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The Role of Cancer Associated Fibroblasts on the Growth of Pancreatic Neuroendocrine Tumors

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BACKGROUND

Pancreatic Neuroendocrine Tumors (pNETs) have a rising incidence rate in the United States with a 23% five-year survival for patients with metastatic disease. These patients have few therapeutic options. The Tumor Microenvironment (TME) is composed of various cell populations interacting to promote tumor development and cancer progression. Among them are Cancer Associated Fibroblasts (CAFs) that are involved in increased cancer cell proliferation, extra cellular matrix remodeling, and treatment resistance. We hypothesize that CAFs contribute to increased pNET growth through factor secretion.

METHODS

A co-culture study was conducted using pNET cell lines (BON-1 & NT-18P) in patient derived serum-depleted fibroblast conditioned media (FCM) isolated from pancreas tumors of grades 1, 2, and 3 (CAF 1, CAF 2, CAF 3 respectively), compared to both serum containing (Normal) and serum depleted (Experimental) controls. Cell proliferation was measured through cell-titer glo assays, clonogenic assays, and cell cycle studies. FCM assessment was performed through media fractionation separated by four molecular weight cut-offs, followed by mass spectrometry for identification of pro-growth factors secreted by CAFs. Finally, bulk RNA sequencing on FCM conditioned cells was used to observe gene expression variances compared to control cells.

RESULTS

FCM conditioned cells demonstrate higher cell proliferation over seven days in comparison to cells grown in serum free control media for both cell lines (NT-18P: +55% for CAF 1, +56% for CAF 2, +60% for CAF 3). FCM treated cells also demonstrated increased colony formation (NT-18P: CAF 2= 33 colonies, CAF 3= 51 colonies) while no measurable colonies formed in the experimental control. There was no statistical difference in tumor cell growth based on grade. NT-18P cells demonstrated an increased number of proliferating cells in G2 mitotic state in FCM conditions (NT-18P: CAF 1= 14% CAF 2= 26%, CAF 3= 14% vs Control= 11%). Media fractionation data showcases highest proliferative rate among cells treated with the highest molecular weight fraction of the FCM (+50 kDa), further validated by mass spectrometry. RNA sequencing analysis is ongoing.

CONCLUSIONS

CAFs contribute to increased growth and proliferation among pNET cells, independent of grade. Our work demonstrates CAF secreted factors with larger molecular weights have a greater influence towards increased cell proliferation. Understanding the role of pNET CAFs may lead to identification of biomarkers that serve as therapeutic targets against pNET development.

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