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CATHEPSIN D IN BREAST CANCER CYTOSOL - FOLLOW-UP AFTER 18 MONTHS

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It has recently been claimed that the concentrations of Cathepsin D (Cath D) in breast cancer cytosol could serve as a prognostic parameter in that disease.
We measured the Cath D concentrations in 71 breast cancer cytosols, which had been obtained during primary surgery between December 1987 and July 1988 and were kept frozen at -40 C until determination.
The assay is based on the binding of two monoclonal antibodies directed against Cath D epitopes. The measuring range of the assay ranged from 0.6 pmol to 120 pmol Cath D/mg cytosol protein (cytprot). Concentrations exceeding 120 pmol/mg cytprot were reassayed after appropriate dilution. The intra- and interassay coefficients of variation of duplicate determinations were always <10%. The assays were kindly provided from ID-CIS, Dreieich, F.R.G..
From these 71 patients 9 developed metastases until February 1990. The Cath D concentrations of these patients were bewteen 18.6 and 76.2 pmol/mg cytprot, with a median of 35 pmol/mg cytprot. The Cath D concentrations of the remaining 62 patients, who did not develop metastases so fer were scattered in wide range from 5.4 to 120.9 pmol/mg cytprot, with a median of 36 pmol/mg cytprot.
No differences could be observed in Cath D concentrations in respect to lymphnode involvement, histology and size of primary tumor. No direct correlation between estrogen or progesterone receptor concentration and Cath D concentration could be detected.
From these preliminary results no difference in Cath D cytosol concentrations could be observed between patients with early metasatases of their disease and patients without metastases at least 18 months after primary surgery.
These data do not support the assumption that the Cath D concentrations of the primary tumor could delineate a group of breast cancer patients with high risk of early recurrence.

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INTERACTIVE EFFECTS OF ESTRADIOL, TPA AND BRYOSTATIN ON GROWTH AND ON THE EXPRESSION OF ESTROGEN RESPONSIVE GENES AND TGF-$\beta$ IN HUMAN BREAST TUMOUR LINES.

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The phorbol ester, TPA (10nM) produced a marked reduction in the growth of MCF7 cells in full growth medium, but had only a small effect on MDA and T47D cells. The effect of TPA on MCF7 cells was partially reversed by bryostatin, suggesting bryostatin does not mimick TPA in this system even though both are believed to act via effects on protein kinase C. When the estrogen receptor +ve MCF7 and T47D cells were maintained in charcoal-stripped serum, the increase in DNA synthesis on stimulation with estradiol was inhibited with 50nM TPA in MCF7 cells but not in T47D cells. The effects of these treatments on the expression of the estrogen responsive genes pNR2 and pNR100(Cathepsin-D) were examined. Rather than preventing transcription of these estrogen responsive genes, TPA alone increased pNR2 and pNR100 mRNA levels in MCF7 cells and the combined effect of estradiol and TPA had a marked synergistic effect in increasing the mRNA levels of these genes. In T47D cells pNR2 transcripts were not detected and the increase in pNR100 expression was not affected by TPA. We conclude that the inhibitory effect of TPA on the growth stimulation of MCF7 cells by estradiol was not due to a general inhibition of the expression of estrogen responsive genes.
An alternative possibility examined was that the inhibitory effects of TPA on MCF7 cells might be due to the stimulation of TGF-$\beta$, acting as an autocrine inhibitory growth factor. Estradiol treatment of MCF7 cells reduced the levels of TGF-$\beta$ mRNA, whereas TPA produced a marked increase. The combined effect of TPA and estradiol furthur increased TGF-$\beta$ mRNA above the levels seen with TPA alone. Bryostatin had little effect on TGF-$\beta$ expression either alone or in combination with estradiol. These observations suggest that the inhibitory effect of TPA on MCF7 cells is due an autocrine inhibition by TGF-$\beta$.

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THE CHROMOSOME 11q13 REGION AND PROGRESSION OF HUMAN BREAST CANCER

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Activation of several oncogenes (neu, myc and int-2) has been described in human breast cancer and may be of interest as potential prognostic marker in breast cancer. We have studied the clinical implications of amplification of genes within the chromosome 11q13 region at different stages of breast cancer development. We found that the int-2 oncogene in this region is amplified in 25/189 (13%) of the cases studied. Two other oncogenes within this region, the bcl-1 and hst-1 oncogene, were always co-amplified with int-2 (25/25 and 20/20 cases, respectively). A fourth oncogene within this 11q13 locus, the sea-1 oncogene, was not amplified in tumors with an int-2 amplification (0/11).
Two other oncogenes, neu on chromosome 17 and myc on chromosome 8, were amplified in 17% and in 8% of cases studied, respectively. Amplification of genes within the 11q13 region did not coincide with amplification of neu and/or of myc: in only one case neu and in another case myc was co-amplified with the oncogenes within the 11q13 region.
Amplification of the 11q13 region was significantly correlated with estrogen receptor positivity ($p<0.04$) and with the presence of lymph node metastases ($p<0.01$). These data suggest that amplification of the chromosome 11q13 region is associated with a distinct tumor type and presumably is indicative, within the group of patients with estrogen receptor positive tumors, of poor prognosis. However, the mean follow-up time of our patients is still too short to associate amplification with tumor recurrence and with patient survival.
Because we did not observe any RNA expression of the int-2 and hst-1 gene in tumors with an 11q13 amplification, we assume that another gene is involved, located within or nearby the oncogenes int-2, hst-1 and bcl-1 of the chromosome 11q13 region. By means of in-gel-renaturation, phenol-emulsion-reassociation-technique, differential cDNA cloning and megabase mapping, we are presently endeavoring to identify this gene.

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REGULATION OF erbB2 AND pS2 EXPRESSION BY ESTROGEN AND ANTIESTROGENS IN HUMAN BREAST CANCER CELLS

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We have studied the regulation of erbB2 and pS2 expression by hormones and antihormones in a human breast cancer cell line ZR-75-1 in vitro and in nude mice. The expression of pS2 gene was studied as a marker for a fuctional estrogen receptor system.

The proliferation of the ZR-75-1 cells was estrogen dependent both in vitro and in vivo. Their growth was inhibited by the antiestrogens toremifene and tamoxifen in a concentration-dependent manner. Withdrawal of estrogen from the culture medium induced the expression of erbB2 mRNA, but upon addition of estrogen ($10^{-8}$M) the mRNA level rapidly declined. The concomitant addition of antiestrogens toremifene or tamoxifen ($6.25\times10^{-8}$M) blocked estrogen action and allowed the reinduction of erbB2 mRNA. In nude mice the results were similar: tumours of ZR-75-1 cells were formed only when estrogen pellet was inserted, but erbB2 expression was low. Concomitant oral administration of toremifene opposed the estrogen-induced downregulation of erbB2 expression. When the estrogen pellet was removed erbB2 expression was induced. Opposite responses were observed in the expression of pS2 mRNA.

The results show that in ZR-75-1 cells estrogen down-regulates the expression of erbB2 gene although it induces the expression of pS2 and activates the proliferation of the cells in vitro and in tumours in nude mice.