5. MOLECULAR EPIDEMIOLOGY OF THYROID CANCER

MARTIN SCHLUMBERGER
Service de Médecine Nucléaire, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif, France

INTRODUCTION
Molecular biology studies have greatly enhanced our knowledge of thyroid tumorigenesis, although their impact in clinical practice is still negligible.

Most benign and malignant thyroid tumors have a monoclonal origin, suggesting that genetic events are responsible for their occurrence (34). These may involve the activation of oncogenes or the inactivation of tumor-suppressor genes. Several genetic abnormalities (point mutations or gene rearrangements) have been evidenced in human thyroid tumors (review in 14,40,53). Several in vitro and in vivo animal models, including transgenic mice that reproduce the human situations, are also available.

ONCOGENES AND THYROID TUMORS
Tyrosine kinase receptors
Growth factors act on the target cell through interaction with specific membrane receptors, some of which belong to the family of tyrosine kinase receptors. The genes encoding these receptors are frequently involved in the pathogenesis of human cancers, including thyroid cancer. Whenever uncontrolled activation of a tyrosine kinase receptor gene occurs, either through overexpression or activating mutations, increased responsiveness to growth factors or ligand-independent gene activation ensues, both of which then activate the signaling pathways downstream. Three tyrosine kinase receptor
genes are known to be associated with the pathogenesis of papillary thyroid cancer: the met gene through overexpression and the ret and irk genes through gene rearrangements.

Ret /PTC oncogene

MOLECULAR BASIS OF Ret/PTC REARRANGEMENTS. The ret proto-oncogene is a 21-exon gene located on chromosome 10q11-2 that encodes a membrane tyrosine kinase receptor. The ret receptor together with the glial cell line-derived neurotropic factor (GDNF) receptor (GFRα1), an extracellular protein tethered to the cell membrane, form a receptor for GDNF. The ret receptor may also combine with other members of the GFRα receptor family, thereby forming receptors for other peptides (artemin, neuturin, persephin). The ret protein is composed of an extra-cellular domain, with a distal cadherin-like domain and a juxta-membrane domain and an intra-cellular domain with tyrosine-kinase activity. The gene is expressed in a variety of neuronal cell lineages including thyroid C cells and adrenal medulla but is not expressed in normal thyroid follicular cells.

Under normal conditions, the ret ligands induce receptor dimerization and tyrosine trans-phosphorylation of the receptor kinase domain, thus activating the pathways downstream. When the gene is mutated, ligand interaction is no longer needed for receptor activation and the downstream pathways are continuously activated: ret/PTC kinase activity promotes interaction with shc, an intermediate in the RAS-RAF-MEK-MAP kinase pathway. Inappropriate activation of this pathway induces abnormal proliferation and differentiation in many human cancers and also induces genomic instability.

Ret activation was first evidenced by transfection experiments and was initially found exclusively in papillary thyroid carcinoma (PTC). The resulting oncogene was thus called ret/PTC (16,18,40). All activated forms of the ret proto-oncogene are due to chromosomal rearrangements in which the 3′ or tyrosine kinase domain of the ret gene is fused with the 5′ domain of a foreign gene. The foreign gene is constitutively expressed, resulting in permanent expression of the rearranged ret gene. These rearranged genes have coiled-coil domains that activate the ret protein through permanent dimerization. They also lack the intracellular juxta-membrane domain that normally exerts a negative regulatory effect on ret tyrosine kinase activity. Finally, the chimeric protein lacks the extracellular and transmembrane domains and is located in the cytosol. Three major forms of the ret rearrangements have been identified in epithelial thyroid tumors:

Ret/PTC1, is formed through an intra-chromosomal rearrangement fusing the ret tyrosine kinase domain to a gene designated H4, whose function is still unknown.
Ret/PTC2, is formed through an inter-chromosomal rearrangement fusing the ret tyrosine kinase domain to a gene located on chromosome 17 that encodes the R1α regulatory subunit of cAMP-dependent protein kinase A.
Ret/PTC3, is formed through an intra-chromosomal rearrangement fusing the ret tyrosine kinase domain to a gene designated ELE1, whose function is still unknown.

In the three major ret rearrangements (ret/PTC1,2,3), the breakpoints of the ret gene are located in the same intronic region, between exons 11 and 12. Several other
ret/PTC rearrangements have been observed that differ because of the location of the breakpoint in the ret gene or because of the partner gene.

Recent studies have shown that the unique spatial proximity of ret and H4 partner genes in the nuclear matrix of thyroid cells (but not in other cell types) may be a major reason for the development of ret rearrangement following exposure to ionizing radiation and may explain why ret/PTC are found exclusively in papillary thyroid carcinomas (30).

**FREQUENCY OF ret/PTC REARRANGEMENTS.** The frequency of ret/PTC rearrangements in papillary thyroid carcinomas occurring in adult patients who never received neck irradiation during childhood varies between 2.5 and 35% among the different series (3,4,11). Ret/PTC1 and ret/PTC3 are the most frequent rearrangements in these tumors and ret/PTC2 is less frequent. Variations in the frequency and type of rearrangements could be due to differences in the geographical origins of the populations studied or in the sensitivity of the method used to detect the rearrangements. In children with sporadic papillary thyroid carcinoma without a history of radiation exposure, the incidence of ret/PTC-positive tumors is similar to that observed in adult patients of the same ethnic background (3,11). Ret/PTC rearrangements are more frequently found in papillary thyroid carcinomas occurring after exposure to ionizing radiation during childhood, due to external irradiation or to the Chernobyl accident (see below).

In patients who did not previously receive neck irradiation, rearranged ret genes were detected only in papillary thyroid carcinomas. All other tumors studied (thyroidal or non thyroidal) were negative for ret/PTC (48). In two series, 15–21% of thyroid adenomas were ret/PTC positive, but the existence of micropapillary thyroid carcinomas cannot be excluded (20).

That ret/PTC rearrangement is found in papillary thyroid microcarcinomas suggests that it is an early event in thyroid carcinogenesis (59). In patients with multifocal disease, diverse ret/PTC rearrangements were found in different tumors from the same patient, indicating that these tumors had arisen through distinct initiating events (54).

A high frequency of ret/PTC rearrangements has also been found in Hürthle cell papillary thyroid carcinomas (7), and in about 10% of poorly-differentiated thyroid carcinomas, demonstrating that these ret-positive tumors derive from papillary thyroid carcinomas (50). On the other hand, ret/PTC-positive tumors lack evidence of progression to undifferentiated tumor phenotypes (55).

Transfection of the ret/PTC1 gene in normal rat thyroid cells resulted in loss of differentiation and of TSH growth dependency. However, cells were totally transformed only after transfection with ret/PTC and mutated ras genes, suggesting that simultaneous activation of several genes is necessary for tumor progression. Transgenic mice in which the ret/PTC1 gene is expressed in thyroid tissue develop papillary thyroid carcinoma, which is histologically identical to the human cancer (24,49). However, in this model all thyroid cells possess the rearrangement but only a few cells give rise to tumors a few months after birth, suggesting that at least one more mutation is needed to give rise to PTC. The use of the ret/PTC3 gene results in a more aggressive histological and clinical behavior. This is consistent with a more aggressive type of human papillary thyroid carcinomas in which ret/PTC3 can be evidenced (42,56).
5. Molecular epidemiology of thyroid cancer

Trk oncogene
The trk proto-oncogene is located on chromosome 1. It encodes a membrane tyrosine kinase receptor for the nerve growth factor (NGF). Trk expression is restricted to peripheral nerve ganglia.

Activated forms of the trk proto-oncogene are the result of chromosomal rearrangements in which the 3' or tyrosine kinase domain of trk is fused with the 5' domain of a foreign gene (17,40). The foreign gene is constitutively expressed giving rise to permanent expression of the rearranged trk gene. These genes have domains that induce trk activation through permanent dimerization. All the breakpoints in these chimeric genes are located in the same trk domain.

Several rearrangements have been found in human thyroid tumors:

N-trk is formed through an intra-chromosomal rearrangement fusing the trk tyrosine kinase domain with the 5' region of the non muscular tropomyosine gene.

Trk-T1 and trk-T2 are formed through fusion of the trk tyrosine kinase domain with the 5' region of the translocated promoter region (tpr) gene.

Trk-T3 is formed through fusion of the trk tyrosine kinase domain with the 5' region of a gene called tag (trk activating gene).

Trk rearrangements have only been found in papillary thyroid carcinomas. Their frequency is lower than that of ret/PTC, ranging from 0 to 10% (3,5,42).

Met oncogene
The met proto-oncogene encodes a membrane tyrosine kinase receptor. Its ligand is the hepatocyte growth factor (HGF) or scatter factor (SF). HGF-SF is a potent mitogen for epithelial cells and promotes cell motility and invasion.

The met proto-oncogene can be activated either as a result of rearrangement with unrelated sequences (this mechanism is not found in thyroid carcinoma) or through overexpression. Overexpression of the met oncogene was found in about 50% of papillary thyroid carcinomas and this may be a factor in metastatic spread (9). Negative or low met oncogene expression has been found in the other histologic types.

A relationship has been found between ras and ret activation and mg overexpression in human thyroid epithelial cells. This overexpression may in turn sustain their growth through the action of HGF secreted by stromal cells.

Defects in the intracellular signaling pathway: ras and b-raf
The ras genes (H-ras, K-ras and N-ras) encode a 21 kD protein (p21) involved in signal transmission from cell membrane receptors to growth factors to the nucleus. The ras gene is activated by point mutations in codon 12 or 61 and sometimes in codon 13 or 59.

Ras mutations were initially found in up to 50% of benign or malignant thyroid tumors and was the most frequent genetic alteration found in these tumors. All three ras genes (H, K and N) were found to be activated at a similar frequency (11–15%) in thyroid tumors. No predominance of mutations in critical codons (12,13 or 61) or in base substitution was reported. The frequency of ras mutations in papillary thyroid
tumors is in general lower than in follicular tumors and varies from 0 to 60% in different series (6,25,35,52,62). In subsequent studies, controversial results were reported and a recent review found that the frequency of ras mutations was lower than initially reported: ras oncogene mutations were found more frequently in follicular carcinomas (34%) than in benign adenomas (19%), codon 61 being the most frequently involved; ras mutations are more rarely observed in other types of thyroid tumors, being present in 11% of papillary thyroid cancers (58).

The tumorigenic role of the ras gene in the thyroid has been studied in normal follicular cells transfected with a mutated ras gene. Under such conditions, follicular cell proliferation is increased and the expression of differentiation markers such as thyroglobulin, thyroperoxidase and NIS is reduced or abolished. Transgenic mice, with ras gene expression targeted at thyroid cells, develop both thyroid hyperplasia and papillary thyroid cancer (44), and follicular adenoma or carcinoma (47), with a decline in the expression of differentiation markers. Mutated ras proteins stimulate cell division, inhibit cell differentiation and cause genomic instability and facilitate additional mutagenic events.

B-raf gene has been found to be activated by mutation in human cancers, in 66% of malignant melanomas and in less than 15% of colon carcinomas. In melanomas, 98% of the mutations are a missence thymine (T) to adenine (A) transversion at codon 1796, resulting in a valine to glutamate substitution at residue 599 (V599E). This mutation was found in 36% and 69% of papillary thyroid carcinomas but was not found in any of the other types of follicular cell derived tumors (8, 26). Moreover, there was no overlap with ret/PTC, ras and b-raf mutations. Thus, the frequent activation at various points of this pathway may be a key event in the pathogenesis of papillary thyroid carcinoma.

**PAX8-PPARγ1 fusion gene**

Cytogenetic studies of follicular carcinomas have evidenced abnormalities in chromosomes 2 and 3 (19), and the molecular basis for a chromosomal translocation t(2; 3)(q13; p25) was recently reported. The chromosome 2q13 breakpoint lies within the coding region of the thyroid transcription factor Pax8, and the 3p25 breakpoint within the coding region of PPARγ isoform 1 (27). Pax8 (Paired Box 8) is a transcription factor that plays a role in thyroid ontogenesis and in the expression of several thyroid specific genes. PPARγ (Peroxisome Proliferator-Activated Receptor gamma) is a transcription factor belonging to the hormone nuclear receptor family. Through dimerization with RXRα (Retinoid X Receptor alpha), PPARγ plays a role in the regulation of lipid metabolism, the inflammatory process, differentiation, the cell cycle and tumorigenesis. The fusion protein consists of PAX8 paired and homeobox binding domains, and PPARγ1 DNA binding, ligand binding, dimerization, and transactivation domains. When the PAX8–PPARγ1 fusion gene was transfected to heterologous cells, it did not transactivate promoter constructs containing PPAR response elements, either alone or in the presence of troglitazone, the PPAR ligand agonist. The fusion construct did however prevent wild-type PPARγ1–mediated transactivation, indicating that it may have a dominant negative effect. This negative effect may inhibit terminal differentiation and growth suppression induced by PPARγ agonists.
The PAX8-PPARγ1 translocation was found in 26%–63% of follicular carcinomas and in 8–13% of follicular adenomas. It was not found in normal thyroid tissues, nor in nodular hyperplasia, papillary, Hürthle cell and anaplastic carcinomas (27,30,38,39).

In one series of follicular carcinomas, 86% revealed either ras (58%) or PPARγ1/PAX8 (30%) mutations, and there was no overlap between these two mutations, PPARγ1/PAX8 rearrangement was almost exclusively found in follicular carcinomas that occurred at a younger age, that were small and widely invasive; in contrast, ras mutations occurred with a similar frequency in both adenomas and follicular carcinomas; these carcinomas occurred at an older age, were larger and were less invasive. All these data suggest two different pathways in follicular tumorigenesis (39).

The translocation is associated with protein expression. Expression is downregulated in some thyroid carcinomas (1) and is overexpressed in some tumors without a detectable PAX8-PPARγ1 translocation, which suggests that PPARγ may have other translocation partners. Indeed, a novel gene, located at 7q34 and provisionally named FTCF (follicular thyroid carcinoma fusion) was recently detected fused to the 5’ region (exons1–6) of the PPARγ1 gene, leading to expression of a FTCF-PPARγ1 fusion transcript and fusion protein.

**Defects in the TSH stimulation pathway: TSH-receptor gene and gsp oncogene mutations**

TSH stimulates follicular cell proliferation and differentiation by binding to a membrane receptor, the TSH-receptor (TSH-R). The TSH-R belongs to the receptor family with 7 transmembrane domains coupled to G proteins. These are heterotrimeric proteins, composed of three sub-units, α, β and γ. Binding of TSH to its receptor stimulates the enzyme adenylate-cyclase, through interaction with a Gs protein, that in turn increases the intra-cellular concentration of cAMP. This acts as a second messenger stimulating protein kinase A (PKA). Activated PKA phosphorylates different target proteins and particularly, the cAMP-responsive transcription factor (cAMP responsive element binding protein, or CREB) in the nucleus. Other pathways may be involved in the intra-cellular transduction of the message.

Several point mutations activating the TSH-R have been described in toxic adenomas with wide variations in frequency (from 10% to more than 80%) between the different series. Such differences may be explained by geographical variations, patient selection but also because different regions of the TSH-R were studied. Most activating mutations are found within or near the third intra-cellular loop, a region implicated in the interaction with the Gs protein. Transfection experiments have shown that the mutated TSH-R is constitutively activated, but differences exist between different mutations in terms of the extent of the increase in basal cAMP, the activation of signal transmission and response to TSH stimulation (57).

Activating point mutations in one of the 2 critical codons of the α subunit of the Gs protein gene (then called gsp) have been found in 7 to 38% of toxic adenomas (46, 57). As a result, mutations were found in these 2 genes in 40–60% of hyperfunctioning adenomas. It may be hypothesized that in negative tumors, alterations in another gene participating in the cAMP pathway may be responsible for the phenotype. Activating
mutations of the TSH-R have also been found in the rare hyperfunctioning follicular carcinomas with high radioiodine uptake and thyrotoxicosis (45).

In transgenic mice, gsp and TSH-R activating mutations have been demonstrated to play a role in the development of hyperfunctioning thyroid tissue. The expression of an A2 adenosine receptor gene (equivalent to TSH-R) in thyroid tissue induces diffuse thyroid hyperplasia and early hyperthyroidism (57). The expression of gsp in thyroid tissue induces focal hyperfunctioning, that is equivalent to that of a human hyperfunctioning nodule with late hyperthyroidism (33).

Activating mutations of gsp and TSH-R have also been found in hypofunctioning benign and malignant follicular thyroid tumors but at a low frequency (<10%) (45, 51). In follicular thyroid carcinomas they are restricted to a subset of tumors with high basal adenylate cyclase activity. These data suggest that gsp and TSH-R mutations may participate in the tumorigenesis of some hypofunctioning thyroid tumors, by conferring a growth advantage to a cellular clone in which another yet unknown genetic alteration has already abrogated the growth-limiting mechanism, which normally down-regulates the response to cAMP (53).

**Tumor suppressor genes and other genetic abnormalities**

Tumor suppressor genes code for proteins that normally inhibit or restrict cell division. They become tumorigenic through loss of function and tend to act in a recessive manner. One allele is usually lost as part of a large deletion of chromosomal material, while the other allele is inactivated by a point mutation.

No genomic abnormalities were found in *Rb*, the retinoblastoma gene. However, transgenic mice with thyroid specific expression of a human papilloma virus, type E7, develop nodular goiter and subsequently papillary and follicular thyroid carcinomas (28). This protein can functionally inactivate the Rb protein, suggesting that the latter acts in the negative control of cell proliferation (14, 53).

A high frequency of inactivating point mutations (22 to 83%) in the *p53* gene were observed in anaplastic but not in differentiated thyroid carcinomas (10, 13, 23). These data suggest that inactivation of the *p53* gene may be a key event in progression from differentiated to anaplastic carcinoma and that this alters cell differentiation. Conversely, the bcl2 protein is expressed in differentiated thyroid carcinomas but is absent in anaplastic tumors (41).

Mutations in the adenomatous polyposis colonic (*APC*) gene probably contribute to the development of thyroid cancer seen in familial adenomatous polyposis, but linkage analysis excluded the APC gene as a rare susceptibility gene for familial papillary thyroid carcinoma.

Germline inactivating mutations in the *PTEN* gene are found in 80% of patients with Cowden’s disease (multiple hamartomas, breast and follicular thyroid tumors) (12). If no hamartomas are present, *PTEN* germline mutations are found in only 5% of the families with breast and thyroid tumors. *PTEN*, the phosphatase and tensin homolog gene is an inhibitor of Akt1, a critical intermediary in several Phosphatidylinositol 3 (P13) kinase signaling transduction pathway. In sporadic follicular thyroid carcinoma, mutations are rare but *PTEN* gene expression is decreased and expression
and phosphorylation of Akt are increased and this may be involved in follicular pathogenesis (43).

Linkage studies have permitted chromosomal mapping of at least 3 syndromes with a preponderance of familial PTC (29). A syndrome of familial PTC together with papillary renal neoplasia has been mapped to 1q21. This syndrome is clinically and genetically distinct from other familial tumor syndromes and is not a variant of familial papillary renal carcinoma caused by inherited activating mutations of the MET proto-oncogene. A familial syndrome characterized by PTC alone has been mapped to 2q21. Two different studies reported genetic linkage to 19p13.2 of a large kindred with different clinical features: in one family all thyroid tumors were oxyphilic (TCO) and many were benign; in the other one no oxyphilic changes were found and all tumors were PTC.

There are also a number of familial disorders potentially related to familial PTC, including familial goiter syndromes, one syndrome located at 14q and another at Xp22. Finally, thyroid nodules may be associated with either hypothyroidism or hyperthyroidism when gene mutations are components of pathways of thyroid metabolism or its regulation.

Loss of genetic sequences has been described in the long arm of chromosome 11 (11q13) in sporadic follicular thyroid tumors and, as described above in the short arm of chromosome 3, but only in follicular carcinomas (19,40).

Simian virus 40 (SV40) large T antigen (Tag) sequences have been detected in several human tumors, and are believed to be the result of SV40 infections. The presence of the Tag region of SV40 has been demonstrated in 66% of papillary thyroid cancer and in 100% of anaplastic thyroid cancer, as well as in normal thyroid tissue adjacent to these tumors (60). The high prevalence of SV40 footprints has been interpreted as a possible participation of this oncogenic virus in the onset/progression of specific thyroid carcinomas. Further studies are needed to understand the role of this finding in thyroid tumorigenesis.

RADIATION-ASSOCIATED THYROID TUMORS

The thyroid gland is highly sensitive to radiation during childhood, the excess relative risk per Gray being 7.7, and 88% of thyroid cancers occurring in these subjects being attributable to radiation. The irradiated thyroid gland is thus an adequate model for the study of radiocarcinogenesis.

Genetic predisposition

Several epidemiological studies have suggested a familial predisposition to developing a thyroid carcinoma after irradiation. Firstly, approximately 3–5% of patients with thyroid cancer, without previous exposure to radiation, have a familial history of the same disease (29). Secondly, when both individuals in sibling pairs were irradiated, the occurrence of thyroid tumors was concordant more often than would have been expected by chance. Thirdly, patients with one radiation-induced tumor (thyroid, salivary, neural, parathyroid) are more likely to develop another tumor than patients with comparable risk factors but who had never had a tumor. This predisposition may
be related to a defect in DNA repair mechanisms, but lifestyle risk factors may also explain some of these epidemiological findings.

**Age at exposure**

Epidemiological studies have shown that the carcinogenic effects of radiation are maximal during early childhood and then decrease rapidly with increasing age. This has been linked to the growth rate of the thyroid gland. Carcinogenesis is a multi-step process, and after the occurrence of a genetic abnormality, several cell divisions are needed for lesions to accumulate and for clonal expansion.

Indeed, a number of experiments in rats have shown that after thyroid exposure to radiation, the risk of developing a thyroid tumor is increased when cell proliferation is stimulated (administration of goitrogens, high or low iodine diet, partial thyroidectomy, TSH stimulation) and decreased when cell proliferation is decreased (hypophysectomy, administration of L-thyroxine). In a recent study in rats, high and low iodine diet both increase proliferation, and both induced thyroid adenomas but no thyroid malignancies occurred. Thus both a mutagenic event (radiation exposure) and increased proliferation rate are needed for the occurrence of thyroid carcinoma (2).

Ionising radiation is less carcinogenic in adults, in whom growth has already been completed: during adulthood, thyroid cell replication rarely occurs (doubling time: 8 years). In contrast, in children thyroid cells are in the process of active replication and this could allow intracellular accumulation of abnormalities that heighten the likelihood of an emerging abnormal clone of transformed cells.

**Genetic abnormalities in radiation-associated thyroid tumors**

Irradiation of the thyroid may directly induce ret/PTC rearrangements. This was found to be the case when ret/PTC rearrangements were induced in a dose-dependent fashion after in vitro irradiation of human cell lines of undifferentiated thyroid cancer (22). Chromosomal loci involved in the ret/PTC1 rearrangement (i.e. ret and H4) are juxtaposed during the interphase in normal human thyroid cells, providing a target for radiation to induce simultaneous double-stranded DNA breaks that lead to erroneous nonhomologous recombination via end-joining (37).

Ret/PTC rearrangements were found in 55–85% of papillary thyroid carcinomas that developed in children who had been exposed to external radiation or contaminated during the Chernobyl accident (4,11,15,21,42,56,61). In both cases, intrachromosomal rearrangements (ret/PTC1&3) were predominant. However, in papillary thyroid carcinomas that emerged early after the Chernobyl accident, the ret/PTC3 form was more frequently observed and was associated with a solid growth pattern and a more aggressive phenotype (56). In contrast, in papillary thyroid carcinoma occurring either after external irradiation or more than 10 years after the Chernobyl accident, ret/PTC1 was the predominant form and was associated with a less aggressive phenotype (classical papillary thyroid carcinoma and diffuse sclerosing variant). Ret/PTC rearrangements were also found in 11–45% of thyroid adenomas that occurred after external irradiation during childhood, and in 52% following exposure in Chernobyl (6,11).
Trk rearrangements were found at a similar low frequency in spontaneous and radiation-associated papillary thyroid carcinomas (3,5,42).

Activating mutations in the ras genes have been found in thyroid tumors from patients with a history of external irradiation, at a frequency similar to that observed in apparently spontaneous tumors (6). In contrast, in tumors that developed in children after the Chernobyl accident, ras point mutations were found in 25% of the follicular tumors but not in papillary thyroid carcinomas (36,61). In spontaneous thyroid tumors, transversions (a base change from purine to pyrimidine or vice-versa) as well as transitions (a base change from purine to purine or pyrimidine to pyrimidine) were detected in the ras genes. In radiation-associated tumors, only transversions were present (6). The exact mechanism of these mutations remains to be determined, but it can be postulated that they arise through an ionizing radiation-induced oxidative lesion, producing 8-OXO-dG which can pair with adenosine during DNA replication (6,53).

The frequency of activating point mutations in the Gqαs and TSH-R genes is low (<10%) in tumors occurring either after external irradiation during childhood and after the Chernobyl accident (6). P53 gene mutations have been detected in a few papillary thyroid carcinomas occurring after external irradiation during childhood and after the Chernobyl accident. These mutations may explain the aggressiveness of some of these tumors (36).

From these data, it can be postulated that radiation may directly lead to DNA strand breaks and ret activation through gene rearrangements. The precise nature of possible secondary genetic events resulting in further progression is unknown.

CONCLUSION

Several conclusions can be drawn from the study of oncogenes and tumor suppressor genes in human thyroid tumors (Table 1):

Alterations of membrane tyrosine kinase receptors (ret/PTC, trk, met) are observed only in papillary thyroid carcinomas; the higher frequency of ret/PTC rearrangements in radiation-associated papillary thyroid carcinoma and also their discovery in radiation-associated follicular adenomas suggest that they may be directly induced by radiation exposure. Met overexpression may be a secondary event.

Activating point mutations of the ras genes are found in 11% of papillary thyroid carcinomas. B-raf mutations were found in 36% and 69%, with no overlap between ret/PTC, ras and b-raf mutations. The activation of this pathway is frequently observed in papillary thyroid carcinomas and may play a determining role in their pathogenesis.

Ras mutations are found in 20% of benign and in 30% of malignant follicular thyroid tumors. B-raf mutations were not found in these tumors. Other genetic abnormalities that may be facilitated by genetic instability induced by ras mutations are needed for tumor progression and to determine the histological type of the thyroid tumor.

PPARγ1/PAX8 translocations were found in malignant and benign follicular tumors. In one series of follicular carcinomas, 86% revealed either ras or PPARγ1/PAX8 mutations. However, there was no overlap between these two mutations and phenotypes associated with each of these mutations were different, suggesting two different pathways in follicular tumorigenesis.
Table 1. Frequencies (%) of genetic alterations in hypofunctioning thyroid tumors, in the absence of previous neck irradiation.

<table>
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<th>Tyrosine kinase receptors:</th>
<th>Papillary carcinoma</th>
<th>Follicular adenoma</th>
<th>Follicular carcinoma</th>
<th>Anaplastic carcinoma</th>
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P53 mutations are observed only in poorly-differentiated or anaplastic thyroid cancers; they play a determining role in progression from differentiated to undifferentiated thyroid carcinomas and in the dedifferentiation process.

TSH-R and Gαs activating point mutations are found in about 60% of hyperfunctioning adenomas; their role in the pathogenesis of hypofunctioning thyroid tumors has not been confirmed.

Several growth factors are overexpressed in thyroid tumors. Paracrine factors such as Fibroblast Growth Factor (FGF1 and 2) are mitogens for thyrocytes, and Vascular Endothelial Cell Growth Factor (VEGF) may play a determining role in tumor neo-vascularisation. Other growth factors may also play a role, such as insulin like growth factor–1 (IGF1), Epidermal Growth Factor or TGFα. Overexpression of these growth factors is believed to be secondary to other oncogenic events.

Other abnormalities may also play a role in thyroid tumorigenesis. DNA methylation is frequently abnormal in thyroid tumors and this may modify gene functions (31). The status of telomerase may be modified: follicular adenomas are telomerase-negative, and about half of papillary and follicular carcinomas are telomerase-positive. Some telomerase-negative cancers maintain telomere length by a mechanism independent of telomerase (32). Other genetic abnormalities may also exist, and deletions have been demonstrated in follicular tumors, possibly indicating the location of yet unknown tumor suppressor genes.

These data may suggest a scheme for epithelial thyroid tumorigenesis. Ongoing studies of the transcriptome and proteome will rapidly increase our knowledge in the field.

REFERENCES

5. Molecular epidemiology of thyroid cancer


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