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Gene expression profile associated with breast cancer metastasizing to bone

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Purpose: The most abundant site of a distant relapse in breast cancer patients is bone but why tumors preferentially relapse to bone is poorly understood. In the present study, we initiated a search for genes that are implicated in bone relapse in breast cancer.

Patients and Methods: We analyzed 107 primary breast tumors that were all lymph-node negative at the time of diagnosis and that all had relapsed. Total RNA isolated from frozen tumor samples was used to gather gene expression data using oligo-microarrays.

Results: A panel of 69 genes was found significantly differentially expressed between patients who relapsed to bone versus those who relapsed elsewhere in the body. The most differentially expressed gene, TFF1, was confirmed by quantitative RT-PCR in an independent cohort ($n = 122$, $p = 0.0015$). Our differentially expressed genes combined with a recently reported gene set relevant to bone relapse in an animal model system, pointed to the involvement of the FGF signaling pathway in preference of tumor cells that relapse to bone. Since patients relapsing to bone may benefit from bisphosphonate therapy, we developed a classifier of 31 genes, which in an independent validation set correctly predicts all bone relapse samples, with a specificity of 50%.

Conclusion: Our study identifies a panel of genes relevant to bone metastasis in breast cancer. The subsequently developed bone relapse classifier could, after thorough confirmation on an extended number of independent samples, and in combination with our previously developed high risk profile, provide a diagnostic tool to recommend adjuvant bisphosphonate therapy in addition to the endocrine or chemotherapy.

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Loss of tumorigenicity of estrogen receptor (ER) beta- expressing breast cancer MCF-7 cells

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Introduction: Proliferation of breast cancer cells is mediated by estrogen receptors (ER). Function and mechanism of action of ERa are well known, however, the role of the second ER form – ERb – discovered in 1996, for growth and treatment remains to be clarified. At the present, opposing scientific conclusions complicate the functional definition of ERb as good or bad prognostic marker. Here we investigated in the context of in vitro and in vivo studies the meaning of ERb expression for the viability and the response to (anti)estrogens of the human mammary carcinoma cell line MCF-7.

Methods: For this purpose the ERa-positive breast cancer cell line MCF-7 was stably transfected with the full-length cDNA of ERb cloned into a GFP-containing expression vector (pEGFP-N1). Proliferation rate and sensitivity to 17b-estradiol, tamoxifen and ICI 182,780 were monitored by MTT assay. Additionally, cell cycle progression was analysed flow cytometrically and the determination of cell cycle mediating proteins, cyclin A and D1, CDK2, p21Waf1/Cip1, p27Kip1 was carried out by Western Blot.

In a second part of the project wild type, empty-vector- as well as ERb-transfected MCF-7 cells were transplanted into nude mice to verify the tumorigenicity of these cells.

Results: Transfection of ERb cDNA resulted in a 30% growth inhibition of MCF-7 cells ($p = 0.043$). Additionally, the in vitro response to 17b-estradiol was reversed: MCF-7/ERb cells did not raise proliferation rate, they were inhibited to 46% by estradiol ($p = 0.016$). However, there was no difference in response to the antiestrogens tamoxifen and ICI 182,780. Importantly, MCF-7/ERb cells did not grow as a tumour in immune deficient mice. We found an ERb-induced decrease of cyclin A and CDK2 expression resulting in a slower transition through S-phase. Modulation of p21Waf1/Cip1, p27Kip1, cyclin D1 and Ki-67 protein level could not be observed.

Conclusion: ERb transfection modified the malignant character of the breast cancer cell line MCF-7 generally and caused an in vitro and in vivo growth inhibition of these cells by decreasing the expression of cell cycle regulators.

These findings may evidence that ERb acts as tumour suppressor and could serve as a good prognostic marker for ERb-positive breast cancer patients. Further investigations are required to prove the idea of exerting ERb expression for therapeutic or even preventive purpose.

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Prophylactic salpingo-oophorectomy in a population of BRCA1 and BRCA2 carriers: experience of the Institut Curie, Paris

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Background: Women with germline *BRCA1* or *BRCA2* mutations have a 60–85% cumulative lifetime risk of invasive breast cancer (BC) and a 15–65% cumulative lifetime risk of epithelial ovarian cancer (OC). Salpingo-oophorectomy (SO) for prevention of ovarian and fallopian-tube cancers in *BRCA1* or *BRCA2* mutation carriers is recommended. The objective of this study is to evaluate the results of this procedure in high risk patients followed in Institut Curie.

Material and Methods: All studied patients (pts) presented a family history of BC and/or OC. Genetic testing was suggested when either two first-degree relatives were affected with cancer: i) at least one with invasive BC before 41 yrs or ii) one with OC at any age, or three 1st- or 2nd-degree relatives from the same lineage affected with invasive BC or OC at any age. All high risk patients who accepted and underwent prophylactic salpingo-oophorectomy between 1994 and 2004 at Institut Curie entered the present retrospective study. All pts were carrying a deleterious *BRCA1* or *BRCA2* mutations, but in 1 case, SO was performed before molecular diagnosis.

Results: Between December 1994 and October 2004, 89 pts with *BRCA1* or *BRCA2* mutations (56 *BRCA1* and 33 *BRCA2* carriers) underwent a prophylactic SO. Fifteen BRCA1 and 2 *BRCA2* carriers have not been previously affected with BC. Forty two *BRCA1* and 31 *BRCA2*-experienced a previous BC history, but at the time of SO, all pts were free of disease from their BC.The number of performed SO was: 9 (10.1%) in 1994–96, 14(15.7%) in 1997–98, 13(14.6%) in 1999–2000, 21 (23.6%) in 2001–2002, and 32 (36%) in 2003–2004. The mean age at the time of SO was 49.01 ± 6.94 yrs for the 56 *BRCA1* carriers and 54.30 ± 7.65 yrs for the 33 *BRCA2* carriers ($p = 0.01$). Histopathology results of SO were: benign lesions in 85 pts (95.5%) and occult carcinomas in 4 (4.5%) pts, *ie*.2 ovarian cystadenocarcinomas and 3 fallopian tube adenocarcinomas (1 associated to ovarian carcinoma). The mean latent period between the announcement of a mutation after *BRCA1/2* testing and SO was 7.57 ± 3.51 months. The median follow-up after SO was 30 months (range 1–106). In all patients, a total local ovarian control was obtained, but 2 pts (5.6%) experienced a metastatic disease. In patients with history of BC ($n = 73$): 12 (16.4%) experienced local recurrence, 4 (5.5%) contralateral BC, and 5 (6.8%) metastatic disease after SO. Of the 89 patients who underwent SO, 86 are still alive: 82 (95%) are free of disease, and 4 presented metastases: 2 of their BC and 2 of their ovarian cancer while 3 pts died from metastases: 2 from BC and one from a pancreatic cancer.

Conclusion: This study shows that risk-reducing SO remains an important option for women at risk for hereditary breast or gynecologic cancer, particularly in patients who previously experienced a breast carcinoma, as asymptomatic ovarian cancers have been found in 4 patients. Longer follow-up is needed to better evaluate the benefits of this procedure, particularly when considering BC occurrence or recurrence.

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Inflammatory breast cancer: at the cross-roads of NFkB and estrogen receptor signalling pathways?

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Introduction: Recently, gene expression profiling of inflammatory (IBC) and non-inflammatory breast cancer (nIBC) indicated that the NFkB pathway is important for the inflammatory breast cancer phenotype.

Materials and Methods: To investigate activation of NFkB in IBC, we performed Real-Time RT-PCR for 8 selected NFkB target genes with a significant, 3-fold differential gene expression profile between IBC and nIBC by cDNA microarrays (VCAM1, CCR5, SOD2, CTSB, IRF7, CD48, IL15 and GBP1) using RNA from 17 IBC and 20 nIBC breast tumours. In addition, immunohistochemistry was performed for all NFkB family members (RelA, RelB, cRel, NFkB1 and NFkB2) on tissue sections from 44 IBC and 46 nIBC specimens. Hot spots with nuclear staining were searched for and 500 nuclei were counted. Immunohistochemical results were validated by NFkB DNA-binding experiments for all NFkB family members using nuclear protein extract from 7 IBC and 10 nIBC tumours.

Results: The expression of all NFkB target genes was significantly elevated in IBC compared to nIBC. Furthermore, we found a statistically elevated number of stained nuclei in IBC compared to nIBC for RelB

($p = 0.038$) and NFkB1 ($p < 0.001$). NFkB DNA-binding data for 4 out of 5 NFkB family members correlated well with the number of stained nuclei: RelA ($R_s = 0.481$; $p = 0.050$), RelB ($R_s = 0.707$; $p = 0.001$), NFkB1 ($R_s = 0.767$; $p = 0.001$) and NFkB2 ($R_s = 0.440$; $p = 0.058$). Transcriptionally active NFkB dimers were found in 17/44 IBC specimens compared to 2/45 nIBC specimens ($p < 0.001$). Within the group of breast tumours with transcriptionally active NFkB, the expression of 7/8 NFkB target genes was significantly elevated compared to the group of breast tumours without transcriptionally active NFkB. The presence of transcriptionally active NFkB dimers was significantly elevated in Estrogen Receptor (ER) negative breast tumours: 16/49 ER- tumours with transcriptionally active NFkB compared to 3/41 ER+ tumours with transcriptionally active NFkB ($p = 0.004$). In this context, ER alpha gene expression data anti-correlated significantly with gene expression data for 7/8 NFkB target genes, and gene expression data for 7/8 NFkB target genes were significantly elevated in ER- breast tumours.

Conclusion: In conclusion, we demonstrated that the NFkB pathway is activated more often in IBC compared to nIBC. Our data suggest a potential cross-talk between the NFkB and ER signalling pathways in breast cancer, potentially contributing to the IBC phenotype.

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The role of aromatase and 17-beta-hydroxysteroid dehydrogenase type 1 mRNA expression in predicting the clinical outcome in human breast cancer

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Introduction: There is a substantial evidence that breast cancer tissue contains all the enzymes responsible for the local biosynthesis of estrogens from circulating precursors. The aromatase enzyme complex is responsible for the conversion of C19 androgens to oestrogens. Also, 17-beta-hydroxysteroid dehydrogenase (17-beta-HSD) type 1 catalyzes the interconversion of estrone to the biologically more potent estradiol. It is well established that increased exposure to local estrogens in postmenopausal women is an important risk factor in the genesis and growth of breast cancer.

The aim of this study was to look at the correlation between aromatase and 17-beta HSD type 1 mRNA expression and clinico-pathological parameters in human breast cancer.

Methods: 127 tumour tissues and 33 normal tissues were analyzed. The levels of transcription of Aromatase and 17-beta HSD type 1 were determined using real-time quantitative PCR. The mRNA expression was normalized against CK19. Levels of expression were analyzed against tumour's stage, grade, nodal status, local relapse, distant metastasis and survival over 10 years follow up period. In addition, the levels were analyzed against estrogen hormone receptors status.

Results: Overall, high expression of aromatase and 17-beta HSD type 1 were correlated with poor survival ($p = 0.0105$ and 0.0182) respectively.

Increased levels of aromatase mRNA expression were positively correlated with progression of the disease as levels were significantly higher in samples of patients who had distant metastasis and local recurrence and/or died of breast related causes when comparing to those who were disease free for > 10 years ($p = 0.0015$). Furthermore, levels of aromatase mRNA expression in patients who died from breast cancer were significantly higher than normal breast tissue ($p = 0.0016$)

We also observed higher levels of aromatase mRNA in tumour samples compared to normal breast tissue. However, the difference did not reach a statistical significance.

There was no correlation between expression level of aromatase and tumour stage, lymph node status and tumour grade. Nonetheless, higher levels were observed in grade 1 tumours compared to normal tissue ($p = 0.01$)

No significant difference in expression of 17-beta HSD type 1 between normal and cancerous tissues was seen. We also noticed an increase in levels correlating with tumour grade. This correlation was statistically significant when we compared grade 1 with grade 2 and grade 1 with grade3 ($p = 0.0031$ and 0.0251 respectively)

Finally, a trend toward increased expression of aromatase is associated with ER + ($p = 0.06$) this trend was not observed in 17 beta HSD Type 1.

Conclusion: Our results suggest that higher levels of enzymes responsible for the local biosynthesis of estrogens in breast cancer patients especially aromatase carries poor outcome. This finding supports the idea that reduction of local estrogen production may improve the outcome of breast cancer in postmenopausal women and aromatase inhibitors would be an ideal modality of treatment.

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Over-expression of activated c-Src in ductal carcinoma in situ predicts disease recurrence at 5 years and correlates with her2 positivity, high tumour grade, comedo histologic type and high proliferation

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Introduction: The oncoprotein Src kinase is downstream of receptor tyrosine kinases HER1 and HER2, and is upregulated in early stages of breast cancer. In vitro and in vivo studies have suggested that increased c-Src activity may promote breast tumour growth and metastasis. Few studies have investigated the expression of activated c-Src in breast cancer subtypes.

Aim: To evaluate the expression of activated c-Src in pure DCIS and invasive breast carcinoma and determine if the level of activated c-Src correlates with clinicopathological factors and predicts tumour recurrence.

Methods: Immunohistochemical expression of activated c-Src was evaluated in 129 women (median age 55 years) with 'pure' DCIS and 43 women with invasive breast carcinoma (median age 53 years) who underwent surgery at one unit. The median follow-up in the DCIS group was 60 months (range 10–155 months) and 65 months in the invasive breast carcinoma group. The level of activated c-Src was scored as 1(low), 2(medium) and 3(high). Estrogen receptor status (ER), HER1, HER2, and Ki67 levels were also measured by immunohistochemistry. In univariate analysis, the log rank test was used evaluate activated c-Src and recurrence-free survival.

Results: See the table.

DCIS characteristics	Level of activated c-Src			p value
	1 (low)	2 (medium)	3 (high)	
Tumour grade (n = 129)				**<0.0005**
Low	6	3	0	
Intermediate	14	20	6	
High	9	26	45	
Histological type n = 123				**<0.0005**
Comedo	2	13	13	
Mixed	7	20	28	
Non-comedo	17	17	6	
HER2 status (n = 114)				**0.002**
Negative (≤1)	13	17	6	
Positive (≥2)	13	26	39	
Ki67 Level (n = 126)				
Mean rank	51.5	59.0	74.9	0.013

In DCIS, but not invasive breast carcinoma, high levels of activated c-Src correlated with HER2 positivity, higher tumour grade, and comedo type DCIS with a high epithelial proliferation (measured by Ki67), but not tumour size, ER status and HER1 expression. Over-expression of activated c-Src was associated with an overall lower recurrence-free survival at 5 years ($p = 0.026$ Log rank). There was a trend towards higher proliferation and activated c-Src in the invasive breast tumours.

Conclusion: Activated Src kinase in DCIS predicts outcome at 5 years and is associated with HER2 positivity, high nuclear grade, comedo-type histology and a high proliferation. Activated c-Src may be important in the early stages of breast tumour development, but in later stages of breast disease, additional molecular events independent of c-Src activation might contribute to further disease progression. Targeting c-Src with intracellular small molecule inhibitors may be therapeutically useful.