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PROGNOSTIC VALENCE OF PROTEIN PRODUCTS OF THE ERB B-GENE FAMILY IN PRIMARY BREAST CARCINOMAS

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The expression of membrane bound receptors for growth factors $p185^{neu}$ (product of erbB2-gene) and EGF-R (epidermal growth factor receptor; erb B1) in tumors is said to indicate poor prognosis. In order to test this hypothesis we examined 280 primary breast cancers for the expression of $p185^{neu}$ and EGF-R using an immunohistochemical methode on paraffin embedded surgical specimens. $P185^{neu}$ and EGF-R were detected in 20% and 22% of tumors, respectively. $P185^{neu}$ detection correlated with tumor size, node involvement and negatively to the estrogen receptor. No correlation was found for EGF-R. Using Kaplan-Meier-survival-analyses (median observation time 62 months) the established prognostic factors (tumor size, node involvement, tumor grade and steroidhormone-receptors) and the expression of $p185^{neu}$ correlated with survival ($p<0.001$) (not EGF-R!). Multivariate analyses indicate that node status is most important for clinical outcome.

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THE THYROID SPECIFIC TRK ONCOGENE FAMILY

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TRK oncogenes are created by chromosomal rearrangements joining the TK domain of the NTRK1 gene (encoding one of the nerve growth factor receptors) to foreign sequences. In human papillary thyroid tumors we have reported TRK oncogene activation in 8 out 52 cases. In three cases the tropomyosin gene has been found responsible for the activation as for the first TRK oncogene described by Barbacid's group. The thyroid TRK oncogenes involving sequences different from tropomyosin have been named TRK-T. Molecular and biochemical studies on TRK-T oncogenes yielded the results below summarized.

A) At least two different genes, TPR and TAG, are involved in TRK-T activation. TPR contributes to the formation of two oncogenes with different structure, TRK-T1 and TRK-T2. TAG, a novel gene that we have mapped on chromosome 3, has been found involved in the generation of TRK-T3.

B) All the TRK oncogenes so far characterized are created by rearrangements involving a 2.9 Kb restriction fragment of the NTRK1 genomic locus.

C) All the TRK oncoproteins are constitutively phosphorylated on tyrosine and their transforming activity correlates with this status. Analysis of the signal transduction pathway triggered by the TRK-T oncoproteins indicated that they are able to interact with the adaptor proteins SHC and GRB2.

D) Upon transfection into PC12, TRK-T1 and TRK oncogenes are able to induce neurites outgrowth thus indicating their ability to mimic the physiological role of the NGF-activated NTRK1 normal receptor.

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A NOVEL APPROACH TO THE STUDY OF CONTROL MECHANISM OF ANGIOGENESIS

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Many lines of evidence show that cancer and many chronic inflammatory diseases are angiogenesis-dependent, suggesting antiangiogenesis as a therapeutic approach against these diseases. We have developed and validated a rat sponge model for the quantitation of neovascularisation using ^{133}Xe clearance wash-out techniqc, in comparison with ^{113}Sn microsphere wash-in technique; the carmine dye method; measurement of haemoglobin and protein levels in the implants and histological and morphometric studies. Using this model, we have been studying the role of cytokines/growth factors, e.g. interleukin 1, IL-6, IL-8, tumour necrosis factor, basic fibroblast growth factor, platelet-derived endothelial cell growth factor and vascular endothelial growth factor on angiogenesis. It has been shown that the angiogenic responses elicited by these cytokines/growth factors can be neutralised by their antibodies or blocked by their receptor antagonists or by selective signalling pathway inhibitors. We have also shown that inflammatory polypeptides substance P and bradykinin and the vasoconstrictor angiotensin II are angiogenic. The neovascular responses produced by these polypeptides were also inhibited by antagonists for their receptor subtypes. The demonstration of specific antagonism suggests that receptor modulation or regulation of the signal transduction pathway of angiogenesis could provide an effective strategy for the management of angiogenic diseases such as cancer. Furthermore, this model has been adapted for mice. The miniaturisation of the sponge model in mice would allow tumour-induced angiogenesis to be studied more widely.

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P53 MUTATIONS IN TWO SETS OF BLADDER CANCER PATIENTS

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Mutations in the p53 tumor suppressor gene are among the early genetic alterations in most of the primary invasive bladder cancer. Denaturing gradient gel electrophoresis (DGGE) assay was used to detect single-base substitutions in exon 6 and in the evolutionary highly conserved regions in exons 5, 7 and 8 of the p53 gene in two groups of urinary bladder cancer patients. In the first group of 28 patients with occupational exposure to asbestos (8 non-smokers and 20 smokers), 24 tumors were classified as grade I-III transitional cell carcinomas and 4 tumors were of other histological types. The second group (n=31) consisted of random bladder cancer cases with transitional cell carcinoma of grade III; the occupational history of the patients was not known. The DGGE analysis of exon 5 through 8 of the paraffin-embedded tissue samples showed mutations in 43% of the tumors from the asbestos exposed group: 65% in exon 6, 7% in exon 7 and 6% in exon 8. Two samples contained two separate mutations. The second group exhibited mutations in 29% of the tumors: 14% in exon 6, 3% in exon 7 and 23% in exon 8; one sample had two mutations. Interestingly, the percentage of the mutations in exon 6 in the tumors of patients exposed to asbestos was high as compared with mutation frequencies reported for other types of human malignancies. The mutation analysis has not yet been completed: we currently perform DGGE analysis of exon 5; and sequencing of the detected mutations is under way. The sequencing results obtained so far have shown codons 280 and 290 as examples of sites of mutations. AGA (Arg) → ACA (Thr) mutation at codon 280 in exon 8 has recently been suggested as a mutational hotspot unique to bladder carcinoma.

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Epidermal Grouth Factor Receptor in Bile Duct Cancer and Hepatolithiasis

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Hepatolithiasis is reported in 5 - 13.3% to be concomittant with cholangiocarcinoma in Taiwan. The epithelium of stone-containing intrahepatic ducts have the potential of producing glandular proliferation . We try to conduct this study to investigate the potential change of malignancy of stone-containing intrahepatic ducts. Immunohisto-chemical PAP method was used for demostrating the epidermal growth factor recept or (EGF-R) in the ductal glands. Usually,the ductal glands could be divided into intra-mural and extra-mural glands. Thirty specimens were used for study, including 11 ducts from the resected hepatocellular carcinoma, as a control; 12 from stone-containing intrahepatic ducts and 7 from bile duct adenocarcinoma. The positive rate of EGF-R in the mucus and serous glands were 25% and 50% in the IHD-S group. 100% in the cancer cell of bile duct adenocarcinoma. In addition, the presence of EGF-R in the ductal gland was also increased by the conspicious inflammatory cells esp. in the intra-mural glands, and stratification of glandular epithelium. The presence of EGF-R was also higher than that of proliferation glands.

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C-KIT PROTO-ONCOGENE AND STEM CELL FACTOR IN GERM CELL TUMORS: PREFERENTIAL COEXPRESSION IN SEMINOMAS.

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The proto-oncogene *c-kit* encodes a transmembrane tyrosine kinase receptor and the stem cell factor (SCF) is its corresponding ligand. The signalling pathway *c-kit*/SCF is important in hematopoiesis, melanogenesis, and spermatogenesis. To investigate the role of *c-kit*/SCF in oncogenesis of germ cell tumors, we have studied immunohistochemically *c-kit* expression in 60 cases of testicular tumors and in normal testes. Of seminoma specimens, 100% (28/28) showed diffusely positive membrane staining; the same pattern of expression was seen in seminoma *in situ*. Non-seminomas were *c-kit* negative in 65% (19/29) or showed cytoplasmatic staining in few tumor cells in 35% (10/29) of the cases, respectively. In 3 mixed germ cell tumors *c-kit* expression was confined to the seminoma component. In normal testes, *c-kit* protein was detected in scattered cells. By Northern blot, *c-kit* gene expression was confirmed to be abundant only in seminomas. In contrast, similar expression of SCF was observed in seminomas, non-seminomas, and normal testis. Our results suggest a role of the *c-kit*/SCF system in oncogenesis of germ cell tumors. In addition, our data suggest that *c-kit*/SCF may participate in autocrine and/or paracrine stimulation of seminoma growth. These results may have biological, diagnostic, and clinical implications.