cell layer to reside as postmitotic neurons in the internal granule layer (IGL) that, like Purkinje cells, express *MYCL* (www.gensat.org; www.cdtdb.neuroinf.jp/CDT/Top.jsp; Allen Brain Atlas). CD133/prominin-positive multipotent progenitors are marked with a "MYC?" because this is suspected but not known. Prom1, prominin 1.

dulloblastoma subgroups arise from distinct cerebellar progenitors. Indeed SHH subgroup medulloblastomas originate from GNPs and overexpress *MYCN*, whereas WNT subgroup medulloblastomas emanate from LRL precursors and overexpress *MYC*. However, attempts to identify precursors of group 3 and group 4 medulloblastomas through enforced expression of *MYC* or *MYCN* into identical cerebellar precursor cells yielded some intriguing differential effects depending on the gene used, which suggests that the two paralogs influence the cellular program in different ways (Kawauchi et al. 2012).

Rapid proliferation of GNPs is mediated by SHH, which is produced by Purkinje cells. By binding to its receptor *PATCHED* (*PTCH1*), SHH directly activates *MYCN* transcription via GLI transcription factors (Kenney et al. 2003). The *MYCN* requirement for cerebellum development was shown in a *Nestin-Cre* transgenic mouse. Conditional deletion of *Mycn* in early neural stem and progenitor cells of this mouse

led to profound cerebellum defects, in part owing to the failure to suppress two cyclin-dependent kinase inhibitory proteins (CKIs), p18[Ink4c] and p27[Kip1] (Knoepfler et al. 2002). Remarkably, these cerebellar defects could be partially rescued by the loss of the two CKIs suggesting that MYCN inhibition of p18[Ink4c] and p27[Kip1] expression is required for proper cerebellar development (Knoepfler et al. 2002; Zindy et al. 2006; Hurlin 2013).

Although *MYC* is not expressed in GNPs, the conditional loss of *Mycn* in neural progenitors induces *Myc* in GNPs, which partially compensates for the loss of *Mycn*, whereas loss of both *Myc* and *Mycn* leads to the complete absence of the EGL and GNPs (Zindy et al. 2006). This functional redundancy implies that *MYC* and *MYCN* may have similar roles. These homologs contain conserved MYC boxes, and similar basic helix-loop-helix (bHLH)-zipper structures that recognize the same DNA motif when dimerized with MAX (see Eisenman 2013; Rahl and Young 2013). Knock-in of

MYCN into the *MYC* locus rescues murine development, strongly suggesting major functional redundancy (Malynn et al. 2000).

However, MYC and MYCN also possess long stretches of nonrelated amino acid sequences, which raises the possibility that they are functionally diverse. Indeed, enforced expression of *MYC* or *MYCN* in *Trp53-null* GNPs induced two distinct groups of MBs: group 3 and SHH, respectively (Kawauchi et al. 2012). Why these two very similar proteins induce two completely different MB groups is not known. Experiments swapping different regions of the *MYC* and *MYCN* proteins to assess which regions are responsible for MB outcome and identifying differences in MYC- versus MYCN-associated cofactors will be required to establish the basis for distinct functions of MYCN and MYC. In addition, chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) could be used to identify different MYC and MYCN genomic-binding sites, which will be highly informative toward understanding the emergence of distinct MBs.

MYC PROTEINS AND THE EPIGENETIC LANDSCAPE IN MEDULLOBLASTOMA

Only recently has a role for epigenetic modifiers in regulating medulloblastoma genesis been suggested. First, high-resolution single-nucleotide polymorphism (SNP) genotyping identified novel genetic events in genes that target H3 lysine 9 (H3K9), a repressive chromatin mark found in postmitotic cells consistent with transcriptional silencing (Northcott et al. 2009b). Second, *MLL2* and *MLL3* mutations were found to be the next most common mutations in a cohort of medulloblastoma after *PTCH1* (Parsons et al. 2011). When WGS was combined with transcriptional profiling, subgroup-specific epigenetic events became apparent (Jones et al. 2012; Northcott et al. 2012a; Pugh et al. 2012; Robinson et al. 2012). Most epigenetic mutations identified in the WNT subgroup can be functionally linked to *CTNNB1*, substantiating that WNT pathway genetic anomalies remain the central driver (Robinson et al. 2012). Similarly, the epigenetic events identified in the SHH subgroup might be expected to cooperate with activating mutations in the SHH signaling pathway. However, it is the overlapping epigenetic events in group 3 and group 4 that will shed light on the mechanism by which these tumors develop; a mechanism that appears to involve the *MYC* genes.

The highest percentage of mutations in histone modifiers was found in *KDM6A*, the histone H3 lysine 27 (H3K27me) demethylase (Fig. 3A) (Northcott et al. 2012a). KDM6A associates with the MLL2 and MLL3 H3-K4 methyltransferases, which are also recurrently mutated along with *CHD7*, and *ZMYM3* whose function further influences H3K4 methylation (Robinson et al. 2012). This clustering of mutations around the machinery that initiates, maintains, and erases H3K27 and H3K4 methyl marks appears to drive a stem cell-like phenotype of preserved H3K27 trimethylation coupled with impaired H3K4 methylation (Fig. 3B). An investigation of other genes that influence these histone signals revealed that EZH2, the H3K27 methyltransferase, was overexpressed in a large subset of groups 3 and 4 medulloblastomas. As expected of enzymes with opposing functions, gain of EZH2 expression was seen in tumors that lacked inactivating *KDM6A* mutations (Robinson et al. 2012). Furthermore, both group 3 and group 4 human tumors and *MYC*-driven group 3 murine MBs had higher levels of H3K27 methylation than SHH or WNT human or murine tumors (Fig. 3C) (Robinson et al. 2012; Campbell and White 2013; Dubuc et al. 2013).

Thus, group 3 and group 4 MBs retain the H3K27 trimethyl marks by loss of the demethylator KDM6A, gain of the methylator EZH2, and interference with H3K4 methylation by mutating *MLL2*, *MLL3*, *ZMYM3*, and *CDH7* (Fig. 3B). The predicted effect is the preservation or promotion of a stem-cell-like state preventing tumor cells from differentiating. The amplification of *MYC* and *MYCN* is mutually exclusive with these recurrent mutations in epigenetic regulators raising the possibility that *MYC* and *MYCN* amplification could affect epigenetic marks that promote maintenance of the undifferentiated state (Fig. 3A) (see Chappell and Dalton 2013).

Indeed *MYC* genes have been implicated in chromatin and epigenetic regulation. They regulate transcription by recruiting histone acetyltransferases (HAT) (McMahon et al. 2000) and other chromatin-modifying factors (Eilers and Eisenman 2008). Changes in MYC levels have been shown to have widespread effects on chromatin marks (Knoepfler et al. 2006). Importantly, MYC has also been shown to induce EZH2 expression in other cancers (Koh et al. 2011; Salvatori et al. 2011). Given that MYC and EZH2 maintain self-renewal, and that binding of MYC induces robust regulation of Sox2 (Lin et al. 2009), it was not surprising to find "stemness" markers such as *Oct4*, *Sox2*, and *Nanog*, all associated with aggressive cancers (Chang and Hung 2012), in mouse group 3 MB cells (Kawauchi et al. 2012).

MYC AND MicroRNAS IN MEDULLOBLASTOMA

MYC genes and microRNAs regulate each other. MicroRNAs (miRs) are 20- to 22-nucleotide-long RNA molecules that repress translation by interacting with the 3′-untranslated region of target mRNAs (He and Hannon 2004). Although the principal consequence of MYC activation is microRNA repression, MYC also induces microRNAs that, in part, mediate its function (see Thomas-Tikhonenko and Psathas 2013).

MYC Regulation of MicroRNAs

MicroRNAs encoded by the *miR-17 ∼ 92* and *miR-106b ∼ 25*, but not the *miR-106a ∼ 363*, clusters are overexpressed specifically in human and mouse SHH MBs (Ferretti et al. 2009; Northcott et al. 2009a; Uziel et al. 2009). The *miR-17 ∼ 92* cluster, also called OncomiR1, was first identified in Eμ-MYC-induced B-cell lymphomas (He and Hannon 2004) and found to be regulated by Mycn in SHH-treated cerebellar neural progenitors (Northcott et al. 2009a) and by MYC proteins in other cancers (Mendell 2008). The *miR-17 ∼ 92* cluster is a member of a family of three clusters including two paralogs, *miR-106b ∼ 25* and *miR-106a ∼*

363, which are encoded on different chromosomes and express microRNAs that share seed sequences (Ventura and Jacks 2009). Consistent with its high expression in SHH MB, enforced expression of the *miR-17 ∼ 92* cluster, together with mutation of $Ptch1^{+/-}$, accelerates the onset and penetrance of SHH MB (Uziel et al. 2009). Although the true direct targets of miRs from the *miR-17 ∼ 92* cluster remain unknown in medulloblastoma, we found that the *miR-17 ∼ 92* cluster, but not the other two paralogs, was dispensable for embryonic cerebellar development but essential for SHH MB development in the mouse because its loss in $Ptch1^{+/-}$ mice completely suppressed tumor development (F Zindy and MF Roussel, unpubl.). Tumor progression can be inhibited by using anti-miR 8-mer tiny locked nucleic acids (LNAs) that target the seed sequence of miR-17/20 and miR-19a/b in SHH MB cells in vitro and in animals bearing flank or cortical SHH MB allografts (Murphy et al. 2013). SHH MBs with the highest expression of these miRs are the most sensitive to LNA treatment, indicating that tumor cells are addicted to miR expression. These data suggest that LNAs targeting the *miR-17 ∼ 92* cluster family could be used therapeutically in SHH MB and other cancers that overexpress members of this cluster family. Besides the *miR-17 ∼ 92* cluster family, the *miR-183 ∼ 96 ∼ 182* cluster was also found in *MYC*-amplified MBs to regulate cell survival, proliferation, and migration (Weeraratne et al. 2012). Interestingly, miR-182 promotes metastasis, a hallmark of aggressive MBs (Bai et al. 2012).

Three other microRNAs are differentially regulated in *MYC*-overexpressing MBs compared to MBs in which *MYC* is not overexpressed (Ferretti et al. 2009). However, whether these microRNAs are directly regulated by *MYC* is as yet unknown.

MicroRNA Regulation of MYC

Assessment of somatic copy number aberrations across 1087 unique primary MBs also identified translocations fusing *MYC* to microRNAs, including *PVT1* (encoding microRNA miR-1204) in group 3 MB, clearly linking

MYC and microRNA expression. In this translocation, the first exon/intron of PVT1 that includes miR-1204 is linked to exons 2 and 3 of *MYC* leading to high levels of expression of both miR-1204 and *MYC* in these MBs (Northcott et al. 2012b). MiR-1204 has been implicated as a candidate oncogene in combination with *MYC* (Shtivelman and Bishop 1989; Carramusa et al. 2007; Northcott et al. 2012b).

MYC can also be regulated by microRNAs in MBs. The miR-33b gene located at 17p11.2, a genomic locus frequently deleted in group 3 MB, was recently found to be up-regulated by lovastatin and, in turn, to repress MYC expression in two human cell lines (DAOY and D283) derived from MYC-driven primary MB patient samples (Takwi et al. 2012). In DAOY cells, loss of miR-512.2 correlated with increased MYC expression (Lv et al. 2012). Primary human medulloblastomas should be similarly investigated.

THERAPEUTIC STRATEGIES FOR MEDULLOBLASTOMA

Since its first identification, MYC has resisted all attempts to be "drugged." However, because deprivation of *MYC* and *MYCN* in group 3 and group 4 models either induce senescence or apoptotic cell death (Swartling et al. 2010; Pei et al. 2012), several strategies are being considered to suppress MYC function either by regulating its expression or preventing its binding to partner proteins.

Because MYC requires MAX for efficient transcription (Blackwood and Eisenman 1991) preventing the interaction between MYC and MAX may suppress MYC function as was shown in MYC-transformed chicken fibroblasts (Berg et al. 2002). Unfortunately, inhibitors of the MYC/MAX interaction have not been suitable for therapy and further efforts are warranted to identify therapeutically viable small molecule antagonists of MYC/MAX dimerization (see Eisenman 2013).

Epigenetic modifiers are being considered as targets for the treatment of different cancers, including MBs. As described above, many epigenetic modifiers that regulate the function of histones, including histone H3, are mutated or overexpressed in human MBs. EZH2 inhibitors have already been shown to be effective in treating lymphomas (McCabe et al. 2012). Given the high expression of EZH2 in many group 3 and group 4 MBs, it will be important to establish the effectiveness of EZH2 inhibitors in preclinical testing in existing mouse models and in primary human xenografts. Histone deacetylase (HDAC) inhibitors, including vorinostat, are currently in clinical trials in pediatric patients with malignant brain tumors, and a recent study found that a novel human group 3 MB cell line was highly sensitive to HDAC inhibitors alone and in combination with radiation (Milde et al. 2012).

Another intensely studied potential therapeutic target is BRD4, a BET-family bromodomain protein, which was identified as the top hit from a shRNA screen of epigenetic modifiers in MYC-driven lymphomas and in acute myeloid leukemia (AML) (Blobel et al. 2011; Zuber et al. 2011a,b). BRD4 is recruited to acetylated histones to stimulate transcriptional elongation and modify chromatin-regulating complexes (Rahman et al. 2011). BRD4 positively regulates *MYC* transcription by binding to the *MYC* promoter. BRD4 inhibition by JQ1, which competitively binds to the acetyl-lysine recognition motif, effectively induced regression of MYC-driven multiple myeloma and B-cell lymphomas (Filippakopoulos et al. 2010; Nicodeme et al. 2010; Delmore et al. 2011). Other BRD4 inhibitors are also being evaluated as potential therapeutics for the treatment of MYC-driven cancers (see Bradner 2013).

Because MYC up-regulates cell metabolism by, for example, increasing folate pathway activity and nucleotide synthesis, small molecule inhibitors of these pathways may also be suitable. This approach will require the screening of libraries of small molecules using MYC-driven mouse models of MB, which should be forthcoming (see Dang 2013).

Finally, microRNAs that are overexpressed or underexpressed in MYC-driven medulloblastoma might be used therapeutically in these tumors. MicroRNAs are not predicted to cross an uncompromised blood–brain barrier and alter-

native modes of delivery, such as nanoparticles or intraventricular administration, should be investigated.

CONCLUDING REMARKS

The relationship of the *MYC* family of genes to medulloblastoma is widespread but subgroup specific. Although promoting proliferation in all the subgroups, the *MYC* genes play different roles in each. Fittingly, these differences appear to reflect the distinct roles *MYC* genes play in hindbrain development. The role in the initiation, maintenance, and progression of the aggressive group 3 medulloblastomas as well as toward the progression of SHH subgroup make the *MYC* genes and their regulators very inviting therapeutic targets for MBs for which current therapies are inadequate.

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