

## Evasion of cell senescence in SHH medulloblastoma

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### ABSTRACT

The mechanisms leading to brain tumor formation are poorly understood. Using *Ptch1*<sup>+/-</sup> mice as a medulloblastoma model, sequential mutations were found to shape tumor evolution. Initially, medulloblastoma preneoplastic lesions display loss of heterozygosity of the *Ptch1* wild-type allele, an event associated with cell senescence in preneoplasia. Subsequently, *p53* mutations lead to senescence evasion and progression from preneoplasia to medulloblastoma. These findings are consistent with a model where high levels of Hedgehog signaling caused by the loss of the tumor suppressor *Ptch1* lead to oncogene-induced senescence and drive *p53* mutations. Thus, cell senescence is an important characteristic of a subset of SHH medulloblastoma and might explain the acquisition of somatic *TP53* mutations in human medulloblastoma. This mode of medulloblastoma formation contrasts with the one characterizing Li-Fraumeni patients with medulloblastoma, where *TP53* germ-line mutations cause chromothriptic genomic instability and lead to mutations in Hedgehog signaling genes, which drive medulloblastoma growth. Here we discuss in detail these 2 alternative mechanisms leading to medulloblastoma tumorigenesis.

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### Introduction



Medulloblastoma is the most common brain tumor in children. According to integrated genomics studies, medulloblastomas can be classified in 4 different molecular subtypes: WNT, SHH, Group 3 and Group 4.<sup>1</sup> Each of these molecular subtypes has specific underlying molecular features, gene expression, demographic characteristics and prognosis.<sup>2</sup> Sonic Hedgehog (SHH) medulloblastomas are characterized by activation of the Hedgehog (Hh) signaling pathway and are often driven by mutations in Hh pathway components.<sup>3</sup> Here we focus on SHH medulloblastoma and discuss how cell senescence shapes the natural history and molecular evolution of this childhood cancer.

### *TP53* mutations and medulloblastoma

For many years, it was thought that *TP53* mutations were infrequent in medulloblastomas<sup>4,5</sup> and that P53 signaling was dispensable for medulloblastoma tumor suppression. However, recent studies reported that *TP53* mutations are frequent in human WNT (with a rate of 16%) and SHH primary medulloblastomas (with a rate of 13% to 21%, depending on the study)<sup>3,6,7</sup> and are indicators of poor prognosis exclusively in SHH medulloblastoma.<sup>7</sup> Moreover, *TP53* mutations have recently been identified as a key event in the pathogenesis of

medulloblastoma recurrence.<sup>8,9</sup> Although the specific roles of P53 in medulloblastoma are still largely unknown,<sup>10</sup> the presence of recurrent *TP53* mutations suggests that P53 signaling plays an important role in driving medulloblastoma tumorigenesis.

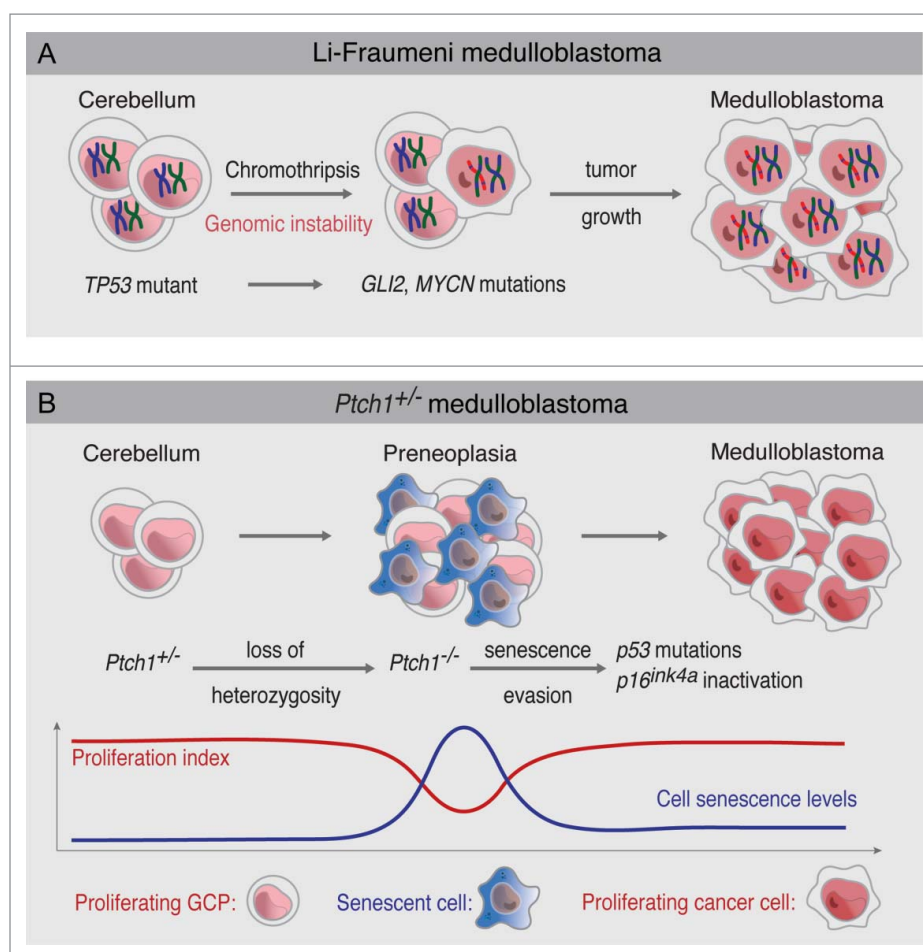
Human genomic studies also established that *TP53* mutations co-exist with mutations or amplifications of Hh signaling components such as *SHH*, *SMO*, *SUFU*, *GLI2* and *MYCN*.<sup>3</sup> These studies, however, do not establish the sequential order of genetic events that lead to medulloblastoma formation and how these mutations correlate with presumptive histopathological stages of medulloblastoma. Nevertheless, patients with Li-Fraumeni syndrome, caused by germ-line mutations in *TP53*, offer an opportunity to study how medulloblastomas arise at the genetic level. Li-Fraumeni patients are cancer-prone and sometimes develop medulloblastoma;<sup>11</sup> therefore, at least for this subset of patients, *TP53* mutations are the first genetic event leading to medulloblastoma formation (Fig. 1A). Notably, most Li-Fraumeni medulloblastomas belong to the SHH subgroup, indicating that *TP53* mutations specifically predispose to SHH medulloblastoma.<sup>12</sup> Because cerebellum granule cell precursors (GCPs) are the cells of origin of SHH medulloblastoma,<sup>13-15</sup> this indicates that GCPs are highly susceptible to transformation in absence of P53. Moreover, these SHH medulloblastomas seem to be the result of chromothripsis, a massive genome rearrangement caused

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**Figure 1.** Two alternative mechanisms of medulloblastoma formation. (A) In Li-Fraumeni patients with germ-line *TP53* mutations, cerebellum GCPs experience chromosomal instability. Chromothriptic events lead to massive chromosomal rearrangements and high levels of amplification in Hh signaling genes such as *GLI2* and *MYCN*. Acquisition of these Hh signaling mutations leads to medulloblastoma growth. (B) In *Ptch1*<sup>+/-</sup> mice, loss of heterozygosity of the *Ptch1* wild-type allele leads to the formation of preneoplasia. Preneoplastic lesions display high levels of cell senescence. Spontaneous *p53* mutations or *p16ink4a* inactivation leads to senescence evasion and progression to advanced medulloblastoma. Proliferation and senescence levels during medulloblastoma formation are indicated.

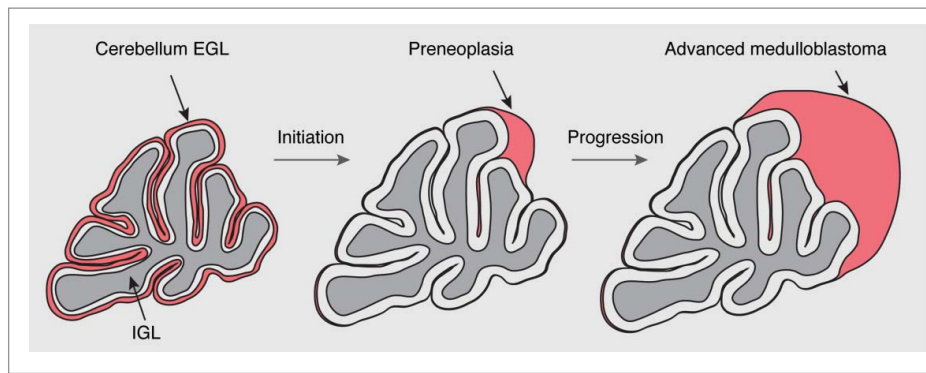
by chromosomal shattering likely occurring in a single event.<sup>16</sup> These chromothriptic events likely lead to mutations in components of Hh signaling, such as *GLI2*, *BOC* and *MYCN* amplifications.<sup>12</sup> Since Hh signaling is the most important mitogenic pathway for GCPs,<sup>17</sup> it is therefore expected that acquisition of Hh pathway mutations efficiently causes SHH medulloblastoma in Li-Fraumeni patients.

Although *p53* knockout mice or mouse models of Li-Fraumeni syndrome do not develop medulloblastoma,<sup>18,19</sup> elegant studies have demonstrated that the inactivation of *p53* together with other DNA repair factors such as *Xrcc4*, *Ligase IV*, *Xrcc2* and *Brca2* leads to Shh medulloblastoma.<sup>20,21</sup> Interestingly, those medulloblastomas also harbored spontaneous mutations in Hh signaling components such as *Ptch1*, *Mycn* and *Gli2*.<sup>20</sup> This result not only highlights how important it is to maintain genomic stability to prevent SHH medulloblastoma formation, but it also indicates that Hh signaling activation seems to be necessary for medulloblastoma formation even when *p53* and DNA repair mechanisms are absent. In summary, germ-line *P53* mutations in both mouse and human cause genomic instability and lead to mutations in Hh signaling components, conducting to medulloblastoma formation (Fig. 1A). This is an interesting paradigm showing that Hh

signaling mutations happen subsequent to *TP53* mutations in Li-Fraumeni syndrome.

Half of the SHH medulloblastomas with *TP53* mutations have a germ-line (Li-Fraumeni) origin and are potentially explained by the mechanism described above.<sup>3,12</sup> However, it is not known how the other SHH medulloblastoma cases (including the ones with somatic *TP53* mutations and the ones without *TP53* mutations) develop and the temporal order in which they acquire their mutations. Another unresolved, yet related, question is whether advanced medulloblastomas arise in a step-wise manner from subclinical precancerous lesions. For many epithelial cancers, the availability of preneoplastic lesions allowed the establishment of tumor progression models with sequential histopathological stages and the molecular changes that characterize them.<sup>22</sup> However, the problem for understanding brain tumor development lies in the inability to detect and obtain precancerous lesions; therefore, genome sequencing of advanced brain tumors only offers a snapshot of the mutations present in advanced tumors but does not show the order in which mutations are acquired during tumor progression.

To address this question, we used *Ptch1*<sup>+/-</sup> heterozygous mice, a well-established model of Shh medulloblastoma



**Figure 2.** Medulloblastoma formation in *Ptch1*<sup>+/-</sup> mice. During postnatal development, granule cell precursors (GCPs) of the cerebellum, the cells of origin of Shh medulloblastoma, are located in the external granule-cell layer (EGL). After their proliferation, most GCPs differentiate, populate the internal granule-cell layer (IGL), and disappear from the EGL after the second postnatal week in the mouse. LOH of the *Ptch1* wild-type allele causes a clonal expansion and leads to the formation of preneoplasia. While most preneoplastic lesions disappear, some of them progress to advanced medulloblastoma. See also Figure 1B.

(Fig. 2). *Ptch1*<sup>+/-</sup> mice develop preneoplastic lesions with high frequency, but only a fraction of those animals develop advanced medulloblastoma.<sup>13,23</sup> Therefore, we hypothesized that an unidentified tumor suppressive mechanism might restrain the progression of medulloblastoma preneoplasia into advanced tumors. We found that apoptosis levels are the same between preneoplastic lesions and advanced medulloblastoma, eliminating apoptosis as an essential tumor suppressor in this model of Shh medulloblastoma. Surprisingly, when we looked at cell senescence, we found that while preneoplastic lesions display high numbers of p21<sup>Cip1</sup> and p16<sup>Ink4a</sup> positive cells (which are effectors and markers of cell senescence and cell cycle arrest<sup>24-26</sup>), advanced medulloblastomas have very low levels of senescence.<sup>27</sup> These high levels of senescence are paralleled by lower levels of proliferation and correlate with loss of heterozygosity (LOH) of the *Ptch1* wild-type allele in preneoplastic lesions (Fig. 1B), suggesting that high levels of Hh signaling may contribute to cell senescence. Using laser capture microdissection, we found that one-third of all advanced medulloblastomas acquired *p53* mutations that were not present in preneoplastic lesions, supporting the notion that *p53* mutations allow senescence evasion and medulloblastoma progression (Fig. 1B). Moreover, we found that engineered *p53* mutations prevent cellular senescence and accelerate medulloblastoma formation, showing that *p53* mutations lead to senescence evasion.<sup>27</sup> Further supporting the idea that senescence evasion is necessary for medulloblastoma progression, advanced tumors without *p53* mutations display low expression of p16<sup>Ink4a</sup> due to promoter methylation. In summary, we found that, contrary to Li-Fraumeni syndrome (where *TP53* germ-line mutations lead to Hh signaling mutations), Hh signaling hyperactivity leads to cell senescence in preneoplastic lesions and creates selection pressure for the inactivation of *p53* or p16<sup>Ink4a</sup>, which allows the progression from preneoplasia to advanced medulloblastoma (Fig. 1B).

Importantly, the finding of spontaneous *p53* mutations is not limited to the *Ptch1*<sup>+/-</sup> model, as we also found spontaneous *p53* mutations in tumors from *Olig1-Gnas* mice, another model of Shh medulloblastoma.<sup>28</sup> This suggests that evasion of senescence could be a hallmark of Shh medulloblastoma.

### Hh and p53 signaling in medulloblastoma

Since *p53* is inactivated during the formation of Shh medulloblastoma (Fig. 1B), this may highlight some possible interactions between *p53* and Hh signaling in the brain. Some studies have explored this relationship using a variety of approaches. Shh, a protein secreted by Purkinje cells of the cerebellum, is the most potent mitogen for GCPs.<sup>17,29,30</sup> Shh also promotes proliferation of neural progenitors of the adult hippocampus.<sup>31</sup> In contrast to these proliferative effects of Shh, *p53* activity suppresses proliferation and self-renewal of adult neural stem cells of the subventricular zone,<sup>32</sup> and this effect might be mediated by p21<sup>Cip1</sup>.<sup>32,33</sup> This may explain why *p53* activity is downregulated during neurogenesis<sup>34</sup> to allow cell proliferation and brain formation. Interestingly, Gli activity has been shown to downregulate *p53* protein levels in cell lines.<sup>35</sup> Specifically, it has been proposed that high levels of Hh signaling in cell lines caused by expression of constitutively active Smo mutants or overexpression of Gli1 and Gli2 leads to an Mdm2-dependent degradation of *p53*.<sup>36</sup> Consistently, low levels of Mdm2 *in vivo* increase *p53* levels, lead to cerebellar hypoplasia and reduce medulloblastoma development in *Ptch1*<sup>+/-</sup> mice,<sup>37</sup> showing that *p53* signaling negatively controls cerebellum growth and implying that *p53* signaling is important for medulloblastoma tumor suppression. Similarly, the proto-oncogene *PPM1D*, a negative regulator of *p53*, is overexpressed in medulloblastomas<sup>38</sup> and increases medulloblastoma formation in mice when overexpressed together with Shh.<sup>39</sup> Together, these findings provide strong evidence that Hh signaling leads to a functional inactivation of *p53* signaling that allows GCP proliferation and, in some instances, medulloblastoma formation. However, the presence of somatic *p53* mutations in *Ptch1*<sup>+/-</sup> medulloblastoma<sup>27</sup> demonstrates that the ability of Shh signaling to functionally suppress *p53* signaling is not always sufficient to inactivate *p53* activity in a tumorigenic context.

For many years, it has been known that *p53* deletion accelerates medulloblastoma formation and increases medulloblastoma incidence in *Ptch1*<sup>+/-</sup> mice;<sup>40</sup> however, the mechanism responsible for this was never investigated. The fact that *p53* mutations, which are acquired spontaneously during medulloblastoma formation, lead to senescence evasion provides an

explanation for the presence of somatic *TP53* mutations in human SHH medulloblastoma.<sup>27</sup>

### Cell senescence as a driver of *p53* inactivation in medulloblastoma

Gli1 or Smo overexpression in cell lines causes high levels of oncogenic stress.<sup>35,36</sup> Recently, we also found that the ligand Shh induces DNA damage in GCPs, an effect that requires the presence of the Hh receptor Boc and CyclinD1.<sup>23</sup> These results support the hypothesis that GCPs are sensitive to replicative stress<sup>41</sup> and that hedgehog signaling likely causes replication stress. It has been demonstrated that oncogene activation leads to oncogene-induced cell senescence (OIS), a tumor-suppressive mechanism that prevents transformation of premalignant lesions into tumors.<sup>42</sup> Moreover, oncogene-induced DNA damage seems to be required for OIS.<sup>43,44</sup> In light of this, the fact that we found cell senescence and *Ptch1* LOH in medulloblastoma preneoplastic lesions<sup>27</sup> suggests that high levels of hedgehog signaling in preneoplasia likely cause high levels of oncogenic stress and this leads to cell senescence. Therefore, we propose that high levels of hedgehog signaling shape the molecular evolution of medulloblastoma by leading to OIS and creating selection pressure to inactivate *p53* in order to evade OIS.

In addition to oncogenes, loss of tumor suppressor genes has been shown to cause OIS.<sup>45</sup> For example, in the context of prostate cancer, *Pten* inactivation causes senescence and leads to the acquisition of *p53* mutations.<sup>46</sup> *Neurofibromin* (*NF1*) loss also leads to Ras-mediated induction of cell senescence.<sup>47</sup> Since we observed a strong association between *Ptch1* loss and cell senescence in medulloblastoma preneoplasia,<sup>27</sup> *Ptch1* seems to be a new tumor suppressor whose absence may be capable of causing OIS.

*TP53* mutations are also present in WNT medulloblastoma.<sup>7</sup> However, the molecular mechanism leading to *TP53* mutations has never been investigated in WNT medulloblastoma. Interesting work has shown that, at least in cell lines, overexpression of the downstream Wnt effector  $\beta$ -catenin leads to *p53* stabilization and activation.<sup>48</sup> Wnt activation in colorectal cancer has been associated with decreased levels of proliferation<sup>49</sup> and accumulation of *p53* protein in early tumor stages,<sup>50</sup> suggesting that Wnt signaling may lead to cell senescence in precancerous lesions and this could be the cause of the acquisition of *TP53* mutations at late stages of colorectal cancer. Additionally, it was shown that  $\beta$ -catenin overexpression causes OIS in thymocytes.<sup>51</sup> We thus speculate that Wnt activation in the brainstem may also lead to changes compatible with cell senescence and this may be an explanation for the presence of *TP53* mutations in advanced WNT medulloblastomas. This is supported by the fact that expression of an active  $\beta$ -catenin mutant in the lower rhombic lip of mice leads to the formation of hyperplastic lesions that only progress to advanced medulloblastomas when *p53* is also deleted.<sup>52</sup>

High-grade astrocytomas frequently harbor *TP53* mutations.<sup>53</sup> It has been shown that low-grade astrocytoma lesions display a DNA damage response<sup>54,55</sup> and that loss of components of the Atm-Chk2-*p53* pathway accelerates astrocytoma development.<sup>55</sup> Although cell senescence was

not interrogated in those reports, another study showed that pilocytic astrocytomas (PA), the most benign type of astrocytomas, display MAPK activation and OIS;<sup>56</sup> interestingly, PA are indolent tumors that display long periods of growth arrest, rarely become high-grade astrocytomas, and do not display *TP53* mutations. This is in agreement with the idea that PA are long-term senescent astrocytic lesions that do not progress.<sup>56</sup>

### Abbreviations

EGL	External Granule-cell Layer
GCPs	Granule Cell Precursors
Hh	Hedgehog
IGL	Internal Granule-cell Layer
OIS	Oncogene-induced senescence
Shh	Sonic hedgehog

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No potential conflicts of interest were disclosed.

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