1 cell line c-myc sequences were on an HSR. Localization of amplified N-myc in 2 cell lines is under investigation. The preliminary results suggest that when an SCLC cell line contains DMs, at least a DM subpopulation carries amplified myc genes. In 4 cases it was possible to study tumours from which the cell lines were derived. In 2 cases, neither the tumour nor the derived cell line showed a myc amplification. In 2 cases both the tumour and the cell line showed amplified myc genes (1x c-myc,1x N-myc), with the higher degree of amplification in the cell line. In conclusion, in SCLC cell lines all the known myc-family genes can be involved in amplification, but not simultaneously. Myc amplification seems to be associated with tumour outgrowth in vivo. Evidently, progression of amplification can occur in vitro.

TYPE IV COLLAGEN DEGRADING COLLAGENASE: MOLECULAR PROPERTIES AND INHIBITORY ANTIBODIES TO THE ENZYME

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A type IV collagen specific collagenase secreted by a highly metastatic mouse tumour (PMT) was purified (Salo et al., J.Biol.Chem., 258: 3058-3063, 1983). The active enzyme was iodinated and found to contain two 65,000 and 60,000 dalton bands on SDS-PAGE without reduction. After reduction only one band of 60,000 daltons was observed. The 65,000 dalton band of the unreduced enzyme was selectively digested by chymotrypsin and trypsin. After digestion with these enzymes, the 65,000 band disappeared and the 60,000 dalton band was the major component. Papain did not degrade the enzyme at all whereas pepsin digested it completely. The results indicate that the enzyme is secreted as a proenzyme, which can be converted to the active form by trypsin and chymotrypsin. Upon reduction both enzyme forms migrated on SDS-PAGE with the same molecular weight of 60,000. Antiserum was raised against the denatured enzyme protein. Following SDS-PAGE of the enzyme after reduction one 60,000 dalton polypeptide was detected by immunoblotting techniques. The antiserum did not detect the native enzyme using the same techniques but accomplished a complete inhibition of enzyme activity. Therefore, the antiserum appears to detect only the trypsin activated and reduced forms of the enzyme. The antiserum has been used to screen expression cDNA libraries and several clones are being characterized.

CULTIVATION OF NORMAL HUMAN EPIDERMAL KERATINOCYTES

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Three published methods to culture human epidermal keratinocytes have been evaluated with regard to: cell isolation technique, cell yield, presence or lack of serum in the growth medium, use of feeder-layer, seeding density, cell attachment and proliferation, fibroblast contamination, cell dissociation and subculturing of primary cultures. Serum-free conditions with or without feeder-layer gave the highest yield of proliferating primary cultures consisting of a more than 99% pure population of keratinocytes that could also be subcultured and passaged at clonal density. The cloning efficiency of these primary cultures was about 0.5% and in the subsequent secondary cultures about 8 to 10%. Two methods, which used media supplemented with up to 20% fetal calf serum, also gave rise to keratinocyte primary cultures. However, serum-supplemented media with time caused both fibroblast overgrowth and enlargement and flattening of the keratinocytes indicating induction of squamous differentiation. Optimized methods yielding large numbers of human mitotically active keratinocytes can be used exprimentally to study growth regulation and carcinogenesis as well as to produce human pseudo-skin in vitro that could be used clinically.

ALKYLATING AGENTS: STUDIES ON MECHANISMS OF RESISTANCE IN DIFFERENT CELL LINES

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Resistance to drugs is a major obstacle in cancer therapy. There is compelling evidence that the cytotoxicity of many alkylating agents is the result of inter- or intrastrand cross-linking formation in DNA. Dianhydrogalactitol (DAG) and Dibromodulcitol (DBD) make interstrand cross-links resulting in 1,6-di-(guanin-7-yl) galactitol in DNA. The kinetics of formation of diguaninyl moieties in DNA and the efficiency and kinetics of repair were studied in parent and DAG

resistant P388 leukaemia, Yoshida sarcoma and BCNU resistant L1210 leukaemia cell lines. The parameters examined vary substantially among the different cell lines. BCNU resistance of L1210 was almost completely overcome by dianhydrogalactitol.

TISSUE DISTRIBUTION OF POLYALKYLCYANO-ACRYLATE NANOPARTICLES CHARGED WITH SPIN-LABLED NITROSOUREA

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The tissue distribution of biodegradable polyalkylcyanoacrylate nanoparticles associated with spin-labled nitrosourea, possessing high antitumour activity has been studied. The investigation has been carried out on C57 black mice with intramusculary implanted Lewis Lung carcinoma after intraperitoneal administration. The localisation of the polymer-carrier into the studied tumour has been estimated by ESR-spectroscopy.

The concentration of the drug carrier in the tumour tissue, 30 min after the administration, is almost constant. The highest concentration was found in the lung tissue of tumour-bearing animals. As intramusculary grafted experimental Lewis Lung carcinoma induces metastasis in the lung, this result can be used for application of the nanoparticles as a reliable drug-carrier of the cytostatic agents for pulmonary metastasis treatment.

INTERFERENCE OF ONCOGENE PRODUCTS WITH THE HORMONE DEPENDENT MMTV-LTR TRANSCRIPTION

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The H-ras and the v-mos oncogene proteins repress the glucocorticoid hormone-dependent transcription of the MMTV-LTR (EMBO J., 5: 2609-2616, 1986). To probe the mechanism of this effect, NIH cells transfected with an MMTV-LTR gene construct or with a chimeric gene construct consisting of the hormone responsive element of the MMTV-LTR (HRE) and the α-globin gene were infected with ras, mos, src or myc expressing retroviruses. Constitutive expression of oncogene protein did not abolish MMTV-LTR inducibility. The kinetics of transcriptional repression of the hormone dependent gene constructs was determined in all cases. Stimulation with dexamethasone and treatment with cycloheximide did not change the pattern of induction and repression of the HRE- α -globin gene transcription in ras- and mos-infected cells. We conclude that (1) the hormone/receptor binding domain is instrumental for the repression of the MMTV-LTR transcription and suggest that (2) the inhibition of the transcription is mediated by a modification of the affinity of activated glucocorticoid receptor to the MMTV-LTR DNA.

TIME DEPENDENCE OF BIOCHEMICAL CHANGES DURING DIFFERENTIATION OF A HUMAN NEUROBLASTOMA CELL LINE, SH-SY5Y

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TPA induces differentiation in the human neuroblastoma cell line SH-SY5Y. The sensitivity of muscarinic receptors to agonist with respect to Ca^{2+} mobilization decreases during the initial 6 hr. After induction of differentiation, the cells loose their processes and a decrease in c-myc expression occurs. The number of muscarinic receptors decreases after this time period followed by the appearance of features of a differentiated phenotype after 24 hr, including long neurite-like processes and an excitable membrane.

IMMUNOHISTOCHEMICAL LOCALIZATION OF S-100P AND NSE IN MALIGNANT MELANOMA

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This study included 30 cases of malignant melanoma. The classification of Clark has been used (LMM, SSMn, NM, ALM). The cells of the following types have been distinguished in morphological pattern: CE-epithelial, CS-spindle and mixed. The PAP method was used formalin-fixed paraffin embedded material. Immunohistochemical localization of S-100P and NSE in cytoplasm of melanocytes were observed. Positive staining was graded on a scale of -,+,++,+++. In the cells of both types S-100P and NSE were spread out within whole cytoplasm. The differences appeared in the intensity of staining: CE: S-100P (+); NSE (++,+++) and CS: S-100P (++,+++), NSE- (+). Immunohistochemistry research of the localization and intensity of S-100P and NSE in melanocytes may assist in the diagnosis