

Papillary Thyroid Carcinoma and RET/PTC Oncogenes

Tiroid Papiller Kanser ve RET/PTC Onkogen

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Abstract

As in the whole world, there is also increasing prevalence of Papillary Thyroid cancer (PTC) in our Country. Activation of cellular oncogenes plays a major role in this increasing prevalence. Rearrangement of the RET gene, also known as RET/PTC rearrangement, is the most common genetic alteration identified to date in thyroid papillary carcinomas. The highest RET/PTC prevalence has been reported as 87% in Belarus after Chernobyl. The genetic changes made important effects not only on etiology and but also sometimes on prognosis. Many factors such as geographical differences, radiation, age and genetic differences are effective on these mutations. In particular RET/PTC-1 appears to be associated with PTC of classical and diffuse sclerosing morphology and RET/PTC3 with solid variant morphology. RET/PTC3 is associated with poorer prognosis and more aggressive tumor behavior and may well be a marker for radiation-induced carcinoma, particularly in children. Ret oncoproteins that are expressed in thyroid carcinomas represent possible targets for therapeutic intervention. Such features suggest the possibility of using specific tyrosine kinase inhibitors to block the Ret oncoproteins signaling. *Turk Jem 2007; 11: 114-8*

Key words: Papillary thyroid carcinoma, RET/PTC

Özet

Tüm dünyada olduğu gibi, Ülkemizde de son yıllarda giderek artan Papiller Tiroid Kanseri (PTC) prevalansı vardır. Artan bu prevalansda hücrel onkogenlerin aktivasyonu ön planda gelmektedir. Bu konuda yapılan genetik araştırmalarda RET reseptör tirozin kinaz (TK) mutasyonları en çok saptanan mutasyonlardır. Bunlar arasında en çok görülen RET/PTC1 ve RET/PTC3'dür. Günümüze dek saptanan en yüksek RET/PTC prevalansı %87 ile Çernobil sonrası Belarus'dan bildirilmiştir. Oluşan bu genetik değişiklikler etiolojide önemli olduğu gibi, ilişkili olmakla beraber prognoz üzerine de önemli etkileri vardır. Bu mutasyonun oluşmasında coğrafi farklılıklar, radyasyon, yaş ve genetik gibi birçok faktör etkili olmaktadır. Oluşan RET/PTC onkoproteinini hedef alan yeni ilaçlar prelinik olarak denenmektedir. Bu RET onkoproteinini sinyalini spesifik tirozin kinaz inhibitörleri ile bloklanması fikrinden doğmuştur. Anahtar kelimeler: Tiroid Papiller Kanseri, RET/PTC. *Turk Jem 2007; 11: 114-8*

Anahtar kelimeler: Tiroid Papiller Kanseri, RET/PTC

Introduction

Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy (1), represents up to 80% of all thyroid cancer (2). PTC is found in a variety of morphologic variants, it usually grows slowly and is clinically indolent, although rare, aggressive forms with local invasion or distant metastases can occur (1).

There are some factors that play role and cause risks in occurrence, typical changes and incidences of the thyroid cancers. Ionizing radiation is the strongest risk factor known for the development of thyroid neoplasia (3,4). Nowadays molecular pathology is very important in thyroid cancers. Mutations are leading factors in cancer occurrence.

RET receptor is fundamentally a tyrosine kinase membrane receptor that is controlled at 10th chromosome. Loss-of-function mutations of RET cause Hirschsprung's disease (HSCR) or colonic aganglionosis (5). Gain-of-function mutations of RET are associated with human thyroid cancer (5). Different forms of RET mutations are found in papillary and medullar thyroid carcinomas (6, 7). The clinical impact of this finding is that family members at-risk of hereditary MTC may be screened by genetic analysis to distinguish those carrying or non-carrying the mutation (6, 7). Rearrangement of the RET gene, also known as RET/PTC rearrangement, is the most common genetic alteration identified to date in thyroid papillary carcinomas (8, 10).

RET gene: It is described by Takahashi in 1985 as a new oncogene in chromosome 10q11.2 region. It codes tyrosine kinase receptors (RTC

family). It primarily originates from neural-crest and is expressed from urogenital cells. Its weight is 170kDa. It controls peripheral neural system, morphogenesis of kidneys and spermatogenesis. In the extracellular part of the RET gene there are 4 cadherin-like and CRD-cysteine rich parts. Transmembrane part is hydrophobic. Cytoplasmic tyrosine kinase (TC) part contains RET 9, RET 43 and RET 51. Cadherin containing part is Ca²⁺ dependent (Figure-1). 27 of the 28 aminoacides (aa) is alike in all life forms. It is in the endoplasmic reticulum as a 150kDa immature protein form. It has three isoforms as a result of alternative bonding with the numbers 9-43-51 at the C side. RET9 and RET51 have different signal patterns. RET51 knockout rats are found normal. In RET 9 there is renal malformation and deficiency in enteric innervations. RET 51 controls life and tubulogenesis (5).

RET signal pattern

GFL (ligand family)-TGF (Transforming growth factor) family have 4 different ligands. (GDNF-glial cell derivative neurotrophic factor, NRTN-nurturin, ARTN-artemin, PSN-persephin) (Figure-2). It has interaction by means of 4 co-receptors (GFR-1 (familial receptor alpha1) –GDNF, GFR-2-NRTN, GFR-3-ARTN, GFR-4-PSN). They control 4 important phosphorylation mechanisms-Tyr905;1015;1062;1096. Their distribution is different in dissimilar cells.

RET-PTC

Somatic chromosomal rearrangement is seen in different rates according to ethnical and geographical differences. RET-TC part makes fusion with 5'-part of the heterologous genes and they compose chimeric RET/PTC oncogenes (5).

RET/PTC Rearrangement- transforming mechanisms

1. Promotor transcription change of the fusion gene-RET expression of the epithelial follicular thyroid cells (unseen in normal).
2. The emergence of the structural active chimeric oncoproteins in the cytoplasmic region.
3. The dimerization caused by the fusion regions and formation of excess amount of RET- kinase activation is the way it happens (5) (Figure 3).

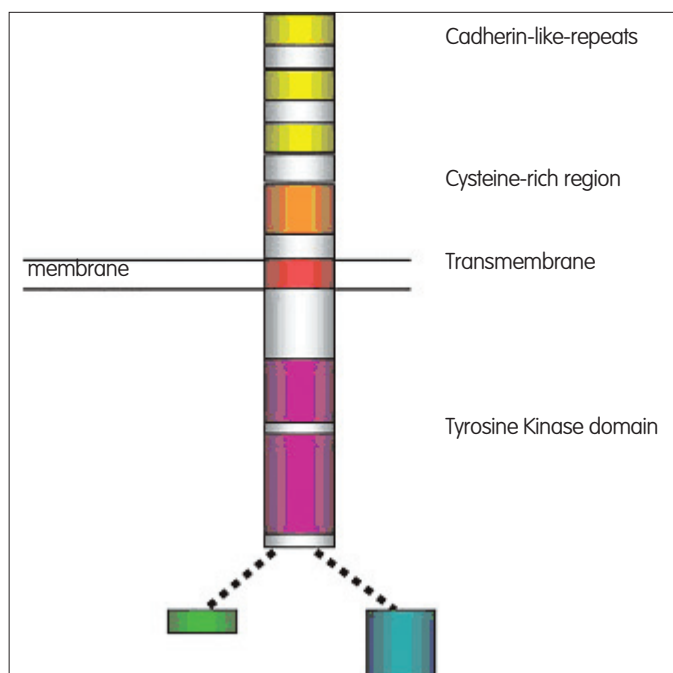


Figure 1. Schematic structure of RET proteins showing the four extracellular cadherin-like repeats, the cysteine-rich region and the transmembrane and split tyrosine kinase domains. The two major isoforms, RET9 and RET51, are indicated. Modified from [5]

Normal-H4- induces apoptosis in thyroid follicular cells. Fusion H4(PTC1) is dominant and has negative effect. It inhibits the apoptosis of the cancer cells. Radiation accelerates the rearrangement. H4(PTC1) and RFG/ELE1 is notified 57-87% in first 8 years. In patients taking therapeutic X-rays the expression begins in hours. Modificatory genes are OPN and CXCR4.

Activation of RET/PTC oncogene and other cellular oncogenes and development of the Papillary Thyroid Cancer

Mutations are the leading factors of the cancer growth. Protein produced by mutation induces and continues monoclonal neoplasm growth by integrating the gene or the genes that control tissue growth and differentiation not only directly but also indirectly. The proteins produced by mutations which causes neoplasms are called as oncogenes. They have dominant characteristics, which means an oncogene in an allele causes cancer even though the other is normal (11). There are now at least 15 types of RET/PTC rearrangements involving RET and 10 different genes (12). The RET/PTC oncogenes, activated forms of the RET proto-oncogene, almost exclusively found in PTC (13). Somatic rearrangement of the tyrosine kinase receptor RET is restricted to papillary thyroid carcinoma (PTC) (14). Rearrangements of the RET receptor tyrosine kinase gene generating RET/PTC oncogenes are specific to papillary thyroid carcinoma (PTC), the most frequent thyroid tumor (15). Reverse transcription-polymerase chain reaction (RT-PCR) perform for RET/PTC1, RET/PTC2, and RET/PTC3 rearrangements and immuno-histochemical staining for the RET gene (16-18). RET/PTC rearrangements are thought to be tumor-initiating events; however, the early biological consequences of RET/PTC activation are unknown (19). In Papillary cancer, we can notice genomic differentiations of RET receptor without detailed definition of function (20). In papillary cancers an inversion and translocation are detected in the genes that synthesis parts of this receptor. Finally mutant receptor proteins occur, therefore RET/PTC1, RET/PTC2, RET/PTC3 receptor mutations are detected in papillary cells. The prevalence of RET/PTC1, RET/PTC2, and RET/PTC3 has been found to vary between 0% and 20% in most series of sporadic (non-radiation-induced) PTCs (21).

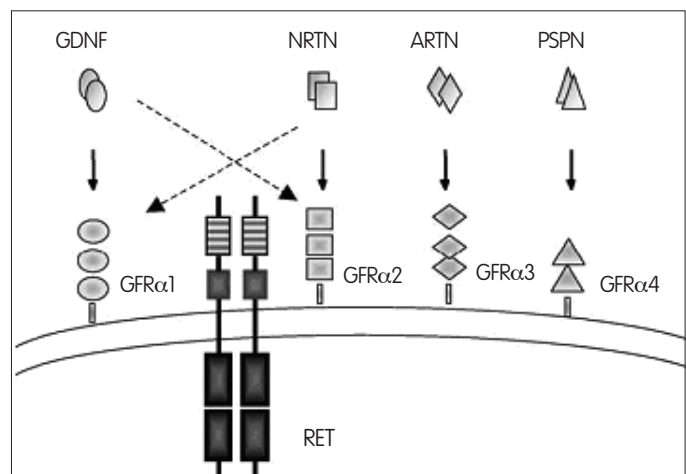


Figure 2. GDNF-family ligands (GFLs) and their receptors. Homodimeric GFLs activate the transmembrane RET tyrosine kinase by binding with high affinity to different GPI-linked GFR α receptors. Binding of the ligand-GFR α complex to RET triggers its homodimerization, phosphorylation of tyrosine residues and subsequent intracellular signaling. Solid arrows indicate the preferred functional ligand-receptor interaction, dotted arrows indicate putative cross-talk. GFR: proteins consist of three, or two in the case of GFR α 4, globular cysteine-rich domains. GFR: GDNF-family receptor- α ; NRTN, nurturin; ARTN, artemin; PSPN, persephin. Modified from [5]

RET/PTC1: The most prevalent rearranged chimeric transcript detected is ret/PTC-1, resulting from fusion of the tyrosine kinase-encoding region of c-ret to the 5' terminal sequences of a gene termed H4. (2). In RET/PTC1 it is noticed as a paracentric inversion between q11.2 and q21 regions of the chromosome 10. RET/PTC1 oncogene activates a pro-inflammatory program, provide a direct link between a transforming human oncogene, inflammation, and malignant behavior (22). Although the RET/PTC1 mutation was frequently identified in Tasmanian (Australia) PTC, there was no clear relationship between RET/PTC1 and recent PTC incidence trends (23). In a comparative study of children with sporadic PTC in the US and children with exposure to radiation in Belarus; solid variant has been detected more common (37%) and RET/PTC3 was the most frequent (58%) in the group exposed to radiation while in the sporadic group papillary pattern was in high levels (70%) and RET/PTC3 was the most common (47%) (24).

In particular, ret/PTC-1 appears to be associated with PTC of classical and diffuse sclerosing (2).

RET/PTC2: It is seen as a translocation in the 17th. chromosome and it is the third frequent mutation.

RET/PTC3: It is second frequent mutation and is caused by a translocation on the ELE-1 gene region of the 10th chromosome. These occur as a result of paracentric inversion (2). Ret/PTC-3 was first described

in children exposed to ionizing radiations a consequence of the Chernobyl accident (2). There are many studies supporting its relation with radiation. The highest rates have been reported in PTCs, which occurred after the Chernobyl accident (overall prevalence 87%) with ret/PTC-3 that is the most common rearrangement detected (58%), this is followed by ret/PTC-1 (16%) and ret/PTC-2 (3%) (2).

The tall-cell variant (TCV) of papillary thyroid carcinoma (PTC), characterized by tall cells bearing an oxyphilic cytoplasm, is more clinically aggressive than conventional PTC (27). RET/PTC3 has been detected in all of the tall cell variants of PTC (27). RET/PTC-3 has been correlated with solid variant morphology in PTC (2, 28). Solid variant is a rare and poorly characterized variant of papillary thyroid carcinoma (29). Ret/PTC-3 has been correlated with poorer prognosis, aggressive tumor behavior, and distinct solid morphology (2). The prevalence of RET/PTC3 activation in PTC is high (%85), and RET/PTC3 is the only type of activation identified in Hong Kong, China (30).

RET/PTC prevalence of specific rearrangements varies according to geographical location (2). Low prevalence rates have been demonstrated in Saudi Arabia (3%), Germany (8%), Japan (only ret/PTC-1 assessed, ranging from 0–9%), and France (11%) (2).

It is particularly common in papillary cancers related with radiation and it has been reported in high levels (87%) in Chernobyl (2) High prevalence of RET tyrosine kinase activation was in Mexican patients with papillary thyroid carcinomas (2, 31). TRK is neural growth factor receptor. It is basically a tyrosine kinase membrane receptor. In some papillary cancers, mutation is also shown in this receptor. The way they cause neoplasia is not definite for both TRK and RET.

WT (Wild-Type) RET: It is common in PTC patients with less differential and more aggressive disease (32). In aggressive PTC TC positivity is detected 45% and it is thought to be an independent risk factor for aggressiveness (32).

Point mutation of RET/PTC and B-raf and N-ras: The RAS-RAF-MEK-ERK-MAP kinase pathway mediates the cellular response to extracellular signals that regulate cell proliferation, differentiation, and apoptosis (33,34). A high (47%) prevalence of RET/PTC rearrangements was found in Hyalinizing trabecular tumor (HTT). By contrast, neither B-raf nor N-ras mutations were found in HTT (35). These findings suggest that N-ras, and B-raf proteins, even if they are RET/PTC, may act along the same signaling cascade, the biological and morphological outcome of their oncogenic activation is not completely overlapping (35).

Mutation of the RAS proto-oncogene occurs in various thyroid neoplasms such as papillary thyroid carcinomas (PTCs), follicular thyroid adenomas and carcinomas. A second genetic alteration frequently involved in PTC is RET/PTC rearrangements (33). Mutated BRAF gene may cooperate with RET/PTC to induce the oncogenesis of PTC (33). There are many types of B-raf mutations defined in thyroid neoplasm. Recently B-raf mutations are also defined in other diseases. Activating mutations of BRAF were reported recently in most melanomas and a small proportion of colorectal tumors (36).

A genetico-clinical association analysis showed a statistically significant correlation between BRAF mutation and development of PTCs of the classic papillary histo-type (37). On the contrary, no link could be detected between expression of BRAF(V599E) and age in diagnosis, gender, dimension, and local invasiveness of the primary cancer, presence of lymph node metastases, tumor stage, and multi focalization of the disease (37).

Activating point mutation of the BRAF gene resulting in V600E (previously designated as V599E) is a common event in thyroid papillary carcinoma that is found in approx 40% of this tumor (34, 36). It has

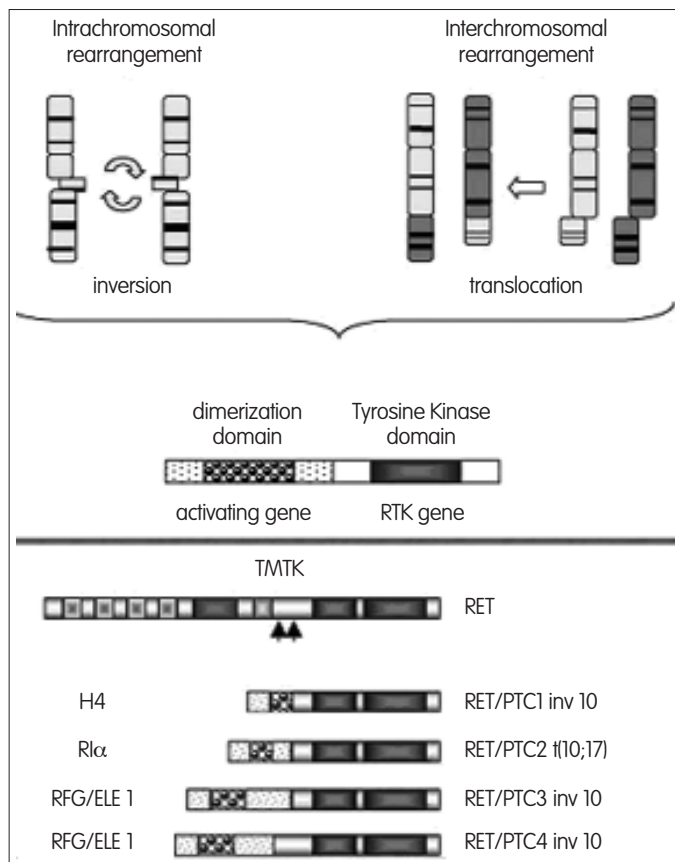


Figure 3. Mechanisms of chromosomal rearrangements generating transforming fusion genes. RET/PTC oncogenes, which are found in papillary thyroid carcinomas, are chimeric genes resulting from chromosomal rearrangements that lead to the fusion of the RET tyrosine kinase domain to different heterologous genes. The rearrangements involving H4, R1α, and RFG/ELE1 partner genes are shown. Arrows indicate breakpoints in papillary thyroid cancer. TM, transmembrane domain; TK, tyrosine kinase domain; CC, coiled-coil domain. Modified from [5]

strong association with classical papillary carcinoma and tall cell and possibly with Warthin-like variants. This mutation occurs also in thyroid that is poorly differentiated and anaplastic carcinomas, usually in the areas containing papillary carcinoma (34, 38). Radiation-induced tumors demonstrated a low prevalence (4%) of BRAF point mutations and high prevalence (58%) of RET/PTC rearrangements. Sporadic papillary carcinomas revealed a clearly distinct pattern, with 37% of tumors harboring BRAF mutations and 20% RET/PTC rearrangements (39). In the PTC tumor both B-raf mutation and RET/PTC occurrence is uncommon (38). Those two occur as alternative events and they are important molecular determinants (38). Previous studies detected ret/PTC rearrangements more frequently in PTC from children than adults, and recent reports describe a high incidence of BRAF T1796A transversion in adult PTC. However, BRAF mutations have not been adequately studied in PTC from young patients (40,41). These results demonstrate a significant difference in the molecular genetic profile of sporadic and radiation-induced thyroid tumors (40-42).

Mutation of the RAS proto-oncogene occurs in various thyroid neoplasms such as papillary thyroid carcinomas (PTCs), follicular thyroid adenomas and carcinomas (33). There is correlation between the follicular variant of PTC and point mutation of RAS in both clinical and pathological characteristics. The follicular variant (FV) of papillary thyroid carcinoma is characterized by a follicular growth pattern and cytological features of papillary carcinoma. ret/PTC rearrangements are common in classic papillary thyroid carcinoma (PTC) and PAX8-PPAR gamma and ras mutations in follicular thyroid carcinoma. Their prevalence in FV has not been established (43). Follicular adenomas and carcinomas show frequently the presence of mutations in one of the three ras genes (44).

Tumor suppressor genes: They prevent tumor growth by suppressing the duplication. Their mutations end up without a lack of this function and the tumor growth accelerates (45). Both of the two alleles must be mutant to cause a carcinogenic effect. P53 is an important suppressor gene and it is also found in the malignant thyroid tumors (44). With immunocytochemical methods P53 is detected in 0-12% of the high-differential thyroid cancers, in 80% of the invasive papillary cancers with lymphatic metastases and in 70% of the anaplastic cancers. In P53 function deficiency, in the thyroid carcinomas of the mice with RET/PTC1 anaplasia and invasiveness increase (46).

The effect of RET/PTC on the prognosis of PTC

Related with the type and frequency of the RET/PTC; including the age of the disease detection, the age of the radiation exposure, cytological variant, sex, lymphatic metastasis, their clinical and epidemiological characteristics and correlations were investigated and some contradictory results were found.

Jiang et al study (14) suggests that the rearrangement of the ret proto-oncogene may be involved in the development of distant metastases in patients with papillary thyroid carcinomas. However, a larger clinical study will be required to verify this observation (13). No difference was found in RET/PTC rearrangements not only between samples taken from irradiated (external x-ray) or not irradiated adult patients but also between children and adults with thyroid cancer of natural occurrence in Belarus and Italy. In this study no significant correlation was observed between the frequency and / or the type (RET/PTC1 or RET/PTC3) of RET/PTC rearrangement and clinical-epidemiological features of the patients such as age at diagnosis, age at exposure, histological variant, gender and tumor-node-metastasis (TNM) categories (47).

Basolo et al. (48) investigated the prognostic meaning of RET/PTC rearrangement on the long term outcome of PTC. No correlation

was found between RET expression and other parameters such as sex, age at diagnosis, tumor class and histological variant (48). In another study from Italy, none of the genetico-clinical analyses showed any significant association between RET/PTC expression and the clinical and pathological features of the cancers (9). We investigated the correlation between RET/PTC oncogene expression and the known prognostic factors of papillary thyroid carcinoma in Korea, but this study failed to prove that RET oncogene expression is associated with alleged prognostic factors (16).

In a study from Mayo Clinic, molecular analyses showed a similar prevalence of RET /PTC rearrangements in solid variant and classical papillary carcinoma (11).

RET/PTC oncoproteins: molecular targets of new drugs

Ret oncoproteins expressed in thyroid carcinomas represent possible targets for therapeutic intervention. Oncogenic activation of the receptor tyrosine kinase encoding RET gene occurs typically by gene rearrangement in papillary thyroid carcinomas (PTC) (49,50). These genetic alterations lead to the expression of deregulated products characterized by ligand-independent activation of the intrinsic tyrosine kinase of Ret. Such features suggest the possibility of using specific tyrosine kinase inhibitors to block the Ret oncoproteins signaling. Inhibition of Ret oncoprotein functions could thereby represent a specific therapeutic approach (49, 50).

The present report summarizes the cellular effects of the arylidene 2-indolinone Ret inhibitor RPI-1 (formerly Cpd1) on the human PTC cell line TPC-1 which spontaneously harbors the RET/PTC1 oncogene. The results provide evidence for that RPI-1 is able to inhibit cell growth and to interfere with Ret/ptc1-driven signaling (49, 50).

These findings in a cellular context that are relevant to the pathological function of RET oncogenes support the role of Ret oncoproteins as useful targets for therapeutic intervention, and suggest RPI-1 as a promising candidate for preclinical development in the treatment of thyroid tumors expressing RET oncogenes (49,50).

Result

After Chernobyl disaster, molecular pathology gain importance in thyroid cancers and studies are held on the role of RET/PTC in PTC. RET's specialty of mutation played an important role in thyroid cancer occurrence. RET/PTC's frequency in PTC differs according to countries and geographical regions. According to the common opinion the effective factors on RET/PTC frequency are; exposure to radiation, age and cytological tumor type, but there some contradictory articles about it. There are also some contradictory results published about the effect of RET/PTC to the prognosis.

As understood from here many ideas will be discussed and many studies are needed about RET/PTC in thyroid cancers.

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