Epigenetic repression of the dopamine receptor D4 in pediatric tumors of the central nervous system.

Abstract:
Epigenetic alterations are common events in cancer. Using a genome wide methylation screen (Restriction Landmark Genomic Scanning-RLGS) we identified the gene for the dopamine receptor D4 (DRD4) as tumor-specific methylated. As DRD4 is involved in early brain development and may thus be involved in developmentally dependent tumors of the CNS in children epigenetic deregulation of DRD4 and its functional consequences were analyzed in vitro. CpG methylation of DRD4 was detected in 18/24 medulloblastomas, 23/29 ependymomas, 6/6 high-grade gliomas, 7/10 CNS PNET and 8/8 cell lines by qCOBRA and bisulfite sequencing. Real-time RT-PCR demonstrated a significantly inferior expression of DRD4 in primary tumors compared to cell lines and non-malignant control tissues. Epigenetic deregulation of DRD4 was analyzed in reexpression experiments and restoration of DRD4 was observed in medulloblastoma (MB) cells treated with 5-Aza-CdR. Reexpression was not accompanied by demethylation of the DRD4 promoter but by a significant decrease of H3K27me3 and of bound enhancer of zeste homologue 2 (EZH2). Knockdown of EZH2 demonstrated DRD4 as a direct target for inhibition
by EZH2. Stimulation of reexpressed DRD4 resulted in an activation of ERK1/2. Our analyses thus disclose that DRD4 is epigenetically repressed in CNS tumors of childhood. DRD4 is a direct target of EZH2 in MB cell lines. EZH2 appears to dominate over aberrant DNA methylation in the epigenetic inhibition of DRD4, which eventually leads to inhibition of a DRD4-mediated stimulation of the ERK1/2 kinase pathway.