302 Poster

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.

303 Poster

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.

304 Poster

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.

305 Poster

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