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Symposium 1

Relevant topics in molecular pathology

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Cancer as a molecular disease of mucins and mucin glycosylation

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Mucins are major glycoproteins of the mucous gels that protect mucosae from environment and are essentially composed by a core protein backbone and 0-linked oligosaccharide attached to serine or threonine. The assembly of a mature mucin implies the coordinated participation of many gene products. Activation of genes coding for the apomucin and for individual glycosyltransferases, with unique donor-substrate specificity, are responsible for the large diversity of the final end product. Interindividual diversity of mucins is determined by the polymorphic nature of mucin genes and by the ABO histoblood group and secretor genes. Further intraindividual, tissue-specific diversity is dependent upon the activation of different mucin and glycosyltransferase genes. Furthermore, consistent alterations of the pattern of mucin expression are observed in cancer tissues.

Our group has been involved in the study of mucins and glycosyltransferases using the following different approaches: i) massive sequencing of complementary DNA libraries from gastric tissues and analysis of ESTs databases to identify new genes involved in the glycosylation pathway. We have so far identified, cloned and demonstrated the enzymatic activity of novel genes of a family of human 34-galactosyltransferases and of a family of 33-galactosyltransferases (1, 2); ii) characterization of mucin genes polymorphism (MUCi and MUC6) in healthy populations and in patients with gastric cancer. Our data show that individuals with a small number of tandem repeats, with smaller glycoprotein products, have an increased riskfor gastric carcinoma development (3, 4); ii) production and characterization of monoclonal antibodies to human mucins using synthetic peptides and *in vitro* GalNAc-glycosylated glycopetides. We have characterized a panel of antibodies to MUCi and produced antibodies to MUC2, MUC5AC and MUC6. Our results show that there are marked alterations in the mucin expression profile observed in intestinal metaplasia. gastric polyps and gastric carcinomas, including the aberrant expression of underglycosylated forms of the MUCi mucin in carcinomas (5-10); and iv) characterization of carbohydrate changes during gastric carcinogenesis.

We found that i) the expression of simple mucin-type carbohydrates is a cancer-associated phenomenon and frequently a marker of cancer progression (11-16); ii) the expression of dimeric sialyl-Lex correlates with venous invasion and poor outcome of gastric cancer patients (17); and ii) we still do not know the meaning of the aberrant expression of histoblood group A antigens and A enzyme in gastric carcinomas of blood group 0 individuals (18).

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Molecular mechanisms in diffuse-type gastric carcinoma: Diagnostic and therapeutic aspects

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Changes in the expression and function of adhesion molecules are important characteristics in the development of gastrointestinal malignancies and might be used in the future as prognostic factors or as new targets in diagnosis and therapy. E-cadherin is a homophilic cell adhesion molecule linking polarized epithelial cells and maintaining the structural integrity of an epithelial monolayer (1). Frequent somatic E-cadherin gene mutations in diffuse type gastric cancer and tumors with markedly diminished or complete loss of homophilic cell-to-cell interactions have previously been demonstrated (2). Partial or complete in-frame deletions of exons expected to be critical for E-cadherin function were detected as well as several point mutations. In about 20% of the cases in-frame deletion of exon 8 or 9 was observed. Very recently, several groups reported germilne E-cadherin mutations predisposing to early onset diffuse-type gastric carcinoma (3-5).

As somatic E-cadherin mutations have been detected in primary tumors and lymph node metastases of gastric cancer patients but were not seen in nontumorous tissues from these patients they

should be attractive targets for cancer diagnosis and therapy. We have investigated whether E-cadherin deletion mutations can be used for rapid tumor cell detection and the generation of tumor cell specific monoclonal antibodies for therapeutic intervention.

Since all somatic E-cadherin messenger RNA (mRNA) deletion mutations identified so far do not interrupt the reading frame, the mutated protein could still be incorporated into the plasma membrane, although part of its extracellular portion is missing. To be able to detect mutated E-cadherin protein but not wild type, we generated monoclonal antibodies in the rat against two frequently encountered deletion mutants, exon 8 and exon 9 deletion. E-cadherin-negative carcinoma cells were transfected with either wild type or mutant E-cadherin complementary DNAs. Extracts from stably transfected cells and from the liver, pancreas, colon, duodenum, stomach, esophagus, lung and kidney were then analyzed by Western blot. A control E-cadherin antibody, AEC (Transduction Laboratories), detects wild type E-cadherin protein in tissues and transfected cells. However, the mutation specific antibodies exclusively label mutant E-cadherin; wild type E-cadherin protein was not detected. In immunofluorescence analysis using the transfected cells and either AEC or the mutation specific antibodies and rhodamine labeled secondary antibodies, mutated E-cadherin protein was targeted at the cell membrane. In immunohistochemical analysis of diffuse type gastric carcinoma specimens (biopsies, primary tumors) previously shown by reverse transcription-polymerase chain reaction (RT-PCR) and direct sequencing to express mutant E-cadherin mRNA, the mutation specific antibodies exclusively label tumor cells while nontumorous epithelial cells on the same tissue sections were not stained. In contrast, immunoreactivity of both tumor and nontumorous cells was seen using the AEC antibody. These results indicate that mutant E-cadherin protein can be detected on tumor cells from biopsies and primary tumors, allowing a discrimination between tumor cells and nontumorous cells since the latter do not express mutant E-cadherin. Preliminary results with a mutation-specific E-cadherin immunotoxin demonstrated reactivity against cells expressing mutant E-cadherin but not against cells expressing normal E-cadherin.

Despite recent advances in the molecular pathology of gastric cancer, translation into the clinic for diagnostic or therapeutic purposes has been less forthcoming. Here we report monoclonal antibodies that specifically recognize mutant E-cadherin protein expressed on the cell surface of gastric carcinoma cells. Our results warrant development of this new tumor marker-monoclonal antibody system for diagnosis and therapy of diffuse type gastric cancer as the mutant E-cadherin epitope is not expressed in noncancerous tissues.

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The RET protooncogene in the diagnostic molecular pathology of thyroid cancer

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The *RET* protooncogene (rearranged during transfection) is located on chromosome lOqil .2 and encodes a transmembrane receptor with cytoplasmic tyrosine kinase activity. *RET* transcripts and protein are expressed in cells of neuroendocrine differentiation, including C-cells, adrenal medulla, parathyroid parenchymal cell precursors, as well as neural ganglions, peripheral nerves and their tumors. The RET protein is a functional receptor for several ligands including the glial-cell-line-derived neurotrophic factor (GDNF), neurturin, artemin and persephin. The ligands use a multisubunit receptor system in which glycosylphosphatidylinositol (GPI)-linked proteins designated GDNF-receptor alpha1-4 (GFRa1-4) and RET function as the ligand-binding and signaling components, respectively (1, 2). Shc and Grb2 appear to be transducing adaptor molecules which are activated by mutated or rearranged RETand by ligand binding. Tyr 1062 on RET is the putative binding site either for PTB or SH2 domains (3) and activated RET induces ELK, cyclic adenosine monophosphate responsive element binding protein and jun-mediated gene expression (4).

The RET protooncogene is involved in the tumorigenesis of medullary thyroid carcinoma (MTC) and papillary thyroid carcinoma (PTC) through activation of its tyrosine kinase either by point mutation or rearrangement.

The role of *RET* in medullary thyroid carcinoma

MTCs comprise 5-10% of all thyroid carcinomas. While a majority of these tumors occur sporadically, about 20% have a familial background. Familial forms of MTC are preceded by bilateral, multicentric C-cell hyperplasia (CCH) and residual CCH adjacent to MTC is considered a useful characteristic to distinguish familial from sporadic forms, however, reactive or secondary CCH has also been found adjacent to tumors of follicular cell origin and various nonneoplastic thyroid lesions (5).

Familial MTC

Several groups have demonstrated that distinct germline mutations in the RET protooncogene are associated with the dominantly inherited cancer syndromes multiple endocrine neoplasia type 2A and 2B (MEN2A and MEN2B) and familial medullary thyroid carcinoma (FMTC). All three syndromes share MTCs as part of the disease phenotype (6). The MEN2A subtype is characterized by the additional occurrence of pheochromocytoma (PCC) and hyperparathyroidism. MEN2A and FMTC is caused by activating mutations in the cysteine-rich extracellular region leading to enhanced dimerization of the RET tyrosine kinase receptor and thus to autophosphorylation. Missense germline mutations in one of six codons for Cys in RETexons 10 (609, 611, 618 and 620) and 11 (630 and 634) have been identified in 97% of MEN2A families and in 87% of FMTC families. In a few FMTC families additional germline mutations have been identified at codons 768, 790, 791, 804 and 891 in RETexons 13,14 and 15, respectively (7). The biological effects of mutations in exons 13 through 15 are thought to