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

R Komminoth

Dept. of Pathology, University of Zürich, Switzerland.

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

alter kinase activity by changing the substrate specificity or the adenosine triphosphate-binding capacity of the receptor or they may activate the receptor by altering its interactions with normal cellular substrates. Interestingly, mutations in codons 618 and 620 can lead to activation as well as to inactivation of the kinase (8). This may explain the combined occurrence of MTOs and Hirschsprung's disease in some MEN2 families.

In MEN2B an activating mutation of the tyrosine kinase core domain has been identified in 94% of patients. This mutation at codon 918 in exon 16 replaces methionine with threonine and causes increased autophosphorylation and alteration of the substrate specificity of the tyrosine kinase. Some rare patients with features of MEN2B have been described who exhibit mutations at codon 883 of *RET* exon 15 (9). In approximately 50% of patients the germline mutation is a *de novo* mutation and nearly always arises on the paternally derived chromosome.

SporadicMTC

Somatic mutations in *RET* have also been found in a proportion of patients with sporadic MTCs and POCs but appear to be rare in other neuroendocrine tumors (10, 11). By far the most common mutation in sporadic tumors involves codon 918 (Met → Thr). This type of missense mutation in exon 16 has been described in 23-85% of sporadic MTCs and in 0-50% of sporadic POCs and some investigators have associated it with a more aggressive phenotype. However, our group, as well as those of others, were not able to confirm these findings and have also demonstrated that there is no association between *RET* codon 918 Met → Thr mutations and malignant PCCs (12). Other groups found additional mutations at codon 768 of exon 13, at codon 883 of exon 15, codon 634 of exon 11 and exon 10 in a small proportion of sporadic MTOs and PCCs. Furthermore, microdeletions causing the loss of Cys residues at codon 630 or 634 and other alterations have been described in a few sporadic tumors.

The differences in mutational frequencies and codons involved that have been reported by various centers may be explained by either regional and environmental factors or technical problems. Thus, in a study of Eng *et al.* (13, 14) microdissected subpopulations from sporadic MTCs and multiple metastases from these tumors were examined and it was found that approximately 80% of sporadic MTCs had at least one subpopulation with the RETcodon 918 Met → Thr mutation.

Analysis of germline DNA for RETmutations reveals the hereditary or sporadic nature of MTOs (15). This DNA analysis can also be performed on DNA extracted from paraffin-embedded tissues (16). In patients with hereditary MTOs, the specific *RET* mutation will be present in both tumor DNA and in all normal tissues and blood cells harboring germline (constitutional) DNA. The absence of a germline RETexon 10, 11, 13-16 mutation appears to rule out MEN2A, 2B or FMTC with a high probability.

Rates of *de nova* mutations in MEN-2A and FMTC appear to be approximately 6% and in MEN2B around 50%. The best policy for evaluating apparently sporadic cases of MTC and POO is still under debate. We and others recommend routine application of *RET* protooncogene testing in all patients with apparent sporadic MTCs and POOs whereas others prefer a more conservative and cost-effective approach in which the histopathological features of surgical specimens and the patient's age at presentation are taken into consideration. Only if the patient is less than 40 years old or has CCH together with MTC or multifocal tumors, should DNA testing be performed. Since a subset of POO may be associated with von Hippel-Lindau disease, patients with familial, multiple, or early onset POO should not only be investigated for *RET* but also for germline VHL gene mutations. Patients with apparent familial cutaneous lichen amyloidosis do not appear to be at risk for MEN2A and do not need to be screened for *RET* mutations.

The role of RET in papillary thyroid carcinoma

The *RET* protooncogene has also been implicated in the causation of PTCs, which compose approximately 80% of all thyroid carcinomas. Several studies have demonstrated that *RET* is activated through somatic rearrangements in a subset of PTCs. The *RETIPTC* oncogenes are rearranged forms of the *RET* protooncogene and encode fusion proteins in which proto-RET tyrosine kinase and C-terminal domains are fused to different donor genes. The respective RET/PTC oncoproteins display constitutive tyrosine kinase activity and tyrosine phosphorylation. At least three forms of the RET/PTC oncogene have been identified; the RET/PTC-1 oncogene (where *c-RET* rearranges with the H4 gene D10S170 on chromosome 10q21)' the RET/PTC-2 oncogene (where *c-RET* rearranges with the regulatory subunit RI_{alpha} of the protein kinase A on 17q23) and the RET/PTC-3 oncogene (where *c-RET* rearranges with the RFG2/Ele1 gene on 10q11.2). Thus, the two most common forms, RET/PTC-1 and RET/PTC-3, both result from a paracentric inversion of the long arm of chromosome 10 (6).

Wide differences (2.5-60%) in frequency of *RET* activation by RET/PTC in the PTCs of different populations have been reported and it is not clear whether these are due to environmental factors, racial differences or technical reasons. However, several studies have shown an association between ionizing radiation and development of PTC. Thus, an increased incidence of PTCs (especially with RET/PTC-3) in children living in contaminated areas around Chernobyl has been reported. Furthermore, it has been shown that RET/PTC activation is present in 77% of occult PTCs (microcarcinomas) (17). Thus, it appears that RET/PTC rearrangement is associated with papillary microcarcinoma, early stage disease and that those tumors rarely progress into poorly differentiated tumors. However, if tumors simultaneously express rearranged *TRK* and RET, the prognosis appears to be worse (18).

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Human *Papillomavirus* detection and cervical cancer screening

J.M.M. Walboomers

Dept. of Pathology University Hospital Vrije Universiteit, Amsterdam, The Netherlands.

Epidemiological and moleCular biologiCal studies have shown that infection with high-risk human *Papillomavirus* (HPV) types is an important etiological factor in the pathogenesis of cervical cancer (1). Findings that are very important for the introduction of high-risk HPV testing in cervical cancer screening programs include:

i) Almost all (>97%) cervical carcinomas contain high risk HPV types and this includes squamous as well as adenocarcinoma.
ii) Applying HPV detection in cervical smears in prospective follow-up studies showed that progression of premalignant cervical intraepithelial neoplasia (CIN) lesions is always associated with the continuous presence of high-risk HPV types (2). No progression was observed in the absence of high-risk HPV types or the presence of non high-risk HPVs.
ii) High-risk HPV prevalence in the screening population of women with normal cytology is about 4%.
iv) Follow-up of women with high-risk HPV positive and cytomorphological normal smears showed that after 3 years 7% of these women had developed cervical intraepithelial neoplasia (CIN) III lesions, In the follow-up of women with high-risk HPV negative smears and normal cytology CIN III lesions were not found. It was calculated that among women with normal cytologythose with high-risk HPVtypes were 116 times (95% CI 13-990) more at risk to develop CIN III in contrast to those without high risk HPVs (3).

Based on these data a new screening strategy for cervical cancer will be discussed in which HPV testing is combined with routine cytology (4, 5).

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