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Metastatic PDAC Cell – Neutrophil Interaction Regulates their Proliferation and Survival

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Metastatic PDAC cell – neutrophil interaction regulates their proliferation and survival

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Abstract

Tumor-promoting inflammation is a hallmark of cancer that contributes to tumor cells' survival and proliferation. Infiltrating leukocytes and pro-inflammatory cytokines released into the tumor microenvironment (TME) often cause this inflammation, which is often pro-tumorigenic. Neutrophils are one of the most abundant types of leukocytes found in circulation. Enhanced neutrophil infiltration into the TME with disease progression was previously observed in my lab. Neutrophils are a pro-tumorigenic and pro-metastatic part of the TME. It has been shown in previous research that neutrophils aid in PDAC progression and metastasis. Although, exact mechanisms of this neutrophil-PDAC interaction remain relatively unknown. The specific objective of this project is to determine the role of tumor-associated neutrophils in PDAC progression and metastasis. Our working hypothesis is that neutrophil-PDAC interaction increases PDAC proliferation, survival, and metastasis. The two neutrophil cell lines that were used in this study were mouse neutrophils, MPRO, and human neutrophils, HL-60. The two human pancreatic cancer cell lines that were used are L3.3 and L3.6. The pancreatic cancer cell line L3.3 is a low metastatic cell line while L3.6 is a high metastatic line. Cancer cells were either treated with neutrophil-conditioned media (indirect interaction), or co-cultured with the neutrophil (direct interaction). MTT assays were performed to analyze proliferation of the cancer cell lines, and a WST assay was performed to analyze the survival of neutrophils. We observed concentration-dependent increase in PDAC cell proliferation following treatment with neutrophil-conditioned media. Similarly, co-culture of PADC cell with neutrophils enhanced their proliferation. We did not observe any difference in neutrophil survival when co-cultured with low-metastatic L3.3 cell. However, neutrophil survival was significantly reduced when co-cultured with L3.6 cells (high metastatic). Together, our data suggest that PDAC-neutrophil interaction differentially modulates of neutrophil and PDAC cells survival/proliferation.

Introduction

PC is difficult to treat and only has a bleak 5-year survival rate of 12% . The pancreas' location often leads to delayed diagnosis, which limits treatment options. Treatment is inhibited further by failure to respond to chemotherapy regimens and developing resistance to chemotherapies. In addition, many patients already have metastases in other organs at the time of diagnosis, further complicating treatment.

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. The long-term objective of these studies is to determine the potential role and significance of tumor-associated neutrophils in malignant transformation and the generation of a pro-inflammatory/angiogenic environment during PC progression, and metastasis and develop novel targeted therapeutics. Previously our laboratory has seen an increase in chemokines, such as those related to leukocyte recruitment and inflammation, and their association with disease progression. Tumor-promoting Inflammation is a hallmark of cancer . Infiltrating cells and molecules in the tumor microenvironment (TME) have been shown as drivers of inflammation, which is often pro-tumorigenic. The TME has also been observed to aid in metastasis greatly. Neutrophils have been shown to have a prominent pro-tumorigenic and pro-metastatic role in TME.

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. My preliminary data indicates that PDAC progression increases depending on specific neutrophil condition media concentrations. In addition, data shows that PDAC increases while neutrophils are present in the tumor microenvironment. However, very little is known about the role of neutrophil-tumor cell interactions in PC pathobiology and the mechanisms behind recruitment, activation, and survival. Our **hypothesis** is *neutrophil-PDAC interaction increases PDAC proliferation, survival, and metastasis.*

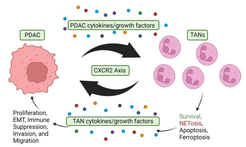

Figure 1. Neutrophil-PDAC interaction in the microenvironment

Experimental Strategy I

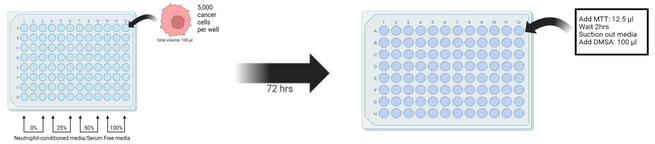

Figure 2. 5,000 cancer cells were incubated for 24 hours so they could adhere to the bottom of their wells. After the 24 hours then the cancer cells were treated with various concentrations of neutrophil-condition media to serum-free media (v/v). The plate was then incubated for 72 hours and an MTT assay was performed to determine prevalence of viable cells in each treatment group.

PDAC cell proliferation modulated in concentration dependent manner by neutrophil-conditioned media

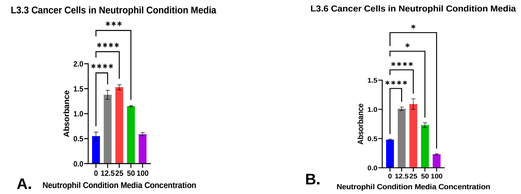

Figure 5. L3.3 **A)** and L3.6 cells **B)** presents bell shape curves on their graphs representing that the L3.3 and L3.6 cell lines had most proliferation while in 25% neutrophil condition media. The results also show that the least amount of cell viability occurred in the 0% and 100% neutrophil condition media signifying that PDAC proliferation is concentration dependent in neutrophil condition media.

Experimental Strategy II

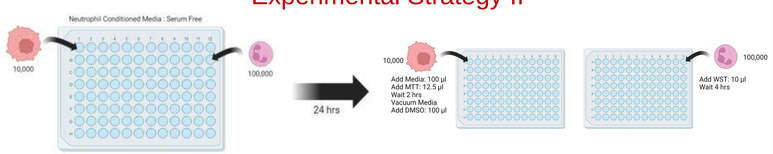

Figure 3. 10,000 cancer cells were added to a 96 well plate then incubated for 24 hours. Then 100,000 neutrophils were added to the wells and incubated for another 24 hours. After this, the neutrophils were then separated onto another plate. Media was readded to the wells containing cancer cells and an MTT assay was performed. WST assay was performed on the plate containing the neutrophils. The assay were performed to determine survival of the cells.

PDAC-neutrophil interaction increases PDAC cell proliferation

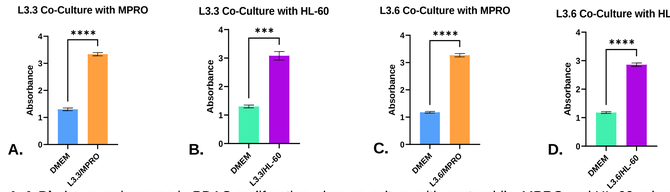

Figure 4. A-D) shows an increase in PDAC proliferation when co-culture with neutrophils, MPRO and HL-60, when compared to DMEM, normal growth media. In conclusion it is shown that the neutrophil-PDAC interaction enhances PDAC proliferation and survival.

PDAC-neutrophil interaction increases L3.6 cell proliferation

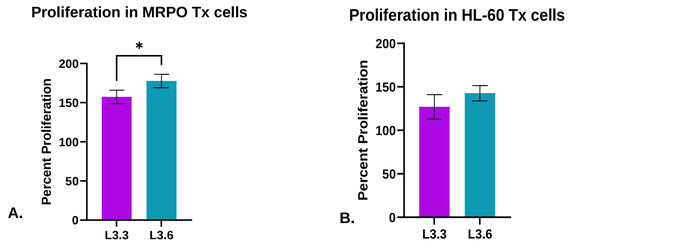

Figure 6. MPRO **A)** and HL-60 neutrophils **B)** show that the L3.6 cell line has the greatest percent proliferation as it is higher than the L3.3 cell line when both with MPRO and HL-60 neutrophils. This is expected because the L3.3 cell line is a low metastatic line as opposed to the L3.6 cell line having a high metastatic line.

Cell line-dependent neutrophil survival mediated by PDAC-neutrophil interaction

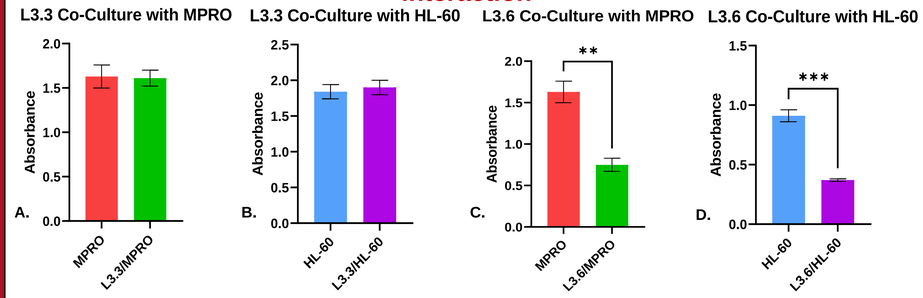

Figure 7. Neutrophils, HL-60 and MPRO, co-cultured with L3.3 cancer cells **A)** and **B)** show no changes in proliferation upon neutrophil-PDAC interaction when compared to cultured alone. **C)** and **D)** decreased survival of HL-60 and MPRO cells co-cultured with L3.6 cells upon neutrophil-PDAC interaction when compared to neutrophils alone.

Summary

- **PDAC survival is modulated in a concentration dependent by neutrophil condition media**
- **PDAC proliferation is increased in PDAC-Neutrophil co-cultures**
- **Neutrophil survival in PDAC-neutrophil co-culture is cell line dependent**

Conclusion

In conclusion, through these two experiments, it is shown that neutrophils have some impact on PDAC. In the first experiment, we learned that PDAC survival is concentration dependent in neutrophil-condition media. Through the co-culture experiment, we see PDAC-neutrophil interaction increases cancer cell proliferation and survival. We also see changes in neutrophil survival upon neutrophil-PDAC interaction that is cell line dependent. My objective for this research was to determine the effects of the neutrophil-PDAC interaction on PDAC cancer cells with different metastatic potentials. This is part of an ongoing project to determine the mechanisms behind the interaction of neutrophils and PDAC cancer cells in the TME. Further research is needed to elucidate the pathways in which PDAC cells and neutrophils interact in order to develop therapeutic interventions for PDAC patients.

References

- Jiang, W., Li, X., Xiang, C., & Zhou, W. (2022). Neutrophils in pancreatic cancer: Potential therapeutic targets. *Frontiers in oncology*, *12*, 1025805.
- Halbrook, C. J., Lyssiotis, C. A., di Magliano, M. P., & Maitra, A. (2023). Pancreatic cancer: Advances and challenges. *Cell*, *186*(8), 1729-1754.
- Hanahan, D. (2022). Hallmarks of cancer: new dimensions. *Cancer discovery*, *12*(1), 31-46.
- Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *cell*, *144*(5), 646-674.
- Reegan Sturgeon, Paran Goel, Caitlin Molczyk, Surindar K. Batra, Rakesh Singh. Neutrophil pro-tumorigenic role in progression and therapy resistance in pancreatic ductal adenocarcinoma [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2023; Part 1 (Regular and Invited Abstracts); 2023 Apr 14-19; Orlando, FL. Philadelphia (PA): AACR; Cancer Res 2023;83(7_Suppl):Abstract nr 1348.