

ESMO
(European Society of Medical Oncology)

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**Search for a Q-RT-PCR gene expression
profile of benign breast tissue associated
with high breast cancer risk**
- Short Report -

Institut Gustave Roussy

Mentor: Dr Suzette DELALOGUE

2007 - 2008

Persons implicated in this work

- **UPRES EA 3535:**

Mrs. Véronique Scott, Research Ingenieur, was involved into the mRNA extraction and Q-RT-PCR analysis of the samples

Pr Gilles Vassal, Head of the UPRES laboratory, and Dr Fabrice André, Head of the Breast Business Unit, have helped monitoring this work

- **Pathology:** Dr Marie Christine Mathieu has supervised this work in its pathological components. Dr Sameh Geah has

- **Centre de Ressources Biologiques:** Mrs. Isabelle Simon was responsible for searching the tumor bank for samples

- **Bio-informatics:** Mr Philippe Dessen, Head of the Bioinformatics lab, Institut Gustave Roussy

- **Transbig Consortium:** Dr Mahasti Saghatchian at IGR

- **Monitoring**

During the academic year 2006/2007 this translational research work was monitored three times per month in IGR: two times during the translational staff every 14 days (chaired by Dr Fabrice ANDRE) and, one time per month, during the DUERCC meeting (chaired by Prof. Martin SCLUMBERGER, Prof. Ellen BENHAMOU, Prof. Jean-Charles SORIA with participation of Dr. Suzette DELALOGÉ).

BACKGROUND AND RATIONALE

One woman in ten currently develops breast cancer in western countries. Each year in the France, breast cancer is diagnosed in more than 40000 women. Despite this substantial burden of disease, however, assessment of breast-cancer risk has received very little attention outside the oncology clinic.

Why evaluate breast cancer risk?

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 and is directly causal. But beside this, very little tools exist to identify patients with substantially higher risk of breast cancer than the general population: it has been known for years that certain histological lesions such as ductal or lobular atypical hyperplasia, confer a 2-5 fold increase of breast cancer risk, but these lesions are rare events.

Breast tissue studies may therefore provide both risk level biomarkers as well as surrogate biomarkers for subsequent prevention strategies.

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 carcinogenetic process by themselves (markers of exposure to a carcinogenetic event)
3. variations in the expression of genes that are markers of the response of the breast tissue to specific carcinogenetic factors (host response)

OBJECTIVES AND PERSPECTIVES

The aim of this work was to identify candidate biomarkers of breast cancer risk in benign breast cancer tissues.

We therefore compared the quantitative mRNA expression of several candidate biomarkers in two retrospective series of breast tissues: the first series was classified as high risk and had been harvested in BRCA1/2 mutation carriers, women diagnosed with atypical hyperplasia or who had developed contralateral or subsequent breast cancer during follow-up. The second series, classified as low-risk, had been harvested in women who only had benign breast disease and did not develop breast cancer during follow-up.

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.

(a) Candidate genes from the literature:

- susceptibility: **BRCA1, BRCA2**
- pathways and function of ER : **ERalpha, ERbeta, PR, GATA3, RAR**
- oncogenes : **Her2, EGF-R**
- apoptosis and DNA repair : **BRCA1, BRCA2, Bcl2, bax**
- proliferation : **cycline B1**
- metabolic regulations : **Cox2, IGF1, IGF1R, IGFBP3**

- **VEGF, TRF1** (hTERT regulator)
- Viral carcinogenesis: **FGF3**
- **caveolin 1**
- Transbig benign vs malignant genes: **FN1, FNBP3, ZRF1, MMP11, KTNC2, 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, FNBP3, ZRF1, MMP11, KTNC2, 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, meeting the following criteria:

- 1) **Benign breast lesions** obtained from biopsy or surgical procedure
- 2) Patients who had been biopsied/operated **with follow-up data available**
- 3) Patients who had given **written informed consent** for the use of their sample for research
- 4) Patients with **frozen material available**
- 5) **≥ 5% epithelial cells** within the sample
- 6) **Sufficient quantity and quality of mRNA** yielded
- 7) 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).

RESULTS

19 cases were selected that met the criteria for high-risk lesions: 5 from BRCA1-mutation carriers, 5 from patients with concomitant atypical hyperplasia, 14 from patients with contralateral cancer or occurrence of breast cancer during follow-up.

23 cases met the criteria for low-risk lesions: typically benign lesions in patients who never developed breast cancer at a minimum of 5-years follow-up (5-17 years, median 8.5).

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

	N	Age (median)	menopausal	% épith cells	Med FU
High risk	20	45 (25-68)	5 (25%)	Med 30%	-
Low risk	22	39 (26-65)	3 (14%)	Med 32%	8.5 yrs

This study compared the ratio of the quantitative level of mRNA expression of 26 candidate genes / level of expression of normal breast (Clontech) in 19 cases defined as “high risk breast tissue” and 23 cases of “low risk breast tissue”:

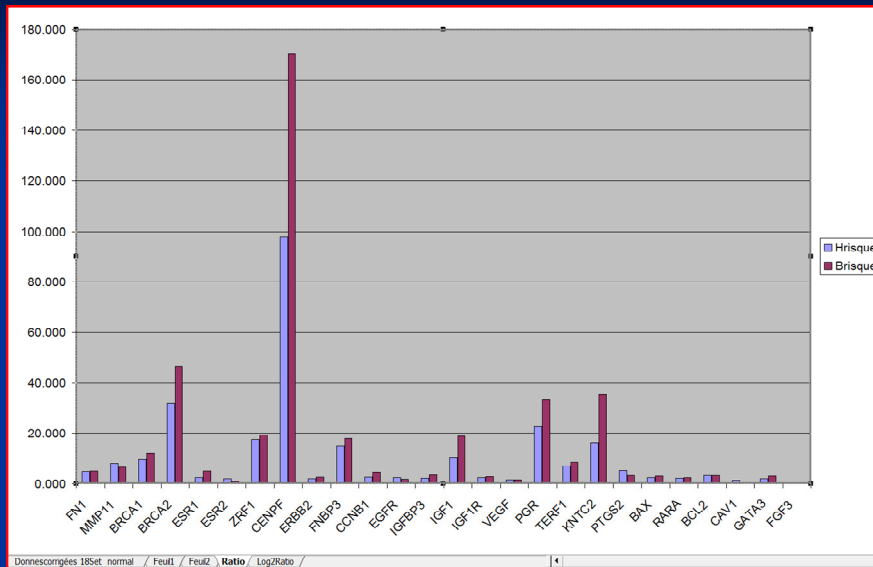
Ratio / sein normal												
		1	0				Tri					
	student test	Haut Risque	Bas Risque	Haut Risque	Bas Risque		student test	Haut Risque	Bas Risque	Haut Risque	Bas Risque	
		Mean	Mean	sd	sd			Mean	Mean	sd	sd	
		Hrisque	Brisque	Hrisque	Brisque							
FN1	4.45E-01	4.839	5.097	0.40	0.25		BAX	3.74E-03	2.449	3.240	0.29	0.26
MMP11	3.60E-01	8.284	6.605	0.82	0.45		ESR2	1.03E-02	2.149	0.925	0.31	0.11
BRCA1	1.23E-01	9.930	12.190	1.20	0.96		CAV1	1.92E-02	1.368	0.542	0.17	0.04
BRCA2	2.23E-02	32.288	46.390	3.04	4.96		BRCA2	2.23E-02	32.288	46.390	3.04	4.96
ESR1	2.27E-02	2.641	5.010	0.32	0.28		ESR1	2.27E-02	2.641	5.010	0.32	0.28
ESR2	1.03E-02	2.149	0.925	0.31	0.11		KNTC2	2.36E-02	16.295	35.454	1.50	3.02
ZRF1	1.35E-01	17.516	19.328	2.23	1.36		CCNB1	3.14E-02	2.703	4.533	0.25	0.20
CENPF	3.98E-02	97.925	169.997	11.54	13.35		CENPF	3.98E-02	97.925	169.997	11.54	13.35
ERBB2	7.98E-02	2.037	2.730	0.16	0.10		IGF1	4.88E-02	10.630	18.911	1.14	1.18
FNBP3	4.98E-02	15.245	17.863	1.79	1.78		GATA3	4.95E-02	2.038	3.173	0.29	0.20
CCNB1	3.14E-02	2.703	4.533	0.25	0.20		FNBP3	4.98E-02	15.245	17.863	1.79	1.78
EGFR	7.29E-02	2.427	1.939	0.30	0.16		EGFR	7.29E-02	2.427	1.939	0.30	0.16
IGFBP3	9.98E-02	2.305	3.693	0.25	0.27		ERBB2	7.98E-02	2.037	2.730	0.16	0.10
IGF1	4.88E-02	10.630	18.911	1.14	1.18		IGFBP3	9.98E-02	2.305	3.693	0.25	0.27
IGF1R	1.60E-01	2.638	3.105	0.35	0.19		BRCA1	1.23E-01	9.930	12.190	1.20	0.96
VEGF	2.29E-01	1.554	1.725	0.21	0.15		ZRF1	1.35E-01	17.516	19.328	2.23	1.36
PGR	1.40E-01	22.739	33.537	2.26	1.67		PGR	1.40E-01	22.739	33.537	2.26	1.67
TERF1	1.93E-01	7.245	8.678	0.82	0.70		PTGS2	1.53E-01	5.373	3.541	0.95	0.24
KNTC2	2.36E-02	16.295	35.454	1.50	3.02		IGF1R	1.60E-01	2.638	3.105	0.35	0.19
PTGS2	1.53E-01	5.373	3.541	0.95	0.24		TERF1	1.93E-01	7.245	8.678	0.82	0.70
BAX	3.74E-03	2.449	3.240	0.29	0.26		RARA	2.06E-01	2.221	2.489	0.31	0.19
RARA	2.06E-01	2.221	2.489	0.31	0.19		VEGF	2.29E-01	1.554	1.725	0.21	0.15
BCL2	4.69E-01	3.484	3.522	0.32	0.16		MMP11	3.60E-01	8.284	6.605	0.82	0.45
CAV1	1.92E-02	1.368	0.542	0.17	0.04		FGF3	4.16E-01	0.000	0.000	0.00	
GATA3	4.95E-02	2.038	3.173	0.29	0.20		FN1	4.45E-01	4.839	5.097	0.40	0.25
FGF3	4.16E-01	0.000	0.000	0.00			BCL2	4.69E-01	3.484	3.522	0.32	0.16

Two genes were the most “predictive” for breast cancer risk: BAX (p=0.004) and ERβ (p=0.01).

CAV1 (p= 0.02), BRCA2 (p= 0.02) and ERα (p=0.02) were of borderline significance.

The distribution of ratios observed (comparing to18S - “house-keeping gene” as a standard gene) is presented to the next graphic:

Distribution des Ratio Bas Risque _ Haut risque (18S)

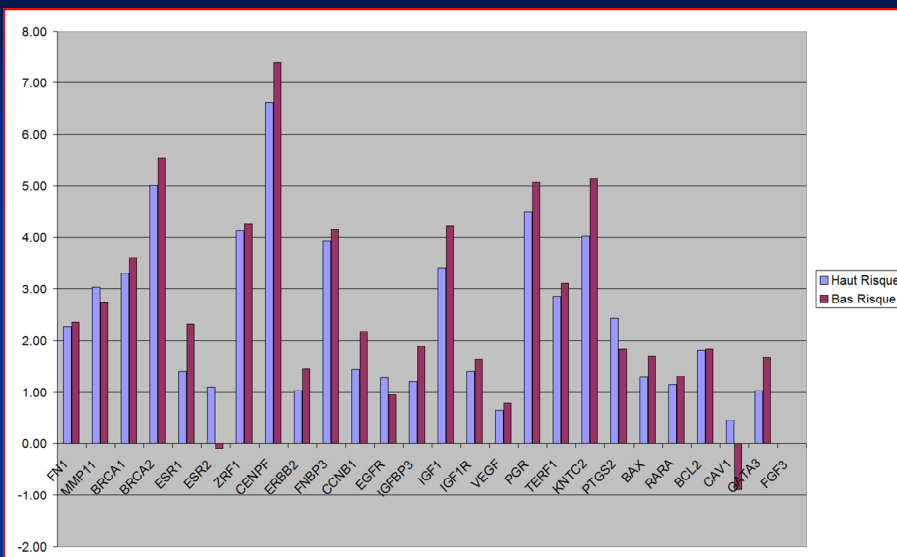


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The Log2 ratios were then established and are presented to the next graphic:

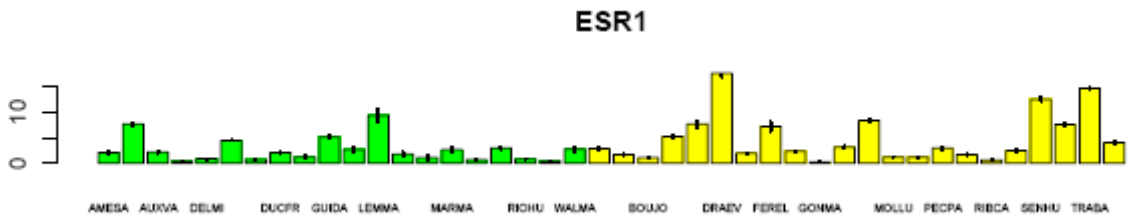
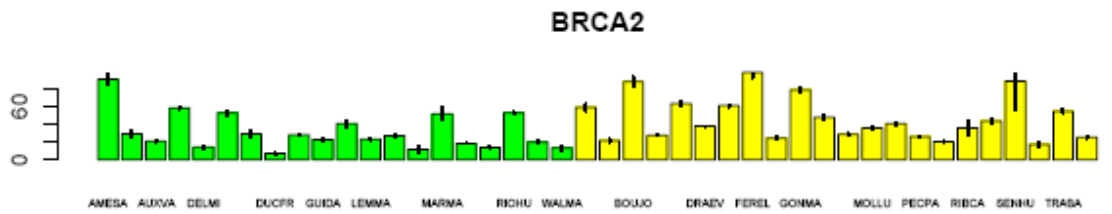
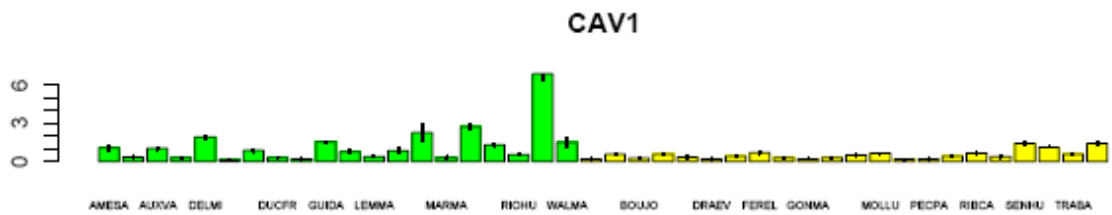
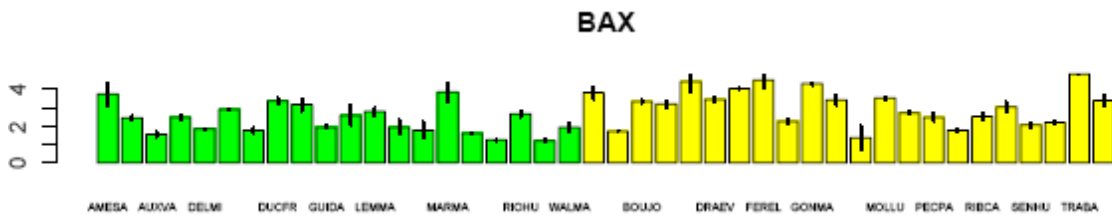
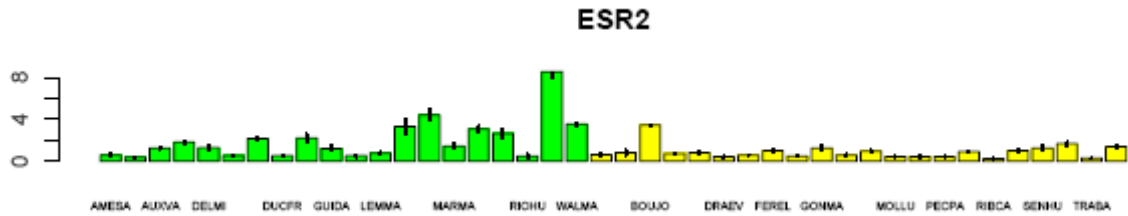
Distribution des Log2Ratio Bas Risque _ Haut risque (18S)



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The distribution of ratios in low risk (yellow) and high risk (green) samples of some of the genes (ESR2, BAX, CAV1, BRCA1, BRCA2) can be visualized on the next graphic:



DISCUSSION

For realisation of this study we needed to establish a database of benign lesions, which did not exist previously in Breast Cancer Unit of Institut Gustave Roussy (IGR).

There was overall a slight important heterogeneity between samples. This is easily explained by the nature of the samples studied: non-clonal benign breast lesions in rather young women (only 25 and 14 % were menopausal respectively in both groups). Indeed the populations were clinically quite homogenous in the nature of the benign lesions and regarding their age. This heterogeneity prompts us to increase the cohort of cases studied.

The Q PCR expression of all genes studied differed from normal breast sample with sometimes very large variations. All analyses were made using the ratio between observed levels of expression and expression of normal breast tissue, beside normalization on 18S. One might however wonder if both categories were of increased risk, because of the discrepancies observed between their tissues and normal breast. This remains possible: indeed patients considered as low-risk have not developed breast cancer at a median follow-up of only 8.5 years and some of them had a slightly increased Gail model risk. We must then be comparing moderate-risk to very-high-risk epithelia. The clinical significance of this comparison remains very important and relevant anyway.

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 showed up as potential markers of risk. This observation invalidates one of our initial hypotheses (find single transformed cells in apparently benign lesions).

In conclusions, our results are interesting and promising. We would like to point out that a validation study needs to be established in order to validate our positive results on identified prediction 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 confers a unique biological phenotype; (2) this phenotype could be identified.

CONCLUSIONS

- 1. This is the first extended study of mRNA expression in benign breast epithelium regarding breast cancer risk**
- 2. This study suggested several genes as candidate biomarkers for the identification of risk breast cancer tissues: ER β and Bax were the most interesting candidates**
- 3. 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**