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Expression of the Ets-2 transcription factor in human breast cancer

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Ets proteins are downstream effectors of Ras-MAPK signaling pathways. Evidence from model systems suggests that Ets-2 may play a role in cellular transformation. This study investigated the *ex vivo* expression pattern of Ets-2 in a large cohort of human breast tissue in an attempt to establish a link between Ets-2 and the formation and progression of human breast cancer.

Reverse transcription PCR, Western blot analysis and immunohistochemical detection were used to compare expression of the Ets-2 transcription factor in human primary breast carcinomas, fibroadenomas and normal breast tissue. Levels were related to ER, PR, HER-2/*neu*, uPA, MMP-2 and MMP-9.

Ets-2 mRNA was detected in 69% of primary carcinomas (n=181), 63% of fibroadenomas (n=43) and 47% of normal breast tissue (n=43). Levels of Ets-2 mRNA were found to correlate with MMP-2 mRNA levels in the carcinomas ($p<0.05$; *Spearman Rank Correlation*). Two forms of the Ets-2 protein were detected, p52 (full length) and p54 (phosphorylated) using Western blotting. Both proteins were increased in primary carcinomas compared to fibroadenoma or normal breast tissue ($p<0.0001$, *Mann Whitney U-test*). These proteins were found to correlate with uPA protein levels in the carcinomas (52 kDa: $p<0.0001$, 54 kDa: $p<0.0005$; *Spearman Rank Correlation*). The Ets-2 protein was localised to the tumour cells in the primary carcinomas and to the epithelial cells surrounding the ducts and lobules in the fibroadenomas and normal breast tissue.

In conclusion, this is the first large scale study implicating a causal role for the Ets-2 transcription factor in human breast cancer.

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Expression of maspin in gastric cancer

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Background: Significant improvements in the analysis of genetic alterations for gastric cancer have been achieved by the recent progress in molecular biology. However, the informations are still insufficient for understanding common pathways for the development and progression of gastric cancer. For the purpose to seek for a candidate gene which regulates tumor progression and metastasis in gastric cancer, we investigated the gene expression profiles using oligonucleotide microarray. We further examined the protein expression of the maspin, which was selected for the lymph node metastasis related gene by the microarray, using immunohistochemistry.

Materials and Methods: Tumor tissue and adjacent normal tissue were obtained from a total of 21 patients with gastric cancer and total RNA was extracted. Total RNA was reverse transcribed to cDNA by using the T7-primer and then examined for gene expression profiles using Affymetrix chip U95Av2 chip set, which includes 12,000 human genes and EST sequences. Formalin fixed and paraffin embedded tumor tissue and lymph nodes were obtained from 34 patients with gastric cancer. The protein expression of maspin was investigated using immunohistochemistry.

Results: In microarray analysis, 25 genes were over-expressed and 2 genes were depressed at least 4-fold in tumor tissue. In further analysis according to lymph node metastasis, expression of maspin, as well as carcinoembryonic antigen and nonspecific cross reacting antigen, was significantly higher in tumors with lymph node metastasis than in those without it. The protein expression of maspin was not observed in normal mucosa. On the conctrary, maspin expression was observed in 29 of 34 tumor tissues. There was a significant correlation between the incidence of maspin positive tumor staining and the maspin mRNA expression levels.
Conclusions: Maspin was selected as lymph node metastasis relating gene in gastric cancer by oligonucleoide microarray and the over- expression of maspin protein was also confirmd by immunohistochemistry. These results suggest that maspin has a potential role for tumor metastasis in gastric cancer.

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A naturally occurring type III variant mutant EGF receptor confers a strong cytoprotective response to ionizing radiation

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Background: Many carcinomas and gliomas frequently express the variant form of EGFR, the type III variant (EGFRvIII), which is known to function as an oncoprotein. We explore the functional consequences of EGFRvIII on cellular responses following radiation and investigate the feasibility of adenovirus- mediated delivery of dominant- negative EGFR-CD533 to inhibit EGFRvIII.

Material and Methods: Chinese hamster ovary cells were used for mechanistical studies on EGFRvIII- mediated cellular radiation responses. To explore the importance of EGFRvIII as a modulator of radiation responses in malignant gliomas, EGFRvIII and EGFR-CD533 were expressed in vitro and in vivo in the cell line U-373 MG through transduction with adenoviral vectors. Radiation responses were studied at the molecular and cellular level including inhibition of the EGFRvIII function through AG-1478, effects on signal transduction cascades, clonogenic survival and apoptosis assay. In vivo studies were carried out with cells pre-transduced with adenoviral vectors before inoculation and with established xenograft tumors expressing intrinsic or adenoviral EGFRvIII and EGFR-CD533 after intratumoral infusions of the adenoviral vectors.Growth delay assays measured radiosensitivity.

Results: 2 Gy resulted in a 4.3-fold increase in EGFRvIII tyrosine phosphorylation in CHO cells.Importantly, this activation led to an immediate 8.5 fold and 3.2 fold activation of MAPK and Akt, respectively. Colony formation and apoptosis assays verified the enhanced relative radioresistance of cells in the presence of EGFRvIII. In vivo studies confirmed the radiation-induced activation of intrinsic EGFRvIII in xenograft tumors, which was completely inhibited through EGFR-CD533. Furthermore, EGFR-CD533 abolished the enhanced clonogenic survival as well as the increased tumorigenicity mediated by EGFRvIII. Growth delay assays demonstrated that EGFR-CD533 expression significantly enhanced radiosensitivity of tumors under conditions of intrinsic as well as adenoviral- mediated EGFRvIII expression.

Conclusions: This work will demonstrate that EGFRvIII is an important modulator of radiation responses conferring a stronger cytoprotective response than EGFR via the MAPK and PI3K pathway. EGFR-CD533 effectively inhibits EGFRvIII function. The dominance of EGFRvIII in cytoprotective responses to radiation needs to be addressed in any therapeutic approach disabling EGFR function for tumor cell radiosensitization.

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In vitro and in vivo efficacy of a HIF-1alpha-antisense oligonucleotide containing locked nucleic acids

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The purpose of this study was to obtain a specific down-regulation of hypoxia-inducible factor 1α (HIF-1α) using antisense oligonucleotides containing locked nucleic acids (LNA).

HIF-1αplays a central role in the mammalian cellular response to hypoxia and the protein is frequently found over-expressed in human cancers. This over-expression is associated with a poor prognosis, resistance to therapy and the formation of metastasis, which makes HIF-1α a potentially interesting molecular target for anticancer therapy.

LNA is a novel class of nucleic acid analogs containing a 2'-O, 4'-C-methylene bridge resulting in an unprecedented high binding affinity toward complementary DNA and RNA. The LNAs obey the Watson-Crick base pairing rules, shows a higher stability toward nucleases and are readily taken up by mammalian cells.

We designed and screened a series of 16-mer antisense oligonucleotides against HIF-1α containing LNAs and phosphorothioate DNAs. Cell cultures were treated for 6 hours with different concentrations of oligonucleotide and 0.5% Lipofectamine 2000 in the medium with or without 10% FCS. After treatments the cells were washed in PBS and medium containing 10% FCS was added. The cell cultures were then exposed to severe hypoxia in incubation bags, using the Anaerocult system, (Merck). Cells were harvested