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Original Research

Enhancer of zeste homologue 2 plays an important role in neuroblastoma cell survival independent of its histone methyltransferase activity

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Abstract Neuroblastoma is predominantly characterised by chromosomal rearrangements. Next to V-Myc Avian Myelocytomatosis Viral Oncogene Neuroblastoma Derived Homolog (MYCN) amplification, chromosome 7 and 17q gains are frequently observed. We identified a neuroblastoma patient with a regional 7q36 gain, encompassing the enhancer of zeste homologue 2 (EZH2) gene. EZH2 is the histone methyltransferase of lysine 27 of histone H3 (H3K27me3) that forms the catalytic subunit of the polycomb repressive complex 2. H3K27me3 is commonly associated with the silencing of genes involved in cellular processes such as cell cycle regulation, cellular differentiation and cancer. High EZH2 expression correlated with poor prognosis and overall survival independent of MYCN amplification status. Unexpectedly, treatment of 3 EZH2-high expressing neuroblastoma cell lines (IMR32, CHP134 and NMB), with EZH2-specific inhibitors (GSK126 and EPZ6438) resulted in only a slight G1 arrest, despite maximum histone methyltransferase activity inhibition. Furthermore, colony formation in cell lines treated with the inhibitors was reduced only at concentrations much higher than necessary for complete inhibition of EZH2 histone methyltransferase activity. Knockdown of the complete protein with three independent shRNAs resulted in a strong apoptotic response and decreased cyclin D1 levels. This apoptotic response could be rescued by overexpressing EZH2ΔSET, a truncated form of wild-type EZH2 lacking the SET transactivation domain necessary for histone methyltransferase activity. Our findings suggest that high EZH2 expression, at least in neuroblastoma, has a survival function

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independent of its methyltransferase activity. This important finding highlights the need for studies on EZH2 beyond its methyltransferase function and the requirement for compounds that will target EZH2 as a complete protein.

1. Introduction

Overexpression of the enhancer of zeste homologue 2 (EZH2) gene has been associated with tumourigenicity in numerous solid tumour types [1–7], and gain-of-function point mutations in the catalytically active SET domain of EZH2 has been recognized in B-cell and T-cell lymphomas [8–16]. Genetic loss-of-function studies have demonstrated a crucial role of EZH2 in the establishment of cell fate decisions in the skin, heart and mammary glands [17]. EZH2 together with suppressor of zeste 12 (SUZ12) and embryonic ectoderm development (EED) forms part of the Polycomb repressive complex 2 (PRC2), which mediates the silencing of genes by trimethylation of lysine 27 on histone H3 (H3K27me3) [18,19]. This H3k27me3 mark has been found in genes that play a key role in cellular processes such as cell differentiation, cell cycle regulation and oncogenesis [20–22]. However, recent studies suggest that EZH2 directly binds to the promoter regions of certain genes and acts as a transcriptional co-activator independent of its histone methyltransferase enzymatic activity [23–25].

Neuroblastoma is a neuroendocrine tumour that arises from the peripheral nervous system [26]. It is the most commonly diagnosed extracranial solid cancer in children, accounting for approximately 15% of all pediatric cancer deaths [27,28]. Chromosome 17q gain, partial loss of chromosome 1p or 11q and v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN) amplification are frequently observed genetic aberrations in neuroblastoma tumours [29]. EZH2 is located on chromosome 7q35, and frequent gains of whole chromosome 7 have been observed in neuroblastoma [30,31]. A functional role for EZH2 in neuroblastoma was reported, whereas EZH2 caused histone hypermethylation in the promoter regions of known tumour suppressor genes CASZ1, CLU, RUNX3 and NGFR resulting in the silencing and downregulation of these genes [32]. In the present study, we show that pharmacological inhibition of EZH2 histone methyltransferase activity [33–36] only causes limited inhibitory effects on cell cycle progression, whereas silencing of the whole protein causes a strong apoptotic phenotype. We overcame apoptosis caused by EZH2 silencing by overexpressing a truncated form of wild-type EZH2 lacking histone methyltransferase activity. These findings highlight the importance of EZH2 for the survival of neuroblastoma cells independent of its histone methyltransferase activity and development of compounds that inhibit EZH2 protein as a whole might be beneficial for the treatment of neuroblastoma patients with high EZH2 expression.

2. Materials and methods

2.1. Patient samples, RNA isolation and profiling

RNA was extracted from 88 tumours with TRIzol (Invitrogen, Carlsbad, CA) following the manufacturer's protocols. RNA concentration and quality were determined using the RNA 6000 Nano assay on the Agilent 2100 Bioanalyzer (Agilent Technologies). Fragmentation of cRNA, hybridization to Human Genome U133 Plus 2.0, microarrays and scanning were carried out according to the manufacturers protocol (Affymetrix Inc, Santa Barbara, CA). Messenger Ribonucleic Acid (mRNA) gene expression data were normalized with the MAS5.0 algorithm within the General Comprehensive Operating System (GCOS) program of Affymetrix Inc. Target intensity was set to 100. All data were analysed using the bioinformatics platform R2 (http://r2.amc.nl). As a reference data set, an RNAseq data set of 498 neuroblastoma tumours was used. Data were derived from Gene Expression Omnibus (GEO) database under number gse 62564 [37].

2.2. Array comparative genomic hybridization (Array CGH) analysis

Array CGH was performed by hybridizing 100-ng genomic DNA to a 180 K platform (Agilent Technologies). DNA was labelled by random priming with CY5-dCTP and CY3-dCTP, respectively, and hybridized at 65 °C for about 17 h. Chips were scanned on an Agilent G2565BA DNA microarray Agilent scanner. Data processing was performed using the bioinformatics platform R2. Circular binary segmentation (CGHcall package in R) was used for scoring the regions of gain, amplification, and deletion. Testing for elevated EZH2 expression of tumours with 7q gain versus no gain tumours was determined using a one-tailed Student's t-test for equal variance.

2.3. Cell culture and compound exposure assays

Classical human neuroblastoma cell lines and neuroblastoma tumour-initiating cell (TIC) lines were