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HORMONAL CONTROL OF BREAST CANCER CELL PROLIFERATION.
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We have focussed on increasing our understanding as to the mechanism of proliferation of normal and neoplastic breast cells. In our recent research we have studied the role of fibroblast growth factors and their receptors in this process. Our research has indicated that both acidic and basic FGF are present in breast tissues with acidic being predominant in breast cancer and we have also demonstrated the presence of receptors for acidic and basic FGF in breast cancer biopsies. To do this we have utilised the technique of polymerase chain reaction and have demonstrated that the majority of biopsies from normal and malignant breast have FGFR1 and FGFR2.

In order to determine whether there are differences between the form of FGFR1 produced by normal and malignant breast cells we have examined the structure of the mRNA using polymerase chain reaction and utilising primers across several regions of the molecule. Essentially these studies have disclosed that breast cancers characterised by greater proportion of the two loop immunoglobulin chain form of FGFR1 and we are currently studying the form of the FGFR2 in these cells.

Regarding therapy we have now shown that basic FGF saporin conjugate can kill cancer cells containing receptors for basic FGF and the therapeutic implication of these conjugates will be discussed.

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SOMATOSTATIN ANALOGUES AND RECEPTORS IN THE TREATMENT OF BREAST AND OTHER CANCERS.
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Somatostatin analogues (SS-A) can be used for *in vivo* imaging by receptor scintigraphy and in the treatment of various types of cancer. Receptors for somatostatin have been found in a great series of various tumor types. In breast cancer SS receptors have been demonstrated in 36-67% of primary tumors and even in 77% when measured by *in vivo* imaging. Patients with SS-R$^+$ tumors have a relatively good prognosis. SS-As may act by indirect endocrine effects (decrease of GH, gastrointestinal hormones, and IGF-I) and by direct growth inhibitory effects via tumor SS-R. In a preliminary clinical randomized study in postmenopausal patients with metastatic breast cancer, using Sandostatin in combination with a new antiprolactin and tamoxifen, we observed interesting endocrine and antitumor effects (11% non-responders). The best therapeutic results of treatment with SS-A have been reported in patients with pituitary adenomas, endocrine tumors and carcinoids. The antitumor efficacy seems related with SS-R density. Poor to moderate effects have been reached in patients with medullary thyroid tumors, pancreatic- and colonca, prostate ca or NSCLC. Side effects are minor. SS-As have also been tested in various experimental cancers such as sarcomas. In conclusion, SS-As form a new endocrine treatment modality with different antitumor efficacy in various types of tumors in the absence of serious side effects.

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Basic fibroblast growth factor and its receptors (bFGF-R) in human breast cancer

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We found bFGF stimulation of proliferation in MCF-7, T-47D, BT-20 cell lines, but not in MDA-MB-231 (Peyrat et al, Cancer Comm. 1992, 3, 329). The first step of bFGF action is its binding to membrane receptors ; we investigated the binding characteristics of bFGF on membranes prepared from these 4 cell lines: with competition experiments, the presence of high-affinity binding sites was demonstrated in each cell type (MCF-7: number of sites (N) = 0.84 pmol/mg membrane proteins, Kd = 0.60 nM ; T-47D: N = 0.90, Kd = 0.55 nM ; BT-20: N = 1.05, Kd = .77 nM ; MDA-MB-231: N = 0.37, Kd = 0.34nM). A second class of lower affinity binding sites was detected only in the two hormone-independent cell lines (BT-20: N = 1.7, Kd = 2.9 nM ; MDA-MB-231: N = 9.1, Kd = 2.7 nM). In a first retrospective study of a series of 38 primary breast cancer biopsies, competitive binding experiments allowed the detection of binding sites in 36/38 breast cancers (Peyrat et al, J Ster Biochem Mol Biol 1992, 43, 87) ; high-affinity binding sites (Kd < 1nM) were present in 19/36 cases and low-affinity binding sites (Kd > 2 nM) were present in 29/36 cases (the two classes of binding sites were present in 12 breast cancers). No relation between bFGF binding sites and node involvement, histologic type or grading of the tumor was evidenced. There were negative correlations (Spearman test) between total (or low affinity) bFGF binding sites and estradiol receptor (ER) ($p = 0.05$) or progesterone receptor (PgR) ($p=0.009$). We recently assayed bFGF-R in a non selected series of 140 primary breast cancers, using saturation of MgCl2 treated microsomal proteins with [^{125}I]-bFGF (10-500 pmol/l) in presence or in absence of an excess of unlabeled bFGF (a gift from Farmitalia). The Scatchard analyses of results revealed a single class of high affinity binding sites (median Kd: 180 pmole/l; range: 14-1370). The median concentration of bFGF-R was 1076 fmol/mg prot. (range: 1-5442). The bFGF-R were positively correlated to ER ($p = 0.0017$) and PgR ($p= 0.004$).

The demonstration of 1) bFGF specific binding sites in breast cancer membranes 2) bFGF growth stimulation of some breast cancer cell lines indicates that this factor could be involved directly in the growth of some breast cancers. The correlations between binding site concentrations and ER and PgR concentrations suggest that bFGF binding sites could be used as differentiation and prognostic factors.

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ANTIPROGESTINS IN THE TREATMENT OF BREAST CANCER

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It is the physiological function of the progesterone receptor in epithelial cells to mediate differentiation and in this way exert growth control. In this presentation we will review data indicating that the progesterone antagonist Onapristone use this function of the progesterone receptor to induce differentiation in progesterone receptor positive (mammary carcinoma) epithelial cells and block the growth of MXT, DMBA, NMU and T 61 tumors. In second line treatment regimens in the MXT, DMBA, and NMU model after a two weeks treatment with tamoxifen the effectiveness of Onapristone on tumor growth was compared with those of a furter treatment with ramoxifen and a high dose treatment with medroxyprogesterone acetate. In this treatment onapristone proved to induce a superior growth inhibition compared to the treatment with tamoxifen and to be as effective as high dose treatment with medroxyprogesterone acetate, which in contrast to Onapristone induced significant side effects. Our panel of indicators of differentiation indcludes a decrease in the volume fraction of undifferentiated epthelial cells, in tumor grading, in the amount of lectin binding sites containing α-ducose and in immunolocalized tenascin expression, as well as an increase in the volume fraction of dysplastic ducts, casein filled vacuoles, of cells in G_1 G_1 of the cell cycle and the proportion of apoptotic nuclei. Interestingly, the growth inhibition ad differentiation of the experimentally induced mammary carcinomas is accompagnied by a strong stimulation of immunolocalized TGF β_1 secreted by the eptithelial cells whereas no effect was detected in the surrounding stromal tissue. Since TGF β is known to be differentiating promoting for most epithelial cells, it was challenging to study if Onapristone might possess inhibitory potential for the carcinogenesis of experimental breast cancer. Finally, we will demonstrate that Onapristone is able to block the growth of the R 3327 H Dunning prostate carcinoma and to induce differentiation if the concentration of progesterone receptors is sufficient.

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AROMATASE INHIBITORS IN THE TREATMENT OF BREAST CANCER.
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Numerous novel modalities of endocrine therapies have rendered obsolete major endocrine ablative surgical procedures. Testololactone and later aminoglutethimide (AGL) have proven effective in postmenopausal patients with advanced breast cancer by reversibly blocking peripheral aromatization of adrenal androgens. Response is clearly related to oestrogen receptor status and randomized studies indicate equal efficacy as with other endocrine treatments like tamoxifen. The latter is used as first-line palliative therapy and for adjuvant purposes due to better tolerance. AGL is a non-steroidal compound which acts by non-selective cytochrome P-450 inhibition thereby interfering with other hormonal pathways (gluco- and mineralocorticoid synthesis and thyroid hormone production). It is unable to efficiently block ovarian aromatase explaining why it can only be used in postmenopausal or castrated women. New analogs in development seem to have greater specificity for peripheral aromatase: CGS 169498 and R83842 have now reached phase II/III levels of clinical development Another class of aromatase inhibitors structurally dependent of the aromatase substrate androstenedione are also developed. Some bind covalently to aromatase leading to its permanent inactivation (enzyme killers). Eventual advantages of these drugs (4 hydroxy-androstenedione and FCE 24304) over AGL await proper randomized comparison. Among future developments combination of potent aromatase inhibitors with other endocrine measures like LHRH analogs, new pure anti-estrogens and anti-progestins will provide means for achieving near total hormonal suppression. Combination with chemotherapy might allow to develop additive and hopefully synergistic associations.

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SOMATOSTATIN RECEPTORS AND CANCER

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The multiple actions of the neuropeptide somatostatin (SS) in the human body are mediated by specific, high affinity SS receptors (SS-R) present in the brain, pituitary, pancreas, gastrointestinal tract, kidney and lymphoid tissue. Not only healthy tissues but also several human cancers are expressing SS-R. SS-R are found in most neuroendocrine tumors, i.e. GH and TSH producing pituitary tumors, endocrine gastroenteropancreatic (GEP) tumors, paragangliomas, pheochromocytomas, MTC and SCLC. SS-R are also expressed in a majority of malignant lymphomas, in several brain tumors (all meningiomas, most astrocytomas), in renal cell carcinomas and in breast tumors. The majority of tumors expressing SS-R are rather differentiated (i.e. astrocytomas vs. glioblastomas), but exception exists (lymphomas). Certain categories of tumors (ovarial tumors, MTC, insulinomas) express SS-R subtypes. In pituitary and GEP tumors, these SS-R are funtional, mediating either hormone secretion inhibition or antiproliferation, and therefore playing a predictive role for assessing the therapeutical efficacy of SS analogs. The high density of SS-R in tumors made it possible to develop an in vivo technique of scanning SS-R positive tumors and their metastases in the patients after i.v. injection of radiolabelled SS analogs. This represents the first example of the clinical use of a small peptide as powerful in vivo diagnostic tool. SS-R may therefore, be considered as a paradigm for further research on the role and the potential diagnostic use of other peptide receptors in pathological states.