Endothelial Cell’s Inflammammasomes and Breast Cancer
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General Abstract

The environment of tumors is critical in cancer formation, progression, and spreading. Inflammation and especially the multi-protein cytokine complexes called inflammasomes are key players in the inflammatory microenvironment. Here, we determined whether inflammasomes were present in vessel cells (i.e., endothelial cells) and the effects of their activation. The consequences for breast cancer are discussed.

Technical Abstract

Background:

• Despite progress in understanding cancer, incidence and associated mortality of breast cancer remain significant.
• Recently, the tumor microenvironment, especially inflammation, has been recognized as a critical mediator of tumor progression and metastasis.
• Besides specific immunity, innate components including the cytokine multi-protein complexes inflammasomes have been detected in immune and other cell types within the tumor microenvironment.

Approach:

• Using known inflammasome activators such as LPS and ATP, we determined the presence of inflammasomes in 2H11 endothelial cells via co-localization of three cytokine proteins: NLRP3, ASC1, and pro-caspase 1.
• After confirming this cytokine presence through confocal microscopy and semi-quantitative dot-blot assays, the effects of inflammasome activation on IL-1ß, IL-18, and VEGF secretion were assessed.

Results:

• Furthermore, the effects of the tumor microenvironment were tested using conditioned media from 4T1 mammary tumor cells.
• Results indicate that following ATP and 4T1 conditioned media + ATP treatments, 2H11 cells secreted significantly more IL-1ß. In contrast, 2H11 cell secretion of IL-18 increased following exposure to LPS+ATP, and LPS treatment alone. VEGF secretion was only significantly increased following LPS+ATP treatment.

Conclusion:

• These data confirm the presence and activity of inflammasomes in 2H11 endothelial cells and suggest that endothelial cells may contribute to the tumor’s inflammatory microenvironment in breast cancer.

Introduction and Significance

• Breast cancer is the most common diagnosed cancer in women within the United States with 30% of diagnosed breast cancers becoming metastatic [1].
• In addition to tumor cells, the breast tumor microenvironment includes various cell types and proteins, including extracellular matrix proteins, that participate in tumor progression.
• While interactions between the local environment and tumor cells are complex, the role of inflammasomes in promoting inflammation through the secretion of IL-1ß and IL-18 has emerged in multiple cancers.[3,4]

• As shown in Fig.3, inflammasomes are protein complexes of NLRP3, ASC1 and pro-caspase 1 leading to the generation of activated caspase 1, which in turn promotes the secretion of the pro-inflammatory cytokines IL-1ß and IL-18. Reduced inflammasome activity correlates with reduced inflammation, in turn, leading to reduced tumor progression.

• Here, the presence of the proteins forming inflammasomes and their activation in vessel cells (2H11) were investigated in vitro. 2H11 cells were assessed following inflammasome activation using known activators and also in the presence of media from 4T1 murine breast tumor cells.

Hypothesis

Vessel cells express inflammatory proteins and participate to the inflammation in breast tumors.

Materials and Methods

Cell and culture conditions: The murine 2H11 cell line was used to mimic endothelial cells. Aggressive stage of human breast cancer was represented by murine mammary 4T1 cells. Cells were grown and cultured in DMEM supplemented with antibiotics, antifungal, and 10% of FBS (Atlanta BioLogic, Atlanta, GA). For experiments, 2H11 cells were incubated with or without 4T1 cell conditioned media for 24 hours at 37°C, 5% CO2. 2H11 cells were treated with or without FBS-free media along (negative control) or with FBS-free media supplemented with LPS (100 ng/mL) or ATP (100 µM) (positive control) or 4T1 conditioned media. Cell lysates were harvested and analyzed by Western blotting, ELISA, and qPCR.

Flow-cytometry & Confocal microscopy: Post-treatment, cells were fixed in formalin and stored at 4°C prior to permeabilization with buffers and immunolabeling with antibodies to NLRP3 (1:500 dilution), caspase 1 (1:100 dilution), and actin (1:500 dilution). Cells were stained and analyzed using flow-cytometry and confocal microscopy. All data were analyzed and processed using the Leica software and the measured point spread function.

Results/Conclusion

Expression and co-localization of three key proteins confirms inflammasome complex formation in endothelial cells.

![Image of inflammasome complex formation](Image)

Breast cancer environment activates 2H11 cell inflammasomes.

![Image of inflammasome activation](Image)

Results/Conclusion

Breast cancer cells activated 2H11 cell inflammasomes leading to the secretion of inflammatory signals.

![Image of inflammatory signals](Image)

Results/Conclusion

As inflammation is key to the clinical progression of breast cancer, an intervention modulating vessel cell pro-inflammation activity may be helpful in preventing breast cancer progression.

Relevance

Data confirm that Vessel cells express inflammatory proteins and contribute to the inflammation in breast tumors.

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References: