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In Silico Analysis of Gene Expression Profile in Human Breast Cancer Cells

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Abstract—*Breast cancer is the most common type of cancer in women and the second leading cause of cancer related deaths, next to lung cancer in both the developed and the developing world. Although only small fractions of breast cancer are truly inherited, mainly they occur due to gene damage, altered function or overexpression. Genes that are over-expressed in the cancerous tissue are of particular interest because over-expression is a trait expected of a gene that is causing the cancer to grow. We have made an attempt to study the molecular changes that occur when a normal breast cell is transformed into a cancer cell since these differences may be the weak factors that can be exploited to fight the cancer. The human breast cancer libraries available in public databases and the online tool Serial Analysis of Gene Expression (SAGE), available at the website for the Cancer Genome Anatomy Project (CGAP) were utilized to identify genes that are over-expressed in breast cancer tissue. Furthermore, various identified differentially expressed genes from human breast cancer cells were checked for their anatomical localized expression in variety of other tissues along with the level of their expression in other tissues using cDNA Northern tools. In the present study, we observed that the genes involved in the control of cell cycle, cell proliferation, apoptosis and extracellular matrix remodeling (proteinases, cysteine proteinases) were highly over-expressed in breast cancer tissues. These over-expressed genes not only highlight the molecular mechanisms involved in breast cancer evolution but also facilitate in generating promising genetic markers linked to development of breast cancer.*

1. INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. It's the result of accumulation of pathologic alterations to a cell's genetic material. Sometimes, the organized process of cell growth and division goes wrong. The genetic material (DNA) of a cell can become damaged or altered, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form even when the body does not need them. The extra cells may form a mass of tissue called a tumor. Breast cancer is responsible for almost 20 percent of all cancer deaths in women. Roughly 180,000 women are diagnosed with this disease each year, of which 44,000 will die, indicating an unmet need for better cancer treatments, in particular for targeted therapies that are more effective and less toxic than traditional cytotoxic chemotherapy. Unlike heart disease, the overall mortality rate for breast cancer has not significantly improved over the past 50 years. The risk of getting breast cancer increases with age, and inherited gene mutations or a family history of breast cancer may increase the risk [1]. Improved diagnostic tools have made it possible to detect breast cancers at early stages, leading to a significant decrease in breast cancer mortality rates over the past decades [2]. Screening methods in use includes physical examination, ultrasound, and mammography. These techniques are effective at detecting slow-growing cancers, but often miss aggressive breast cancer in its early stages.

One approach to the discovery of novel diagnostic and prognostic markers and therapeutic targets is to compare the gene expression profiles of normal and cancerous cells and identify genes or subsets of genes with expression levels that correlate with tumor stage. Such genes may furthermore provide attractive targets for novel therapies in our efforts to overcome these devastating diseases. In this study, the various molecular changes that occur when a normal cell is transformed in to a cancer cell were investigated. Serial analysis of gene expression (SAGE), an alternative comprehensive gene expression profiling technique that does not require a prior knowledge of the transcripts present in the cells was used to compare the expression of thousands of genes in breast cancer tissue. Thus, it allows for the identification of novel transcripts, making it particularly suitable for the discovery of new molecular targets [3].

2. METHODOLOGY

The Cancer Genome Anatomy Project (CGAP), a program of the National Cancer Institute (NCI) was used to study the molecular changes that occur in a cancer cell. To identify molecular-level differences, the genes that were differentially expressed in normal breast cells and cancerous breast cells were studied using *Serial Analysis of Gene Expression* (SAGE) libraries available in public databases. The statistical parameter F=2, P=0.05 were selected for gene search. Over expressed genes related to breast cancer were noted and their chromosome location; normal function, family name and the altered functions if any were investigated and recorded. NCBI website was used for this and rsID of the genes of interest was searched. Anatomical localized expression of various identified differentially expressed genes and the levels of

expression of these genes in normal and cancerous tissue were measured using SAGE Anatomic viewer and SAGE/cDNA Virtual Northern.

3. RESULTS AND DISCUSSION

Genes were compared for their expression in normal and cancerous cells and those which showed over-expression were selected. More than thousand over-expressed genes in the breast tissues were analyzed and out of them twenty genes showing low value of P (< 0.01) were selected for further studies.

CGAP data for genes S100A7, FASN and CRIP1 genes were found in cancer libraries but not in the normal library. They were found 1283, 10544 and 799 times in the pool of genes from the cancerous tissue. S100A7, FASN and CRIP1 were found to be highly over-expressed in breast cancer. Similarly the genes; MMP9, NPY1R, Hs66705, PPIA, AA224053, DB480425, DN992173 and others were found in the pool of genes from the cancerous tissue (**Table 1**).

SAGE Anatomic viewer and SAGE Virtual Northern: Anatomical localized expression of few identified differentially expressed genes further revealed the over-expression (indicated by pink color) of MMP9 and S100A7 genes in breast cancer tissue but not with other types of cancers. The gene expression level measurements also showed S100A7 gene to be over-expressed in breast cancerous tissue as compared to normal breast cancer tissue. Other genes like MMP9, NPY1R, and Hs.667053 also showed high expression levels in breast cancerous tissue (**Table 2**). The twenty genes showing over-expression in the breast tissue: MMP9, S100A7, FANS, CRIP, DCD NPY1R, RHPN1, PPIA Hs.667053, CF139360, BU663123, Hs.560423, AA224053, Hs.541414, Hs.704721, DB480425, Hs.676248, AI393188, DN992173 and BE957760 were further analyzed on the basis of position and expression pattern. Some of these have been studied.

MMP-9: Matrix metalloproteases (matrix metalloproteinase, MMPs) are involved in degradation of a wide range of extracellular metalloproteases molecules and a number of bioactive molecules. MMPs have been implicated in the degradation of basement membrane, which is the first step of metastasis**.** Various studies have shown that metastatic dissemination of tumour cells requires degradation of extracellular matrices by several families of proteases, including metalloproteinases. The extracellular proteases are differentially expressed in various tissue types and in many diseases such as cancer the up-regulation of MMPs has been linked with cancer. Over-expression and activation of MMP-9 predicted a higher stage of hormone-sensitive ductal breast carcinoma. Down-regulation of the endogenous inhibitor of MMP-9, tissue inhibitor of metalloproteinase 1, and translocation of the transcription factor nuclear factor-κB in tumours may have an appreciable role in the over-expression of MMP-9 [4]. Not surprisingly, inhibition of these protumorigenic enzymes in animal models of metastasis has shown impressive therapeutic effects. Evaluation of MMP-9 expression may provide valuable information about breast cancer treatment [5].

S100A7: the protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. This protein lacks calcium binding ability in one EF-hand at the N-terminus. The protein is widely over-expressed in invasive estrogen receptor (ER) α-negative breast cancers. S100A7 has been shown to be over-expressed in breast cancers at sites of necrosis in tumour tissues as well as in the nasal fluid during allergic inflammatory reactions [6, 7, 8, 9]. S100A7 over-expression enhances NF-κB-mediated MMP-9 secretion in MDA-MB-231 cells, indicating its role in enhanced invasiveness (10). Nuclear factor-kappa B (NF-κB) is an essential transcription factor that not only modulates cellular responses to stress but also plays a pivotal role in inflammation, immunity, cell cycle growth and survival. S100A7 is among the most highly expressed genes in pre-invasive breast cancer, is a marker of poor survival when expressed in invasive disease, and promotes breast tumour progression in experimental models [11, 12]. Although a number of putative functions have been proposed for S100A7, its biological role whether S100A7 contributes to breast cancer growth or metastasis remains to be defined.

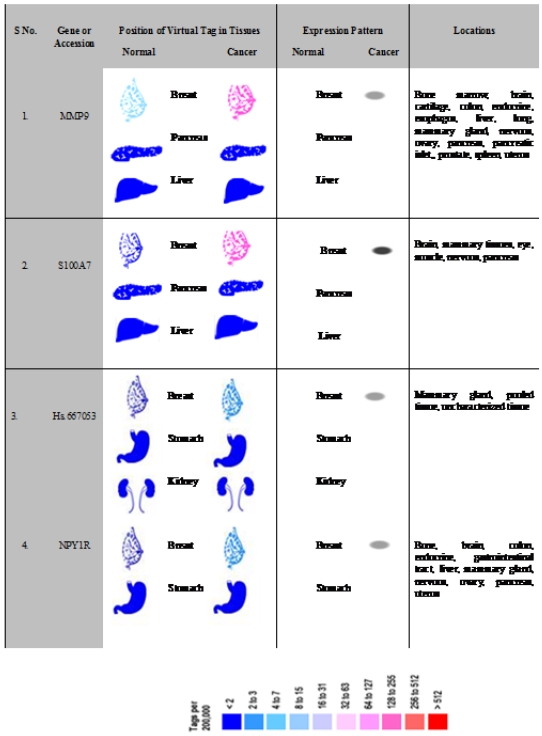
NPY1R: NPY1R belongs to the G-protein-coupled receptor superfamily. The encoded transmembrane protein mediates the function of neuropeptide Y (NPY), a neurotransmitter, and peptide YY (PYY), a gastrointestinal hormone. The encoded receptor undergoes fast agonist-induced internalization through clathrin-coated pits and is subsequently recycled back to the cell membrane. Activation of Y1 receptors may result in mobilization of intracellular calcium and inhibition of adenylate cyclase activity. NPY-Rs expression has been reported in primary breast cancer, where Y1R expression predominated as compared to Y2R, which is predominant in non-neoplastic breast. The neoplastic condition of breast tissue may thus induce a switch of expression from Y2R to Y1R. Moreover, a functional interplay between estrogen and YIR has been reported in a human breast cancer responsive to this steroid, where estrogen was found to increase Y1R expression, which in turn negatively regulates estrogen-stimulated cell proliferation. Chronic stress is associated with elevated levels of sympathetic neurotransmitter (norepinephrine and neuropeptide Y: NPY) release and immunosuppression. The expression of NPY receptors has been reported in human breast carcinomas. Recently, activation of the NPY Y5 receptor was shown to stimulate cell growth and increase migration in human breast cancer cells [13].

Hs.667053/ M41: The M41 gene contains oestrogen response elements, one of which is associated with Alu repeats. M41 mRNA is shown to be expressed at significantly higher level in human breast cancer specimens than in normal human breast and benign lesions.

Table 1: Analysis of differential gene expression in the human breast cancer libraries Tag

	Gene or Accession	Libraries		Tags		Tag Odds Normal : Cancer	P
		Normal	Cancer	Normal	Cancer		
TACCCTATGT	MMP9	0	3	0	44	0	0.00
GAGCAGCGCC	S100A7	3	17	7	1283	0.01	0.00
TGATCTCCAA	FASN	16	26	66	10544	0.1	0.00
TCCACGATGC	NPY1R	0	11	0	44	0	0.00
TTTGGGCCTA	CRIP1	13	27	49	799	0.1	0-00
AAGCATCAGC	DCD	0	3	0	44	0	0.00
GAGCGTTTTG	PPIA	0	2	0	33	0	0.00
CAGGTCTCCC	RHPN1	0	9	0	32	0	0.00
TACCCATTTC	Hs.667053	0	8	0	26	0	0.00
TGCTGCGACA	BU663123	0	2	0	32	0	0.00
GATGCGTAAT	Hs.704721	0	2	0	31	0	0.00
TGGATGGTGA	DB480425	0	3	0	93	0	0.00
TAATGTAGGT	Hs.676248	0	2	0	42	0	0.00
CAACAATAGC	AI393188	0	9	0	19	0	0.01
CCAGAATAAT	Hs.560423	0	2	0	24	0	0.00
CTGGAGTTCT	DN992173	0	2	0	79	0	0.00
AAATGTAGGT	BE957760	0	3	0	40	0	0.00
GCGGGTGTTT	Hs.541414	0	14	0	92	0	0.00
GCCGGCTCAT	AA224053	0	4	0	14	0	0.05
GTGAGGCACC	CF139360	0	2	0	19	0	0.010

Table 2: Anatomical localized expression and levels of expression of few differentially expressed



In carcinomas, M41 gene up-regulation is associated with the development of the malignant cell. M41 mRNA has been reported to be produced by the carcinoma cells of breast cancer specimens using *in situ* hybridization and using a PCR assay [14]

DCD: Dermicidin (DCD) is an antimicrobial gene secreted by human eccrine sweat gland onto the skin as a part of innate host defence of the immune system. It encodes a secreted protein which is further processed into mature peptides of distinct biological activities. The C-terminal peptide is constitutively expressed in sweat and has both antibacterial and antifungal activities. The N-terminal peptide, also known as diffusible survival evasion peptide, promotes neural cell survival. DCD exhibits proteolytic activity, phosphatise activity and binds IgG. A glycosylated form of the N-terminal peptide has been reported to be associated with cachexia (muscle wasting) in cancer patients which is often seen during end-stage cancer. Based on its function and restricted expression pattern in normal adult tissues, DCD is a candidate cancer therapeutic target. The secreted nature and extracellular mechanism of DCD action make it even more attractive for such a purpose [15].

FASN: Fatty acid synthase (FASN) is a multifunctional enzyme that is essential for the endogenous synthesis of long-chain fatty acids from its precursors acetyl-CoA and malonyl-CoA. Breast cancer cells endogenously synthesize 95% of fatty acids de novo, despite having adequate nutritional lipid supply. This phenomenon helps in enhanced cell proliferation, survival, chemoresistance and metastasis [16]. However, the exact role of the major lipogenic enzyme fatty acid synthase (FASN) as cause, correlate or facilitator of breast cancer remains unidentified. FASN has been reported to inhibit the intrinsic pathway of apoptosis and has been recently proposed as a direct target of p53 family members, including p63 and p73. Increased expression of FASN has emerged as a phenotype common to most human carcinomas. FAS expression is an indicator of poor prognosis in breast and prostate cancer, is found elevated in the blood of cancer patients, and its inhibition by cerulenin and C75 chemical has been reported to be cytotoxic to human cancer cells [17]. FASN and the fatty acid synthesis pathway provide a number of avenues of future exploration applicable to the diagnosis, prognosis, treatment, and prevention of human cancer.

CRIP1: Cysteine-rich intestinal protein 1 (CRIP1) belongs to the LIM/double zinc finger protein family, which includes cysteine- and glycine-rich protein-1, rhombotin-1, rhombotin-2, and rhombotin-3. Human CRIP1, primarily a cytosolic protein, was cloned in 1997 using RT-PCR of human small intestine RNA and oligonucleotides whose sequence was derived from the human heart homolog of this protein [18] and has been shown to be over-expressed in several tumor types, including breast, cervical, prostate, pancreatic, and colorectal cancers but its prognostic impact and its role in cellular processes, particularly in breast cancer, are still unclear [19]. CRIP1 has been reported to act as a tumour suppressor in proliferation and invasion processes since the lack of CRIP1 expression in breast cancer tissue is significantly associated with a worse prognosis for patients and low endogenous.

4. CONCLUSION

A number of factors have long been thought of to be associated with increased risk of cancer like stress, hormones, obesity and chemicals or carcinogens; it is the result of interplay between genetics and environment. Out of more than thousands genes analyzed, twenty genes were found to show overexpression profile in breast cancer tissue. Most prominent ones include: MMP9, S100A7, FASN, NPY1R, CRFP1 and Hs.667053. S100A7 gene is involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100A7 over-expression strongly induces NF-κB promoter activity which leads to up-regulation of several downstream target genes, including MMP-9 and thus enhancing breast tumor growth by activating proinflammatory and metastatic pathways. MMP9 gene products, extracellular proteases cause the degradation of basement membrane which is the first step of metastasis and also might have a role in expression of estrogen and progesterone receptors. Estrogen is responsible for the increased expression of NPY1R, which in turn negatively regulates estrogen-stimulated cell proliferation. Chronic stress is also associated with elevated levels of expression of NPY1R gene products sympathetic neurotransmitter (norepinephrine and neuropeptide Y: NPY) release and furthermore to immunosuppression. In cancerous cells, over-expression of FASN gene is observed as it synthesizes lipids *de novo* which are required for cell proliferation, survival and metastasis. Further, it highlights the role of obesity in driving common cancer. FASN also inhibits the intrinsic pathway of apoptosis. CRIP1 acts as a tumor suppressor and during the cancerous cell proliferation is over-expressed. In the present study we observed that the genes involved in the control of cell proliferation, apoptosis and extracellular matrix remodeling (proteinases, cysteine proteinases) were over-expressed and their function altered. The identification and functional role of these over-expressed genes in breast cancer tissue is of high relevance not only for the potential value as early prognostic biomarkers but also because they may provide insight into mechanisms and pathways of relevance in breast cancer progression. Most importantly, this analysis also gives insight into the genomic identification of significant targets of breast cancer. Additional analysis and validation of the identified genes will be required to determine the clinical value, and to determine whether they may constitute novel targets for translational research.

5. ACKNOWLEDGEMENT

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