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IN VITRO PROPAGATION OF PRENEOPLASTIC HEPATOCYTES INITIATED IN VIVO BY DIETHYLNITROSAMINE

R. Kerler and H.M. Rabes

Continuous administration of the carcinogen diethylnitrosamine (DEN) to rats in the drinking water (5 mg/kg/die) induces hepatocellular carcinomas after about 4 months (H.M. Rabes, W. Szymkowiak, Cancer Res. 39:1298-1304, 1979). During the preneoplastic latency period enzyme-aberrant foci occur, which develop by clonal growth from a single initiated cell (Rabes et al., Cancer Res. 42: 3220-3227, 1982) and are regarded as precursor lesions for hepatic nodules and carcinomas. A large variety of alterations has been described for these cells by morphological methods. However, in order to perform biochemical studies on alterations in these cells during early stages of hepatocarcinogenesis it appears necessary to select this cell population from other normal hepatocytes and to propagate them in vitro to obtain a sufficiently enriched population for specific analysis.

Liver of Wistar AF/Han rats, fed DEN for 60 days, were perfused in situ in the animal with oxygenized collagenase solution (0.05% in Mg^{2+} -free Hanks buffer, 30 min., 37°C) (P.O. Seglen, Methods Cell Biol. 13:29-83, 1976). A cell suspension was obtained which contained about 90 % living cells as determined by trypanblue exclusion. The cells were seeded in Falcon plastic flasks in a density of approximately 10^4 cells/ml in a medium composed of TCM 199 and NCTC 135 with addition of glucose, lactalbumine hydrolysate, essential aminoacids and 10 % fetal calf serum. During 2 weeks after seeding most adult normal hepatocytes are lost under these conditions, while preneoplastic hepatocytes start growing very slowly in a focal pattern as small epithelial monolayer plaques. These cells can be harvested and used for biochemical investigations concerning early stages of preneoplasia. In a few of these plaques cells proliferate faster within the first 7 weeks of culture and give rise to transformed foci with a biological behaviour characteristic of tumor cells: they grow with a short cell cycle time; in suspension culture they develop into multicellular spheroids; in soft agar these cells are able to form colonies; after i.p. injection into thymusaplastic nude mice they produce hepatocellular carcinomas.

The fact that enzyme-aberrant putative preneoplastic liver cells are endowed with an increased growth potential can thus be used for selection and propagation of these cells in vitro. Different preneoplastic stages can be delineated between initiation and final tumorigenicity and can be used for the investigation, by means of biochemical and cytogenetic methods, of sequential alterations at different stages of carcinogenesis.

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EFFECT OF N-ETHYL-N-BUTYL-NITROSAMINE ON THE ESOPHAGEAL MUCOSA OF THE RAT - HISTOMETRIC INVESTIGATION OF EARLY TUMOR STAGES.

H.U. Sons, F. Borchard

80 male Wistar rats received an oral application ad libitum of N-ethyl-N-butyl nitrosamine in a concentration of 0.18 g per litre of drinking water. By a maximum of 112 days after the commencement of exposure to the carcinogen, papillomas of the esophagus appeared. The mucosal changes were compared by microscopy with the results from a control group of 10 male Wistar rats of the same age which had not been exposed to the carcinogen, these examinations being performed at three intervals (up to 48 days, up to 93 days, and up to 112 days after the start of exposure to the carcinogen). The following histomorphometric data were determined with the aid of a Leitz ocular micrometer: the width of the epithelium, the length of the papillary body, the thickness of the horny layer, the number, length and maximum width of the nuclei, and the number of mitoses.

The earliest changes found were an increase in the thickness of the epithelium and the horny layer, and an elongation of the papillary bodies and a broadening of the parabasal cell layer. Later there was a significant thickening of the non-papillomatous epithelium - with a substantial increase in the rates of mitoses in all the epithelial layers -, as well as enlargement of the nuclei, especially in the middle and upper layers of the epithelium, and a thickening of the partly parakeratotic horny layer. Some of the papillomatous changes conformed with moderate epithelial dysplasias. Because the exposure duration was too short, no invasive carcinomas were observed, even with the larger tumors.

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EFFECT OF HYPERTHERMIA ON THE OXYGENATION OF MULTICELLULAR TUMOR SPHEROIDS

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Changes in tumor oxygenation during hyperthermia are mainly elicited by temperature-induced variations in both cellular respiration and O_2 supply via blood flow. To study heat-dependent changes in tumor oxygenation at constant O_2 supply conditions multicellular spheroids were used as in vitro-models of tumor microregions.

Spheroids of EMT6/Ro-cells were cultured in stirred media. Oxygen tension (PO_2) profiles in these tumor spheroids were recorded with a special microelectrode technique (Mueller-Klieser and Sutherland, Cancer Res. 42:237, 1982). Cellular aggregates with diameters of 400-600 μm were subjected to heat treatment at 40, 42 and 44 °C. Before heating and 20, 40, 60 and 80 minutes after attainment of the respective temperature PO_2 profiles were registered in each individual spheroid investigated. Heating of spheroids for 60 or 80 minutes at 40 °C resulted in a 30% decrease of the PO_2 in the center of the cell aggregates. Warming up to 42 or 44 °C for 20 minutes led to a pronounced increase in the central PO_2 . During maintenance of 42 °C for up to 80 minutes there was only a gradual decline in central PO_2 . In contrast, the corresponding values returned to the control level after heating spheroids for 80 minutes at 44 °C. Theoretical analysis of the experimental data revealed that the changes in the central PO_2 registered were caused by inverse variations of the O_2 consumption rate Q within these spheroids.

The results obtained show that hyperthermia in tumor tissue may be associated with an increase in Q at moderate temperatures which may partly counteract the concomitant enhancement of O_2 delivery through elevated blood flow under these conditions. A transient reduction in Q may occur at higher temperatures partially compensating for a restricted O_2 supply via blood flow. Apparently, there is an adaptation of cellular metabolism to heat at 44 °C. The biological mechanism of this adaptation is not known to date and, hence, needs further evaluation.

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ANTITUMOR ACTIVITY OF P-AMINOBENZOYL-O-PHENYLENEDIAMINE (GOE 1734) ON THE MXT MAMMARY TUMOR

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P-aminobenzoyl-O-phenylenediamine (Goe 1734) is a new N-phenylbenzamide derivative with unknown mechanism of action. In rapidly proliferating transplanted tumor models Goe 1734 was ineffective, whereas slowly proliferating tumors, especially autochthonous models, showed significant response. We investigated Goe 1734 on the MXT mammary tumor in different stages of tumor development. a: early stage; start of daily treatment one day after s.c. inoculation of 10 mg tumor tissue; dosage: 3 x 20, 2 x 30, or 1 x 60 mg/kg. b: advanced stage; start of daily treatment 12 days after s.c. inoculation of 30 mg tumor tissue. At this time point the mean tumor volume was about 400 mm³; dosage: 1 x 30, 2 x 30 or 1 x 60 mg/kg. Treatment was given per os. In early and advanced stages tumor growth inhibition was significant (>60%) at optimal dosage compared to untreated controls. For instance: Early: T/C = 0.32 after 2 x 30 mg/kg daily x 12 days; advanced: T/C = 0.38 after 60 mg/kg daily x 12 days. (T/C = ratio of mean tumor volume in treated animals to controls). The results are interesting since the significant activity of Goe 1734 in autochthonous mammary tumors can also be observed in the transplanted model (volume doubling time about 4 days), which offers some advantages for further studies, especially with regard to expenditure.

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