p53 and Medulloblastoma

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Our understanding of medulloblastoma biology has increased dramatically over the past decade, in part a result of the recognition that there exists tremendous intertumoral heterogeneity not apparent by morphology alone. A particular area that significantly changed our approach to medulloblastoma has been an increased understanding of the role of p53. A role for p53 in medulloblastoma has been established over the past 20 years, however, not until recently has its significance been identified. Recent developments in the understanding of intertumor heterogeneity has clarified the role of *TP53* mutations, as the importance of *TP53* mutations is highly dependent on the molecular subgroup of medulloblastoma, with *TP53* mutant Sonic Hedgehog medulloblastomas forming an extremely high-risk group of patients. As such, there is now a tremendous push to understand the role that p53 plays in treatment resistance of medulloblastoma. In this review, we will summarize the current understanding of p53 in medulloblastoma drawn primarily from recent advances in integrated genomics.

Medulloblastoma is the most common malignant brain tumor of childhood. It arises in the cerebellum with an incidence of ~0.74 per 100,000 person-years (CBTRUS 2011; Ostrom et al. 2015). Current therapy for medulloblastoma consists of maximal safe surgical resection followed by adjuvant external beam irradiation and chemotherapy (Gajjar et al. 2006; Packer et al. 2006; Ramaswamy et al. 2011). Clinical risk stratification relies on extent of surgical resection, presence of leptomeningeal dissemination, and age, to classify patients as high or average risk (Zeltzer et al. 1999). This is based

largely on a morphological classification, in which the majority of tumors look identical. As such, historically, medulloblastoma has been considered a single clinicopathological entity, and treated as such.

Advances in integrated genomics over the past decade have yielded tremendous insight into the biology of medulloblastoma. Specifically, it is now well accepted that medulloblastoma actually comprises four distinct entities, termed molecular subgroups (Taylor et al. 2012). These four subgroups termed WNT, Sonic Hedgehog (SHH), and group 3 and group

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4 have distinct transcriptional profiles, genetics, demographics, recurrence patterns, and outcomes (Northcott et al. 2009, 2011, 2012a,b; Remke et al. 2011a,b; Kool et al. 2012; Dubuc et al. 2013; Ramaswamy et al. 2013). WNT tumors are characterized by activation of the WNT pathway, occur primarily in noninfant children, and have an excellent prognosis. WNT tumors are characterized genetically by frequent mutations in exon 3 of *CTNNB1*, which encodes β -catenin and monosomy 6 (Ellison et al. 2011; Northcott et al. 2012c). SHH tumors are characterized by activation of the SHH pathway and can occur across all ages with an intermediate prognosis. Genetically, they frequently harbor mutations activating the SHH pathway, such as in *Patched1*, *SUFU*, and *Smoothened*. In addition, they commonly harbor amplifications of *GLI2* and *MYCN*, along with activating mutations in the *TERT* promoter (Jones et al. 2012; Northcott et al. 2012a,c; Remke et al. 2013). SHH medulloblastoma are currently the focus of targeted therapies with smoothened inhibitors (Gajjar et al. 2013; Shou et al. 2015). Group 3 medulloblastoma occur most frequently in infants and young children who are regularly metastatic at diagnosis, have a poor prognosis and in ~20% of cases harbor amplifications of the *MYC* oncogene (Cho et al. 2011; Taylor et al. 2012). Group 4 medulloblastoma are the most common subgroup, comprising >40% of cases. Group 4 medulloblastoma is frequently metastatic at diagnosis, harbors tandem duplications of *SNCAIP*, amplifications of *MYCN*, and in >80% of cases harbors isochromosome 17q (Northcott et al. 2011, 2012c). As such, the identification of these four biological subgroups has fundamentally changed our understanding of medulloblastoma, whereby it is now accepted that medulloblastoma is a very heterogeneous entity and most genomic and biological observations of medulloblastoma need to be interpreted in the context of subgroup specificity.

One particular example has been the role of *TP53* and its protein product p53 in medulloblastoma. Over the past three decades, as with most other malignancies, there has been tremendous interest in the role of p53 in the on-

cogenesis of medulloblastoma. Indeed, there have been numerous conflicting reports in the past; however, in the context of rapid genomic advances, there has recently been a surge in our understanding of the role of p53. Although p53 has been investigated thoroughly from a clinical, genomic, and functional perspective, its role across and within medulloblastoma subgroups remained relatively unknown. Herein, we will summarize the current knowledge of p53 in medulloblastoma including challenges and future directions.

SOMATIC AND GERMLINE ABERRATIONS IN *TP53*

The initial observation that led to the investigation of the role of p53 in medulloblastoma came from reports that, in >40% of medulloblastoma samples, 17p is frequently deleted as part of isochromosome 17q (Bigner et al. 1988; Friedman et al. 1988; Biegel et al. 1989, 1992). Indeed, this led to the notion that loss of 17p was associated with the pathogenesis of medulloblastoma, which drove several efforts to identify *TP53* mutations across cohorts of medulloblastoma. These initial efforts were relatively disappointing as very few somatic mutations were identified across small cohorts of medulloblastoma patients, particularly those patients with 17p loss. This suggested that an alternative tumor suppressor was present on 17p and that 17p loss and *TP53* mutations are unrelated (Adesina et al. 1994; Pfaff et al. 2010). The link between *TP53* mutations and the pathogenesis of medulloblastoma was revisited later when it was identified that individuals with Li–Fraumeni syndrome, which is caused by a germline mutation in *TP53*, develop medulloblastoma at a much higher frequency than the general population (Kleihues et al. 1997; Barel et al. 1998). This suggested that loss of p53 likely had a role in the pathogenesis of medulloblastoma; however, this association is relatively rare. Moreover, the prognostic relevance of *TP53* mutations in medulloblastoma was unknown, and as such routine sequencing of *TP53* was reserved for those patients with a strong family history of Li–Fraumeni syndrome.

The prognostic value of *TP53* mutations has recently been the subject of several reports, with conflicting publications suggesting very different roles for p53 in medulloblastoma. An institutional cohort of 108 consecutive medulloblastoma patients from the Hospital for Sick Children suggested that somatic, dominant negative *TP53* mutations are almost universally fatal (Tabori et al. 2010). Indeed, in this study, none of the *TP53*-mutated patients harbored loss of 17p or had an *MYC* amplification, and only one patient had an *MYCN* amplification. Most importantly, the majority of patients were clinically average risk, and time to progression was less than a year in all cases. This was the first suggestion that *TP53* mutational status was an independent predictor of poor outcome in medulloblastoma. However, two conflicting reports, one from Heidelberg and another from Newcastle, suggested a very different prognostic role, whereby *TP53* mutations were commonly associated with mutations in exon 3 of *CTNNB1*, which is associated with activation of the WNT pathway (Pfaff et al. 2010; Lindsey et al. 2011). Medulloblastoma patients harboring WNT-activated tumors have an excellent prognosis, suggesting that *TP53*-inactivating mutations on their own do not confer a poor survival.

Further insight into the prognostic role of *TP53* mutations in medulloblastoma came from recent integrated genomic analyses. Indeed, when accounting for this heterogeneity, the apparent contradictory prognostic roles of *TP53* mutational status across studies can be resolved. A combined report reconciled these two observations through the analysis of a large cohort of 553 medulloblastomas. This study revealed that the prognostic value of somatic *TP53* mutations is subgroup dependent. Specifically, patients with WNT tumors harboring somatic *TP53* mutations have an excellent prognosis. However, those patients with SHH tumors harboring somatic *TP53* mutations had a dismal prognosis (Zhukova et al. 2013). *P53* mutations were almost never observed in patients with group 3 and 4 tumors, in which isochromosome 17q is a common aberration; these findings agree with and confirm previous reports. Within the WNT subgroup, the majority

of *TP53*-mutated cases were of the classic histology; however, large-cell and/or diffuse anaplastic histology was commonly observed in the *TP53*-mutated SHH cases. The age distribution of *TP53* mutations is also intriguing. *TP53* mutations are commonly observed in the childhood age group (4–16 yr) and rarely observed in infants and adults. In the childhood age group, germline mutations of *TP53* are frequently observed in SHH medulloblastoma and, as such, associate Li–Fraumeni syndrome with SHH medulloblastoma (Kool et al. 2014).

The majority of treatment failures in SHH medulloblastoma are associated with *TP53* mutations. Indeed, this group of medulloblastoma constitutes a very high-risk group and is the focus of international efforts for new and novel therapies (Ramaswamy et al. 2015). A potential explanation for the poor prognosis in *TP53*-mutated SHH relates to radiation resistance, as the majority of SHH medulloblastoma relapse locally in the tumor bed (Ramaswamy et al. 2013). Indeed, the majority of patients with *TP53* mutations are children over age 3, and as such receive upfront radiation (Zhukova et al. 2013, 2014; Kool et al. 2014; Ramaswamy et al. 2015). However, it is unclear whether *TP53* mutations confer radiation resistance, or radiation confers genomic instability in *TP53*-mutated tumors. In fact, there has been some suggestion that survival of patients with *TP53* mutant SHH medulloblastoma is possible with a de-escalation of therapy and omission of radiation and/or alkylating agents (Rausch et al. 2012; Kool et al. 2014). This has not, however, been clinically shown and requires further study in the context of controlled clinical trials. Defects in p53 at relapse have also been recently described (Hill et al. 2015; Poschl et al. 2015). Although somatic nucleotide variant (SNV) type *TP53* mutations are not observed at diagnosis in group 3 and 4 medulloblastoma, they can be observed at relapse, and are usually associated with *MYC* amplification. Although typical, dominant negative *TP53* SNVs are seldom or never seen in group 3 or group 4 medulloblastoma, these two subgroups often do have complete loss of one copy of *TP53*, usually in the setting of deletion the entire arm of chromosome 17p as part

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of isochromosome 17q. The combination of p53-MYC defects results in short survival post-recurrence. The role of *TP53* at relapse requires further study. However, the emergence of *TP53* mutations at relapse implicates a role for p53 in radiation resistance and treatment failure.

GENOMIC LANDSCAPE OF P53-MUTATED MEDULLOBLASTOMA

Several integrated genomic studies have revealed significant insight into the genomic landscape of *TP53*-mutated tumors. *TP53* mutations associated with WNT pathway activation are still commonly observed to have bland genomes, with the exception of monosomy 6, which is a frequent cytogenetic aberration in WNT subgroup tumors (Jones et al. 2012; Northcott et al. 2012a; Pugh et al. 2012; Robinson et al. 2012). It has previously been suggested that β -catenin protein is down-regulated by activated p53, and that excess β -catenin promotes accumulation of transcriptionally active p53 (Damalas et al. 1999; Sadot et al. 2001; Levina et al. 2004). This implies that, in the presence of activated β -catenin, there is a selective pressure for loss of *TP53* to promote a proliferative state. The excellent prognosis for children with *TP53*-mutated WNT tumors also suggests that somatic *TP53* inactivation in this context does not result in radiation resistance.

A recent next-generation sequencing study of SHH medulloblastoma casts further light on the role of *TP53* mutations in medulloblastoma (Kool et al. 2014). In this study, it was again suggested that *TP53* mutations are frequently found in childhood, but not infant or adult SHH tumors, as the frequency of germline mutations in *TP53* is higher in children aged 3–16 yr. Moreover, *TP53* mutations infrequently occur with *PTCH1*, *SMO*, or *SUFU* mutations, which are known to activate the SHH pathway; rather, *TP53* mutations are more commonly observed in the context of downstream lesions, such as *MYCN* and *GLI2* amplification (Kool et al. 2014). This strongly suggests that *TP53*-mutated SHH tumors are unlikely to respond to upstream SHH pathway inhibitors, such as the smoothened inhibitors GDC-449

and LDE-225, which are currently in early phase clinical trials for SHH-activated medulloblastoma (Rudin et al. 2009; Gajjar et al. 2013). As *TP53*-mutated SHH tumors constitute a very high-risk group, and account for the majority of treatment failures within the SHH subgroup, novel therapies beyond SMO inhibition are required to improve outcomes in this group (Ramaswamy et al. 2015). Interestingly, 17p loss was a commonly observed event in the context of *TP53* mutations. However, the significance of this is not currently clear.

The chromosomal landscape of *TP53*-mutated SHH tumors is also unique. Unlike the bland genomes observed in *TP53*-mutated tumors of the WNT subgroup, genomic instability is a hallmark of *TP53*-mutated SHH tumors, particularly germline mutations (Rausch et al. 2012). A detailed next-generation sequencing study of a germline *TP53*-mutated SHH tumor showed an association with the presence of chromothripsis, a genomic state characterized by catastrophic DNA rearrangements as a result of chromosome shattering (Rausch et al. 2012). In this study, chromothripsis was a frequent occurrence in *TP53*-mutated SHH medulloblastomas, whereas it was nonexistent in WNT medulloblastoma. This was extended to three additional *TP53*-mutated SHH samples that revealed multiple chromosomes showing chromothripsis. Chromothripsis-associated rearrangements were frequently associated with *MYCN* and *GLI2* amplifications, although chromosome 2 was not affected by chromothripsis. Recurrent fusions, such as the *PVT1*–*MYC* fusion in group 3 medulloblastoma or the *C11orf95*–*RELA* fusion observed in supratentorial ependymoma as a result of chromothripsis, were not observed in the SHH samples (Northcott et al. 2012c; Parker et al. 2014). Chromothripsis is also commonly observed in *TP53*-mutated AML suggesting a common mechanism in certain *TP53*-mutated cancers (Rausch et al. 2012). Although the role of chromothripsis in medulloblastoma initiation is unclear, the association between germline *TP53* mutations and chromothripsis suggests that the *TP53* mutation precedes the massive shattering and chromosomal rearrangements. This

provides a possible explanation for the absence of chromothripsis in WNT-activated medulloblastoma in that *TP53* mutations are a late event found in association with mutations of exon 3 of *CTNNB1*. The observation that chromothripsis was present in *Ptch*^{+/-};*Tp53*^{-/-} mice further strengthens the link between chromothripsis and p53 in SHH medulloblastoma (Wetmore et al. 2001; Rausch et al. 2012).

The role of p53 defects in group 3 and 4 medulloblastoma is unclear. Although 17p loss is a commonly observed event in the context of isochromosome 17q, SNVs of *TP53* have not been observed at diagnosis in either of these subgroups. As such, the role of 17p loss is in these two subgroups is unclear, as is the question of whether loss of one copy of *TP53* helps to drive clonal selection of cells lacking one copy of chromosome 17p. Negative regulators of p53 have been shown to have aberrant expression in group 3 and 4 medulloblastoma. One such example is *WIP1*, an oncogene present on 17q, which when highly expressed, inhibits p53 (Castellino et al. 2008). *WIP1* is highly expressed in group 3 and 4 medulloblastoma suggesting a role for p53 defects in these two subgroups. However, the extent of a role for p53 in the tumorigenesis of group 3 and 4 medulloblastoma is unclear and requires further study.

PRECLINICAL MODELS OF p53 DEFICIENT MEDULLOBLASTOMA

The development of numerous preclinical models has provided further insight into the role of p53 defects in medulloblastoma tumorigenesis. Several preclinical medulloblastoma models have been developed, most of which rely on inactivation of *Ptch1* (Goodrich et al. 1997; Wu et al. 2011, 2012). *Ptch1* heterozygosity is sufficient to promote tumorigenesis. However, the incidence of medulloblastoma in *Ptch*^{+/-} mice is <20% (Goodrich et al. 1997; Wetmore et al. 2000; Wu et al. 2011). When *Ptch1* loss is combined with *Tp53* loss (i.e., *Ptch*^{+/-};*Tp53*^{-/-}), mice have a tumor incidence exceeding 90%; it has been suggested that genomic instability is promoted by p53 loss, which accelerates the incidence of tumors

(Wetmore et al. 2000, 2001; Wu et al. 2011; Rausch et al. 2012). p53 loss in isolation is insufficient to drive medulloblastoma formation. However, *Tp53*^{-/-} mice do form other tumor types, such as lymphoma (Jacks et al. 1994; Wetmore et al. 2001; Lee et al. 2007; Wu et al. 2012). As such, it is unclear if p53 loss is exclusively a cooperating event in murine medulloblastoma formation. *Sufu* mutations are frequently observed in infant medulloblastoma. However, *Sufu* loss alone is insufficient to promote medulloblastoma formation. When *Sufu* loss is combined with p53 loss, SHH-driven tumor formation was frequently observed. p53 loss has been shown to accelerate tumorigenesis in a variety of genetic backgrounds, including Rb loss, Lig4 loss, Xrcc4 loss, Cdkn2a loss, and Sleeping Beauty under the Math1 promoter (Frank et al. 2000; Marino et al. 2000; Lee and McKinnon 2002; Kawauchi et al. 2012).

WNT-driven medulloblastoma can also be induced through combined mutant *Ctnnb1* and *Tp53* loss when crossed to Blbp-Cre (Gibson et al. 2010). This model results in medulloblastoma formation in up to 15% of mice, suggesting that loss of p53 is required for transformation of progenitor cells in the lower rhombic lip, the presumed cell of origin of WNT tumors. Indeed, this combined with the relatively frequent occurrence of *TP53* mutations in human WNT tumors suggests a role for both *CTNNB1* and *TP53* in the pathogenesis of WNT medulloblastoma.

p53 defects also have an important role in murine non-WNT/SHH tumors. Sequencing of tumors from the MYCN-driven mouse model (Glt1-tTA/TRE/MYCN-Luc) revealed spontaneous p53 mutations within the DNA-binding domain in 83% of tumors examined (Swartling et al. 2010; Hill et al. 2015). Combining MYC expression with p53 defects also resulted in robust medulloblastoma formation. Two allograft models of MYC-driven medulloblastoma have been generated, and these transcriptionally resemble group 3 medulloblastoma. Both models rely on loss of p53, one requires a *Tp53*^{-/-} background and the second relies on inactivation of p53 using a dominant negative allele. In both models, MYC expression

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alone, in the absence of p53 inactivation, was insufficient to result in medulloblastoma formation, suggesting a role for p53 defects in sporadic murine models of medulloblastoma

THERAPEUTIC TARGETING OF *TP53* IN MEDULLOBLASTOMA

The subset of patients with *TP53* mutant SHH medulloblastomas represents one of the biggest challenges in pediatric neuro-oncology. These patients account for the majority of treatment failures within the SHH subgroup, and they are also the subset of SHH patients least likely to respond to targeted SHH inhibitors. *TP53*-mutated SHH medulloblastomas commonly harbor downstream genetic events, including *MYCN* and *GLI2* amplifications, which will likely result in resistance to current SHH inhibitors (e.g., vismodegib and sonidegib, which target Smoothened) in phase II clinical trials (Pfaff et al. 2010; Tabori et al. 2010; Gajjar et al. 2013; Kool et al. 2014; Shou et al. 2015). As such, successful therapeutic targeting of this group will require the identification of SHH pathway inhibitors, which act downstream from either *MYCN* or *GLI2*. One possible option is arsenic trioxide, which is a *GLI2* inhibitor, and a second option is bromodomain inhibitors, which target SHH transcriptional output (Kim et al. 2013; Tang et al. 2014). A recent report also suggested that in an *MYCN*-driven mouse model with spontaneous p53 mutations, Aurora Kinase inhibitors, such as Alisertib, may confer a survival advantage (Hill et al. 2015). Another intriguing therapeutic possibility in *TP53*-mutated SHH tumors is lithium. Lithium is an inhibitor of GSK3 β , a negative regulator of the WNT pathway, and as such can mimic canonical WNT activation (Stambolic et al. 1996). Thus, one potential therapeutic option is the restoration of radio-sensitivity of *TP53*-mutated medulloblastoma through the administration of lithium (Zhukova et al. 2014). All of these therapeutic options require investigation in phase I clinical trials so their potential can be validated. Another therapeutic option being explored in non-*TP53*-mutated medulloblastoma is pharmaco-

logical activation of the wild-type copy of p53. One agent in particular, nutlin-3, has been shown using in vitro and in vivo systems, to block the interaction of MDM2 and p53, thus restoring p53 function (Kunkele et al. 2012). MDM2 expression has been shown to be increased in medulloblastoma, compared with normal cerebella, suggesting that this is a promising therapeutic strategy in non-*TP53*-mutated medulloblastoma (Kunkele et al. 2012).

CONCLUSIONS AND FUTURE DIRECTIONS

Our understanding of the role of p53 in medulloblastoma has increased dramatically over the past decade. However, despite these advances, the role of p53 in medulloblastoma initiation, progression, and maintenance is largely unknown and future advances will likely uncover indirect roles for p53-associated proteins in the pathogenesis of group 3 and 4 medulloblastoma. Advances in integrated genomics have brought p53 back to the forefront in the study of medulloblastoma biology. For children with SHH medulloblastoma, it is now clear that *TP53* mutations harbor significant prognostic utility and comprise one of the highest risk subsets of medulloblastoma patients. As such, pre-clinical efforts across many groups is centered squarely on developing novel therapeutics for this very high-risk group of medulloblastoma. Moreover, the identification of this group will also allow more robust preclinical modeling of dominant negative *TP53* mutations, which will also lead to the ability to the implementation of preclinical trials for novel therapies.

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REFERENCES

- Adesina AM, Nalbantoglu J, Cavenee WK. 1994. *p53* gene mutation and *mdm2* gene amplification are uncommon in medulloblastoma. *Cancer Res* **54**: 5649–5651.
- Barel D, Avigad S, Mor C, Fogel M, Cohen IJ, Zaizov R. 1998. A novel germ-line mutation in the noncoding region of the *p53* gene in a Li–Fraumeni family. *Cancer Genet Cytogenet* **103**: 1–6.
- Biegel JA, Rorke LB, Packer RJ, Sutton LN, Schut L, Bonner K, Emanuel BS. 1989. Isochromosome 17q in primitive neuroectodermal tumors of the central nervous system. *Genes Chromosomes Cancer* **1**: 139–147.
- Biegel JA, Burk CD, Barr FG, Emanuel BS. 1992. Evidence for a 17p tumor related locus distinct from *p53* in pediatric primitive neuroectodermal tumors. *Cancer Res* **52**: 3391–3395.
- Bigner SH, Mark J, Friedman HS, Biegel JA, Bigner DD. 1988. Structural chromosomal abnormalities in human medulloblastoma. *Cancer Genet Cytogenet* **30**: 91–101.
- Castellino RC, De Bortoli M, Lu X, Moon SH, Nguyen TA, Shepard MA, Rao PH, Donehower LA, Kim JY. 2008. Medulloblastomas overexpress the *p53*-inactivating oncogene *WIP1/PPM1D*. *J Neurooncol* **86**: 245–256.
- CBTRUS. 2011. CBTRUS Statistical Report: Primary brain and central nervous system tumors diagnosed in the United States in 2004–2007. Central Brain Tumor Registry of the United States, Hinsdale, IL.
- Cho Y-J, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, et al. 2011. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol* **29**: 1424–1430.
- Damalas A, Ben-Ze'ev A, Simcha I, Shtutman M, Leal JF, Zhurinsky J, Geiger B, Oren M. 1999. Excess β -catenin promotes accumulation of transcriptionally active *p53*. *EMBO J* **18**: 3054–3063.
- Dubuc AM, Remke M, Korshunov A, Northcott PA, Zhan SH, Mendez-Lago M, Kool M, Jones DT, Unterberger A, Morrissy AS, et al. 2013. Aberrant patterns of H3K4 and H3K27 histone lysine methylation occur across subgroups in medulloblastoma. *Acta Neuropathol* **125**: 373–384.
- Ellison DW, Kocak M, Dalton J, Megahed H, Lusher ME, Ryan SL, Zhao W, Nicholson SL, Taylor RE, Bailey S, et al. 2011. Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. *J Clin Oncol* **29**: 1400–1407.
- Frank KM, Sharpless NE, Gao Y, Sekiguchi JM, Ferguson DO, Zhu C, Manis JP, Horner J, DePinho RA, Alt FW. 2000. DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the *p53* pathway. *Mol Cell* **5**: 993–1002.
- Friedman HS, Burger PC, Bigner SH, Trojanowski JQ, Brodeur GM, He XM, Wikstrand CJ, Kurtzberg J, Berens ME, Halperin EC. 1988. Phenotypic and genotypic analysis of a human medulloblastoma cell line and transplantable xenograft (D341 Med) demonstrating amplification of *c-myc*. *Am J Pathol* **130**: 472–484.
- Gajjar A, Chintagumpala M, Ashley D, Kellie S, Kun LE, Merchant TE, Woo S, Wheeler G, Ahern V, Krasin MJ, et al. 2006. Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): Long-term results from a prospective, multicentre trial. *Lancet Oncol* **7**: 813–820.
- Gajjar A, Stewart CE, Ellison DW, Kaste S, Kun LE, Packer RJ, Goldman S, Chintagumpala M, Wallace D, Takebe N, et al. 2013. Phase I study of vismodegib in children with recurrent or refractory medulloblastoma: A pediatric brain tumor consortium study. *Clin Cancer Res* **19**: 6305–6312.
- Gibson P, Tong Y, Robinson G, Thompson MC, Curre DS, Eden C, Kranenburg TA, Hogg T, Poppleton H, Martin J, et al. 2010. Subtypes of medulloblastoma have distinct developmental origins. *Nature* **468**: 1095–1099.
- Goodrich LV, Milenkovic L, Higgins KM, Scott MP. 1997. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**: 1109–1113.
- Hill RM, Kuijper S, Lindsey JC, Petrie K, Schwalbe EC, Barker K, Boulton JK, Williamson D, Ahmad Z, Hallsworth A, et al. 2015. Combined MYC and P53 defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* **27**: 72–84.
- Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA. 1994. Tumor spectrum analysis in *p53*-mutant mice. *Curr Biol* **4**: 1–7.
- Jones DT, Jager N, Kool M, Zichner T, Hutter B, Sultan M, Cho YJ, Pugh TJ, Hovestadt V, Stutz AM, et al. 2012. Dissecting the genomic complexity underlying medulloblastoma. *Nature* **488**: 100–105.
- Kawauchi D, Robinson G, Uziel T, Gibson P, Reh J, Gao C, Finkelstein D, Qu C, Pounds S, Ellison DW, et al. 2012. A mouse model of the most aggressive subgroup of human medulloblastoma. *Cancer Cell* **21**: 168–180.
- Kim J, Aftab BT, Tang JY, Kim D, Lee AH, Rezaee M, Kim J, Chen B, King EM, Borodovsky A, et al. 2013. Itraconazole and arsenic trioxide inhibit Hedgehog pathway activation and tumor growth associated with acquired resistance to smoothened antagonists. *Cancer Cell* **23**: 23–34.
- Kleihues P, Schauble B, zur Hausen A, Esteve J, Ohgaki H. 1997. Tumors associated with *p53* germline mutations: A synopsis of 91 families. *Am J Pathol* **150**: 1–13.
- Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, Cho YJ, Koster J, Schouten-van Meeteren A, van Vuurden D, et al. 2012. Molecular subgroups of medulloblastoma: An international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, group 3, and group 4 medulloblastomas. *Acta Neuropathol* **123**: 473–484.
- Kool M, Jones DT, Jager N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, et al. 2014. Genome sequencing of SHH medulloblastoma

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- predicts genotype-related response to smoothened inhibition. *Cancer Cell* **25**: 393–405.
- Kunkele A, De Preter K, Heukamp L, Thor T, Pajtlér KW, Hartmann W, Mittelbronn M, Grotzer MA, Deubzer HE, Speleman F, et al. 2012. Pharmacological activation of the p53 pathway by nutlin-3 exerts anti-tumoral effects in medulloblastomas. *Neuro Oncol* **14**: 859–869.
- Lee Y, McKinnon PJ. 2002. DNA ligase IV suppresses medulloblastoma formation. *Cancer Res* **62**: 6395–6399.
- Lee Y, Kawagoe R, Sasai K, Li Y, Russell HR, Curran T, McKinnon PJ. 2007. Loss of suppressor-of-fused function promotes tumorigenesis. *Oncogene* **26**: 6442–6447.
- Levin E, Oren M, Ben-Ze'ev A. 2004. Downregulation of β -catenin by p53 involves changes in the rate of β -catenin phosphorylation and Axin dynamics. *Oncogene* **23**: 4444–4453.
- Lindsey JC, Hill RM, Megahed H, Lusher ME, Schwalbe EC, Cole M, Hogg TL, Gilbertson RJ, Ellison DW, Bailey S, et al. 2011. TP53 mutations in favorable-risk Wnt/Wingless-subtype medulloblastomas. *J Clin Oncol* **29**: e344–e346; author reply e347–e348.
- Marino S, Vooijs M, van Der Gulden H, Jonkers J, Berns A. 2000. Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev* **14**: 994–1004.
- Northcott PA, Nakahara Y, Wu X, Feuk L, Ellison DW, Croul S, Mack S, Kongkham PN, Peacock J, Dubuc A, et al. 2009. Multiple recurrent genetic events converge on control of histone lysine methylation in medulloblastoma. *Nat Genet* **41**: 465–472.
- Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, et al. 2011. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* **29**: 1408–1414.
- Northcott PA, Jones DT, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, et al. 2012a. Medulloblastomics: The end of the beginning. *Nat Rev Cancer* **12**: 818–834.
- Northcott PA, Korshunov A, Pfister SM, Taylor MD. 2012b. The clinical implications of medulloblastoma subgroups. *Nat Rev Neurol* **8**: 340–351.
- Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, Stutz AM, Korshunov A, Reimand J, Schumacher SE, et al. 2012c. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature* **488**: 49–56.
- Ostrom QT, de Blank PM, Kruchko C, Petersen CM, Liao P, Finlay JL, Stearns DS, Wolff JE, Wolinsky Y, Letterio JJ, et al. 2015. Alex's Lemonade Stand Foundation infant and childhood primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. *Neuro Oncol* **16**: x1–x36.
- Packer RJ, Gajjar A, Vezina G, Rorke-Adams L, Burger PC, Robertson PL, Bayer L, LaFond D, Donahue BR, Marymont MH, et al. 2006. Phase III study of craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma. *J Clin Oncol* **24**: 4202–4208.
- Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, Lee R, Tatevossian RG, Phoenix TN, Thiruvengadam R, et al. 2014. C11orf95–RELA fusions drive oncogenic NF- κ B signalling in ependymoma. *Nature* **506**: 451–455.
- Pfaff E, Remke M, Sturm D, Benner A, Witt H, Milde T, Von Bueren AO, Wittmann A, Schottler A, Jorch N, et al. 2010. TP53 Mutation is frequently associated with CTNNB1 mutation or MYCN amplification and is compatible with long-term survival in medulloblastoma. *J Clin Oncol* **28**: 5188–5196.
- Poschl J, Koch A, Schuller U. 2015. Histological subtype of medulloblastoma frequently changes upon recurrence. *Acta Neuropathol* **129**: 459–461.
- Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, et al. 2012. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* **488**: 106–110.
- Ramaswamy V, Northcott PA, Taylor MD. 2011. FISH and chips: The recipe for improved prognostication and outcomes for children with medulloblastoma. *Cancer Genet* **204**: 577–588.
- Ramaswamy V, Remke M, Bouffet E, Faria CC, Perreault S, Cho YJ, Shih DJ, Luu B, Dubuc AM, Northcott PA, et al. 2013. Recurrence patterns across medulloblastoma subgroups: An integrated clinical and molecular analysis. *Lancet Oncol* **14**: 1200–1207.
- Ramaswamy V, Remke M, Adamski J, Bartels U, Tabori U, Wang X, Huang A, Hawkins C, Mabbott D, Laperriere N, et al. 2015. Medulloblastoma subgroup-specific outcomes in irradiated children: Who are the true high-risk patients? *Neuro Oncol* doi: 10.1093/neuonc/nou357.
- Rausch T, Jones DT, Zapotka M, Stutz AM, Zichner T, Weischenfeldt J, Jager N, Remke M, Shih D, Northcott PA, et al. 2012. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. *Cell* **148**: 59–71.
- Remke M, Hielscher T, Korshunov A, Northcott PA, Bender S, Kool M, Westermann F, Benner A, Cin H, Ryzhova M, et al. 2011a. FSTL5 is a marker of poor prognosis in non-WNT/non-SHH medulloblastoma. *J Clin Oncol* **29**: 3852–3861.
- Remke M, Hielscher T, Northcott PA, Witt H, Ryzhova M, Wittmann A, Benner A, von Deimling A, Scheurlen W, Perry A, et al. 2011b. Adult medulloblastoma comprises three major molecular variants. *J Clin Oncol* **29**: 2717–2723.
- Remke M, Ramaswamy V, Peacock J, Shih DJ, Koelsche C, Northcott PA, Hill N, Cavalli FM, Kool M, Wang X, et al. 2013. TERT promoter mutations are highly recurrent in SHH subgroup medulloblastoma. *Acta Neuropathol* **126**: 917–929.
- Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, et al. 2012. Novel mutations target distinct subgroups of medulloblastoma. *Nature* **488**: 43–48.
- Rudin CM, Hann CL, Laterra J, Yauch RL, Callahan CA, Fu L, Holcomb T, Stinson J, Gould SE, Coleman B, et al. 2009. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med* **361**: 1173–1178.
- Sadot E, Geiger B, Oren M, Ben-Ze'ev A. 2001. Down-regulation of β -catenin by activated p53. *Mol Cell Biol* **21**: 6768–6781.

- Shou Y, Robinson DM, Amakye DD, Rose KL, Cho YJ, Ligon KL, Sharp T, Haider AS, Bandaru R, Ando Y, et al. 2015. A five-gene hedgehog signature developed as a patient pre-selection tool for hedgehog inhibitor therapy in medulloblastoma. *Clin Cancer Res* **21**: 585–593.
- Stambolic V, Ruel L, Woodgett JR. 1996. Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr Biol* **6**: 1664–1668.
- Swartling FJ, Grimmer MR, Hackett CS, Northcott PA, Fan QW, Goldenberg DD, Lau J, Masic S, Nguyen K, Yakovenko S, et al. 2010. Pleiotropic role for *MYCN* in medulloblastoma. *Genes Dev* **24**: 1059–1072.
- Tabori U, Baskin B, Shago M, Alon N, Taylor MD, Ray PN, Bouffet E, Malkin D, Hawkins C. 2010. Universal poor survival in children with medulloblastoma harboring somatic *TP53* mutations. *J Clin Oncol* **28**: 1345–1350.
- Tang Y, Gholamin S, Schubert S, Willardson MI, Lee A, Bandopadhyay P, Bergthold G, Masoud S, Nguyen B, Vue N, et al. 2014. Epigenetic targeting of Hedgehog pathway transcriptional output through BET bromodomain inhibition. *Nat Med* **20**: 732–740.
- Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, et al. 2012. Molecular subgroups of medulloblastoma: The current consensus. *Acta Neuropathol* **123**: 465–472.
- Wetmore C, Eberhart DE, Curran T. 2000. The normal patched allele is expressed in medulloblastomas from mice with heterozygous germ-line mutation of patched. *Cancer Res* **60**: 2239–2246.
- Wetmore C, Eberhart DE, Curran T. 2001. Loss of *p53* but not *ARF* accelerates medulloblastoma in mice heterozygous for patched. *Cancer Res* **61**: 513–516.
- Wu X, Northcott PA, Croul S, Taylor MD. 2011. Mouse models of medulloblastoma. *Chin J Cancer* **30**: 442–449.
- Wu X, Northcott PA, Dubuc A, Dupuy AJ, Shih DJ, Witt H, Croul S, Bouffet E, Fuhs DW, Eberhart CG, et al. 2012. Clonal selection drives genetic divergence of metastatic medulloblastoma. *Nature* **482**: 529–533.
- Zeltzer PM, Boyett JM, Finlay JL, Albright AL, Rorke LB, Milstein JM, Allen JC, Stevens KR, Stanley P, Li H, et al. 1999. Metastasis stage, adjuvant treatment, and residual tumor are prognostic factors for medulloblastoma in children: Conclusions from the Children's Cancer Group 921 randomized phase III study. *J Clin Oncol* **17**: 832–845.
- Zhukova N, Ramaswamy V, Remke M, Pfaff E, Shih DJ, Martin DC, Castelo-Branco P, Baskin B, Ray PN, Bouffet E, et al. 2013. Subgroup-specific prognostic implications of *TP53* mutation in medulloblastoma. *J Clin Oncol* **31**: 2927–2935.
- Zhukova N, Ramaswamy V, Remke M, Martin DC, Castelo-Branco P, Zhang CH, Fraser M, Tse K, Poon R, Shih DJ, et al. 2014. WNT activation by lithium abrogates *TP53* mutation associated radiation resistance in medulloblastoma. *Acta Neuropathol Commun* **2**: 174.

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