The role of OTX2 in medulloblastoma

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General introduction
Medulloblastoma as a heterogeneous disease

Histology and clinical aspects of medulloblastoma

Medulloblastoma is a malignant embryonal tumor arising from the cerebellum (Figure 1). With an incidence just below 1 per 100,000 children, it is the most common malignant brain tumor in children [1, 2]. Boys have a higher incidence than girls (7.5 and 4.8 per 1,000,000 boys and girls respectively) [3] and the incidence peaks at 7 years of age [4]. However, tumors also occur in adults, albeit far less frequent than in children [5-7].

Figure 1. Sagittal (A) and axial (B) section of a MRI scan of a patient with a medulloblastoma. Adapted from Figure 1 of Crawford et al. 2007 [3].

The 5-year overall survival rate of medulloblastoma patients has increased over the past decades from 30% [8] to 60%-80% [3, 9, 10]. This is largely a result of improved treatment strategies, which currently consist of surgical resections, radiation therapy and chemotherapy. However, many children still die from this disease or suffer from long-term side effects of the treatment they receive [11-15].

In the current risk stratification, patients are stratified as average risk if the patient is older than 3 years, has no metastatic disease and has a near-total resection (<1.5 cm of residual disease). Both metastatic disease and residual disease have been shown to decrease outcome [16]. Younger children are classified as high risk, because no radiotherapy is performed in that age group. This risk stratification is however limited in its prediction of the outcome for the patients.

Based on the histology of the tumor, the pathological classification of the World Health Organization recognizes classical medulloblastoma and 4 variants thereof [5]. Classical medulloblastomas represents the majority of tumors (60 – 80%). These tumors are characterized by a sheet of small, round, densely packed cells with hyperchromatic nuclei and limited cytoplasm [5]. The nodular/desmoplasmic medulloblastomas, which are the most common of the variants, are defined by dense nodules of differentiated neurocytic cells
surrounded by high proliferating undifferentiated cells with intercellular reticulin [5]. When these tumors mainly consist of these nodules, the tumor is classified as a medulloblastoma with extensive nodularity. Large cell medulloblastomas are characterized by large cells with prominent nucleoli and more cytoplasm compared to classic medulloblastomas. The last variant, anaplastic medulloblastoma, shows enlarged, tightly packed pleomorphic nuclei showing angulation, mounding and wrapping. In light of the clinical outcome, the tumors with either large cell or anaplastic histology are associated with the worst outcome, while patients with desmoplastic/nodular medulloblastomas have a slightly improved outcome [17, 18]. However, the clinical significance is limited and precise classification is difficult, as multiple features can be found within one tumor. Although the overall survival of medulloblastoma patients has increased, there is still much to gain from better risk stratification and improved treatment. Towards that end, the biology of the tumor needs to be better understood to recognize the molecular events that are at the genesis of the tumor.

**Biology of medulloblastoma**

The first clues of the molecular pathways potentially involved in medulloblastoma tumorigenesis came from familial cases. In cancer predisposition syndromes, including Gorlin, Turcot, Li-Fraumeni, blue rubber-bleb nevus, Nijmegen Breakage and Rubinstein-Taybi syndrome, patients have an increased risk to develop medulloblastoma [19, 20] [21-26]. As the molecular events in these tumors have been characterized, their role in medulloblastoma has also been investigated.

Germline mutations in *PTCH1* are the most common cause of Gorlin syndrome. Patients mostly develop basal cell carcinoma, but also have an increased risk for developing medulloblastoma [27]. The mutations in *PTCH1* result in an aberrant activation of the sonic hedgehog (SHH) pathway, which plays a critical role in normal development of the cerebellum. Besides somatic *PTCH1* mutations, mutations in other components of the SHH pathway, including *PTCH2, SMO* and *SUFU*, which all cause SHH pathway activation, also occur in sporadic medulloblastoma [28-33]. A range of mouse models for medulloblastoma have been developed based on activated SHH signaling [34].

Patients with Turcot syndrome caused by *APC* mutations also have an increased risk of developing medulloblastoma. When the tumor suppressor *APC* is mutated, it is unable to halt the activity of the wingless (WNT) pathway, causing aberrant activation of this pathway. In sporadic medulloblastoma *APC* mutations are rare [35]. However, in 2-10% of all medulloblastoma, activating mutations are found in *CTNNB1* (*beta-catenin*), which is
a downstream effector of the WNT pathway [35-41]. These mutations enable CTNNB1 to escape degradation, to localize in the nucleus and to function as a transcriptional activator of genes, including MYC and CCND1. Other aberrations within the WNT pathway, affecting AXIN1 or AXIN2, have also been reported in medulloblastoma [38, 42]. Recently, the first mouse model for medulloblastoma with an aberrant WNT signaling has been described [43].

The frequency of mutations of TP53, which causes Li Fraumeni syndrome, has been variable within medulloblastoma literature, ranging from 0 to 10% [44-48]. Mutations are mostly found in metastatic disease, aggressive tumors or recurrences after treatment [48, 49]. Interestingly, this poor prognosis has not been validated in other studies, where somatic P53 mutations commonly co-occur with CTNNB1 mutations in tumors, thereby predicting a favorable outcome for the patients [46, 50]. In contrast, however, medulloblastomas with germline P53 mutations always show activation of SHH signaling and are characterized by massive chromosome rearrangements which occur in a one-step event called chromothripsis [51]. Often these DNA rearrangements lead to high-level amplifications of genes activating the SHH pathway. Although the overall frequency of P53 mutations in human medulloblastoma is low, many mouse models of medulloblastoma are derived in p53 null background [34].

Cytogenetic analyses of medulloblastoma also provided candidates for medulloblastoma biology. The most common genetic aberration in medulloblastoma is loss of chromosome arm 17p. This is often coinciding with a gain of chromosome arm 17q in the form of an isochromosome 17q [52-57]. Concomitant with these gains, chromosome arm 17p is lost. Many genes in this region have been proposed to play a role in medulloblastoma, such as TP53, REN, MNT and HIC. However, it is unclear how the reduced expression of these genes contributes to medulloblastoma formation, as the other allele of these genes seems mostly unaffected [48]. Also, a second hit of the remaining 17p has not been identified. A possible explanation could be haploinsufficiency of genes on 17p. Alternatively, a mild increased expression of genes on 17q might be beneficial for the tumor, as i17q seems to occur more often in recurrent tumors than in primary tumors [58]. Other major cytogenetic aberrations include monosomy of chromosome 6, loss of 9q and gains of 1q and 7 [1, 54, 55, 59-61].

Cytogenetic analyses also commonly find amplifications of members of the MYC family. MYC and MYCN amplification are most frequent (3-10% each) [10, 54, 55, 62-64], but MYCL amplifications also occur [65, 66]. Other amplifications have been found in medulloblastoma as well, but their frequencies are much lower. Examples include OTX2 ([67-70], ERBB1 and ERBB2 [71-73], TERT [74], GLI1 and GLI2 [75], cell cycle genes such as MDM2 [71], CDK4, CDK6 [65], CCND1, CCNE2 [65] and histone modifying genes such as JMJD2B, JMJD2C and
MYST3 [65, 76]. Homozygous deletions have been described for CDKN2A (p16) [77] and several histone modifying genes such as DMBT1, EHMT1, SMYD4, L3MBTL3, SCML2 [65, 78].

Finally, a number of genes and pathways have been investigated for (in)activating mutations. However, only sporadic mutations have been reported for NRAS [79], CXCR4 [80], P75 [81], PIK3CA [82], PMS2 [83], BRCA2 [84], MSH6 [85], NBS [86-88] and MXI [89]. Recently, the first genome-wide screening for mutations in medulloblastoma has been published. Besides mutations in genes such as PTCH1 and CTNNB, a number of mutations have been reported in histone modifying genes, including MLL, MLL2, MLL3, SMARCA4, ARID1A and KDM6B [90].

**Molecular classification of medulloblastoma**

Although a large number of genes, pathways and genomic events have been implicated by genetics and cytogenetics in medulloblastoma, the relation between the different affected genes remains to be elucidated. Most studies only investigated a particular gene, pathway or genomic event, thus co-occurrence of these aberrations could not be determined. Furthermore, most studies were performed on relatively small cohorts. As medulloblastoma is a very heterogeneous disease, these cohorts differ greatly on various parameters such as age, gender, histology, metastases at diagnostic and survival. Thus, individual studies were biased, resulting in a high variability between studies.

Recent studies in large well-described cohorts using genome-wide expression profiling together with additional techniques, such as comparative genome hybridization (CGH) or single nucleotide polymorphism (SNP) array and mutation analyses, were performed to define distinct molecular subgroups of medulloblastoma [41, 61, 75, 91, 92]. Even though these studies varied in the number of proposed subtypes, classification of the tumors is comparable. Recently, the authors of these studies reached a consensus, defining 4 major subgroups of medulloblastoma: WNT, SHH, group 3 and group 4 (Figure 2) [93, 94].

The SHH and WNT subgroup comprises 28% and 11% of medulloblastoma incidences, respectively [41, 61, 75, 91, 92, 94]. These subgroups are characterized by activating aberrations in SHH or WNT signaling pathways, both of which have been previously implicated in medulloblastoma. Besides mutations in proteins of the SHH pathway, SHH subgroup tumors are also characterized by frequent MYCN or GLI amplifications and loss of 9q. These tumors have a higher incidence in infants and adults and mostly comprise the desmoplastic histology. Metastases are rare and infants have a good prognosis [10]. WNT subgroup tumors are characterized by frequent monosomy of chromosome 6 [40]. These
tumors occur mostly in older children and are associated with good clinical outcome and no metastases. The molecular studies revealed that aberrations in the SHH and WNT pathway are mutually exclusive. However, as both subgroups cluster away from other subgroups in all published studies, these tumors still share common characteristic, such as a NOTCH and PDGF signaling signature.

Compared to the SHH and WNT subgroups, group 3 as well as group 4 tumors are highly enriched for 17q gains (mostly in the form of i(17q)). Furthermore, these tumors more often metastasize than SHH and WNT subgroup tumors [41, 61, 75, 91, 92, 94]. Also, these tumors contribute to the unequal distribution of gender in medulloblastoma. While the distribution in WNT and SHH is equal, group 3 and 4 tumors occur twice more often in boys. More interesting, tumors of female patients often show a loss of X, suggesting X chromosome associated events.

The group 3 and 4 tumors showed no clear signature associated with a signaling pathway, but
rather with differentiation. Subgroup 3 is characterized by expression of genes associated with photoreceptor and GABAergic differentiation. These tumors also have an increased incidence of MYC amplification, which is associated with a bad prognosis. Group 4 tumors express neuronal and glutameric markers. In general these tumors have a higher proportion of 17q aberrations than the tumors with a photoreceptor signature. Due to the similarities between group 3 and 4 tumors, individual tumors were previously found to switch groups when using different clustering methods [61, 75].

The non-WNT/SHH subgroups are less well defined compared to the WNT and the SHH subgroups. This might be caused by the presence of heterogenous populations of tumor cells, resulting in a more gradual transition between subgroups. As mixed populations occur in a single tumor, these differentiation signatures seem to reflect events in tumor progression rather than tumor initiation [91]. In contrast to WNT and SHH tumors, the molecular mechanism of tumorigenesis is unclear, although they represent 2/3 of all tumors.

Although molecular classification has revealed the complexity of medulloblastoma as a heterogeneous disease, it also highlights the fact that we know little of the molecular mechanisms involved in the majority of tumors. A potential oncogene involved in group 3 and 4 tumors is \textit{OTX2}.

**OTX2 and medulloblastoma**

\textit{OTX2 as a transcription factor}

\textit{Orthodenticle homeobox 2, OTX2}, encodes a transcription factor containing a bicoid-like homeodomain. Together with \textit{OTX1, DMBX1} and \textit{CRX, OTX2} is a human homologue of \textit{Drosophila orthodenticle (otd)} [95]. In the human genome, the \textit{OTX2} gene is located on chromosome 14q22.3 and consists of a least 5 exons, of which 3 are coding (Figure 3A). Different transcripts have been reported, which use different transcription start sites. A proximal transcription start site is located in exon 3, while more distal start sites were located within exon 1 and 2. Studies in mice have shown that these different promoter regions play a role in temporal and spatial expression of \textit{Otx2} in development [96].

Although many different mRNAs have been reported, only 2 isoforms are known for the encoded protein (289 and 297 amino acids). Alternative splicing of the second coding exon causes these different isoforms. This splicing event is well conserved among other species, including mouse, rat, dog and even fly [97, 98]. Both isoforms have a homeodomain at the
N-terminus, while the proline, serine and threonine-rich C-terminal transactivation domain contain a SIWSPA conserved motif and 2 tandem tail motifs. Expression of the shorter isoform has been associated with neuronal tissue [99] (Figure 3B)

**OTX2 in embryonic and cerebellum development**

In early mouse embryogenesis, *Otx2* is expressed within the whole epiblast and visceral endoderm. After the onset of gastrulation, expression is maintained in the epiblast, the anterior visceral endoderm and in leading cells of primitive streak. Later in gastrulation, *Otx2* is expressed in a number of specific regions, such as node derivatives, anterior definitive endoderm, rostral axial mesendoderm and the anterior neuroectoderm [100, 101]. This *Otx2* expression pattern in embryonic development is highly conserved across the different species, including chicken and zebrafish [102-105]

*Otx2* expression is essential in early development, as it is required for correct patterning of the embryo as well as regionalization and lineage specification in the brain [106]. Homozygous *Otx2* knockout mice die during embryogenesis, due to developmental abnormalities. This includes the absence of the forebrain and midbrain [107, 108]. The localization of the cerebellum is also dependent of *Otx2* expression, although it is not expressed within the region that will eventually form the cerebellum. *Otx2* expression marks the border between the midbrain and the hindbrain region. Repression of *Otx2* by ectopic expression of *Fgf8* causes cerebellum-like structures in the midbrain region [109, 110]. Conversely, silencing of *Fgf8* resulted in activation of *Otx2* in the hindbrain and thereby reduced cerebellum

At later stages of development, Otx2 is also expressed in the cerebellum itself. In rat, Otx2 is detected from E16/E17 in the rhombic lip (germinal trigone) [111, 112]. From E19, Otx2 was detected in both the external granular layer and the emerging internal granular layer. At P5, Otx2 is expressed in a gradient, with the highest expression in the posterior regions. Otx1 is expressed in the more anterior cerebellum [112]. In adult rat, only weak cerebellar expression is detected in the granular layer [111].

In mice, Otx2 is first detected in the cerebellar region between E10.5 – E11.5, when a few cells of the rhombencephalon express Otx2 [113]. Otx2 protein is detected at E14.5 in the rhombic lip, while between E15.5 and E17.5 the protein is detected in external granular layer. Like in rat, Otx2 expression forms a gradient with the highest expressing in the posterior regions. At P2, Otx2 is also detected in the emerging internal granular layer. Otx2 mRNA is detected in both the external and internal granular layer at P7, while other structures like the Purkinje cell layer or white matter remain negative [114]. In later stages, P15 and P22, when the external granular layer is dissolved, some Otx2 expression is still found in the internal granular layer [114]. However, immunohistochemical staining only showed very weak staining in the caudal internal granular layer at P15 [113].

In humans, OTX2 expression is first detected in the cerebellar rhombic lib at fetal week 13, while the protein was not detected at fetal week 9 [70, 115]. At week 23, OTX2 is detected in a small number of mitotic cells in the external granular. From 26 weeks onwards, OTX2 is also detected in the internal granular layer. Interestingly, Purkinje cells and dentate nuclei showed cytoplasmic staining at this stage. Whether this protein was derived from mRNA expressed in these cells is unclear. In retinal development, Otx2 can be produced by one cell and than transported to other cells in distant cell layers [116]. No OTX2 expression was detected in the cerebellum postnatal [70].

Otx2 expression in granular progenitor cells seems essential for a proper development of the cerebellum. Experiments, in which Otx2 was conditionally knocked out at different stages of embryonic development, revealed that if Otx2 was knocked out at E16.5 in mice, the mesencephalon and anterior cerebellum developed normally, while the posterior cerebellum showed reduced development and folding [117]. Knockout at earlier stages resulted in more general changes in the brain regionalization.

**DNA-binding and targets**

As a transcription factor, OTX2 can bind to promoters or enhancer elements to regulate gene expression. The DNA binding-domain of OTX2 recognizes specific DNA sequences, the so-called DNA binding sites. Based on resemblance of OTX2 to the Drosophila bicoid gene, it
was hypothesized that OTX2 would have a similar binding sequence [118, 119]. Indeed, the bicoid-binding sequence, TAATCC, or strongly resembling sequences have been discovered in the promoters of OTX2 target genes like Tnc, Rbp3, Wnt5a, Gnrh1, DCT, RAX, [118-123].

Although this approach to discover an OTX2-binding site in promoters was biased by the assumption that this sequence should resemble that of bicoid, this DNA-binding sequence has been validated. Based on an in vitro binding sequence assay, the TAATCC core sequence showed the strongest potential for binding OTX2 [124]. However, the same authors also revealed binding to other sequences, such as TAAGCC, although these interactions were weaker. Hence, TAATCC seems the most potent OTX2-binding site, but other sequences also enable OTX2 binding.

OTX2 can bind to DNA as a dimer. This dimerization is thought to require a palindromic binding site or two closely spaced binding sites, but Briata et al. reported that one site is sufficient for dimerization [118]. Chatelain et al. argued that this was an in vitro artifact and that multiple binding sites are required for dimerization, because each site can only be bound by a single OTX2 [124]. They conclude that OTX2 normally binds as a monomer, but that dimerization occurs when two sites are closely spaced. In line with this idea, heterodimerization of OTX2 with homologue DMBX1 has been reported to occur on the TAATCCGATTA sequence [125]. As CRX and OTX1 are even more related to OTX2 and share the binding specificity to TAATCC related sequences, heterodimerization has also been implied for these proteins [126].

Besides a difference in affinity, it is currently unclear whether there is a biological relevance for the different sequences to which OTX2 can bind. Similarly, no functional difference is known for monomeric and dimeric binding of OTX2. The existence of tandem binding sites and (inverted) palindromic binding sites in promoter regions has not been linked to specific regulatory features.

Protein interaction
OTX2 binding to DNA by itself does not regulate gene expression. To enable gene regulation, OTX2 needs to interact with other regulatory proteins, as mutations and deletions outside the DNA binding domain of OTX2 reduce gene activation by OTX2 without altering the DNA binding [124]. Furthermore, overexpression of DNA binding domain-lacking recombinant OTX2 proteins inhibited normal OTX2 gene activation.

The precise protein complex that interacts with OTX2 on the promoter to regulate gene
expression, is unknown. However, a number of physical interactions between OTX2 and other regulatory proteins have been reported. TLE4, MEIS2, MITF, SOX9, SOX2, LHX1 (LIM1) and FOXA2 (HNF3B) were all shown to bind OTX2 via immunoprecipitation [123, 127-134]. Gene activation by OTX2 was repressed by FOXA2 or TLE4 binding, while LHX1, MEIS2, MITF, SOX2 and SOX9 binding to OTX2 enhanced promoter activity [128, 131]. Because these studies are limited to in vitro regulation of selected promoters, the importance and frequency of these interactions in vivo are unknown.

Based on yeast two-hybrid studies or on described binding to OTX1 or CRX, there are several other potential candidates for direct interaction. These include ALX4, ATP5, ATXN1, ATXN7, C2ORF65, CDC25A, CDC25C, CHIC2, DMRT2, EMX1, HOXB13, KAT2A, KRTAP4-12, MDF1, MIXL1, NRK, NLR, PSMC5, RBPMS, RPL24, SP1, SP3, SP4, TLX3, ZBTB3, [132, 133, 135-140]. Some of these candidates, like ATP5, RBPMS, CDC25A, CDC25C and RPL24, have no direct role in transcriptional regulation. However, several of these genes are potential transcriptional regulators. Especially the direct interaction of OTX2 with ATXN7 and KAT2A (GCN5) is worth noting. Both proteins bind CRX and binding to OTX2 has been reported, although no evidence of direct binding with OTX2 was shown [141]. If OTX2 indeed recruits ATXN7 and GCN5, this implies that OTX2 activates genes expression via histone acetylation. Although not a transcriptional regulator, OTX2 was also shown to bind EIF4E, a protein involved in initiation of mRNA translation [142]. Even though it is unclear what the functional consequences of this binding are, this might implicate a non-nuclear function for OTX2.

OTX2 in disease
A role for OTX2 has been implicated in a number of diseases affecting the eyes or the pituitary gland, including syndromic microphthalmia 5 (MCOPS5), combined pituitary hormone deficiency-6 (CPHD6), early-onset retinal dystrophy and pituitary dysfunction, bipolar disorder and branchiootoorenal syndrome. In a patient with branchiootoorenal syndrome and oculoauriculovertebral spectrum features, OTX2 was duplicated due to complex chromosomal rearrangements [143]. In all other reported cases, OTX2 was found heterozygously mutated [99, 124, 144-152]. In vivo studies of these aberrant OTX2 proteins revealed that the mutations either abolished DNA binding or reduced transactivation. In none of the reported cases it was investigated whether these mutations also caused cerebellar malformations.

OTX2 in medulloblastoma
OTX2 was first identified by Michiels et al. as highly expressed in medulloblastoma compared to normal cerebellum by SAGE analyses [153]. They observed that expression of
OTX2 was a common event in medulloblastoma. Many subsequent studies confirmed the overexpression of OTX2 in medulloblastoma, both on mRNA and protein level (Figure 4) [67-70, 154]. The percentage of OTX2 expressing tumors ranges between 60 – 85% among cohorts. The percentage is dependent on the number of SHH subtype tumors within the cohort, which is correlated with the age distribution and histology [61, 70]. Yokato et al. remarked that OTX2 expression was absent in Ptch-/- mice derived tumors [154]. Our group has previously published that both desmoplastic tumors and very young or older patients showed less OTX2 protein expression [70]. In hindsight these observations are in line with the absence or low OTX2 expression in tumors of the SHH subgroup, which are enriched for desmoplastic histology and occur more frequent in very young children and adults [61]. The first evidence for an oncogenic role of OTX2 was provided by the discovery of OTX2 amplifications in medulloblastoma using digital karyotyping [68, 69]. The human medulloblastoma cell lines D425, D458 and D487 all contained over 10 copies of OTX2. As D425 and D458 were both derived from the same patient, the amplification of OTX2 seemed tumor specific rather than an artifact of cell culture. Indeed, recent FISH analysis of the tumor tissues from which D425, D458 and D487 were derived, revealed amplification of OTX2. Furthermore, amplifications of the OTX2 locus were shown for 8 out of 42 primary tumors [68]. Other papers have confirmed the amplifications in the cell lines, but not in tumors [67, 69, 70]. Recent analyses using SNP arrays revealed 10 focal gains (< 1 Mb) and 11 large 14q gains in 201 primary tumors and 12 cell lines, including the amplifications in D425 and D458 [67].

The clinical significance of OTX2 gains remains to be fully explored. Adamson et al. observed that the amplifications were restricted to the non-WNT, non-SHH subtypes and to tumors with anaplastic features [67]. They suggest a relation between OTX2 gain and survival, but because of low numbers of tumors combined with histological bias no clear conclusion can be made. Currently, no other genomic or genetic aberrations, such as mutations or translocations, of OTX2 have been reported [68, 70] (Bunt unpublished). In vitro manipulation confirmed the oncogenic function of OTX2 in medulloblastoma. Transient silencing of OTX2 expression in medulloblastoma cell lines using siRNA by Di et al. resulted in reduced cell proliferation in cells with endogenous OTX2 expression [69]. These results were later reconfirmed by the same group using both siRNA and lentiviral shRNA. Overexpression of OTX2 in immortalized rat kidney epithelium cells (RK3E) resulted in increased colony forming potential and enabled tumor formation in mouse brain. Overexpression in a medulloblastoma cell line lacking endogenous OTX2 expression increased the proliferation rate, while transduction of D425 cells with OTX2 shRNA reduced the growth of xenografts intracranially [155].
Scope of this thesis

With increasing knowledge of the biology of medulloblastoma, it is clear that this malignant brain tumor is very heterogeneous at the molecular level. One of the implied oncogenes in medulloblastoma, the transcription factor OTX2, is highly and specifically expressed in over 75% of all medulloblastomas. Therefore this gene is an interesting candidate for targeted therapy. However, little is known about the function and downstream partners of this gene in medulloblastoma.

In this thesis, the role of OTX2 and its direct target genes in medulloblastoma have been investigated. The effect of inducible ectopic expression of OTX2 in medulloblastoma cell lines without endogenous OTX2 will be addressed in Chapter 2. Subsequently, growth inhibition and neuronal-like differentiation after silencing of OTX2 in a medulloblastoma cell line with high endogenous OTX2 levels will be described as well as the direct targets of OTX2 (Chapter 3). Next, the DNA binding characteristics of OTX2 in medulloblastoma will be investigated (Chapter 4). In Chapter 5, the relationship between OTX2 expression and histone modifications in medulloblastoma will be explored. Finally, the relevance of OTX2 in medulloblastoma will be discussed.

Figure 4. Expression of OTX2 in medulloblastoma. (A) OTX2 is highly expressed in over 70% of all primary medulloblastoma. OTX2 is mainly expressed in the WNT group as well as in group 3 and 4, while the SHH subgroup has little to no expression. (B) OTX2 (brown) is expressed specifically in tumor cells and not in the surrounding normal tissue. Figure B adapted from De Haas et al 2006 [70].
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