Evaluation of STAT3 Signaling in Macrophages Using a Lentiviral Reporter System

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Background

Despite tremendous advances in breast cancer treatment over the past few decades, breast cancer remains the most commonly diagnosed cancer and remains the second leading cause of cancer-related death in American women.

Breast cancer growth requires complex interactions among a diverse cell population at the tumor site. We are interested in studying how the tumor microenvironment (TME) promotes tumor growth, with the ultimate goal of identifying novel therapeutic targets for breast cancer patients. Our lab is particularly interested in tumor-associated macrophages (TAMs) because these cell types have been shown to be a negative prognostic factor in breast cancer patients. Therefore, when designing new breast cancer treatments, it is essential to consider TAMs.

In studying the mechanisms behind breast cancer tumorigenesis, the Signal Transducer and Activator of Transcription (STAT) signaling pathway, specifically STAT3, has garnered increasing interest in recent years due to the fact that it has been found to be constitutively activated in up to 70% of breast cancers (3). Normally, STAT3 signaling is induced by cytokines or growth factors and modulates the expression of key mediators of inflammation and the immune response (4). STAT3 transcriptional activity is controlled by several mechanisms. This pathway promotes tumor proliferation and survival, angiogenesis, invasion and metastasis, tumor-promoting inflammation, and immune evasion. This pathway is seen as a promising target for new breast cancer therapies.

As anticipated from the role of STAT3 as an oncogenic transcription factor, our lab has previously found that inhibition of STAT3 in primary tumor nodules leads to reduced tumor cell proliferation and tumor burden. In contrast, we have also found that loss of STAT3 in myeloid cells leads to a worse prognosis in mice as well as faster tumor growth and proliferation (5). Therefore, STAT3 signaling in the myeloid lineage, particularly TAMs, is suppressing tumorigenesis. Therefore, it is fundamental to understand the role and mechanism of STAT3 signaling within the TME, in addition to the tumor cells, prior to implementing breast STAT3 inhibitors.

Aim 1: Transcription of the STAT3-GFP, TATA-GFP, and EFS-GFP control reporter plasmids in RAW 293 T cells

This reporter system can be used to evaluate STAT3 activity in live cells by reporting downstream transcription events, instead of relying on cell-surface marker identification. The primary goal of my project is to utilize these plasmids to ensure the STAT3 reporter is functional and specific. Then, we can study/treat these plasmids into various macrophage cell lines.

We hypothesize that by using this STAT3 reporter, we will be able to accurately visualize activated STAT3 signaling in TAMs in vivo by monitoring GFP expression. This will allow us to characterize the expression profiles of these TAMs within the breast cancer tumor microenvironment. Ultimately, we anticipate that STAT3 signaling will be diminished in macrophages that are associated with the tumor cells.

Aim 2: Measure STAT3-GFP reporter expression response to cancer secreted cytokines

We will validate the reporter with the canonical activator of STAT3 signaling, IL-6. Then, we will stimulate with conditioned media from HES72T cells (hybrid negative breast cancer cell line) to assess if factors secreted from the tumor cells impact STAT3 signaling in macrophages.

The results from our initial naked DNA transfection into HEK 293 T cells revealed proper functionality of the EFS-GFP and TATA-GFP plasmids, which demonstrated high and low levels of GFP expression, respectively. The STAT3-GFP plasmid displayed less GFP expression when compared to the TATA-GFP transfected cells.

Lentiviral Transfection/Transduction

Day 1: Plate viral packaging cells (HEK 293T)

Day 2: Transfect cells

• R7G/Raux vector (Reverse Transcripase)
• MDC/rSIV vector (Envelope Vector)
• pGPEG vector (Packaging Vector: Gap and Pol)
• Lentiviral vector (STAT3-GFP, TATA-GFP, EFS-GFP)

Transfection

Plate RAW cells and evaluate response to L-AgNPS717 Conditioned Media

Preliminary Lentivirus Transduction

EFS-GFP Transduced RAW Cells

With our preliminary lentiviral transduction, we were able to successfully transduce the EFS-GFP plasmid into the RAW cells. We were not able to visualize any GFP expression in the STAT3-GFP or STAT3-GFP transduced cells, both when unstimulated or stimulated with Conditioned Media from our human breast cancer cell line (HES72T) or L-AgNPS. Either these plasmids were not successfully transduced, or there was not enough GFP expression to be observed.

Future Directions

• Optimize EFS-GFP, TATA-GFP, and STAT3-GFP lentiviral vectors into various cell types.
  • RAW cells (immortalized macrophase cell line)
  • HES72T cells (human breast cancer cell line)
  • BMDCs (bone marrow derived macrophage)
  • THP-1 (human immortalized macrophage-like cell)

• Determine Lentivirus Multiplicity of Infection (MOI)

• Optimize amount of virus needed for each cell type, amount of cells, and exposure time to the virus

• Determine transduction efficiency.
  • P24 ELISA or dual reporter

• Quantify GFP expression levels by Flow Cytometry

• Use Recombinant-activated cell sorting (FACS) to isolate positively transfected cells containing the EFS-GFP, TATA-GFP, and STAT3-GFP reporters.

• Determine what factors in conditioned media of HES72T cells stimulate STAT3 signaling.

• Create lentivirus with new plasmids that contain antibiotic resistance gene for more stable transductions in vivo.

Conclusion

STAT3 is a critical transcription factor in the development and growth of breast cancer, and knowledge of its mechanism of action within TAMs is critical for the development of new therapeutic strategies. We were able to successfully transduce the STAT3-GFP, TATA-GFP, and EFS-GFP plasmid reporters into HEK 293 T cells, where we were able to confirm their functionality in vitro. A lentiviral transduction system was successfully created for the EFS-GFP plasmid, and future optimization will allow for the development of STAT3-GFP and STAT3-GFP plasmid reporters. Ultimately, we will be able to establish a methodology of evaluating STAT3 signaling in macrophages in vitro, and translate that to in vivo studies within TAMs.

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References: