Comparative transcriptomic and proteomic analysis of lasofoxifene and fulvestrant, in an ERα-mutated metastatic breast cancer explant model, identifies potential molecular mechanisms underlying differential treatment efficacy

#1694

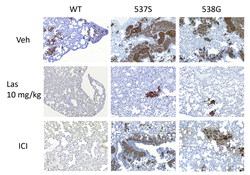
M. Lainé [1], D. A. Tieri[1], C. Zhu[2], I. Raman[2], G. Chen[2], B. S. Komm[3], G. L. Greene[1]
[1] University of Chicago
[2] UT Southwestern
[3] Sermonix Pharmaceutical

BACKGROUND

Metastatic breast cancer (mBC) is one of the leading causes of death among women. Mutations in estrogen receptor alpha (ERα) are acquired as the disease becomes resistant to current therapies, leading to tumor cell metastasis to distant organs. A combination of lasofoxifene (Las) and the CDK4/6 inhibitor abemaciclib has shown promising results in the ELAINE 2 trial, with significantly increased progression-free survival and good tolerability in mBC patients with activating ERα mutations who have progressed on endocrine/CDK4/6 therapy, including fulvestrant.

In mouse studies, Las, as well as the Las plus palbociclib (Pal) combination, resulted in a significant reduction in primary tumor burden and metastasis compared to fulvestrant (Ful) and Ful plus Pal treatment. On the right we show that treatment with Las at 10 mg/kg reduces the number of the lung metastases in xenograph model of MCF7 WT, MCF7 Y537S and MCF7 D538G (Breast Cancer Res. 2021 May 12;23(1):54).

In this study we investigated the transcriptomic and proteomic differences between Las and Ful treatment on MCF7 Y537S xenograph model using Nanostring GeoMx-DSP. Difference between DCIS and IDC are also investigated.



RESULTS

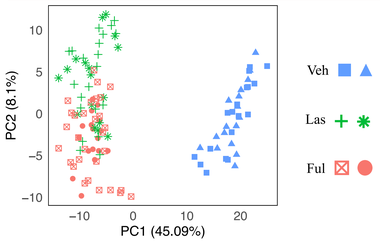


Figure 1: PCA analysis of differentially regulated genes after Limma correction shows more difference between Vehicle and Fulvestrant or Lasofoxifene treated mice than between Lasofoxifene and Fulvestrant, even though Las is a SERM and Ful is a SERD

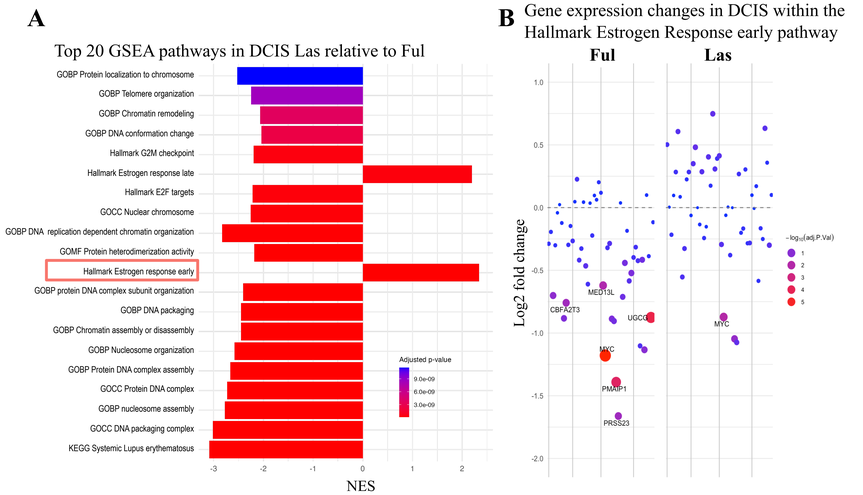
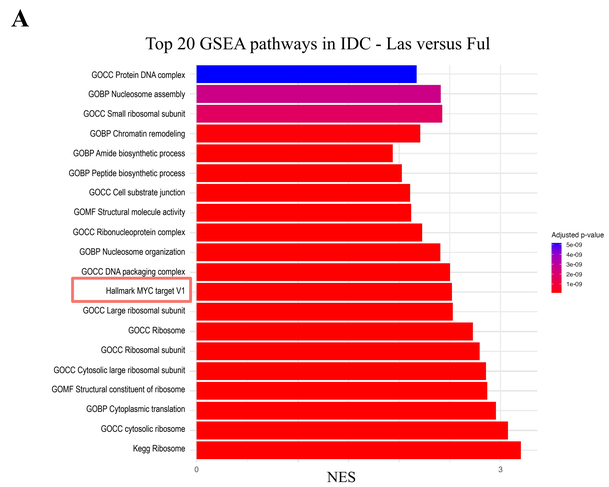


Figure 2: **(A)** Top 20 GSEA pathway analysis performed on differentially regulated genes in DCIS tumor cells comparing Ful and Las treatments. The pathway analysis was conducted using various human gene sets. The overall response to Ful, compared with Las, showed enrichment of gene sets involved in DNA and chromatin processing.
(B) Focused analysis of the *Hallmark Estrogen Response Early* pathway, comparing relative gene expression under Ful and Las treatments versus vehicle control, significant genes are labeled. This plot highlights the pattern differences for the transcriptional effects of Las and Ful treatments. In this pathway Myc is down regulated in both treatments. Ful down regulates more genes than Las.



B Gene expression changes in IDC within the Hallmark MYC target v1 pathway

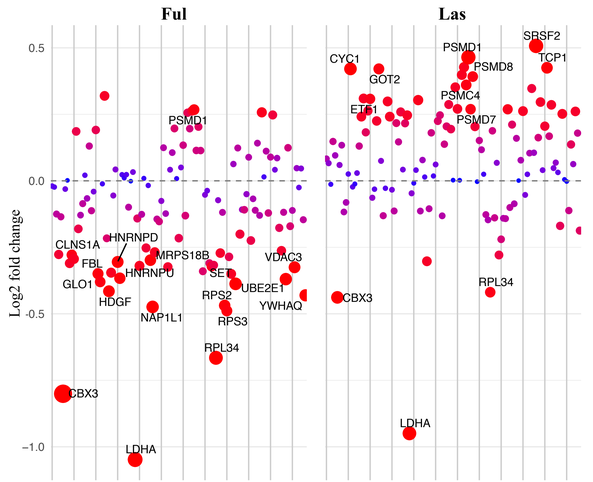


Figure 3: **(A)** Top 20 GSEA pathway analysis performed on differentially regulated genes in IDC tumor cells comparing Ful and Las treatments. The pathway analysis was conducted using various human gene sets. The overall response to Las, compared with Ful, showed enrichment of gene sets involved in DNA and chromatin processing but mainly in ribosomal function, translation and more ribosomal structural organization.
(B) Because ERα can activate and regulate MYC, we focused on the analysis of the *Hallmark MYC target v1* pathway, comparing relative gene expression for Ful and Las treatments versus vehicle control; significant genes are labeled. These results suggest that Ful downregulates multiple MYC-associated genes involved in metabolism and protein synthesis, showing a broader suppression of MYC-driven programs. Las, in contrast, upregulates genes linked to mitochondrial activity, proteasome function, and RNA processing, suggesting a more active MYC-responsive transcriptional profile.

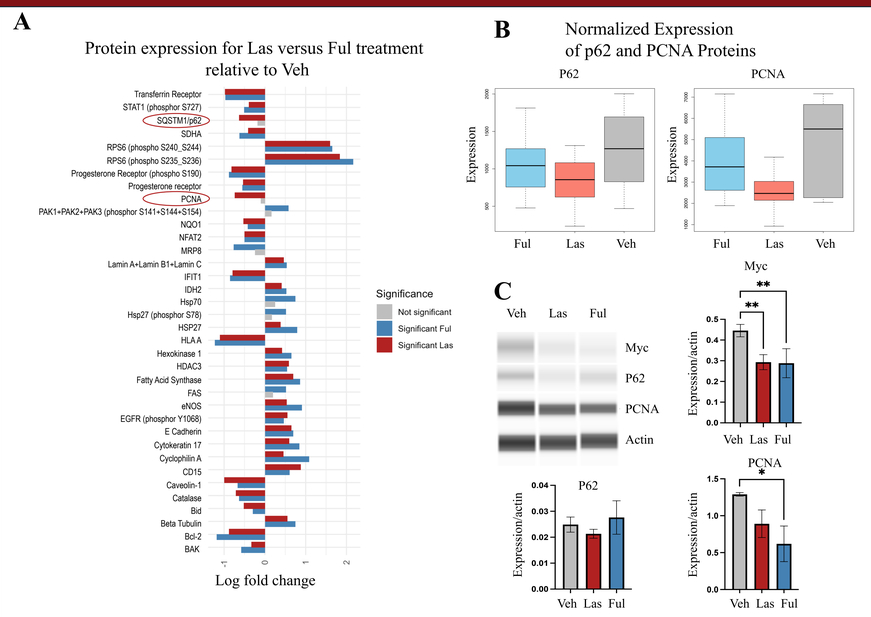


Figure 4: (A) Fold change of protein expression compared to Veh measured via GeoMx-DsP for Las or Ful treatment in IDC samples. Color bars show significant protein levels for either Las (red) or Ful (blue) treatment. P62 and PCNA are the only proteins significantly down regulated under Las treatment. Both proteins are commonly upregulated in breast cancer and are associated with cell proliferation. Conversely, suppression of p62 has been shown to inhibit tumor growth and metastasis.
(B) Normalized quantile expression of the GeoMx-DSP data for each treatment (Ful, Las and Veh) showing reduced p62 and PCNA expression for Las treatment.
(C) Confirmation of down regulation of p62, PCNA and Myc protein levels in MCF7 Y537S mutant cells on a western blot following 3 days of treatment with Las or Ful. The western blot shows 1 representative gel and the histograms represent the mean +/- standard deviation of three biological replicates. * p<0.05, **p<0.01 determined by one-way ANOVA.

METHODS

FFPE slides from xenograft model bearing the ERα Y537S mutation (MCF7 ERα Y537S mutant) were analyzed using GeoMx-DSP technology to investigate differences in transcriptional and protein expression between Las, Ful and Veh treatments. We also examined differences between ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). GeoMx-DSP was performed at the UT Southwestern microarray core facility in Dallas TX. Bioinformatics was performed at the Center for Research Informatics at University of Chicago. For western blot analysis, MCF7 ERα Y537S cells were treated for 3 days in 5% stripped DMEM; cells were lysed and processed using the WES technology.

CONCLUSIONS

- Both Las and Ful treatments have similar overall effects on the transcriptome and proteome.
- In DCIS, Ful shows a stronger impact on transcriptomic pathways related to chromatin remodeling, DNA-protein complexes, and DNA packaging.
- In IDC, Las treatment affects not only chromatin processing but also ribosomal function.
- Las and Ful each modulate gene set pathways differently, resulting in distinct patterns when compared to vehicle treatment.
- At the protein level, most proteins respond similarly to both treatments, except p62 and PCNA, which are significantly downregulated by Las in IDC samples.
- The clinical efficacy of Las in metastatic breast cancer may not be fully explained by the subtle transcriptomic and proteomic differences between Las and Ful, suggesting that additional mechanisms may contribute to its therapeutic benefit.
- Work is under way to further identify any key differences that distinguish the observed clinical efficacy of lasofoxifene versus fulvestrant.