Therapy Resistant Breast Cancer Thrives: Notch 4 and Transcription of Estrogen Responsive Genes

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Background

- In the United States, almost 1 in 8 women will be diagnosed with breast cancer at some point in their lives. Of these cases, approximately 80% will be estrogen receptor-α positive breast cancer[1].
- Women with ERα + breast cancers are given endocrine therapy to interrupt the production of estrogen within the body, but some patients develop a resistance to this therapy due to continued signaling among pathways. One such pathway is the Notch signaling pathway. If this signaling continues, resistant cells continue to survive, which can spell disaster – and possibly even death – for the patient (poor prognosis and survival)[2].
- It has been previously determined that in the presence of Notch1, estrogen responsive genes *pS2, CD44, VEGFa, CCND1* and *C-MYC* are upregulated.
- NNotch4 is active at a very low level in the presence of estrogen but becomes highly activated when cells are treated with anti-estrogens[3].
- It is hypothesized that Notch4 contributes to the continued signaling and expression of estrogen responsive genes within the body[4]. However, the mechanism through which this signaling occurs remains unknown.
- Our research seeks to understand the mechanism by which Notch4 contributes to the continued transcription of estrogen-responsive genes, specifically which estrogen- and Notch1- responsive genes are upregulated and whether the estrogen receptor and Notch4 Intracellular Domain (ICD) are present at the promoter region of these genes in the absence of estrogen.

Methods

Cell Culture

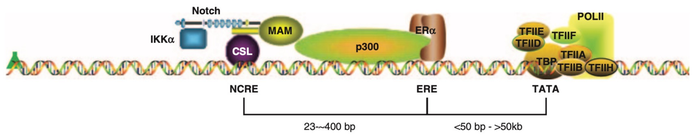
- MCF-7/N4ICD-HA cells were seeded and cultured in estrogen-free media for 72 hours. Following this period, doxycycline was added, and the cells were incubated for a further 12 hours. RNA was then harvested from the cells using the Omega E.Z.N.A. Total RNA Kit®.

Real Time RT-PCR

- cDNA was produced using the qScript® method and the GeneAmp® PCR System 7000. Following laboratory protocol and using SYBR Select Master Mix, RT-PCR was run using previously confirmed primers to detect the expression of *CD44, VEGF*α , *CCND1* and *C-MYC*.

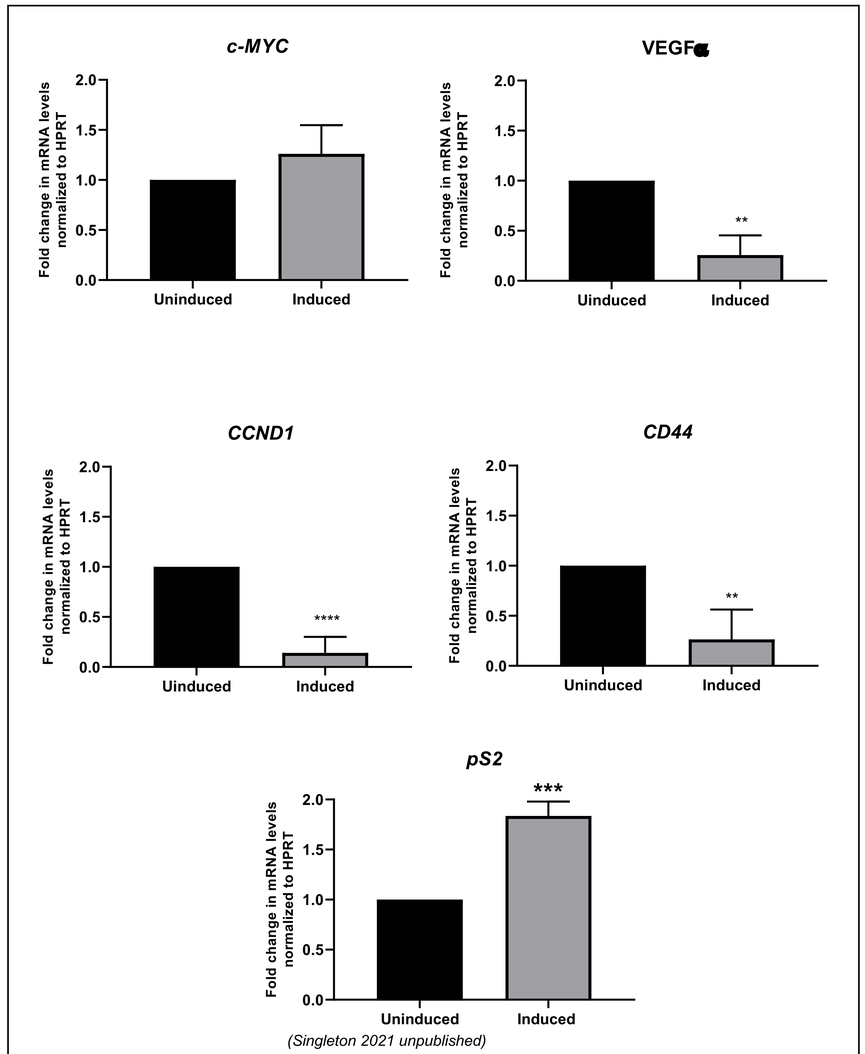
Quantitative ChIP

- Quantitative ChIP was previously performed in the lab using antibodies for ERα and HA following laboratory protocol.

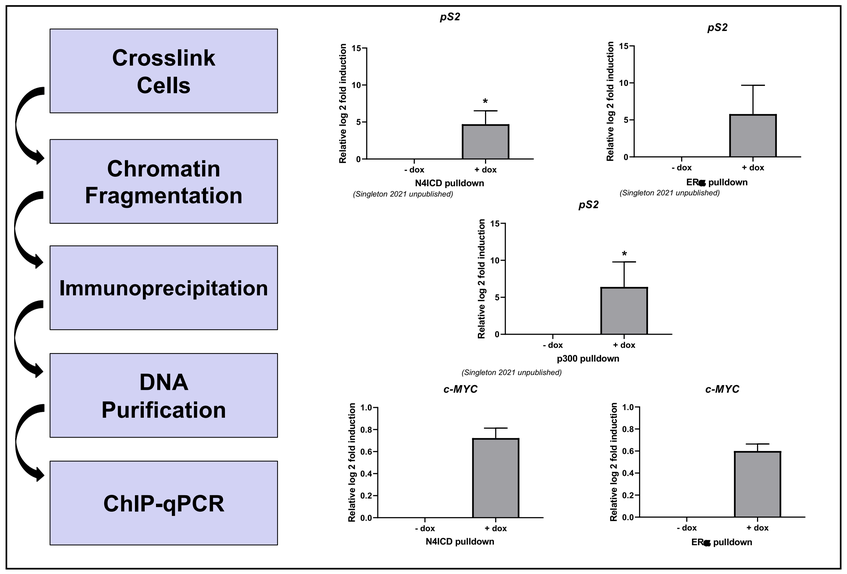


Model of the Notch/Erα crosstalk pathway. The full mechanism of this crosstalk remains unknown, as is whether this interaction is direct[1].

Expression of Notch and ERα Responsive Genes



ChIP Studies



Conclusion & Next Steps

Conclusion

pS2 and *c-MYC* are upregulated in the presence of Notch4. In addition, ChIP-PCR indicates that Notch4 and the estrogen receptor are present at the promoter region of these genes. The remaining genes are not upregulated, which may be due to the interaction between Notch1 and Notch4; this relationship requires further study.

Future Studies

- Studies of *c-MYC* need to be repeated to determine expression and the presence of Notch 4 at the promoter region.
- Further study of other possible upregulated targets, such as *GREB1* and *ABCA3*
- The results from this work may indicate a necessary ChIP-Seq study which would allow a more comprehensive view of all the genes targeted by Notch4 under estrogen-deprived conditions.
- Understanding the Notch4 mechanism will allow further studies to explore ways to disrupt Notch signaling, allowing for the issue of endocrine therapy resistance to be addressed and eventually solved.

References

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