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14. ABSTRACT

The innate immune system is critical for the early detection of invading pathogens and for initiating cellular host defense counter measures, which include the production of type I interferon (IFN). Immune dysfunction develops in patients with many cancer types and may contribute to tumor progression and failure of immunotherapy. This study will investigate the role of a newly identified IFN-mediated innate immunity pathway in immune surveillance, and the inactivation of a critical component of the pathway, STING, as a mechanism for immuo-escape in the development and progression of breast cancer. We propose a pilot study to test this hypothesis, that innate immunity pathway involved STING plays a role in purging pre-neoplastic cells and suppression of STING is a mechanism for escape of breast cancer cells from immune surveillance. Over the course of the study, we determined that STING was absent from many human breast cancer cell lines, at both the RNA and protein level. This caused the lack of IFNβ secretion in response to intracellular DNA. In human breast cancer tissue, we observed the presence of human retroviral elements and up-regulation of a number of immune response genes, as compared to normal human tissue.

15. SUBJECT TERMS
Breast cancer, innate immunity, STING

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1) ABSTRACT: The innate immune system is critical for the early detection of invading pathogens and for initiating cellular host defense counter measures, which include the production of type I interferon (IFN). Immune dysfunction develops in patients with many cancer types and may contribute to tumor progression and failure of immunotherapy. This study will investigate the role of a newly identified IFN-mediated innate immunity pathway in immune surveillance, and the inactivation of a critical component of the pathway, STING, as a mechanism for immuo-escape in the development and progression of breast cancer. We propose a pilot study to test this hypothesis, that innate immunity pathway involved STING plays a role in purging pre-neoplastic cells and suppression of STING is a mechanism for escape of breast cancer cells from immune surveillance. Over the course of the study, we determined that STING was absent from many human breast cancer cell lines, at both the RNA and protein level. This caused the lack of IFNβ secretion in response to intracellular DNA. In human breast cancer tissue, we observed the presence of human retroviral elements and up-regulation of a number of immune response genes, as compared to normal human tissue.

2) SUBJECT TERMS: Breast cancer, innate immunity, STING

3) INTRODUCTION: One in five human cancers are caused by infection, usually viral infection, as recently stated by the International Agency Escape from innate immunity is an essential step in the development of breast cancer. One strategy of immune escape of cancer cells is the suppression of IFN-inducing signaling. Recently, a critical component of the innate immune system that responds to DNA-based microbes, STING (stimulator of interferon genes) has been identified. STING is critical for the induction of IFN by DNA pathogens after infection, and the pathway will purge the infected cells by IFN-induced program cell death to terminate further propagation of the microbes.

Based on multiple public database gene expression arrays, STING appears to be expressed in most normal cell types, but the expression is highly variable and often absent in cancers, including breast cancer. Interferon signaling is defect in breast cancer but the mechanism is unknown. This leads to an idea that loss of STING may be a possible molecular defect in innate immunity that allows the escape of tumor cells from immune surveillance. Thus, we postulate a hypothesis that the STING innate immunity pathway plays a role in immune surveillance of breast cancer cells in addition to its anti-microbe function, and suppression of STING expression is an immune escape mechanism of breast cancer cells.

4) BODY: The objective of the proposed study will be to profile human breast cancer tissue for STING expression, and to explore the role of STING in breast cancer. We will further elucidate why STING is absent in some cancers but not others. We will correlate STING expression with its localization within the tumor and the downstream inflammatory response that its expression causes.

To accomplish this objective, we will analyze the presence of STING in breast cancer cell lines by Western blot. The localization of STING in human tumors will be analyzed by immunohistochemistry. IFNβ expression in breast cancer cells will be determined by ELISA. Finally, the inflammatory response and the presence of viral genes in breast cancers will be analyzed using nanoString® technology through a custom-designed genechip.

At the RNA level, STING is absent in 38 of 44 breast cancer cell lines tested (Figure 1A). As compared to control human mammary epithelial cells (HMECs), STING expression was only greater in HCC1937 and MDA157 breast cancer cell lines. At the protein level, STING expression was lower in all breast cancer cell lines tested as compared to HMEC controls (Figure 1B). In all breast cancer cell lines tested, IFN stimulatory DNA did not cause secretion of IFNβ, likely due to the decreased expression or altogether absence of STING (Figure 1C).

Since the experiments described above utilize transformed cell lines that may have inherent defects in the innate immune system pathways, we next aimed to examine immune gene regulation in human breast cancer tissue. Using our in-house designed nanoString chip, we observed the presence of Human Endogenous Retroviral (HERV) genes in RNA from human breast tumors and an up-regulation of immune response genes, as compared to normal human mammary tissue (Figure 2). However, we did not observe any correlation with viral expression levels and an inflammatory response. To do this, higher numbers of samples would likely be needed, which could be acquired by collaborating with our on-campus tumor bank.

5) KEY RESEARCH ACCOMPLISHMENTS:

- Quantified STING mRNA levels in 44 human breast cancer cell lines
- Examined STING protein levels in 10 human breast cancer cell lines
- Determined the levels of IFNβ secretion in response to polyI:C and ISD stimulation in 9 human breast cancer cell lines
- Examined levels of viral genes present in human breast cancer tissue, along with levels of immune response genes

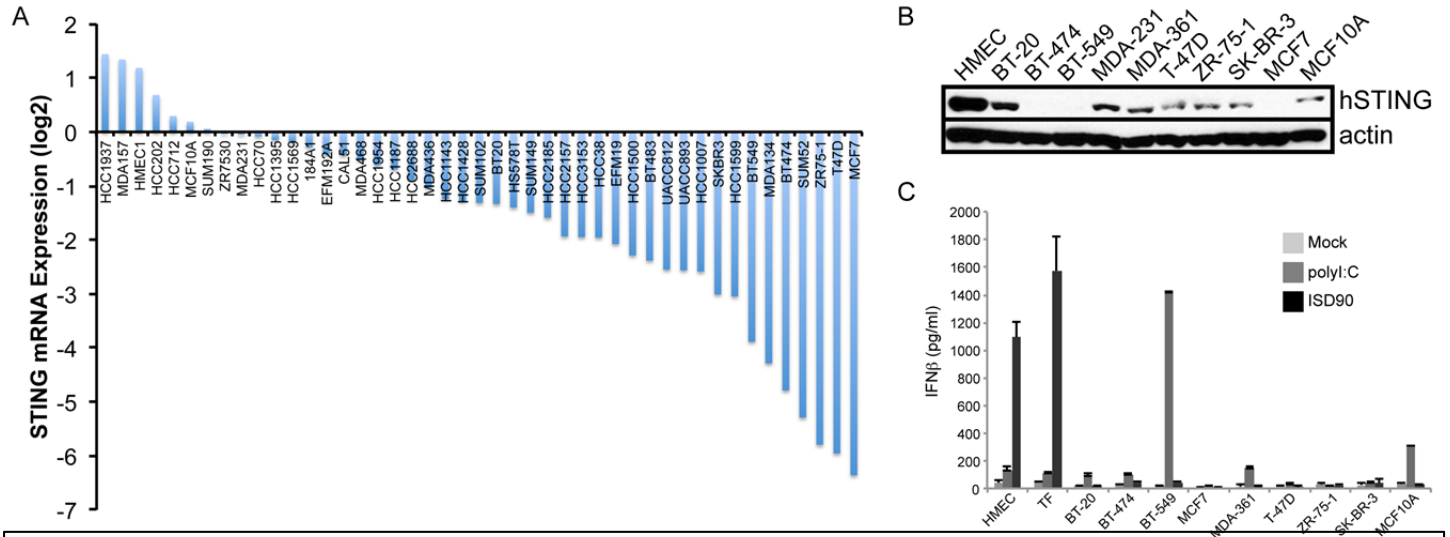

Figure 1 – A) Levels of STING mRNA in 44 breast cancer cell lines. B) STING protein levels in breast cancer cells lines is lower than in HMECs. C) IFNβ ELISA of breast cancer cell lines treated with polyI:C (an RNA mimic) or interferon stimulating DNA (ISD90). Only control cell lines (HMEC and telomerase fibroblasts (TF)) respond to DNA.

6) REPORTABLE OUTCOMES: A post-doctoral fellow in my lab competed for and received a institutional T32 training grant in Translational Breast Cancer Research (T32CA119929). We have obtained tissue from 10 human breast tumors, which have been useful in developing, quality checking, and building the informatics pipeline for our in-house designed nanoString chip.

7) CONCLUSIONS: We are beginning to understand the role that STING plays in oncogenesis. Firstly, STING is absent or down-regulated in many breast cancer cells lines, at both the mRNA and protein level. Furthermore, the DNA-sensing pathway, where STING is essential, is non-fucntional in these cells lines. Work by collaborators has shown that STING is required for $CD8^{+}$ T cell responses. This would help us understand why STING is absent in many breast cancer cell lines. However, preliminary data also shows that $STING^{-/-}$ mice are more resistant to epithelial cell tumorgenesis. It could be that the presence of STING is causing chronic inflammation that is conducive to a higher oncogenic potential. We have started to examine how the immune response is regulated in primary breast cancer tissue. However, our preliminary findings did not reveal any significant correlation between breast cancer subtype and immune response regulation. If we stimulated the primary cells with DNA, we may observe a loss in immune response signaling, whereas normal mammary epithelial cells are able to mount immune response signaling.

8) REFERENCES: N/A

9) APPENDICES: N/A

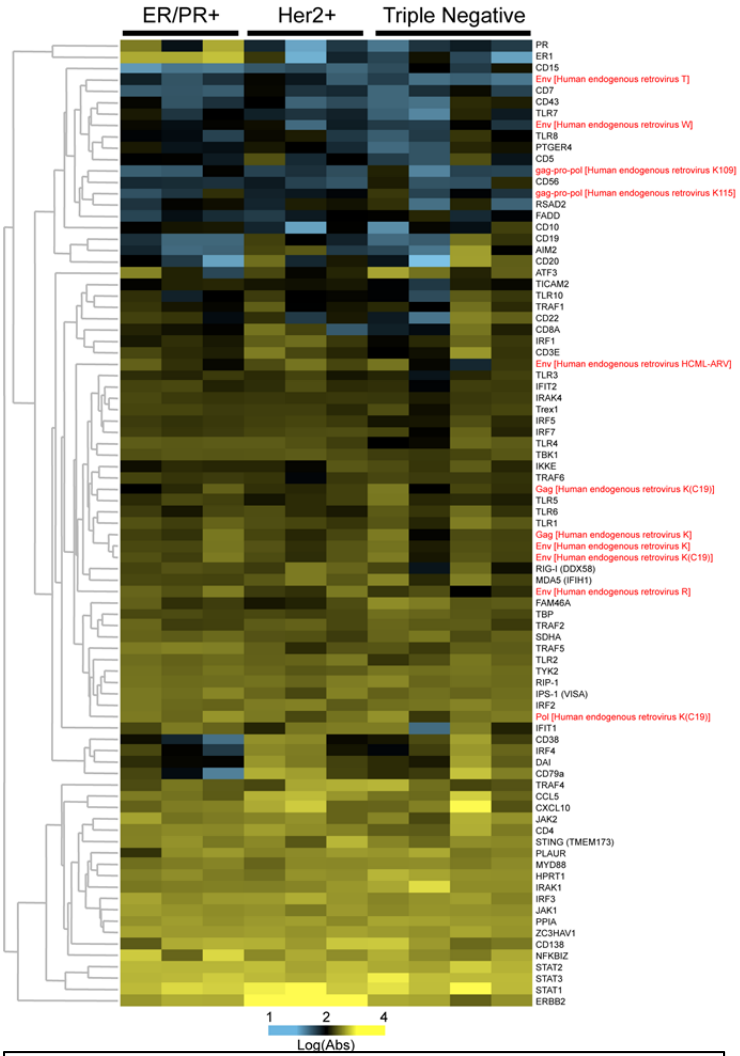

Figure 2 – Transcriptional profiling of 10 human breast cancers belonging to the ER/PR+, Her2+, or Triple Negative subtype. Immune response genes are shown in black and HERV genes are shown in red. Ratio data is compared to normal human mammary tissue.