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Targeting soft tissue sarcoma cancer stem cells improves doxorubicin sensitivity in vitro

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Soft tissue sarcomas (STS) are rare tumors encompassing over 70 distinct histopathological subtypes that share a common treatment strategy comprising surgical resection, radiation, and chemotherapy in certain situations. Disease progression and failure to respond to anthracycline based chemotherapy, a standard first-line agent, is associated with poor outcomes. Recurrence and chemo-resistance represent significant barriers to improving patient survival.

Cancer stem cells are a specialized type of cell that persist and repopulate tumors after treatment and drive resistance in various forms of cancer. We are interested in the contribution of STS-CSCs to doxorubicin chemo-resistance in complex-karyotype STS. Specifically, we hypothesized the presence of a common genetic signature across unique STS subtypes involved in CSC-regulation that could be targeted to improve the efficacy of existing treatment regimens. To identify and isolate STS-CSCs, we used the Aldefluor assay coupled with fluorescent activated cell sorting (FACS). High aldehyde dehydrogenase (ALDH) activity is a well-established marker of stem cell populations. The Aldefluor assay fluorescently labels cells with high and low ALDH activity as bright and dim, representing CSCs and non-CSCs, respectively.

We first used the Aldefluor assay to profile the abundance of CSCs in five complex-karyotype STS cell culture models, including dedifferentiated liposarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma. In order to gain insight into the molecular pathways active in STS-CSCs, Aldefluor-bright and -dim populations were isolated by FACs and analyzed by RNA-sequencing. Differential gene expression analysis identified a small subset of commonly upregulated genes among the STS cell lines tested and shared across CSCs. Gene-set enrichment analysis of upregulated genes in STS-CSCs further identified a signature for Enhancer of Zeste homolog 2 (EZH2), part of the polycomb repressive complex 2 (PRC2) and a histone methyltransferase responsible for H3K27 methylation. As an epigenetic modulator, increased EZH2 expression and PRC2 activity functions to decrease activity of genes involved in growth suppression, thereby conferring oncogenic activity. At present, EZH2 can be targeted with the small molecule tazemetostat, an FDA-approved treatment for metastatic and locally advanced epithelioid sarcoma as well as follicular lymphoma.

To evaluate the possibility of EZH2-mediated differences in chromatin accessibility between CSCs and non-CSCs, we performed ATAC (assay for transposase-accessible chromatin)-seq analysis. In ATAC-seq, a transposase inserts sequencing adapters into areas of open chromatin, but not in areas of inaccessible chromatin, such as regions with increased histone methylation. In this way, areas of more accessible chromatin generate increased numbers of sequencing reads, which can be compared between different cell lines and conditions. ATAC-seq analysis of Aldefluor bright and dim cells isolated from five STS cell lines identified numerous areas of shared, differentially accessible chromatin unique to CSCs. We further evaluated differential chromatin accessibility surrounding transcription start-sites in CSCs and non-CSCs. Comparison of ChIP (chromatin immunoprecipitation)-sequencing data specific for the histone methylation target of EZH2 (H3K27me3) with our ATAC-seq data showed a strong correlation between datasets, indicating that EZH2 is involved in regulating chromatin in a CSC-specific manner.

In order to test the effects of EZH2 inhibition on STS-CSCs, we generated doxorubicin resistance STS cell lines by serial selection with increasing concentrations of doxorubicin. We identified a positive correlation between CSC abundance and doxorubicin IC_{50} in doxorubicin resistant cell lines by Aldefluor assay and soft-agar colony formation assays. Co-treatment of doxorubicin and tazemetostat was not only synergistic in the parent cell lines, but rescued doxorubicin chemosensitivity in resistant lines. These data confirm the presence of shared genetic programs across distinct subtypes of STS that are unique to CSCs and amenable to therapeutic targeting.