Cancer genetics

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Identification of a three differentialy expressed genes in breast cancer

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Purpose: By using the Differential Display (DD) we describe here the identification of a novel gene that is differentially expressed in human breast cancer cell lines. Further we describe the identification of two differentially expressed, but known genes.

Methods/Results: The method of DD is based on differentially expressed messenger RNA's between close related cell populations. Eight human tumor cell lines (breast, gastric, colon, lunge, uterus, melanoma, neuroepithelioma, oral cavity carcinomas) where used to select differentially expressed genes by DD. Using one 18 bp arbitrary primer in combination with a 21 bp anchored primer we identified a cDNA clone that is over expressed in MCF 7 breast cancer cell lines. Cloning and sequencing analysis identified a novel cDNA sequence of 380 base pairs. Northern analysis were used to verify the DD results and indicated an approximately 1.5 Kb mRNA overexpression in MCF 7 cells. The reamplification of two additional differentially expressed cDNA's identified known genes, one encoding for the estrogen receptor protein the other one for the calcium binding protein family member.

Conclusion: The DD is a useful technique for the identification of novel genes or differentially expressed genes in different cell populations. By using the DD method we describe here the identification of two known genes and a novel breast cancer associated gene that showed higher intensity of amplification in MCF 7 breast cancer cells compared to all other tumor cell lines listed.

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Expression of the human *p73* gene, a *p53*-homologue, is downregulated in invasive breast carcinomas

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Breast carcinomas (BC) show a frequent loss of heterozygosity (LOH) in the 1p36-33 chromosomal region where the *p73* gene resides. This gene showing high identity with the p53 DNA binding domain (DBD) was tested for its putative tumor suppressor role in breast epithelium (BE) carcinogenesis. The studied tissues were normal BE (n = 22), benign breast lesions (BBL) (n = 18), *in situ* carcinoma (ISC) (n = 10) and invasive BC (n = 67).

Results: Immunohistochemical studies revealed a conspicuous p73 nuclear staining of normal epithelial and myoepithelial mammary cells forming acini and ducts as opposed to total lack of p53 staining in the same tissues. Compared to adjacent normal BE, invasive BC exhibited a decrease of p73 nuclear immunostaining whereas ISC showed a significant increase, pointing out an association between loss of p73 function and invasiveness of transformed epithelial cells. *P73* gene LOH was found in 7/24 informative BC (29%) *versus* 0/7 in BE adjacent to BC and 0/10 in BBL. *p73* DBD sequencing of the 5 BC with *p73* LOH and 2 BC lacking LOH, all revealed lack of *p73* gene mutations but rare *p53* mutations (2/7, 29%). mRNA levels as measured by semi quantitative RT-PCR were found to be lower in BC than in normal BE.

Conclusion: Although not showing canonical properties of the *p53* tumor suppressor gene, the p73 gene elicits properties of tumor suppression in breast epithelium.

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Survival and ERB-B2 expression of cytokeratin-18 (CK18) bone marrow micrometastases (BMM) in stage I–III breast cancer

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Purpose: The detection of BMM in breast cancer patients is associated with poor prognosis. Since such cells have been shown to frequently overexpress the $p185^{erb\text{-}B2}$ oncoprotein, which has been reported as independent predictor of poor prognosis the analysis of primary breast tumors, we investigated whether the detection of $p185^{erb\text{-}B2}$ expression on BMM can be used to identify breast cancer patients with stage I–III disease who are at high risk for decreased survival.

Methods: We analysed the rate of expression of $p185^{erb\text{-}B2}$ oncoprotein on CK18-positive tumor cells which were detected in BM aspirates from 52 primary breast cancer patients with stage I–III disease, using an immunocytochemical double-labelling technique. In all patients, follow-up information on a median of 64 months (range, 8–143) of survival was available. Survival rates (for which only cancer-related deaths were considered) were assessed in patients with and without $p185^{erb\text{-}B2}$-positive BMM.

Results: A mean number of 40 CK 18-positive tumor cells (range, 1–612) per 4×10^5 BM cells and patient was detected in double-labelling for $p185^{erb\text{-}B2}$ and CK18. These cells co-expressed $p185^{erb\text{-}B2}$ in 31 (60%) of 52 patients. The survival rate was 35% in this group, whereas in the 21 patients with $p185^{erb\text{-}B2}$-negative micrometastasaes, the survival rate was 67% (P = 0.032 by the log-rank test). Considering important variables for multivariate statistics, expression of $p185^{erb\text{-}B2}$ on BMM was the only independent predictor of decreased survival in this analysis. The relative risk for early cancer-related death was 2.8 (95% CI, 1.1–7.0; P = 0.029).

Conclusions: The expression of $p185^{erb\text{-}B2}$ on BMM identifies stage I–III breast cancer patients with a poor overall survival.

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Breast cancer and vitamin D receptor polymorphism

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Purpose: Recently, an association between prostate cancer and vitamin D receptor (VDR) polymorphism could be demonstrated. As breast cancer (BRCA) growth is influenced by vitamin D *in vitro* and *in vivo*, we investigated the distribution of the TaqI (T/t), BsmI (B/b) and ApaI (A/a) polymorphisms.

Methods: DNA was extracted from white blood cells of 249 BRCA patients and 184 healthy controls. VDR genotypes were determined by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion of the PCR product.

Results: The VDR genotype distribution as depicted in the following table was statistically not different between BRCA patients and normal controls:

	BB	Bb	bb	TT	Tt	tt	AA	Aa	aa
BRCA (n = 249)	18%	46%	36%	38%	47%	15%	27%	50%	23%
Control (n = 184)	18%	47%	35%	37%	55%	8%	28%	19%	

However, carriers with tt were at significant higher risk than subjects with TT or Tt (odds ratio 2.01; 95% confidence limits 1.04–4.09, $p < 0.05$).

Conclusions: The preliminary results indicate an association between breast cancer and VDR polymorphism. Subanalyses for haplotypes will be done to confirm these data.