

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 experimentally 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