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# Bioinformatics analysis of basal and luminal cancer for diagnostic biomarkers

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## Abstract

There are more than 1.15 million cases of breast cancer diagnosed worldwide annually. Only a few reliable prognostic and predictive factors are currently applied in clinical practice to the treatment of breast cancer patients. The simultaneous evaluation of the expression of thousands of genes is a capability of DNA microarrays. Recent preliminary studies suggest that gene expression profiling using a DNA microarray can potentially provide patients with newly diagnosed breast cancer with independent prognostic information. An overview of the uses of microarray techniques in breast cancer is provided in this study and compare basal and luminal BC using the microarray aspect at two levels of miRNA and genes. Our results showed that 221 gene probes could be a significant differential expressed genes in breast cancer and only seven of miRNAs were differentially expressed. The genes represented in ETS1, ZEB1, EMP1, PRSS23, TFPI, NAV2, ACSL4, IGFBP3, TUBB2A, NPNT and MYB were highly differential expressed in basal breast cancer compared to luminal breast cancer and these genes were interacted with hsa-miR-429, hsa-miR-34a and hsa-miR-200b which these miRNAs were downregulated in basal breast cancer. These genes and miRNAs could be a diagnostic or prognostic biomarker need investigation in further study.

**Keyword: Breast cancer, Bioinformatics, Microarray, Biomarker**.

## Introduction

Cancer is a disease that is the result of the uncontrollable growth of abnormal cells. Abnormal cells perform unusual functions rather than the known function within the human body. The growth of normal cells is controllable, while the growth pattern of abnormal cells is uncontrollable. Within a momentary period, the number of abnormal cells in the body increases which in turn creates further complications for proper body functions. Cancerous cells can target any organ in a functional body; organs such as skin, lungs and breast are commonly affected.

Breasts contain mammary glands and are recognized not only as an important organ but also as a paired structure. They are present in both males and females yet are more important in females following puberty. The accessory gland of the female reproductive system's primary function is to produce milk. Breast cancer is a condition in which cells in the breast grow out of control. Various types of breast cancer can originate, depending on which cells are adversely affected.

The differentiation of breast cancer into molecular subgroups according to patterns of gene expression (Perou *et al*., 2000) has enhanced our knowledge of the diversity of breast cancer. In situ hybridization (ISH) and immunohistochemistry (IHC) are frequently employed as a substitute for gene expression investigations (Blows et al., 2010 ; Engstrom *et al* .; 2013 and Sung *et al*., 2016) and they largely mirror the genetic subtypes (Callagy et al., 2003). Human epidermal growth factor receptor 2 (HER2) and the proliferation marker Ki67 expression can be used to further categorize luminal (estrogen and/or progesterone receptor positive) tumors into three categories. Tumors that are not luminal can be categorized into two subtypes: HER2 and triple-negative (TN). TN can be further separated into the basal phenotype and the non-basal or five-negative phenotype based on the expression of basal markers (Blows et al., 2010 ; Engstrom *et al* .; 2013; Ishitha *et al*., 2016 and Nielsen *et al* .,2004). It has been extensively investigated how basal markers are expressed in TN breast tumors.

Studying long-term survival, however, has revealed that luminal breast cancers can recur for many years after the initial diagnosis. To differentiate between treatment and follow-up, additional prognostic categorization is necessary due to the heterogeneity of luminal cancers (Hennigs *et al*., 2016). Studies on microarrays in relation to cancer try to categorize cancer patients into homogenous categories, look for

differentially expressed genes in tumors with various characteristics, or forecast the prognosis of patients. Using breast cancer as an example, we address the theories driving these studies, their power, and the validity and clinical application of the findings. Based on microarray data and cluster analysis, a number of subtypes of breast cancer have been identified (Perou *et al*., 2001). In this study, we hope to identify a particular marker that may be utilized to differentiate between basal and luminal cells, and perhaps this marker can be used as a therapeutic target.

## Materials and Methods

**Acquisition of microarray data:** Microarray gene expression and miRNA expression data were obtained from the NCBI Gene Expression Omnibus (GEO) ftp (https://www.ncbi.nlm.nih.gov/geo/). NCI-60 gene expression data (GSE32474) and miRNA expression data (GSE26375) were retrieved for basal and luminal breast cancer. Gene probes or miRNAs with a fold change of >4 or <-4 and P<0.01 for basal in comparison to luminal breast cancer over the median value were selected for further analysis .

**Functional analysis and pathway enrichment analysis:** The differentially expressed genes (DEGs) from NCI-60 were submitted to STRING-db (https://string-db.org/ ) online tool to build a network for up-regulated genes and down regulated genes. The network generated from STRING-db was exported to CluGo app that plugs in cytoscape software for both up regulated genes and down regulated genes to determine pathway that each group may participate by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. DAVID (http://david.ncifcrf.gov/) has been used as online tool that can perform a functional analysis for discovering the relationships among the selected gene sets (Stekel, D. 2003). by determining the biological process .

**Analysis of miRNA-target mRNA network :**The selected differentially expressed miRNA (DEMs) were imported to miRNET (https://www.mirnet.ca/) a platform can search the target genes based on the conserved sites matching the seed regions of miRNAs. The miRNA-target network was constructed by using both DEGs and DEMs but the generated network was reduced to handle the network properly.

## Results and Discussion

**Identification of DEGs :** The identification of genes from basal breast cancer was performed on NCI-60 cell line panel (GSE32474) of DNA microarray. A total of 221 gene probes were identified with significant differential expression in breast cancer (Appendix 1). Among them, 142 gene probes with higher expression levels and 79 gene probes with lower expression levels in basal breast cancer cells [log2 (fold change) >4 or log2 (fold change) <−4, adj.P<0.01]. The up-regulated and down-regulated genes (DEGs) with adj.P<0.01 were plotted in volcano plot (Figure 1).

**Gene Ontology function and KEGG pathway analyses:** The biological processes where DEGs participate were determined using DAVID Gene Functional Classification Tool. These processes were significantly involved in the positive regulation of epithelial to mesenchymal transition, response to estradiol, neuron migration, cellular protein metabolic process and extracellular matrix organization (the top 5 biological processes) (Figure 2). Furthermore, the DEGs were divided into two groups represented in upregulated and downregulated DEGs and each group was imported into cytoscape software to determine the pathways which each group using KEGG pathway. The upregulated DEGs were primarily involved in transcriptional mis-regulation in cancer, TGF- beta signaling pathway, cellular senescence etc. (Figure 3A).

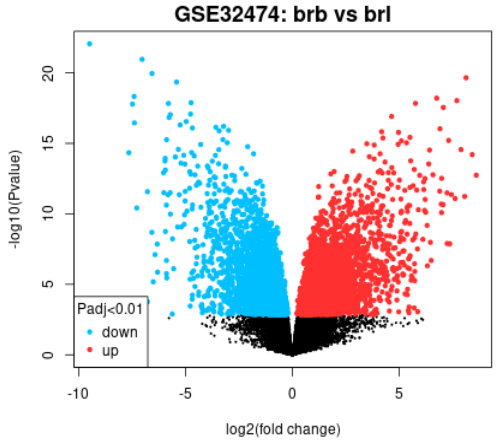


**Figure 1. The volcano plot of differential expression genes with Padj < 0.01 highlighted with red and blue colors in comparison to not significant genes with black color**.

However, the downregulated DEGs were significantly participated in Estrogen-dependent gene expression, Signaling by Receptor Tyrosine Kinases, Signaling by Nuclear Receptors, ESR-mediated signaling, Signaling by ERBB4, Proteoglycans in cancer and PI3K-Akt signaling pathway (Figure 3B).

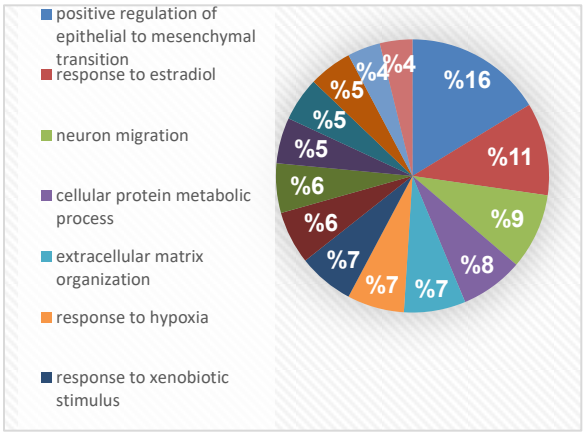


**Figure 2. The gene ontology of biological processes for DEGs scaled by fold enrichment. All biological processes were significant adj.P < 0.01.**

**Analysis of miRNA-target mRNA network:** There were 889 miRNA probes identified from GSE26375 for breast cancer. Out of these miRNAs only five miRNAs were significantly related to basal breast cancer in comparison with luminal one. Those five miRNAs were divided into Four downregulated miRNAs (hsa-miR-429, hsa-miR-34a, hsa-miR-200b, and hsa-miR-200a) and only one upregulated miRNA (hsa-miR-146a). Collectively, the five miRNAs were further analyzed by miRNET platform to determine the relationship between these miRNAs from miRNA expression data (GSE26375) and DEGs from NCI-60 gene expression data (GSE32474). Interestingly, the downregulated miRNAs represented in hsa-miR-429, hsa-miR-34a, hsa-miR-200b have interacted significantly with protein members of DEGs list (ETS1, ZEB1, EMP1, PRSS23, TFPI, NAV2, ACSL4, IGFBP3, TUBB2A, NPNT and MYB) (Figure 4). These results revealed that current miRNA downregulation may be linked to some of the downregulated or upregulated DEGs (Table 1).

Through the simultaneous analysis of tens of thousands of expressed genes, microarray technology has improved molecular understanding of cancer biology. A unique molecular classification of breast cancer was discovered when a series of breast tumors were hierarchically clustered based on a set of intrinsic genes that were expressed differently in various individuals (Perou et al., 2000).

The basal-like, HER2-positive, luminal-A, and luminal-B subtypes of human breast cancer are included in the so-called intrinsic molecular classification of the disease. These subtypes have distinctive pathological characteristics and clinical outcomes: HER2-positive breast cancer is erbB2/HER2 gene amplified and is associated with poorer outcomes when untreated. Both luminal-A and luminal-B breast cancers are ER-positive, though luminal-B cancers have worse outcomes. Basal-like breast cancer is primarily triple-negative, lacking expression of ER, progesterone receptor (PR), and normal erbB2/HER2 gene copy number. Using cDNA microarrays to profile the gene expression of 65 breast cancer samples from 42 different patients, Perou and colleagues' key study first established molecular profiles of breast cancer (Perou *et al*., 2000).

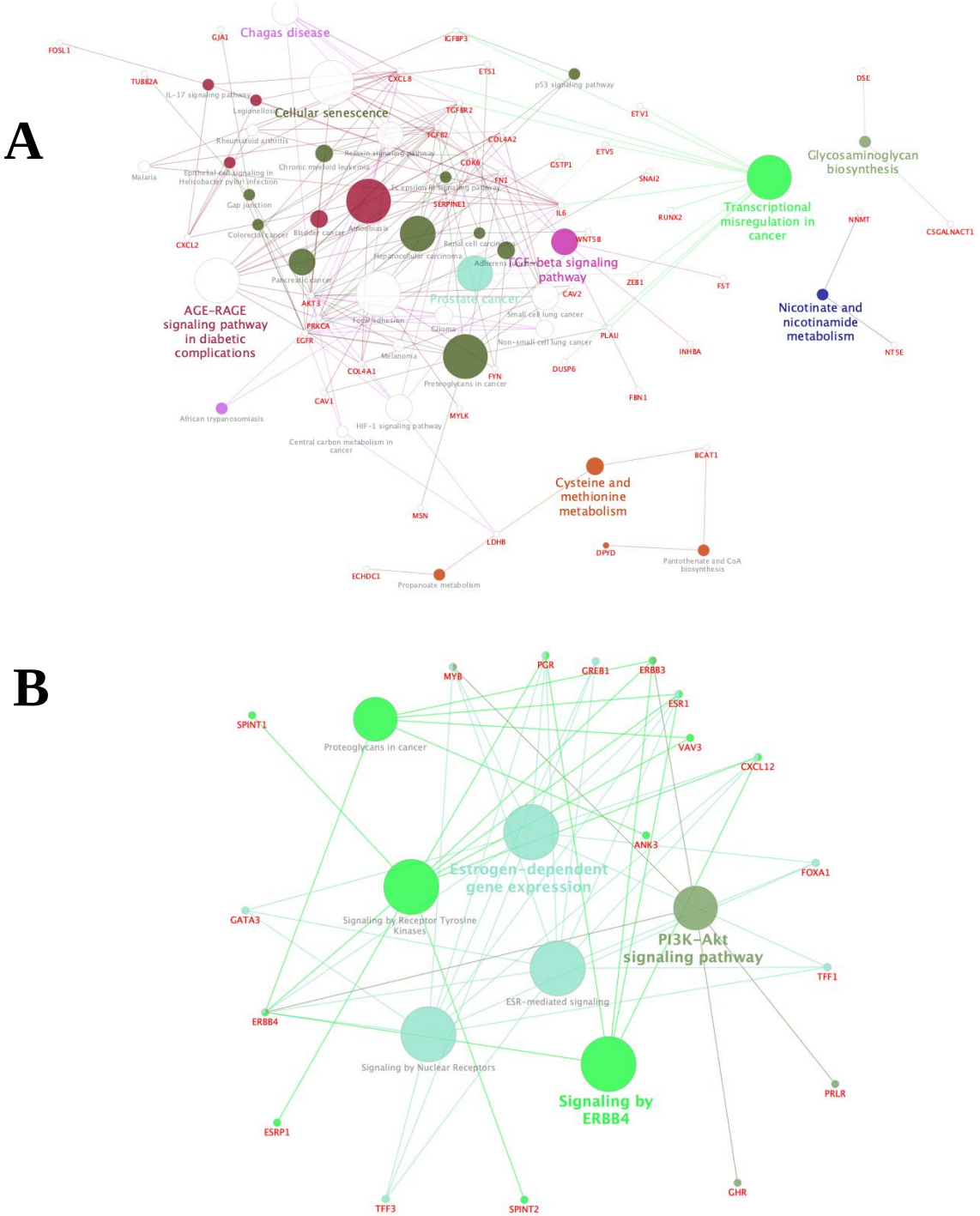


**Figure 3. The pathways network analysis for DEGs. A) upregulated DEGs pathways. B) Downregulated DEGs pathways.**

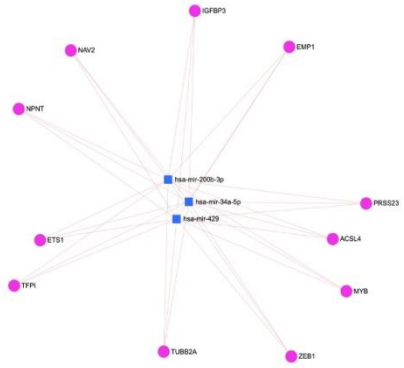


**Figure 4. The network analysis for miRNAs interaction with upregulated and downregulated DEGs.**

**Table 1. The fold change of DEGs that interact with miRNAs**

| Gene name | logFc |
|---|---|
| ETS1 | 6.7613471 |
| ZEB1 | 6.4264317 |
| EMP1 | 6.1540808 |
| PRSS23 | 4.7045537 |
| TFPI | 4.481085 |
| NAV2 | 4.4485306 |
| ACSL4 | 4.2970303 |
| IGFBP3 | 4.2019184 |
| TUBB2A | 4.096885 |
| NPNT | -5.2494323 |
| MYB | -5.7119466 |

The sample size was increased to 78 breast tumors (including 40 from the initial publication) in a later investigation by the same team employing hierarchical clustering and an intrinsic gene set of 456 cDNA clones. The discovery of three subtypes within the luminal cluster (47 cancers): luminal A (32 tumors), luminal B (five tumors), and luminal C (10 tumors) was made possible by increasing the sample size (Sørlie et al., 2001). While luminal C was further separated from luminal A and luminal B by strong expression of a group of genes shared with basal-like and HER2-positive subtypes, but of unknown function, luminal B and luminal C tumors showed decreased expression of ER-related genes compared with luminal-A cancers (Sørlie et al., 2001).

Luminal-A and luminal-B subtypes have been replicated in numerous gene expression studies. Both subtypes exhibit luminal cytokeratins 8/18, ER, and genes linked to ER activation like CCND1 (cyclin D1), as well as expression patterns resembling those of the luminal epithelial component of the breast. The primary molecular difference between the two luminal subtypes is that luminal B typically expresses more proliferative genes and less ER-related genes (Sørlie et al., 2001; Sørlie et al., 2003 and Brenton et al., 2005). Even though only 10% of tumors were HER2-positive by immunohistochemistry, Luminal-B tumors exhibit increased expression of growth receptor signaling genes (Loi *et al*.,2005). Approximately 20% of luminal-B breast cancers were HER2-positive by immunohistochemistry, according to a review of several gene expression studies (Wirapati *et al*., 2008). A clinically meaningful classifier of luminal-B breast cancer should exclude HER2-positive breast cancers because they are treated very differently from HER2-negative breast cancers (Sotiriou C and Pusztai L 2009). The luminal-B subtype is identified by immunohistochemistry in about 30% of HER2-positive tumors. Immunohistochemistry or ESR1 gene expression has also shown that the majority of tumors are ER-positive ( Sørlie et al., 2003; Sotiriou C and Pusztai L 2009). The clinical significance of whether the intrinsic molecular classification classifies an ER-positive breast cancer with overexpression of HER2 as HER2-positive or as luminal B remains to be determined.

In our study, the genes that significantly expressed in basal breast cancer tissue and interact with the set of miRNAs under the current study were ETS1, ZEB1, EMP1, PRSS23, TFPI, NAV2, ACSL4, IGFBP3, TUBB2A, NPNT and MYB. Some of These genes have a well-known role in breast cancer specifically.

ETS1 (transcription factor) has initially been characterized as the proto-oncogenic transcription factor that contributes to tumor angiogenesis and invasiveness in cancer cells (Fujimoto *et al*., 2002 ; Hsing *et al*., 2020). Although ETS1 has demonstrated dichotomous roles as an oncogene and a tumor suppressor gene in a variety of cancers, its function in the tumorigenesis of breast cancer is still unknown (Kim *et al*., 2020). Breast cancer progression and embryonic development are both greatly aided by zinc finger E-box binding homeobox 1 (ZEB1) (Wu et al., 2020). Interestingly, this gene controls the expression of numerous oncogenic and tumor-suppressive miRs, including miR-34a. ZEB1 expression down-regulates miR-34a expression to drive pro-actin cytoskeletal remodeling, and gains invasive and metastatic capabilities (Ahn *et al*., 2012). Our study comes with the same findings and

this can be considered a strong evidence of in silico substantial role in cancer studies.

The peripheral myelin protein (PMP22) gene family includes the epithelial membrane protein 1 (EMP1). EMP1 has been identified as a c-MYC target (Bredel *et al*., 2005), is highly expressed in undifferentiated cells (Ben-Porath et al., 2005), and has been linked to several cancers, including breast cancer (Sun et al., 2014) and nasopharyngeal cancer (Sun *et al*., 2014). Particulrly in hormone-related cancers like endometrial and breast cancer, EMP2 has been regarded as an oncogene. The current study reported this gene could be an oncogene in basal breast cancer in comparison to luminal breast cancer. However, Serine protease PRSS23 is a newly discovered protein that has been associated with tumor progression in various types of cancers. Interestingly, PRSS23 is coexpressed with estrogen receptor α (ERα), which is a prominent biomarker and therapeutic target for human breast cancer (Chan *et al*., 2012).

Tissue factor (TF) pathway inhibitor-1 (TFPI) is a plasma serine protease inhibitor, which is mainly known for its role in the coagulation cascade, being responsible for the modulation of TF induced blood coagulation. TFPI has anti-tumor effects in breast cancer cell lines (Stavik et al., 2013). While other study demonstrated the overexpression of TFPI was linked with breast cancer and may has role in lymphatic spread (Sierko *et al*., 2010). Neuron navigator 2 (NAV2) is one of three members of the neuron navigator family. NAV2 also regulates the cytoskeleton, which affects cell-cell and cell-matrix adhesion, facilitating tumor cell invasion and metastasis (Nakaya *et al*., 2008). One of the studies of NAV2 on breast cancer found the decreasing of NAV2 expression causes to increase the tumor size and also in risk of grade disease with high levels(Kholdi, *et al*., 2017).

One of the five mammalian long chain acyl-CoA synthetases isoforms, ACSL4, condenses a fatty acid with a molecule of coenzyme A to create a thioester, which activates the fatty acid for further metabolism. One of many biomarkers for an aggressive breast cancer phenotype and/or hormonal intervention resistance is ACSL4 (Wu *et al*., 2013). The insulin-like growth factor (IGF) system is essential for healthy growth and development because of the powerful proliferative and antiapoptotic effects that these growth factors have. Estrogen receptor (ER) expression and IGFBP-3 expression were found to be negatively correlated in early studies looking at IGFBP-3 expression in breast cancer cell lines (Kamila *et al*., 2015). Tubulin proteins, on the other hand, form dynamic microtubules and are essential cellular building blocks. TUBB2A was connected to cellular growth, motion, and adhesion (Shin *et al*., 2020). The downregulated Nephronectin (NPNT) is an extracellular matrix protein originally identified in the embryonic kidney. NPNT was identified as upregulated gene in breast cancer tissues compared to normal breast tissues (Wang *et al*., 2018). Our results showed this gene is significantly downregulated in basal breast cancer in comparison to luminal breast cancer and this is could be a diagnostic marker for basal breast cancer specifically not for breast cancer in general. Although, the transcription factor MYB (encoded by MYB) is the prototype member of the family, which includes MYBL1 (encoded by MYBL1) and MYBL2 (encoded by MYBL2). MYB positively regulates promoters of key cell proliferation genes, such as Myc, and it can also work as a transcriptional repressor. This gene considered as proto-oncogene but can play as potential tumor suppressor role in Luminal Breast Cancer (Thorner *et al*., 2010).

This study can be considered as the first report comparing basal and luminal BC using microarray aspect at two levels of miRNA and genes. The list of the genes and their expression reported in our data was controversial and needed more concerted data from in vivo experiments to be validated in diagnosis and treatment for basal breast cancer.

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