RET rearrangements in archival oxyphilic thyroid tumors: New insights in tumorigenesis and classification of Hürthle cell carcinomas?

Petra B. Musholt, MD, Florian Imkamp, MD, Reinhard von Wasielewski, MD, Kurt W. Schmid, MD, *and* **Thomas J. Musholt, MD,** *Hannover, Essen, and Mainz, Germany*

Background. *Oncocytic carcinomas (Hürthle cell carcinomas [HCCs]) are commonly considered a subgroup of follicular thyroid carcinomas (FTCs). Recent characterization of a subgroup of "Hürthle cell" papillary thyroid carcinomas (PTCs) was based on the identification of PTC-specific RET hybrid oncogenes in HCCs.*

Methods. *We examined 27 HCCs, 4 oxyphilic FTCs, 5 oxyphilic PTCs, 2 poorly differentiated carcinomas arising from HCCs (HCC-UTCs), and 16 oxyphilic adenomas. Total RNA was extracted from paraffin-embedded thyroid neoplasms by a novel macrodissection technique that uses a cylindric punch. After reverse transcription–polymerase chain reaction–based screening for RET rearrangements, the samples were tested for all known RET/PTC 1 to 11 hybrids with the use of artificially constructed chimeric sequences as controls.*

Results. *The elimination of C cells by punching dissection significantly reduced RET wild-type expression. RET hybrid oncogenes (7x RET/PTC1, 1x RET/PTC1L, 2x RET/PTC3, 5 uncharacterized RET/PTCx) were demonstrated in 7 of 27 HCCs, in 0 of 4 oxyphilic FTCs, in 4 of 5 oxyphilic PTCs, in 1 of 2 HCC-UTCs, and in 3 of 16 oxyphilic adenomas.*

Conclusion. *Our results suggest that the expression of rearranged RET hybrid oncogenes (1) is present in a similar percentage of HCCs when compared with the literature on nonoxyphilic PTCs, (2) defines PTC-like HCCs better than histomorphologic characterization, (3) excludes HCCs as a subgroup of FTCs, and (4) may play a role in the early tumorigenesis of oncocytic tumors. (Surgery 2003;134:881-9.)*

From the Departments of Visceral & Transplantation Surgery, Clinical Chemistry, Pathology, Hannover University Medical School, Hannover, Germany; the Department of Pathology, Essen University Hospital, Essen, Germany; and the Department of General & Abdominal Surgery, Johannes Gutenberg University, Mainz, Germany

OXYPHILIC TUMORS OF THE THYROID gland are composed of large ovoid, thyroglobulin-producing cells with a granular, brightly eosinophilic (ie, acidophilic), mitochondrion-rich cytoplasm that arises from thyroid epithelial cells (Hürthle or Askanazy cells, oncocytes). Malignant oxyphilic neoplasms that demonstrate vascular invasion or capsular penetration are referred to as Hürthle cell carcinomas (HCCs) and comprise approximately 3% of all thyroid malignancies.[1] With respect to the often mixed cell type of thyroid tumors, the term Hürthle cell neoplasm should be restricted to tumors with more than 75% of oxyphilic cells.[2] HCCs commonly are considered a subgroup of follicular thyroid carcinomas (FTCs)[3] but have also been described as a separate thyroid cancer entity.[2] Papillary variants of HCCs were recognized mainly histomorphologically on the basis of the presence of papillary architecture, because diagnostic nuclear features of papillary thyroid carcinoma (PTC) may be obscured by the nuclear hyperchromasia that accompanies Hürthle cell metaplasia.[4]

Oxyphilic thyroid carcinomas are heterogenous neoplasms that display a wide range of biologic behavior. Every endocrine center will be able to report, on the one hand, HCC patients who die of highly aggressive, hematogenously metastatic tumors and, on the other hand, patients who live many years with slow-growing tumors and lymphatic

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Reprint requests: Petra B. Musholt, MD, Hannover University Medical School, Clinical Chemistry, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.

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metastases. Generally, HCCs are believed to follow a clinically aggressive course similar to FTCs, rather than the more indolent course that is observed in most patients with PTCs.[4,5] The prediction of disease progression, selection of the appropriate extent of surgery, and assessment of the usefulness of radio-iodine treatment in patients with HCC remain subjects of ongoing disputation.[6]

Because no histomorphologic parameters seem to be reliably related to biologic behavior and clinical outcome of oxyphilic carcinomas of the thyroid gland, the question arose whether distinct molecular genetic properties in these tumors might serve as prognostic factors. A Toronto group suggested that the subset of so-called Hürthle cell PTCs displayed the same biologic behavior as nonoxyphilic PTCs: a more indolent clinical course that is associated with frequent lymph node metastases.[4,7] The nonmorphologic, but solely molecular, characterization of Hürthle cell PTCs was based on the identification of a somatic chromosomal translocation that is found exclusively in PTCs (RET/PTC rearrangements).[7,8]

Adult follicular epithelium physiologically does not express the membrane-bound tyrosine kinase receptor RET that is involved in signal transduction. Through replacement of *RET* gene sequences that code for the extracellular domains of the RET protein by promoter regions of different ubiquitously expressed genes, the intracellular tyrosine kinase domain of RET is expressed constitutively and activated oncogenically. To date, 11 different fusion partner genes are reported to form (because of variable break-points) at least 15 different *RET* hybrid oncogenes, which are designated as RET/PTC 1 to 11, 1L, and Δ3(3r1, 3r2, 3r3), respectively.[9,10]

The aim of our study was the assessment of the frequency and type of RET/PTC rearrangements in histologically carefully classified thyroid neoplasms with cell oxyphilia. Because of the rarity of the tumors and consequently the lack of fresh-frozen tissue samples, we undertook the development of an analytic assay for the identification of *RET* rearrangements in archival tissue. When a reverse transcription–polymerase chain reaction (RT-PCR)–based technique is used for RET/PTC analysis in archival tissues, 3 problems must be addressed: (1) Total RNA extracted from formalin-fixed, paraffin-embedded tissue usually consists of short RNA fragments of not more than 300 base pairs, which necessitates appropriate PCR design. (2) The wild-type RET receptor is expressed in parafollicular C cells, which are dispersed throughout the thyroid gland. This RET messenger RNA expression may affect RT-PCR analysis for RET/PTC hybrids and therefore should be minimized or eliminated by dissection of the tumor and removal of C-cell–containing normal thyroid tissue. (3) A comprehensive search for *RET* rearrangements ideally should include not only all 15 known hybrid oncoproteins (of which only RET/PTC 1, 2, and 3 are detected in a considerable number of specimens) but should also allow for the detection of yet unknown RET/PTC chimeras. To address the aforementioned technical challenges, we developed a novel technique of macrodissection of archival tissue and redesigned our previously published RT-PCR protocol for the detection of *RET* rearrangements.[10] In the absence of appropriate positive controls for most *RET* chimeric oncogenes, artificial hybrid sequences of all known chimeras were generated.

METHODS

Patients and tissues. Oxyphilic tumors of the thyroid gland that had been diagnosed in routine pathologic reports from 1986 to 2000 and selected by the availability of paraffin tissue and clinicopathologic data only were re-evaluated independently by 2 pathologists (R.vW., K.W.S.). The group consisted of white patients who originated predominantly from the northern part of Germany, which is an area of moderate iodine deficiency. All patients (36 female patients, 18 male patients; age at primary surgery, 53 years [range 13.2 to 74.3 years]) underwent surgical procedures in the endocrine surgical clinic of the Hannover University Medical School.

The tumors were subdivided histologically, according to the criteria listed in Table I, into the following groups: oxyphilic adenomas (oxyAd), solid oncocytic malignancies designated as HCCs, oxyphilic FTCs (oxyFTC), oxyphilic PTCs (oxyPTC), and poorly differentiated thyroid carcinomas that arise from HCCs (HCC-UTCs; Fig 1). Overall, our molecular studies were performed on 69 tissues from 54 patients: 16 oxyAd (including 2 multifocal adenomas, 16 tissue blocks), 27 HCCs (including 26 widely invasive and 1 minimally invasive, 35 tissue blocks), 4 oxyFTCs (including 1 minimally invasive, 5 tissue blocks), 5 oxyPTCs (8 tissue blocks), 2 HCC-UTCs (4 tissue blocks), and 1 Hashimoto thyroiditis with oxyphilic metaplasia (diagnosed in proximity to a medullary thyroid carcinoma in the same patient).

In 1 patient, 1 of multiple oxyAds and a minimally invasive oxyFTC were studied. In 2 cases of HCC, both the primary neoplasm and a tumor recurrence were examined (3 and 7 years after primary surgery, respectively). In 10 cases (5 HCCs, 1 oxyFTC, 3 oxyPTCs, and 1 HCC-UTC), tissue specimens that were taken from different tissue blocks (ie, different

Table I. Carcinomas with oxyphilic metaplasia

Hürthle cell carcinoma	*OxyPTC*	*OxyFTC**
>75% oncocytes, predominantly solid/trabecular architecture or nesting of tumor cells	Papillary architecture (papillas, formations of folds, or intraluminal protrusions) and/or (focally) characteristic nuclear features (eg, ground glass nuclei, longitudinal grooves, nuclear pseudoinclusions, optical clearing of the chromatin)	>30% follicular architecture, (eg, nodules of back-to-back follicles, follicles with [partially] sustained colloid storage)

*This subdivision of oxyphilic follicular thyroid carcinomas was introduced for the purpose of our molecular pathologic studies to differentiate these FTC-resembling tissues from oncocytic malignancies without distinct follicular features.

tumor areas) were subjected independently to molecular analysis. In the HCC-UTC that showed 2 neighboring, apparently distinct, tumor areas, both the HCC tumor area and the anaplastic tumor area were examined. In 4 cases (3 HCC, 1 oxyPTC) fresh-frozen tumor tissue was available in addition to archival specimens. A sporadic medullary thyroid carcinoma that harbored the wild-type *RET* gene and normal thyroid tissues (2 cases) and a tall cell PTC without chromosomal *RET* rearrangement served as positive and negative controls, respectively.

Macrodissection and RNA extraction. A novel method of macrodissection of formalin-fixed, paraffin-embedded thyroid cancers that omitted contamination by stromal tissue and normal follicular epithelium including C cells was established (unpublished results). In brief, areas that solely contained tumor tissue were marked on histologic slides and the corresponding paraffin tissue block (Fig 2). Several tissue cylinders were obtained from the centers of tumor areas with a punching tool, re-embedded in paraffin, cut into multiple 20-μm sections, and collected in a small vial. Total RNA was extracted from these tissue sections by proteinase K digest and subsequent phenol-chloroform-isopropanol precipitation.

In addition to archival tissue, fresh-frozen tumor tissue was available in 4 cases. After the malignancy was confirmed histologically in each specimen, total RNA extraction was performed according to standard procedures with the use of TRIZOL reagent (Invitrogen, Karlsruhe, Germany).

RT and expression analysis. Standard first-strand synthesis was carried out with random hexamers and SuperScript II reverse transcriptase (Invitrogen). To screen for *RET* rearrangements in general, a triplex PCR was designed (unpublished results) that simultaneously amplified 2 short RET complementary DNA (cDNA) fragments: first, the tyrosine kinase domain (91 bp); second, the extracellular domain (70 bp). Both sequences were located distant to the 2 known fusion areas within the *RET* proto-oncogene (exons 11 and 12), thereby potentially allowing the detection of chimeras that carry yet unknown *RET* breakpoints. Third, a slightly longer, intron-spanning cDNA fragment of the housekeeping gene GAPDH (147 bp) was co-amplified to ensure the appropriate quality of the RNA that was used and to serve as an internal control for a successful PCR. cDNA samples negative for *RET* chromosomal rearrangements were evaluated at least 5 times by screening PCR analysis.

Samples indicative for *RET* rearrangements were subjected to a hybrid-specific, breakpoint-spanning PCR analysis that used sense oligonucleotides of the respective 5′ fusion partner gene and, except for RET/PTC 4 with a break-point in *RET* exon 11, one and the same antisense oligonucleotide located in *RET* exon 12 (unpublished results). Each tissue specimen was tested at least 10 times for the most common rearrangements (RET/PTC 1 and 3, respectively) and 3 times for all other known *RET* chimeras (RET/PTC 1L, 2, Δ3, 4 to 11).

Artificial RET/PTC hybrids. Artificially constructed chimeric sequences represented all 15 known RET/PTC hybrid oncogene variants. In brief, the chimeras were generated by PCR amplification of the 5′ sequence of the respective fusion partner gene and of the appertaining 3′ *RET* sequence that flank the breakpoint of the 2 fused genes, with the use of oligonucleotides with attached restriction sites for *Eco*RI (promoter gene, sense primer) and *Hin*dIII (*RET*, antisense primer; Fig 3). Site-directed mutagenesis that used overlapped extension PCR introduced a cleavage site for the rare restriction enzyme *Aat*II at the fusion region (Fig 4). Successful cloning into the pBlueScriptII $KS^{+/-}$ phagemid (Stratagene, La Jolla, Calif) was verified by PCR amplification with standard T3 and T7 oligonucleotides and direct sequencing (MWG Biotech, Ebersberg, Germany). DNA of vectors that carry the RET/PTC hybrid sequences was diluted in water and served as the positive control for PCR assays that amplified specific *RET* chimeras.

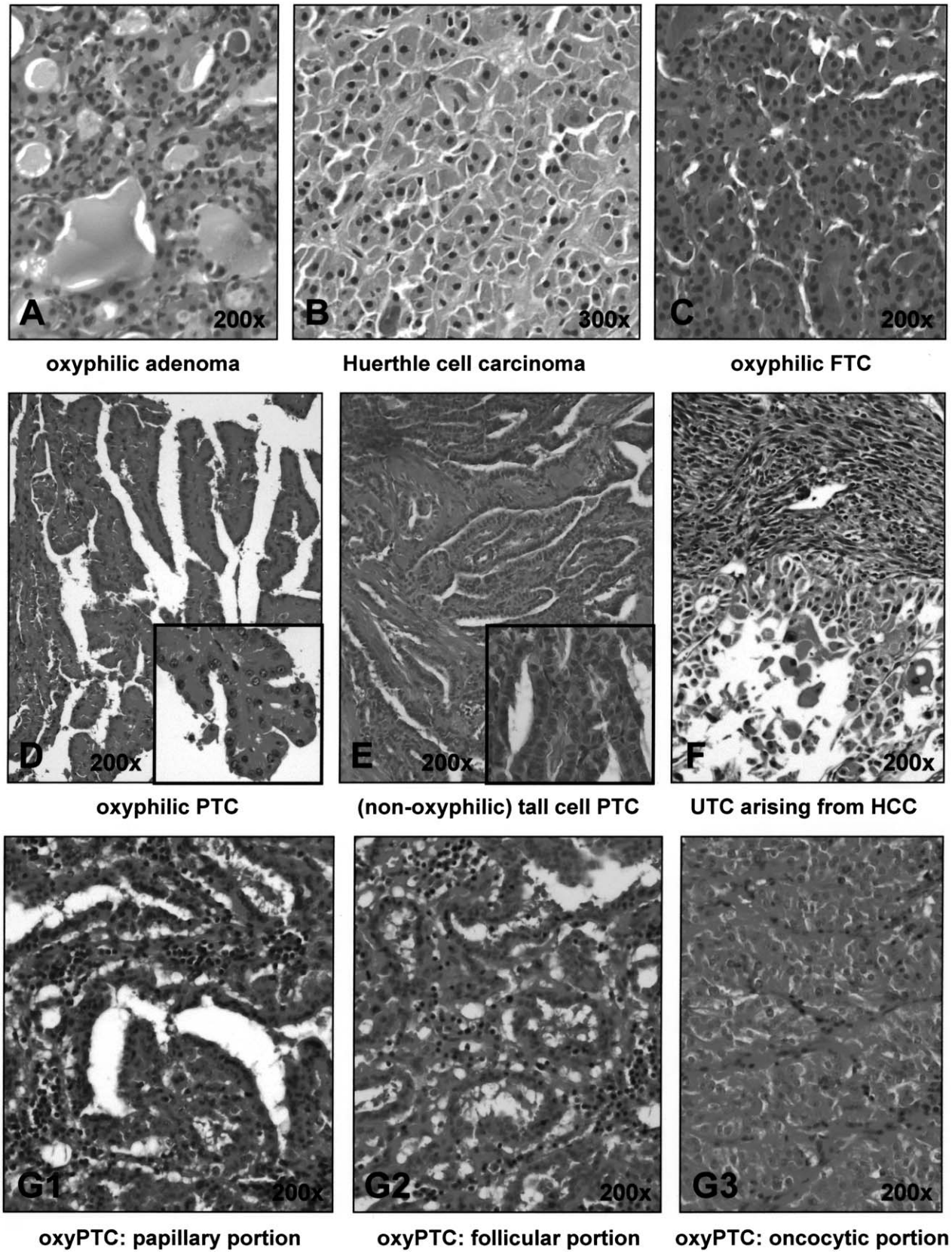

Fig 1. **A**, OxyAds comprise of adenocytes with brightly eosinophilic, granular cytoplasm. The tumors show no evidence of vascular invasion or capsular penetration. **B**, HCCs are malignant oxyphilic neoplasms that

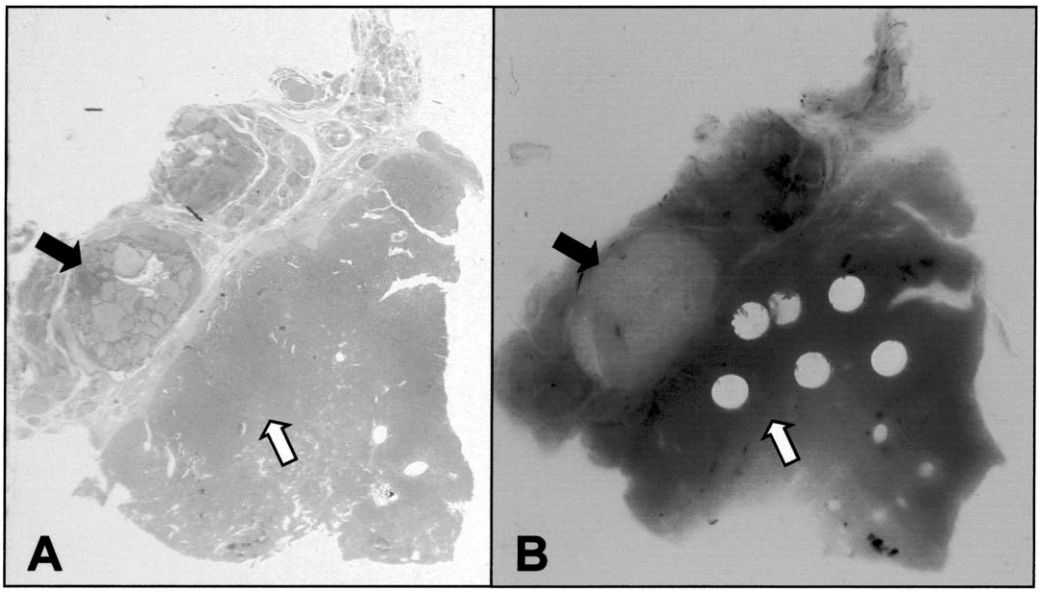

Fig 2. An eosin-hematoxylin–stained thyroid tissue slide (**A**) and the corresponding paraffin tissue block (**B**) that harbored an HCC (*white arrow*) and a non-oxyAd (**black arrow**) are shown. After the identification of solid oxyphilic tumor areas without evidence of remaining normal thyroid tissue, a punch was used to harvest 6 tissue cylinders of 1-mm diameter each. The macrodissected paraffin-embedded tissue cylinders that consisted of almost pure tumor tissue were subjected to molecular analysis.

RESULTS

Amplification of the housekeeping gene GAPDH was successful in 63 of 69 specimens examined, which thereby confirmed sufficient quality of the RNA that was extracted from formalin-fixed, paraffin-embedded tissue. In 3 of the 6 samples that showed weak GAPDH amplification, another tissue sample of the same patient was available for analysis. As expected, amplification products of both the RET tyrosine kinase domain and the extracellular domain were generated in specimens of medullary thyroid carcinoma and, weaker, in C-cell–containing normal thyroid tissue, which was consistent with the expression of the wild-type RET receptor. The macrodissected specimens that were derived from oxyphilic tumors, however, demonstrated very weak PCR products of the extracellular RET domain in only a few cases. Therefore, wild-type RET expression by contaminating C cells was almost completely eliminated after macrodissection of the tumor samples with the use of our punching technique (results not shown).

In 3 of 16 oxyADs (19%, 1 multifocal), 7 of 27 HCCs (26%), 0 of 4 oxyFTCs, 4 of 5 oxyPTCs, 1 of 2 HCC-UTCs, and in the oxyphilic thyroiditis, PCR failed to amplify the extracellular RET domain but demonstrated the expression of the tyrosine kinase domain, which indicated a rearrangement of the *RET* gene. Analysis for the specific hybrid oncogene sequences identified the RET/PTC 1 chimera in 1 of 3 oxyAds, in 4 of 7 HCCs, in 1 of 4 oxyPTCs, in the 1 HCC-UTC, and in the oxyphilic thyroiditis, while the hybrid RET/PTC1L was detected in 1 HCC. The RET/PTC 3 chimera was found in 1 HCC and 1

are composed of large ovoid, mitochondrion-rich oncocytes. **C**, The subgroup of oxyFTCs comprises oxyphilic carcinomas that show follicular structures in more than 30% of the tumor. **D**, OxyPTCs were defined as oxyphilic carcinomas that were composed of papillary architecture and/or show (focally) characteristic nuclear features of PTCs. **E**, Particular care was taken to distinguish oxyphilic PTCs from (predominantly) tall cell PTCs, which also exhibit intense eosinophilic staining. **F**, One of the poorly differentiated oxyphilic tumors that were studied seemingly consisted of 2 different adjacent neoplasms ("collision tumor"): a solid HCC and an anaplastic carcinoma, spindle cell type. However, closer examination revealed common nuclear features in both tumor areas that allowed the assumption of an undifferentiated thyroid carcinoma (UTC) that arose from an HCC. **G**, Four of 5 oxyPTCs that were examined were non-uniform differentiated tumors with mixed architecture: papillary structures (**G1**), areas of follicular growth patterns (**G2**), and areas of tumor cells with pronounced eosinophilic cytoplasm and nuclei with PTC characteristics (**G3**).

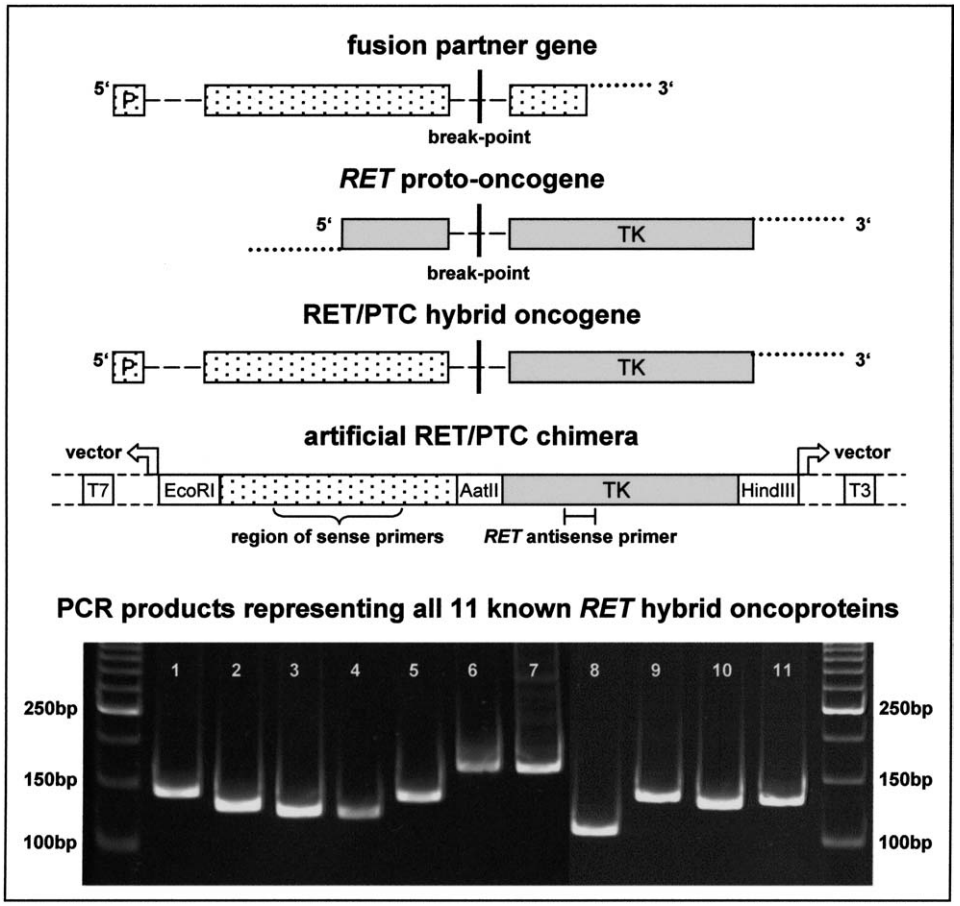

Fig 3. Schematic illustration of the generation of artificial RET/PTC hybrid sequences. The breakpoint for a chromosomal rearrangement of a fusion partner gene (coding for 1 of the rearranged proteins that are expressed ubiquitously in thyroid tissue) and of the *RET* proto-oncogene are depicted. The variants RET/PTC11 and Δ3 are not shown. Except for RET/PTC4 with a breakpoint in intron 10, the breakpoint within *RET* is located in intron 11, fusing exon 12 (which codes for the tyrosine kinase [*TK*] domain) to exonic sequences of the fusion partner gene. The resulting fused RET/PTC hybrid oncogene in vivo carries the promoter (*P*) of the fusion partner gene, which leads to the activation of the chimeric gene and to subsequent nonphysiologic expression of the RET tyrosine kinase domain. Sequences that span the breakpoints of the genes were amplified, and cleavage sites for the restriction enzymes *Eco*RI, *Aat*II, and *Hin*dIII were introduced by site-directed mutagenesis. After restriction digests, the cleaved products were ligated to form the artificial hybrid cDNA sequence that was cloned into a vector. The cloned chimeric inserts were reamplified and separated on a 6% polyacrylamide gel.

oxyPTC. The type of *RET* rearrangement was not determined in 2 oxyAds, 1 HCC, and 2 oxyPTCs; the samples were designated to carry a RET/PTC x chimera (Table II). None of the 4 oxyFTCs that were examined exhibited chromosomal *RET* translocations. The *RET*-rearranged HCC-UTC was a poorly differentiated oxyphilic carcinoma with few typical oncocytic cells and insular architecture.

Of 10 cases in which molecular analysis of up to 3 different tumor areas was carried out, the malignancies of 5 patients did not harbor *RET* hybrid oncogenes, including both distinct areas of the HCC-UTC "collision tumor." In 3 of 4 cases with divergent results for the presence of *RET* translocations, the missing amplification of the RET tyrosine kinase domain in 1 of 2 to 3 tumor areas that were examined was accompanied by failed GAPDH amplification in the respective sample, which pointed to RNA degradation rather than focal RET/PTC expression. The latter was also the case in 2 patients in whom both the primary and the recurrent tumor were examined, which explains the result of a RET/PTC-negative primary and RET/PTC-positive recurrent tumor and vice versa.

Analysis of fresh-frozen tumor tissue (4 specimens) confirmed the hybrid oncogene that was identified in the corresponding paraffin-tissue in 3 cases and allowed for the identification of the

RET/PTC 3 chimera in another rearrangement-positive sample in which the specific PCR for the hybrid oncogene had failed.

CONCLUSION

The heterogeneous group of oxyphilic or oncocytic neoplasms of the thyroid gland has been a matter of ongoing controversy regarding the histologic classification, the assessment of clinical behavior, and treatment recommendations. Initially classified as a subgroup of FTCs, the lack of follicular architecture and hematogenous spread in most oxyphilic malignancies prompted several authors to describe HCCs as a separate entity that arise from thyrocytes in addition to papillary, follicular, and anaplastic tumors.[2] Cases in which oxyphilic metaplasia is observed along with typical follicular or papillary growth patterns or cases in which eosinophilic tall cell PTCs are misdiagnosed as oxyphilic lesions have further complicated the classification of these tumors (Fig 1). In our series, malignancies that were described as FTCs with cell oxyphilia on routine histologic examination were found to have papilla-like formations and/or typical nuclear features of PTCs when carefully re-evaluated. Even within the group of HCCs, the divergent biologic behavior of subgroups of tumors has led to disagreement about clinical demeanor and treatment recommendations.[11]

The discussion about the nature and clinical course of HCCs gained new aspects from molecular analyses; the latter implied that subtypes of these thyroid tumors can be characterized genetically rather than histomorphologically. A Toronto group suggested the subgroup of "Hürthle cell PTCs," which have a rather indolent clinical course comparable to nonoxyphilic PTCs, on the basis of the finding of chromosomal rearrangements of the *RET* proto-oncogene in these neoplasms.[7,8] Cheung et al[7] demonstrated the 3 most common *RET* hybrids (RET/PTC 1, 2, and 3) in 15 of 19 HCCs by Southern hybridization technique. Our study demonstrated the expression of *RET* chimeric oncogenes in 26% of the HCCs that were examined. These oxyphilic tumors therefore harbored a sporadic genetic aberration that was specific for papillary-type neoplasms, despite a complete lack of histomorphologic features of a PTC. The percentage of RET/PTC rearrangements in about one fourth of the HCCs that were studied is comparable to frequencies that are detected in PTCs from patients without a history of irradiation.[9] Higher frequencies of RET chimeras have been observed when immunohistochemistry methods have been applied; however, one has to be aware of the variability in the evaluation of RET immunostains and of over-interpretation of focal and/or weak staining as positive expression of the RET oncoprotein.[12]

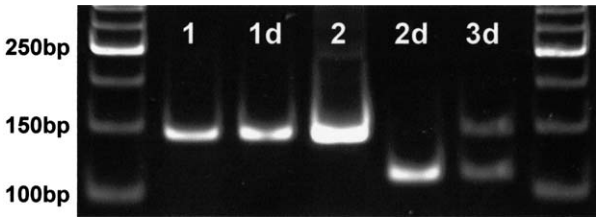

Fig 4. RET/PTC 1 amplification products of a rearrangement-positive HCC (*1*), of a vector that carries the artificial RET/PTC 1 chimera (*2*), and of the tumor sample mixed with a few copies of vector DNA (*3d*) are presented (145 bp). After restriction digest with *Aat*II (*d*), only the artificial sequence (in which the rare *Aat*II restriction site was introduced) is cleaved, which results in a 113-bp fragment and an (invisible) 32-bp fragment. Therefore, truly RET/PTC 1-positive specimens are easily distinguishable from specimens that were contaminated accidentally with the artificially constructed hybrids during laboratory experiments.

In our study, none of the 4 oxyphilic tumors that were characterized by considerable follicular features carried *RET* chimeric oncogenes (as 4 of 5 oxyphilic tumors with papillary features did). In a comprehensive molecular analysis of 16 nonoxyphilic sporadic FTCs, we found neither *RET* nor *NTRK1* rearrangements (unpublished results), although in our Hannover patient cohort 14% (15/119 patients) of the PTCs[10] and 26% (7/27 patients) of the HCCs harbored *RET* chimeras. To our knowledge, *RET* chromosomal rearrangements have not been described in follicular or anaplastic thyroid carcinomas.

In summary, whereas the presence of sporadic *RET* translocations seems to allow for the classification of a tumor as a PTC-like neoplasm, the absence of *RET* hybrid oncogenes is not sufficient to rule out a papillary phenotype. The finding of RET/PTC hybrids in HCCs may not only classify the rearrangement-harboring tumors as Hürthle cell PTCs but may also imply that all HCCs without specific follicular architecture are PTC-like neoplasms.

The isolation of RNA from archival, paraffin-embedded tumor tissues opens up important specimen resources for molecular biologic analysis of thyroid neoplasms. Nevertheless, the quality and fragment length of the extracted RNA are crucial when applying RT-PCR techniques, and (especially negative) results have to be verified by repetition, which may mandate several hundred nanograms of total RNA. When the expression of the *RET* proto-oncogene in thyroid tissues is examined, one has to

Table II. Frequency and type of RET/PTC rearrangements in oxyphilic tumors

Tumor	*Rearrangements (n)*	*RET/PTC 1 (n)*	*RET/PTC 3 (n)*	*RET/PTC x (n)*
OxyAd (n = 16)	3 (19%)	1	—	2
HCC (n = 27)	7 (26%)	4+1(1L)	1	1 (Focal)
OxyFTC (n = 4)	—	—	—	—
OxyPTC (n = 5)	4	1	1	2
HCC-UTC (n = 2)	1	1	—	—

be aware that wild-type expression of this tyrosine kinase receptor in normal C cells, which are dispersed throughout the thyroid gland, may hamper molecular studies significantly. Dissecting pure tumor tissue by methods such as laser capture microdissection[12] or by scraping tissues off glass-slides with microscope and scalpel is cumbersome and supplies only small amounts of dissected tissue. Our novel, simple macrodissection technique with a punching tool that stamps cylinders of marked tumor from paraffin blocks delivered sufficient amounts of C-cell–depleted tissue. Total RNA that was isolated from archival tissues usually is fragmented and partially degraded, which explains the need for repeated RT-PCR analysis and for an internal control by co-amplification of a housekeeping gene. In 6 of 54 tumors that were examined, the screening was indicative for a rearrangement of the *RET* gene, but the hybrid-specific PCRs did not reveal the nature of the chimera (RET/PTC x). In 1 of these cases, fresh-frozen tissue of the same tumor was available for analysis, and an RET/PTC 3 expression was easily and reproducibly detected, which confirmed the lower quality of paraffin-extracted RNA. Because the screening method allowed for the detection of every kind of rearrangement that involved the RET tyrosine kinase domain, the remaining RET/PTC x hybrids represent either chimeras in which our assay failed to identify correctly 1 of the 15 known hybrid oncogene variants or represent chimeras that harbor yet unknown breakpoints or fusion partner genes.

Facilitated by our macrodissection technique that allowed for the selection of several distinct areas of the same tumor, we looked for focal RET/PTC expression. Except for 1 specimen, all divergent results for the presence of hybrid oncogenes were accompanied by insufficient RNA quality of the rearrangement-negative samples. The HCC displaying expression of a RET/PTC x chimera in only 1 of 2 examined tissue cylinders was a uniformly solid oncocytic tumor without unusual or mixed histopathologic features.

The detection of *RET* rearrangements in an oxyphilic Hashimoto-like thyroiditis and in 19% of the oxyADs that were studied underlines the hypothesis that the oncogenic activation of the tyrosine kinase receptor is an early event in the process of malignant transformation. Studies of transgenic mice that carry the RET/PTC 1 oncogene imply that RET hybrid oncoprotein expression is a causative, but not solely sufficient, genetic alteration in the multistep carcinogenesis of thyroid malignancies.[13] Fusco et al[14]described RET/PTC oncoproteins in 65% of hyperplastic or adenomatous thyroid lesions with borderline morphologic signs of papillary cancer but with no signs of invasive growth patterns. With laser capture microdissection, the oncogenic *RET* activation was shown to be restricted to tumor areas with papillary cytologic alterations. The detection of RET/PTC rearrangements in benign lesions does not justify the interpretation of the entire nodule as a PTC but may allow the designation as a papillary carcinoma precursor lesion. Therefore, the simple presence of a RET/PTC chimera in an oxyAD does not serve as a reliable marker for a histomorphologically overlooked PTC but may exclude the diagnosis of a FTC.

In conclusion, our results corroborate the usefulness of molecular analysis for RET/PTC rearrangements in archival specimens for the facilitation of the classification of oxyphilic thyroid tumors. Characterization of HCCs with molecular-genetic, rather than histomorphologic, criteria provides improved impartiality and comparability. From the genetic point of view, the fact that *RET* rearrangements occur with a similar degree of frequency in HCCs and nonoxyphilic PTCs excludes the classification of all Hürthle cell tumors as a subgroup of FTCs and even questions the classification of these carcinomas as a separate entity. In the future, molecular analysis of other thyrocyte-specific markers in addition to *RET* rearrangements may facilitate the characterization of individual oxyphilic tumors as either PTC-like or FTC-like neoplasms.

Since the manuscript was accepted for publication, in June 2003 Saenko et al[15] described a novel *RET* tumorigenic rearrangement, Δrfp/ret (numbered in order of publication, RET/PTC12) which was identified in the papillary thyroid carcinoma of an externally irradiated patient.

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DISCUSSION

Dr Alan P. B. Dackiw (Toronto, Ontario, Canada). Do you have any data in your RET/PTC-positive oxyphilic carcinomas postoperatively of the avidity in these tumors for radioactive iodine? Characteristically, these carcinomas do not have an avidity for radioactive iodine or are less avid. In your RET/PTC-positive patients, was there any difference in their avidity response to radioactive iodine in that patient population?

Dr Musholt. As I pointed out, we do not have completed chart review and collection of follow-up data on all patients whose tissues were examined. Although it was hard work to gather the molecular data, the next step certainly is to search for clinical characteristics of the RET/PTC-positive HCCs. We will follow your suggestion and look into differences in radioiodine uptake.

Dr Martha A. Zeiger (Baltimore, Md). We currently use pathologic evidence as the gold standard for the classification of thyroid tumors. However, I think there is plenty of evidence already in the literature that many of these tumors overlap in terms of genetic alterations and that perhaps there may exist a thyroid tumor progression model like the one described for colon cancer. Furthermore, we know that lymphomas have been reclassified by genomic classification.

What are your thoughts about the possibility of reclassification of thyroid tumors in the future and whether indeed we are looking at a tumor progression model?

Dr Musholt. I think our results and the results of groups studying this issue before clearly state that HCCs are not a subgroup of FTCs. HCCs used to be classified as some subtype of FTC, but molecular evidence strongly suggests that this is not the case.

The question is whether HCCs should be reclassified as a subgroup of PTCs. If you find RET/PTC rearrangements in HCCs without any histologic evidence of papillary architecture, I would be encouraged to reclassify this tumor as a Hürthle cell PTC. What do you do with a rearrangement-negative oxyphilic tumor? As you pointed out, the RET/PTC translocation might be only 1 of the underlying molecular alterations, and further studies on this subject are necessary. In general, the classification of thyroid carcinomas with molecular-genetic, rather than histomorphologic, characterization is tempting because of the improved impartiality.

Dr Allan Siperstein (Cleveland, Ohio). I think that the molecular characterization is going to become increasingly more important. You speculated that outcome might be related. Did the frequency of the mutations vary by tumor stage? Also, did you have the opportunity to look at lymph node metastases or distant metastases to see whether their pattern of *RET* mutations corresponded with the primary tumor?

Dr Musholt. We have not yet undertaken a correlation of molecular and clinical data on all of the patients that we included in the study. RET/PTC-positive HCCs comprised T2, T3, and T4 tumors with a range of nodal involvement and even distant metastasis, and I cannot yet report on clinical characteristics. However, we have a hint that the tumors we classified as oxyFTCs display a lower tumor stage without lymphatic spread, but earlier recurrences.