pronounced in cells of neural origin, but not strictly confined to any certain subset of cells. The foetal expression patterns may reflect the types of tumours preferably expressing the N-myc gene during post-natal life.

POTENTIAL MARKERS OF CELLULAR DIFFERENTIATION AND NEOPLASIA IN THE URINARY BLADDER

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Using the rat bladder-MNU carcinogenesis model, attempts are being made to establish a relationship between changes in selected properties of bladder urothelium and stroma and their biologic behaviour placing particular emphasis on changes during neoplastic transformation in luminal plasma membrane, intermediate filament (viz. cytokeratin), and extracellular matrix properties. Extra criteria for defining structure-function correlations in normal and abnormal differentiation involve: (a) histopathological and ultrastructural markers; and (b) probes targetting selected cell surface antigens and glycoconjugates, keratin proteins and extracellular matrix components.

The results, based on luminal surface and vertical tissue section analysis document a broad spectrum of changes in the course of differentiation, hyperplasia and neoplasia. Mapping studies of overt neoplastic change suggest associated 'field changes' of apparently uninvolved bladder mucosa. The data is interpreted within a conceptual model of stromal-epithelial regulatory/deregulatory processes in bladder.

KARYOTYPIC CHANGES IN HUMAN MALIGNANT MELANOMA

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Chromosome studies of the six melanoma patients were performed on direct preparations from primary and/or metastatic tumour explants. Abnormalities in ploidy demonstrated by the addition or loss of normal chromosomes and other structure rearrangements were noted in all cases. In one of the primary lesions 30% cells showed in direct karyotype analysis evidence of gene amplification in form of homogeneously staining region (HSR) on chromosome 6 at band q16 as well as two other characteristic chromosomes (7p14+ and 14q32+) among numerous defined and undefined markers. No double-minute bodies (DMs) were observed in primary tumour specimens. Cells from primary cultures, cell lines and cell strains from both primary lesion and metastatic node shared the same alterations, suggesting their origin from a common precursor.

HETEROTRANSPLANTATION OF HUMAN ENDOMETRIAL TUMOURS TO NUDE MICE

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Established tumour lines of human endometrial tumours are few, because of low take rate and tumour heterogeneity. Thirteen specimens of human endometrial tumours were transplanted. Twelve were biopsy samples of varying histology obtained at curettage, one was a metastasis, obtained from paraaortic lymph nodes at laparotomy.

Serial transplantation was successful in five cases and two tumours grew 2 respectively for 1 passage.

Thymidine incorporation into DNA in the successfully transplanted tumours was increased during the first passages. The take rate was 100% in the fifth and subsequent passages. Twelve original tumours had detectable cytosol estrogen receptor and eight had detectable progesterone receptor content.

The success of heterotransplantation seems to be based initially upon the tumour subtype and progesterone receptor content.

AMPLIFICATION OF myc-FAMILY GENES IN SMALL CELL LUNG CANCER (SCLC) CELL LINES

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We studied 18 SCLC cell lines established in Groningen. Myc-family gene amplification was found in 11 cell lines: 7x c-myc, 2x N-myc, 2x L-myc. Out of 8 cell lines with a myc amplification, 7 appeared to contain numerous DMs. In situ hybridization was carried out to find the location of the amplification. In 3 cell lines we found amplified c-myc on DMs, in

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