

SEARCH FOR A Q-RT-PCR GENE EXPRESSION PROFILE OF BENIGN BREAST TISSUE ASSOCIATED WITH HIGH BREAST CANCER RISK

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BACKGROUND

Recent developments in the ability to predict and alter breast-cancer risk warrant a new look at the role of assessment of this risk in primary care. Breast tissue studies may therefore provide both risk level biomarkers as well as surrogate biomarkers for subsequent prevention strategies (5). These markers might be:

1. variations in the expression of genes directly involved in the breast cancer susceptibility by themselves (genetic background, germline variations)

2. variation in the expression of genes involved in the carcinogenic process by themselves (markers of exposure to a carcinogenic event)

3. variations in the expression of genes that are markers of the response of the breast tissue to specific carcinogenic factors (host response).

In 5% of breast cancer cases, a leading germ-line mutation of either BRCA1, BRCA2, or other rare predisposing genes such as PTEN or P53, can be evidenced (1) and is directly causal. However, in all other cases, the direct cause of breast cancer remains totally unknown (2). Apart from germline mutations in BRCA1 and BRCA2 genes (which accounts for 1 person in 500-800 in the general population), most factors identified as risk factors individually confer a low to moderate increase of breast cancer risk and have no major relevance for specific individual counselling.

We hypothesized that some of the genes might have value for the identification of high-risk tissues: either because they might be involved in carcinogenic pathways, or because they highly discriminate between cancer and benign, they might be able to identify epitheliums where transformed cells are already present.

OBJECTIVES

The aim of this work was to identify candidate biomarkers of breast cancer risk in benign breast cancer tissues. As stated above, we hypothesized for the purpose of this study women who are at high risk of developing breast cancer whatever the leading cause share common markers of breast cancer risk in their breast tissue and we aimed at identifying them. The results of this study might give further insight into benign-malignant transition in breast epithelium and provide prognostic profiles among benign breast lesions for use in a prospective evaluation.

MATERIALS AND METHODS

We investigated, by a quantitative RT-PCR method, genes potentially involved in breast benign-malignant transition, which could eventually be markers for subsequent breast cancer risk among normal/benign breast tissues.

- Selection of genes

(a) Candidate genes from the literature: Susceptibility: BRCA1, BRCA2, Pathways and function of ER1: Eralpha, ErbB1, PR, GATA3, RAR, Oncogenes: Her2, EGFR, Apoptosis and DNA repair: BCL2, BRCA2, Bcl2, Bax, Proliferation: cyclin B1, Metabolic regulations: Cox2, IGF1, IGF1R, IGF1R3, VEGF, TRF1 (TERT regulator), Viral carcinogenesis: FGFR3, Cavdin-1, TRANSBIG benign-vs. malignant genes: FN1, FBNP3, ZRF1, MMP11, KTN2C, CENPF

(b) Candidate genes from the Transbig Consortium data

The six genes significantly deregulated in cancer versus benign lesions in a confirmatory trial are tested: FN1, FBNP3, ZRF1, MMP11, KTN2C, CENPF

- Case selection

We searched two databases of 262 and 676 patients with frozen samples of normal and benign breast tissue available in the breast tissue bank of our Institution, for samples meeting the following criteria:

- Benign breast lesions obtained from biopsy or surgical procedure

- Patients who had been biopsied/operated in our Institution with follow-up data available

- Patients who had given written informed consent for the use of their sample for research purposes

- Patients with frozen material available
 - > 5% epithelial cells within the sample
 - Sufficient quantity and quality of mRNA yielded
 Be either classified as high risk (BRCA1 or BRCA2 germline mutation, concomitant atypical hyperplasia, contralateral breast cancer or occurrence of breast cancer during follow-up) or low-risk (totally benign lesion in a patient who did not develop subsequent breast cancer at a minimum of 5-years follow-up).

At the beginning 65 patients from two exhaustive databases (262 + 676 = 938 patients) have been selected because they met the inclusion criteria. Out of them, for 5 patients we did not find frozen samples in IGR's tissue bank (7.7 %). From remaining 60 patients from our list with existing frozen tissues, the extraction of RNA has been realized in 48 patients (70.77 %). The remaining 14 frozen samples could not be used for administrative and legal reasons (patients' written consent statements missing). The satisfying quality and quantity of the extracted RNA have been found in 42 samples (64.62 % of all samples in database, 91.3 % of total samples with RNA extraction). These 42 patients have been analyzed in our study.

RESULTS

Table 1. The characteristics of the patients included in this study and of their lesions are summarized in the next table:

	N	Age med	Menopausal	% cell epith	Med FU
High risk	19	45 (25-68)	5 (26%)	Med 30%	
Low risk	23	39 (26-65)	3 (13%)	Med 32.5%	8.5 yrs

Table 2. P-value (t-test) for distribution ratio for 2 classes (high risk - low risk) :

Ratio / sein normal	T				Tn						
	student test	Haat	Risque Bas	Risque Haut	student test	Haat	Risque Bas	Risque Haut			
FN1	4.45E-01	4.638	5.007	0.403	0.25	BAX	3.74E-03	2.449	3.240	0.29	0.26
MMP11	3.60E-01	6.294	4.605	0.502	0.45	ESR2	1.83E-02	2.149	0.925	0.11	0.11
BRCA1	1.25E-01	9.930	12.369	1.20	0.96	CAV1	1.83E-02	1.368	0.540	0.17	0.64
BRCA2	2.32E-02	32.288	46.369	3.04	4.96	BRCA2	2.32E-02	32.288	46.369	3.04	4.96
ESR1	2.27E-02	2.641	3.059	0.32	0.26	ESR1	2.27E-02	2.641	3.059	0.32	0.26
ESR2	1.83E-02	2.149	0.925	0.11	0.11	KTN2C	2.36E-02	16.295	26.446	1.56	2.02
ZRF1	1.36E-01	17.536	19.338	2.23	1.36	CENB1	3.14E-02	2.703	4.533	0.25	0.20
CENPF	3.86E-02	97.765	109.997	11.54	13.21	CENPF	3.86E-02	97.765	109.997	11.54	13.21
ERBB2	1.02E-02	2.027	2.702	0.16	0.16	IGF1	4.88E-02	10.630	18.911	1.14	1.68
FBNP3	4.88E-02	15.245	17.803	1.79	1.78	GATA3	4.88E-02	2.038	3.175	0.29	0.20
CENB1	3.14E-02	2.703	4.533	0.25	0.20	FBNP3	4.88E-02	15.245	17.803	1.79	1.78
IGF1	2.29E-02	2.477	1.899	0.26	0.16	EGFR	7.29E-02	2.437	1.939	0.28	0.16
IGF1R3	9.86E-02	3.305	3.693	0.25	0.27	ERBB2	7.29E-02	2.437	2.739	0.16	0.10
IGF1	4.88E-02	10.630	18.911	1.14	1.68	IGF1R3	9.86E-02	3.305	3.693	0.25	0.27
IGF1R	1.80E-01	2.026	1.952	0.21	0.19	BRCA1	1.25E-01	9.930	12.369	1.20	0.96
VEGF	2.29E-01	1.564	1.725	0.21	0.15	IGF1R	1.36E-01	17.536	19.338	2.23	1.36
PGR	1.45E-01	22.776	33.557	2.26	1.67	PGR	1.45E-01	22.776	33.557	2.26	1.67
TRF1	1.33E-01	1.245	0.670	0.02	0.20	PTGS2	1.33E-01	5.373	3.541	0.65	0.24
KTN2C	2.36E-02	16.295	26.446	1.56	2.02	IGF1R	1.36E-01	17.536	19.338	2.23	1.36
PTGS2	1.33E-01	5.373	3.541	0.65	0.24	TRF1	1.33E-01	1.245	0.670	0.02	0.20
BAX	3.74E-03	2.449	3.240	0.29	0.26	RARA	1.80E-01	2.021	2.489	0.11	0.16
RARA	2.86E-01	2.271	2.489	0.31	0.19	VEGF	2.29E-01	1.564	1.725	0.21	0.15
BCL2	4.88E-01	3.444	3.252	0.52	0.36	MMP11	3.60E-01	6.294	4.605	0.45	0.45
CAV1	1.83E-02	1.368	0.540	0.17	0.64	FGF3	4.16E-01	0.000	0.000	0.00	0.00
GATA3	4.88E-02	2.038	3.175	0.29	0.20	FN1	4.45E-01	4.638	5.007	0.40	0.25
FGF3	4.16E-01	0.000	0.000	0.00	0.00	BCL2	4.88E-01	3.444	3.252	0.52	0.36

Diagram 1. Distribution of ratio low risk - high risk (comparing to 18S - "house-keeping" gene) :

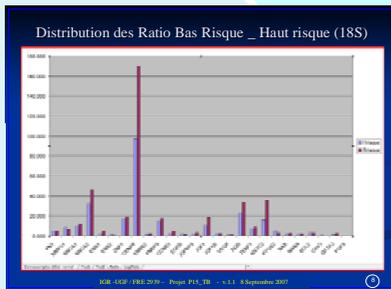
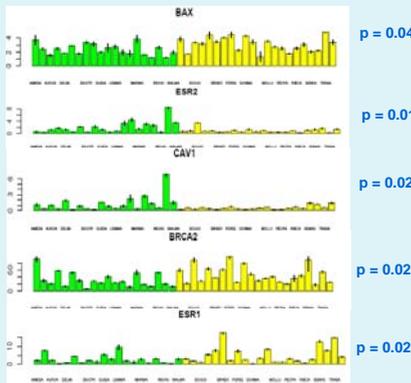


Diagram 2. Distribution of ratio low risk - high risk of the predictive genes (BAX, ESR2, CAV1, BRCA2, ESR1)



DISCUSSION

This study suggested two genes to be good candidates as biomarkers capable of identifying high risk breast cancer tissue: Bax (p=0.003) and ESR2 (p=0.01). Cav1 and BRCA2 had borderline significance (p=0.02) and may be also candidates to be explored in an extended part of this study. On the opposite, none of the genes from the Transbig profile (all of them being highly discriminant between benign and malignant lesions in our experience) (75, 76) showed up as potential markers of risk. This observation invalidates one of our initial hypotheses (find single transformed cells in apparently benign lesions). Oncogenes such as EGFR, VEGF, or Her2 were not differently regulated in both risk profiles. Proliferation did not differentiate both risk groups, an observation that was expected regarding the high proliferation levels of certain totally benign lesions in both groups. As well, none of the genes involved in metabolic pathways and shown as markers of risk in blood were predictive of breast cancer risk in this study. Our results are interesting and promising. We would like to point out that a validation study needs to be established (and is actually performing) in order to validate our positive results on identified predictive genes. The results of this study might give further insight into benign-malignant transition in breast epithelium and provide a prognostic set of biomarkers among benign breast lesions that may be suitable for prospective validation and use. With this validation study we should be able to confirm our hypotheses: (1) breast cancer risk presents a unique biological phenotype; (2) this phenotype could be identified.

CONCLUSION

- This is the first extended study of mRNA expression in benign breast epithelium regarding breast cancer risk

- This study suggested several genes as candidate biomarkers for the identification of risk breast cancer tissues: ERB and Bax were the most interesting candidates

- We will first extend this retrospective series in order to have stronger data, and a prospective study will then be held, based on these results

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