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“Novel Insights into Dedifferentiated Liposarcoma Pathogenesis: Evaluating the Tumor-Promoting Role of IL6/GP130 Signaling via MDM2 Upregulation”

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ABSTRACT

Introduction: Dedifferentiated liposarcoma (DDLPS), an aggressive high grade liposarcoma subtype, is characterized by amplification of MDM2 and wild-type TP53. Given that the response to chemotherapy is less than satisfactory, cytotoxic chemotherapy and radical surgery remain the mainstay treatment methods; however, local recurrence is frequent and can affect quality of life and overall survival. Clearly, there are ongoing needs for more effective treatments based on an in-depth understanding of underlying liposarcoma pathogenesis. Recent studies have shown that adipose tissue is largely implicated as a driver of multiple cancer types by releasing pro-inflammatory factors such as interleukin-6 (IL6). In these malignancies, IL6 was shown to bind to soluble IL6Rα and membrane-bound glycoprotein 130 (GP130), resulting in activation of disease-promoting pathways. Interestingly, it has been shown that activation of GP130 results in elevated MDM2 transcription levels in colorectal cancer. Given that DDLPS tends to arise in the fat-rich region of the retroperitoneum and that MDM2 amplification occurs consistently in this disease, the investigation of the role of the GP130 pathway in DDLPS tumorigenesis is especially relevant. Therefore, the overall goal of this study is to investigate the GP130 signaling cascade in DDLPS and the potential role of preadipocytes as sources of IL6 for retroperitoneal DDLPS. The exploration of this pathway in DDLPS might lead to the discovery of new deregulated pathways that will hopefully have an impact on patients in the clinic.

Methods: Protein levels were measured by western blot while gene expression was assessed using quantitative real-time polymerase chain reaction (qRT-PCR). Growth (MTS) and viability analyses were performed to measure the impact of IL6 or GP130 knockdown on DDLPS cell lines. GP130 knockdown was performed using siRNA. The in vitro impact of small molecule GP130 inhibitor SC144 on DDLPS viability was determined using
MTS, and fluorescent microscopy. The effects of SC144 on apoptotic levels were assessed using measuring annexin V and propidium iodide (PI) levels by flow cytometry. Preadipocyte (PreAdip) cells served as the normal control in all in vitro viability and survival investigations. Soluble IL6 levels were measured using enzyme-linked immunosorbent assay (ELISA). For direct co-culture studies, RFP-tagged DDLPS were plated in the absence or presence of PreAdip cells, and visualized over time using the IncuCyte Zoom live-imaging fluorescent microscope. Boyden chambers were utilized for indirect trans-well co-culture analyses; endpoint was performed using trypan blue exclusion. Experiments using PreAdip-derived conditioned media (CM) served as secondary methods to investigate the impact of PreAdip secreted factors on DDLPS growth. Anti-IL6 monoclonal antibody MAB206 was added to DDLPS growth media to determine PreAdip secretion of IL6 in CM studies. In vivo studies were performed using the Lipo246 xenograft model, and tumors were processed for molecular and histological analyses.

**Results & Conclusions:** Our results showed that GP130 is overexpressed in DDLPS tissue and cell lines compared to the normal adjacent tissue and PreAdip controls, respectively. PreAdip cells secrete higher levels of IL6 than DDLPS cell lines, confirming previously published data that showed high expression of IL6 in PreAdip. The addition of IL6 (10ng/mL) increased cellular growth and migration in DDLPS. Culturing DDLPS in the presence of PreAdip or PreAdip-derived CM increased cancer cell growth, suggesting that PreAdip release cancer-promoting factors. Use of anti-IL6 monoclonal antibody MAB206 decreased the growth effects of PreAdip-derived CM, proposing that IL6 may be a main tumor-promoting factor released by PreAdip cells. Blockade of GP130 with 625 nM SC144 in the presence of IL6 inhibited DDLPS viability after 96 hours; however, PreAdip cells demonstrated tolerance to SC144 with an EC50 dose of 6.332 µM. In vivo analysis revealed decreased tumor volume upon treatment with SC144 as compared to the vehicle control, signifying the importance of GP130 signaling for disease progression. Interestingly, inhibition of GP130 also reduced STAT1 and STAT3 activation, and MDM2 expression in DDLPS cells,
suggesting the potential role of GP130 as an upstream regulator of MDM2 transcription. Taken together, these data suggest a novel PreAdip:DDLPS axis in which preadipocytes release IL6, which may promote DDLPS pathological aggressiveness by activating the GP130 pathway and ultimately leading to the upregulation of MDM2.
RECENT ABSTRACTS AND PRESENTATION


Abstract for poster presentation, 2015 OSUMC Translational Therapeutics Retreat, Columbus, Ohio, October 2015.
RECENT PUBLICATIONS


AWARDS AND HONORS

National Cancer Institute S.P.O.R.E. Diversity Supplement Grant (U54)

Future of Research (F.O.R.) Symposium Travel Award

University Fellowship

FUTURE PLANS

My ultimate goal is to make as great a positive impact on patient lives as possible. I hope to do so by using the knowledge gained during graduate school and obtaining a medical degree to advance disease research and information dissemination. By working as a physician scientist, I plan to practice medicine while leading a research laboratory. This will hopefully lead to the substantial change I someday hope to make.