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TUMORIGENICITY OF A HIGH TITER RETROVIRUS CARRYING THE HUMAN erbB PROTO-ONCOGENE

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By inserting a normal human EGF receptor (hEGFR) cDNA into a retroviral vector, I have generated a high titer retrovirus ($3x10^7$/ml). Infected NIH3T3 cells express 400,000 hEGFR/cell. In the absence of EGF cells grew like controls: with 20 ng/ml EGF, they grew twice as fast and reached a higher saturation density. The dose-dependent growth response in serum-free medium can be used as a highly sensitive bioassay to quantitate EGF and TGFα. The EGFR virus induced EGF-dependent foci of morphologically transformed cells and EGF independent foci in cells expressing also TGFα. In the presence of EGF, transformed cells formed foci in soft agar. Nude mice, which contain endogenous EGF, inoculated with cells expressing high levels of hEGFR developed tumors with a 55 days latency; the latency was shorter (35 dd) when the mice were given exogenous EGF (5 ug daily). Thus high levels of EGFR can induce ligand-dependent transformation and contribute to tumorigenicity. These results suggest that the high number of EGFR found in a variety of human tumors may play a direct role in the pathogenesis of these diseases. (In coll. with D.Lowy, I.Pastan)

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REDUCED HLA-A,B,C EXPRESSION IN TUMORIGENIC v-RAF TRANSFECTED HUMAN UROTHELIAL CELLS.

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We have previously demonstrated that tumorigenic (TGrIII) human urothelial cells grown in vitro have a markedly decreased expression of HLA-A,B,C antigens as compared to non-tumorigenic (TGrII) human urothelial cell lines. In this study we have used the immunofluorescence test and a standard NIH HLA-typing assay to demonstrate that after transfection of the HCV29 cell line (TGrII) with the v-raf oncogene, changes in morphology and tumourigenicity was accompanied by a decreased expression of HLA-A,B,C. Treatment of the tumorigenic transfected cells with α-interferon increased the expression of HLA-A,B,C antigens by 0,5 - 1,5 fold.
We conclude that there exists an inverse relationship between HLA-A,B,C expression and tumorigenicity in human urothelial cell lines.

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IN VIVO/IN VITRO STUDIES OF TRANSPLACENTAL ETHYLNITROSOUREA (ENU) CARCINOGENESIS IN RATS.

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The transplacental induction of neurogenic tumors by ENU treatment of pregnant rats has previously been described by several authors. In the present investigation the development of malignancies in other tissues was studied in vivo and in vitro. Malignancy was defined morphologically, by sc transplantation into newborn syngeneic hosts and into nude mice, and by the ability of the cells to invade and destroy cocultured fragments of embryonic chick hearts.

Rats treated in utero with a single pulse of ethylnitrosourea (ENU) at a dose of 60 mg/kg maternal body weight developed malignant tumors of the central and peripheral nervous system after 214 ± 56 days.

In addition to the neurogenic tumors microscopy revealed small cell lung carcinomas in several rats and lymphatic neoplasia in a few.

By explantation at various times after ENU exposure but before the development of tumors malignant transformation was seen after the cultivation of brain, liver, skin and bladder tissues for 294 ± 71 days.

Serial studies of transplanted tumors and in vitro propagated cells showed that tumorigenic and invasive properties do not necessarily develop simultaneously.

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ESTABLISHMENT AND CHARACTERIZATION OF HUMAN UROTHELIAL CELL LINES.

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Explant cultures were prepared from 55 non-malignant and 63 malignant human urothelial biopsies. Primary growth was seen in the course of 1-2 weeks from about 75% of the biopsies irrespective of their origin. Secondary growth for more than 4 passages was seen in 33 and 44% respectively.

The established cell lines were classified into four grades of transformation (TGr0-TGrIII) according to their morphology, life span, invasiveness in cocultures with normal tissue, and tumorigenicity in nude mice.

The characterization of these four categories of cells includes studies of cell size and shape, membrane structure, karyotype, restriction fragment length polymorphism (RFLP) of the α-globin gene, isoenzyme pattern, growth pattern, DNA repair and angiogenic potential.

Special studies have been carried out by S. Ottesen of the HLA expression of these cell lines.