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High Resolution Genomic Profiling Identifies Prognostic DNA Copy-Number Aberrations and Novel Oncogenic Pathways in Pediatric Neoplasias

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Survival rates of children with brain tumors and leukemias have significantly improved during the last decades due to developments in diagnostic procedures, multimodal therapies and supportive care. However, these two groups of malignancies remain the leading cause of cancer-related deaths in children. To date, outcome prediction is primarily based on clinical variables, such as patient age, type of neoplasia, stage and localization. However, since novel insights in genetic and biological features of these neoplasias are gained, these might also be useful to further improve risk stratification, therapeutic procedures and thus, outcome of these patients.
To identify novel DNA copy-number alterations and genes relevant to cancer biology, array-based comparative genomic hybridization (array-CGH) was performed in 66 low-grade astrocytomas (LGA), 80 medulloblastomas, 10 supratentorial primitive neuroectodermal tumors and 73 precursor T-cell lymphoblastic leukemias (T-ALL), followed by gene expression studies and functional analyses of promising candidate genes.
In LGA, duplication of the *BRAF* proto-oncogene was the most frequent genomic aberration, and tumors with *BRAF* duplication showed significantly increased *BRAF* mRNA expression as compared to tumors without duplication. Both the stable silencing of BRAF through shRNA lentiviral transduction and pharmacological inhibition of MEK1/2, the immediate downstream phosphorylation target of BRAF, inhibited proliferation of cultured tumor cells derived from LGA. These findings identify aberrant activation of the MAPK pathway due to gene duplication or mutation of *BRAF* as a hallmark pathomechanism in LGA and suggest inhibition of the MAPK pathway as a novel treatment strategy.
Further, a model for molecular risk stratification was proposed from an array-CGH screen conducted in 80 medulloblastoma samples. Fluorescence in situ hybridization (FISH) analyses for chromosome arms 6q, 17p, and 17q and the *MYC* and *MYCN* loci were carried out in an independent validation set (n=260). Genomic alterations were correlated with clinical, histological, and survival data. Gain of 6q and 17q and genomic amplification of *MYC* or *MYCN* were each associated with poor outcome in the array-CGH study. In contrast, all patients with 6q-deleted tumors survived. Given these findings, the following hierarchical molecular staging system was defined: (1) *MYC/MYCN* amplification, (2) 6q gain, (3) 17q gain, (4) 6q and 17q balanced, and (5) 6q deletion. The prognostic value of this staging system was investigated by FISH analysis in an independent validation cohort (n=260). By adding molecular markers to clinical risk factors, a large proportion of patients (72 of 260 patients; 30%) at high risk for relapse and death could be identified, who would otherwise be considered standard risk by application of clinical variables alone.
Noteworthy, DNA copy-number alterations in T-ALL frequently harbored genes which are regulators or downstream intermediates of the TGF-β or the PI3K-AKT pathways and were identified in 25/73 (34%) and 21/73 (29%) of the patients, suggesting that these pathways play a key role in T-ALL leukemogenesis. Furthermore, a deletion at 6q15-16.1 was identified in 9 of 73 (12%) patients. Deletions of 6q15-16.1 were associated with poor early

treatment response. This deletion encompasses the *CASP8AP2* gene. CASP8AP2 regulates apoptosis suggesting a possible functional link between the clinical effect of the deletion and the molecular mode of action.