heterogeneity of sialyl-Le^a carrying glycoproteins in two more cell lines than COLO 205, namely SW1116 and LoVo. Peaks that showed reactivity in the Ma552/C50 assay, indicating the presence of sialyl-Le^a carrying MUC1 mucins, were found in the spent medium and cell extract from SW1116 as well as from COLO 205.

S20.16

Characterisation of Model Systems for Studying the Aberrant Glycosylation of the MUC1-1 Gene Product

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The polymorphic epithelial mucin (PEM), coded for by the MUC1 gene is expressed on the luminal surface of many simple epithelial cells. Like other members of the mucin family, PEM contains a large domain which consists of tandemly repeated amino acids. This domain acts as a scaffold for the *O*-linked carbohydrate. PEM is upregulated in pregnant and lactating human mammary gland and in many adenocarcinomas. In addition, in malignant cells the molecule appears to be aberrantly glycosylated resulting in shorter sugar side-chains leading to the exposure of cryptic peptide epitopes such as that recognised by the antibody, SM3. In order to study the mechanisms underlying this aberrant glycosylation it is necessary to have an *in vitro* model system. To this end we have developed, by SV40 T antigen immortalisation, a cell line (MTSV1-7) which has many characteristics of normal mammary epithelial cells (Bartek *et al.*, 1991, PNAS 88, 3520 – 3524). MTSV1-7 does not grow in soft agar or form tumours in nude mice and, unlike breast carcinoma lines, forms ordered structures in collagen gels (Berdichevsky and Taylor-Papadimitriou, 1991, *Exp. Cell Res.*, 194, 267 – 274). Furthermore, by the criterion of antibody binding, MTSV1-7 appears to glycosylate the mucin like a normal cell. We are now in the process of purifying the MTSV1-7 mucin to confirm its carbohydrate composition by mass spectrometry (in collaboration with Professor Anne Dell). We intend to use this cell line and a breast carcinoma cell line to study the mechanisms responsible for the aberrant glycosylation of tumour cells.

S20.17

Different Expression of Mucin Cores in Human Colorectal Adenocarcinoma of the Non-Mucinous and Mucinous Type

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The non-mucinous and the mucinous adenocarcinoma of the colon differ not only morphologically but also in the prevalence of alterations in the Ki-*ras*, c-*myc* and *p53* genes. In this study the expression of the mucin cores MUC1 and MUC2 was investigated in these two histologic types of the colorectal adenocarcinoma.

Method: Formalin-fixed and paraffin-embedded tissues of 36 non-mucinous and 31 mucinous adenocarcinomas were tested by immunohistochemistry using the monoclonal antibodies BC3 and CCP58 directed against the MUC1 and MUC2 protein core, respectively. The percentage of stained cells was evaluated and compared with the staining of the normal colonic tissues. The expression was qualified as low (<25% stained cells), moderate (25 – 50% cells) or high (50 – 100% cells).

Results: In 73% of normal colorectal tissues the expression of MUC1 was low. Malignant transformation results in a high expression of MUC1 in the majority of both non-mucinous (83%) and mucinous (84%) carcinomas i.e. the prevalence of MUC1 protein core overexpression does not differ in the two types of tumors. By contrast, the expression of MUC2 protein core, low in 80% of normal colorectal tissues, was differently altered in malignant tissues: 77% of mucinous tumors exhibited high MUC2 expression versus 25% of non-mucinous carcinomas. Mucinous tumors which did not overexpress MUC2 protein core belonged to the group overexpressing MUC1 protein core. Those mucinous tumors which did not overexpress MUC1 protein core (16%) belonged to the group overexpressing MUC2 protein core.

Conclusions: Malignant transformation of the colon induces with similiar frequency the overexpression of MUC1 protein core in mucinous and non-mucinous carcinomas. MUC2 protein core is highly overexpressed only in mucinous carcinomas, suggesting distinct genetic alterations in the genesis of these tumors.

S20.18

Alterations in the Expression of Mucin and Fucosyltransferase Genes after Malignant Transformation of Human Colonic Mucosa

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The mucin-bound carbohydrate structure sialyl-Le^x is over-expressed in more than 90% of colorectal carcinomas. Recent results indicate that it is located on MUC-1 protein core and its biosynthesis *in vitro* is catalysed by at least two fucosyltransferases FTIII and FTV. We investigated the influence of colonic malignant transformation on the expression of the mucin genes MUC-1, MUC-2, and the fucosyltransferase gene FTIII.

Method: mRNA was isolated from fresh normal or malignant colonic tissue of six patients, digested with DNAse to remove contaminating DNA and applied for reverse transcriptase-polymerase chain reaction (RT-PCR) for 25 – 44 cycles. The amounts of the amplified DNA from tumor or normal tissue were determined in the linear range of the reaction by scanning gel photographs and related to the amount of pyruvat dehydrogenase amplified in parallel.

Results: Malignant transformation induced a 2 – 4 fold increased expression of the MUC-1 gene in 2 patients and a 2 – 4 fold decrease in 3 patients. In one patient the MUC-1 expression was not altered in the tumor. The MUC-2 gene expression was 2 – 4 fold increased in 1, decreased in 3 and not altered in 2 patients. The FTIII expression was increased 2 fold in 2 and decreased 2 fold in 2 patients. It remained unaltered in 2 patients.

Conclusion: These results indicate that malignant transformation induces discordant mucin and fucosyltransferase