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Enhanced expression of epidermal growth factor receptor correlates with alterations of chromosome 7 in human pancreatic cancer

(karyology)

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ABSTRACT **Recently, the gene for the epidermal growth factor (EGF) receptor has been mapped to chromosome 7p, the short arm of chromosome 7 [Shimizu, N., Kondo, I., Gamou, M. A., Behzadian, A. & Shimizu, Y. (1984) *Somatic Cell Mol. Genet.* 10, 45–53]. Utilizing EGF binding in saturation studies, karyology, and cDNA hybridization experiments, we have sought to determine whether there is a correlation between dosage or alteration of chromosome 7 and enhanced expression of EGF receptor in cultured human pancreatic carcinoma cells. Saturation binding studies with ^{125}I-labeled EGF were performed at 4°C with four established human pancreatic cancer cell lines: T_3M_4, PANC-1, COLO 357, and UACC-462. Analysis of binding data revealed enhanced numbers of EGF receptors in all four cell lines. Chromosome banding analysis revealed clonal structural alterations of chromosome 7p in the cell lines T_3M_4, PANC-1, and COLO 357, whereas UACC-462 displayed multiple copies of chromosome 7. Hybridization studies using a radiolabeled EGF receptor cDNA probe failed to demonstrate DNA sequence amplification in any cell line but confirmed the presence of EGF receptor mRNA in these cells in approximate proportion to EGF receptor number. Our results suggest that enhanced expression of EGF receptor in human pancreatic cancer can be associated with either structural or numerical alterations of chromosome 7.**

Pancreatic cancer is the fifth leading cause of cancer death in the United States, with a one-year overall survival rate of only 12% (1). Relatively little is known concerning the molecular mechanisms that regulate malignant pancreatic growth. However, the exocrine pancreas has receptors for epidermal growth factor (EGF) (2). The EGF receptor is a protein with intrinsic kinase activity leading to autophosphorylation at tyrosine residues (3). This kinase exhibits strong sequence homology with the product of the avian erythroblastosis virus v-*erbB* oncogene (4). Further, tyrosine phosphorylation is a relatively uncommon biological phenomenon that is effected by a small group of growth-promoting polypeptides and by the products of certain oncogenes (5). It has therefore been suggested (4) that this reaction is involved with the regulation of cellular growth. Although EGF exerts long-term trophic effects on the exocrine pancreas (6) and enhances pancreatic protein synthesis at concentrations as low as 42 pM (7), it is not known whether alterations in EGF binding or action are implicated in pancreatic carcinogenesis.

We now report the results of EGF binding, karyology, and molecular genetic analysis of four human pancreatic carcinoma cell lines. Our data suggest that these cell lines overexpress the EGF receptor in association with either structural or numerical alterations of chromosome 7.

MATERIALS AND METHODS

Cell Culture. PANC-1 human pancreatic carcinoma cells were obtained from the American Type Culture Collection. T_3M_4 (8) and COLO 357 (9) cells were obtained from R. S. Metzgar (Duke University). Pancreatic cell line UACC-462 was established in the Tissue Culture Core Laboratory of the University of Arizona Cancer Center (UACC). The growth properties and detailed karyology of this line will be presented elsewhere. A-431 cells were obtained from B. Ozanne (University of Texas, Dallas). Cells were routinely propagated in monolayer culture at 37°C in a 5% CO_2/95% air atmosphere. PANC-1, A-431, and COLO 357 cells were grown in Dulbecco's modified Eagle's medium (DMEM), T_3M_4 cells were grown in RPMI 1640 medium, and UACC-462 cells were grown in modified Leibovitz' L-15 medium (10). Media were supplemented with antibiotics and 10% newborn calf serum.

EGF Binding Studies. Biologically active EGF was prepared from mouse submaxillary glands and purified by reversed-phase high-performance liquid chromatography (11). The most abundant EGF species thus isolated, α-EGF, was iodinated by the chloramine-T method to a specific activity of 80–120 μCi/μg (12) (1 Ci = 37 GBq). For measurement of binding, cells that were 75–80% confluent were washed once in DMEM supplemented with 20 mM Hepes (pH 7.4) (binding medium) and incubated at 4°C in binding medium containing labeled ligand. Nonspecific binding, determined in the presence of 1000-fold excess unlabeled EGF (1–6 μg/ml), never exceeded 5% of total binding, except at very high ligand concentrations in saturation studies. Incubations were stopped by washing cells six times in Hanks' balanced salts solution containing 0.1% bovine serum albumin.

Chromosomal Analysis. G- and Q-binding analyses of all four cell lines were performed as described (13). All results are expressed according to ISCN recommendations (14).

cDNA Hybridization Studies. DNA was extracted from PANC-1, T_3M_4, COLO 357, UACC-462, and A-431 cells and analyzed by the method of Southern (15). The DNA was digested with *Eco*RI, fractionated by electrophoresis in agarose gels, and blotted onto Nytran membranes (Schleicher & Schuell). The filters were probed as described (16), using the 2.4-kilobase EGF receptor cDNA pE7, which was derived from A-431 cells (17) and obtained from G. Merlino (National Cancer Institute). RNA was isolated from the same cell lines by the guanidium isothiocyanate method (18). Serial dilutions (1:2) of each RNA were spotted onto nitrocellulose

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Abbreviation: EGF, epidermal growth factor.

Table 1. Alterations of chromosome 7p in pancreatic cell lines

Line	Modal number	Copies of chromosome 7p		EGF receptors	
		Abnormal	Normal	No. per cell	K_d, nM
T_3M_4	56	2.0	2.0	1.2×10^6	10.81
PANC-1	58	1.0	4.0	4.0×10^5	6.77
COLO 357	53*	2.0*	1.0*	2.5×10^5	1.56
UACC-462	54	0	5.0	8.5×10^4	1.62

Binding of ^{125}I-labeled EGF was assayed under equilibrium conditions as indicated in *Materials and Methods*. Karyology was performed as described (13), according to ISCN recommendations (14).
*From ref. 9.

filters and probed with pE7 as above, except that prehybridization and hybridization were carried out in 50% (vol/vol) formamide at 42°C.

RESULTS

EGF Binding Characteristics. To determine the number of cell-surface EGF receptors, binding of ^{125}I-labeled EGF was assayed at 4°C. At this temperature, maximal binding to T_3M_4 and PANC-1 cells occurred at 6 hr, and to COLO 357 and UACC-462 cells, at 4 hr (data not shown). Binding studies with various concentrations of ^{125}I-labeled EGF were therefore carried out for either 6 or 4 hr, respectively. Scatchard analysis (19) of these saturation data demonstrated the presence of a single order of binding sites in all four cell lines, with the indicated K_d values (Table 1). The data also demonstrated the following approximate number of cell-surface EGF receptors per cell: T_3M_4 cells, 1.2×10^6; PANC-1 cells, 4×10^5; COLO 357 cells, 2.5×10^5; and UACC-462 cells, 8.5×10^4 (Fig. 1, Table 1).

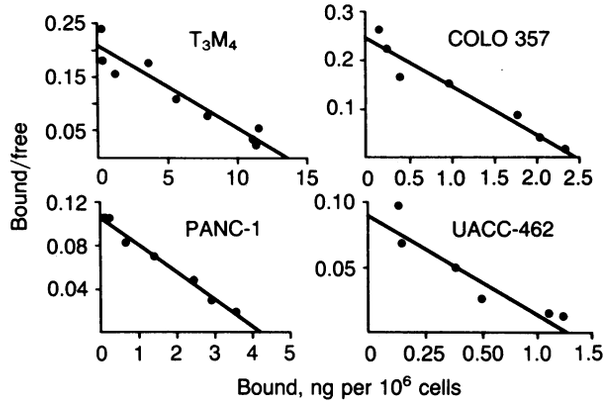

FIG. 1. Scatchard analysis of binding data. T_3M_4 and PANC-1 cells were incubated for 6 hr, and COLO 357 and UACC-462 cells were incubated for 4 hr, with various concentrations (2–150 ng/ml) of ^{125}I-labeled EGF at 4°C. Data for each cell line are the means of duplicate or triplicate determinations from three separate experiments.

Chromosomal Analysis. G- and Q-banding analyses revealed several clonal karyotypic alterations in all four cell lines, including structural and numerical alterations of chromosome 7 (Table 1, Fig. 2). Karyotypic information on COLO 357 has been published (9) and has been confirmed in our laboratory (data not shown). Structural alterations of

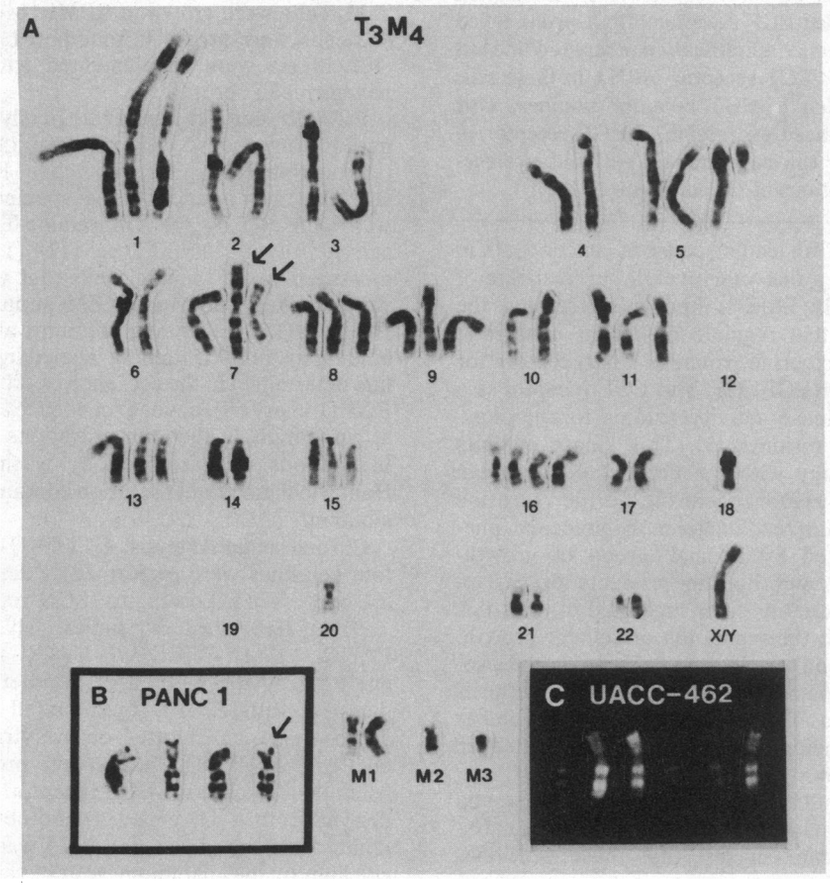

FIG. 2. Documentation of chromosome 7 abnormalities in human pancreatic carcinoma cell lines. (*A*) G-banded karyotype of cell line T_3M_4, demonstrating a reciprocal translocation of chromosome 7 [rcpt(7;7)(p11;q11.2)], as indicated by the arrows. (*B*) G-banded chromosomes 7 from the PANC-1 cell line; arrow shows a deletion of 7p at band p15. (*C*) Q-banded, normal-appearing chromosomes 7 from the cell line UACC-462.

chromosome 7p were detected in three cell lines: T_3M_4, rcpt(7;7)(p11;q11.2); PANC-1, del(7)(p15); COLO 357, i(7p) and t(7;10)(p15;q11). UACC-462 did not display any structural alteration of chromosome 7 but exhibited an average of five copies of this chromosome per cell.

Molecular Genetic Analysis. Southern blotting analysis failed to reveal DNA sequence amplification in any of the four pancreatic cancer cell lines (Fig. 3). In contrast, in the same series of experiments, A-431 cells showed the expected amplification of the EGF receptor gene. This cell line also exhibited a marked overabundance of EGF receptor mRNA (Fig. 4). UACC-462 cells had the lowest levels of this mRNA, and PANC-1 cells had intermediate levels, whereas T_3M_4 and COLO 357 cells had the highest levels among the pancreatic cell lines (Fig. 4).

DISCUSSION

Several lines of evidence suggest that an overabundance of EGF receptors may be a characteristic feature of certain human cancers. First, primary biopsy samples from glial (20), epidermoid (21), and pulmonary (22) malignancies in humans have an increased number of EGF receptors. Second, the presence of a large number of EGF receptors is associated with enhanced metastatic potential and tumor invasiveness in human breast (23) and bladder (24) cancers. Third, EGF receptor overexpression also occurs in cultured human carcinoma cells, including A-431 epidermoid cells (17), SCC-15 squamous cells (25), MDA-468 breast cancer cells (26), SK-MG-3 astrocytoma cells (27), and a number of melanoma cell lines (28).

We have determined that, in comparison to normal pancreatic tissue (2), four different cultured human pancreatic carcinoma cells have an overabundance of EGF receptors. None of these cell lines exhibited an amplification of the EGF receptor gene. In contrast, A-431 (17), MDA-468 (26), HN-5 (25), and SK-MG-3 (27) cells have significant amplification of this gene. Gamou *et al.* (29) reported that the UCVA-1 pancreatic carcinoma cell line also has a significant increase in the number of EGF receptors. Taken together, these results suggest that EGF receptor overexpression may be a common finding in human pancreatic malignancies and that increased gene dosage is not the only mechanism through which a cell may overexpress this receptor.

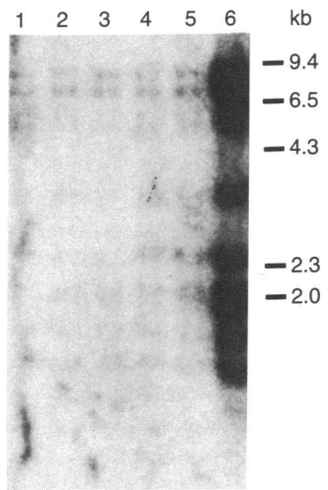

FIG. 3. Southern blot analysis. DNA (10 μg) from each cell line were digested with *Eco*RI and probed with pE7. Lanes: 1, normal human lymphocytes (used as a negative control); 2, T_3M_4 cells; 3, COLO 357 cells; 4, PANC-1 cells; 5, UACC-462 cells; 6, A-431 cells (used as a positive control). Positions of size markers [kilobases (kb)] run in parallel are at right.

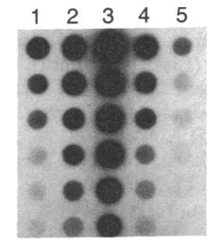

FIG. 4. RNA dot-blot analysis. Serial 1:2 dilutions of total RNA (5 μg in each initial well) were spotted onto nitrocellulose filters and probed with pE7. Rows: 1, PANC-1 cells; 2, COLO 357 cells; 3, A-431 cells; 4, T_3M_4 cells; 5, UACC-462 cells.

Enhanced expression of the EGF receptor in A-431 cells is most often associated with a translocation on the short arm of chromosome 7 (30), whereas in melanoma cells it correlates directly with an increase in the number of copies of this chromosome (28). In the present study, three of the four pancreatic cell lines exhibited structural alterations involving 7p. In PANC-1 and COLO 357 cells, the same band region of chromosome 7 was involved in a structural aberration. Further, COLO 357 as well as T_3M_4 cells exhibited isochromosome or translocation formation resulting in two copies of 7p sharing a common centromere. In contrast, the cell line with the largest number of copies of chromosome 7 (UACC-462) did not exhibit structural abnormalities of 7p and had the fewest EGF receptors. This cell line also exhibited the lowest EGF receptor mRNA level. These observations indicate that 7p11→7p15 is the region most consistently overrepresented in pancreatic cancer cells displaying enhanced expression of EGF receptors. However, all four cell lines exhibit additional chromosomal alterations that may contribute to modulation of EGF receptor expression.

The differences in EGF receptor mRNA levels between the various cell types tended to parallel the differences in EGF receptor number. However, there were some discrepancies, which may reflect variations in mRNA stability, receptor protein half-life, or receptor distribution between the cytoplasm and cell surface at 4°C. Irrespective of the reasons for these differences, the observation that a number of human pancreatic carcinoma cell lines overexpress the EGF receptor in association with alterations of chromosome 7p suggests that the gene coding for this receptor may be of biological significance in pancreatic tumors.

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