IV - b

AUTOCRINE STIMULATION OF BREAST CANCER CELL GROWTH BY ESTROGEN REGULATED PROTEIN(S)

H. Rochefort, F. Capony, G. Cavalié-Barthez, M. Chambon, M. Garcia, G. Freiss, V. Cavaillès, M. Morisset, I. Touitou and F. Vignon
Unité d'Endocrinologie Cellulaire et Moléculaire, (INSERM U 148) and University of Montpellier, 60 Rue de Navacelles, 34100 Montpellier France.

Cancer cells acquire the ability to make and to respond to their own growth factor (autocrine mechanism) while normal cells require more frequently signals from other cells (paracrine mechanism). The human mammary cancer cell lines (MCF7, T47D, etc...) are good system to study the mechanism by which hormone regulate their growth, since they contain estrogen and progesterone receptors and their proliferation is increased by estrogens and decreased by antiestrogens and progestins.

We have shown that glycoproteins present in conditioned media are able to stimulate the growth of resting MCF7 cells when prepared from estrogen treated MCF7 cells but not from control cells. In this conditioned medium, there are several proteins and peptides which are putative autocrine mitogens to mediate the effect of estrogen on cell growth. We have focused on a 52.000 daltons (52 K) glycoprotein (Cell, 20, 352, 1980) which is specifically increased by estrogen and decreased by progestins and antiestrogens. Several high affinity monoclonal antibodies to the partially purified secretory 52 K protein have been produced. This protein has been detected by immunoperoxydase staining in epithelial cells of some breast cancers but not in normal mammary gland and in normal or tumoral endometrium. In benign mastopathia, the 52 K protein was significantly more abundant in ductal hyperplasia and cysts, but absent in lobular and non proliferative diseases, suggesting its interest as a marker of high risk mastopathia.

By immunoaffinity, the 52 K protein has been purified to homogeneity in the medium and in the cell extract. The protein is N-glycosylated with high mannose oligosaccharide chains bearing mannose 6 phosphate signals. The 52 K protein is the precursor of cellular proteins (48 K and 34 K) which are routed intracellularly in lysosomes via mannose 6-P receptors. Estrogen increase the production of the precursor which is secreted into the medium. The protein can then be partially uptaken and processed via mannose 6-P receptors. The purified 52 K protein is also able to stimulate the growth of estrogen deprived MCF7 cells (F. Vignon et al., Endocrinology, April 1986) and displays a lysosomal enzymatic activity.

We conclude that estrogen may stimulate the growth of breast cancer cells indirectly via the secretion of growth factors and/or of certain enzymes such as the 52 K protein which are able to act as autocrine mitogen. Cloning of the protein is in progress to specify its structure and possible function in cancerogenesis.

IV - c

CLINICAL SIGNIFICANCE OF GROWTH FACTOR RECEPTORS IN BREAST AND BLADDER CANCER

Adrian L. Harris, R. Sainsbury, G. Needham, D. Neal, D. Veale, K. Smith, R. Hall, J. Farndon and L. Hirst
Cancer Research Unit and Department of Clinical Oncology, University of Newcastle upon Tyne, Newcastle upon Tyne, U.K.

High affinity epidermal growth factor receptors (EGFr), K_D 10^{-9} to 10^{-10} can be demonstrated in human primary breast tumours. They are inversely correlated with oestrogen receptors ($^{4}/_{53}$ ER+ve tumours had EGFr; $^{31}/_{51}$ ER-ve tumours had EGFr). EGFr was highly correlated with poorly differentiated tumours on the Bloom grading system ($^{13}/_{48}$ grade I, II had EGFr; $^{32}/_{60}$ grade III had EGFr). The EGFr were functional as shown by EGF stimulated tyrosine kinase activity. $^{2}/_{42}$ tumours had only the internal domain of the EGFr detectable. There was a reciprocal relationship of stromal fibroblast EGFr to tumour EGFr, suggesting paracrine interactions. The reciprocal relationship of ER and EGFr was demonstrated in 7 breast cancer cell lines. In the early follow-up of patients in the above studies, $^{12}/_{15}$ early relapses were in EGFr+ve tumours.

In bladder cancer, there is a similar relationship of EGFr to poor differentiation ($^{18}/_{21}$ poorly differentiated tumours were EGFr+ve, $^{10}/_{27}$ moderately differentiated tumours were EGFr+ve) and invasion ($^{7}/_{24}$ tumours were EGFr+ve; $^{21}/_{24}$ invasive tumours were EGFr+ve). In both bladder and breast cancers there is evidence for genomic DNA rearrangements in the region of the EGFr gene. The overexpression of EGFr in the worse prognosis, more poorly differentiated and invasive bladder and breast tumours provides a novel target for therapy. We have initiated studies in patients using EGFr antibodies to target therapy in bladder cancer.

V - a

POSSIBLE ROLE OF GASTROINTESTINAL HORMONES IN THE DEVELOPMENT OF GASTRIC AND PANCREATIC CANCER

C.B.H.W. Lamers
Dept. of Gastroenterology and Hepatology, University Hospital, Leiden, The Netherlands.

Gastrointestinal hormones exert trophic actions on various tissues of the gastrointestinal tract. Animal studies have suggested that gastrointestinal hormones may be involved in the development of cancer of the stomach and the pancreas. Although some studies suggest a direct carcinogenic effect of gastrointestinal hormones, it is more likely that these hormones act by sensitizing cells to carcinogens. Gastrin, a hormone produced by the gastric antrum, is known to exert trophic effects on parietal and chief cells of the stomach. This hormone may also be involved in the development of gastric cancer, since the carcinogenic effect of MNNG is promoted by hypergastrinaemia. Furthermore, hypergastrinaemia is also involved in the development of carcinoid tumours of the gastric body. Cholecystokinin, a hormone produced in the upper small intestine with trophic effects on the pancreas, is suggested to be involved in pancreatic carcinogenesis. Raw soya flour, a diet known to increase plasma cholecystokinin concentrations, induces hypertrophy, nodular and adenomatous hyperplasia, dysplasia and cancer of the pancreas in rats. Similar findings are found after chronic treatment of rats with cholecystokinin. Furthermore, both a raw soya flour diet and administration of cholecystokinin promote the carcinogenic effect of nitrosamine-derivates on the pancreas in experimental animals. It is concluded that gastro-intestinal hormones may play a role in the development and growth of tumours of the stomach and the pancreas. Further studies are needed to determine whether inhibition of this tumour promoting effect is of therapeutic value.

V - b

SOMATOSTATIN ANALOGS IN THE TREATMENT OF VARIOUS EXPERIMENTAL TUMORS

A. V. Schally, T. W. Redding, R.-Z. Cai, and J. I. Paz
VA Medical Center and Tulane University School of Medicine, New Orleans, LA, USA

In tests in animal models, various octapeptides of somatostatin were shown to possess significant antitumor activity. Analog Ac-p-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-15) in doses of 3 µg bid, inhibited the growth of estrogen and prolactin dependent MT/W9A mammary adenocarcinoma in Wistar/Furth rats (9). In Dunning R3327H model of prostate cancer in Copenhagen-Fisher rats, modern superactive octapeptide analogs of somatostatin including D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, (analog RC-121), significantly reduced the weight and volume of prostate cancers and given in combination with D-Trp-6-LH-RH microcapsules potentiated the effect of the latter. Both analogs, D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160) in doses of 2.5 µg bid inhibited the growth of W.D. ductal pancreatic tumors in golden hamsters in agreement with results obtained earlier with less potent analogs of somatostatin. The analogs Ac-p-Cl-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2 (RC-15) and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160-2H) in doses of 2-5 µg bid appeared to have antitumor activities, in short-term studies, as shown by more than 50% increase in survival rate in mice bearing the Dunn osteosarcoma in short-term studies. Our results from the animal tumor models support the contention that a new approach based on analogs of this type could become a useful addition to the present methods of treatment of certain endocrine-dependent or hormone-sensitive tumors.