Targeting DNMT3B Reveals an Epigenetic Vulnerability in Chondrosarcoma

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Significance and Hypothesis

Chondrosarcoma is the second most common primary malignant bone tumor and remains highly resistant to conventional chemotherapy and radiotherapy, highlighting the need for new therapeutic strategies. Chondrosarcomas demonstrate a low mutational burden and copy number variation at the genetic level, suggesting that epigenetic mechanisms might drive oncogenesis. Epigenetic dysregulation, particularly aberrant DNA methylation, has emerged as an important driver of tumor progression in many cancers, including chondrosarcoma. The methylated epigenome is coordinated by class of proteins called DNA methyl transferases (DNMTs). DNMT3B particularly plays an important role in normal chondrocyte survival and proliferation, however the functional role of DNMT3B in chondrosarcoma is unclear. We hypothesized that DNMT3B is the major driver of aberrant methylation in chondrosarcoma and is critical for chondrosarcoma oncogenesis, identifying it as a novel therapeutic target in this disease.

Innovation

We investigated the biological and therapeutic significance of DNMT3B in chondrosarcoma using integrated bioinformatic and experimental approaches.

Approach

Publicly available gene expression datasets were re-analyzed to evaluate the association of DNMT3B expression with prognosis in chondrosarcoma. Furthermore, previously established chondrosarcoma cell lines were utilized to determine the impact of genetic ablation and pharmacologic inhibition of DNMT3B on chondrosarcoma growth and migration. Prognostic outcomes were analyzed using Kaplan-Meier method and unpaired student's T-test was used for comparative in vitro proliferation and migration studies. Statistical significance was defined as $P < 0.05$.

Results

Analysis of public datasets revealed that elevated expression of DNMT3B is associated with significantly worse overall survival in chondrosarcoma patients (Figure 1A). Consistently, chondrosarcoma cell lines showed markedly increased DNMT3B expression compared with normal articular chondrocytes. Pharmacologic inhibition of DNMT3B significantly reduced CS cell viability, migration, and invasion while having minimal effects on normal chondrocytes. Although DNMT inhibitors such as Nanaomycin A and 5-azacytidine effectively suppressed CS cell growth, no synergistic effect was observed when combined with IDH inhibition. Furthermore, DNMT3B inhibition was far more potent in restricting chondrosarcoma growth than IDH inhibitors in IDH mutant tumors. shRNA-mediated knockdown confirmed that DNMT3B depletion suppresses chondrosarcoma proliferation (Figure 1B-C). Mechanistically, DNMT3B inhibition induced expression of peroxisome proliferator activated receptor gamma (PPARγ), potentially rendering the cells more sensitive to naturally occurring PPARγ agonists. Notably, combined treatment with a PPARγ agonist and DNMT inhibitor produced a synergistic anti-proliferative effect in chondrosarcoma cells *in vitro* (Figure 3).

Discussion and Impact

Aberrant DNA methylation has been proposed as a major driver of chondrosarcoma oncogenesis. These studies demonstrate an oncogenic dependency on DNMT3B expression and enzymatic activity for chondrosarcoma proliferation. These studies provide a rational foundation for DNMT3B inhibition as a novel therapeutic strategy in human chondrosarcoma.

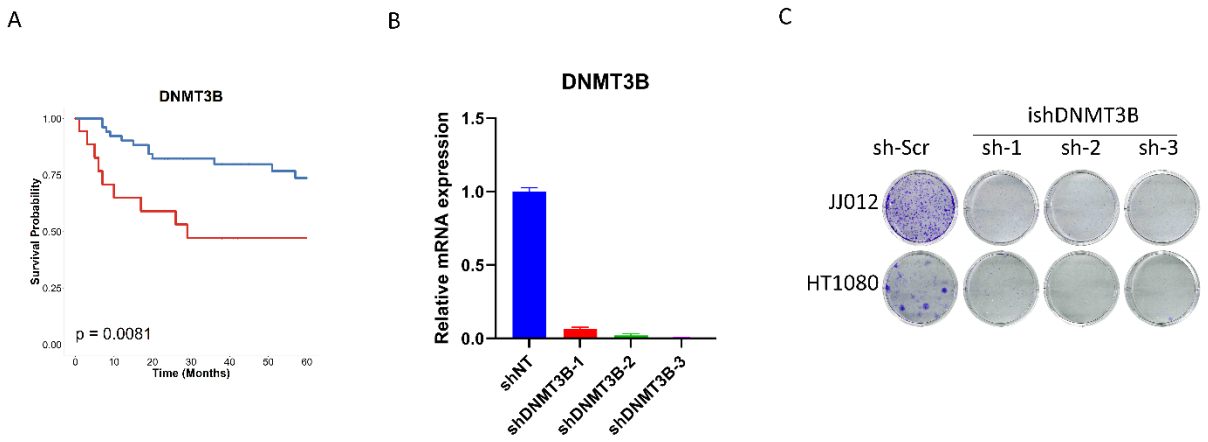

Figure 1. (A) Kaplan-Meier curves showing overall survival of DNMT3B. Patients were stratified by the top quartile (Top 25%, red) versus others (blue) based on gene expression levels. P values were calculated using the log-rank test. (B) qPCR analysis of DNMT3B mRNA levels in JJ012 cells. (C) Representative images of colony formation assays in 2 chondrosarcoma cell lines after induction of shRNAs targeting DNMT3B or a scrambled control (shScr).

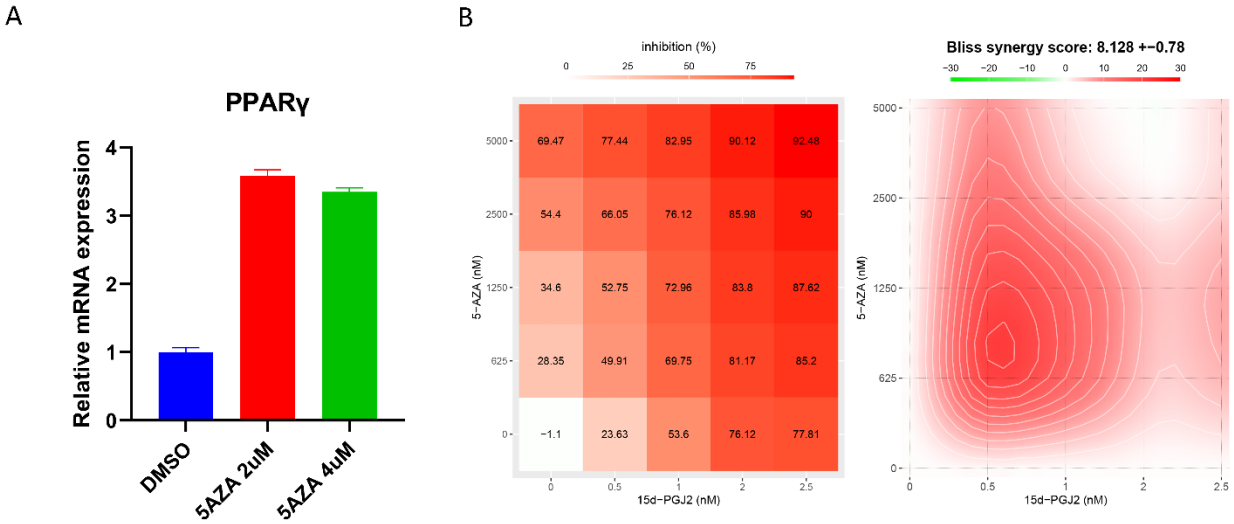

Figure 2. (A) qPCR analysis showing the mRNA expression levels of PPARγ in JJ012 cells with the DNMT inhibitor 5-azacytadine . (B) Quantitative evaluation of synergistic effects between 5-AZA and 15d-PGJ2, a naturally occurring agonist of PPAR-gamma. Bliss score indicates a synergistic interaction between 5-Azacytidine and 15d-PG_2 in JJ012 cells.