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Thesis abstract

Investigation into the biology of human Malignant Rhabdoid Tumour

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Malignant Rhabdoid Tumour (MRT) is a rare paediatric cancer of the kidney and CNS that is resistant to current treatment protocols. Prognostic outcomes remain poorest in infants under the age of one, yet frequency of MRT is highest in these patient cohorts. MRT is genetically characterised by homozygous inactivation of the *SMARCB1* gene, a critical subunit of the SWI/SNF chromatin-remodelling complex. Excluding *SMARCB1*, Next-Generation studies have revealed that there are limited additional recurrent genetic events present, implicating epigenetic deregulation in the pathogenesis of MRT. The ability of Histone deacetylase inhibitors (HDACi) to mimic the histone acetylation functions of the SWI/SNF complex in SMARCB1-null cells provides rationale for investigating the therapeutic potential of HDACi in MRT. Sustained treatment of human MRT cell lines with low-dose Panobinostat (LBH589) led to cell growth arrest and changes in cellular morphology. Transcriptional profiling of three independent MRT cell lines following 21-day treatment by Illumina Human HT-12 v4 Expression BeadChip revealed a marked increase in the induction of neural, renal, muscle and osteoblast differentiation pathways. Additionally, sustained low-dose LBH589 treatment *in vivo* resulted in tumour growth arrest associated with tumour calcification detectable by X-ray imaging. Histological analysis of LBH589-treated tumours revealed significant areas of ossification, confirmed by Alizarin Red staining. Immunohistochemical analysis demonstrated increased TUJ1 and PAX2 staining suggestive of neuronal and renal differentiation, respectively. These data suggest a SMARCB1-dependent SWI/SNF function important for lineage maturation. Re-expression of SMARCB1, in G401 MRT cells under a 4-hydroxytamoxifen inducible vector, phenocopied low-dose LBH589 treatment leading to cell growth inhibition, senescence and terminal differentiation *in vitro* and *in vivo*, suggesting mechanistic similarity. EZH2 is a core subunit of the transcriptional repressive complex, PRC2, which confers transcriptional silencing via the addition of methyl groups to Lysine 27 of Histone 3 (H3K27me^3). Coincidentally, EZH2 is a known HDAC recruiter and SMARCB1 transcriptional target, implicating EZH2 as a potential mechanistic candidate. Intriguingly, EZH2 expression and H3K27me^3 were drastically reduced following sustained low-dose LBH589 treatment in MRT cells. Corresponding reduction of EZH2 and H3K27me^3 was observed in G401 cells following re-expression of SMARCB1. Sustained siRNA knockdown of EZH2 in MRT cells resulted in reduced cell growth and cellular morphology changes associated

with differentiation and senescence. Q-PCR profiling revealed similar genetic signatures in EZH2 knockdown cells as those seen in low-dose sustained LBH589 and SMARCB1 re-expressed MRT cells. Unexpectedly, treatment of MRT cells with the EZH2-catalytic domain inhibitor, GSK126, had a moderate effect on EZH2 expression and partially reduced $H3K27me^3$ and cell growth at doses 1nM-10µM. Excitingly, MRT cells treated in combination with low-dose LBH589 and GSK126 demonstrated a greater reduction in cell growth, *in vitro* and *in vivo*, compared to single agent controls, revealing a synergistic relationship. These data suggest EZH2 is an important mediator of MRT proliferation and differentiation and provide evidence for dual therapeutic targeting of EZH2 with low-dose HDACi in MRT.

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