Amplification of c-Ki-ras-2 oncogene sequences in human carcinoma of pancreas

Ismail Parsa, Parviz, M. Pour and Cathleen, M. Cleary

State University of New York, Health Science Center at Brooklyn, Brooklyn, NY, and The Eppley Institute for Research in Cancer and Allied Diseases Omaha, NE, U.S.A.

(Received 12 December 1986; Accepted 19 June 1987)

Key words: Carcinogenesis

Summary

c-Ki-ras-2 sequences were visualized in paraffin embedded sections from normal adult human pancreases and 24 carcinomas of pancreas by an in situ hybridization technique. A biotinylated 1 kbp EcoRI fragment of pHiHi3 DNA was used as probe and the oncogene was visualized as one or two large grains of reaction products produced in more than 9% of normal pancreas nuclei by streptavidin-peroxidase complex and diaminobenzidine tetrachloride. Its amplification in pancreatic carcinomas was detected as one or more large grains in 54% of the nuclei. In addition, tumor cells showed small nuclear and cytoplasmic grains scarcely seen in normal pancreatic cells. The differential transcriptional activity of this oncogen in cancer cells and the adjacent normal pancreatic cells on the same section was evident in sections from 5 cases where normal pancreas was present.

Introduction

Pancreatic carcinoma in humans occurs late in life and is considered to be a multiphasic event. The activation of normal proto-oncogenes by structural or regulatory changes is suggested to contribute to the progression of this event. The presence of mutations in human pancreatic carcinomas was first reported by ito et al. (1984). The presence of ras oncogenes designated as c-Ki-ras-2 and c-Ki-ras-3 in a number of tumors was described by Brodbelt et al. (1985) and Koutsoukou et al. (1986). The amplification of these oncogenes and their expression in pancreatic carcinomas was also observed (Acheson et al., 1987; Parsa et al., 1987). The c-Ki-ras-2 is amplified in 54% of pancreatic carcinomas. The differential transcriptional activity of this oncogen in cancer cells and the adjacent normal pancreatic cells on the same section was evident in sections from 5 cases where normal pancreas was present.

Correspondence to: Ismail Parsa, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY, U.S.A.

Supported in part by grants No. CA 38955 and CA 22682 from National Cancer Institute.

0169-4197/88/$03.50 © 1988 Elsevier Science Publishers B.V. (Biomedical Division)
oncogenes belonging to the ras family has been demonstrated in several human solid tumors and cell lines [1-7]. Activation of the c-Ki-ras oncogene in particular, has been shown in human pancreatic carcinoma and in carcinoma-derived cell lines [7-9]. Activation and amplification of c-Ki-ras-2 oncogene and the chromosomal localization of this gene by in situ hybridization in one of the chemically induced human transplantable carcinoma cell lines were recently reported by Parsa et al. [10-12]. The present report describes the amplification of c-Ki-ras gene sequences in 24 cases of human pancreatic carcinoma evidenced by in situ hybridization on paraffin-embedded sections.

Materials and Methods

**Human Pancreas.** Sections of Bouin's fixed adult pancreas from cadaveric donors aged 12–69 years without pancreatic disease were used as control.

**Carcinoma.** Pancreatic tumor tissues were obtained during laparotomy (13 cases) or at autopsy (12 cases). Formaldehyde-fixed paraffin embedded sections, 4–6 μm in thickness, were stained with hematoxylin and eosin for diagnosis and the adjacent sections were included in this study. Histologically, tumors were of well differentiated glandular type in 11 cases, anaplastic in 4 cases, and of mixed histology, containing both glandular areas and less differentiated anaplastic areas in 10 cases.

**c-Ki-ras-2 Probe.** Clones containing pHiHi-3 were grown in LB broth (Gibco, NY, U.S.A.) and the plasmid's DNA was amplified by treating the cells with 170 μg/ml chloramphenicol for 15 h. The plasmid DNA was isolated by centrifugation in CsCl, treated with DNase free RNase and further purified by centrifugation. The EcoRI digest of plasmid DNA was electrophoresed on 1% agarose gel and the 1.0 kbp fragments were electroeluted, ethanol precipitated and used as probes [14].

**Nick translation.** The probe was nick translated according to Rigby et al. [13] except that for in situ hybridization biotinylated deoxynucleotides were substituted for the radioactively labelled nucleotides in the routine procedure. The nick translation mixture contained 400 μg DNase I, 24 units of DNA polymerase I, 5.0 mM MgCl₂, 8 mM sodium phosphate, 4% glycerol, 80 mM dithiothreitol, 80 μg/ml of bovine serum albumin, biotinylated dUTP and dCTP (Enzo Biochem, NY, U.S.A.) and unlabeled dATP and dGTP at a concentration of 25 mM each in 50 mM Tris-HCl (pH 8.0). In addition 2 μCi of tritium-labeled dATP (New England Nuclear) was included as an indicator for nucleotide incorporation and for chromatographic purification. The probe was incubated in this mixture at 14°C for 2 h. The reaction was stopped by the addition of disodium ethylenediamine tetraacetate (EDTA) to a concentration of 20 mM and incubation at 65°C for 10 min followed by ice bath. The mixture was filtered through a Sephadex-G 50 (Sigma Chemical) column and the nick-translated fraction was used for in situ hybridization.

**In situ hybridization.** Sections were deparaffinized and hydrated in decreasing etha-