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Gene Expression Signatures of Ductal Breast Carcinoma Shows Differentially Expression of Cell Cycle, Proliferation and Apoptosis Related Genes

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Abstract— **Breast cancer is one of the most common malignant diseases among women in western countries. Ductal carcinoma of the breast is a pre-invasive malignant lesion that is curable. However, undetected or untreated tumors can progress to invasive breast cancer. Therefore, understanding of molecular changes in this tumor and determining of diagnostic and prognostic biomarkers will be valuable for early diagnosis and predict of prognosis in this disease. In this study, we investigated differentially expressed genes in ductal carcinoma of breast. Moreover, we also investigated probable mechanisms that abrogated in these lesions. The results have shown that there are 96 upregulated and 110 downregulated gene in ductal breast cancer compared normal breast. Analyzing of this gene list revealed that upregulated genes are significantly enriched cell cycle term. However, downregulated genes are enriched regulation of cell proliferation and apoptosis terms.**

Index Terms— **Ductal Breast Carcinoma, gene expression, in silico**

I. INTRODUCTION

Breast cancer is one of the most commonly diagnosed malignant tumor among women in developing and developed countries [1]. However, this disease is not in a single form and there are different types with varying morphological features and genomics content [2]. In this respect, gene expression analysis in breast cancer has been used extensively for the identification of breast cancer molecular subtypes [3].

Development of effective tools such as DNA microarrays for monitoring gene expression on a large scale has resulted in the discovery of gene networks and regulatory pathways in tumor processes [3]. Disease biomarkers are routinely having been used for diagnostic and prognostic purposes. In this respect, gene expression profiling is a powerful tool for identification of novel biomarkers, since allows measurement of thousands mRNAs in a single experiment [4-6].

A significant amount of microarray data has been produced and deposited in publicly available data repositories recently, including the Gene Expression Omnibus (GEO) [7] Array Express Archive [8] and Oncomine [9]. These databases allow researchers to the discovery of diagnostic and/or prognostic gene signatures by using *in silico* approaches.

In this study, we investigated gene expression datasets from normal breast and ductal breast cancer samples using the oncomine database. Further, we also performed a gene term enrichment to obtain characteristics of the set of differerentially expressed genes. The results have shown that 96 up regulated and 110 down regulated genes are differentially expressed in ductal cancer of the breast. Moreover, the results also that while up-regulated genes are related to cell cycle, down-regulated genes are related to the regulation of cell proliferation and apoptosis, in ductal breast cancer.

Differentially expressed genes and abrogated pathways in cancers are targets for diagnostic and prognostic approaches. Therefore, this article suggests novel putative biomarkers as well as drug targets for ductal breast carcinoma and should be investigated further.

We suggested that the list of differentially expressed genes and abrogated mechanisms which reported in this study may be useful for finding of novel biomarkers, since abrogated signaling mechanisms and differentially expressed genes in cancer may be useful for finding of novel diagnostic and/or prognostic biomarkers.

II. METHOD

The Oncomine database (http://oncomine.org) was used to compare the transcriptomics datasets of the ductal breast cancer and normal breast tissues. Only mRNA as data type and clinic specimens as sample type were selected for further analysis. To compare the gene expression in a tumor type to its normal counterpart, gene expression data from the same study, performed with the same methodology, were used as described previously [10-12]. The gene expression data were log-transformed and median-centered per array, and the standard deviation was normalized to one per array [9]. The ductal breast cancer array sets used in this study are summarized in Table 1.

TABLE I: Arrays used in this study

	Dataset	Reference
1	Perou Breast	[13]
2	Richardson Breast 2	[14]
3	Sorlie Breast	[15]
4	Sorlie Breast 2	[16]

Two filters were used to find the differentially expressed genes in ductal breast cancer compared to the normal breast. In the first filter, 4 ductal breast cancer microarray sets were compared, and the top 500 up or down regulated genes with average p -value < 0.0001 were selected. In the second filter, each of these filtered genes was assessed manually for

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differentially expression in each array. A gene was considered differentially expressed when its mean expression value in the tumor samples was significantly lower than that of the normal tissue counterpart using a t-test ($p<0.0001$), and the fold of induction was ≥2 in least two independent arrays (≥ 50% of 4 arrays). The web based "Database for Annotation, Visualization and Integrated Discovery" (DAVID) (http://david.abcc.ncifcrf.gov) tool was used to determine enriched gene ontology terms within the differentially expressed gene lists.

III. RESULTS AND DISCUSSION

Ductal carcinoma of the breast is a pre-invasive malignant lesion where have been found in the lining of the breast milk duct without invasion of the basement membrane [17, 18]. Although ductal carcinoma of breast is considered as a malign tumor that is curable, undetected or untreated lesions can develop into invasive breast cancer and spread to the surrounding breast tissue [19]. Invasive ductal breast carcinoma is the most common type of breast cancer [20]. However, there are no effective markers to predict which cases will go on to become invasive cancers and which ones won't. Therefore, new diagnostic and prognostic markers needed for early diagnosis of disease and identify patients who are at the highest risk of developing metastases [20].

Gene-expression signatures of primary breast tumors may be useful to identify novel diagnostic and prognostic markers [2]. These expression profiles have been extensively used in some countries [21, 22] and support classic prognostic markers to obtain more accurate prognostic information [23].

In this study, we examined microarray data from normal breast and ductal breast cancer samples using the oncomine database, to determine differentially expressed genes. The results have shown that 96 up and 110 down regulated genes are differentially expressed genes in ductal breast cancer compared to normal breast (Table II).

TABLE II: Differentially expressed genes in ductal breast carcinoma.

Upregulated Genes	MARCH6, TTC17,CLIC1, SLMO2, ENG1, PLAUR, ARL3, BLVRA, ISG15, CDCL9, PDAP1, CKS2, POLE2, NAT1, DRAP1, MAPK13, OAS1, PSMA3, CEACAM6, LSM1, ODF2, BCCIP, EBNA1BP2, KRT8P12, GALC, IFITM1, TULP4, RSAD2, RBBP6, SHFM1, PCNA, PSMB8, PSMA2, UBE2L6, PDCD10, EPRS, LILRB2, THUMPD3, MAL2, OCIAD2, LAGE3, SDC1, INHBA, CHCHD2, CISD1, PPP2R2C, STRBP, CORO1A, COX6C, PPIB, HNRPLL, MNAT1, IFI30, ARNT2, NME1, PPP4C, LSM3, IFI6, CDC123, PSMD10, ADAM15, GARS, NDUFS5, EBI3, KNTC1, ZNF165, IFIT1, DTYMK, CBWD5, CBWD1, RAB22A, BLZF1, SP110, CTSS, RSPH1, IFT88, COX4NB, KPNA2, CD37, ZFP62, SFPQ, ESR1, FLI1, LYPD1, RAC2, CISD2, RFWD2, C2, PAFAH1B3, HAX1, TOP2A, TXNDC9, TCEB2, BOLA2, CNIH4, ZNF92
Downregulated Genes	IGFBP6, EDNRB, CABC1, MGLL, SORBS2, DCT, DPT, FBLN1, FAT1, KANK1, PCSK7, ITM2A, KDM6B, GABARAPL1, CYGB, PFKFB3, SLC12A4, GABRE, SLC25A42, PDGFRL, KRT17, ANTXR2, PHF17, PRELP, RPS23, ABLIM1, MORN1, MRPL27, VAMP2, JUN, SDC2, CXCL1, EPB41L4B, METTL7A, FOLR1, ZHX3, CUL5, PDZD2, FGFR1, PROS1, EGFR, SLC25A1, GPM6B, GFAP, BTD, GRAMDIC, DUSP6, LRP1, PTRF, SMARCA2, ARHGEF17, SKI, SDK2, PIM1, CLIC5, ALDH1A2, MFAP4, LRRC56, CASP4, SFRP1, DST, IGF2, LZTS1, DPYSL2, HYAL2, VIM, LGALS3, FAM13B, CIB2, WASF3, RBPMS, CBX6, NRN1, ALB, CAV1, GNAI1, ACBD4, PIK3R1, ARHGEF10, PPARA, TPM2, TMTC1, PITPNA, NR1H2, CDH13, PTPRE, FAM13A, ATF3, CALD1, STAT5B, TXNIP, NCOR1, PPP2R1B, ACSL1, RARB, ANXA1, IGJ, KANK2, LONRF1, ADH1B, ZBTB4, EPS15, ARHGAP20, C5orf4, ARHGEF6, CCL14, BNIP3L, EIF2B1, AOC3, TMX4

DAVID was used to perform GO Term Enrichment analysis to obtain characteristics of the set of significant genes. This analysis provides a list of gene functions, which are over-represented in a gene set. Analysis of the 96 up-regulated genes in ductal breast cancer with the DAVID functional annotation tool (GOTERM BP FAT) resulted in the identification of "cell cycle" as significantly "enriched term" ($p<0.0001$). 15 genes were appointed to this GO term, showing a significant enrichment (3.4 times) in the corresponding biological processes (Table III). Analysis of the 110 down-regulated genes in ductal breast cancer with DAVID resulted in the identification of 5 terms ($p<0.0001$). All the enriched terms were related to regulation of cell proliferation and apoptosis (Table III).

TABLE III: Functional enrichment of differentially expressed genes in ductal breast carcinoma. While upregulated genes are involved in cell proliferation and apoptosis, downregulated genes are related to the cell proliferation and apoptosis.

	Term	Count	Fold	P-value	Genes
Upregulated	GO:0007049~cell cycle	15	3,486598	6,79E-05	DTYMK, KNTC1, BCCIP, PSMB8, ARL3, PSMA2, INHBA, MNAT1, RSPH1, MAPK13, PSMD10, PSMA3, CDC123, CKS2, KPNA2
Downregulated	GO:0042127~regulation of cell proliferation	20	18,34862	3,997518	EGFR, CXCL1, TXNIP, FGFR1, CAV1, IGFBP6, STAT5B, ANXA1, IGF2, SKI, EDNRB, CDH13, ALDH1A2, CUL5, CCL14, ATF3, JUN, RARB, SMARCA2, DPT
	GO:0042981~regulation of apoptosis	17	15,59633	3,326044	EGFR, TXNIP, ARHGEF6, STAT5B, PIM1, ANXA1, ARHGEF17, IGF2, EDNRB, CDH13, CUL5, CASP4, SFRP1, ALB, JUN, BNIP3L, RARB
	GO:0043067~regulation of programmed cell death	17	15,59633	3,293275	EGFR, TXNIP, ARHGEF6, STAT5B, PIM1, ANXA1, ARHGEF17, IGF2, EDNRB, CDH13, CUL5, CASP4, SFRP1, ALB, JUN, BNIP3L, RARB
	GO:0010941~regulation of cell death	17	15,59633	3,281153	EGFR, TXNIP, ARHGEF6, STAT5B, PIM1, ANXA1, ARHGEF17, IGF2, EDNRB, CDH13, CUL5, CASP4, SFRP1, ALB, JUN, BNIP3L, RARB
	GO:0008285~negative regulation of cell proliferation	11	10,09174	4,793146	CXCL1, ALDH1A2, CDH13, CAV1, CUL5, JUN, IGFBP6, SKI, RARB, SMARCA2, DPT

I. CONCLUSION

In our study, we found that the genes that involved in cell cycle, proliferation and apoptosis are differentially expressed in ductal breast carcinoma compared to normal breast. Therefore, further investigation of these genes and corresponding pathways may open new perspectives for diagnosis and treatment of disease.

In silico studies are powerful approaches to the confirmation of established laboratory-based works as described previously [24]. These studies will support experimental approaches to finding new diagnostic/prognostic biomarkers by using various tools such as Oncomine. Although this article focuses only on the gene expression changes in ductal breast cancer, *in silico* approaches that were used in this study can be used to investigate gene expression changes in various malignant and pre-malignant lesions.

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