Regulation of tRNA transcription by glucocorticoid receptor in breast cancer cells

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Introduction and Hypothesis

Transcription factor activity of steroid hormone nuclear receptors (**NRs**) are intimately associated with the development and progression of breast and other cancers, with both pro- and anti-tumor effects, although the exact mechanisms by which they balance these remains poorly understood.

Previous work revealed a novel axis of progesterone receptor (**PR**) gene regulation through RNA polymerase III (**POL III**) in breast cancer cells. ChIP-seq studies identified increased PR occupation of POL III-regulated tRNAs in progesterone vs vehicle treated cells.

POL III transcribes genes for tRNAs and certain other small non-coding RNAs, pointing to a potential role of PR and other NRs in regulating essential processes like translation.

Other NRs besides PR are of interest for the progression of breast and other cancers, such as glucocorticoid receptor (**GR**), a ubiquitous protein that regulates numerous cellular processes. Breast and other cancer patients are often given glucocorticoids to manage symptoms of chemotherapy, so we are interested in relevant effects of GR on the underlying biology of the cancer cells.

Our short-term goal is to determine association of GR at tRNA genes and the impact on transcription, and long-term to determine how these associations impact cell phenotype.

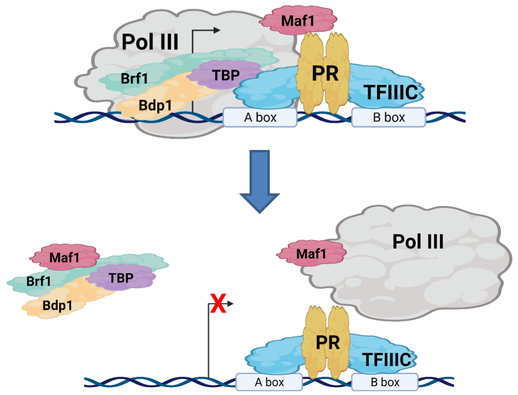

Figure 1. Working model of PR regulation of POL III activity. PR occupation of POL III-transcribed genes promotes POL III complex removal from the gene, decreasing tRNA transcription.

Results

GR is expressed in breast cancer cell lines

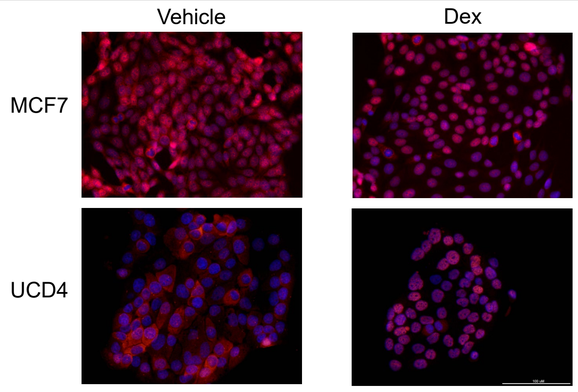

Figure 2. Immunocytochemistry with GR antibody (red) and DAPI (blue) in breast cancer cells treated for 1 hour with vehicle (EtOH) or GR agonist dexamethasone (Dex, 1 uM). GR is localized to the cytosol with vehicle and moves to the nucleus with Dex treatment.

Results

Association of GR at tRNA genes in MCF7 cells

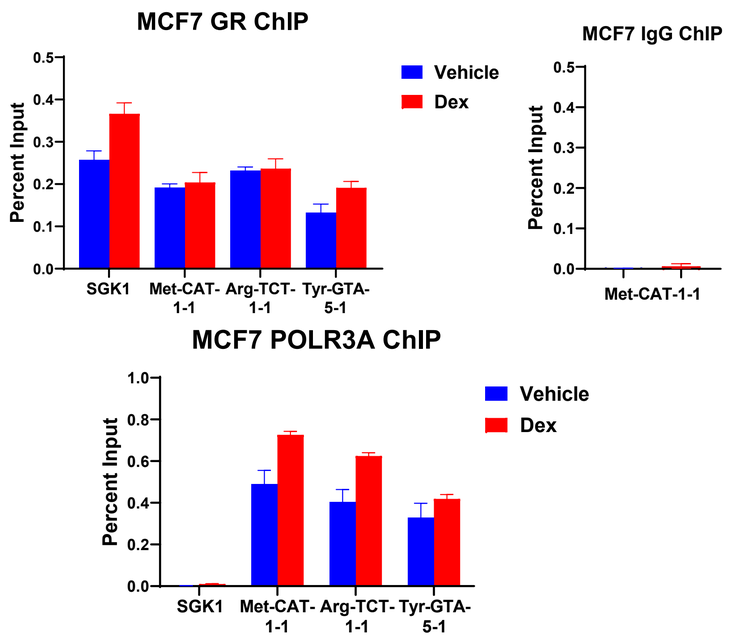

Figure 3. ChIP-qPCR for tRNA genes in MCF7 cells treated for 1 hour with vehicle (EtOH) or 1 uM Dex. Immunoprecipitation with GR, POLR3A, and IgG antibodies normalized to input. POLR3A serves as a positive control for occupancy at tRNA genes. Example IgG negative control shown for Met-CAT-1-1 gene. SGK1 promoter serves as a positive control for Dex-induced GR occupancy. n = 2 samples each

Pre-tRNA transcripts decrease with Dex treatment in UCD4 cells

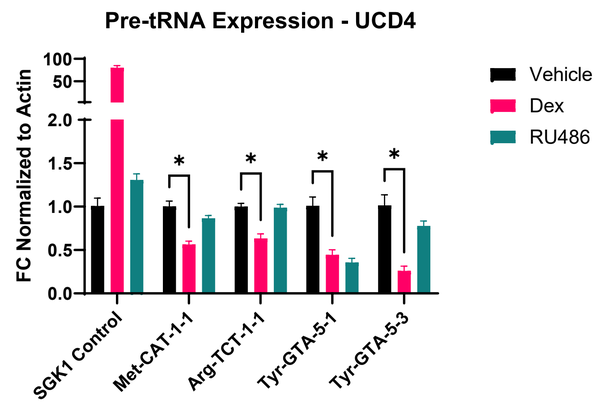

Figure 4. RT-qPCR for pre-tRNAs from UCD4 cells (n = 3) treated with vehicle (EtOH), 1 μM Dex, or 1 μM RU-486 (GR antagonist) for 72 hours. SGK1 mRNA serves as a positive control for Dex-induced GR transcriptional activity

BrU-Seq for sequencing of nascent tRNA transcripts

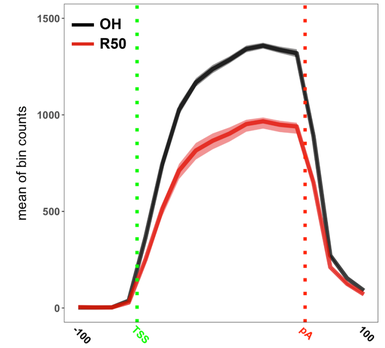

Figure 5. Global tRNA transcription measured with BrU-sequencing. T47D cells were treated with vehicle (OH) or 10 nM R5020 (PR agonist) for 1 hr, followed by 4 mM BrU for 30 min. BrU IP and sequencing was performed by Bentley Lab. Counts shown for 82 tRNAs shown to bind PR and POLR3A in ChIP.

Future Directions

- **Sequencing nascent tRNA transcripts**
  - Sequencing tRNAs is an active field of research
  - Pulse labeling with Bromouridine(BrU) followed by BrU-IP and sequencing (BrU-seq) can quantify nascent tRNA transcripts
  - This approach allows rapid assessment of hormone treatment impact on nascent tRNA transcripts (Figure 5)
- **Necessity of DNA binding by receptors for POL III interaction**
  - PR(C587A) and GR(R493A) mutants lack the ability to bind DNA
- **Effects of GR on tRNA production in PR negative cells/tissues**
  - GR is ubiquitous in normal tissue and many cancers, unlike sex steroid NRs
  - GR impact on tRNAs can be assessed in triple-negative breast cancer which lacks PR and ER

References

1. Finlay-Schultz J, Gillen AE, Brechbuhl HM, Ivie JJ, Matthews SB, Jacobsen BM, Bentley DL, Kabos P, Sartorius CA. Breast Cancer Suppression by Progesterone Receptors Is Mediated by Their Modulation of Estrogen Receptors and RNA Polymerase III. Cancer Res. 2017 Sep 15;77(18):4934-4946. PMID: 28729413
2. Vilasco M, Communal L, Mourra N, Courtin A, Forgez P, Gompel A. Glucocorticoid receptor and breast cancer. Breast Cancer Res Treat 130, 1–10 (2011).
3. Banuelos J, Shin SC, Lu NZ. A hotspot in the glucocorticoid receptor DNA-binding domain susceptible to loss of function mutation. Steroids. 2015 Apr;96:115-20. doi: 10.1016/j.steroids.2015.01.022. PMID: 25676786;