Elke Katrin Pfaff
Dr. med.

Prognostic and Predictive Value of *TP53* Mutations in Pediatric Medulloblastoma

Promotionsfach: DKFZ (Deutsches Krebsforschungszentrum)
Doktorvater: Prof. Dr. Peter Lichter

Medulloblastoma (MB) constitutes the most common malignant pediatric brain tumor and therefore is one of the leading causes of cancer-related mortality in this age group. Even though considerable therapeutic improvements were achieved during the last three decades, the five-year overall survival rate of approximately 70% for standard and high-risk MB patients is still poor. Commonly used staging systems based on clinical parameters like patient age, metastatic stage at diagnosis, extend of surgery and histopathology do not adequately account for the tumors' heterogeneous genetic background. Therefore, future risk stratification models might be complemented by information about distinct genomic alterations of the tumor allowing for a more precise outcome prediction.
Accordingly, this study was conducted to investigate a potential prognostic and/or predictive value of the *TP53* mutation status in MB.
In a large cohort of 310 primary MB samples *TP53* mutations were confirmed to constitute an infrequent event in MB occurring in less than 7% of cases. Results of p53-immunostaining revealed a strong positive correlation between p53-immunopositivity of the tumor and *TP53* mutation. Concerning the concomitance of *TP53* mutations with other distinct genomic aberrations, notably, *TP53* mutations were not restricted to a certain cytogenetic MB subgroup. However, *TP53* mutations occurred significantly more frequently in MB featuring a high-level *MYCN* amplification as well as in tumors of the prognostically favorable WNT-subgroup. The latter group was further characterized by monosomy 6 and *CTNNB1* mutation. On the other hand, loss of 17p (the chromosome locus of the *TP53* gene), which is a common alteration in MB, was not associated with *TP53* mutations. Two tumors in the context of Li-Fraumeni Syndrome (LFS), which were included in this study, showed a higher genomic instability defined by a higher frequency of copy number aberrations compared to MB with sporadic *TP53* mutation. *TP53* wild-type tumors, however, featured the lowest number of copy number aberrations.
Notably, patient outcome (defined by progression-free survival and overall survival) was not worse for patients with *TP53*-mutated MB compared to patients with *TP53* wild-type tumors. Even when excluding *CTNNB1*-mutated tumors from the survival analysis (which are known to be associated

with a favorable prognosis), no significant difference was observed between patients with *TP53*-mutated and *TP53* wild-type tumors, respectively. Distinct therapy regimen applied may account for controversial results in different studies concerning the correlation of *TP53* mutation status and patient outcome (e.g. application of high-dose cyclophosphamide in a cohort of Tabori and colleagues compared to lomustine in the cohort of this study).

Cell viability assays after treatment of MB cell lines with either 4-hydroperoxycyclophosphamide (4-HC) or lomustine (CCNU) revealed a wider range of calculated values for the half maximal effective concentration (EC_{50}) of the single cell lines after treatment with 4-HC than after treatment with CCNU. Measuring the formation of DNA double-strand breaks (DSB) via γH2AX focus assay after treatment with the alkylating agents could confirm the findings that various MB cell lines showed distinct suspectibility to the alkylating agents 4-HC and CCNU without any correlation with the *TP53* mutation status being observed.

Taken together, the results of this study indicate that sporadic *TP53* mutations in MB constitute a late event in tumorigenesis and are not *per se* associated with an aggressive tumor phenotype. In fact, presumably earlier events determine the tumors' affiliation to distinct cytogenetic MB subgroups and are therefore decisive for prognosis. Taking different treatment regimen into account, a potential predictive value of the *TP53* mutation status dependent on the cumulative dose of alkylating agents applied should be investigated in prospective studies.