The role of OTX2 in medulloblastoma

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Discussion
Discussion

**OTX2 as an oncogene in medulloblastoma**

OTX2 expression has been implicated in the biology of medulloblastoma since 1999 [1], but until recently little was known about its molecular role in medulloblastoma. In recent years, OTX2 has been established as an oncogene expressed in the majority of medulloblastoma [2-9]. This thesis describes the biological effect of OTX2 manipulation in medulloblastoma, showing that OTX2 promotes proliferation and inhibits differentiation. Furthermore, downstream molecular studies like the identification of target genes by expression profiling and ChIP-on-chip assays revealing the effects of OTX2 on H3K27 methylation have been performed.

**Drivers of OTX2 expression**

Although OTX2 has been recognized as an oncogene and a marker for the majority of medulloblastoma, little is known of the upstream regulation of OTX2 expression. OTX2 is focally gained in about 20% of all medulloblastoma [2, 10] and high level amplifications of OTX2 (>100 copies) have also been reported but are less frequent [2, 4, 8-10]. Still frequent increased copy numbers do not explain why high overexpression of OTX2 is found in over 70% of all medulloblastomas. Furthermore, there are no reports suggesting genomic aberrations in the promoter region of OTX2.

Even though the spatial and temporal expression from the various promoter regions of OTX2 have been studied, little is known about the transcription factors regulating OTX2 expression in medulloblastoma [11-16]. For the WNT subgroup of medulloblastoma, the aberrant activation of beta-catenin (CTNNB1) may drive OTX2 expression. Based on studies in brain and eye development, OTX2 can be a direct target of the WNT pathway [12, 13, 16, 17]. For the Group 3 and 4 medulloblastomas there are currently no candidate transcription factors known. OTX2 itself can regulate its promoter in an autoregulatory loop, providing another potential explanation for the high OTX2 expression [18, 19].

**Clinical value of OTX2**

As a clinical marker, OTX2 immunohistochemical staining was associated with classical medulloblastoma, but has no further prognostic value [8]. In hindsight, OTX2 is a marker highly expressed in all medulloblastoma, except for the SHH subgroup. This subgroup contains many desmoplastic tumors, which explains the association of OTX2 with classical medulloblastoma. In the other three subgroups, WNT, Group 3 and Group 4, OTX2 expression...
is very high in all tumors and expression levels do not show any correlation with clinical parameters. However, based on this high expression in many tumors and the absence of expression in normal brain, OTX2 is an interesting candidate for targeted therapy.

Potential drugs against OTX2 are retinoic acids. In embryonic development, OTX2 promoter activity can be suppressed by all-trans retinoic acid [20]. Subsequently, several studies have shown that OTX2 expressing cell lines are sensitive to different retinoic acids and OTX2 mRNA and protein levels are reduced after treatment [3, 9, 21]. Of the different retinoic acids tested, 9-cis retinoic acid seems to be most potent in suppressing OTX2. The effect of retinoic acid is dependent on OTX2 expression, as overexpression of OTX2 enables cells to overcome the sensitivity for retinoic acids [3, 6]. In flank xenografts treated with 9-cis retinoic acid, tumor growth is{Lock, 2011 #3249}decreased and differentiation is induced, similar to our OTX2 silencing experiment in D425 cells [22].

Intracranial xenografts of D425 cells however did not respond to 9-cis retinoic acid treatment [3]. The authors suggest this is caused by an opposing effect of FGF signaling, which can be reduced by drugs targeting this pathway. However the authors only used D425 cells, which have a high level amplification of OTX2, and did not assess the level of knockdown in the tumors. These cells might be less sensitive to retinoic acid-induced promoter repression than cells without amplification. Furthermore, the local concentration of retinoic acid might be insufficient to be effective. More extensive studies are needed to investigate retinoic acids as a drug against OTX2 expressing tumors. The newly described mouse models for WNT might be very useful in this context [23].

Targeting transcription factors in tumors is difficult. Besides directly targeting of OTX2 expression, there are two alternative approaches for therapy. First of all, downstream targets of OTX2 can be targeted. We discovered that many mitotic cell cycle genes are direct targets of OTX2 [5, 6]. Among those genes are AURKA, MELK, or CENPE, for which drugs are available [24-27] Alternatively, the genes required for oncogenic acceleration by OTX2 could be targeted. As we showed by inducible overexpression of OTX2 in MED8A and DAOY cells, OTX2 overexpression alone leads to senescence [5]. In contrast, Adamson et al found that stable overexpression in non-neoplastic rat kidney cells (RK3E) or transient transfection of OTX2 in medulloblastoma cell line MHH1 induced proliferation [2]. This suggests that OTX2 requires a specific cellular context in order to drive proliferation. Understanding more about the molecular difference between these cells may provide novel targets to switch OTX2 function in cancer cells.
Photoreceptor genes as targets of OTX2

As previously described, OTX2 is expressed within 3 molecular subgroups of medulloblastoma (WNT, Group 3 and 4), which show many differences at the molecular level. Group 3 tumors show many genes that have been described as targets of OTX2 in retinal development, such as RBP3, TULP3, RP1 and GNGT2. These genes seem genuine targets in medulloblastoma, as they were downregulated in D425 cells after OTX2 silencing and most of these genes bind OTX2 in their promoters. However, these genes are not expressed within the WNT group or most of Group 4. However, there is a subset of Group 4 tumors that express photoreceptor genes as well as neuronal genes that are markers of Group 4. Cho et al performed immunohistochemistry on these tumors using CRX (photoreceptor gene) and GRM8 (neuronal gene). Interestingly, they found that these genes are expressed within distinct populations in these tumors. Assuming that OTX2 is expressed within both cell types, co-factors seem to be required to alter the targets within each cell type. As photoreceptor genes are normally not expressed within the developing cerebellum, they may represent a progression within the tumor cells with a co-factor that turned on activation of photoreceptor genes by OTX2. Elucidating this process may provide us with important knowledge about the biology of OTX2 positive medulloblastoma as it is currently unknown whether these photoreceptor-specific genes have any role in medulloblastoma tumorigenesis.

Understanding the role of OTX2 in medulloblastoma

We are only slowly starting to understand the role of OTX2 in medulloblastoma. Based on recent data using medulloblastoma cell lines, OTX2 dependence is evident. Unfortunately, the cell lines with OTX2 expression most likely represent only Group 3 tumors, especially those with MYC amplification. Additional experiments using the mouse model for WNT group tumors [23] might be helpful to further underline the importance of OTX2 in medulloblastoma.

As we have identified both in our overexpression and silencing cell models, OTX2 can induce proliferation by direct regulation of cell cycle genes. However, this function is context dependent, as overexpression to physiological levels causes oncogenic stress. A potential partner in crime is the MYC oncogene. Within a subset of Group 3 tumors MYC is amplified and high levels of MYC are also present in all WNT tumors and some of the Group 3 and Group 4 tumors. MYC is known to drive G1-S cell cycle, while OTX2 seems to play a bigger role in the G2-M part of the cell cycle. Interestingly, OTX2 also seems to directly affect MYC protein levels, as OTX2 silencing in D425 results in a small decrease in MYC mRNA, but a large decrease in MYC protein level. A MYC stabilizing effect might be one of the advantages of OTX2 expressing tumors. Furthermore, our analyses of both OTX2 and MYC binding in
D425 cells clearly distinguish a set of shared targets, which comprise of highly expressed genes which are enriched for medulloblastoma and progenitor cell specific genes.

In regard to the interaction between MYC and OTX2 in medulloblastoma, the recently described MYC driven mouse models are intriguing [22, 28]. Although in both described models the resulting tumors are most similar to Group 3 tumors, they might not be representative for Group 3 tumors. First of all, only half the Group 3 tumors highly express MYC. Secondly, in the mouse models the tumors do not express Otx2 or any photoreceptor genes, which are characteristic for this subgroup. In both described models, tumors only arise within a P53 defective background [22, 28], while in human primary tumors P53 mutations are not associated with MYC tumors [29, 30]. Furthermore in one model described by Kawauchi et al the cells where also Cdkn2c null, while in the model from Pei and colleagues a degradation resistant MYC mutant was used (refs). Therefore even though these mouse models most resemble Group 3 tumors they do not really fully capture the molecular background of Group 3. Most likely the similarity is only based on the expression of MYC and its target genes. It would be interesting for instance to combine these mouse models with the recently described mouse models, which overexpresses Otx2 in the hindbrain, but do not form tumors [31].

Mode of function

Although we know now more about the targets of OTX2 and the effect of overexpression or silencing of OTX2 in medulloblastoma, the exact function of OTX2 remains unclear. OTX2 binds to many promoters in medulloblastoma cells, of which only a small part is regulated after either ectopic expression or silencing. We can speculate that therefore only part of its bound genes can be considered transcriptional targets in the classical sense. An alternative function of OTX2 might lie within control of the global histone modification landscape, thereby allowing or preventing regulation of genes by their cognate transcription factors. Indeed, we found that OTX2 has a distinct effect on H3K27 methylation. The OTX2-bound promoters display a bivalent-like state of histone modifications, with high levels of the activating H3K4me3 and H3K9ac marks and intermediate levels of the repressive H3K27me3 mark. Upon silencing of OTX2, the H3K27me3 levels strongly decrease, while H3K4me3 and H3K9ac levels remain unchanged. Nevertheless, of the OTX2-bound genes, only 7% shows an increased expression and also 7% a decreased expression after OTX2 silencing. The H3K27me3 mark is therefore strongly reduced in OTX2-bound promoters, but expression can go up, down or remain unchanged. This suggests that the primary effect of OTX2 is to maintain moderate H3K27 levels at the OTX2-bound promoters. When OTX2 is silenced and no longer can bind to these promoters, the H3K27me3 disappears and genes can be regulated by specific transcription factors.
Reference List