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DNMT3B is a novel target in high grade chondrosarcoma

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Background: Chondrosarcomas are a clinically and histologically heterogeneous group of malignancies characterized by the presence of neoplastic cartilaginous tissue. Complete surgical resection is currently the only curative modality. Despite surgical excision, nearly 50% of patients with high grade lesions experience local recurrence or distant metastases. As conventional chemotherapy and radiation therapy are ineffective in chondrosarcoma, the development of new therapeutic strategies is of major importance. Alterations in global DNA methylation is an epigenetic process involved in the pathogenesis and progression and many tumors, including in chondrosarcoma. DNA methylation is primarily mediated by the action of DNA methyltransferase (DNMT) enzymes. In particular, DNMT3B is the predominant isoform in articular chondrocytes, and its expression is critical to chondrocyte differentiation, survival, and homeostasis. However, a role for DNMT3B in supporting chondrosarcoma survival and proliferation has not previously been explored.

Purposes: The purpose of this study was to evaluate the expression of DNMT3B across histologic grades of primary human chondrosarcoma. Furthermore, this study explores chondrosarcoma dependence on DNMT3B activity by targeting DNMT3B with a previously established small molecule inhibitor. Collectively, this study aims to identify epigenetic regulation as a novel therapeutic target in treating chondrosarcoma.

Patients and Methods: Twenty-five surgically resected human chondrosarcoma samples were acquired and histologically graded by a board-certified musculoskeletal pathologist. In total, there were five samples of each of the following histologic grade: benign enchondroma, grade I, grade II, grade III, and dedifferentiated chondrosarcoma. DNMT3B expression level was evaluated in each specimen by immunohistochemistry. Simultaneously, previously established chondrosarcoma cell lines (SW1353 and JJ012) were treated with Nanaomycin A, a small molecule inhibitor of DNMT3B. The effect of DNMT3B inhibition on chondrosarcoma cell survival and proliferation was determined by ATP viability assay and BrdU uptake.

Results: Immunohistochemistry staining for DNMT3B revealed low expression levels in benign enchondroma and grade 1 chondrosarcoma lesions, but high expression levels in grade 2, grade 3, and dedifferentiated tumors (Fig. 1A-B). Consistent with these results, qPCR analysis for gene expression revealed significantly higher DNMT3B expression in SW1353 and JJ012 chondrosarcoma cells as compared to healthy human articular chondrocyte controls (Fig. 1C). Inhibition of DNMT3B with Nanaomycin A significantly reduced chondrosarcoma cell viability (Fig. 1D) and proliferation (Fig. 1E) *in vitro*.

Conclusions: In this study, we show increased expression of DNMT3B in more aggressive chondrosarcoma tumors relative to lower grade lesions. Through its role in modifying the tumor epigenetic landscape, DNMT3B might be a critical factor in supporting many features of high-grade chondrosarcoma, including more robust proliferation, prolonged survival, invasion, and metastasis. Additionally, these data suggest that DNMT3B might be a viable diagnostic and prognostic aid in predicting tumor behavior, especially in cases where histologic grade is not immediately clear. Furthermore, we show that targeting DNMT3B with small molecule inhibitor Nanaomycin A limits chondrosarcoma cell viability and proliferation *in vitro*. A direct causal link between DNMT3B expression, DNA methylation landscape, and chondrosarcoma oncogenesis remains incompletely elucidated and will be the focus of future work. These preliminary findings, however, provide early support for the development of epigenome modulating therapies for the systemic treatment of chondrosarcoma.

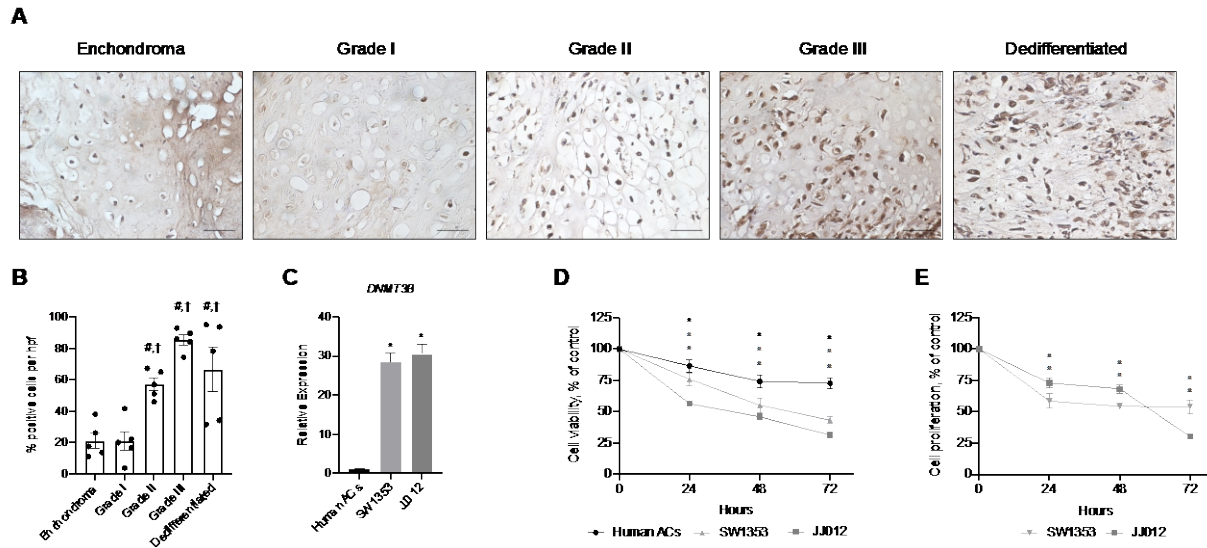


Figure 1: (A) Representative immunohistochemistry images demonstrating increased DNMT3B expression with histological grade in human chondrosarcoma samples and (B) corresponding quantification. Scale bars: 50 μm . #, t , $p < 0.05$ vs. Enchondroma and Grade 1, respectively, via one-way ANOVA with *post-hoc correction*. (C) qPCR analysis demonstrating increased DNMT3B expression in SW1353 and JJ012 chondrosarcoma cells relative to healthy human articular chondrocyte controls. (D) ATP cell viability assay results demonstrating reduced SW1353 and JJ012 cell viability over time with 0.5 μM Nanaomycin A treatment. (E) Cell proliferation assay results by BrdU uptake demonstrating reduced SW1353 and JJ012 cell proliferation over time with 0.5 μM Nanaomycin A treatment. *, $p < 0.05$ via two-tailed Student's t -test ($n = 3$).