Lost in Translation? A Systematic Database of Gene Expression in Breast Cancer

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Key Words

Breast cancer • Adenocarcinoma • Proteomics •
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Abstract

Ten years of translational research in breast cancer (BC) using high-throughput technologies such as cDNA arrays have produced an impressive amount of results. However, it is difficult for a single researcher to overview these results, since no critical synthesis is provided in the literature. This is a meta-analysis of gene expression in BC. Thirteen translational studies fulfilled the inclusion criteria, involving 553 BC patients and 79 controls. Large cohorts of patients and well-defined samples were rare. A total of 1,350 genes were reported at least once to be deregulated in BC. Out of these findings, 1,212 (90%) were not confirmed by other authors. A cohort of 138 genes of particular interest remained for further analysis. Of these, 79 were consistently reported to be overexpressed in BC, 41 to be underexpressed and in 18 cases results were contradictory. The most frequently reported deregulated genes in BC include: GATA binding protein 3, arylamine N-acetyltransferase, Myb-related protein B and zinc transporter SLC39A6 precursor (overexpressed); cadherin-3 precursor, keratin type I cytoskeletal 17 and type II cytoskeletal 5 (underexpressed). These genes obviously correlate with the presence and/or development of BC. More efforts should be devoted to the establishment of common standards in translational BC research.

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Introduction

Breast cancer (BC) is the most common malignant tumor and the leading cause of cancer death in women [1]. Histologically, most BC are adenocarcinomas, and their classification is classically taking into consideration the cell type (as in apocrine carcinoma), the type of secretion (as in mucinous carcinoma), architectural features (such as papillary carcinoma) and spreading characteristics (as in inflammatory carcinoma) [2]. This traditional knowledge is now being augmented by genetic studies: an important discovery in BC was that germ mutations in 2 genes (BRCA1 and BRCA2) are responsible for approximately two thirds of familial BC [3]. Better molecular understanding of BC has also permitted the emergence of novel target-directed therapies. For example, overexpression of human epithelial growth factor receptor type 2 (HER2, also referred to as HER2/neu or ErbB-2) occurs in about one fourth of invasive BC. Strategies to target HER2, such as the monoclonal antibody trastuzumab, are effective in treating this subgroup of BC [4].

BRCA and trastuzumab might be only the first chapters of a long story. Novel technologies in translational research have provided powerful tools for further describing, classifying and understanding BC. In particular, over the past decade, DNA microarrays have allowed the measurement of mRNA levels across thousands of genes simultaneously [5]. Microarrays can be applied to analyze gene expression, DNA sequence variation (including the discovery of single nucleotide polymor-

Table 1. Studies analyzed

Author	Year	Sample origin	Sample preparation	Tumor patients	Controls
Amatschek et al. [8]	2004	cancer tissue and unpaired normal tissue	whole tissue frozen	20	22, various tissues, pooled
Nishidate et al. [9]	2004	cancer tissue	laser microbeam microdissection	81	–
Zhao et al. [10]	2004	cancer tissue and unpaired normal tissue	laser capture microdissection	61	3
Garcia et al. [11]	2005	cancer tissue and paired (?) normal tissue	whole tissue frozen	15	5
Sorlie et al. [12]	2001	cancer tissue and unpaired normal tissue	whole tissue frozen	81	4
Sorlie et al. [13]	2003	cancer tissue and unpaired normal tissue	whole tissue frozen	38	4
Bertucci et al. [14]	2000	cancer tissue and unpaired normal tissue	macrodissected tissue frozen	34	8
Pollack et al. [15]	2002	cancer tissue and cell lines	whole tissue frozen	44 + cancer cell lines	–
Sotiriou et al. [16]	2003	cancer tissue	whole tissue frozen	99	–
Turashvili et al. [17]	2007	cancer tissue and paired normal tissue	laser capture microdissection	20	10
Grigoriadis et al. [18]	2006	cancer tissue and unpaired normal tissue	beads-based enrichment	50	10
Di Cristina et al. [19]	2007	cancer tissue	whole tissue frozen	10	–
Axelsen et al. [20]	2007	cancer cell lines and normal tissues	pooled	cancer cell lines	13, various tissues, pooled

phisms), DNA copy number variation, loss of heterozygosity, DNA methylation and transcription factors. The resulting gene expression profiles have provided molecular pathologists with new information on molecular variation between normal and diseased breast tissue, but also on the variability between different BC and between different individual samples.

In the meantime, terabytes of gene expression data are available to the research community. The number and pace of publications in BC make it difficult, if not impossible, for the individual researcher to keep an overview in his own domain of expertise. Moreover, contradictory results have been reported and there is an increasing number of publications on methodological problems related to DNA array studies. Thus, some researcher might feel 'lost in translation'.

Our group has already gained expertise in merging gene expression data in human cancer [6, 7]. The aim of the present meta-analysis was to screen the literature for DNA array studies in BC, to select appropriate studies, to merge their results and to compare/contrast these findings, with the hope of identifying molecular key players in BC.

Materials and Methods

A Medline search of studies published in English using the key words 'breast cancer', 'gene expression' and 'normal' with the limit 'human' was performed. A total of 2,027 abstracts were found. Abstracts of all reports obtained were reviewed and a related article search was performed. Relevant studies were selected by 2 authors (N.S. and M.A.R.), based on compliance with the follow-

ing requirements: (1) sample origin: human tissue or human sera or human-derived cell lines; (2) highly paralleled transcriptomics studies; (3) quantitative results on overexpression or underexpression available. Surveys concerning only selected genes and findings based on animal models were not considered. Studies focusing on prognostic factors, therapy response, metastasization and prognostic assessment were excluded.

Gene expression data were categorized as upregulated, down-regulated or expressed. Then, data were entered into an Excel (Microsoft Corporation, Redmond, Wash., USA) working sheet. No threshold was defined for up- or downregulation and therefore genes defined as differentially expressed might be only slightly altered in expression. All statistics provided are descriptive and obtained with the built-in tools of the Excel software.

Among various transcriptomics technologies, only cDNA array studies were selected, while serial analysis gene expression studies, for example, were discarded. Results from 13 studies were available [8–20]. When indicated, corresponding supplementary files were downloaded from the publisher's website, as indicated by the authors, and this information was incorporated into the database. This data warehouse was then merged into a single file. SwissProt primary accession numbers were retrieved online (<http://www.expasy.ch>) between July and October 2007. Gene accession numbers were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed>; search 'nucleotide'). In case of doubt, an Entrez cross-database search was added (<http://www.ncbi.nlm.nih.gov/sites/gquery?term=XXX>). Data were then sorted by SwissProt primary accession numbers and double records were merged.

These 13 studies used various samples. Eight surveys analyzed human cancer tissues and unpaired normal tissues [8, 10–14, 17, 18], 2 studies tested only tumor tissue [9, 16], 2 studies compared results from human cancer tissues and tumor cell lines [15, 19], and a single survey examined tumor cell lines and normal tissue [20].

The total number of tumor patients profiled in all studies was 553. For comparative analysis, 79 samples from normal tissues obtained at surgery (for example during reduction mammoplasty) were analyzed. An overview of all studies is provided in table 1.

Table 2. Genes upregulated in human breast cancer

Accession No.	SwissProt	Entry name	Name	References															
				8	9	10	11	12	13	14	15	16	17	18	19	20			
AA789233	P23771	GATA3_HUMAN	GATA binding protein 3																
D90041	P18440	ARY1_HUMAN	arylamine N-acetyltransferase 1																
X14420	P10242	MYBB_HUMAN	Myb-related protein B																
X52022	Q13433	S39A6_HUMAN	zinc transporter SLC39A6 precursor																
AF065388	P02452	CO1A1_HUMAN	collagen alpha-1(I) chain [precursor]																
L12350	P08123	CO1A2_HUMAN	collagen alpha-2(I) chain precursor																
AW949747	P02461	CO3A1_HUMAN	collagen alpha-1(III) chain precursor																
D90041	P12111	CO6A3_HUMAN	collagen alpha-3(VI) chain [precursor]																
AA456878	O60635	TSN1_HUMAN	tetraspanin-1																
J02874	P35442	TSP2_HUMAN	thrombospondin-2 [precursor]																
D13665	P15090	FABPA_HUMAN	fatty acid-binding protein, adipocyte																
D28137	Q15063	POSTN_HUMAN	periostin precursor																
U77180	P16671	CD36_HUMAN	platelet glycoprotein 4																
M29541	P17861	XBPI_HUMAN	X-box-binding protein 1																
Y14690	Q14534	ERG1_HUMAN	squalene monooxygenase																
X57527	Q15012	LAP4A_HUMAN	lysosomal-associated transmembrane protein 4A																

Genes quoted 3 times and more are given. Dark grey = Upregulated; bright grey = expressed.

Results

A total of 1,350 genes were reported at least once to be deregulated in BC. Of these findings, 1,212 (90%) were reported only once, in other words they were not confirmed by other authors. A cohort of 138 genes of particular interest remained for further analysis. Of these, 79 were consistently reported to be overexpressed in BC, 41 to be underexpressed and in 18 cases the results were contradictory.

Upregulated Genes in Human BC

Of 79 genes that were consistently reported to be upregulated in BC, 1 gene was found in 6 studies [GATA binding protein 3 (GATA3)], 3 genes in 4 studies [arylamine N-acetyltransferase (NAT) 1, Myb-related protein B (B-Myb), zinc transporter SLC39A6 precursor] and 12 genes in 3 studies (collagen alpha-1(I) chain [precursor], collagen alpha-2(I) chain precursor, collagen alpha-1(III) chain precursor, collagen alpha-3(VI) chain [precursor], tetraspanin-1, thrombospondin-2 [precursor], fatty acid-binding protein, adipocyte, periostin precursor, platelet glycoprotein 4, X-box-binding protein 1, squalene monooxygenase, lysosomal-associated transmembrane protein 4A). These upregulated genes are shown in table 2. Moreover, 63 further genes were reported by 2 authors to

be upregulated (online suppl. table 1, www.karger.com/doi/10.1159/000123849).

Downregulated Genes

Of 41 genes that were consistently reported to be downregulated in BC, 3 were found in 4 studies (cadherin-3 precursor, keratin type I cytoskeletal 17 and keratin type II cytoskeletal 5). Six genes were reported by 3 authors to be downregulated (chitinase 3-like 2, lactadherin precursor, pleckstrin homology-like domain family A member 1, tripartite motif-containing protein 29, bullous pemphigoid antigen 1 and activating transcription factor). These downregulated genes are shown in table 3. Moreover, 32 further genes were reported by 2 authors to be downregulated (online suppl. table 2, www.karger.com/doi/10.1159/000123849).

Deregulated Genes, Contradictory Results

The expression of numerous further genes has been reported to be deregulated in human BC. However, results reported are contradictory so that these genes appear to be up- or downregulated in different cancers and/or individuals, possibly in subgroups with typical phenotype. These genes are coding, for example, mucin-like protein 1, annexin A1, tumor necrosis factor and various surface antigens, all proteins with possible involvement

Table 3. Genes downregulated in human breast cancer

Accession No.	SwissProt	Entry name	Name	References															
				8	9	10	11	12	13	14	15	16	17	18	19	20			
X63629	P22223	CADH3_HUMAN	cadherin-3 precursor																
Z19574	Q04695	K1C17_HUMAN	keratin, type I cytoskeletal 17																
M21389	P13647	K2C5_HUMAN	keratin, type II cytoskeletal 5																
U58514	Q15782	CH3L2_HUMAN	chitinase 3-like 2																
U58516	Q08431	MFGM_HUMAN	lactadherin precursor																
AK026181	Q8WV24	PHLA1_HUMAN	pleckstrin homology-like domain family A member 1																
L24203	Q14134	TRI29_HUMAN	tripartite motif-containing protein 29																
M69225	Q03001	BPA1_HUMAN	bullous pemphigoid antigen 1, isoforms 1/2/3/4/5/8 [fragment], dystonin																
L19871	P18847	ATF3_HUMAN	activating transcription factor 3																

Genes quoted 3 times and more are given. Dark grey = Downregulated; bright grey = expressed.

in carcinogenesis, differentiation, immune response or the metastasization process. These genes are summarized in online supplement table 3 (www.karger.com/doi/10.1159/000123849).

Discussion

The aim of the present meta-analysis of gene expression in human BC was to screen the literature for DNA array studies in BC, to select appropriate studies, to merge their results, and to compare/contrast these findings, with the hope of identifying key molecular players in BC.

Our study confirms that a number of studies applying cDNA array technologies in BC are available, increasing rapidly in numbers, with 173 studies published in the last 12 months. However, our first important observation is that translational studies using cDNA arrays comparing normal tissue with tumor tissue in large cohorts of patients are rare. Only 13 translational studies fulfilled the inclusion criteria, involving 553 BC patients and 79 controls. This figure appears very low, considering that BC is the most common cancer in women.

Well-characterized samples were also rare. The normal human breast consists of ductal epithelium and surrounding stroma. Breast development requires extensive penetration of the ductal epithelium into the stroma. Thus, it can be expected that analysis of whole tissue biopsies of the breast without separating epithelial from stromal components will induce a significant variability in genome-wide translational research results. This ex-

pectation is confirmed by experimental gene expression results: after laser capture microdissection, the stroma and epithelium clustered according to the tissue type and not the presence of tumor [21]. Against this background, it appears disappointing that of the 13 large-scale studies selected for the present meta-analysis, as much as 9 were performed using frozen samples obtained from whole tissue biopsies (stroma and epithelium together) or surgical specimen. Only 4 studies used enriched epithelial cells, 3 by laser microdissection [9, 17], 1 with beads immunoselection [18]. This is surprising, since cell enrichment techniques have been well validated in BC [21, 22].

A further problem is the definition of normality for comparison purpose and the availability of such control tissue. In BC research, it is obviously a problem to obtain normal tissue. Breast stroma is evolving during pregnancy, lactation and involution, making the definition of normal tissue difficult. In the present meta-analysis, the normal samples represented only 14% of the number of tumor samples. This low figure is likely to be explained by the small size of BC and by a trend towards breast-preserving surgery. Perhaps, this also explains why most cDNA array studies in BC, and also the most cited, compare different tumor phenotypes (for example, survival prediction [23], estrogen receptor status [24], nulliparous vs. multiparous [25], response to neoadjuvant therapy [26], molecular portraits [27] and metastasization [28]) rather than tumor with controls. At the end of the day, the information available on specific modifications in gene expression in BC is scarce.

A further problem is the bias induced by interindividual variability on gene expression results. Samples from different individuals are expected to show variation in gene expression due to a number of factors, such as difference in tissues, interindividual variation and clinical differences. When these factors were analyzed, the primary source of variation could be attributed to differences between tissues and between individuals [21]. However, only a single, recent study compared paired samples (normal vs. tumor) obtained in single patients, and this only in half the patients (10 of 20) [17].

In spite of all these methodological limitations, this meta-analysis could identify a panel of 138 genes of particular interest in BC, since they are reported to be regularly deregulated in cDNA array studies.

Upregulated Genes

The most frequently reported deregulated gene in BC is GATA3. GATA3 is a transcriptional activator highly expressed by the luminal epithelial cells in the breast. Patients whose tumors are expressing low GATA3 levels have significantly shorter overall and disease-free survival when compared with those whose tumors had high GATA3 levels. GATA3 had independent prognostic significance above and beyond conventional variables. This result confirms an independent identification of GATA3 as a BC prognostic marker by global gene expression meta-analysis [29].

Arylamine N-acetyltransferase, a product of the NAT 2 gene, is an enzyme important in carcinogen metabolism via activation and detoxification pathways [30]. NAT2 genetic polymorphism has been reported to be associated with development of BC [31].

B-Myb is a transcription factor. Myb has been known for 20 years for being a protooncogene that can be activated by retroviral actions [32]. B-Myb is broadly expressed in rapidly dividing mammalian cells. B-Myb has been shown to promote cell survival by activating antiapoptotic genes and could be implicated in tumorigenesis by regulating gene expression [33].

Zinc transporter LIV-1 (SLC39A6) is a specialist protein controlling the level of intracellular zinc in cells. Zinc plays a vital role in controlling the cellular processes of the cell, such as growth, development and differentiation. SLC39A6 is estrogen regulated and present in increased amounts in estrogen receptor-positive BC as well as in tumors that spread to the lymph nodes. Resistance to antiestrogen drugs such as tamoxifen and fulvestrant often occurs in advanced BC and is associated with differential expression of individual LIV-1 family members [34].

Downregulated Genes

Cadherin-3 precursor is downregulated in BC. Loss of expression of the intercellular adhesion molecule E-cadherin frequently occurs in invasive lobular breast carcinomas as a result of mutational inactivation [35]. Cadherins are hormonally regulated cell adhesion proteins that carry out physiological roles during mammary development but have pathological effects when deregulated. E-cadherin expression is irreversibly lost in invasive lobular BC, serving both as a tumor suppressor and an invasion suppressor. Ductal BC involves complex, reversible, epigenetic modulation of multiple cadherins. Transcriptional regulators of E-cadherin have been identified that induce epithelial-to-mesenchymal transitions [36].

Keratins are broadly used in immunohistochemistry laboratories for characterizing adenocarcinomas and defining their organ of origin. Cytokeratin 17 is expressed in the myoepithelium of extralobular ducts of the normal breast tissue, but is not expressed in the vast majority of BC, which do not contain myoepithelial markers (keratin 17 and vimentin) [37]. Cytokeratin 5 is expressed in the basal cells of the breast epithelium. The basal phenotype (expression of cytokeratin 5 and cytokeratin 14) has similar immunohistochemical characteristics with myoepithelial cells of the breast and has poor outcome [38, 39].

Thus, the genes identified with this meta-analysis of gene expression in BC are key players in the development and/or phenotype of BC. This is a proof of principle for integrating results from cDNA array studies obtained by different authors with independent samples under various experimental conditions.

We believe that performing such meta-analysis of gene expression is a powerful approach for mining the terabytes of data available from the hundreds of gene expression studies in BC. However, this meta-analysis also shows that much more effort should be devoted to the cohort of patients analyzed, to the control tissues and to sample enrichment procedures, as well as to the establishment of common data standards in translational BC research.

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