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Identification of Differentially Expressed Genes in Papillary Thyroid Carcinomas With and Without Rearrangements of the Tyrosine Kinase Receptors RET and/or NTRK1[1]

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Background. **The transforming capacities of *RET* and/or *NTRK*1 chimeric oncogenes as well as the molecular background of non-rearranged papillary thyroid carcinomas (PTCs) remain to be elucidated. To assess altered gene expression, we examined PTCs with and without tyrosine kinase receptor rearrangements by mRNA differential display (DD).**

Materials and methods. **Six of 13 PTCs examined harbored *RET* chimeras (3× RET/PTC1, 1× RET/PTC3) and/or *NTRK*1 chimeras (2× trk, 1× TRK-T3, 2 unknown TRK hybrids). The method of DD analysis was refined by a novel fragment-recovery technique using a high-performance fluorescence scanner.**

Results. **Of 500 up- or down-regulated mRNA transcripts, 19 selected fragments were recovered, cloned, sequenced, and identified. The accuracy and high degree of reproducibility of the method was demonstrated. Differential expression of gene products with potential association to cell proliferation or tumor progression was observed, such as 14-3-3beta and Rab27a. Moreover, several gene products with unknown functions were demonstrated in PTCs bearing *RET* or *NTRK*1 hybrids *versus* rearrangement-negative PTCs, including a homologue of the Ig kappa light chain constant region.**

Conclusions. **Candidate transcripts with presumed tumorigenic potential in other solid tumors may prove to be relevant in the progression of PTCs, too. Most promising is the isolation of several differentially expressed, yet unknown, genes that may open new insights in the pathogenesis or progression of PTC.**

Key Words: **papillary thyroid carcinoma; differential gene expression; chromosomal rearrangements; genetic alterations; mRNA differential display.**

INTRODUCTION

Papillary thyroid carcinomas (PTCs) are the most frequent thyroid malignancies. Although the tumors are generally associated with a favorable prognosis, a wide spectrum of biological behavior can be observed that is most likely based on varying genetic backgrounds of the respective neoplasms.

Different genetic alterations have already been described in thyroid tumors: alterations of the thyroid-stimulating hormone receptor (TSHR) and of the membrane-bound G protein—both leading to a constitutive activation of the adenylate cyclase-protein kinase A (PKA) pathway—have been identified in benign neoplasms, especially in hyperfunctioning adenomas, as well as in congenital hyperthyroidism and in a subset of differentiated thyroid carcinomas [1, 2]. However, additional molecular alterations seem to be required for malignant transformation of the thyrocyte following activation of the aforementioned oncogenes. The retinoblastoma (Rb) gene and especially the TP53 gene are tumor-suppressor genes that are inactivated in some thyroid cancers with predominantly poorly differentiated phenotype [3, 4] (Fig. 1).

Starting about 15 years ago, rearrangements of the *RET* proto-oncogene were detected in papillary carci-

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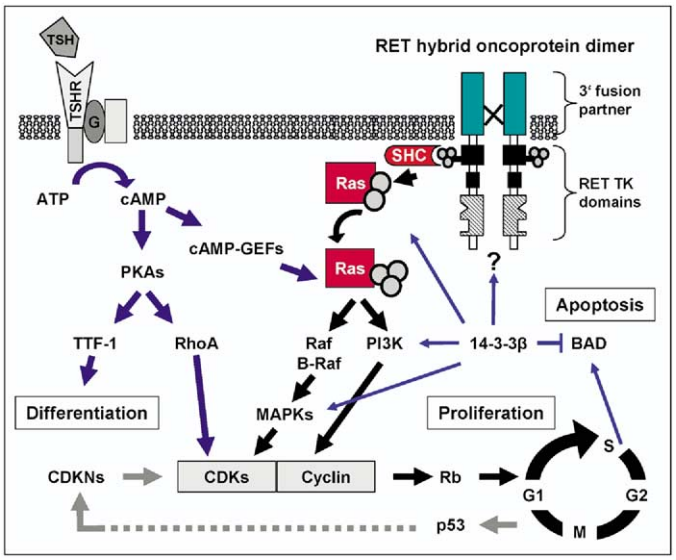

FIG. 1. Selected signaling and modulating pathways in the oncogenesis of papillary thyroid carcinomas (PTCs) are illustrated. The physiological proliferation of the thyroid cell is initiated through activation of the TSH receptor (TSHR). Following binding of the thyroid growth hormone TSH, G-protein coupled to the receptor is activated leading to a well-established signal cascade involving protein kinases A (PKAs). Thus, the hormone signal is transmitted to the nucleus where the transcription of proteins necessary for the cell-cycle progression is initiated. As a result, the cyclin-dependent kinases (CDKs) are activated that "flip the switch" in favor of the progression to DNA synthesis and mitosis. Intact feedback loops controlling cell proliferation and tissue integrity—here partially depicted by the action of p53 and the inhibitors of the cyclin-dependent kinases (CDNKs)—will limit the number of cell divisions. Alterations of TSHR or G-protein, resulting in increased mitoses, are predominantly described in thyroid adenomas. Similarly, unphysiological activation of the hybrid RET receptor—following chromosomal rearrangement with the promoter of a fusion partner gene (RET/PTC1-12)—leads to tyrosine kinase (TK) activity, which initiates cell proliferation through the Ras pathway involving Raf, B-Raf, and phosphatidylinositol 3-kinase (PI3K). Mitogenic gain-of-function mutations of the *RAS* oncogene or of *BRAF* are also reported for PTCs. 14-3-3β protein (whose mRNA transcript we found to be overexpressed in carcinomas) interacts with proteins of several levels of the Ras signal cascade. Hypothetically, up-regulation of 14-3-3β will result in increased mitosis by activation of Ras or its downstream pathway and will halt apoptosis by inhibition of the pro-apoptotic protein BAD. (Color version of figure is available online.)

nomas of the thyroid gland and the specificity of these findings for PTCs were proven [5–7]. Only solitary observation of *RET*-rearranged follicular thyroid carcinomas (FTCs) are published [8]. The oncogenic activation of a chimeric *RET* gene is the result of translocations between the *RET* locus 10q11.2 and another chromosome or the result of an inversion of chromosome 10. Thus, the tyrosine kinase (TK) domain of the *RET* proto-oncogene is fused to the amino terminus of one of several ubiquitously expressed genes which donate the promoter region for the resulting chimera. The replacement of the extracellular domain of the RET receptor in somatic cells leads to constitutive and ectopic expression of the resulting hybrid oncoproteins which causes an increased tyrosine kinase activity in tissues physiologically lacking expression of RET. To date, 11 different fusion partner genes are reported forming at least 15 different *RET* hybrid oncogenes, designated RET/PTC 1, 1L, 2, 3, Δ3, 4–12 [5, 9; Imkamp and Musholt, submitted for publication, 2006]. Some RET/PTC variants are formed by variable intronic breakpoints in *RET* (RET/PTC 4) or in the partner genes (RET/PTC 1long, Δ3 = 3r2 and 3r3), respectively.

In a linear signaling cascade, the rearranged RET receptor induces a Ras-dependent activation of B-raf, stimulating cell proliferation and matrix invasion of the respective thyrocytes [10] (Fig. 1). Congruously, either (1) a RET/PTC rearrangement or (2) an activating point mutation within *RAS* or (3) a *BRAF* oncogene mutation can be detected in up to 70% of papillary tumors of the thyroid gland [11, 12]. Combined genetic alterations of *RET*, *RAS*, and *BRAF* have only rarely been described [13].

Comparable to *RET*, the proto-oncogene encoding for the tyrosine kinase receptor NTRK1 (synonymously designated TrkA), a transmembrane receptor for the nerve growth factor, is rearranged and oncogenically activated in PTCs, resulting in the chimeric oncogenes trk, TRK-T1, -2, -3 [14, 15].

Rearrangements of the TK domains of the receptors RET and NTRK1, respectively, have been observed in 0–61% of all non-radiation-induced PTCs and in up to 90% of patients with a history of radiation exposure (i.e., therapeutic external beam irradiation to the neck, Chernobyl-associated radio-iodine contamination, etc.) [14, 16, 17].

Expression of *RET* or *NTRK*1 hybrid oncogenes is considered an early event in the process of malignant transformation. Similar to the *RAS* oncogene, the constitutive activation of *RET* and *NTRK*1 leads to cell proliferation but may not be sufficient to induce malignant growth in every case [18, 19]. Recent data suggest that specific hybrid oncogenes display different malignant potential that is associated with distinct morphological phenotypes of the papillary thyroid tumors [5]. The most frequently expressed hybrid oncogene, RET/PTC1, is predominantly found in the classic PTC variant and in papillary microcarcinomas. These PTCs are usually less aggressive and account for the good prognosis of the malignancy in general [14]. On the contrary, PTCs with solid histological pattern or tall-cell variants of PTC—which are associated with aggressive behavior—predominantly express the RET/PTC3 hybrid oncoprotein also found in 80% of the tumors from the Chernobyl area [20, 21].

To summarize, the transforming capacities of the RET/PTC and NTRK1 chimeric receptors in papillary thyroid cancers as well as the molecular background of non-rearranged PTCs remain to be elucidated. There-