Gene expression analysis and interpretation for HER2 +ve breast cancer

**Introduction**

Breast cancer is a disease marked by genetic and clinical diversity, presenting multiple subtypes. Typically, these subtypes are classified into four categories determined by the immunohistochemical expression of hormone receptors: estrogen receptor-positive (ER+), progesterone receptor-positive (PR+), human epidermal growth factor receptor-positive (HER2+), and triple-negative breast cancer (TNBC). [1]

HER2-positive breast cancer accounts for 15–20% of all breast cancer cases. It is identified by the overexpression of the HER2 protein, determined through immunohistochemistry (IHC) status or fluorescence or chromogenic in situ hybridization (FISH/CISH) assessing HER2 gene copy number [2]. This type of breast cancer has a relatively poor prognosis. Therefore, identifying any differentially expressed genes and performing pathway analysis may result in identifying druggable targets that are associated with HER2 expression. Currently, trastuzumab is the drug of choice for adjuvant chemotherapy for HER2-positive breast cancer patients [3], but identifying other proteins that are created from overexpressed genes may result in identifying additional druggable targets.

A recent study conducted a genomic analysis of 64 HER2-positive breast cancer genomes, categorizing them into four subgroups (Groups A, B, C, and D) based on genomic features such as somatic mutations, copy-number changes, and structural variations. Subgroups A and B are estrogen receptor-positive (ER+) and closely aligned with the luminal B intrinsic subtype. They are characterized by low tumor protein p53 (TP53) values, as well as amplification of cyclin D1 (CCND1) and ribosomal protein S6 Kinase B1 (RPS6KB1). In contrast, groups C and D are estrogen receptor-negative (ER−) and show proximity to the HER2-positive intrinsic subtype with high TP53 expression[4]. This shows us that it is possible to identify subtypes within traditionally categorized groups that can allow for the use of precision medicine in identifying optimal chemotherapeutic agents or better predicting prognosis. Differential gene analysis can be the first step in identifying these subtypes especially if it is combined with data regarding response to chemotherapeutic agents and survival data.

**Methods**

Differential Gene Analysis: Helps identify the genes that are upregulated and downregulated in HER2 positive vs negative breast cancer. This will better help understand the pathology behind HER 2 positive breast cancer thus assisting in identifying druggable targets. Helps in the quantification and statistical analysis of systematic changes between HER2-positive and HER2-negative breast cancer, DESeq2 is an R package that employs negative binomial generalized linear models. This tool facilitates the examination of differential expression, particularly between HER2-positive and HER2-negative cases, in contrast to variations within the HER2-positive category. The estimates of dispersion and logarithmic fold changes within DESeq2 incorporate data-driven prior distributions.

Pathway enrichment analysis identifies biological functions that are overrepresented in a group of genes more than would be expected by chance and ranks these functions by relevance. Helps identify the presence of parallel pathways to the affected gene.

**Results**

There is an upregulation of 205 genes and a downregulation of 951 genes so HER2 seems to be more potent at downregulating genes as compared to upregulated pathways. The top 10 genes that have been up and downregulated has been listed below.

**Up-regulated :**

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1. **CSN2**

CSN2 or casein 2 gene upregulation makes sense in breast cancer as studies have shown that pregnancy hormones (i.e. oestradiol and progesterone) induce robust upregulation of Casein 2 (Csn2) in both pre and post-pregnancy mammary organoid cultures. [5] This is relevant as women with higher lifetime exposure to oestrogen and progesterone either endogenously via being nulliparous( lack of single or multiple postmenopausal amenorrhea), early menarche and late menopause, or exogenously via HRT are at higher risk of developing breast cancer. [6] However, its association with the HER2 subtype is intriguing. I am yet to find any papers that explore this relationship.

1. **KRT1**

The upregulation of other genes in the KRT family like KRT19,18 etc have been observed in HER2 positive breast cancer as some of them like cytokeratin19 induced by HER2/ERK binds and stabilizes HER2 on cell membranes. Perhaps KRT1 has a similar function. The use of shRNA to silence KRT19 led to increased ubiquitination and destabilization of HER2. Additionally, the application of a KRT19 antibody resulted in the downregulation of HER2 and a decrease in cell viability. These findings offer a novel target for HER2-positive breast cancers. [7]

Another way to think about it could be that the ERBB2 gene encodes for a member of Epidermal growth factor (EGF) receptor family. Epidermal growth factor (EGF) is a prevalent mitogenic factor known for promoting the proliferation of various cell types, particularly fibroblasts and epithelial cells. [8] So, a ERBB2 +/ amplified cell may upregulate KRT1 to increase the production of keratin which is the main component of an epithelial cell.

**Down-regulated :**

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1. **CPLX2**

Interestingly, a study on HCC (Hepatocellular carcinoma) showed that CPLX2 is significantly upregulated in HCC and is associated with poor overall survival. CPLX2 accomplishes this by modulating ferroptosis and apoptosis through the NRF2 pathway in human hepatocellular carcinoma (HCC) cells. [9] Perhaps, the downregulation of CPLX2 has a protective function in breast cancer but its effect is outweighed by the other genetic aberrations.

1. **SRM3B**

A study was able to use 3 salivary proteins as markers to differentiate TNBC (triple negative breast cancer) patients from healthy subjects. One of these proteins is submaxillary androgen-regulated protein 3B (SMR3B). [10] The downregulation of this gene in HER2 +ve cases is indicative of the fact that this marker can also be used to differentiate between HER2+ve and TNBC as well. Further studies regarding its viability to differentiate TNBC against ER and PR+ ve BC should be conducted. If these salivary biomarkers are produced well in advance of the onset of BC, they could potentially be used as a screening tool.

**Pathway enrichment (up):**

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1. **HSA04080: Neuroactive ligand-receptor interaction**

This pathway is incredibly broad and progesterone was mentioned a couple of times along with FSH( Follicle Stimulating Hormone), GnRH(Gonadotropin hormone-releasing hormone) which are all part of the ovulation cycles and regulate the cyclical release of estrogen and progesterone in the body. Higher lifetime exposure to estrogen and progesterone has been proven to increase risk of breast cancer [6]. This could be why this specific pathway is enriched.

1. **HSA04151: PI3K-Akt signalling pathway**

HER2 serves as a powerful activator of downstream pathways, notably the PI3K/Akt pathway, which acts as a central regulator of cell growth and survival. [11] The potential mechanisms of trastuzumab's actions include inhibition of the upregulated PI3K-AKT pathway[12], and the effectiveness of the drug could be due to an auxiliary effect that it has on this pathway. Perhaps identifying more drugs that directly target this pathway may have better outcomes for the patient.

**Pathway enrichment (down):**

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1. **HSA04024: cAMP signalling pathway**

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cAMP signalling pathway is downregulated which does not sync up with current literature as we move downstream and expect an down-regulation of PI3K-Akt pathway, Hedgehog pathway and decreased cell survival due to increased rate of apoptosis. However, according to current literature and previous analysis of upregulated pathways there is actually a up-regulation of PI3K-Akt pathway. Therefore, this finding requires additional evaluation.

A study has shown that upregulating this pathway can actually improve patient outcomes by decreasing the risk of metastasis. Activation of the cAMP signalling pathway has demonstrated the ability to hinder the migration and motility of various cell types. An efficient method for selectively triggering cAMP signalling is by inhibiting cyclic nucleotide phosphodiesterases (PDEs). Inhibitors of phosphodiesterases (PDEs) have been observed to impede the migration of breast cancer cells, indicating their potential as valuable therapeutic targets for preventing breast cancer metastasis. [13]

1. **HSA05165: HPV infection**

According to the pathway analysis, there is supposed to be a downregulation of HPV infection pathway but according to certain studies, there is actually a upregulation or positive correlation between HPV infection and the development of breast cancer. The initiation of breast cancer carcinogenesis is hypothesized to involve HPV infection as an early trigger, potentially acting synergistically with other environmental factors. The confirmation of an association between HPV and breast cancer remains uncertain. The presence of HPV DNA in breast cancer samples has generated conflicting data, and a precise and clarified mode of HPV transmission to the breast is still unknown. [14]

**PCA**

A graph of red and black dots

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PCA was performed with the variance stabilized transformed values and the above figure was obtained. There does not seem to a clear dichotomy in terms of the HER2=ve and HER2-ve cases.

**GITHUB LINK :**

https://github.com/AlinNavas/Differential-Expression-Analysis/blob/main/Differential%20expression%20analysis%20for%20HER2%20positive%20breast%20cancer%20cases.R

References

1. Shaath, H., Elango, R. and Alajez, N.M. (2021) ‘Molecular Classification of Breast Cancer Utilizing Long Non-Coding RNA (lncRNA) Transcriptomes Identifies Novel Diagnostic lncRNA Panel for Triple-Negative Breast Cancer’, *Cancers*, 13(21), p. 5350. Available at: <https://doi.org/10.3390/cancers13215350>.
2. Sareyeldin, R.M. *et al.* (2019) ‘Gene Expression and miRNAs Profiling: Function and Regulation in Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Breast Cancer’, *Cancers*, 11(5), p. 646. Available at: <https://doi.org/10.3390/cancers11050646>.
3. Greenblatt, K. and Khaddour, K. (2023) ‘Trastuzumab’, in *StatPearls*. Treasure Island (FL): StatPearls Publishing. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK532246/> (Accessed: 20 December 2023).
4. Ferrari, A. *et al.* (2016) ‘A whole-genome sequence and transcriptome perspective on HER2-positive breast cancers’, *Nature Communications*, 7, p. 12222. Available at: <https://doi.org/10.1038/ncomms12222>.
5. Ciccone, M., Trousdell, M. and Dos Santos, C. (2020) ‘Characterization of Organoid Cultures to Study the Effects of Pregnancy Hormones on the Epigenome and Transcriptional Output of Mammary Epithelial Cells’, *Journal of Mammary Gland Biology and Neoplasia*, 25, pp. 1–16. Available at: <https://doi.org/10.1007/s10911-020-09465-0>.
6. Shah, R., Rosso, K. and Nathanson, S.D. (2014) ‘Pathogenesis, prevention, diagnosis and treatment of breast cancer’, *World Journal of Clinical Oncology*, 5(3), pp. 283–298. Available at: <https://doi.org/10.5306/wjco.v5.i3.283>.
7. Ju, J. -h *et al.* (2015) ‘Cytokeratin19 induced by HER2/ERK binds and stabilizes HER2 on cell membranes’, *Cell Death and Differentiation*, 22(4), p. 665. Available at: <https://doi.org/10.1038/cdd.2014.155>.
8. Wong, R.W.C. and Guillaud, L. (2004) ‘The role of epidermal growth factor and its receptors in mammalian CNS’, *Cytokine & Growth Factor Reviews*, 15(2–3), pp. 147–156. Available at: <https://doi.org/10.1016/j.cytogfr.2004.01.004>.
9. Li, H. *et al.* (2023) ‘CPLX2 Regulates Ferroptosis and Apoptosis Through NRF2 Pathway in Human Hepatocellular Carcinoma Cells’, *Applied Biochemistry and Biotechnology*, 195(1), pp. 597–609. Available at: <https://doi.org/10.1007/s12010-022-04135-9>.
10. Giri, K., Maity, S. and Ambatipudi, K. (2022) ‘Targeted proteomics using parallel reaction monitoring confirms salivary proteins indicative of metastatic triple-negative breast cancer’, *Journal of Proteomics*, 267, p. 104701. Available at: <https://doi.org/10.1016/j.jprot.2022.104701>.
11. Iqbal, Nida and Iqbal, Naveed (2014) ‘Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications’, *Molecular Biology International*, 2014, p. 852748. Available at: <https://doi.org/10.1155/2014/852748>.
12. Kute, T. *et al.* (2004) ‘Development of Herceptin resistance in breast cancer cells’, *Cytometry. Part A: The Journal of the International Society for Analytical Cytology*, 57(2), pp. 86–93. Available at: <https://doi.org/10.1002/cyto.a.10095>.
13. Dong, H. *et al.* (2015) ‘Inhibition of breast cancer cell migration by activation of cAMP signaling’, *Breast Cancer Research and Treatment*, 152(1), pp. 17–28. Available at: <https://doi.org/10.1007/s10549-015-3445-9>.
14. Dong, H. *et al.* (2015) ‘Inhibition of breast cancer cell migration by activation of cAMP signaling’, *Breast Cancer Research and Treatment*, 152(1), pp. 17–28. Available at: <https://doi.org/10.1007/s10549-015-3445-9>.