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Identification of CD146 as a marker of tumor-propagating cells in human sarcomas leads to novel treatment opportunities

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BACKGROUND: Tumor-propagating cells (TPCs) are believed to drive cancer initiation, progression and recurrence. These cells are characterized by enhanced tumorigenicity and self-renewal capacity. We previously used a functional dye-efflux assay to identify side population (SP) cells from sarcomas which have stem-like properties. However, no cell surface markers for TPCs have been identified in primary human sarcomas.

PURPOSE: Our aim was to identify a robust cell surface marker that can isolate TPCs in primary human sarcomas and identify signaling pathways that are activated in TPCs, and which could be targeted for therapy.

PATIENTS AND METHODS: Undifferentiated pleomorphic sarcoma (UPS) and osteosarcoma tumor specimens were obtained from patients fresh at the time of open biopsy or surgical resection but prior to any radiation or chemotherapy. Tumor samples were dissociated and enzymatically digested to single cell suspensions, exposed to Hoechst 33324 dye and separated by flow cytometry into side population (SP) and non-side population (NSP) cells. SP and NSP cells were analyzed for the expression of 235 cell surface markers using a high throughput flow cytometry cell surface antigen screen. Markers with an enrichment of ≥ 2-fold were selected for testing in vivo by injection into NOD-scid immunodeficient mice and observed for 24 weeks for tumor growth. Gene expression profiling was performed for SP vs NSP and $CD146^+$ vs $CD146^-$ cell fractions using the Illumina HT-12 v4 platform and gene set enrichment analysis (GSEA) was applied for identification of over- or underexpressed signaling pathways. Gene expression was analyzed by quantitative real-time reverse transcription PCR (qPCR). The role of Notch signaling on tumor xenografts was investigated by treating NOD-scid mice with the Notch γ-secretase inhibitor DAPT or vehicle control by intraperitoneal injection for 22 days. For TGF-β signaling, mice were treated with LY2109761, a TGF-β receptor type I inhibitor or control, by oral gavage for 4 weeks. Xenograft growth was analyzed for tumor size and weight, expression of gene pathway members, as well as self-renewal through serial transplantation assays.

RESULTS: Using a high throughput cell surface antigen screen, we identified 5 markers highly enriched on the surface of side population (SP) cells from primary human osteosarcoma and undifferentiated pleomorphic sarcoma (UPS), which have TPC characteristics. In vivo xenograft growth and serial transplantation assays were performed to test the tumorigenic potential of these markers. Only $CD146^+$ cell fractions were highly tumorigenic, and could also sustain tumor growth over multiple passages.

Since CD146 is also a marker of pericytes, we stained frozen primary tumor tissue sections with CD31 and CD146 to distinguish between blood vessels and tumor cells. The location of CD146+ cells in UPS and osteosarcoma was visualized using immunofluorescence. CD146+ cells were present both near blood vessels and within the tumors, indicating that a population of tumor cells, unrelated to the vasculature are expressing CD146. Furthermore, we found that CD146+ and SP cells represent both distinct and overlapping populations of TPCs. Transcriptional profiling of CD146+ and SP cells followed by GSEA analysis revealed common activation of several signaling pathways including TGF-β and Notch. Inhibition of the Notch pathway using a γ-secretase inhibitor, or TGF-β signaling with a type I receptor inhibitor, significantly reduced tumor growth, self-renewal and expression of gene pathway members in both osteosarcoma and UPS.

CONCLUSIONS: The results of this study suggest that CD146 can be used successfully as a cell surface marker to identify a TPC fraction in sarcomas, and that identification of signaling pathways specifically upregulated in this tumor cell population can potentially be targeted leading to novel therapeutic options to reduce tumor recurrence for patients with high grade sarcomas.