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Investigation of Notch and TP53 Signaling in Pediatric Medulloblastoma

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Medulloblastoma is the most common malignant brain tumor during childhood with a considerably high mortality rate of about 30%. Many survivors suffer from severe treatment induced sequelae. To date, risk stratification consists of clinical and partly histological markers, which do not reflect the true heterogeneity of medulloblastomas regarding tumor biology. Hence, a better understanding of the underlying molecular pathomechanisms is needed to improve current outcome prediction models and therapy options.

For further elucidating the molecular pathogenesis of primary medulloblastoma, mutational analysis of *Notch1* and *Notch2* receptor, immunohistochemical analysis of Notch2 protein expression and lentiviral knockdown of the *Notch2* gene in a medulloblastoma cell line (D425) were performed. We carried out sequencing of *Notch1* in 20 tumor samples, but did not detect any activating mutations. In conclusion, activating mutations of the *Notch1* gene do not appear to be frequent in primary medulloblastoma. For *Notch2*, we identified a previously undescribed nucleotide exchange (P2359A) located in exon 34, a “hot-spot” region for activating mutations of *Notch1* in T-cell acute lymphoblastic leukemia (T-ALL). Overall, the nucleotide exchange (P2359A) was detected in five out of 250 sequenced primary medulloblastoma samples (2% frequency), but was also identified in the germline DNA of one patient. Thus, the nucleotide exchange (P2359A) seems to be a rare nucleotide polymorphism, which might be associated with the pathogenic development of medulloblastoma. Analyzing Notch2 protein expression immunohistochemically in 185 medulloblastoma samples on a tissue microarray (TMA), we could not detect a significant correlation between overall or progression-free survival and Notch2 protein expression. When performing a lentiviral-mediated knockdown of the *Notch2* gene in a medulloblastoma cell line (D425), we observed impaired proliferation and a change in cell shape suggesting a central role of *Notch2* in terms of proliferation.

In the second part of the thesis, a cancer-prone family suffering from Li-Fraumeni syndrome (LFS) with atypical tumors is genetically characterized. LFS is a familiar cancer syndrome that is often linked to germline mutations of the *TP53* tumor-suppressor gene. Sequence analysis of blood samples from all family members revealed a germline *TP53* mutation (R248W) in three family members of two generations. In this family, all carriers of the

mutation suffered from brain tumors as their primary malignancies. The index patient was diagnosed with an anaplastic medulloblastoma harboring an unusual complex genomic profile. Genome-wide DNA copy-number analysis of the medulloblastoma displayed six distinct high-level genomic amplifications, spanning for example the *MYCN*, *GLI2*, *API5* and *COSP3* genes. The second tumor of the index patient, an extrarenal rhabdoid tumor, showed a novel high-level amplification of the *MYC* oncogene. The father of this patient suffered from a myxopapillary ependymoma (WHO °I), while a brother died of an early relapse of a choroid plexus carcinoma. Investigating this LFS-familiiy with atypical tumors, we detected novel oncogene amplifications. Familial cancer syndromes are generally rare, however these aberrations could also be of importance in the pathogenesis of similar sporadic tumors.