

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Transcription Factor Targets as Treatment for Medulloblastoma

Mehdi H. Shahi and Elva Díaz

*Department of Pharmacology, UC Davis School of Medicine, Davis, CA
U.S.A.*

1. Introduction

Pediatric brain tumors are more complex disorders than other cancers due to gaps in our understanding of their cellular origins, insufficient drug therapy, and complications arising from the blood brain barrier. Nevertheless, in the last decade, there has been significant improvement in the treatment of these tumors although the survival rate is still low. Thus, new innovative approaches to treat these malignant tumors are warranted. Among pediatric brain tumors, medulloblastoma is the most common and contributes significantly to the high mortality rate among children throughout the world. Defects in Sonic Hedgehog (Shh) signal transduction are major contributors to brain tumor development, especially medulloblastoma. In this book chapter, we review recent publications that identified new Shh downstream target genes that are implicated in medulloblastoma development. The products of these genes represent novel therapeutic targets that might lead to innovative approaches to treat brain tumors.

2. Medulloblastoma biogenesis

Medulloblastoma is a member of the family of cranial primitive neuroectodermal tumors (PTEN) (Wechsler-Reya and Scott, 2001). Medulloblastoma cells are small, round, undifferentiated cells that are often located near the cerebellum. Their morphological properties and physical location are most consistent with cerebellar granule neuron precursor (GNPs), and thus, medulloblastomas are believed to originate from transformed GNPs that have failed to differentiate and continue to proliferate (Wechsler-Reya and Scott, 2001). For example, mutations in the Shh signaling pathway lead to persistent GNP proliferation and a failure to differentiate and ultimately medulloblastoma development (Wechsler-Reya and Scott, 2001). However, tumor biogenesis is a complex process that involves many genes and signaling pathways and recent studies indicate that subtypes of medulloblastoma have distinct molecular and cellular origins. For instance, mutations in the Wnt pathway lead to a discrete subtype of medulloblastoma that arise outside of the cerebellum (Gibson et al., 2010). Therefore, it is very difficult to treat tumors with one drug or pathway inhibitor. Furthermore, Shh signal transduction is important for a wide variety of cellular processes during normal development; therefore, drugs that interfere with Shh signal transduction might lead to neurodevelopmental defects. In addition, Shh downstream target genes are cross-regulated by multiple, independent pathways including Notch and

Wnt pathways. Thus, developing novel therapeutics to downstream target genes allows the ability to inhibit selectively multiple signal transduction pathways, to provide added efficacy in disease treatment, and to ameliorate possible deleterious side effects associated with inhibition of Shh signal transduction in normal development.

2.1 Sonic hedgehog signal transduction pathway

Binding of Shh to the Patched (Ptch1) receptor relieves the inhibition of another transmembrane receptor Smoothened (Smo) (Ingham and McMahon, 2001) (Figure 1). Upon Smo activation, members of the Gli family of transcription factors translocate to the nucleus and bind to the promoter of many target genes such as Ptch1, D Cyclins, and Nmyc (Browd et al., 2006; Yoon et al., 2002). In the cerebellum, Shh is produced by Purkinje neurons to promote proliferation of GNPs during the first week of postnatal development (Wechsler-Reya and Scott, 1999). Shh signalling regulates proliferation of GNPs in part via expression of Nmyc (Kenney et al., 2003; Knoepfler et al., 2002; Oliver et al., 2003). Mutations in the Shh signal transduction pathway are thought to underlie the etiology of medulloblastoma that arise from transformed GNPs that fail to cease proliferation during development (Wechsler-Reya and Scott, 2001). For example, mice with a heterozygous deletion of the Shh negative regulator Ptch1 develop tumors that resemble human medulloblastomas (Goodrich et al., 1997). The focus of this book chapter is the discovery of new Shh downstream target genes that are implicated in medulloblastoma development.

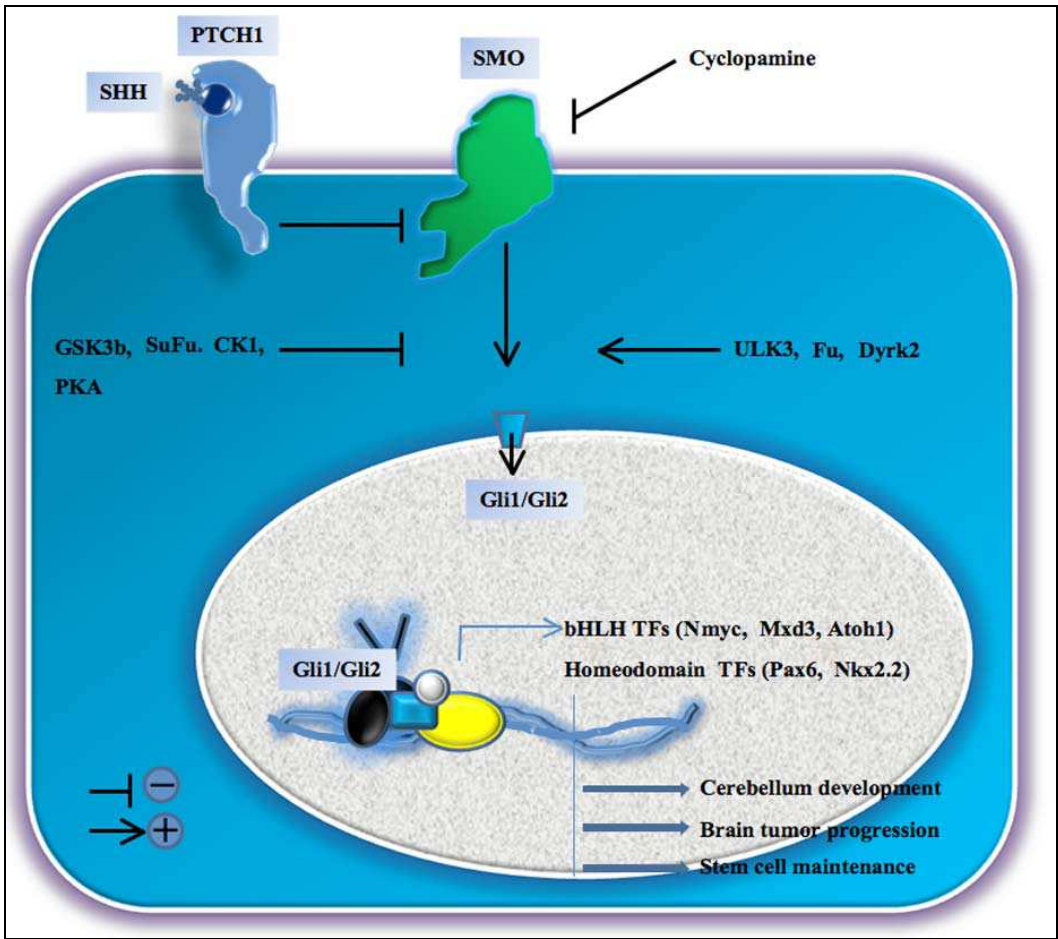


Fig. 1. The Sonic Hedgehog Signal Transduction Pathway

2.2 Cancer stem cell hypothesis

Current research suggests that a small subset of cells within the tumor mass with the capability of tumor regeneration persist even after aggressive therapies including surgery, radiation, and chemotherapy. These cells behave like stem cells and are hypothesized to be tumor stem cells (TSCs) with renewal capacity (Singh et al., 2004). Therefore, the current outlook supports the need for new approaches to target these TSCs for complete tumor eradication or at least to overcome tumor recurrence and increase the survival of the patient. A complete understanding of the biology of TSCs and the genes and pathways involved is vital towards the development of novel therapeutics to treat brain tumors. It has been reported that Oct-4, BMP (bone morphogenic protein), Janus family kinase, Notch, Shh and Wnt signaling regulate stem cell renewal (Taipale and Beachy, 2001). Among these pathways, Shh is thought to be the major contributor to brain tumor development, especially medulloblastoma, and Shh shows crosstalk with multiple stem cell renewal pathways including Wnt and Notch (Sengupta et al., 2007) (Figure 2), suggesting that mutations in the Shh pathway might lead to the development of TSCs and ultimately tumorigenesis. Indeed, a recent report has shown that a subset of medulloblastoma cells derived from *Ptch1* heterozygous mice are cancer stem cells, which are capable of initiating and propagating tumors (Ward et al., 2009). However, the exact mechanism of how TSCs are regulated by Shh signaling is not well explored. This book chapter will review mechanisms of newly identified Shh downstream target genes in stem cell biology to provide insight into the role in medulloblastoma biogenesis.

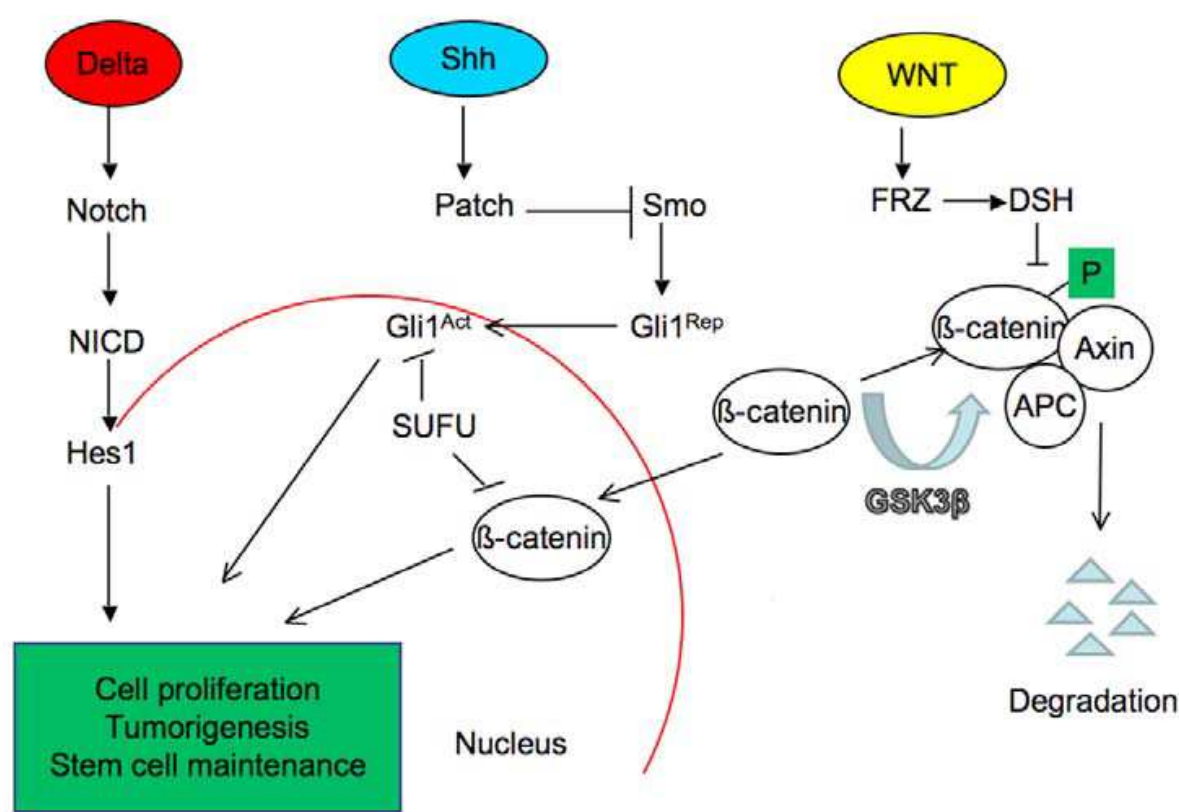


Fig. 2. Crosstalk between Sonic Hedgehog, Wnt, and Notch signal transduction pathways

3. Transcription factors implicated in Sonic Hedgehog Signal Transduction in Neural Tube

Pax6 and Nkx2.2 are homeobox transcription factors and their involvement within the Shh signaling pathway is well defined in the development of the neural tube (Briscoe et al., 2000); however, their role has only recently been explored in brain tumor development. Interestingly, both Pax6 and Nkx2.2 are targets of the major Shh downstream transcription factor Gli1 in medulloblastoma cell lines and tissues (Shahi et al., 2010). Moreover, both genes have been implicated in stem cell maintenance (Hu et al., 2009; Zhang et al., 2010); therefore, it would be fruitful to understand these genes in the context of Shh signaling in TSC-mediated medulloblastoma biogenesis.

3.1 Nkx2.2

Nkx2.2 (also known as NKX2B and NKX2-2) is a member of the homeobox transcription factor family NK-2 originally identified in *Drosophila* (Kim and Nirenberg, 1989). The Nk-2 homeodomain transcription factor family has four members: Nkx2.1, Nkx2.1, Nkx2.3 and Nkx2.5. These transcription factors show specific binding to the consensus sequence T(C/T)AAGTG (Chen and Schwartz, 1995; Tsao et al., 1994), a unique feature compared with other homeodomain proteins. The Nk-2 transcription factor family is defined by three domains which are conserved in all members: a) Homeodomain: 60 amino acid, helix-turn-helix motif, binds to DNA; b) Transcriptional repressor-domain (TN-domain): located at the N-terminal region of the protein; and c) Nk-2 specific domain (Nk2-SD): located at the C-terminal region of the protein (Lints et al., 1993). Nkx2.2 is a nuclear transcription factor and transport into the nucleus is mediated by nuclear localization signals (NLS) of which Nkx2.2 has two located within the homeodomain (Hessabi et al., 2000).

3.1.1 Role of Nkx2.2 in nervous system development

The role of Nkx2.2 during patterning of the ventral neural tube is well defined. Nkx2.2 is expressed in a gradient induced by Shh signaling to facilitate the subdivision of the ventral neural tube into five progenitor domains: p0, p1, p2, pMN (motor neuron) and p3 (Briscoe et al., 2000). It has been shown that the Gli transcriptional activator binds to the enhancer region of Nkx2.2 in differentiating neural progenitors (Vokes et al., 2007). Interestingly, another homeodomain transcription factor, Pax6 (discussed below), is modulated by Shh signaling to define ventral neural tube progenitor cell fate and it is repressed by Nkx2.2 at the boundary of pMN and p3 (Briscoe et al., 2000). After the establishment of ventral neural progenitor cell fate, expression of both homeodomain transcription factors Pax6 and Nkx2.2 is maintained to promote the transition of neural progenitors to specific types of postmitotic neurons: v0, v1, v2, MN and v3 (Briscoe et al., 2000). However, this stage of development is independent of the Shh target transcription factors Gli1 and Gli2, suggesting that later stages of Shh signaling act through other transcription factors (Ding et al., 1998).

3.1.2 Role of Nkx2.2 in brain cancer

Nkx2.2 transcription factor expression was surveyed in astrocytic and oligodendroglial tumors of low to high grade (Riemenschneider et al., 2004). In general, Nkx2.2 expression was high in the low grade tumors (class II and III - anaplastic astrocytoma) compared to high grade glioblastoma multiforme (class IV-GBM) (Riemenschneider et al., 2004). Nkx2.2 is also expressed in medulloblastoma cell lines and primary tumor samples and Nkx2.2

expression is dependent on Shh-Gli1 pathway (Shahi et al., 2010). Upon knockdown of Gli1 in medulloblastoma cell line with short interfering RNAs (siRNA), Nkx2.2 expression was reduced compared to control negative siRNA treated cells (Shahi et al., 2010), suggesting that Gli1 positively regulates Nkx2.2 expression. The expression pattern of Nkx2.2 in astrocytoma samples was found to be present in most of the astrocytoma cell lines while expression in tumor samples was low or absent except for some low grade (class III) astrocytoma tumor samples (Shahi et al., 2010). This high expression of Nkx2.2 in some of the low grade astrocytoma tumor samples is consistent with previous results of Riemenschneider and colleagues. In contrast, knockdown of Gli1 in the high grade astrocytoma cell line U87MG showed no change in expression pattern compared to control cells (Shahi et al., 2010), suggesting that other signaling pathways or transcription factors regulate expression of Nkx2.2 in astrocytoma. The expression of Nkx2.2 was low in the majority of neuroblastoma cell lines analyzed (72%) compared to normal control cells (Shahi et al., 2011).

3.1.3 Role of Nkx2.2 in stem cells

The origin and progression of brain tumors is a complex process that is not very well understood. However, recent advances in tumor biology research suggest that the tumor mass originates from a small number of atypical stem cells called TSCs. TSC renewal is regulated by many pathways including BMP, Notch, Shh and Wnt (Ponnusamy and Batra, 2008; Taipale and Beachy, 2001). A recent study indicated that high expression of Nkx2.2 inhibits the self-renewal characteristics of glioma TSCs that underlies the high grade tumor GBM and switches cell fate towards oligodendroglial differentiation (Muraguchi et al., 2011). This study is also supported by previous work in which human embryonic stem cells (hESCs) were induced by Shh signaling to express Nkx2.2 to form pre-oligodendrocyte precursor cells (Hu et al., 2009). These results support a negative role of Nkx2.2 in TSC proliferation and suggest that promoting the inhibitory role of Nkx2.2 is a possible therapeutic target strategy to cause TSCs to switch their fate from a proliferative nature to a differentiated state.

Interestingly, Nkx2.2 is positively regulated by the graded expression of Shh in neural tube development (Briscoe et al., 2000). However, its expression pattern in medulloblastoma, astrocytoma and neuroblastoma cell lines and primary tumor samples indicates that it may be down-regulated at least partially by Shh signaling, although other signaling pathways and transcription factors might also play a role (Shahi et al., 2010; Shahi et al., 2011). Regardless, its high expression in low grade astrocytic tumors compared to high grade tumors suggest that it would be a good marker for the prognosis of patients to define their astrocytic tumor grade (Riemenschneider et al., 2004).

3.2 Pax6

Pax6 (paired box gene 6) is a homeodomain transcription factor of which there are 9 members in this family (Pax1-9). Pax6 is highly conserved in vertebrates and is the most studied among the Pax family members for its vital role in neuronal fate determination (Mansouri et al., 1996). The vertebrate Pax6 gene encodes three isoforms: canonical Pax6, Pax6(5a) and Pax6(Δ PD). The canonical Pax6 has a paired domain (PD) at the N-terminus connected via a linker region to the paired type homeodomain (HD). The C-terminus contains a proline/serine/threonine (P/S/T) domain. Both PD and HD domains bind to

DNA whereas the P/S/T domain has transactivation activity. Isoform Pax6 (5a) is formed with the insertion of 14 residues in the PD, which affects its DNA binding activity. The linker region encodes three alternative translation start codons to generate Pax6 isoforms known as Pax6 (Δ PD) or pairless isoforms that lack a PD.

3.2.1 Role of Pax6 in nervous system development

Pax6 plays a crucial role for the establishment of ventral progenitors and specification of the ventral interneuron and motor neuron cell fates in response to graded Shh signaling in the ventral spinal cord and hindbrain. There is a low-to-high gradient of Pax6 expression from ventral to dorsal in the ventral neural tube in response to graded Shh signaling (Ericson et al., 1997). Interestingly, when Pax6 expression is suppressed during Shh signaling, expression of other transcription factors appears in the neural tube for specification of ventral neurons. After the closure of the neural tube, progenitors are destined to become forebrain, midbrain or hindbrain and the demarcation boundary is determined by the graded expression pattern of Pax6 (Mastick et al., 1997).

3.2.2 Role of Pax6 in brain cancer

Pax6 contributes significantly to neurogenesis and it is expressed throughout the ventricular zone and in the external granular layer of the developing cerebellum. High expression of Pax6 in transformed rat fibroblasts suggests that it might function as a proto-oncogene (Maulbecker and Gruss, 1993). Furthermore, Pax6 showed high expression in 78% of medulloblastoma samples (Kozmik et al., 1995). In glioma tumor samples, expression of Pax6 was high in low grade tumors (class III-anaplastic astrocytoma) and low in high grade class IV-GBM (Zhou et al., 2003). Zhou and colleagues suggest that high expression of Pax6 is favorable for patient survival compared to low expression, indicating that Pax6 would be a good prognostic marker for the transition of low grade to high grade glioma. In a recent study, Shahi and colleagues showed that Pax6 expression is regulated by Shh-Gli1 signaling in medulloblastoma and astrocytoma cell lines and primary tumor samples (Shahi et al., 2010). Knockdown of Gli1 in medulloblastoma cells led to decreased Pax6 expression compared to control negative siRNA (Shahi et al., 2010), suggesting that Gli1 upregulates Pax6 expression in medulloblastoma. Interestingly, medulloblastoma cell lines and primary tumor samples showed high expression of Pax6 compared to normal control samples (Shahi et al., 2010). Similarly, expression of Pax6 in siRNA-mediated Gli1 knockdown in astrocytoma cells led to increased Pax6 expression compared to control negative siRNA (Shahi et al., 2010), suggesting that Gli1 might also downregulate Pax6 expression in astrocytoma. Moreover, most astrocytoma and primary tumor samples showed low expression of Pax6 compared to normal controls (Shahi et al., 2010), supporting this possibility. Intriguingly, this differential regulation of Pax6 expression in medulloblastoma and astrocytoma cells suggests that Shh signaling elicits distinct outcomes for the growth of these tumors (Shahi et al., 2010). In neuroblastoma cell lines, Pax6 expression was low compared to normal control samples (Shahi et al., 2011). Moreover, another independent study revealed that Pax6 behaves like a tumor suppressor gene to inhibit the invasiveness of glioma tumors (Mayes et al., 2006). Pax6 was found to contain a hemizygous deletion in its 5'-region in subependymoma tumor (Maekawa et al., 2010), suggesting that Pax6 may switch between an oncogene and tumor suppressor and that this switching activity is variable among tumors. In favor of this hypothesis, another transcription factor, Atoh1 (discussed below), behaves as an oncogene in medulloblastoma and a tumor suppressor gene in colorectal cancer and Merkel cell carcinoma (Bossuyt et al.,

2009; Flora et al., 2009). Atoh1 is regulated by different signaling pathways in these tumors and its differential regulation is thought to underlie its oncogenic or tumor suppressive activity (Bossuyt et al., 2009; Flora et al., 2009). Therefore, Pax6 might only be a good therapeutic target in tumor types for which it behaves as an oncogene such as medulloblastoma.

3.2.2 Role of Pax6 in stem cells

Treatment of embryonic stem cells with the soluble signaling molecules BMP4 and Wnt3a induced the generation of cerebellar granule and Purkinje cell fates (Su et al., 2006). Pax6 expression was correlated with these differentiated cells (Su et al., 2006), suggesting that Pax6 functions to promote cerebellar cell fate and thus might play a role in medulloblastoma biogenesis. Pax6 also plays an indispensable role in the proliferation and expansion of the adult mammalian retinal stem cells (Xu et al., 2007). Pax6 is also important for the specification of neuroectoderm (NE) from human embryonic stem cells (hESCs) (Zhang et al., 2010). Moreover, overexpression of the isoforms Pax6 and Pax6 (5a) but not Pax6 (Δ PD) initiate differentiation of hESCs, although, Pax6 appears to be the main factor to determine the NE specification (Zhang et al., 2010). Interestingly, during the specification of NE from hESCs, Pax6 inhibits the expression of pluripotent genes; however, the mechanism of this inhibition is not known (Zhang et al., 2010). It would be intriguing to explore the role of specific Pax6 isoforms in brain tumorigenesis and the contribution of these isoforms to the stem cell origin of brain tumors.

4. Transcription factors involved in sonic hedgehog signal transduction in cerebellum

In the cerebellum, Shh is produced by Purkinje neurons to promote proliferation of GNPs during the first week of postnatal development (Wechsler-Reya and Scott, 1999). Shh signalling regulates proliferation of GNPs in part via expression of the bHLH (basic helix-loop-helix) transcription factor Nmyc (Kenney et al., 2003; Knoepfler et al., 2002; Oliver et al., 2003). Mutations in the Shh pathway are thought to underlie the etiology of medulloblastoma that arise from transformed GNPs that fail to cease proliferation during development (Wechsler-Reya and Scott, 2001). Interestingly, recent studies have implicated two additional transcription factors, Atoh1 (formerly known as Math1) and Mxd3 (formerly known as Mad3) in the Shh signalling pathway that regulate GNP proliferation and medulloblastoma biogenesis.

4.1 Atoh1

Atonal (Atoh1) was originally identified in *Drosophila* and is highly conserved (other known names; Hath1, Math1 and bHLHa14). Atoh1 belongs to the family of bHLH transcription factors. The protein consists of a basic domain that recognizes specific E-box sequences (5'CANNTG-3') while the two helices interact with other bHLH protein to form heterodimers (Krizhanovsky et al., 2006). Atoh1 plays a key role in specification of cerebellar cell fates (Ben-Arie et al., 1996). In the past few years, evidence has been mounting for a role of this gene in cerebellar brain tumor development.

4.1.1 Role of Atoh1 in nervous system development

The role of Atoh1 in cerebellum is well defined and its regulated expression is important for granule cell development (Ben-Arie et al., 1997). Mice lacking Atoh1 fail to form granule

cells and are born with a cerebellum that is devoid of an external germinal layer (Ben-Arie et al., 1997). Overexpression of Atoh1 interferes with differentiation and causes irregularity in the maturation of granule cells (Helms et al., 2001). Interestingly, expression of Atoh1 and Neurogenin (another bHLH transcription factor) sub-divides the lower rhombic lip into discrete presumptive cell fates and this subdivision is dependent on Pax6 expression (Landsberg et al., 2005). The phenotype of Atoh1 overexpressing transgenic mice indicates that Atoh1 downregulates expression of Pax6 (Helms et al., 2001). Moreover, misexpression of Atoh1 in neural precursors is lethal for the proper development of central nervous system (Isaka et al., 1999).

4.1.2 Role of Atoh1 in brain tumors

Atoh1 plays a critical role in the coordination of proliferation and differentiation of GNPs (Ben-Arie et al., 1997). Therefore, this transcription factor might also be involved in medulloblastoma tumorigenesis. In support of this possibility, double mutant mice of poly [ADP-ribose] polymerase 1 (PARP-1)^{-/-}/p53^{-/-} mice developed medulloblastoma with high expression of Atoh1 (Tong et al., 2003), suggesting a role as an oncogene. Interestingly, the expression pattern of Atoh1 is distinct in adult and childhood medulloblastoma. In adult medulloblastoma, Atoh1 is expressed at high levels compared to childhood medulloblastoma (Salsano et al., 2004). The origin of these two categories of medulloblastomas is controversial; however, its expression provides further support for the role of Atoh1 in medulloblastoma development. Atoh1 is regulated by Shh signaling in GNPs (Kenney and Rowitch, 2000) and its expression is downregulated upon Shh signaling inhibition (Romer et al., 2004), suggesting that Atoh1 might function in medulloblastoma development. Indeed, deletion of Atoh1 disrupts Shh signaling in the developing cerebellum and prevents medulloblastoma (Flora et al., 2009), supporting an oncogenic role. Interestingly, Atoh1 appears to act directly on the Gli2 transcription factor in the Shh signaling pathway to regulate medulloblastoma biogenesis (Flora et al., 2009). It is well known that Atoh1 binds to E-box elements within target genes and accordingly Atoh1 binds to E-box sequences located in the second intron of Gli2 (Flora et al., 2009). This finding represents a new paradigm of the role of Shh signaling in cerebellum and medulloblastoma because thus far, Gli1 has been thought to be the major transcription factor of the Shh pathway. Gli2 was considered a secondary transcription factor that acts as an activator only in the absence of Gli1 (Bai and Joyner, 2001). The regulation of Gli2 by Atoh1 suggests the possibility that either Atoh1 acts on Gli2 in a Gli1-independent manner or that Gli1 regulates the expression of Atoh1 and subsequently Atoh1 acts on Gli2 to promote tumorigenesis.

Ayrault and colleagues demonstrated that co-expression of Atoh1 and Gli1 in primary GNPs cells from postnatal cerebella of healthy C57BL/6 mice transformed into TSCs (Ayrault et al., 2010). Less than 200 TSCs were capable to induce medulloblastoma in the brain of transplanted naïve mice (Ayrault et al., 2010), suggesting that both Atoh1 and Gli1 cooperate to transform GNPs into TSCs. However, Ayrault and colleagues were unable to see the induction of Gli2 expression in response to Atoh1 induced expression in primary GNPs in contrast to Flora and colleagues. The authors suggest that this discrepancy is due to different genetic models for their experiments and warrants further research into the interaction of Atoh1 with Shh mediated transcription factors Gli1 and Gli2 in the development of medulloblastoma.

Although it has been suggested that Atoh1 acts as an oncogene and a possible target of Shh signaling to promote medulloblastoma development, it is important to note that other

studies suggest that Atoh1 functions as a tumor suppressor gene in other cell types. For example, Atoh1 regulates proliferation and apoptosis in colorectal cancer and Merkel cell carcinoma with the induction of Jun N-terminal kinase (Ntrk1) signaling pathway (Bossuyt et al., 2009).

4.1.3 Role of Atoh1 in stem cells

Many markers have been determined for the identification of TSCs including CD133, Nestin and BMI-1. Cells expressing these selective markers have the capability to generate tumors when xenotransplanted into nude mice. Among these markers, CD133⁺ expressing cells are considered potent medulloblastoma propagating cells. However, a recent study showed that Atoh1⁺ and CD15⁺/SSEA-1 expressing cells have a higher-fold capability of generating medulloblastoma in Ptch1 mutant mice compared to Atoh1⁻/CD15⁻ cells (Read et al., 2009). Intriguingly, CD133⁺ cells were unable to generate medulloblastoma in Ptch1 mutant mice (Read et al., 2009), suggesting that cells expressing high Atoh1⁺ and CD15⁺ are specified to promote proliferation and to inhibit apoptosis and differentiation. Thus, it will be intriguing to understand how Atoh1 regulates TSCs that give rise to medulloblastoma.

4.2 Mxd3

Mxd3 (previously known as Mad3) is a member of the bHLH transcriptional regulators (Hurlin et al., 1995) to which Nmyc also belongs. In the classical model, Myc and Mad proteins form heterodimers with the cofactor Max and bind to E-box sequences to activate or repress, respectively, transcription (Grandori et al., 2000). Thus, it has long been thought that Mad/Max complexes function to antagonize Myc/Max complexes by competitive binding to DNA and the promotion to a Mad-dependent differentiation cell state from a Myc-dependent proliferative state. Myc is an established oncogene; thus, Mad proteins were originally hypothesized to function as tumor suppressor genes. However, recent studies for Mxd3 challenge the current model.

4.2.1 Role of Mxd3 in nervous system development

Mxd3 is transiently upregulated in GNPs during postnatal cerebellum development and it fails to be downregulated in *weaver* mice in which GNPs fail to exit the cell cycle (Diaz et al., 2002). In cultured GNPs, Mxd3 is essential for Shh-dependent GNP proliferation and Mxd3 is upregulated in response to Shh signaling (Yun et al., 2007). Mxd3 regulates GNP proliferation in part via expression of Nmyc (Yun et al., 2007). Intriguingly, Mxd3 is predicted to interact directly with Nmyc to promote GNP proliferation (Barisone et al., 2008), suggesting that Mxd3 and Nmyc function as part of a feedback loop to regulate GNP proliferation. Similar to other Mad family proteins, mice with a targeted deletion of Mxd3 are viable; however, Mxd3 null mice exhibit increased sensitivity to radiation-induced apoptosis (Queva et al., 2001). The effect of Mxd3 deletion in postnatal cerebellum development was not explored in this mouse line. Interestingly, Mxd3 expression in immature B cells has been shown to induce cell proliferation without differentiation, a phenotype of leukemia (Gore et al., 2010), suggesting that Mxd3 might also regulate proliferation of other types of cancer cells.

4.2.2 Role of Mxd3 in brain cancer

Like Nmyc, Mxd3 is upregulated in tumors and pre-tumor cells from Ptch1 heterozygous mice (Yun et al., 2007) and Mxd3 is expressed in human brain tumors including

glioblastoma and medulloblastoma (Barisone et al., 2008). Together, these studies suggest that Mxd3 might also function with Nmyc to regulate tumor biogenesis. Furthermore research is required to address this intriguing possibility.

4.2.3 Role of Mxd3 in stem cells

Mxd3 has not been studied extensively and there are no published reports regarding a role for Mxd3 in regulation of stem cells. However, given the fact that Mxd3 and Nmyc function together to regulate GNP proliferation and are predicted to physically associate with each other, it is likely that Mxd3 functions with Nmyc to regulate stem cell proliferation. Additional experiments are necessary to test this interesting possibility.

Location	Function	Pathway	References
Ventral progenitors of the spinal cord	Transcription factor	Sonic hedgehog Pax6	Ericson et al., 1997; Briscoe et al., 2000
Astrocytic and oligodendroglial tumors	Tumor suppressor	Sonic hedgehog	Riemenschneider et al., 2004; Shahi et al., 2010
Medulloblastoma	Oncogene	Sonic hedgehog	Shahi et al., 2010
Neuroblastoma	Tumor suppressor	Sonic hedgehog	Shahi et al., 2010
Human embryonic stem cells	Transcription factor	Sonic hedgehog	Hu et al., 2009
Oligodendroglial cells	Transcription factor	Sonic hedgehog	Danesin et al., 2006; Muraguchi et al., 2010

Table 1. Summary of Nkx2.2 function in neural progenitor cells and tumors.

Location	Function	Pathway	References
Ventral progenitors of the spinal cord	Transcription factor	Sonic hedgehog Nkx2.2	Ericson et al., 1997
Medulloblastoma	Oncogene	Sonic hedgehog	Kozmik et al., 1995; Shahi et al., 2010
Glioma	Tumor suppressor	Sonic hedgehog	Zhou et al., 2003; Mayes et al., 2006; Shahi et al., 2010
Neuroblastoma	Tumor suppressor	Sonic hedgehog	Shahi et al., 2011
Subependymoma tumors	Tumor suppressor	PTEN, SOX	Maekawa et al., 2010
Embryonic stem cells	Transcription factor	BMP4, Wnt3a, Atoh1	Sue et al., 2006; Zhang et al., 2010
Retinal stem cells	Transcription factor	Sonic hedgehog, Sufu	Xu et al., 2007; Cwinn et al., 2011

Table 2. Summary of Pax6 function in neural progenitor cells and tumors

Location	Function	Pathway	References
Cerebellum	Transcription factor	Sonic hedgehog Pax6	Ben-Arie et al., 1997; Kenny and Rowitch 2000; Helms et al., 2006; Flora et al., 2009
Medulloblastoma	Oncogene	Sonic hedgehog Gli2	Tong et al., 2003; Salsano et al., 2004; Briggs et al., 2008; Flora et al., 2009
Tumor stem cells	Transcription factor	Sonic hedgehog	Ayrault et al., 2010; Read et al., 2009
Neural stem cells	Transcription factor	Sonic hedgehog	Isaka et al., 1999; Hu et al., 2010
Colorectal cancer and Merkel cell carcinoma	Tumor suppressor	Jun N-terminal kinase	Bossuyt et al., 2009

Table 3. Summary of Atoh1 function in neural progenitor cells and tumors

Location	Function	Pathway	References
Neural progenitor cells	Transcription factor	Unknown	Queva et al., 2001
Cerebellar granule cell precursors	Transcription factor	Sonic hedgehog	Yun et al., 2007
Medulloblastoma	Oncogene	Sonic hedgehog	Yun et al., 2007; Barisone et al., 2008
Immature B cells	Transcription factor	Id2	Gore et al., 2010

Table 4. Summary of Mxd3 function in neural progenitor cells and tumors

5. Conclusion

While many questions still remain, our understanding of the pediatric brain tumor medulloblastoma has become clearer in the past few years. Mounting evidence indicates that TSCs are the major contributor to propagate medulloblastoma and that there are many pathways that contribute to TSC maintenance. However, the precise mechanism by which these TSCs are regulated by these signaling pathways to promote tumorigenesis is not well understood. Therefore, additional studies are warranted to understand these pathways. However, the identification of new transcription factors that contribute to TSC proliferation and tumor biogenesis is a major advance in our understanding of this disease. These new transcription factors represent exciting new therapeutic targets for treatment since it is likely that a multitude of downstream molecules can be targeted with a single drug.

6. Acknowledgment

We would like to acknowledge members of the Diaz lab for discussion about the ideas discussed in this chapter. Research in the Diaz lab is funded by grants to E.D. from the National Science Foundation and the NIH Director’s New Innovator Award Program.

7. References

- Ayrault, O., Zhao, H., Zindy, F., Qu, C., Sherr, C.J., and Roussel, M.F. (2010). Atoh1 inhibits neuronal differentiation and collaborates with Gli1 to generate medulloblastoma-initiating cells. *Cancer Res* 70, 5618-5627.
- Bai, C.B., and Joyner, A.L. (2001). Gli1 can rescue the in vivo function of Gli2. *Development* 128, 5161-5172.
- Barisone, G.A., Yun, J.S., and Diaz, E. (2008). From cerebellar proliferation to tumorigenesis: new insights into the role of Mad3. *Cell Cycle* 7, 423-427.
- Ben-Arie, N., Bellen, H.J., Armstrong, D.L., McCall, A.E., Gordadze, P.R., Guo, Q., Matzuk, M.M., and Zoghbi, H.Y. (1997). Math1 is essential for genesis of cerebellar granule neurons. *Nature* 390, 169-172.
- Ben-Arie, N., McCall, A.E., Berkman, S., Eichele, G., Bellen, H.J., and Zoghbi, H.Y. (1996). Evolutionary conservation of sequence and expression of the bHLH protein Atonal suggests a conserved role in neurogenesis. *Hum Mol Genet* 5, 1207-1216.
- Bossuyt, W., Kazanjian, A., De Geest, N., Van Kelst, S., De Hertogh, G., Geboes, K., Boivin, G.P., Luciani, J., Fuks, F., Chuah, M., *et al.* (2009). Atonal homolog 1 is a tumor suppressor gene. *PLoS Biol* 7, e39.
- Briscoe, J., Pierani, A., Jessell, T.M., and Ericson, J. (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101, 435-445.
- Browd, S.R., Kenney, A.M., Gottfried, O.N., Yoon, J.W., Walterhouse, D., Pedone, C.A., and Fuhs, D.W. (2006). N-myc can substitute for insulin-like growth factor signaling in a mouse model of sonic hedgehog-induced medulloblastoma. *Cancer Res* 66, 2666-2672.
- Chen, C.Y., and Schwartz, R.J. (1995). Identification of novel DNA binding targets and regulatory domains of a murine tinman homeodomain factor, nkx-2.5. *J Biol Chem* 270, 15628-15633.
- Diaz, E., Ge, Y., Yang, Y.H., Loh, K.C., Serafini, T.A., Okazaki, Y., Hayashizaki, Y., Speed, T.P., Ngai, J., and Scheiffele, P. (2002). Molecular analysis of gene expression in the developing pontocerebellar projection system. *Neuron* 36, 417-434.
- Ding, Q., Motoyama, J., Gasca, S., Mo, R., Sasaki, H., Rossant, J., and Hui, C.C. (1998). Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. *Development* 125, 2533-2543.
- Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T.M., and Briscoe, J. (1997). Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell* 90, 169-180.
- Flora, A., Klisch, T.J., Schuster, G., and Zoghbi, H.Y. (2009). Deletion of Atoh1 disrupts Sonic Hedgehog signaling in the developing cerebellum and prevents medulloblastoma. *Science* 326, 1424-1427.
- Gibson, P., Tong, Y., Robinson, G., Thompson, M.C., Curre, D.S., Eden, C., Kranenburg, T.A., Hogg, T., Poppleton, H., Martin, J., *et al.* (2010). Subtypes of medulloblastoma have distinct developmental origins. *Nature* 468, 1095-1099.
- Goodrich, L.V., Milenkovic, L., Higgins, K.M., and Scott, M.P. (1997). Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* 277, 1109-1113.

- Gore, Y., Lantner, F., Hart, G., and Shachar, I. (2010). Mad3 negatively regulates B cell differentiation in the spleen by inducing Id2 expression. *Mol Biol Cell* 21, 1864-1871.
- Grandori, C., Cowley, S.M., James, L.P., and Eisenman, R.N. (2000). The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu Rev Cell Dev Biol* 16, 653-699.
- Helms, A.W., Gowan, K., Abney, A., Savage, T., and Johnson, J.E. (2001). Overexpression of MATH1 disrupts the coordination of neural differentiation in cerebellum development. *Mol Cell Neurosci* 17, 671-682.
- Hessabi, B., Schmidt, I., and Walther, R. (2000). The homeodomain of Nkx2.2 carries two cooperatively acting nuclear localization signals. *Biochem Biophys Res Commun* 270, 695-700.
- Hu, B.Y., Du, Z.W., Li, X.J., Ayala, M., and Zhang, S.C. (2009). Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects. *Development* 136, 1443-1452.
- Hurlin, P.J., Queva, C., Koskinen, P.J., Steingrimsson, E., Ayer, D.E., Copeland, N.G., Jenkins, N.A., and Eisenman, R.N. (1995). Mad3 and Mad4: novel Max-interacting transcriptional repressors that suppress c-myc dependent transformation and are expressed during neural and epidermal differentiation. *EMBO J* 14, 5646-5659.
- Ingham, P.W., and McMahon, A.P. (2001). Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 15, 3059-3087.
- Isaka, F., Ishibashi, M., Taki, W., Hashimoto, N., Nakanishi, S., and Kageyama, R. (1999). Ectopic expression of the bHLH gene Math1 disturbs neural development. *Eur J Neurosci* 11, 2582-2588.
- Kenney, A.M., Cole, M.D., and Rowitch, D.H. (2003). Nmyc upregulation by sonic hedgehog signaling promotes proliferation in developing cerebellar granule neuron precursors. *Development* 130, 15-28.
- Kenney, A.M., and Rowitch, D.H. (2000). Sonic hedgehog promotes G(1) cyclin expression and sustained cell cycle progression in mammalian neuronal precursors. *Mol Cell Biol* 20, 9055-9067.
- Kim, Y., and Nirenberg, M. (1989). Drosophila NK-homeobox genes. *Proc Natl Acad Sci U S A* 86, 7716-7720.
- Knoepfler, P.S., Cheng, P.F., and Eisenman, R.N. (2002). N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. *Genes Dev* 16, 2699-2712.
- Kozmik, Z., Sure, U., Ruedi, D., Busslinger, M., and Aguzzi, A. (1995). Deregulated expression of PAX5 in medulloblastoma. *Proc Natl Acad Sci U S A* 92, 5709-5713.
- Krizhanovsky, V., Soreq, L., Kliminski, V., and Ben-Arie, N. (2006). Math1 target genes are enriched with evolutionarily conserved clustered E-box binding sites. *J Mol Neurosci* 28, 211-229.
- Landsberg, R.L., Awatramani, R.B., Hunter, N.L., Farago, A.F., DiPietrantonio, H.J., Rodriguez, C.I., and Dymecki, S.M. (2005). Hindbrain rhombic lip is comprised of discrete progenitor cell populations allocated by Pax6. *Neuron* 48, 933-947.

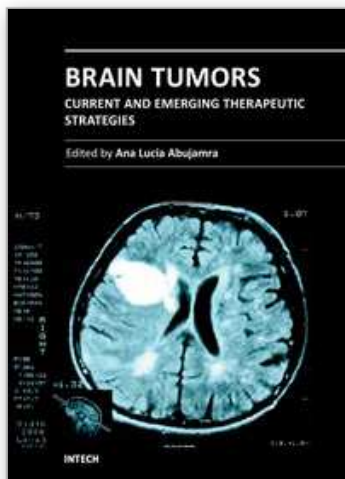
- Lints, T.J., Parsons, L.M., Hartley, L., Lyons, I., and Harvey, R.P. (1993). Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. *Development* 119, 969.
- Maekawa, M., Fujisawa, H., Iwayama, Y., Tamase, A., Toyota, T., Osumi, N., and Yoshikawa, T. (2010). Giant subependymoma developed in a patient with aniridia: analyses of PAX6 and tumor-relevant genes. *Brain Pathol* 20, 1033-1041.
- Mansouri, A., Hallonet, M., and Gruss, P. (1996). Pax genes and their roles in cell differentiation and development. *Curr Opin Cell Biol* 8, 851-857.
- Mastick, G.S., Davis, N.M., Andrew, G.L., and Easter, S.S., Jr. (1997). Pax-6 functions in boundary formation and axon guidance in the embryonic mouse forebrain. *Development* 124, 1985-1997.
- Maulbecker, C.C., and Gruss, P. (1993). The oncogenic potential of Pax genes. *EMBO J* 12, 2361-2367.
- Mayes, D.A., Hu, Y., Teng, Y., Siegel, E., Wu, X., Panda, K., Tan, F., Yung, W.K., and Zhou, Y.H. (2006). PAX6 suppresses the invasiveness of glioblastoma cells and the expression of the matrix metalloproteinase-2 gene. *Cancer Res* 66, 9809-9817.
- Muraguchi, T., Tanaka, S., Yamada, D., Tamase, A., Nakada, M., Nakamura, H., Hoshii, T., Ooshio, T., Tadokoro, Y., Naka, K., *et al.* (2011). NKX2.2 suppresses self-renewal of glioma-initiating cells. *Cancer Res* 71, 1135-1145.
- Oliver, T.G., Grasfeder, L.L., Carroll, A.L., Kaiser, C., Gillingham, C.L., Lin, S.M., Wickramasinghe, R., Scott, M.P., and Wechsler-Reya, R.J. (2003). Transcriptional profiling of the Sonic hedgehog response: a critical role for N-myc in proliferation of neuronal precursors. *Proc Natl Acad Sci U S A* 100, 7331-7336.
- Ponnusamy, M.P., and Batra, S.K. (2008). Ovarian cancer: emerging concept on cancer stem cells. *J Ovarian Res* 1, 4.
- Queva, C., McArthur, G.A., Iritani, B.M., and Eisenman, R.N. (2001). Targeted deletion of the S-phase-specific Myc antagonist Mad3 sensitizes neuronal and lymphoid cells to radiation-induced apoptosis. *Mol Cell Biol* 21, 703-712.
- Read, T.A., Fogarty, M.P., Markant, S.L., McLendon, R.E., Wei, Z., Ellison, D.W., Febbo, P.G., and Wechsler-Reya, R.J. (2009). Identification of CD15 as a marker for tumor-propagating cells in a mouse model of medulloblastoma. *Cancer Cell* 15, 135-147.
- Riemenschneider, M.J., Koy, T.H., and Reifenberger, G. (2004). Expression of oligodendrocyte lineage genes in oligodendroglial and astrocytic gliomas. *Acta Neuropathol* 107, 277-282.
- Romer, J.T., Kimura, H., Magdaleno, S., Sasai, K., Fuller, C., Baines, H., Connelly, M., Stewart, C.F., Gould, S., Rubin, L.L., *et al.* (2004). Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/-)p53(-/-) mice. *Cancer Cell* 6, 229-240.
- Salsano, E., Pollo, B., Eoli, M., Giordana, M.T., and Finocchiaro, G. (2004). Expression of MATH1, a marker of cerebellar granule cell progenitors, identifies different medulloblastoma sub-types. *Neurosci Lett* 370, 180-185.
- Sengupta, A., Banerjee, D., Chandra, S., Banerji, S.K., Ghosh, R., Roy, R., and Banerjee, S. (2007). Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. *Leukemia* 21, 949-955.

- Shahi, M.H., Afzal, M., Sinha, S., Eberhart, C.G., Rey, J.A., Fan, X., and Castresana, J.S. (2010). Regulation of sonic hedgehog-GLI1 downstream target genes PTCH1, Cyclin D2, Plakoglobin, PAX6 and NKX2.2 and their epigenetic status in medulloblastoma and astrocytoma. *BMC Cancer* 10, 614.
- Shahi, M.H., Schiapparelli, P., Afzal, M., Sinha, S., Rey, J.A., and Castresana, J.S. (2011). Expression and epigenetic modulation of sonic hedgehog-GLI1 pathway genes in neuroblastoma cell lines and tumors. *Tumour Biol* 32, 113-127.
- Singh, S.K., Hawkins, C., Clarke, I.D., Squire, J.A., Bayani, J., Hide, T., Henkelman, R.M., Cusimano, M.D., and Dirks, P.B. (2004). Identification of human brain tumour initiating cells. *Nature* 432, 396-401.
- Su, H.L., Muguruma, K., Matsuo-Takasaki, M., Kengaku, M., Watanabe, K., and Sasai, Y. (2006). Generation of cerebellar neuron precursors from embryonic stem cells. *Dev Biol* 290, 287-296.
- Taipale, J., and Beachy, P.A. (2001). The Hedgehog and Wnt signalling pathways in cancer. *Nature* 411, 349-354.
- Tong, W.M., Ohgaki, H., Huang, H., Granier, C., Kleihues, P., and Wang, Z.Q. (2003). Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53(-/-) mice. *Am J Pathol* 162, 343-352.
- Tsao, D.H., Gruschus, J.M., Wang, L.H., Nirenberg, M., and Ferretti, J.A. (1994). Elongation of helix III of the NK-2 homeodomain upon binding to DNA: a secondary structure study by NMR. *Biochemistry* 33, 15053-15060.
- Vokes, S.A., Ji, H., McCuine, S., Tenzen, T., Giles, S., Zhong, S., Longabaugh, W.J., Davidson, E.H., Wong, W.H., and McMahon, A.P. (2007). Genomic characterization of Gli-activator targets in sonic hedgehog-mediated neural patterning. *Development* 134, 1977-1989.
- Ward, R.J., Lee, L., Graham, K., Satkunendran, T., Yoshikawa, K., Ling, E., Harper, L., Austin, R., Nieuwenhuis, E., Clarke, I.D., *et al.* (2009). Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma. *Cancer Res* 69, 4682-4690.
- Wechsler-Reya, R., and Scott, M.P. (2001). The developmental biology of brain tumors. *Annu Rev Neurosci* 24, 385-428.
- Wechsler-Reya, R.J., and Scott, M.P. (1999). Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron* 22, 103-114.
- Xu, S., Sunderland, M.E., Coles, B.L., Kam, A., Holowacz, T., Ashery-Padan, R., Marquardt, T., McInnes, R.R., and van der Kooy, D. (2007). The proliferation and expansion of retinal stem cells require functional Pax6. *Dev Biol* 304, 713-721.
- Yoon, J.W., Kita, Y., Frank, D.J., Majewski, R.R., Konicek, B.A., Nobrega, M.A., Jacob, H., Walterhouse, D., and Iannaccone, P. (2002). Gene expression profiling leads to identification of GLI1-binding elements in target genes and a role for multiple downstream pathways in GLI1-induced cell transformation. *J Biol Chem* 277, 5548-5555.
- Yun, J.S., Rust, J.M., Ishimaru, T., and Diaz, E. (2007). A novel role of the Mad family member Mad3 in cerebellar granule neuron precursor proliferation. *Mol Cell Biol* 27, 8178-8189.

- Zhang, X., Huang, C.T., Chen, J., Pankratz, M.T., Xi, J., Li, J., Yang, Y., Lavaute, T.M., Li, X.J., Ayala, M., *et al.* (2010). Pax6 is a human neuroectoderm cell fate determinant. *Cell Stem Cell* 7, 90-100.
- Zhou, Y.H., Tan, F., Hess, K.R., and Yung, W.K. (2003). The expression of PAX6, PTEN, vascular endothelial growth factor, and epidermal growth factor receptor in gliomas: relationship to tumor grade and survival. *Clin Cancer Res* 9, 3369-3375.

IntechOpen

IntechOpen



Brain Tumors - Current and Emerging Therapeutic Strategies

Edited by Dr. Ana Lucia Abujamra

ISBN 978-953-307-588-4

Hard cover, 422 pages

Publisher InTech

Published online 23, August, 2011

Published in print edition August, 2011

Brain Tumors: Current and Emerging Therapeutic Strategies focuses on tumor models, the molecular mechanisms involved in the pathogenesis of this disease, and on the new diagnostic and treatment strategies utilized to stage and treat this malignancy. A special section on immunotherapy and gene therapy provides the most up-to-date information on the pre-clinical and clinical advances of this therapeutic venue. Each chapter in Brain Tumors: Current and Emerging Therapeutic Strategies is authored by international experts with extensive experience in the areas covered.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mehdi H. Shahi and Elva Díaz (2011). Transcription Factor Targets as Treatment for Medulloblastoma, Brain Tumors - Current and Emerging Therapeutic Strategies, Dr. Ana Lucia Abujamra (Ed.), ISBN: 978-953-307-588-4, InTech, Available from: <http://www.intechopen.com/books/brain-tumors-current-and-emerging-therapeutic-strategies/transcription-factor-targets-as-treatment-for-medulloblastoma>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen