

# 20 Years of *RET/PTC* in Thyroid Cancer: Clinico-Pathological Correlations

## ABSTRACT

The *RET/PTC* oncogene has been isolated almost twenty years ago. During these years, the research has given a final answer to several questions. In fact, it has been demonstrated that: a) *RET/PTC* is an early event in the process of thyroid carcinogenesis and has a critical role in the generation of the papillary carcinoma; b) *RET/PTC* activation is essentially restricted to the papillary histotype and to the Hürthle thyroid tumors; c) its incidence increases after exposure to radiations. However, some questions have not found a final answer yet: a) which is the real frequency of *RET/PTC* activation? Likely it is around 20%, but this point is still questionable; b) which other gene modifications are required to lead a thyroid cell carrying a *RET/PTC* oncogene to the malignant phenotype?, and c) is there any correlation between *RET/PTC* activation and clinical parameters? We hope that these questions will have a clear answer in the near future. (**Arq Bras Endocrinol Metab 2007;51/5:731-735**)

**Keywords:** Thyroid; Carcinoma; *RET/PTC*; Transformation

## RESUMO

### Vinte Anos de *RET/PTC* no Câncer de Tiróide: Correlações Clínico-Patológicas.

O oncogene *RET/PTC* foi isolado há quase 20 anos atrás. Durante esses anos de pesquisa várias questões foram solucionadas. Na verdade, já foi demonstrado que: a) o *RET/PTC* é um evento precoce no processo da carcinogênese da tiróide e tem um papel crítico na geração do carcinoma papilífero; b) a ativação do *RET/PTC* está restrita essencialmente ao histotipo papilar e aos tumores de Hürthle; c) sua incidência aumenta após a exposição à radiação. Entretanto, algumas questões permanecem ainda sem uma resposta final: a) qual é a real frequência de ativação do *RET/PTC*? Provavelmente em torno de 20%, mas este número ainda é questionável; b) quais outras modificações gênicas são necessárias para transformar uma célula tiroideana que possui oncogene *RET/PTC* no fenotipo maligno?; e c) existe alguma correlação entre a ativação do *RET/PTC* e parâmetros clínicos? Esperamos que essas questões sejam solucionadas em futuro próximo (**Arq Bras Endocrinol Metab 2007;51/5:731-735**)

**Descritores:** Tireóide; Carcinoma; *RET/PTC*; Transformação

IN 1987, NATURE PUBLISHED the first report of a new oncogene activated in human papillary thyroid carcinomas (PTC). The DNA extracted from the tumors of five PTC patients and from two corresponding lymph-nodal metastases gave rise to transforming *foci* when transfected onto the murine NIH3T3 fibroblasts. Molecular analysis of the tertiary foci originating from these positive samples showed the same Alu pattern after digestion with restriction enzymes, indicating that the same oncogene was activated in all of the tumors positive at the transfection assay (1). After 3 years, this oncogene was molecularly cloned: it was a chimeric gene generated by the fusion of the RET tyrosine kinase domain (TK) with the 5' ter-

## revisão

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minal region of a new gene that was denominated H4 (now CCDC6). This oncogene was denominated RET/PTC depending on the gene involved in this rearrangement and the thyroid cancer histotype from which it was isolated (2). In the following years, at least 10 types of RET/PTC variants have been isolated: all of them consist in the fusion of the TK domain of RET with other genes that provide to the chimeric gene the promoter and the 5' coding region (3). However, apart from RET/PTC1 and RET/PTC3, all the other variants do not seem to have a significant role in PTCs since they have been isolated just in rare cases. In the case of RET/PTC3, the TK domain of RET is fused to the RFG gene (also designated *Elle1/ARA70/Ncoa4*) (4). A chromosomal inversion [inv (10) (q11.2q21)] accounts for the generation of RET/PTC1 (5), whereas a cytogenetically undetectable paracentric inversion within 10q11.2 accounts for the generation of the RET/PTC3 oncogene (3).

The inappropriate expression of RET/PTC in the thyroid follicular cells (where the RET proto-oncogene is not expressed), the mislocalization of the RET TK from the membrane to the cytoplasm, the absence the extracellular regulatory region of RET and the presence of coiled-coil domains in the RET-partner coding sequences, that favor the dimerization process, account for the oncogenic activity of the chimeric RET/PTC gene.

After the identification and the cloning of the RET/PTC oncogene, several questions have been raised.

### **IS RET/PTC INVOLVED IN THYROID CARCINOGENESIS OR IS IT JUST AN ASSOCIATED EVENT?**

RET/PTC oncogene is able to transform the rat thyroid cells PC Cl 3 (3), which show all the typical markers of thyroid differentiation. In fact, the PC RET/PTC cells change morphology, are no longer dependent on TSH for growth and lost all of their differentiated functions. However, they were not able to grow in soft agar and they were not tumorigenic when injected into athymic mice. This result is consistent with the low aggressiveness most frequently shown by human papillary carcinomas.

Moreover, transgenic mice expressing a TG-mediated expression of RET/PTC oncogenes develop thyroid papillary carcinomas with features very similar to those shown by human PTCs with the presence of nuclear grooves and ground glass cells (6). Therefore, we can certainly assess that the RET/PTC oncogene has a critical role in thyroid carcinogenesis.

### **IS RET/PTC ACTIVATION AN EARLY OR LATE EVENT IN THE PROCESS OF THYROID CARCINOGENESIS?**

The results obtained during these years clearly demonstrate that RET/PTC oncogene activation is an early event in the process of thyroid carcinogenesis. In fact, immunohistochemical and RT-PCR analyses first allowed the detection of RET/PTC activation in a high percentage of occult thyroid carcinomas (7). Moreover, RT-PCR analysis on the RNA extracted by Laser Capture Microdissection (LCM) from the papillary cells has allowed the detection of RET/PTC activation in thyroid nodules with incomplete morphological evidence of papillary carcinoma (8).

### **WHICH IS THE PREVALENCE OF RET/PTC IN PAPILLARY THYROID CARCINOMAS, AND IS IT SPECIFIC FOR THIS TUMOR TYPE?**

Two studies have shown RET/PTC1 rearrangements in hyalinizing trabecular variant of papillary carcinoma suggesting that this tumor represents a variant of papillary carcinoma rather than a separate entity (9). Conversely, almost all the data published so far exclude RET/PTC activation in adenomas, follicular carcinomas, and anaplastic thyroid carcinomas (ATC) (10).

Two different studies evidenced a significant number of RET/PTC activation, mainly RET/PTC1, in Hürthle thyroid cell adenomas and carcinomas, characterized by the presence of oncocytes (also called Askanazy or oxyphil cells), but not in Hürthle hyperplastic nodules (3,11). The absence of RET/PTC activation in Hürthle hyperplastic lesions suggests that RET/PTC activation might represent a secondary event in the generation of Hürthle neoplasms.

A long-standing controversy exists with respect to the prevalence of RET/PTC in papillary thyroid carcinomas. Indeed, the reported frequency of RET/PTC in papillary carcinomas in different studies varies from 0 to 87% (12). In part this is likely due to geographic variability. However, this cannot serve as the only explanation since a striking variability in the frequency has been reported in the same geographical regions (8 and 85 in Australia, 5 and 22 in Canada). In the USA, the five largest series report the frequency of RET/PTC ranging from 11 to 43% (12).

It is very interesting the work that has been recently done by the group of Nikiforov analyzing the same tumors with five different techniques. According to their data, it is likely that a "low-sensitive"

RT-PCR and Southern blot are the most reliable techniques to detect RET/PTC activation. By the use of these techniques the cases of PTC positive for RET/PTC are about 20% (12). This result is consistent with that reported by Santoro et al. (13).

Several reports have described multiple activation of the RET/PTC isoforms in the same patient. These findings are more frequent in radiation-induced tumors. These results would account for a plurifocal origin of thyroid papillary carcinomas occurring in genetically predisposed individuals. Alternatively, RET/PTC may also be in several cases a common secondary event in the process of thyroid carcinogenesis.

### **DOES RET/PTC ACTIVATION OCCUR IN NON-THYROID NEOPLASIAS? AND WHY DOES IT OCCUR JUST IN THE THYROID?**

The results obtained so far seem to exclude the presence of RET/PTC in the neoplasias of tissues other than thyroid, at least in a significant number of cases. In fact, the analysis of more than five hundred tumors of different origin for RET/PTC activation gave negative results. Moreover, in the period ranging between 1980 and 1990, a huge number of DNAs originating from different neoplasias has been analyzed by many laboratories around the world, in a NIH3T3 transfection assay: there has not been any report of RET/PTC activation in non-thyroid neoplastic tissues. The group of Nikiforov proposed a possible explanation for this thyroid specificity. This group showed that, while RET and H4 loci are about 30 megabases apart in the linear map of chromosome 10, they frequently juxtapose in nuclei of thyroid cells but not in other cell types. This contiguity would provide the structural basis for the nonhomologous recombination of the two genes (14).

### **IS RET/PTC ACTIVATION ASSOCIATED WITH RADIATION EXPOSURE?**

A sharp increase in the incidence of pediatric thyroid papillary cancer with a high prevalence of the solid-follicular tumors was documented after the Chernobyl power plant explosion. All of the studies performed analyzing these PTCs show a strong correlation between the solid variant PTC and the RET/PTC3 activation (3,16). This result has been confirmed by transgenic RET/PTC3 mice: they develop PTCs with solid features (3). It is also noteworthy that a signifi-

cantly higher prevalence of RET/PTC3 activation was observed in the most heavily contaminated areas suggesting a preferential formation of this type of rearrangement after high radiation doses. The analysis of PTC, which developed after a long latency period in Belarusian children showed a 1:1 ratio of RET/PTC3 and RET/PTC1. This suggests that RET/PTC3 may be typical for radiation-associated childhood PTC with a short latency period, whereas RET/PTC1 may be a marker for later-occurring PTC of radiation-exposed adults and children (17). Two different studies performed in France and the USA on radiation-associated thyroid tumors originated after therapeutic radiations show RET/PTC rearrangements at a very high rate. However, in contrast with the radiation-associated tumors originated from the atomic accident of Chernobyl, the most frequently detected chimeric gene was RET/PTC1 instead of the RET/PTC3 (3).

### **IS RET/PTC ACTIVATED IN HASHIMOTO'S THYROIDITIS?**

Two studies have shown RET/PTC1 activation in a significant number of Hashimoto's thyroiditis (HT) cases, whereas another study detected no RET rearrangements in Hashimoto's (18). Another group describes RET/PTC rearrangement in non-malignant thyroid tissue associated to lymphocytic thyroiditis (LT), whereas it was absent in the pure LT areas. A very recent study shows that sixty-eight percent (15 of 22) of HT were positive by FISH analysis (19).

Therefore, the activation RET/PTC in HT is still controversial. In our opinion it does not appear unexplainable: a RET/PTC activation in thyroid cells would not be able to induce the malignant phenotype in these cells. However, the presence of RET/PTC cells might be able to evoke a strong immunological response. A secondary molecular event, that means another gene alteration, could determine the appearance of the thyroid cancer. This hypothesis seems supported by the presence of lymphoid infiltration in thyroids of transgenic mice carrying the RET/PTC oncogenes. An alternative hypothesis may be that free radicals production, cytokine secretion, cellular proliferation and other events related to the HT inflammation trigger the occurrence of the RET/PTC rearrangement in follicular cells "predisposed" to it by an unstable chromatin conformation. Similar mechanisms may underlie the occurrence of a low level of RET rearrangements as a secondary phenomenon in papillary carcinoma subsets.

### DOES RET/PTC ACTIVATION CORRELATE WITH SOME CLINICAL DATA?

Several studies have tried to associate the presence of a rearranged RET with clinical parameters. Some studies have found a certain tendency of the association of RET/PTC activation with lymphatic involvement in otherwise low-risk patients of young age, lower recurrence rate and improved survival with small tumors (3). Another study by Puxeddu et al. (20) did not show any correlation between RET/PTC activation and age, sex, tumor size, staging, number of neoplastic foci, and histological subtype. It is likely that these studies have been all impaired by the lack of a unique and validated technique to detect RET/PTC rearrangements.

### WHICH GENE ALTERATIONS ASSOCIATE TO RET/PTC TO ACHIEVE THE COMPLETE MALIGNANT PHENOTYPE IN HUMANS?

This question does not have any answer yet. The model of the RET/PTC transgenic mice suggests that other gene alterations are involved in the process of the RET/PTC-induced carcinogenesis. In fact, thyroid neoplasias do not occur in all of the RET/PTC transgenic animals, and they appear only after long latency period (7-10) months, and not all the thyroid cells go towards the neoplastic phenotype. This clearly means that other molecular events are required for the development of thyroid carcinomas.

### CONCLUSIONS

RET/PTC activation represents a feature of a significant number of PTC where it represents in most of the cases a very early event, with no or very low incidence in adenomas, follicular and anaplastic carcinomas. Transgenic animal models clearly support a critical role of RET/PTC in the process of thyroid carcinogenesis. Even though several isoforms of RET/PTC have been found, RET/PTC1 and RET/PTC3 are the most frequent forms, prevailing the first in the classical papillary subtype, the second in the solid subtype. The RET/PTC activation has been found also in Hürthle adenomas and carcinomas, where it may represent, at odds with PTC, a secondary event. No clear association has been found between RET/PTC activation and clinical features, likely because of the lack of a unique and validated technique to detect RET/PTC rearrangements.

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