Cultured cell lines have been used to study the regulation of mucin gene expression *in vitro* facilitating the study of the biology of mucin-producing cells.

In conclusion, it is now possible to establish the mucin phenotype of tumors at the molecular level. This information allows a better study of the alterations of cell differentiation occurring during neoplastic transformation as well as the role of mucins in tumor progression.

39

CELL SURFACE BOUND MUCINS AND TUMOR PROGRESSION

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We have investigated the role of episialin, a surface bound mucin also known as EMA, PEM, CA 15-3 etc. encoded by the MUC1 gene, in tumor progression. The molecule has an extended rod-like structure protruding more than 200 nm above the plasma membrane. The expression of the molecule is usually more than ten times that in normal epithelia as determined by *in situ* hybridization and can be extremely high on metastatic cells present in pleural effusions. Episialin overexpression strongly reduces cell-cell and cell-matrix adhesion. The anti-adhesion properties of the molecule are due to the extreme length of the molecule since genetically modified molecules with a reduced length did not exhibit the anti-adhesion effect. E-cadherin/episialin double transfectants showed that episialin can prevent E-cadherin mediated cell-cell adhesion. Decreased E-cadherin mediated cell-cell interactions are known to promote invasion. Episialin overexpression is expected to have the same effect. Indeed, episialin overexpression promoted invasion in matrigel. Episialin overexpression at the cell-stroma boundary in primary breast cancers caused large "clefts" between the stroma and tumor cells. These results suggest that episialin has the same anti-adhesion properties *in vivo*. Episialin also interfered with immune recognition. Melanoma transfectants expressing high levels of episialin were less susceptible to lysis by LAK cells and allogeneically stimulated T-lymphocytes. The same transfectants had a significantly higher propensity to form lung metastases after i.v. injection in nude mice than episialin negative revertants of the same clones. Episialin may protect the tumor cells against NK cells and/or episialin expressing cells are more likely to metastasize as a result of the decreased cell-cell interactions. Our results strongly suggest that episialin overexpression is an important factor in tumor progression.

40

IMMUNE RESPONSE TO THE POLYMORPHIC EPITHELIAL MUCIN (PEM)

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Several tumor associated antigens have been shown to be able to induce an immune response. The identification of such antigens and the effector mechanisms involved is a first step for the development of useful cancer vaccines. PEM is a glycoprotein localized on the luminal surface of most simple epithelia. In cancer cells it is overexpressed and undergoes a process of aberrant glycosylation. Cryptic epitopes within the core protein of the extracellular domain are therefore exposed and could be a target for an immune response. An MHC unrestricted T cell response mediated by CD8 cells was described and explained by the particular structure of the molecule made up of tandem repeats (TR). Lymphocytes from tumor draining lymph nodes from patients with gynecological malignancies were utilized to study both the humoral and cellular immune responses of these patients. The human antibodies produced by these patients were directed against different epitopes within the TR sequence of PEM and were able to recognize the tumor associated glycoforms of the molecule. T cell clones were isolated that were able to proliferate in the presence of specific peptides corresponding to the TR of PEM presented by autologous B cells. The functional analysis of these clones revealed a Th phenotype. The possible contribution of T helper cells in generating and maintaining anti-tumor immunity opens new possibilities for effective immunological approaches in cancer therapy.

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41

THE MUC1 GENE PRODUCT, PEM AS A TARGET ANTIGEN IN CARCINOMAS

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Most glandular epithelial cells lining ducts express on their apical surface a transmembrane glycoprotein the polymorphic epithelial mucin (PEM) which is heavily O-glycosylated. PEM is overexpressed in more than 90% of breast and ovarian carcinomas and in some lung and colon tumours. Although the sequence of the MUC1 gene coding for the core protein is not altered in the cancer associated mucin, the O-glycans which are added are shorter and more heavily sialylated. This results in the appearance of novel carbohydrate epitopes and the unmasking of core protein epitopes which lie between the O-glycosylation sites. The increased expression of an antigenically distinct molecule, which is no longer restricted in its expression to the apical surface appears to induce both B and T cell responses in some breast, ovarian and pancreatic cancer patients.

To compare immunogens based on the MUC1 gene or its product, and to optimise antigen presentation, syngeneic and transgenic mouse models have been developed. In the syngeneic model, intramuscular injection of cDNA coding for the MUC1 gene (driven by the actin promoter) given before injection of MUC1-expressing mouse tumour cells can inhibit tumour growth. In the animals rejecting the tumour, cytotoxic T cells can be isolated and their development correlates to some degree with effective tumor immunity, while the appearance of circulating antibodies does not. In a mouse transgenic for the human MUC1 gene, PEM expressing tumours are rejected when antigen presentation is enhanced by expression of the B7 molecule.