Paracrine adipocyte signaling promotes colorectal cancer growth in vitro

Nivetha Ramasamy

Purpose: To identify candidate mediators of paracrine signaling between adipocytes and colorectal cancer cells contributing to the obesity-cancer link.
Background: The worldwide prevalence of obesity has nearly tripled over the last few decades and is a major public health concern. Obesity-associated inflammation in adipose tissue correlates with colorectal tumorigenesis and tumor proliferation. The altered secretome of obesity-associated adipose tissue also affects colon cancer growth and development. Hence, we sought to determine the role of adipocyte-derived secreted factors in mediating the tumor-promoting paracrine signaling observed in obesity.
Methods: Adipocytes were isolated from the gonadal fat pad of C57BL/6J male mice fed either a low-fat (10 kcal% fat) or high-fat (60 kcal% fat) diet and cultured for 24 hours. Cytokine production from the cultured adipocytes was quantified using the Bio-Plex Pro mouse chemokine 33-plex panel. To determine if adipocyte-derived metabolites or growth factors promote cancer cell proliferation, human SW620 colon cancer cells were grown for 24 hours in conditioned media and cell viability was assessed using the MTT assay. To test whether a subset of these cytokines directly impacted cancer cell growth, SW620 cells were cultured with or without the addition of 20 ng/mL recombinant IL-6 or 50 ng/mL CCL5 to the culture media. Proliferation was measured using the MTT assay and gene expression was assessed via qPCR.
Results: Adipocyte-conditioned media contained 19 detectable cytokines, with CCL2, CCL7, and CCL5 being the most abundant. IL-6, CCL12, CCL11, and CXCL16 were all significantly elevated (p2.0) in adipocyte-conditioned media from obese relative to lean mice. SW620 cells demonstrated a >20% increase in proliferation when grown in conditioned media from adipocytes isolated from either low-fat or high-fat fed mice. CCL5 stimulation for 24 hours resulted in a ~17% increase in SW620 cell viability when cultured under replete (10% FBS) growth factor conditions, but a ~8% decrease under reduced (0.5% FBS) growth factor conditions. IL-6 stimulation did not impact SW620 cell growth. To understand the downstream cell signaling effects of IL-6 and CCL5 stimulation, we performed qPCR analysis on EMT and Wnt signaling markers in SW620 cells after 4-hour cytokine stimulation in media without growth factors (0% FBS). IL-6-stimulated cells exhibited a significant increase in *SOCS3* and *PTHLH* while CCL5-stimulated cells exhibited a significant decrease in *TCF7L1*. **Conclusions:** Overall, these data indicate tumor-promoting paracrine signaling from adipose tissue may potentiate colon cancer growth in part via adipocyte-derived cytokine production. Further work to delineate the mechanisms through which such paracrine signaling acts will be important to develop interventions to reduce the growing burden of obesity-driven colon cancer.