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Abstract:

Malignant tumors are inherently pro-inflammatory, and infiltrating leukocytes are thought to be critical for tumor maintenance and progression. Infiltrating cells and molecules driving tumor-associated inflammation have considerable potential as therapeutic targets, yet this area remains relatively under-explored. Neutrophils are the most prevalent leukocyte in the innate immune system and have been shown to play an essential role in cancer progression. Previously our lab has reported that as pancreatic ductal adenocarcinoma (PDAC) disease progresses, there is an increase in the infiltration of neutrophils. The specific objective of this study is to determine the role of neutrophil-PDAC cell interaction in therapy resistance. We analyzed whether direct neutrophil-PDAC cell interaction modulates their survival and whether it is dependent on therapy resistance. We used two human and murine neutrophil cell line models (MPRO and HL60) and two isogenic parent CD18/HPAF (C) and gemcitabine-resistant variant CD18/HPAF-R (CGR). Neutrophil and PDAC cells were co-cultured for 24 h, and their proliferation/survival was determined using WST and MTT assays. We observed that PDAC cancer cell proliferation increased when co-cultured with neutrophils. Moreover, cell free-neutrophil conditioned media modulated PDAC cell proliferation in a concentration-dependent (v/v) manner. We observed significantly higher proliferation in therapy-resistant CGR cell lines. Furthermore, our data suggest neutrophils co-cultured with therapy-resistant CGR cells had higher survival compared to neutrophils co-cultured with parent C cells. Together our data demonstrate that neutrophil-PDAC cell interaction modulates their proliferation and survival.

Introduction:

Pancreatic Cancer (PC) holds the reputation as one of the deadliest types of cancer, which can largely be attributed to its meager survival expectancy of 3.5-5 years. Located deep in the abdomen, the pancreas' concealed position leaves patients susceptible to a late diagnosis in which the tumor has often already metastasized or developed resistance to routine therapies. Therapy resistance is a trademark quality strongly associated with a more malignant form of PC, PDAC. PDAC makes up 85% of all PC cases. The overwhelming absence of successful treatment options for PDAC is concurrent with the aggressive nature of the disease.

PDAC is treated as a never-healing wound in the body, naturally attracting inflammatory entities. Pro-inflammatory cytokines and leukocytes, specifically neutrophils, contribute to creating a tumor microenvironment (TME) that resultantly promotes tumor cell proliferation and survival. Such infiltrating cells and molecules remain the focus of potential therapeutic targets but remain largely unexplored. Previous research in our laboratory has determined that CXCR2, a pro-tumorigenic chemokine receptor, has proven to have substantial implications on therapy resistance. CXCR2 directs the recruitment of tumor-promoting leukocytes, specifically showing an increase in neutrophil recruitment- a known driver of pro-tumorigenic processes such as inflammation. Neutrophils are the most common leukocyte and have proven to play an intrinsic role in cancer progression. The presence of neutrophils has been implicated in promoting therapy resistance in PDAC. Here, we report that the products created by neutrophils play a role in promoting tumor cell proliferation. Neutrophil-lymphocyte ratio (NLR) is now an established indication of prognosis- the higher the NLR, the lower the chances of survival.

At present, this research aims to discover the specific role tumor-associated neutrophils play in malignant transformation and the development of a pro-inflammatory/angiogenic TME during PDAC progression. Such discoveries will aid in understanding therapy resistance, allowing for novel treatment options to be created. The role of neutrophil-PDAC interaction on PDAC proliferation and therapy resistance was tested by tumor cells undergoing growth at varying concentrations of neutrophil-conditioned media (NCM) to serum-free (SF) media (v/v). Our preliminary data from both CD18/HPAF (C) cancer cell lines and CD18/HPAF GemR (gemcitabine resistant) (CGR) cell lines showed that cancer cells treated at a higher concentration of NCM displayed a decreased survival when compared to SF. Such findings allow the inference that certain ratios of NCM to cancer cells aid in tumor cell proliferation. Furthermore, cancer cells were grown in a co-culture alongside MPRO (mouse) neutrophils and HL-60 (human) neutrophils in which the data reported increased survival of cancer cells in co-culture. The central hypothesis is that neutrophils play a distinct role in PDAC progression by promoting tumor cell proliferation, survival, and therapy resistance.

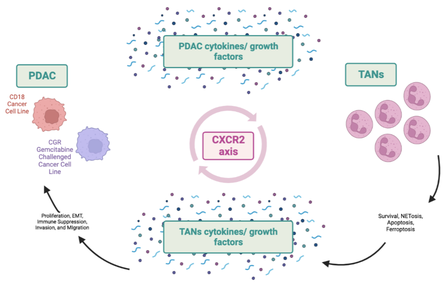

Figure 1: Neutrophil-PDAC interaction in the microenvironment.

Experimental Strategy

Co-Culture with HL-60 and MPRO Neutrophils

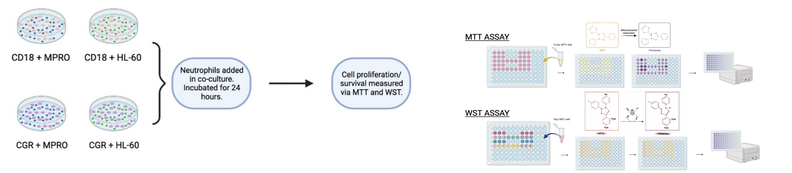

Figure 2: Cancer cells were seeded overnight and then treated with MPRO (mouse) and (HL-60) human neutrophils for 24hrs. Survival of cells and neutrophils determined by MTT and WST assay, respectively.

PDAC-Neutrophil Interaction Enhances Cell Proliferation

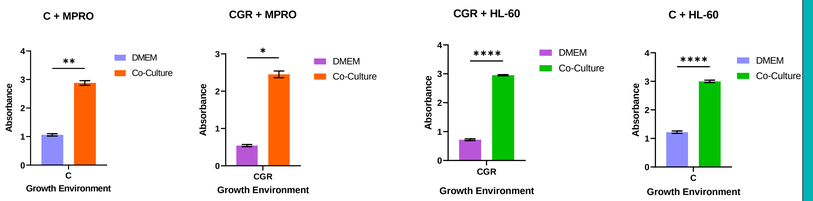

Figure 3: Resulting cell proliferation for C and CGR lines post co-culture with neutrophils. Control groups of each cancer cell line grown in cancer cell media (DMEM) are included in contrast. Across both data sets, C and CGR cell lines show significantly higher survival in co-culture treatment. It becomes clear that the proximity of neutrophils themselves displays an association with the increase in cancer cell proliferation in PDAC.

Higher Cell Proliferation in Therapy Resistant PDAC Cells

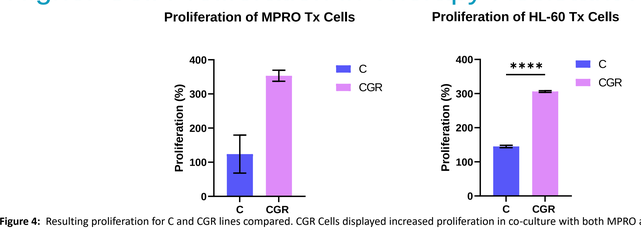

Figure 4: Resulting proliferation for C and CGR lines compared. CGR Cells displayed increased proliferation in co-culture with both MPRO and HL-60 neutrophils. Percent proliferation analysis of MPRO treatment measured C: 123.8%, CGR: 353.3%. Percent proliferation analysis of HL-60 treatment measured C: 145%, CGR: 306%. Thus, we report that therapy-resistant PDAC cells are more successful in proliferation and survival in co-culture with neutrophils.

Cell Line-Dependent Neutrophil Survival During PDAC-Neutrophil Interaction

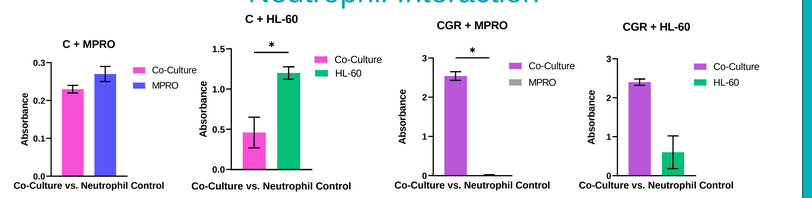

Figure 5: Results of WST assay measuring the survival of *neutrophils* co-cultured with C and CGR cancer cell lines for 24 hours. Experimental data include the survival of MPRO (mouse) and HL-60 (human) neutrophils against C and CGR cell lines. WST-1 assay was performed to determine the neutrophil dysfunction/ survival after 24 hours. The data collected shows less survival of neutrophils undergoing co-culture with C cancer cells in comparison to controls. Therapy-resistant PDAC cells prove to aid in the survival of neutrophils as CGR + MPRO, and CHR + HL-60 graphs demonstrate a clear increase in neutrophil survival.

Experimental Strategy

CD18 and CD18 GemR Cell Lines Treated at Varying Concentrations of Neutrophil Conditioned Media

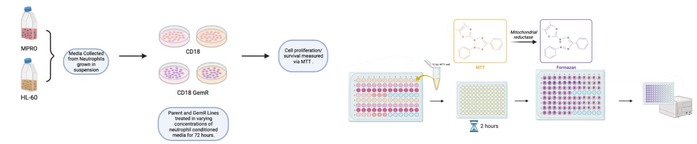

Figure 6: PDAC cell lines, C and CGR, were treated at varying concentrations of neutrophil-conditioned media to SF media (v/v) for 72 hours. Survival was measured using an MTT assay.

PDAC Survival in Neutrophil Conditioned Media is Concentration-Dependent

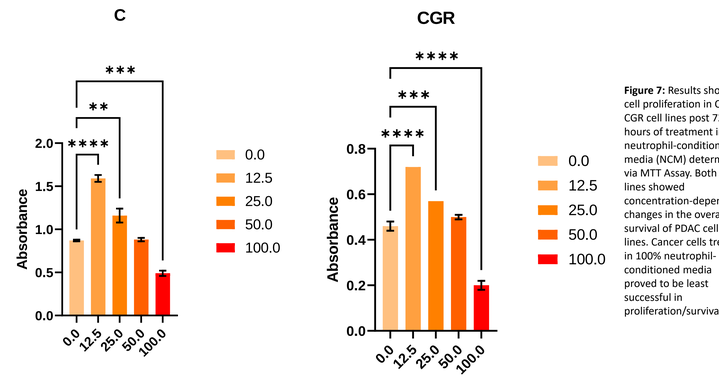

Figure 7: Results show cell proliferation in C and CGR cell lines post 72 hours of treatment in neutrophil-conditioned media (NCM) determined via MTT Assay. Both cell lines showed concentration-dependent changes in the overall survival of PDAC cell lines. Cancer cells treated in 100% neutrophil-conditioned media proved to be least successful in proliferation/survival.

Summary:

- The interactions between PDAC cells and neutrophils result in increased PDAC cell proliferation.
- CGR cancer cells co-cultured with MPRO and HL-60 displayed higher cell proliferation than the parent C cell line.
- Neutrophil survival is more successful in CGR co-culture.
- Survival in both C and CGR cancer cell lines is modified in a concentration-dependent manner when treated with neutrophil-conditioned media.

Conclusions:

The progression of PDAC is modulated by PDAC-neutrophil interaction. The proximity of neutrophils themselves displays an association with enhanced cell proliferation in both C and CGR cell lines. As expected, therapy-resistant PDAC cells display higher proliferation in comparison to the parent C line. Finally, neutrophil survival is cell-line-dependent, observing decreased survival among the parent C cell line and increased survival among the therapy-resistant CGR cell line irrespective of neutrophil type.

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