Can DNA Methylation Profiling Diagnose Soft-tissue Sarcoma and Classify Histologic Subtypes and Grades?

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Disclosures: Nothing to declare.

INTRODUCTION: The rarity and heterogeneity of soft-tissue sarcoma have made it difficult to study its development, diagnosis, and treatment. A clear classification of the subtype and grade is important for predicting prognosis and establishing management strategies. However, this may be challenging. Thus, we need a new method to help diagnose soft-tissue sarcoma in conjunction with traditional methods. Genetic alterations can be found in some subtypes of the soft-tissue sarcoma, but many others show dysregulated gene expression attributed to epigenetic changes, such as DNA methylation status. Thus, we asked: (1) Can DNA methylation profiling diagnose soft-tissue sarcoma and differentiate histologic subtypes? (2) Can it be used to classify each grade?

METHODS: We performed a retrospective medical record review, and the DNA methylation profiling of patients with a soft-tissue sarcoma treated between 2019 and 2022. Ethical approval for this study was obtained from the Institutional Review Board (Project number VC22SISI0072). All tissue samples were collected intra-operatively. Thirty-four soft-tissue sarcoma and five normal tissue samples in the Human Biobank with clinical data were included in this study. Genomic DNA was extracted from frozen tissue, and DNA methylation profiles were obtained using an Illumina MethylationEPIC kit (Illumina). Genomic annotation of DNA methylation sites and hierarchical cluster analysis were performed to interpret results from DNA methylation profiling results. The t-test was utilized to analyze differential methylation probes. Benjamini-Hochberg adjusted pvalue calculations were used to account for bias resulting from evaluating thousands of methylation sites.

RESULTS SECTION: The most common histologic subtypes were leiomyosarcoma (n = 8), and liposarcoma (n = 7). The tumor grade was Fédération Nationale des Centres de Lutte Contre Le Cancer (FNCLCC) grades 1, 2, and 3 in 3, 15, and 16 patients, respectively. DNA methylation profiling demonstrated differences between soft-tissue sarcoma, and normal tissue, with 72 sites having an adjusted p-value of < 0.000001. Most sarcomas were distinguished from normal samples using hierarchical cluster analysis (Heatmap 1). Among the 72 sites, the majority exhibited a hypermethylation pattern in sarcoma, with only 2 sites showing a hypomethylation pattern. Differences in DNA methylation were observed between leiomyosarcoma and liposarcoma at a total of 32,630 positions, where 25,100 sites exhibited hypermethylation patterns in liposarcoma than leiomyosarcoma. Two and 155 sites had adjusted p-values of less than 0.0001, and 0.001, respectively. The two groups were prominently distinguishable (Heatmap 2). A significant distinction was observed between grade 1 or 2, and grade 3 in hierarchical cluster analysis (Heatmap 3A). A total of 34 sites showed significant differences in DNA methylation status. Twenty-four sites exhibited hypermethylation in grade 1 or 2 than grade 3. Differences in DNA methylation patterns between grade 1 and grade 2 or 3 sarcomas were observed at 144 sites and were evident (Heatmap 3B).

DISCUSSION: There were some limitations to our study. First, the total sample size was small, and the number of normal samples was particularly limited. Only five paired tissue samples were obtained from patients with soft-tissue sarcoma, not from normal healthy individuals. Nevertheless, considering the adjusted p-values of less than 0.0001 in statistical analyses for 18,534 CpG sites, there was a clear difference in DNA methylation patterns between soft-tissue sarcoma and healthy control samples. Second, we did not compare soft-tissue sarcoma with benign soft-tissue tumors. DNA methylation profiling may be able to diagnose soft-tissue sarcoma, differentiate histologic subtypes, and classify the tumor grades.

SIGNIFICANCE/CLINICAL RELEVANCE: This research suggests that DNA methylation analysis could play a supportive role in achieving accurate diagnosis and effective treatment. Future in vivo and in vitro studies analyzing specific genes with altered methylation status with more soft-tissue sarcoma samples of different grades and subtypes could contribute to understanding the significance of DNA methylation status in soft-tissue sarcomas, such as tumorigenesis.

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IMAGES AND TABLES: