Genetic background of carcinogenesis in the thyroid gland

Andrzej Lewiński & Katarzyna Wojciechowska

Department of Endocrinology and Metabolic Diseases, Medical University of Lodz,
Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland

Correspondence to: Andrzej Lewiński, MD., PhD.,
Department of Endocrinology and Metabolic Diseases,
Medical University of Lodz,
Rzgowska St. No. 281/289, 93-338 Lodz, POLAND
PHONE: +48 (42) 271-17-15
FAX: +48 (42) 271-13-43
EMAIL: alewin@csk.umed.lodz.pl

Submitted: 2007-02-27 *Accepted: 2007-03-05*

Key words: **thyroid gland; carcinogenesis; protooncogenes; DNA methylation; LOH; point mutations; structural aberrations; suppressor genes; cyclins D1 and E; sporadic and hereditary cancers**

Neuroendocrinol Lett 2007; **28**(2):77–105 **PMID:** 17435680 NEL280207R01

Abstract

The process of carcinogenesis is permanently one of the most interesting and significant issues for researchers in different fields of medicine. Therefore, we attempted to bring closer the problem of neoplastic transformation in the thyroid gland. This article covers the latest data about genetic factors, involved in thyroid carcinogenesis. We have presented results of the most recent studies referred to molecular biology of thyroid neoplasms. We have demonstrated not only the genetic background of cancers, derived from the thyroid follicular cell, but also genetic aspects related to medullary thyroid carcinoma and some benign thyroid lesions.
The review describes DNA methylation disturbances and the mutations in thyrotropin receptor and G protein genes. Furthermore, we introduce the results of studies performed at our laboratory, concerning mutations in the following protooncogenes: *RAS, RET, Trk, MET*, and *BRAF*. Also, we present our data, regarding the loss of heterozygosity (LOH) in the short arm of chromosome 3. Additionally, we discuss overexpression of cyclin *D1* gene in benign and malignant thyroid lesions. Previous studies performed at our laboratory indicate the role of IGF-I in the pathogenesis and invasiveness of thyroid cancers.
The review indicates that progress in genetics of the thyroid cancer is extremely rapid.

INVITED REVIEW

INTRODUCTION

Carcinogenesis is a multistage process, preceded by an initiation of neoplastic transformation at the level of single cell. Taking into account the view about monoclonal origin of neoplasms, it should be kept in mind that, in order – for a single cell – to be able to undergo complete neoplastic transformation, it must have mutations fixed in – at least – a few, or perhaps in several genes. The inactivation of suppressor genes and the activation of oncogenes play the key role in the neoplastic transformation of the cell (Figure 1).

Protooncogenes are defined as genes, which undergo expression in normal cells; they play an essential role in the regulation of growth processes, as well as in the differentiation and maturation processes of cells. Protooncogenes become oncogenes in result of change in DNA sequence, which – in turn – is caused by, at least, one point mutation or by more extensive changes of DNA sequence. Cellular protooncogenes have their fixed localisation in chromosomes, constituting about 1% of all genes in normal cells. Similarly to the majority of protein encoding genes, protooncogenes occur in the haploid genome in single copies [50].

The following mechanisms of protooncogene activation have been found:

a) point mutation,
b) amplification,
c) inversion,
d) translocation and
e) insertion.

The point mutation within protooncogene changes the information for protein coding. The modified protein can induce one or more changes, which accompany the development of the neoplasm.

Amplification consists in multiplication of the number of gene copies. The number of copies of certain genes in neoplasms amounts to several, several dozen and even – up to over 100 copies of single gene.

The mechanism of inversion is a rotation of chromosome fragment by 180 degrees with respect to its initial position. There are pericentric inversions, in which centromere is the point of rotation, and paracentric ones, in which the rotation proceeds around a point which is not centromere.

Translocation consists in a displacement of protooncogene to chromosomal site, which is under the regulatory effect of another gene expression.

Insertion is defined as an incorporation of strong transcriptive signals of promoter type or of, so called, transcription enhancing sequences into the region which proceeds protooncogene or, sometimes, also into the region adjacent to the protooncogene end.

Oncoproteins, encoded by oncogenes, are divided into nuclear and cytoplasmic. With respect to their localisation, as well as to the structural and functional protein properties of protooncogene products, seven classes of oncoproteins have been distinguished:

a) growth factors,
b) receptor and non-receptor proteins, revealing the activity of tyrosine kinase,
c) receptor proteins without tyrosine kinase activity,
d) G-proteins, connected with cytoplasmic membrane,
e) proteins revealing serine kinase activity,
f) cytoplasmic receptors,
g) proteins involved in gene transcription [50].

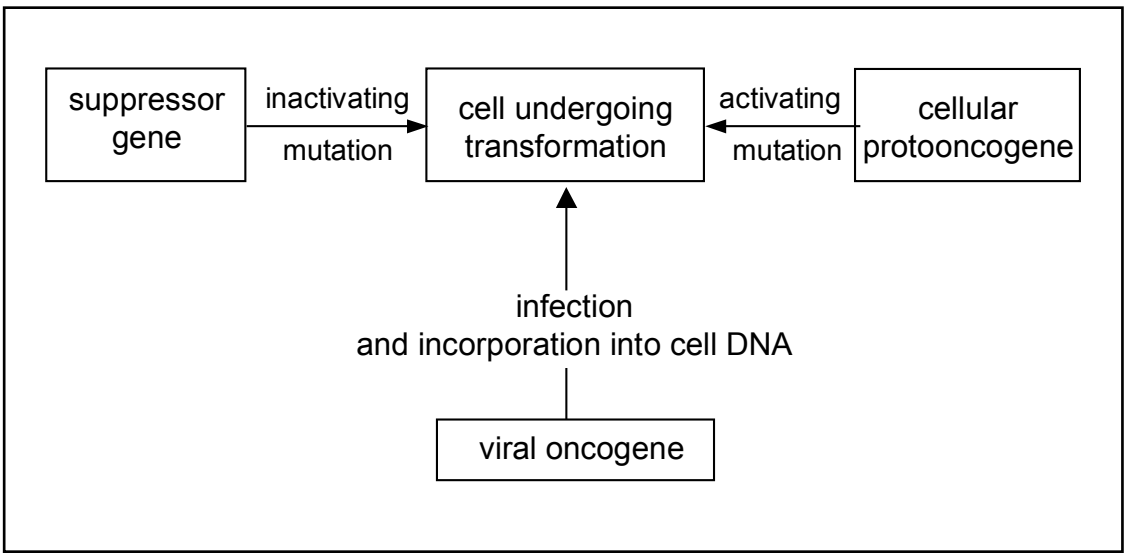

Figure 1. Changes of the activity of protooncogenes and suppressor genes, leading to neoplastic transformation.

Thyroid carcinoma is the most frequently observed neoplasm of endocrine glands. In the majority of cases, it is derived from thyroid follicular cell (TFC) and – in almost 90% of cases – it is a differentiated carcinoma (DTC) of papillary or follicular type (PTC-papillary thyroid carcinoma, FTC – follicular thyroid carcinoma) [98].

In addition, medullary thyroid carcinoma (MTC), originating from the parafollicular cell (C cell) of the thyroid gland, should be discussed, this type of cancer being rarely observed. The hereditary predisposition to MTC has been fairly well documented – about 25% of cases of MTC are related to germinal mutations of *RET* gene and occur as multiple endocrine neoplasia type 2 (MEN 2), including MEN 2A, MEN 2B, or as familial medullary thyroid carcinoma (FMTC) [53]. These problems will be discussed in detail in further parts of this article.

Contrary to MTC, DTC is – most often – sporadic carcinoma. The hereditary form of DTC is found very rarely as familial non-medullary thyroid carcinoma (FNMTC), as well as part of certain hereditary neoplastic syndromes. Out of all DTCs, only about 0.1% of thyroid cancers are associated with familial adenomatous polyposis (FAP) of the colon or with the, so-called, Cowden's syndrome (see further in the text).

Thyroid neoplasms, derived from TFC, provide an attractive research model for studies in molecular genetics and cytogenetics, since they include a broad spectrum of phenotypes, from benign (adenomas) to highly malignant (undifferentiated cell carcinoma). Below, we provide a review of the genetic background of different types of thyroid carcinoma [98].

GENETIC BACKGROUND OF THYROID CARCINOMAS DERIVED FROM THE FOLLICULAR CELL OF THIS GLAND

Disturbances of DNA methylation and oncogenesis

DNA methylation disturbances can also contribute to neoplastic transformation of cells in its early stage. Together with the four main nitric bases in DNA, there can also be untypical bases, including 5-methylcytosine (5-mC), which stands for 3–6% of the total cytosine content in vertebrates [1,111]. DNA methylation is – at its basis – a mutagenic process because 5-mC easily deaminates into thymine. If resulting thymines are not enzymatically cut off and replaced by cytosines, point mutations occur.

It should also be noted that DNA methylation process has also a number of positive functions in the cell, including among others, stabilisation of DNA structure and chromosome X inactivation, as well as it is responsible for the phenomenon of genomic "imprinting". The presence of 5-mC as the fifth base in DNA chain increases the information content in the fragments of the same length.

Disturbances of the normal pattern of DNA methylation are most probably either the causes of or they remain in a close association with the process of development of neoplasms. It has been found in a number of neoplasms that generalised demethylation is accompanied by a local hypermethylation in regions with a high density of CpG (deoxycytidine-phosphate-deoxyguanosine) dinucleotides, containing cytosine in methylated form.

In turn, DNA methylation disorders in the developmental processes of thyroid neoplasms are not clearly understood. DNA methylation disorders in TFCs can probably involve genes for RAS proteins, for Gs proteins, as well as other gene sites, among others, the promoter regions of suppressor genes. For example, disturbances have been found in the normal pattern of DNA methylation within the H-*RAS* oncogene in cells from both benign and malignant thyroid neoplasms (hypermethylation of regions with CpG dinucleotides) [109]. The recent analysis also revealed hypermethylation of suppressor gene *RASSF1A* in *BRAF (v-raf murine sarcoma viral oncogene homolog B1)*-mutation negative PTCs [183]. While studying the cellular lines of PTC and FTC, both hypomethylation of DNA and hypermethylation of DNA within the first exon of *p16INK4A* gene were reported [12]. Additionally, DNA hypermethylation of that suppressor gene was observed in some follicular thyroid adenomas (FTA), PTC, FTC, poorly-differentiated carcinomas and undifferentiated thyroid cancers [183].

The proccess of hypermethylation has also been studied as a therapeutic target for antineoplastic agents (azanucleosides: decitabine and its ribonucleoside analogue – 5-azacytidine). Clinical trials of both azanucleocides have been performed [42].

G proteins

G proteins (guanine binding proteins) have the status of universal links between membrane receptors, which receive extracellular signals, and cellular effector proteins. The majority of data on the functioning of G proteins have resulted from studies on Gs proteins, i.e., a subgroup of proteins with an ability to stimulate adenylyl cyclase. It has been observed that mutation changes, occurring in genes which encode these proteins, especially in the subunit α-encoding gene, lead to their excessive activation [39,152].

The follicular cells, in which α chain mutations of Gs proteins have occurred, can present with an activity of adenylyl cyclase, leading to an excessive stimulation of cellular growth and thyroid hormone secretion. Mutations in Gs protein α chain have been found in cells of clonal hyperfunctioning thyroid adenomas, as well as in some malignant tumours of the thyroid gland.

Recent studies confirm the lack of thyrotropin receptor (TSH-R) and Gs protein α chain mutations in TSH-R antibody negative Graves' disease patients and the observation that somatic gain-of-function TSH-R

mutations are present in hyperfunctioning adenomas and goitres but not in non-functioning thyroid lesions [46]. However, it is believed that the mutational activity of Gsα protein is not the solely cause of either nodule formation or hyperfunctioning adenoma development, and that the participation of a broad complex of signal transduction regulating mechanisms should be presumed at the pathogenetic base of these lesions.

Thyrotropin receptor (TSH-R)

Receptors for TSH in the thyroid gland belong to the family of receptors associated with G proteins; they can also be regarded as potential oncogenes. The receptor for TSH consists of an extracellular part (7 transmembrane domains), connected by extra- and intracellular loops, and of a cytoplasmic "tail"; it can undergo conformational changes in result of mutational substitution of amino acids.

Somatic mutations of the TSH-R-encoding gene have been found, according to different authors [40,134] – in cells of 20–80% of studied hyperfunctioning adenomas. Substitutions of amino acids were observed above all in the third cytoplasmic loop of TSH-R in a number of clonic hyperactive adenomas of the thyroid gland and were associated with position 619 (Asp→Gly) and position 623 (Ala→Ile) [50].

Some authors underline the frequency of TSH-R mutations to be much higher among inhabitants of regions with marked iodine-deficiency, i.e., with ioduria below 50 μg/day [40]. Also, very recent studies confirmed that activating mutations of the TSH-R or Gs protein α chain genes were not involved in high iodine uptake thyroid carcinoma [14].

It should be mentioned here that the gene mutation of TSH-R, occurring in cells of the germinal line, results in the inherited hyperthyroidism, leading to familial forms of this disease. Congenital hyperthyroidism, associated with mutation of this type, can manifest itself either in the infancy or in the childhood, being characterized by the occurrence of toxic diffuse goitre or toxic adenoma, as well as by a severe clinical course of the disease [80].

RAS protooncogenes

The formation process of precursor cell can be explained by mutations, activating protooncogenes of the *RAS* family: N-*RAS*, K-*RAS* 1, H-*RAS*, and K-*RAS* 2. These protooncogenes have a fixed location; in man, they are found on chromosomes 1, 6, 11, and 12, respectively. The mutations in codons 12, 13 or 61 convert these protooncogenes into active oncogenes [104]. The *RAS* protooncogenes encode p21 membrane protein which presents with an internal activity of GTP-ase. Products of the protooncogenes are involved in the intracellular transduction of the signal, affecting the proliferation and differentiation of different cells. The p21 protein manifests structural and functional features of G proteins located in the cellular membrane. Mutations of the *RAS* protooncogenes are met in various neoplasms and with varying incidence.

It seems that the activation of *RAS* protooncogenes is but only one of many stages in the process of thyroid tumour development, possibly being the initiation stage [40]. However, this activation can also occur at the stage of neoplastic promotion and progression. It has been observed that the incidence of *RAS* oncogene activations is not the same in various histopathological types of thyroid neoplasms. In Lemoine *et al.*'s studies [94], the incidence rate amounted to 80% for the neoplastic cells of follicular type and to 20% for the cells of papillary type. In subsequent studies, different incidence rates were reported by those and other authors (Table 1). These differences depend on the histopathological type, the extent of differentiation, and environmental factors, e.g., iodine supplementation in diet.

It is interesting that the incidence rate of *RAS* protooncogene mutations in cells of FTA and of FTC was much higher in regions with confirmed iodine deficiency than in iodine-rich regions, being 85% and 17%, respectively, for FTAs, and 50% and 10%, respectively, for FTCs [156]. This observation suggests that in regions with iodine deficiency and – in consequence – with goitre endemia, the activation of *RAS* protooncogenes plays an important role in the initiation and growth of follicular tumours.

Table 1. Activation of ras oncogene in neoplasms derived from follicular thyroid cell*.

Adenoma	CARCINOMA			Literature
	follicular	papillary	undifferentiated	
8/24	8/15	2/10	6/10	Lemoine *et al.* [93]
6/24	0/3	3/14	—	Namba *et al.* [125]
6/13	1/1	8/13	1/1	Suarez *et al.* [162]
0/9	2/14	1/15	—	Karga *et al.* [79]
14/25	4/16	0/22	—	Shi *et al.* [156]
0/19	5/21	0/31	4/16	Manenti *et al.* [108]

*Based on: Pierotti *et al.* [136]

The occurrence of point mutations in H-*RAS*, K-*RAS*, and N-*RAS* oncogenes in PTC has also been analyzed at our laboratory. The presence of the two point mutations was confirmed in tumour specimens obtained from 29 cases of PTC. The first point mutation was a heterozygous single base substitution (GAA/CAA) at codon 31 of K-*RAS* oncogene, resulting in change from the glutamic acid to glutamine. It should also be noted that this new mutation has not been described before in medical literature. The second alteration was a single base substitution (CAA/CAC) at codon 61 of N-*RAS* oncogene, resulting in an amino acid change from histidine to glutamine. The polymorphism of H-*RAS* oncogene was also found in one probe [35].

The investigations *in vitro* have proved that the activation of *RAS* oncogene leads to the loss of differentiation markers such as: thyroglobulin (Tg), thyroid peroxidase (TPO), TSH-R and to increased proliferation of cultured TFC. However, the activation of *RAS* oncogene is not sufficient alone to cause malignant transformation of TFCs [13,118,138], but probably indirectly leads to increased chromosomal instability and next genetic disorders.

A problem has arisen what factor is necessary for the transformation of adenoma into carcinoma. Some reports have recently been published, suggesting that the role in question may be held by *PAX-8/PPAR*γ rearrangement. The *PAX-8* (paired-box containing transcription factor-8) gene (*locus* 2q13), belonging to the family encoding the transcription factors of organogenesis, plays the key role in differentiating TFCs. In turn, PPARγ (peroxisome proliferator-activated receptor gamma, *locus* 3p25) belongs to nuclear receptors, which form heterodimers with RAR nuclear receptors for retinoic acid. The proteins encoded by *PPAR*γ are involved in the control of lipid-carbohydrate metabolism and – in a broader aspect – in the control of cell proliferation and differentiation. In result of t(2:3)(q13:p25) translocation, fusion gene is formed and – next – the corresponding protein, encoded by that gene [88,129]. PAX8-PPARγ chimeric protein may inactivate the wild type PPARγ, which is a tumour suppressor [88,129,189]. Up to now, it has not been documented whether PAX8-PPARγ takes part in dedifferentiation of FTC, however, the high incidence rate of these rearrangements in poorly-differentiated or undifferentiated carcinomas has been recorded [129].

RET protooncogene

The product of *RET* protooncogene (*locus* in 10q11.2 region) presents with tyrosine kinase activity. The expression of *RET* protooncogene plays an important role in the embryogenesis of mammals, regulating the growth, migration, and differentiation of neural crest cells. The cells of neural crest, which are derived from the neuroectoderm of the prosencephalon, mesencephalon and rhombencephalon, participate in the development and differentiation of a number of tissues in growing human embryo. The product of *RET* protooncogene is a membrane protein, built of an extracellular part, with a region rich in cysteine, a transmembrane and an intracellular part, containing two domains of tyrosine kinase.

The significance of the particular codons of *RET* protooncogene will be discussed in detail in further parts of the present review. Herein, it is to be mentioned that, while either germinal point mutations or somatic point mutations, deletions or insertions within *RET* gene are responsible for the development of MTC (hereditary vs. sporadic, respectively), *RET*-activating chromosomal rearrangements (paracentric inversions within long arms of chromosome 10 or translocations between chromosome 10 and other chromosomes) are the initiating factors in PTC; *RET*/PTC rearrangements are, however, found only in the part of PTC cases (see further in the text of this article).

Structural aberrations in the pathogenesis of PTC related to RET protooncogene

So far, 11 oncogenic sequences of *RET*/PCT have been identified and described. All of them result from chromosomal rearrangements which link *RET* tyrosine kinase domain with 5' end of other genes. Continuous expression in the cell is a common feature of all *RET*/PTC fusion genes, however, their functions are mostly unknown. Fusion proteins demonstrate a constitutive activity of the RET tyrosine kinase domain, while the *RET* gene sequences, encoding extracellular and transmembrane domains, are lost and the fusion protein alone floats in cytoplasm [97].

Rearrangements of *RET* gene have been found almost exclusively in PTCs. To be sure, the presence of *RET*/PTC forms has been ascertained in only a small number of cases of FTAs, however, only in case of PTC it has been found that the activation of *RET* gene is a clonal event, i.e., significant and specific for this type of thyroid neoplasm.

It should be noted here that the above-mentioned oncogenic sequences of *RET*/PTC lead to the development of hybrid genes, which are characterised by an overproduction of RET protein. On the other hand, in MTC with point mutations in particular exons of the *RET* gene, production of abnormal RET protein is observed.

The *RET*/PTC1 sequence results from paracentric inversion of chromosome 10, inv(10)(q11.2q21). This type of inversion leads to fusion of the 3' end of the tyrosine domain of *RET* protooncogene with the 5' end of the gene, marked by H4 probe (*locus D10S170*) [for review see 97] (Figure 2).

The *RET*/PTC2 sequence results from a mutual translocation between chromosomes 10 and 17, t(10;17)(q11.2; q23). This type of translocation leads to a fusion of the 3' end of the tyrosine/kinase domain of *RET* protooncogene with the 5' end of the gene on chromosome 17,

encoding the *RI*α regulating subunit for A protein kinase [for review see 97] (Figure 3).

The *RET*/PTC3 sequence originates in result of the fusion of the 3' end of the tyrosine/kinase domain of *RET* protooncogene with the 5' end of *ELE1* gene, also known as RFG (ret fused gene). It is a paracentric inversion within the long arm of chromosome 10 [97] (Figure 4). Moreover, some variants of *RET*/PTC3 were identified, in which the part belonging to *ELE1* was shorter of 144pb (*RET*/PTC3r2*)* or of 18pb (*RET*/PTC3r3*)* from that in the typical form [97].

The *RET*/PTC4 sequence is formed in result of the fusion of the 3' end of tyrosine-kinase domain of *RET* protooncogene with the 5' end of *ELE1* gene. The displacement between the tyrosine/kinase domain of *RET* protooncogene and *ELE1* gene took place in a different location than it was in case of *RET*/PTC3 [97] (Figure 4).

Summing up, the *RET*/PTC1, *RET*/PTC3 and *RET*/PTC4 are formed in result of intrachromosomal rearrangement (a paracentric inversion within the long arm of chromosome 10).

Interestingly enough, the *RET*/PTC2 is not the only case of interchromosomal translocations among *RET*/PTC rearrangements. All the other known forms of the rearrangements in question are translocations; thus, *RET*/PTC5 (*GOLGA* 5 gene, encoding the autoantigen of Golgi apparatus, located on chromosome 14), *RET*/PTC6 (*HTIF1* gene, located on chromosome 7), *RET*/PTC7 [*RFG7* gene (*HTIF1*-related gene*)* located on chromosome 1)], *RET*/PTC8 (*RFG8* gene, located on chromosome 18), *RET/KTN1* gene, encoding kinectine, located on chromosome 14), *RET/ELKS* (*ELKS* gene located on chromosome 12) and *RET/PCM-1* (*PCM-1* gene, located on chromosome 8) originate in result of translocations between chromosome 10 and different other chromosomes. The fusion is located within intron 11 of the *RET* gene (on the area of ab. 2kb) between the two domains: transmembrane and TK1 [97].

Additionally, it should be recalled that in cases of PTC, developed in children after the accident at the Chernobyl nuclear power station, the *RET*/PTC3 and *RET*/PTC1 sequences were the ones most often identified (58% and 16%, respectively) [130]. Very recent study analyzed the prevalence of *RET*/PTC1 and *RET*/PTC3 in thyroid tumours from liquidators (workers who cleaned up after the accident in Chernobyl), and showed that liquidators, similarly to children exposed to Chernobyl fallout, presented a high prevalence of *RET*/PTC3. This finding suggests that irradiation exerts the same effect, regardless of age [41].

As mentioned above, ionizing radiation is an inducing factor for *RET*/PTC rearrangements. An evaluation of a dose-dependent induction of *RET*/PTC rearrangements in TFC after the *in vitro* exposure to γ-radiation was performed [24]. The occurrence of *RET*/PTC1 (over 80% of all rearrangements) and *RET*/PTC3 rearrangements after exposure to the lowest dose of γ-radiation has been documented. That observation provided additional evidence for a strong correlation between radiation exposure and genetic events in PTC [24].

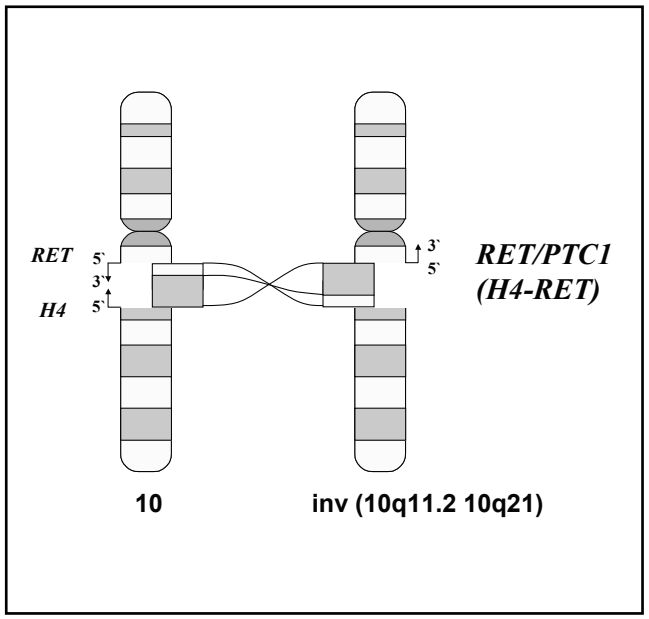

Figure 2. A schematic presentation of the paracentric inversion, leading to formation of *RET*/PTC1 oncogenic sequence.

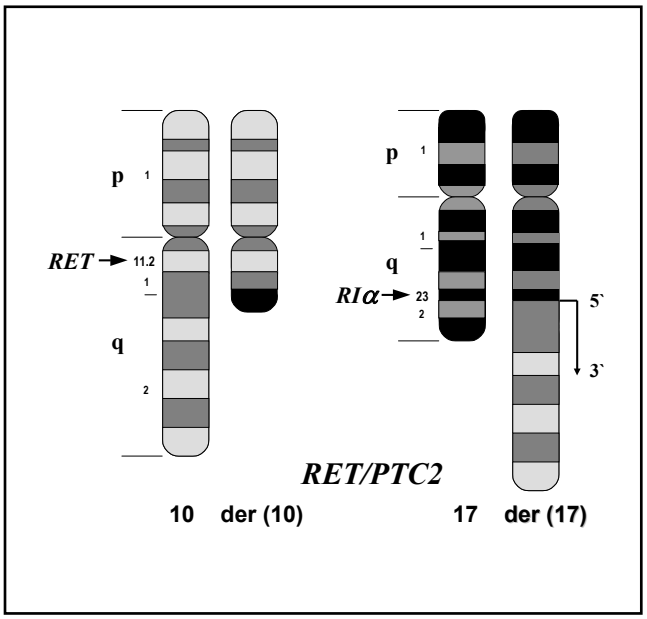

Figure 3. A schematic presentation of the mutual translocation between chromosome 10 and 17, leading to the formation of oncogenic sequence *RET*/PTC2.

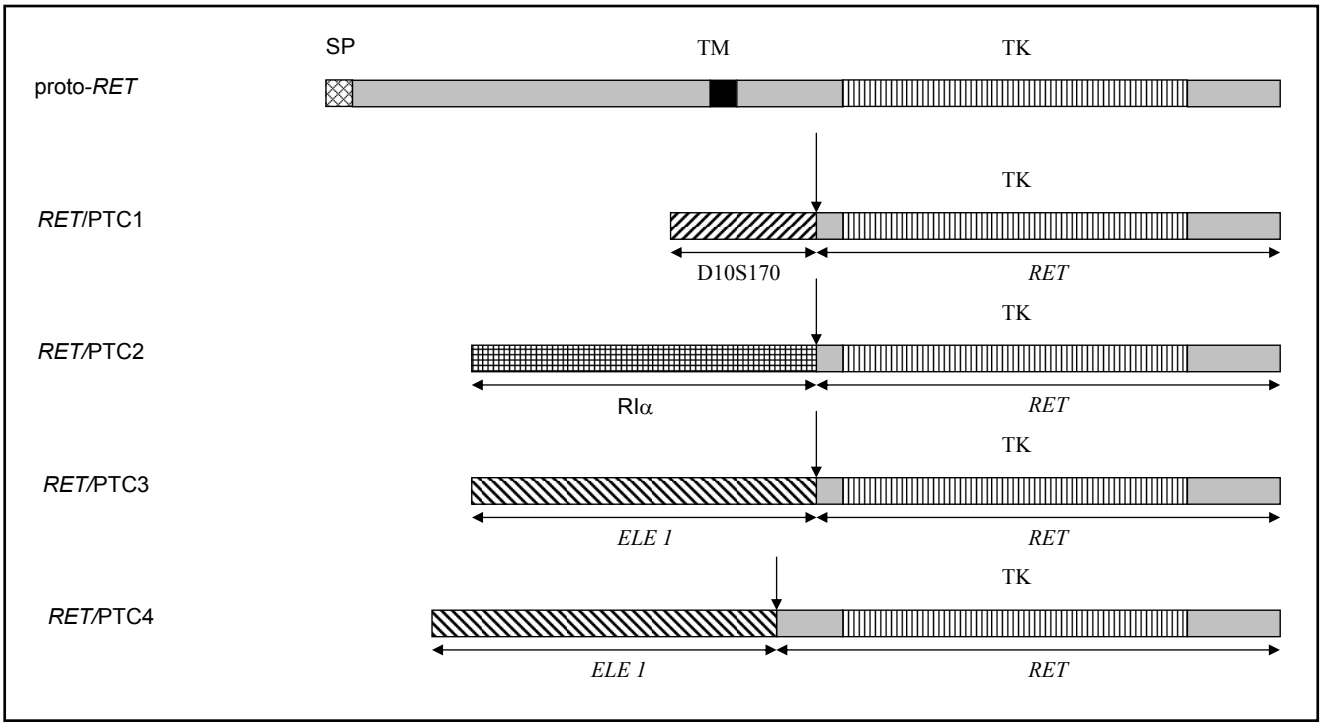

Figure 4. A schematic presentation of RET protein, a product of *RET* protooncogene and of the products of the oncogenic sequences *RET*/PTC 1, 2, 3, and 4 (based on: Suarez [161]). The signalling sequence (SP), the transmembrane part (TM), and the domain of tyrosine kinase (TK) are presented within the RET protein. The domain of tyrosine kinase (TK) and the incomplete products of *H4 (D10S170)*, *ELE1*, and *RIα* genes are schematically presented within the products of oncogenic sequences of the *RET*/PTC family. The arrows mark fusion points.

Trk oncogene in pathogenesis of PTC

The formation of oncogenic sequences of protooncogenes from the *Trk* family (Figure 5), the protein products of which demonstrate tyrosine kinase activity, may lead to a transformation of neoplastic cells towards PTC. The *NTRK1* protooncogene, in its unchanged ("wild") form, encodes protein from the family of receptors for the nerve growth factor (NGF). In man, the gene *loci* of *NTRK1* are localised within two regions of chromosome 1 (1q23-24, 1q32-41).

The oncogenic sequence of *Trk* results from an intrachromosomal inversion within the long arms (q) of chromosome 1, including the *NTRK1* gene, what facilitates the formation of a new hybrid gene. In result of this chromosomal aberration, a fusion takes place between the 3'-end of the tyrosine/kinase domain of *NTRK1* gene with the 5'-end of the non-muscular tropomyosin gene, localised in 1q31 region. The oncogenic sequence of *Trk* is the most frequent one and characteristic for the "spontaneous" PTC, i.e., PTC without previous irradiation history [136,161].

Three oncogenic sequences from the *Trk* family (*Trk*-T1, *Trk*-T2, *Trk*-T3) in PTC cells were identified (Figure 5) [68].

The oncogenic *Trk*-T1 and *Trk*-T2 sequences, which differ from each other by the molecular size, are formed in result of fusion of the 3'-end of the tyrosine kinase domain of *NTRK1* protooncogene (as it has already been mentioned, this fragment of the gene is a part of *Trk* oncogene) with the 5'-end of the translocated promoter region (*TPR*) gene. The *TPR* gene, localised in 1q25 region, encodes the protein which – probably – is a component of the cytoskeleton. It has been postulated that the deletions, inversions and mutual translocations, occurring between a pair of chromosomes 1, lead to revealing of these oncogenic sequences [68].

The *Trk*-T3 oncogenic sequence results from the fusion of the 3'-end of the tyrosine kinase domain of *NTRK1* protooncogene with the 5' end of *Trk* fused gene (*TFG*) or of *Trk* activating gene (*TAG*) [68].

The *TFG* (*TAG*) gene, which encodes the formation of a cytoplasmic protein (of molecular weight – 68 kDa), was localised on chromosome 3. Therefore, the oncogenic sequence of *Trk*-T3 results from mutual chromosomal translocation t(1;3) [68].

The resulting hybrid oncogenes are found in PTCs with variable frequency, depending on examined population. Estimatation of the frequency of rearrangements of *RET* and/or *NTRK1* protooncogenes in PTC in the Polish population and evaluation the possible relationships between presence of *RET* oncogenes and such parameters, as patient's age, gender, histopathological variant of tumour and clinical staging was performed at

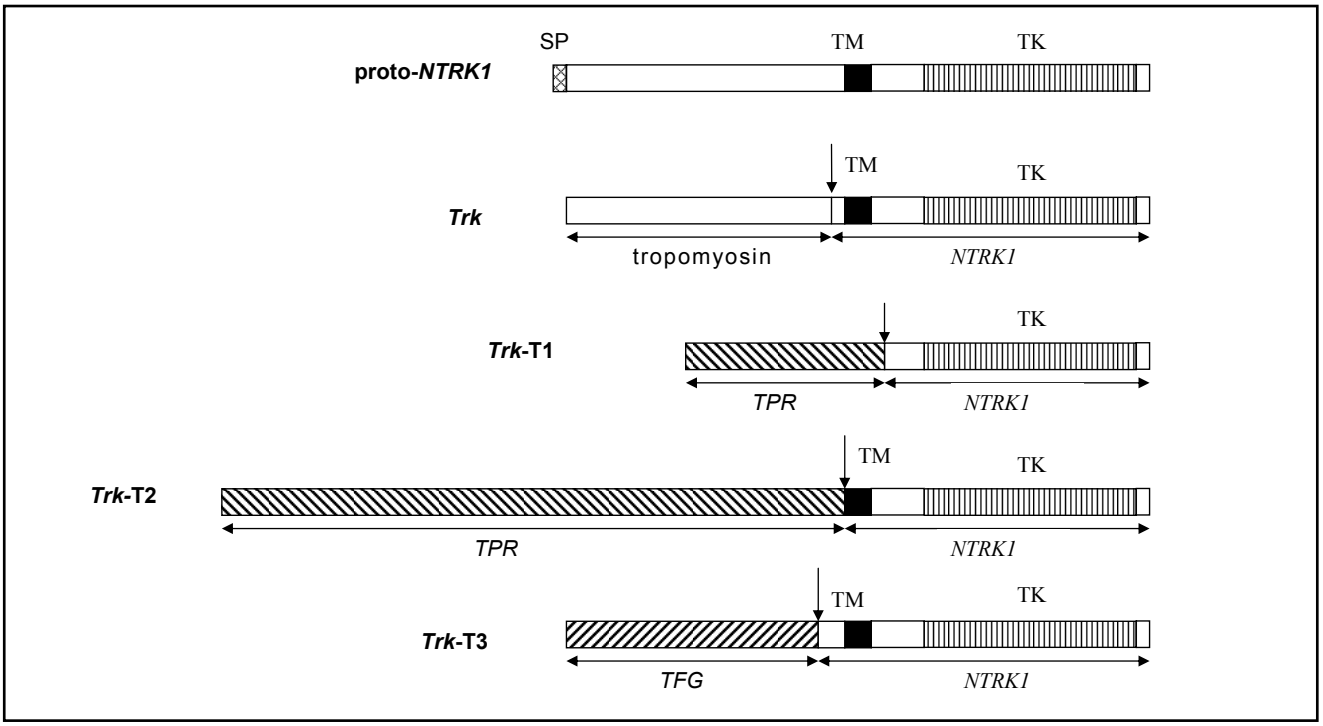

Figure 5. A schematic presentation of the *NTRK1* protooncogene product and of the products of oncogenic sequences, *TRK, TRK-T1, TRK-T2,* and *TRK-T3* (based on: Suarez [161]). The signalling sequence (SP), the transmembrane part (TM), and the domain of tyrosine kinase (TK) are presented within NTRK1 protein. The domain of tyrosine kinase (TK) and the incomplete products of the gene for tropomyosine, *TPR* gene, and *TFG* gene are presented within the products of the oncogenic sequences of *TRK* family. The arrows mark fusion points.

our laboratory. Expression analysis of *RET* and *NTRK1* was carried out by duplex reverse transcription-polymerase chain reaction (duplex RT-PCR) and OneStep RT-PCR, respectively, in tumour tissues obtained from 33 patients with PTC. Rearrangements of the *RET* protooncogene (*RET*/PTC1, *RET*/PTC2, *RET*/PTC3) were detected in 7 out of 33 PTCs (21%) and rearrangements of *NTRK1* [*Trk*-T1 and Trk(*TPM3*)] were detected in 4 out of 33 examined samples (12%). In none of examined cases, did the *RET* and *NTRK1* rearrangements occur in the same sample. No correlations were found between *RET*/PTC sequences and patients age, gender, the histopathological variant of PTC and assignment to particular stage in clinical staging system (TNM Staging, the University of Chicago Clinical Class, and Ohio State University Staging) [17]. The frequency of *RET*/PTC rearrangements in PTC in the Polish population was studied earlier by other authors and it was estimated as 27% [179]. In our study cited above [17], we have identified *RET*/PTC rearrangements with similar frequency of 21%. It is worth considering the period of time when both studies have been performed (i.e., the first study during 1995-2000 and our present study during 2001–2004), taking into account their relation in time to the introduction of obligatory model of iodine prophylaxis in Poland (1997). Hypothetically, normalization of iodine supply could also have affected gene rearrangements (and, possibly the oncogene-derived hybrids) but, unfortunately, neither *RET* nor *NTRK1* gene rearrangements in PTC in the Polish population had exclusively been examined in the time period before the obligatory iodine prophylaxis was introduced, thus making the comparison impossible.

MET protooncogene and PTC formation

The *MET* protooncogene (*locus* in 7q21–q31 region) encodes the formation of the transmembrane receptor protein, the ligand of which is the hepatocyte growth factor – scatter factor (HGF-SF), a multifunctional polypeptide cytokine. HGF-SF is a potential mitogen for epithelial cells and a promotor of cellular migration and invasion. The *MET* receptor protein is a heterodimer, built of two subunits – α and β (molecular weight – 190 kDa), linked by a bisulphide binding. The α subunit (molecular weight – 50 kDa) is localised in the extracellular region, being affected by a marked glycation. The β subunit (molecular weight – 143 kDa) consists of an extracellular part, with the domain participating in ligand binding, a transmembrane fragment and a cytoplasmic part with tyrosine kinase domain. The functionally mature receptor is formed from a precursor of molecular weight equal to 170 kDa, which undergoes glycation and extracellular enzymatic cutting by urokinase [177].

The activation of *MET* oncogene can be the result of:
1) gene amplification, what leads to an overexpression of its product,
2) disorders of posttranslating mechanisms,
3) dimerisation of the receptor, or
4) the expression of the receptor precursor, which has not been enzymatically cut into two subunits [43,117].

An overexpression of *MET* protein has been reported in cells of about 50% of the studied carcinomas, mainly in cells from PTCs [43]. In cases of the remaining histological types of thyroid tumours, the expression of *MET* protein was either low or absent.

At our laboratory, c-*MET* oncogene expression in 18 samples of PTC – compared with macroscopic normal tissue samples from the same patients – has been analyzed. Three times higher relative expression of c-*MET* oncogene has been demonstrated in PTC samples, when compared with normal thyroid tissue. It has also been shown that in some cases of examined paired tissues the expression of oncogene c-*MET* has been proved only in PTC samples, but not in normal thyroid tissue from the same patients [36].

The overexpression of *MET* oncogene is usually associated with the presence of PTC with high histological and clinical degree of malignancy and the resulting invasiveness.

Moreover, the presence of *MET* gene mutation has been confirmed in metastases of human thyroid carcinoma. Mutation induces alteration of intracellular adhesion and leads to the increased motility and invasiveness of cancer cells. In conclusion, the overexpression of *MET* oncogene and MET protein has been confirmed in cancer cells of tumours and metastases. This also suggests that the *MET* oncogene could be a promising molecule to target with pharmacological therapy, impairing tumour cell metastatic potential [106].

It should also be noted that *MET* point mutation, located in exon 14 at the position 3 223, changing the codon 1 010 from ACT to ATT and leading to substitution of threonine with isoleucine, has been relatively frequent in DTC. However, the correlation between the presence of this mutation and clinical and molecular significance is still unclear [176].

It should be stressed here that the identification of papillary microcarcinoma, a separate form of PTC in clinical pathology, has been a step forward in the definition of factors, prognostically significant for this type of neoplasm; one of them might be – in certain cases of papillary microcarcinoma – *MET* oncogene.

BRAF oncogene

The *BRAF* gene (official name: *v-raf murine sarcoma viral oncogene homolog B1*) encodes B-type RAF protein – MEKK [*Mitogen activated protein kinase(MAPK)/Extracellular signal-regulated protein kinase(ERK) Kinase Kinase*], one of the elements of the ERK/MAPK kinase pathway that plays a key role in cell proliferation, differentiation and apoptosis [31] (Figure 6)

The *BRAF* gene mutation has been shown to cause the continuous uncontrolled activation of kinases pathway, what induces excessive proliferation and transformation into cancer cells. There have been few recognized mutations in the *BRAF* gene:
- exon 15, T1799A: V600E [89] (formerly, position 1796 and residue 599) – in *melanoma* and thyroid carcinoma [in PTC and in anaplastic thyroid carcinoma (ATC) derived from PTC];
- exon 15, TG1799-1800AA: V600E – the lymph node metastasis of PTC [173];
- exon 15, T1800-1802GAAdel and T1799A or T1799-1801TGAdel: K601del – the lymph node metastasis of PTC [133];
- exon 15, A1802G, K601E – FTA and follicular variant of PTC [159,158,169,170];
- exon 15, TGA1799-1801del, VK600-1E – solid variant of PTC [171];
- inv (7) q21-22q34 – the radiation-induced PTC [29];
- exon 15, C1787G : L596V – the non-small cell lung cancer – NSCLC (the cellular line NCI-H2087) [38];
- exon 11, G1403C: G468A – the non-small cell lung cancer – NSCLC (the cellular line NCI-H1395) [38].

The mutation of the *BRAF* gene has also been detected in PTC and a poorly-differentiated or undifferentiated thyroid cancer derived from PTC.

The occurrence of *BRAF* mutation in PTC, according to different authors, is varied within the range from 29% to 69% cases of PTC (Table 2). The occurrence of *BRAF* mutation in PTC in Polish population was recorded in our laboratory by the real-time PCR reaction in 5 out of 13 cases of PTC (38,5%) and SSCP (single-strand conformation polymorphism) analysis showed the *BRAF* mutation in 13 out of 33 cases of PTC (39%) [18].

The most frequent genetic event in *BRAF* gene in thyroid cancer is a point mutation, being a transversion of thymine to adenine at nucleotide 1799 (T1799A), resulting in valine (V) to glutamic acid (E) substitution in the evolutionary conservative segment of the BRAF protein chain in the position 600 (V600E) (Figure 7). Mutation is believed to mimic the phosphorylation in activation segment by the contiguity of glutamic acid COOH residue that leads to stimulation of the site of the regulated phosphorylation at serine 599 [33,61,82].

The *BRAF* mutation in thyroid cells has probably a direct influence on the histopathological phenotype of

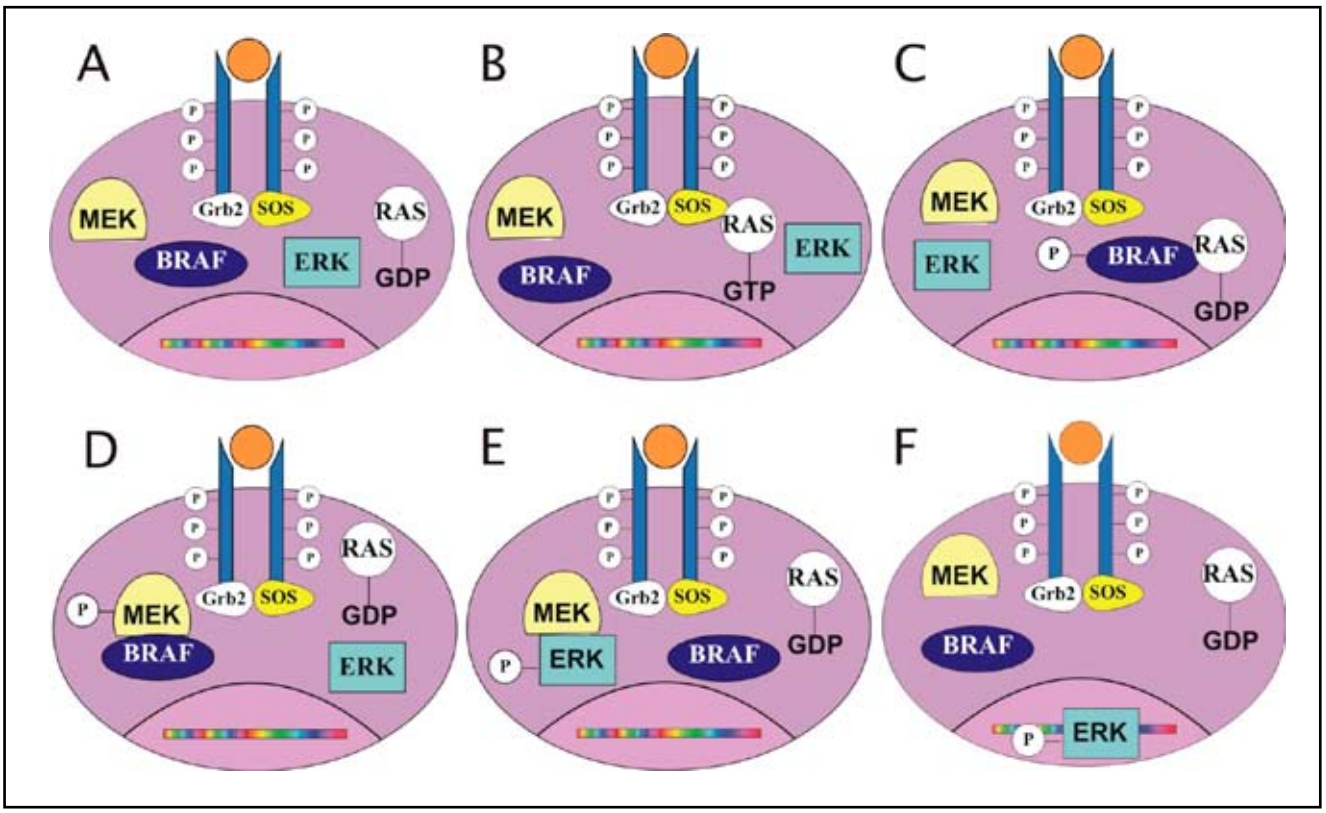

Figure 6. The MAPK pathway. Once activated, receptors by binding the ligand(A) activate the protein RAS (B), which in turn binds BRAF (C) by inducing a conformational change and activation and promote activation of the MEK (D) and then ERK (E). The activation of the MAPK pathway results in synthesis of DNA (F).

the malignant thyroid tumours. V600E mutation of the *BRAF* gene is most often found in the tall-cell variant of PTC (6 of 6 cases), more seldom – in classic variant (28 of 53 cases) and the most seldom – in follicular variant (2 of 30 cases) [132]. Trovisco *et al.* [169] have recently demonstrated the *BRAF* mutation in 6 out of 8 cases of Warthin-like PTC, 28 out of 53 PTC (classic variant), 6 out of 15 PTC (oncocytic variant with conventional growth pattern) and 1 of 3 PTC (tall-cell variant). Recently, a new mutation of the *BRAF* gene – A1802G, resulting in substitution of lysine with a glutamic acid in codon 601 (K601E), has been reported. It is characteristic for FTA and for the follicular variant of PTC (4 of 54 cases) [159,169,171]. It has also been proved that V600E mutation of *BRAF* gene leads to about 2.5 times higher BRAF kinase activity than K601E mutation [175]. Another new mutation of the *BRAF* gene results from triplet deletion – TGA1799-1801del – and has been found in solid variant of PTC [171]. The deletion leads to conversion of codons 600 (GTG) and 601 (AAA) into a single one (GAA) and brings about a substitution of valine and lysine by glutamic acid in the protein BRAF activation segment – VK600-1E [171].

Table 2. The occurrence of BRAF mutation in PTC and ATC according to different authors.

CARCINOMA		Literature
papillary	undifferentiated	
69% (24/35)	—	Cohen *et al.* [33]
53% (40/76)	0% (0/7)	Fukushima *et al.* [61]
46% (23/50)	—	Soares *et al.* [159]
38% (21/56)	—	Xu *et al.* [186]
38% (39/104)	60% (3/5)	Nikiforowa *et al.* [132]
36% (28/78)	—	Kimura *et al.* [82]
29% (49/170)	33% (2/7)	Namba *et al.* [126]
38.5% (5/13)	—	Brzeziańska *et al.* [18]
13% (2/6)	35% (6/17)	Soares *et al.* [158]

BRAF mutations were more often confirmed in tumours diagnosed in older patients, with extrathyroidal extension, lymph node metastases and distant metastases, therefore presenting III and IV clinical stage of the disease [132]. However, according to Fugazzola *et al.* [60], V600E mutation tended to be associated only with bigger volume and extension of the tumour and with lymph node metastases. Trovisco *et al.* [170] have proved that the mean age of patients with classic variant of PTC, harbouring V600E *BRAF* mutation, is higher when compared with age of patients bearing PTC without V600E *BRAF* mutation.

A very recent report has shown a new genetic alteration of *BRAF* gene, resulting from either T1800_T1802 GAA del in addition with T1799A translocation or from T1799_T1801 TGA del; these aberrations brought about lysine deletion at position 601 (K601 del) in three (3) cases of matched lymph node metastases but not in primary tumours [133]. Similarly to these findings, Vasko *et al.* [173] have confirmed a new lymph node PTC metastasis – specific tandem *BRAF* mutation TG1799-1800AA, leading to the change of codon 600 and resulting in V600E mutation of *BRAF* gene. It is possible that the presence of *BRAF* mutation in lymph node-metastasized PTC tissues predisposes to novel *BRAF* alterations in lymph node and – this way – a survival of PTC cells and subsequent metastases to next lymph nodes.

It is supposed that the radiation is not an initiating factor of the aforementioned *BRAF* point mutation [131,185]. This *BRAF* point mutation is characteristic for sporadic and there is higher occurrence of chromosomal rearrangements of *BRAF* in the radiation-induced carcinomas [64]. Also it is worth noting that Ciampi *et al.* [29] have revealed the intrachromosomal inversion in radiation-induced PTCs. The fusion of the *A-kinase anchor protein 9* gene (*AKAP9*) with C-terminal encoding region of *BRAF* gene resalts from paracentric inversion [inv (7) (q21-22q34)] of the long arm of chromosome 7.

Mutation of the *BRAF* gene was also examined in benign lesions of the thyroid gland. However, in case of autonomously functioning thyroid nodules (AFTN), the presence of *BRAF* mutation has not been confirmed [83]. Furthermore, Krohn and Paschke [85] did not confirm the existence of *BRAF* mutations in cold thyroid nodules.

It is worth recalling that the detection of the mutation is usually performed, using molecular biology methods, for example PCR-SSCP (PCR-single-strand conformation polymorphism), PCR-RFLP (PCR-restriction fragments length polymorphism) and sequencing, as well as the real-time PCR method [76]. The material is obtained from specimens, collected during operations or from fine-needle aspiration biopsies (FNAB). It seems that the detection of the *BRAF* mutation in material from FNAB will improve the preoperative diagnostics of thyroid tumours [32,184]. In near future, the detection of V599E mutation (currently – V600E) from blood plasma will be an important step forward in thyroid carcinoma diagnosing [174].

On the whole, there are wide expectations related to the introduction of BRAF protein inhibitors, as an extremely selective method of thyroid cancer treatment. Studies have already been performed, focused on the clinical use of MEK inhibitor – C1-1040 – in neoplastic lesions [116]. It has been proved that the application of U0126 – the inhibitor of MEK phosphorylation – causes suppression of the cellular line with the *BRAF* mutation growth. Currently used MEK inhibitors decelerate the growth of colon tumours *in vivo* [154]. Recently, BAY-439006 has been introduced by Bayer Co. and it is in III stage of clinical trials. Bay 43-9006 seems to be a promising one as it has an excellent safety profile in humans and the effectiveness in inhibiting RAF kinases [11,92]. Treatment with this compound can inhibit cell proliferation and induce apoptosis in *melanoma* cells with *BRAF* mutation.

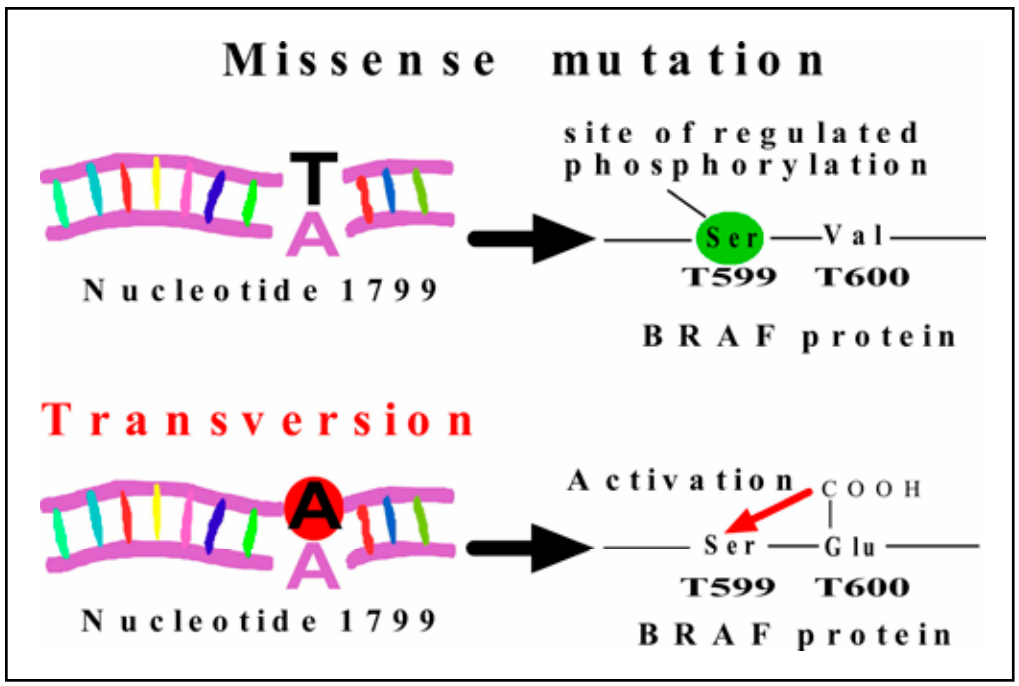

Figure 7. Molecular mechanism of the V600E point mutation

Correlation between BRAF, RAS mutations and RET rearrangements

BRAF mutation causes not only uncontrolled stimulation of the kinase pathway but also most probably diminishes the sensibility of the stimulation by RAS protein. The very recent analysis revealed that hypermethylation of suppressor gene *RASSF1A* is found only in *BRAF* mutation negative PTCs [185]. Furthermore, other studies have not confirmed the coexistence of mutations of *RAS* and *BRAF* genes in thyroid carcinoma.

Also study, performed by Domingues *et al.* [44] on *RET/PTC* rearrangements and *BRAF* V600E mutation in thyroid aspirates from FNAB – proved the mutual exclusivity among these common genetic aberrations. In only one case both genetic alterations have been demonstrated (case of patients with breast cancer and lung metastasis). Other studies also suggest that there is no overlap between *BRAF* and *RET/PTC* rearrangements [142,159,82].

On the other hand, Xu *et al.* [186] have suggested that *BRAF* cooperates with *RET/PTC* and Frattini *et al.* [58] has demonstrated the similar global gene expression profiles of PTCs, harbouring *BRAF* mutation or *RET/PTC* rearrangements. The explanation of this outcome could be based on the fact that both BRAF and RET proteins are related to the same proliferation pathway in cells.

It has also been proved that gene expression is a consequence of the adequate mutation and this fact may have clinical and diagnostic significance. At the same time, it predicts success for therapies designed to prevent the consequences of these mutations. An analysis of gene expression in PTC (*BRAF*, *RET/PTC1*, *RET/PTC*3, K-*RAS*, H-*RAS*, N-*RAS*) has segregated PTC cases into three groups which reflect tumour morphology and mutational status [67].

It is worth noting that *BRAF* mutation predisposes to other genetic alterations, leading to the more aggressive course of PTC; however, it is probably not sufficient – acting alone – to transform PTC to ATC [132,155].

Furthermore, Quiros *et al.* [145] demonstrated the overlap between *BRAF* mutation and *P53* mutation (but not *RAS* and *RET/PTC* oncogenes). This finding supports the thesis that *BRAF* mutation favours accumulation of *P53* mutations in DTC that progresses to ATC.

Recent views on the hereditary forms of DTC (familial differentiated thyroid carcinoma – FDTC; familial non-MTC – FNMTC)

The term DTC designates both PTC and FTC. At the same time, it requires a certain amount of consideration why the familial form of DTC should be understood as PTC only. Although the FNMTC term concerns the familial non-medullary thyroid carcinoma, it is, in fact, identified with the familial PTC. Despite strong clinical suggestions, neither gene nor a group of genes, responsible for the familial form of DTC, has yet been identified. Familial DTC is diagnosed in families with history of, at least, 2 cases of this neoplasm [95].

Already in the 70ties of 20th century, the first reports appeared in literature about familial aggregation of malignant thyroid neoplasms, observed after exclusion of cases of MTC. Familial aggregation in DTC, not included into other hereditary neoplastic syndromes, was described for the first time by Nemec *et al.* [127]. It is believed that from 3 to 6% of patients with DTC reported some incidence of the same neoplasm among 1st degree relatives.

As mentioned before, no gene has been identified which would be responsible for the familial form of DTC. Some reports have suggested – most probably – the role of *locus* 1q21 for the familial PTC gene, which has been shown to undergo segregation together with papillary renal carcinoma, resulting in the, so-called PTC-PRN (papillary thyroid carcinoma – papillary renal neoplasia) syndrome [107]. Another proposed gene-candidate for the familial form of PTC of oxyphilic type (thyroid cell oxyphilia – *TCO* gene) was the gene localised in the short arm of chromosome 19 (*locus* 19p13.2) [23]. Other studies demonstrated a possible role of *MNG1* (multinodular goitre 1) gene, localised in chromosome 14 and responsible for the familial form of thyroid nodular goitre (NG) [9,10,128]. However, in a large multicentre study, an evaluation of association of the two latter genes with the familial PTC did not reveal any significant correlation [115]. Recently, *locus* 2q21 has been proposed as a site which is conjugated with the familial PTC, especially of follicular type [114,167].

Some hereditary neoplastic syndromes, in which the inherited form of DTC occurs, should be shortly characterized. Cowden's syndrome is a hereditary syndrome, in which an increased incidence of thyroid carcinoma, especially of follicular type (in 10% of patients), is observed. It is characterized by the presence of multiple hamartomas, disorders of the osseous system and of a 50-percent risk of breast carcinoma. Thyroid diseases, are found in 2/3 of the patients with Cowden's syndrome. The phosphatase and tensin homologue, deleted on chromosome ten (*PTEN*) gene, responsible for Cowden's diseases, belongs to suppressor genes and is located on the short arm of chromosome 10 – this gene will be discussed in detail in further part of this article [150].

In patients with familial adenomatous polyposis (FAP), multiple polyps of the colon develop, some of which undergo neoplastic transformation [160]. This syndrome is inherited in autosomal dominant manner and is the cause of 1% of all cases of adenocarcinomas of the colon. The *APC* (adenomatous polyposis gene) gene, responsible for FAP, is located within the long arm of chromosome 5 (*locus* 5q21) and consists of 15 exons. The protein product of *APC* gene is built of 2843 amino acids. This protein, in its central domain, is linked with β-catenine, belonging to adhesive molecules of the E-cadherin group. This protein facilitates degradation

of β-catenine, what prevents activation of the genes participating in proliferation. The deletions and insertions of base pairs were the most frequently found mutations within the *APC* gene, the majority of those mutations being localised at the 5' end of gene. Mutations of *APC* gene and disturbances of the gene expression were also observed in the neoplasms originating from TFC. It is assumed that PTC, accompanying FAP in about 1.2% of cases, is an extragastrointestinal manifestation of this pathological syndrome, since somatic mutations of the *APC* gene were either rarely or not at all found in cells of the sporadic PTC [21,25]. Patients with FAP showed a rare histopathological subtype of PTC – the cribriform-morular variant (CMV) [78,91,168]. Strong protein expression and genetic aberration of β-catenin have also been confirmed in this subtype of PTC [187].

Turcot's syndrome is another hereditary syndrome, which results from mutation in the *APC* gene or in *MLH1* or *PMS2* genes, involved in DNA repair. It is characterized by multiple polyps of the colon, which – similarly as in FAP syndrome – undergo neoplastic transformation [15]. Additionally, neoplasms of other localisations [malignant neoplasms of the central nervous system (CNS), stomach, the small intestine] are found in these patients.

Thyroid carcinoma has also been described in patients with Peutz-Jeghers' syndrome, which develops in result of mutation in *STK11* gene, encoding the protein from the group of serine-treonine kinases [3,72,188]. This syndrome belongs to hereditary diseases from the lentigioma group. It is characterized by multiple pigmented naevi of mucous membranes and by the occurrence of polyps of the gastrointestinal tract, which – however – are very rarely susceptible to neoplastic transformation.

Carney's syndrome belongs to endocrine adenomatosis syndromes. In the analysis of conjugations, two loci: 2p.16 and 17q22-24 were found, undergoing segregation with the syndrome, however, no gene responsible for the syndrome development has been characterized yet. In the syndrome, beside characteristic pigmentary changes of the lentigioma type, hypermelanosis and blue naevi, myxoid (mucous) tumours of internal organs are diagnosed, especially of the heart. Out of endocrine tumours, autonomous adrenal adenomas, leading to Cushing's syndrome, are most often recognised. Thyroid carcinomas are rare, with the most frequent – PTC.

Multiple endocrine neoplasia of type 1 (MEN 1) is a syndrome with an autosomal dominant inheritance, which includes germinal mutations, causing function loss in one of the suppressor gene alleles. The *MEN 1* gene is located within the long arm of chromosome 11 and encodes menin, a 610-amino acid nuclear protein. The function of menin has not yet been recognised in detail; it is only known that menin is a suppressor protein, linked with transcription factors. Disturbances in *MEN 1* gene function lead to development of various neoplasms of the endocrine system, e.g., adenomas of the parathyroid glands, pancreatic islets and the pituitary. Although thyroid tumours, derived from TFCs, are not frequent, the probability rate of PTC is higher in those patients than that in general population [149].

3p chromosome and follicular thyroid carcinoma

In the last decade, a cytogenetic and molecular evidence has been provided for the loss of genetic material in the short arm of chromosome 3 (3p) in cells of FTC. A loss of heterozygosity (LOH) in the 3p21-24.3 region of certain FTCs was observed. Probably, the suppressor gene is located at this region, the normal function of which is protection against the transformation of FTA into FTC, while a loss of the gene favours such a malignant transformation.

The genetic consequences of LOH within the short arms of 3 (3p21) chromosome are a subject of intensive research; as it is known, an inactivation of genetic material in this localisation is one of the most frequently observed changes in solid tumours, including FTCs.

Recently, a new suppressor gene (*RASSF1A*), present in this *locus*, has been characterised. It has been documented that in non-differentiated thyroid carcinomas in men, a simultaneous inactivation of *RASSF1A* gene and of p16 gene is the one, most frequently observed. The inactivation of *RASSF1A* gene is accomplished via hypermethylation [96,151]. Participation of RASSF1A protein in apoptic signal transferring, microtubule stabilization and regulation of cell cycle have been confirmed [37]. Methylation *de novo* of *RASSF1A* promoter has very frequently been shown in different carcinomas (lung cancer, breast cancer, prostate cancer, renal cancer, pancreatic cancer, liver cancer, thyroid cancer) and leads to silencing of expression of RASSF1A protein [37]. Methylation of *RASSF1A* promoter has been confirmed in both benign and malignant lesions of thyroid gland, indicating that this could be an early event in thyroid tumour pathogenesis and progression. The presence of *RASSF1A* methylation could be useful early prognostic factor of carcinoma [124].

It should be emphasized that *THR*β (thyroid hormone receptor beta) protooncogene is located in 3p24.3 region, the expression of which leads to the synthesis of receptor proteins of many hormones, including triiodothyronine (T_3). Most probably, the suppressor gene is located at the similar region. This observation prompted us to study the loss of genetic material at that localisation in PTC cases. However, studies performed by our research group failed to demonstrate the LOH presence in *locus* 3p24.3 (*THR*β gene) in 22 out of 23 studied patients with follicular variant of PTC (96%) [19].

Participation of different suppressor genes and of CCND1 or CCNE genes in the pathogenesis of thyroid neoplasms

As it has already been mentioned, out of the genes, the function of which is associated with the process of carcinogenesis, cell growth inhibiting genes are distinguished, called either suppressor genes or anti-oncogenes. The most recent reports indicate that the process of carcinogenesis is strongly associated with disturbances of the cellular cycle regulation. The determination of cellular division occurs usually at the end of G1 phase of the cellular cycle in the, so-called, internal (restrictive) cell cycle checkpoint (G1/S). The transition through the G1/S checkpoint regulates a number of proteins, among others, serine/treonine protein kinases (cdk2, cdk4, cdk6), known as cyclin-dependent kinases, cyclins D and E, as well as the products of suppressor genes, i.e., of *p53* gene, *Rb* (retinoblastoma) gene and *p16INK4A*.

A number of publications have been issued during the recent decade, reporting on the observed incidence rates and types of mutations, occurring in these genes, located in cells from various neoplasms, including neoplasms of the thyroid gland [69,77,144,153]. The presence or absence of protein products of these genes in neoplastic cells was also analyzed by means of immunohistochemical methods [55,157].

P53 gene

The mutations, occurring in *P53* suppressor gene (*locus* in 17p13.1 region) are the most frequently observed molecular changes in human neoplasms. The majority of *P53* gene mutations, found in human neoplasms, are mutations of the missence type, i.e., such mutations which cause one amino acid to be substituted by another one. The most numerous mutations in *P53* gene were observed in codons 175, 248, and 273. New hotspots were identified at codons 179, 195, 196, 213, 217, 249, 254, 278, 281, and 298 in breast tumours by a DNA microarray method [165]. The products of non-mutated *P53* gene are suppressor proteins and only a mutation occurring within the gene in question can equip p53 protein with oncogenic properties.

One of the main functions of p53 protein in normal cells is genome stability maintenance by cell cycle regulation at the level of gene transcription. The control of DNA defects is the second important role, held by normal p53 protein. It has been observed that, following the effect of a DNA impairing factor exerted onto cell, e.g., ionising radiation, the quantity of normal p53 protein considerably increases. G1 phase is then extended in order to enable repairs of damages before the S phase of the cell cycle is entered. Normal p53 protein is also an important regulator of apoptosis process, i.e., a programmed cell death. In this process, p53 protein controls the expression of apoptosis genes, among others, of *Bax* and *Bcl-2* genes, by activation and inhibition, respectively.

It is presumed that the transformation of DTC into poorly-differentiated or undifferentiated carcinoma has a genetic background. In cells of poorly-differentiated or undifferentiated thyroid carcinoma, the observed incidence rate of mutations within *P53* gene remains – according to different authors – between 60 and 90%. A performed sequencing revealed a mutation of the transition type – G:C→A:T in codon 273 of *P53* gene. On the other hand, in immunohistochemical studies, the observed percent of cells revealing a positive nuclear reaction for p53 protein, was 14.3% for FTC, 21.1% for PTC and as much as 80% for undifferentiated carcinomas [27]. It should be noted that the frequency of mutations within *P53* gene, as observed in cells from malignant neoplasms of the thyroid gland, did not always correlate with the overexpression of p53 protein.

The role of *P53* inactivation in dedifferentiation in TFCs has experimentally been proven. Transgenic mice with expression of *RET*/PTC1 and *RET*/PTC3 developed PTC, but after crossing them with P53 –/– mice, the new strain of mice underwent dedifferentiation to ATC [90,139]. Additionally, the recovery of wild-type *P53* expression in cultured anaplastic thyroid cells caused the decrease of the proliferation rate and return of TPO and PAX-8 expression and TSH stimulation responding [48,119].

Rb gene (retinoblastoma)

The *Rb* gene is localised on chromosome 13q14 and consists of 27 exons. This gene encodes the formation of nuclear phosphoprotein (pRb) (molecular weight of 105–110 kDa) which manifests suppressor activity. The inactivation of *Rb* gene suppressor function results from deletion, insertion, or point mutations of the gene. In turn, Rb protein demonstrates an ability to bind viral oncoproteins, e.g., SV 40 virus, human E1A adenoviruses, *papilloma* E7 viruses, the consequence of which is its inactivation [178].

The molecular mechanisms, by which Rb protein controls the proliferation of cells, affecting the G1/S phase of the cellular cycle, can be summarized in the following way: 1) the unphosphorylated form of Rb protein binds the transcription factor of the E2F family; 2) a gradual phosphorylation of Rb protein is made by a complex, consisting of cdk4 and cdk6 kinases and of D1 cyclin; 3) the phosphorylated Rb protein releases the E2F transcription factor; 4) the E2F activates the process of transcription of a number of genes, among others, of cyclin E and of itself (regulation of its own expression), responsible for crossing the barrier between the G1 phase and S phase of the cell cycle [178].

A deletion of exon 21 of *Rb* gene was found in the majority of cells of malignant thyroid tumours. The percent of positive cells for Rb protein was significantly lower.

Recent studies showed that the Rb protein overexpression in FTC did not differ significantly from FTA (p=0,486), however confirmed the existence of mutual regulation mechanism of Rb and cyclin D1 protein ex-

pression, which are observed in cells from various carcinomas [52].

P16INK4A gene

The *P16INK4A* gene, also called *CDKN2A* or *MTS1* (multiple tumour suppressor 1), which encodes p16INK4A protein, is localised on chromosome 9p21. The loss of genetic material in the 9p21 region was observed in cells of many neoplasms. Besides the deletion of the entire gene and point mutations, *INK4A* gene is also inactivated in result of cytosine methylation in the region rich with CpG pairs, located in the promoter region of this gene [12].

P16INK4A protein forms complexes with cdk4 and cdk6 kinases, in which cyclins of D class (D1, D2, D3) play the role of regulating subunits. The basic function of p16INK4A protein in the regulation of the cell cycle is realised by the cdk4 binding, competitive for D1 cyclin. In this way, p16INK4A protein inhibits the phosphorylation process of Rb protein, performed by cyclin-dependent cdk4 and cdk6 kinases. Thus, p16INK4A protein takes part in the regulation of the cell cycle together with the *Rb* gene product [30] (see earlier in the text) and cyclin D1 [120].

High immunohistochemically-detected expression of P16INK4A protein in FTC suggests that the altered expression pattern of P16INK4A may disturb the regulatory mechanism of TFC cell cycle and plays a significant role in the formation of benign neoplasm and their malignant counterparts derived from TFC [51].

nm23 genes

The *nm23-H1* and *nm23-H2* genes (*locus* 17q21-22) are regarded to be suppressor genes in the mechanism of metastases of neoplastic cells originating from various tissues. The *nm23-H1* and *nm23-H2* genes encode subunit A and subunit B, respectively, of nucleotide-diphosphatic B kinase (NDPK) (EC 2.7.4.6), taking part in the metabolism of purine nucleotides. The protein products of these genes demonstrate 88% homology and their physiological role is reduced to the regulation of proliferation, differentiation and intercellular adhesion. Moreover, it has been observed that the transcript (mRNA) of *nm23-H2* gene is identical with the transcript of PuF transcription factor gene, which is an activator of *c-myc* oncogene promoter [109]. It has been observed that the allelic mutations of *nm23-H1* gene and/or the decreased expression of the product of that gene were correlated with the degree of clinical malignancy and the ability to form distant metastases [71,148].

The level of *nm23-H1* gene expression did not differ significantly between the cells derived from NG and cells of DTC [193]. On the other hand, the decrease in expression of the *nm23-H1* gene, but not of the *nm23-H2* gene in PTC cells of patients with metastases to lymph nodes has been demonstrated [2]. However, the relationships between nm23-H1 immunoreactivity and age, gender, differentiation status, local recurrence or distant metastases have not been observed [190].

Molecular abnormalities associated with the development and progression of epithelial neoplasms of the thyroid gland, derived from TFC are presented in the Figure 8.

PTEN and PIK3CA genes

The *PTEN* gene located in chromosome 10q22-23 encodes tyrosine phosphatase which shows a suppressive function (inhibits cell growth), opposite to tyrosine kinase [63]. Germinal deletion of this gene is associated with Cowden's syndrome [16]. It has been observed that the *PTEN* can dephosphorylase the *PIK3CA* gene product – phosphatidylinositol 3-kinase (PIP3) [63]. Phosphatidylinositol 3-kinase is essential in kinase B transport (AKt1, PKB) to cell membrane, where it is phosphorylased and it activates kinases pathway [16,150].

The *PIK3CA* gene is located in chromosome 3 (3q26.3) and encodes PIP3. The somatic mutations in catalytic subunit of *PIK3CA* are frequent in various tumours (of the colon, stomach, breast, ovary and brain). *PIK3CA* mutations have also been confirmed in undifferentiated thyroid carcinoma, markedly less common in DTC. The possibility of use of *PIK3CA* gene as a potential target in therapy of ATC is still studied [66,150].

Cyclin D1 gene (CCND1) and cyclin E gene (CCNE)

The cyclins D1 and E are the essential proteins controlling the cell cycle. The overexpression of these proteins causes the disorders of cell cycle and leads to uncontrolled proliferation. The *CCNE* gene, located in chromosome 19 (19q13.1), encodes cyclin E [4]. It has been suggested that expression of cyclin D1 and E, as well as of cyclin-dependent kinase inhibitors – p27 and p21, and their subcellular localization may be a useful adjunct in differentiating benign from malignant thyroid neoplasms and in predicting tumour behaviour [135].

The *PRAD1* (*CCND1*) gene located in *locus* 11q13 encodes cyclin D1. The role of cyclin D1 in proliferative change of thyroid gland is not clear yet. The expression of cyclin D1 has not been found in normal thyroid tissue. On the other hand, the overexpression of cyclin D1 has been confirmed in neoplastic thyroid tissue by immunohistochemical examination [52]. Increased expression of cyclin D1 has been proved in poorly-differentiated and undifferentiated thyroid carcinoma when compared with DTC. This fact suggests that cyclin D1 takes part in the progression of a thyroid carcinoma and could have prognostic significance. The investigation of cyclin *D1* gene expression in neoplastic lesions of thyroid (PTC, MTC, FTA) in the Polish population has been performed using the RQ real-time PCR method. Our research group have proved the increased level of cyclin *D1* gene expression in neoplastic thyroid tissue (FTA, PTC, MTC), when compared with control thy-

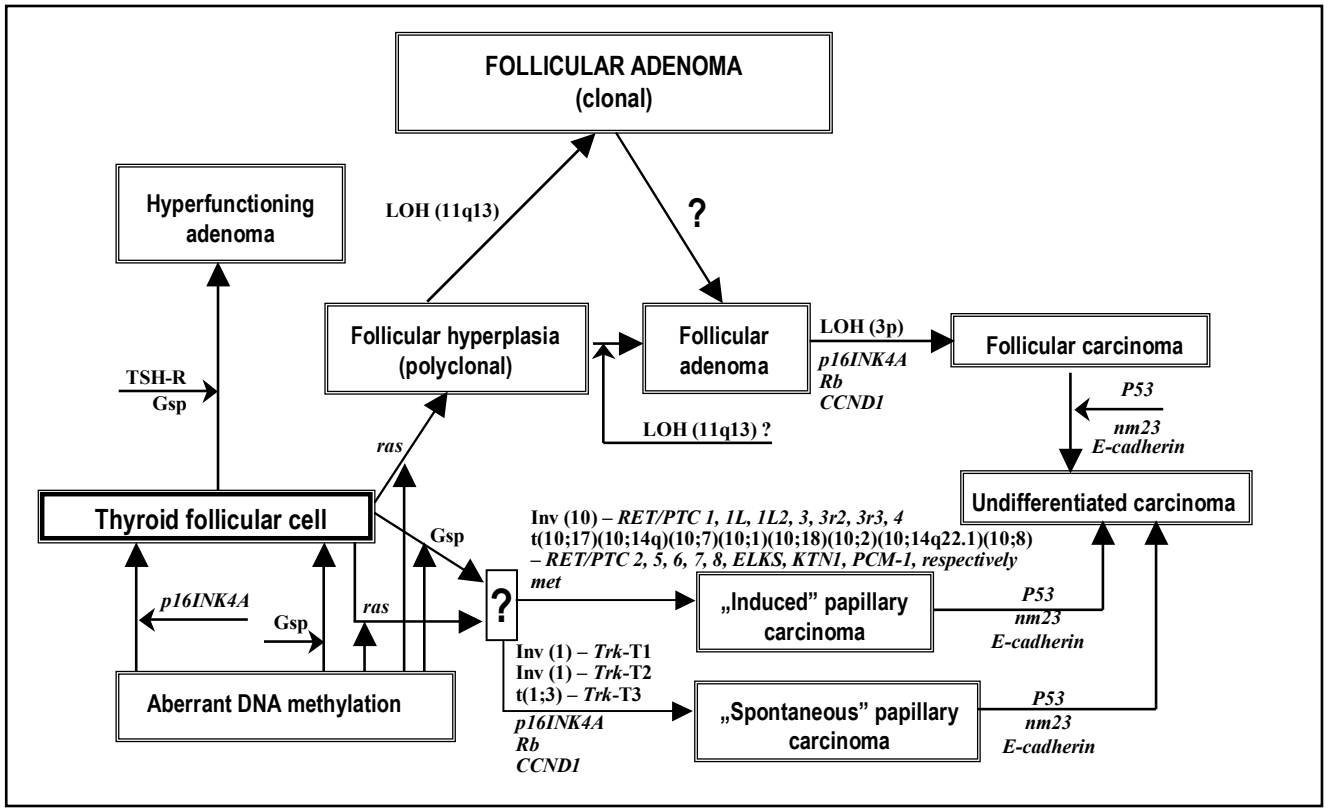

Figure 8. Molecular abnormalities associated with the development and progression of epithelial neoplasms of the thyroid gland, derived from the follicular cell of this gland. The figure presents proved and assumed molecular changes (based on: Fagin [47], Farid et al. [49], and Suarez [161] in our own modification). The abnormalities of genes above the arrows have the primary role in subsequent phases of the thyroid neoplasm development.
Gsp – Gs protein, TSH-R – receptor for TSH, *ras, met, RET, Trk* – protooncogenes; *P53, Rb, p16INK4A, nm23* – suppressor genes; *CCND1* – D1 cyclin gene; LOH – loss of heterozygoticity; inv(10) – paracentric inversion within chromosome 10; t(10;17), t(1;3) – translocations; 11q13 – striated region on the long arm of chromosome 11; 3p – short arm of chromosome 3, E-cadherin – an adhesive molecule. Inactivating mutations or microdeletions (LOH), involving suppressor genes or activating mutations (*ras, met*) or structural aberrations (*RET*/PTC, *Trk)* of protooncogenes are necessary for the transition from one phase to another (following the direction of arrows).

roid tissue. The observed differences in the level of gene expression among studied tumours have not been statistically significant (p>0.05). The correlation between the level of *CCND1* gene expression and histopathological type of tumour has not been recorded [20].

Involvement of other genes and factors in the process of thyroid carcinogenesis

CTNNB1 gene

The *CTNNB1* gene encodes a multifunctional cytoplasmic protein β-catenin. Beta-catenin is an adherens junction protein. Adherens junctions are critical for the establishment and maintenance of epithelial layers. β-catenin links together E–cadherin with α–catenin. E-cadherin is a transmembrane protein which is involved in intracellular adhesion. Beta-catenin influences transcriptional activity of *c-myc* and *c-fos* genes, as well as *CCND1* gene. Accumulation of β-catenin in the cell nucleus and continuous activation of the above mentioned genes, resulting from point mutation in exon 3 of *CTNNB1* gene, have been observed in many tumours [172]. This mutation has been confirmed in 7 out of 28 (25%) of poorly-differentiated and in 19 out of 29 (66%) of undifferentiated thyroid carcinomas [65,112]. Other studies have also confirmed observation that cell differentiation degree in FTC and in metastases of PTC are positively correlated with expression of E-cadherin [105].

Thyroid hormone receptor (TR or THR)

The *TR* gene is a cellular homologue of virus oncogene *v-erbA*. Alternative splicing of the primary transcripts of two T_3 genes (*TR*α and *TR*β) divide into four isoforms α1, α2, β1, β2. Abnormal *TR* gene and protein expression has been noticed in various neoplasms; THR receptor has an important role in carcinogenesis. Triiodothyronine influences almost all the processes which have been disturbed in cancerous cell (regulation of proliferation, differentiation and apoptosis). Furthermore, the cooperation between TR and other protooncogenes (*c-Jun*, *Mdm2*), as well as suppressor gene (*P53*) has been proved.

Additionally, the LOH chromosomal regions with *loci* of the *TR* gene allele have been observed in thyroid carcinoma. The "missense" point mutations of *TR* gene, resulting in changing receptor function as a transcription activator, have been confirmed in neoplasms of the liver, kidney, thyroid and pituitary gland. Puzianowska *et al.* [143] have demonstrated that the mean expression level of *TR*β mRNA and *TR*α mRNA is markedly reduced, whereas the level of proteins TRβ and TRα is considerably increased in PTC tissues, when compared with normal thyroid tissue.

The transformation of TFCs into cancer cells was proved in the experiment with knock-in mutant mice ($TR\beta^{PV/PV}$), bearing a mutation (PV) of the thyroid hormone receptor gene [81]. TRßPV mutation was originally identified in patients suffering from thyroid hormone resistance syndrome. It has been shown that both the presence of mutation in two alleles of *TR* gene or mutation in one allele, together with inactivation of wild type allele of gene, leads to FTC [81]. The obtained results allowed the authors to postulate a tumour suppressor role for thyroid hormone receptor in a mouse model of thyroid carcinogenesis [81].

The demonstration of suppressor role for *TRß* opens new therapeutic perspectives for *TRß* isoforms analogs and its native ligand T_3. Silencing of the *TRß* gene by hypermethylation or by chromatic modifying agents could help to understand the mechanism of suppressive function of this gene. Further studies will explain the presence and function of various factors involved in suppressive signal, transferring to the cell nucleus [86].

C-myc and c-fos genes

The *c-myc* and *c-fos* genes code nuclear proteins which are components of the transcription complexes, regulating the expression of specific genes. Their products participate in the transduction of mitogenic signal on the pathway of the, so-called, "rapid response". Point mutations, amplifications or translocations of these genes into another chromosomal region lead to their activation and an increase of expression. Differences in the expression of these genes were noted, depending on the degree of differentiation among thyroid neoplasms [113]. It is thought that the disturbed expression of the *c-myc* and *c-fos* genes, as observed in cells of the carcinomas derived from the TFC, is not a primary change but a secondary consequence of the formed tumour phenotype [182].

Neither the presence nor the absence of c-myc protein, as localised by the immunohistochemical method, has any significant meaning in the prognosis of the follicular tumours [5].

Growth factors, cytokines and neuropeptides

The effect of growth factors on cell proliferation in the thyroid gland and the assessment of a potential role of these factors in the development of neoplastic changes, are the subject of studies at a number of research centres. Out of many growth factors, cytokines and neuropeptides, which may affect the proliferation processes in normal TFCs and the growth of benign and malignant neoplasms of the thyroid gland, an attention is focused on the activity of EGF, as well as of IGF-I, IGF-II, TGF-α, TGF-β, FGF, TNF-α, interferons, interleukins and somatostatin. A review of reports on this vast subject was presented in other publications [99,102].

Studies in our laboratory were performed in order to examine the expression of *IGF-I* gene and of genes for *IGF-binding proteins (IGFBP)-1, -2, -3, -4* in human thyroid cells from NG, FTA and FTC, cultured in monolayers. The experiment has demonstrated that in cells derived from benign lesions (NG, FTA) the expression in question was evidently lower than in cells of malignant tumours (FTC). Concluding, the differences in the expression of *IGF-I* and *IGFBP 1–4* genes between benign (NG, FTA) and malignant (FTC) lesions may allow for differentiation between these two types of lesions by molecular biology techniques [100].

Additionally, further research on the influence exerted by exogenous IGF-I on the expression of *IGF-I* and *IGFBP-1, -2, -3, -4* genes in cells from NG and from different human thyroid carcinomas (PTC, ATC, MTC) was performed; IGF-I revealed a stimulatory influence on the expression of its own gene, that effect being the most expressed in ATC cells. These facts indicate an important role of IGF-I in the pathogenesis and invasiveness of the examined malignant neoplasms [101].

Thyroglobulin (Tg) and thyroid peroxidase (TPO)

In normal thyroid cells, the biosynthesis of iodotyrosines and iodothyronines depends on the availability of iodine, the appropriate activity of TPO, the effectiveness of the system which generates hydrogen peroxide, and on the normal structure of Tg. In turn, TSH is regarded to be the main regulator of thyroid growth and of active iodine transportation. While in autoimmunological diseases of the thyroid gland, antibodies are observed against TSH-R**,** Tg, and TPO [62,75], in conditions of iodine deficiency the incidence of *RAS* family oncogene mutations increases [156].

Only few convincing data – at least so far – have been found, which would indicate direct effects of disturbed *Tg* and *TPO* gene expression on the neoplastic transformation of TFC.

The transcription factors, TTF-1 (thyroid transcription factor-1) and PAX-8, are the main regulators of the promoter expression of *Tg* and *TPO* genes, respectively. The neoplastic transformation of thyroid cells *in vitro*, by means of the *RAS* viral oncogene, was associated with the loss of expression of *Tg* and *TPO* genes and with a decreased cellular uptake of iodine [57].

The gene expression of *TSH-R*, *Tg*, *TTF-1* and *PAX-8* has been compared. In well-differentiated cell lines (WRO), the expression of all four genes has been documented; on the contrary, in the anaplastic cell lines

(ARO) only the expression of *TTF-1* and the reduced expression of *TSH-R* have been confirmed [28].

Telomerase

Telomerase is a specialized ribonucleoprotein polymerase that regulates the synthesis of telomerase repeats at chromosome ends. Much evidence has been accumulated indicating that telomerase activity is markedly repressed in normal human somatic tissues but reactivated in cancers and immortal cells, what suggests that the increased telomerase activity plays a role in carcinogenesis and cell immortalization. Telomerase activity is detected in the majority of thyroid cancers at advanced stages, frequently in PTC, even at early stages, and less frequently – in benign and hyperplastic tissues. These results suggest that telomerase reactivation plays a role during thyroid cancer development as an early indicator of the progress [26].

Chromosomal aberrations

If chromosomal aberrations in neoplastic diseases of the hematopoietic system are significant not only in the diagnostics but also in prognosing, then the to-date's cytogenetic observations of solid tumours do not allow for an explicit determination of the role of the aberrations found in clinical prognoses. In certain types of neoplastic tumours (including thyroid tumours), attempts have been undertaken to correlate the occurrence of a given chromosomal aberration (one or many) with the further clinical course of the disease.

Till now, nobody has succeeded to determine the extent, by which the chromosome aberrations, found in cells from tumour tissue cultures, affect the process of initiation, promotion or progression of these changes *in vivo*. Neither do we know to what extent are the observed aberrations secondary changes, caused by metabolic disorders in the already neoplastically transformed cells, and what is the character of the selection process into normal and aneuploid cells in the conditions of tissue culture.

In the cytogenetics of neoplasms, a rule has been accepted that only clonic abnormalities of chromosomes should be submitted to analysis. In case of thyroid tumours, it should be noticed that clonic chromosome aberrations were found for benign tumours in approximately 20% of cases. In that material, trisomy of chromosome 7 was the one most frequently observed among numerical aberrations, while structural aberrations involved most often the 19q13 chromosome [8].

A number of chromosome aberrations were found in cells from FTA. The most frequent clonic aberrations are associated with the presence of additional chromosomes, pairs 5, 7, and 12 [147]. A deletion in the long arm of 11q13 chromosome was sporadically observed in the cells from FTA [110].

As it has already been mentioned, the loss of genetic material in the short arm of 3(3p) chromosome may be associated with the transition of the benign form of follicular neoplasm into the undifferentiated thyroid carcinoma.

The structural aberrations of chromosomes, observed in PTC of the thyroid gland, concerned mainly chromosomes 10 and 17. An attention is to be pointed onto the clonic structural aberrations, namely: inv(10)(q11.2;q21) and t(10;17)(q11.2;q23) which, as it has been mentioned before, lead to the formation of oncogenic sequences: *RET*/PTC1 and *RET*/PTC2, respectively. Many other chromosomal aberrations were also observed in the cells of PTC, e.g., trisomy of chromosome 5 [70] and trisomy of chromosome 12 [164]. In turn, structural and numerical aberrations of chromosome 7 were associated with the occurrence of PTC, characterised by high histological and clinical malignancy [166].

The chromosome aberrations, as observed in undifferentiated thyroid carcinoma, manifested a complex character. The dominating forms included polyploid karyotypes and chromosome translocations, including the Robertsonian type (i.e., translocations within the pairs of acrocentric chromosomes – 13, 14, 15 (Group D) and 21, 22 (Group G). Also observed are dicentric and ring chromosomes, as well as "double minute" chromosomes [136]. The latter term denotes tiny chromosomes, appearing in metaphases in the numbers from several to several hundred. They are mainly observed in neoplastic cells, however, their presence has also been reported in cells submitted to the activity of certain mutagenic compounds. It is assumed that the "double-minute" chromosomes are a cytological effect of gene amplification [103].

Destabilization of the genome can lead to progresion of thyroid carcinoma to poorly-differentiated carcinomas and ATC. The loss of specific chromosomal regions containing *loci* for suppressor genes favours neoplastic transformation. Chromosomal abnormalities in DTC, as well as in poorly- and undifferentiated thyroid carcinomas have been noticed in the following regions: gains – 5p15, 5q11-13, 8q23, 19p, 19q and deletions – 8p and 22q; additionally, other gains – 1p34-36, 6p21, 9q34, 17q25, and deletions – 20q 1p11-31, 2q32-33, 4q11-13, 6q21 and 13q21-31 have been observed only in poorly- and undifferentiated thyroid carcinomas; finally, there have been several aberrations characteristic solely for undifferentiated thyroid carcinoma: gains – 3p13-14 and 11q13, as well as losses of genetic material in region 5q11-31 [181].

Studies of Polish authors from Gliwice and Gdańsk showed that the incidence rate of LOH was very similar in PTC and FTC, particularly in case of patients over 45 years old; LOH examinations were carried out using 14 highly polymorphic markers, previously described as frequently lost in thyroid tumours [180].

S100A4 gene

The *S100A4* gene located in chromosome 1 (1q21) encodes the calcium-binding protein A4 (or calcium placental proteins CAPL). The S100A4 protein is associated with cell proliferation and progression of neoplastic processes. The overexpression of *S100A4* gene has been observed in the most advanced thyroid carcinomas and lymph node metastases. Furthermore, this overexpression has been associated with very poor prognosis of the thyroid carcinoma. The results of performed studies suggest that *S100A4* gene overexpression could be the prognostic marker of thyroid carcinoma and potential target for therapeutic intervention [192].

It is worth noting that the expression of S100A4 protein has not been observed in normal TFCs and FTA cells, however, the presence of S100A4 has been shown in PTC cells and papillary *microcarcinoma* [74]. The invasive types of FTC more often demonstrate overexpression of S100A4 than the minimally invasive ones. In addition, in case of undifferentiated thyroid carcinoma, the overexpression of S100A4 protein has been proved in 61.9% of examined cases, however, the incidence rate has been lower than in PTC [74]. Unfortunately, the correlation between expression of S100A4 and clinical and pathological features of PTC has not been confirmed, although, it has been demonstrated that S100A4 protein plays an important role in PTC pathogenesis and it could be a new specific marker of differentiation of thyroid cells [74].

Further investigations, searching for a correlation between overexpression of *S100A4* gene and invasion or metastases, presented the increased S100A4 protein expression in 24 out of 28 cases of PTC node and distant metastases (86%) in contrast to the normal thyroid tissue and benign multinodular goitre. It should also be noticed that the expression of S100A4 protein has considerably been more intensive in invading fronts than in central portions of primary tumour and metastases. It has also been shown that the level of *S100A4* mRNA in node metastases is considerably higher, comparing to primary tumour [191].

In order to find new early diagnostic screening markers of the material from thyroid FNAB, the microarray technology has been performed. The overexpression of *S100A4* gene in PTC has been demonstrated [141].

MEDULLARY THYROID CARCINOMA – GENETIC ASPECTS AND SCREENING TESTS

Medullary thyroid carcinoma is a malignant neoplasm, derived from parafollicular cells (C) and occurring in two forms: sporadic and hereditary (Table 3). The hereditary form of MTC is found either as a component of multiple endocrine neoplasia type 2A and 2B (MEN 2A and MEN 2B) or – without any other endocrinopathies – as non-MEN FMTC.

MEN 2A, MEN 2B, and FMTC are three clinically different neoplastic syndromes, characterised by autosomal, dominating inheritance. The formation of these neoplasms is associated with point mutations within *RET* protooncogene, which is located on chromosome 10. In result of this type of mutations, an abnormal RET protein is produced. A strong correlation has been demonstrated between the type and location of mutations in *RET* protooncogene and the phenotype of the disease in question. The hereditary form of MTC, when compared with the sporadic one, presents with certain differences in clinical pathology, including – among others – bilateral localisation in the gland, the occurrence in younger age, and development on the basis of previously existing foci of C cell hyperplasia.

The diagnosis of the hereditary form of MTC can be made if two members of the same family are affected by this disease, or if MEN 2 syndrome is present in the patient with MTC. Molecular definition of hereditary MTC consists in an identification of germinal mutation of *RET* protooncogene. All the known mutations are missence mutations and lead to a substitution of cysteine by another amino acid. Diagnosis of a RET mutation in patients with MEN 2 syndrome enables presymptomatic genetic testing of other members of family, and leads to early reaction to prevent death and serious morbidity from MTC [73].

RET protooncogene and its protein product

The *RET (rearranged during transfection)* was discovered by Takahashi *et al.* [163] in cells of the NIH 3T3 line transfected with human DNA, isolated from lymphocyte-derived lymphoma.

RET protooncogene (*locus* in 10q11.2 region) contains 20 exons and includes more than 60 kb of genomic DNA. The human *RET* protooncogene encodes the formation of RET protein, i.e., membrane receptor, demonstrating tyrosine kinase activity. The receptors belonging to this group, form a family of transmembrane tyrosine-spe-

Table 3. Approximate prevalence rates of sporadic and hereditary MTC among all types of this carcinoma.
(Ponder [137]; Ferenc *et al.* [53])

Medullary thyroid carcinoma	%
Sporadic form	75
Hereditary form:	
- MEN 2A	15
- MEN 2B	3
- familial (FMTC)	7

cific protein kinases, which play the role of receptors for growth factors.

It has been shown that the glial cell-derived neurotrophic factor (GDNF) is a ligand for RET. However, the extracellular part of RET protein does not have any area to bind the ligand. Another molecule is required for signal transmission, the molecule being called GDNF receptor α (GFR-α). In other words, the RET molecule plays the role of a coreceptor for the GDNFR-α which, in complex with GDNF, activates the RET molecule. Subsequent years have brought discoveries of three other proteins which are ligands for RET: neurturine (NRTN), persefine (PSPN) and arthemine (ARTN). They are structurally similar to GDNF (GDNF family of proteins) [6].

It has recently been documented that the growth arrest-specific gene 1 (*Gas1*) shows high structural similarity to the GDNF and compromises RET-mediated GDNF-dependent survival effects by modifining signalling downstream of RET [22].

The schematic organisation of *RET* protooncogene in juxtaposition with RET protein is presented in Figure 9. The exons 1–10 encode the extracellular part of RET protein which contains – beginning from the amine end (NH_2) – a signalling sequence and, then – a domain, demonstrating a similarity to proteins from the cadherin family and a conservative region, rich in cysteine; this cysteine-rich region plays presumably an essential role in the determination of the three-dimensional structure of the extracellular part of RET protein, while cysteine residues themselves take part in the receptor dimerisation process. The transmembrane part of RET protein is encoded by exon 11. Exons 12–20 encode the intracellular part of RET protein, which contains two regions presenting with tyrosine kinase activity (TK1 and TK2). The interkinase region (TK), consisting of 27 amino acids, separates TK1 region from that of TK2.

Medullary thyroid carcinoma – the sporadic form

Somatic mutations of *RET* protooncogene are associated with sporadic incidence of MTC. In 23–67% of this form of MTC, mutations were noted in codon 918 (exon 16), leading to the replacement of methionine by threonine (ATG–ACG) [45]. The mutations comprised only the DNA, which originated from tumour tissue, while, for example, they have been not found in the DNA from peripheral blood lymphocytes. Less often, the mutations concerned codons 768 and 883, resulting from replacements of glutaminic acid by asparaginic acid and alanine by phenyloalanine, respectively. It was also observed that the already mentioned mutation in codon 918 of *RET* protooncogene, increases the development rate and the spread out of this form of MTC, deteriorating the prognosis.

Also, there are other – extremely rare – somatic mutations of *RET* protooncogene, resulting in a sporadic MTC, i.e., deletions or insertions of *RET* sequence fragment (usually in exons 10 and 11).

Lately, a new mutation has been disclosed in Czech patients with sporadic MTC, namely – triplesomatic mutation Gly911Asp, Met918Thr, and Glu921Lys in exon 16 and double-somatic mutation Val591Ile in exon 10, with a concomitant somatic mutation Met918Thr in exon 16 [45]. There are also data suggesting that single-nucleotide polymorphisms (SNPs) could act as genetic modifiers in the development of some forms of hereditary and sporadic MTC [7,34].

Cases of the sporadic MTC form always demand of a detailed clinical evaluation, since some of them may be assigned to the MEN 2A syndrome (either incomplete family history or a *de novo* mutation which occurred in the parental generation) or turn out to be the classical form of familial MTC, as discussed in further parts of this article. An analysis of *RET* protooncogene mutations in genomic DNA is decisive for the final diagnosis.

Medullary carcinoma – associated with type 2A multiple endocrine neoplasia (MEN 2A)

The MEN 2A syndrome is a rare, hereditary neoplastic syndrome, in which MTC develops in all the affected patients, while pheochromocytoma is found in 50% of the patients, and either parathyroid hyperplasia or adenoma is observed in about 5–10% of the patients.

Approximately 92% of MEN 2 cases are caused by mutations in exons 10, 11, 13–16 of the RET protooncogene. There exists inter- and intrafamilial phenotypic variability among the MEN 2 families, even when the disease is caused by the same RET mutation, suggesting a role for genetic modifiers, such as polymorphisms/haplotypes [54].

Characteristic for MEN 2A is a change of the base in one of codons: 609, 611, 618, 620 (exon 10) or 634 (exon 11) caused – in the region of RET protein rich in cysteine – a substitution of that amino acid with another amino acid. It is to be recalled that the region rich in cysteine is located close to the transmembrane domain of RET protein (Figure 9).

In patients with the MEN 2A syndrome, each mutation of *RET* protooncogene in codon 634, which substitutes cysteine by another amino acid, predisposes to the development of pheochromocytoma. In turn, the specific mutation in this codon (TGC–CGC), causing the replacement of cysteine by arginine, highly correlates with the occurrence of either parathyroid hyperplasia or adenoma/as of parathyroid glands.

Primary hyperparathyroidism in the MEN 2A syndrome is usually characterised by mild hypercalcemia and by a mostly asymptomatic clinical course.

In general, no other developmental abnormalities are associated with the MEN 2A syndrome, however, in certain families, two clinical variants of this syndrome can be observed: 1) with cutaneous lichen amyloidosis, 2) with Hirschsprung's disease [87,146]. Hirschsprung's disease (*megacolon*) is caused by point mutations of *RET* gene or by deletion of the entire gene; the disease

can occur in sporadic, as well as in hereditary form. If it coexists with the MEN 2A syndrome, these cases are always associated with mutations in codons 618 or 620 of *RET* gene. The pathogenesis of *megacolon* is related to the lack of nerve plexuses of the submucous and muscular layer, especially of ganglion cells. From the clinical point of view, this disease is characterized by strong constipations, connected with meteorism, often leading to intestinal obstruction and ileus, already in the neonatal period.

Medullary carcinoma – associated with type 2B multiple endocrine neoplasia (MEN 2B)

In about 40–50% of MEN 2B cases, MTC coexists with adrenal pheochromocytoma. Extensions of this pathological process onto the parathyroid glands are observed either rarely or never in the MEN 2B syndrome. Thus, consequently, no hyperparathyroidism is observed in the MEN 2B syndrome. Medullary thyroid carcinoma, associated with the MEN 2B syndrome, generates local metastases in cervical and mediastinal regions (lymph to the nodes), and distant metastases to the liver, the lungs or bones.

Unlike MEN 2A syndrome, does the MEN 2B syndrome – beside medullary carcinoma and pheochromocytomas – reveal other characteristic features, first of all, neuromas and gangliomas occur (mainly mucous neuromas, often localized on the lips, on the tongue, and in the colon), together with marphanoid features of body build (long, thin limbs, the abnormal upper to lower body part ratio, frequent dislocations of femoral bone epiphysis, Gothic palate).

Also very characteristic is an increased number of autosomal ganglia and hyperplasia of autonomic nerve fibres in intestinal wall, what leads to disorders of motor intestinal activity. The association between MEN 2B and its most common *RET* mutation, colonic dysmotility, and low density of substance P (SP) in the colonic circular muscle has been presented. Larger numbers of patients need to be studied to investigate whether low SP is primarily associated with the constipation or *RET* mutation and if it is a common feature of MEN 2B [84].

In more than 90% of patients with the MEN 2B syndrome, a substitution of single base in codon 918 (exon 16) of *RET* protooncogene was identified in genomic DNA, leading in each case to the replacement of ATG by ACG. The mutation in codon 918 of this protooncogene

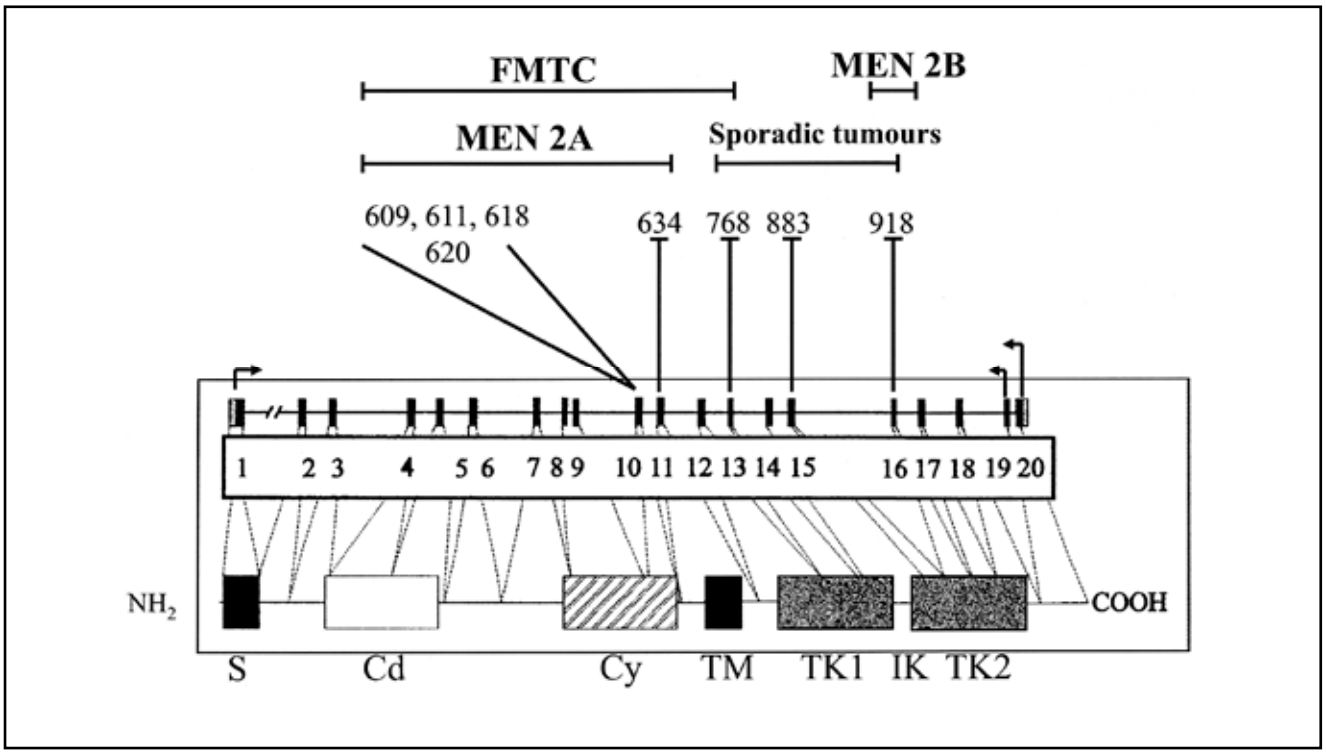

Figure 9. The structure and mutations of *RET* protooncogene, identified in patients with sporadic and hereditary form of MTC (following Mulligan and Ponder [123]).
The exons of *RET* protooncogene (1 to 20) are presented as black rectangles. The dotted lines link the exons with associated RET protein domains. The mutated codons of *RET* protooncogene and the phenotypes, associated with each mutation, are demonstrated above the map of exons. Within RET protein – the gene product – the following elements are presented: the signalling sequence (S), the homologous sequence to cadherins (Cd), the region rich in cysteine (Cy), the domain in the transmembrane part (TM), the domain of tyrosine kinase (TK1 and TK2) and the interkinase domain (IK).

seems to affect the specificity of cytoplasmic substrates of RET protein tyrosine kinase. No mutations in exons 10 and 11 were observed in patients with MEN 2B [140].

Non-MEN familial MTC – FMTC

According to Mulligan *et al.* [121], the familial form of MTC includes such cases of this neoplasm, which are found in families (even through several generations, affecting minimum 4 persons in a family), in which MTC has been diagnosed without any symptoms of adrenal pheochromocytoma or of hyperplasia and adenoma of parathyroid glands (i.e., without any symptoms of MEN 2).

A number of hereditary MTCs remain unclassified because it often cannot be decided if, in case of hereditary MTC without any other symptoms, the observed pathology is not the MEN 2A syndrome, in which adrenal tumours may occur later on, usually 10 years after MTC. Therefore, it is possible to wrongly diagnose FMTC instead of MTC as a part of the MEN 2A syndrome.

Both FMTC and MTC coexisting with MEN 2A syndrome are characterized by the development of neoplastic foci in both thyroid lobes. It is to be noted that FMTC occurs later on, i.e., in age of 40–50 years, when compared with MTC in the MEN 2A syndrome (in age of 20–30 years).

In more than 80% of patients with FMTC, mutations in genomic DNA concerned the same five codons (609, 611, 618, 620, 634), similarly as in the MEN 2A syndrome. However, the percentage distribution of mutations in FMTC was almost the same for codons: 618 (35%), 620 (25%), and 634 (30%) [121,122,123]. In turn, other authors observed a mutation of *RET* protooncogene in codon 618 in 50% of examined patients with FMTC [59].

At present, it is not yet possible to differentiate FMTC from MTC coexisting with the MEN 2A syndrome, at the molecular level.

It is intriguing why mutations in the same codons of *RET* protooncogene, while causing the same substitutions of amino acids, lead to the manifestation of clinical symptoms, which are different in the MEN 2A syndrome and in FMTC. Perhaps, it is either an effect of an additional mutation within the *RET* protooncogene or in another closely located site, or – an effect of tissue-specific varying gene expression. In turn, an attempt to explain the problem on the basis of genomic imprinting, i.e., the influence of either maternal or paternal *RET* allele (depending on the origin of mutation) on the enhancement of mutation effect in cells of adrenal medulla and/or parathyroid glands, requires further studies.

In few patients with FMTC, just as it is reported for some MEN 2A patients, cases are observed, which are accompanied by Hirschsprung's disease; these cases are always associated with mutations in codons 618 or 620.

Screening examinations for mutations in families with an increased risk for MTC

The screening examinations, searching for the hereditary form of MTC, include:

1) family history with genealogical analysis,
2) clinical examination, as well as USG-guided cytological examination (FNAB),
3) DNA analysis and biochemical tests.

The goals of DNA analysis are the following:

a) differentiation of the sporadic (non-hereditary) MTC from the hereditary form of this neoplasm,
b) an identification of mutated *RET* gene carriers in families with increased risk, i.e., in those with history of hereditary MTC.

In screening examinations towards the hereditary form of MTC, the genomic DNA is analyzed, being most often isolated from peripheral blood lymphocytes. The most important determination in biochemical studies is serum calcitonin concentration, especially in dynamic test with pentagastrin. Pentagastrin administration (in a dose of 0.5 μg/kg b.w.) is followed by calcitonin concentration measurement by the immunoradiometric assay (IRMA), before the test and then, after 2, 5 and 10 or 15 minutes from pentagastrin administration.

In each patient with diagnosed MTC, its hereditary forms (MEN 2A and 2B) and FMTC – are to be excluded.

The diagnosis of MTC, regardless of its histological form, allows to decide on possibly the most radical thyroidectomy to be performed; in these cases, the extent of performed surgery is directly associated with further therapeutic prognosis. In cases with confirmed presence of mutations of *RET* protooncogene (asymptomatic carriers), radical thyroidectomy is postulated already at the age of 6 years (in cases of FMTC and MEN 2A) and even immediately after diagnosis of *RET* mutation in cases of MEN 2B (in the latter syndrome, genetic examination should be performed as soon as by the age of 12 months).

Figure 10 presents the scheme of diagnostic management, applied to identify cases of hereditary MTC among family members of the proband in whom MTC has been diagnosed on the basis of FNAB and histopathological studies. DNA is isolated from peripheral blood lymphocytes and tumour tissue of patients with diagnosed MTC (probands), in order to look for mutations in *RET* protooncogene. Using the PCR technique, an amplification of a studied DNA fragment is possible. In molecular diagnostics of MTC, a combination of PCR with SSCP and/or sequencing methods is applied.

The presence of mutation only in DNA from tumour tissue indicates the sporadic form of MTC. In this situation, no further biochemical tests are required.

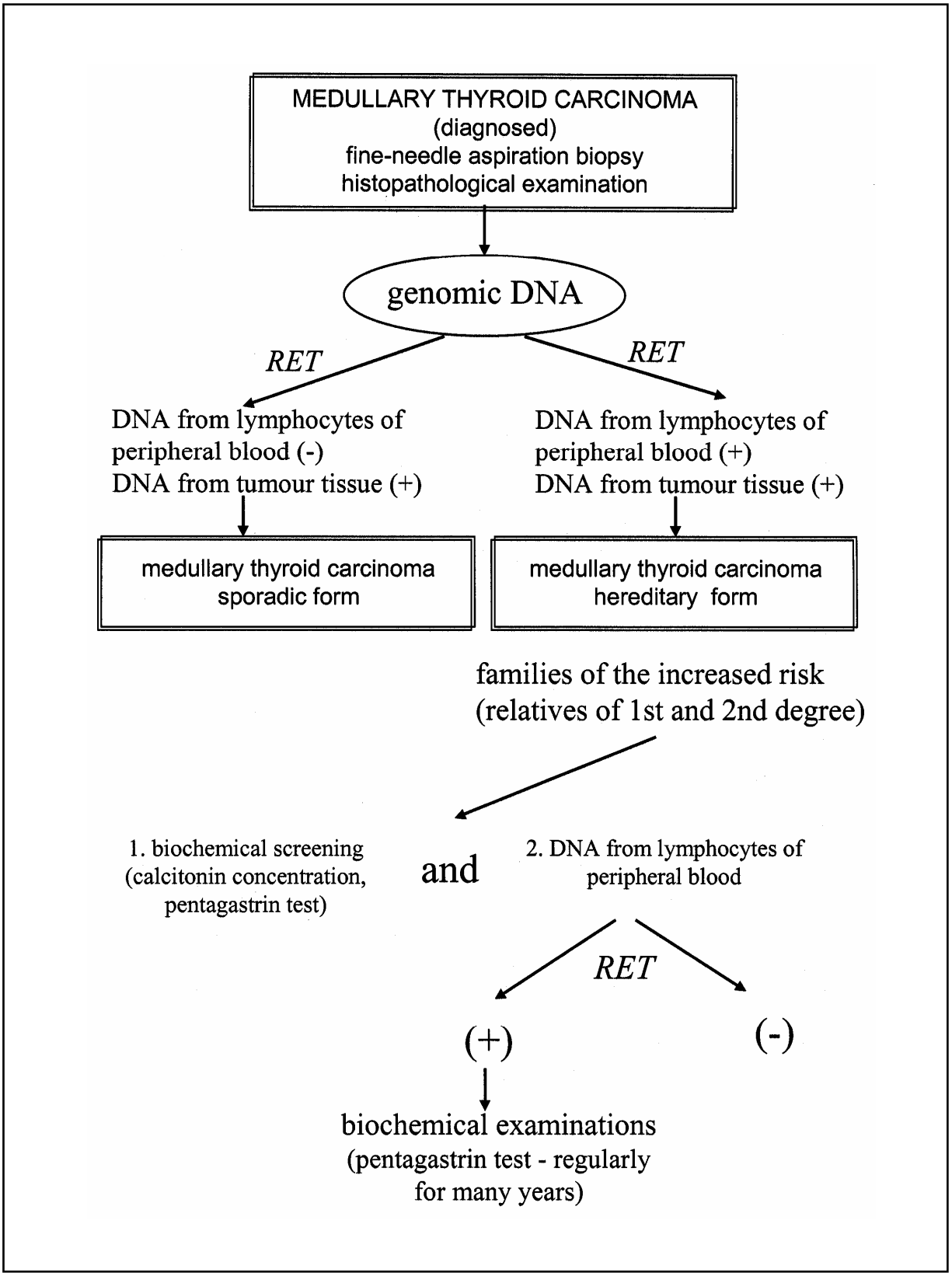

Figure 10. The proposed diagnostic management to differentiate between the sporadic and hereditary forms (FMTC, MEN 2A, MEN 2B) of medullary thyroid carcinoma (based on: Forster-Gibson and Mulligan [56], in our own modification).

Pentagastrin stimulation test, performed in patients with this form of MTC, serves the purpose of assessing the radicality of performed surgery, as well as it may be used for postoperative follow up.

The presence of mutations in DNA from peripheral blood lymphocytes, as well as in DNA from tumour tissue of patients with diagnosed MTC, indicates the hereditary form of MTC. In such situation, screening tests are to be performed in family members of the proband (the 1st and the 2nd degree of relationship), including serum calcitonin level determination, pentagastrin stimulation test and an evaluation of DNA from peripheral blood lymphocytes. The presence of mutations in DNA from peripheral blood lymphocytes in a family member of the proband is an indication to periodic (every 6 months) – but repeated for many years – monitoring with the use of pentagastrin test.

Summing up, it should be concluded that the genetics of thyroid neoplasms is now the most dynamically developing branch of thyroidology.

REFERENCES

1 Adams RL, Hanley A, Rinaldi A. DNA demethylation in erythroleukaemia cells FEBS Lett. 1990; **269**: 29–31.
2 Arai T, Yamashita T, Urano T, Masunaga A, Itoyama S, Itoh K, Shiku H, Sugawara I. Preferential reduction of nm23-H1 gene product in metastatic tissues from papillary and follicular carcinomas of the thyroid. Mod Pathol. 1995; **8**: 252–256.
3 Aretz S, Stienen D, Uhlhaas S, Loff S, Back W, Pagenstecher C, McLeod DR, Graham GE, Mangold E, Santer R, Propping P, Friedl W. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. Hum Mutat. 2005; **26**: 513–519.
4 Ashworth LK, Batzer MA, Brandriff B, Branscomb E, de Jong P, Garcia E, Garnes JA, Gordon LA, Lamerdin JE, Lennon G, Mohrenweiser H, Olsen AS, Slezak T, Carrano AV. An integrated metric physical map of human chromosome 19 Nat Genet. 1995; **11**: 422–427.
5 Auguste L-J, Masood S, Westerband A, Belluco C, Valdermma E, Attie J. Oncogene expresion in follicular neoplasms of the thyroid. Am J Surg. 1992; **164**: 592–598.
6 Baloh RH, Tansey MG, Johnson EM Jr, Milbrandt J. Functional mapping of receptor specificity domains of glial cell line-derived neurotrophic factor (GDNF) family ligands and production of GFRalpha1 RET-specific agonists. J Biol Chem. 2000; **275**: 3412–3420.
7 Baumgartner-Parzer SM, Lang R, Wagner L, Heinze G, Niederle B, Kaserer K, Waldhausl W, Vierhapper H. Polymorphisms in exon 13 and intron 14 of the RET protooncogene: genetic modifiers of medullary thyroid carcinoma? J Clin Endocrinol Metab. 2005; **90**: 6232–6236.
8 Belge G, Rouge L, Soares J, Bruckmann S, Thode B, Fonseca E,, Clode A, Bartnitzke S, Castedo S, Bullerdiek J. Cytogenetic investigations of 340 thyroid hyperplasias and adenomas revealing correlations between cytogenetic findings and histology. Cancer Genet Cytogenet. 1998; **101**: 42–48.
9 Bevan S, Pal T, Greenberg CR, Green H, Wixey J, Bignell G, Narod SA, Foulkes WD, Stratton MR, Houlston RS. A comprehensive analysis of MNG1, TCO1, fPTC, PTEN, TSHR, and TRKA in familial nonmedullary thyroid cancer: confirmation of linkage to TCO1.J Clin Endocrinol Metab. 2001; **86**: 3701–3704.
10 Bignell GR, Canzian F, Shayeghi M, Stark M, Shugart YY, Biggs P, Mangion J, Hamoudi R, Rosenblatt J, Buu P, Sun S, Stoffer SS, Goldgar DE, Romeo G, Houlston RS, Narod SA, Startton MR, Foulkes WD. Familial nontoxic multinodular thyroid goiter locus maps to chromosome 14q but does not account for familial nonmedullary thyroid cancer. Am J Hum Genet 1997; **61**: 1123–1130.
11 Bollag G, Freeman S, Lyons JF, Post LE. Raf pathway inhibitors in oncology. Curr Opin Investig Drugs. 2003; **4**: 1436–1441.
12 Boltze C, Zack S, Quednow C, Bettge S, Roessner A, Schneider-Stock R. Hypermethylation of the CDKN2/p16INK4A promotor in thyroid carcinogenesis. Pathol Res Pract. 2003; **199**: 399–404.
13 Bond JA, Wyllie FS, Rowson J, Radulescu A, Wynford-Thomas D., In vitro reconstruction of tumour initiation in a human epithelium. Oncogene. 1994; **9**: 281–290
14 Bourasseau I, Savagner F, Rodien P, Duquenne M, Reynier P, Guyetant S, Bigorgne JC, Malthiery Y, Rohmer V No evidence of thyrotropin receptor and G(s alpha) gene mutation in high iodine uptake thyroid carcinoma Thyroid. 2000; **10**: 761–765.
15 Broaddus RR, Lynch PM, Lu KH, Luthra R, Michelson SJ. Unusual tumors associated with the hereditary nonpolyposis colorectal cancer syndrome. Mod Pathol. 2004; **17**: 981–989.
16 Bruni P, Boccia A, Baldassarre G, Trapasso F, Santoro M, Chiappetta G, Fusco A, Viglietto G. PTEN expression is reduced in a subset of sporadic thyroid carcinomas: evidence that PTEN-growth suppressing activity in thyroid cancer cells mediated by p27kip1. Oncogene. 2000; **19**: 3146–3155.
17 Brzeziańska E, Karbownik M, Migdalska-Sęk M, Pastuszak-Lewandoska D, Włoch J, Lewiński A. Molecular analysis of the *RET* and *NTRK1* gene rearrangements in papillary thyroid carcinoma in Polish population. Mutat Res. 2006; **599**: 26–35.
18 Brzeziańska E, Migdalska-Sęk M, Pastuszak-Lewandoska D, Wojciechowska K, Lewiński A. Incidence assessment of *BRAF* V599E mutation in papillary thyroid carcinoma in Polish population. (Abstract P-02-15). Endokrynol Pol - Polish J Endocrinol. 2005; **56**: 560–561.
19 Brzeziańska E, Pastuszak-Lewandoska D, Cyniak-Magierska A, Lewiński A. Molecular analysis of loss of heterozygosity (LOH) at 3p24.3 locus (*c-erA*β gene) in papillary thyroid carcinoma. (Abstract). Endokrynol Pol - Polish J Endocrinol. 2005; **56:** 370.
20 Brzeziańska E, Pastuszak-Lewandoska D, Cyniak-Magierska A, Lewiński A. Ocena ekspresji cykliny D1 w nowotworach tarczycy jako marker inwazyjności nowotworu [(Estimation of cyclin D1 expression in thyroid neoplasms as a neoplasmatic invasive marker) (In Polish)]. Endokrynol Pol – Polish J Endocrinol. 2006; **57**(suppl. A): 106.
21 Bulow C, Bulow S. Is screening for thyroid carcinoma indicated in familial adenomatous polyposis? The Leeds Castle Polyposis Group. Int J Colorectal Dis. 1997; **12**: 240–242.
22 Cabrera JR, Sanchez-Pulido L, Rojas AM, Valencia A, Manes S, Naranjo JR, Mellstrom B. GAS1 is related to the GDNF family receptors alpha and regulates ret signaling. J Biol Chem. 2006; **281**: 14330–14339.
23 Canzian F, Amati P, Harach R. A gene predisposing to familial thyroid tumors with oxyphilia maps to chromosome 19p13.2. Am J Hum Genet.1998; **63**: 1743–1748.
24 Caudill CM, Zhu Z, Ciampi R, Stringer JR, Nikiforov YE Dose-dependent generation of RET/PTC in human thyroid cells after in vitro exposure to gamma-radiation: a model of carcinogenic chromosomal rearrangement induced by ionizing radiation. J Clin Endocrinol Metab. 2005; **90**: 2364–2369.
25 Cetta F, Pelizzo MR, Curia MC, Barbarisi A. Genetic and clinicopathological findings in thyroid carcinomas associated with familial adenomatous polyposis. Am J Pathol. 1999; **155**: 7–9.
26 Cheng A-J, Lin J-D, Chang T, Wang T-CV. Telomerase activity in benign and malignant human thyroid tissues. Br J Cancer. 1998; **77**: 2177–2180.
27 Chen R-X, Masuda T, Fujimori K, Naganuma H, Takaya K, Mori Y, Nagura H. High-risk group of differentiated thyroid carcinoma with p53 overexpression and local recurrence. Thyroidology Clin Exp. 1998; **10**: 125–130.

28 Chen ST, Shieh HY, Lin JD, Chang KS, Lin KH. Overexpression of thyroid hormone receptor beta1 is associated with thyrotropin receptor gene expression and proliferation in a human thyroid carcinoma cell line. J Endocrinol. 2000; **165**: 379–389.

29 Ciampi R, Knauf JA, Kerler R, Gandhi M, Zhu Z, Nikiforova MN, Rabes HM, Fagin JA, Nikiforov YE. Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. J Clin Invest. 2005; **115:** 94–101.

30 Clurman BE, Groudine M. The *CDKN2* tumor-suppressor locus- a tale of two proteins. N Engl J Med.1998; **338**: 910–912.

31 Cobb MH. MAP kinase signaling pathways. Promega Notes Magazine 1996; **56**: 37–42.

32 Cohen Y, Rosenbaum E, Clark DP, Zeiger MA, Umbricht CB, Tufano RP, Sidransky D, Westra WH. Mutational analysis of BRAF in fine needle aspiration biopsies of thyroid: a potential application for the preoperative assessment of thyroid nodules. Clin Can Res. 2004; **10**: 2761–2765.

33 Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, Beller U, Westra WH, Ladenson PW, Sidransky D. BRAF mutation in papillary thyroid carcinoma. J Natl Can Inst. 2003; **95:** 625–627.

34 Costa P, Domingues R, Sobrinho LG, Bugalho MJ. RET polymorphisms and sporadic medullary thyroid carcinoma in a Portuguese population. Endocrine. 2005; **27**: 239–243.

35 Cyniak-Magierska A, Brzeziańska E, Pastuszak-Lewandoska D, Januszkiewicz J, Lewiński A. Analiza występowania mutacji punktowych w onkogenach z rodziny *RAS* w raku brodawkowatym gruczołu tarczowego [Analysis of the prevalence of family *RAS* oncogenes point mutations in papillary thyroid carcinoma (in Polish)]. Abstract, Endokrynol Pol - Pol J Endocrinol. 2004; **55**: 98–99.

36 Cyniak-Magierska A, Brzeziańska E, Pastuszak-Lewandoska D, Migdalska-Sęk M, Lewiński A. Ocena ekspresji onkogenu c-MET w raku brodawkowatym gruczołu tarczowego [Analysis of *c-MET* oncogene expression in papillary thyroid carcinoma (In Polish)]. Abstract, Endokrynol Pol - Pol J Endocrinol. 2004; **55**: 109.

37 Dammann R, Yang G, Pfeifer GP. Hypermethylation of the cpG island of Ras association domain family 1A (RASSF1A), a putative tumor suppressor gene from the 3p21.3 locus, occurs in a large percentage of human breast cancers. Cancer Res. 2001; **61**: 3105–3109.

38 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature. 2002; **417:** 949–954.

39 Dhanasekaran N, Heasley LE, Johnson GL. G protein-coupled receptor systems involved in cell growth and oncogenesis. Endocr Rev. 1995; **16**: 259–270.

40 Derwahl M. Editorial: TSH receptor and Gsα gene mutations in the pathogenesis of toxic thyroid adenomas – a note of caution. J Clin Endocrinol Metab. 1996; **81**: 2783–2785.

41 Di Cristofaro J, Vasko V, Savchenko V, Cherenko S, Larin A, Ringel MD, Saji M, Marcy M, Henry JF, Carayon P, De Micco C. ret/PTC1 and ret/PTC3 in thyroid tumors from Chernobyl liquidators: comparison with sporadic tumors from Ukrainian and French patients. Endocr Relat Cancer. 2005; **12**: 173–183.

42 Digel W, Lubbert W. DNA methylation disturbances as novel therapeutic target in lung cancer: preclinical and clinical results Crit Rev Oncol Hematol. 2005; **55**: 1–11.

43 Di Renzo MF, Olivero M, Ferro S, Prat M, Bongarzone I, Pilotti S, Belfiore A, Costantino A, Vigneri R, Pierotti MA, *et al.* Overexpression of the c-MET/HGF receptor gene in human thyroid carcinomas. Oncogene. 1992; **7**: 2549–2553.

44 Domingues R, Mendonca E, Sobrinho L, Bugalho MJ. Searching for RET/PTC rearrangements and BRAF V599E mutation in thyroid aspirates might contribute to establish a preoperative diagnosis of papillary thyroid carcinoma. Cytopathology. 2005; **16**: 27–31.

45 Dvorakova S, Vaclavikova E, Sykorova V, Duskova J, Vlcek P, Ryska A, Novak Z, Bendlova B. New Multiple Somatic Mutations in the RET Proto-oncogene Associated with a Sporadic Medullary Thyroid Carcinoma. Thyroid. 2006; **16**: 311–316.

46 Elgadi A, Frisk T, Larsson C, Wallin G, Hoog A, Zedenius J, Norgren S. Lack of mutations in the TSHr and Gsalpha genes in TSHr antibody negative Graves' disease. Exp Clin Endocrinol Diabetes. 2005; **113**: 516–521.

47 Fagin JA. Genetic basis of endocrine disease 3. Molecular defects in thyroid gland neoplasia. J Clin Endocrinol Metab. 1992; **75**: 1398–1400.

48 Fagin JA, Tang SH, Zeki K, Di Lauro R, Fusco A, Gonsky R. Reexpression of thyroid peroxidase in a derivative of an undifferentiated thyroid carcinoma cell line by introduction of wild-type p53. Cancer Res. 1996; **56**: 765–771.

49 Farid NR, Shi Y, Zou M. Molecular basis of thyroid cancer. Endocr Rev. 1994; **15**: 202–232.

50 Ferenc T, Lewiński A. Udział czynników genetycznych w patogenezie nowotworów tarczycy wywodzących się z komórki pęcherzykowej tego gruczołu [Participation of genetic factors in the pathogenesis of thyroid neoplasms derived from the follicular cell of this gland (In Polish]. Endokrynol Pol – Polish J Endocrinol. 1999; **50** (suppl. 1 to no. 4): 61–90

51 Ferenc T, Lewinski A, Lange D, Niewiadomska H, Sygut J, Sporny S, Jarzab B, Salacinska-Los E, Kulig A, Wloch J. Analysis of P161NK4A protein expression in follicular thyroid tumors. Pol J Pathol. 2004; **55**: 143–148.

52 Ferenc T, Lewinski A, Lange D, Niewiadomska H, Sygut J, Sporny S, Jarzab B, Salacinska-Los E, Kulig A, Wloch J. Analysis of cyclin D1 and retinoblastoma protein immunoreactivity in follicular thyroid tumors. Pol J Pathol. 2005; **56**: 27–35.

53 Ferenc T, Lewiński A, Pastuszak-Lewandoska D. Rak rdzeniasty tarczycy - zagadnienia genetyczne i badania przesiewowe [Medullary thyroid carcinoma – genetic problems and screening tests (In Polish)]. Endokrynol Pol - Polish J Endocrinol. 1997; **48** (suppl. 2 to no. 2): 177–188.

54 Fernandez RM, Navarro E, Antinolo G, Ruiz-Ferrer M, Borrego S. Evaluation of the role of RET polymorphisms/haplotypes as modifier loci for MEN 2, and analysis of the correlation with the type of RET mutation in a series of Spanish patients. Int J Mol Med. 2006; **17**: 575–581.

55 Figge J, Bakst G, Weisheit D, Solis O, Ross JS. Image analysis quantitation of immunoreactive retinoblastoma protein in human thyroid neoplasms with a streptavidin-biotin- peroxidase staining technique. Am J Pathol. 1991; **139**: 1213–1219.

56 Forster-Gibson CJ, Mulligan LM. Multiple endocrine neoplasia type 2. Eur J Cancer. 1994; **13**: 1969–1974.

57 Francis-Lang H, Zannini M, De Felice M, Berligieri MT, Fusco A, Di Lauro L. Multiple mechanism of interference between transformation and differentation in thyroid cells. Mol Cel Biol. 1992; **12**: 5793–5880.

58 Frattini M, Ferrarion C, Bressan P, Balestra D, De Cecco L, Mondellini P, Bongarzone I, Collini P, Gariboldi M, Pilotti S, Piarotti MA, Greco A. Alternative mutations of *BRAF*, *RET* and *NTRK1* are associated with similar but distinct gene expression patterns in papillary thyroid cancer. Oncogene. 2004; **23**: 7436–7440.

59 Frilling A, Happner W, Eng C, Mulligan LM, Raue F, Broelsch CE. Presymptomatic genetic screening in families with multiple endocrine neoplasia type 22. J Mol Med. 1995; **73**: 229–233.

60 Fugazzola L, Mannavol D, Cirello V, Vannucchi G, Muzza M, Vicentini L, Beck-Peccoz P. BRAF mutations in an Italian cohort of thyroid cancers. Clin Endocrinol. 2004; **61:** 239–243.

61 Fukushima T, Suzuki S, Mashiko M, Ohtake T, Endo Y, Takebayashi Y, Sekikawa K, Hagiwara K, Takenoshita S. *BRAF* mutations in papillary carcinomas of the thyroid. Oncogene. 2003; **22:** 6455–6457.

62 Furmaniak J, Smith BR. Immunity to the thyroid-stimulating hormone receptor. Springer Semin Immunopathol. 1993; **3**: 143–146.

63 Furnari FB, Huang HJ, Cavenee WK. The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. Cancer Res. 1998; **58**: 5002–5008.

64 Fusco L, Viglietto G, Santoro M. A new mechanism of BRAF activation in human thyroid papillary carcinomas. J. Clin. Invest. 2005; **115:** 20–23.
65 Garcia-Rostan G, Camp RL, Herrero A, Carcangiu ML, Rimm DL, Tallini G. Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. Am J Pathol. 2001; **158**: 987–996.
66 Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle-Teijeiro J, Santoro M. Mutation of the PIK3CA gene in anaplastic thyroid cancer. Cancer Res. 2005; **65**: 10199–10207.
67 Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, Zhu Z, Ciampi R, Roh M, Shedden K, Gauger P, Doherty G, Thompson NW, Hanash S, Koenig RJ, Nikiforov JE. Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. Oncogene. 2005; **24:** 6646–6656.
68 Greco A, Miranda C, Pagliardini S, Fusetti L, Bongarzone I, Pierotti MA. Chromosome 1 rearrangements involving the genes TPR and NTRK1 produce structurally different thyroid-specific TRK oncogenes. Genes Chromosomes Cancer. 1997; **19**: 112–123.
69 Greenblatt MS, Benett WP, Holstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res. 1994; **54**: 4855–4878.
70 Herrmann ME, Talpos GB, Mohamed AN, Saxe A, Ratner S, Lalley PA, Wolman SR. Genetic markers in thyroid tumors. Surgery. 1991; **110**: 941–948.
71 Hiasa Y, Konishi N, Tao M, Matsuda H, Tsumatani K, Nakamura Y, Kitahori Y, Ozono S, Hirao Y, Okajima E. Different expression of nm23-H1 and nm23-H2 protein with proliferative activities in various sizes of human renal cell carcinoma. Oncology. 1996; **53**: 79–87.
72 Houlston RS, Stratton MR. Genetics of non-medullary thyroid cancer. Quart J Med. 1995; **88**: 685–693.
73 Hubner RA, Houlston RS Molecular advances in medullary thyroid cancer diagnostics. Clin Chim Acta. 2006; **370**: 2–8.
74 Ito Y, Yoshida H, Tomoda C, Uruno T, Miya A, Kobayashi K, Matsuzuka F, Kakudo K, Kuma K, Miyauchi A. S100A4 expression is an early event of papillary carcinoma of the thyroid. Oncology. 2004; **67**: 397–402.
75 Janota-Bzowski M, Czarnocka B, McIntosh RS, Asghar MS, Watson PF, Kemp H, Pastuszko D, Łyczkowska A, Carayon P, Weetman AP. Comparison of immunodominant regions of TPO defined by murine anti-human TPO monoclonal antibodies and recombinant anti-TPO IgGκ Fab fragments from patients with Hashimoto's thyroiditis. Endokrynol Pol – Polish J Endocrinol 1997; **48**: 237–247.
76 Jarry A, Masson D, Cassagnau E, Parois S, Laboisse C, Denis MG. Real-time allele-specific amplification for sensitive detection of BRAF mutation V600E. Mol Cell Probes.2004; **18**: 349–352.
77 Jones C, Shaw JJ, Wyllie FS, Gaillard N, Schlumberger M, Wynford-Thomas D. High frequency deletion of the tumour suppressor gene p16INK4a (MTS1) in human thyroid cancer cell lines. Mol Cell Endocrinol. 1996; **116**: 115–119.
78 Kameyama K, Mukai M, Takami H, Ito K. Cribriform-morular variant of papillary thyroid carcinoma: ultrastructural study and somatic/germline mutation analysis of the APC gene. Ultrastruct Pathol. 2004; **28**: 97–102.
79 Karga H, Lee J-K, Vickery AL, Thor A, Gaz RD, Jameson JL. *Ras* oncogene mutations in benign and malignant thyroid neoplasms. J Clin Endocrinol Metab. 1991; **73**: 832–836.
80 Karges B, Krause G, Homoki J, Debatin KM, de Roux N, Karges W. TSH receptor mutation V509A causes familial hyperthyroidism by release of interhelical constraints between transmembrane helices TMH3 and TMH5 J Endocrinol. 2005; **186**: 377–385.
81 Kato Y, Ying H, Willingham MC, Cheng SY. A tumor suppressor role for thyroid hormone beta receptor in a mouse model of thyroid carcinogenesis. Endocrinology. 2004; **145**: 4430–4438.
82 Kimura ET, Nikiforova MN, Zhu Z, Knauf JA; Nikiforov YE; Fagin J A. High prevalence of *BRAF* mutations in thyroid cancer: genetic evidence for constitutive activation of RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res. 2003; **36:** 1454–1457.
83 Kimura ET, Vanvooren V, van Sande J, Nikiforov YE, Fagin JA. Autonomously functioning thyroid nodules are not associated with BRAF mutations. Clin Endocrinol. 2004; **60:** 394–396.
84 King SK, Southwell BR, Hutson JM. An association of multiple endocrine neoplasia 2B, a RET mutation; constipation; and low substance P - nerve fiber density in colonic circular muscle. J Pediatr Surg. 2006; **41**: 437–442.
85 Krohn K, Paschke R. BRAF mutations are not an alternative explanation for the molecular etiology of ras-mutation negative cold thyroid nodules. Thyroid. 2004; **14:** 359–361.
86 Kohrle J. Guard your master: thyroid hormone receptors protect their gland of origin from thyroid cancer. Endocrinology. 2004; **145**: 4427–4429.
87 Kouvaraki MA, Shapiro SE, Perrier ND, Cote GJ, Gagel RF, Hoff AO, Sherman SI, Lee JE, Evans DB. RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. Thyroid. 2005; **15**: 531–544.
88 Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA. PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma. Science. 2000; **289**: 1357–1360.
89 Kumar R, Angelini S, Czene K, Sauroja I, Hahka-Kemppinen M, Pyrhönen S, Hemminki K. BRAF mutations in metastatic melanoma; a possible association with clinical outcome. Clin Cancer Res. 2003; **9**: 3362–3368.
90 La Perle KM, Jhiang SM, Capen CC. Loss of p53 promotes anaplasia and local invasion in ret/PTC1-induced thyroid carcinomas. Am J Pathol. 2000; **157**: 671–677.
91 Lee S, Hong SW, Shin SJ, Kim YM, Rhee Y, Jeon BI, Moon WC, Oh MR, Lim SK. Papillary thyroid carcinoma associated with familial adenomatous polyposis: molecular analysis of pathogenesis in a family and review of the literature. Endocr J. 2004; **51**: 317–323.
92 Lee JT, McCubrey JA. BAY-43-9006 Bayer/Onyx. Curr Opin Investig Drugs. 2003; **4**: 757–763.
93 Lemoine NR, Mayall ES, Stringer B, Wynford-Thomas D. High frequency of *ras* oncogene activation in all stages of human thyroid tumorigenesis. Oncogene. 1989; **4**: 159–164.
94 Lemoine NR, Mayall ES, Wyllie FS, Farr CJ, Hughes D, Padua RA, Thurston V, Williams ED, Wynford-Thomas D. Activated *ras* oncogenes in human thyroid cancers. Cancer Res. 1988; **48**: 4459–4463.
95 Leprat F, Bonichon F, Guyot M, Trouette H, Trojani M, Vergnot V, Longy M, Belleannee G, de Mascarel A, Roger P. Familial nonmedullary thyroid carcinoma: pathology review in 27 affected cases from 13 French families. Clin Endocrinol. 1999; **50**: 589–594.
96 Lewiński A. Postępy w endokrynologii [Advances in endocrinology (In Polish)]. Med. Prakt. 2003; **1–2**: 90–100.
97 Lewiński A, Ferenc T. Karcinogenéza v štítnej žľaze - účasť genetických factorov. In: Kreza A, Langer P, Klimeś I, Stárka L, Payer J, Michálek J. Všeobecná a klinická Endokrinólogia [Carcinogenesis in the thyroid gland – participation of genetic factors. In: General and Clinical Endocrinology (In Slovak)], Academic Electronic Press, Bratislava; 2004. p. 285–297.
98 Lewiński A, Ferenc T, Sporny S, Jarząb B. Thyroid carcinoma: diagnostic and therapeutic approach; genetic background (review). Endocrine Regul. 2000; **34:** 99–113.
99 Lewiński A, Gesing A. Czynniki wzrostowe gruczołu tarczowego i ich udział w patogenezie raka tarczycy [Thyroid growth factors and their involvement in the pathogenesis of thyroid carcinoma (In Polish)]. Endokrynol Pol – Polish J Endocrinol. 1995; **46** (suppl. 2 to no. 3): 11–27.
100 Lewiński A, Marcinkowska M, Brzeziańska E, Tantush AS, Włoch J, Brzeziński J. Effect of Exogenous Insulin-like Growth Factor-I (IGF-I) on the Expression of the IGF-I Gene and the Genes of IGF-Binding Proteins (IGFBPs) 1–4 in human thyroid cells from nodular goiter, follicular adenoma and follicular carcinoma cultured in monolayers. J Endocr Genet. 2003; **3:** 141–149.

101 Lewiński A, Marcinkowska M, Brzeziańska E, Włoch J, Brzeziński J. Expression of insulin-like growth factor I (IGF-I) gene and of genes for IGF-binding proteins 1, 2, 3, 4 (IGFBP-1–IGFBP-4) in non-neoplastic human thyroid cells and in certain human thyroid cancers. Effect of exogenous IGF-I on this expression. Endocr Res. 2004; **30:** 47–59.

102 Lewiński A, Pawlikowski M, Cardinali D. Thyroid growth-stimulating and growth-inhibiting factors. A review. Biol Signals.1993; **2**: 313–351.

103 Limon J, Mitelman F. Znaczenie aberracji chromosomowych w litych guzach nowotworowych człowieka [The significance of chromosomal aberrations in solid neoplastic tumors in humans (In Polish)]. Pat Pol. 1994, **45**: 1–15

104 Liu RT, Hou CY, You HL, Huang CC, Hock-Liew, Chou FF, Wang PW, Cheng JT. Selective occurrence of ras mutations in benign and malignant thyroid follicular neoplasms in Taiwan. Thyroid. 2004; **14**: 616–621.

105 Liu Y, Jiang C, Tan Y. [Pathological study on the expression of cell adhesion molecules and metastasis suppressor gene in thyroid follicular carcinoma and papillary carcinoma] (In Chinese)]. Zhonghua Bing Li Xue Za Zhi. 2002; **31**: 322–326.

106 Lorenzato A, Olivero M, Patane S, Rosso E, Oliaro A, Comoglio PM, Di Renzo MF. Novel somatic mutations of the MET oncogene in human carcinoma metastases activating cell motility and invasion Cancer Res. 2002; **62**: 7025–7030.

107 Malchoff CD, Sarfarazi M, Tendler B, Forouhar F, Whalen G, Malchoff DM. Familial papillary thyroid carcinoma is genetically distinct from familial adenomatous polyposis coli. Thyroid. 1999; **9**: 247–252.

108 Manenti G, Pilotti S, Re FC, Della Porta G, Pierotti MA. Selective activation of *ras* oncogenes in follicular and undifferentiated thyroid carcinomas. Eur J Cancer. 1994; **30A**: 987–993.

109 Martinez R, Venturelli D, Perrotti D, Veronese ML, Kastury K, Druck T, Huebner K, Calabretta B. Gene structure, promoter activity, and chromosomal location of the DR-nm23 gene, a related member of the nm23 gene family. Cancer Res. 1997; **57**: 1180–1187.

110 Matsuo K, Tang SH, Fagin JA. Allelotype of human thyroid tumors: loss of chromosome 11q13 sequences in follicular neoplasms. Mol Endocrinol. 1991; **38**: 1–14.

111 Matsuo K, Tang SH, Zeki K, Gutman RA, Fagin JA. Aberrant deoxyribonucleic acid methylation in human thyroid tumors. J Clin Endocrinol Metab. 1993; **77**: 991–995.

112 Miyake N, Maeta H, Horie S, Kitamura Y, Nanba E, Kobayashi K, Terada T. Absence of mutations in the beta-catenin and adenomatous polyposis coli genes in papillary and follicular thyroid carcinomas. Pathol Int. 2001; **51**: 680–685.

113 McDermott MT. Oncogenes and thyroid cancer. In: Falk SA, editor. Thyroid disease: Endocrinology, Surgery, Nuclear Medicine, and Radiotherapy. Philadelphia: Lippicott-Raven Publishers; 1997. p. 231–239.

114 McKay JD, Lesueur F, Jonard L, Pastore A, Williamson J, Hoffman L, Burgess J, Duffield A, Papotti M, Stark M, Sobol H, Maes B, Murat A, Kaariainen H, Bertholon-Gregoire M, Zini M, Rossing MA, Toubert ME, Bonichon F, Cavarec M, Bernard AM, Boneu A, Leprat F, Haas O, Lasset C, Schlumberger M, Canzian F, Goldgar DE, Romeo G. Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21. Am J Hum Genet. 2001; **69**: 440–446.

115 McKay JD, Williamson J, Lesueur F, Stark M, Duffield A, Canzian F, Romeo G, Hoffman L. At least three genes account for familial papillary thyroid carcinoma: *TCO* and *MNG1* excluded as susceptibility loci from a large Tasmanian family. Eur J Endocrinol. 1999; **141**: 122–125.

116 Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified as a mutational target. Biochem Biophys Acta. 2003; **1653:** 25–40.

117 Michelin S, Daya-Grosjean L, Sureau F, Said S, Sarasin A, Suarez HG. Characterization of a c-met proto-oncogene activated in human xeroderma pigmentosum cells after treatment with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Oncogene. 1993; **8**: 1983–1991.

118 Monaco C, Califano D, Chiappetta G, *et al.* Mutated human Kirsten ras, driven by a thyroglobulin promoter, induces a growth advantage and partially dedifferentiates rat thyroid epithelial cells in vitro. Int J Cancer. 1995; **63**: 757–760.

119 Moretti F, Farsetti A, Soddu S, Misiti S, Crescenzi M, Filetti S, Andreoli M, Sacchi A, Pontecorvi A. p53 re-expression inhibits proliferation and restores differentiation of human thyroid anaplastic carcinoma cells. Oncogene. 1997; **14**: 729–740.

120 Motokura T, Arnold A. Cyclins and oncogenesis. Biochim Biophys Acta. 1993; **1155**: 63–78.

121 Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JBJ, Gardner E, Ponder MA, Frilling A, Jackson SN, Lehnert H, Heumann HPH, Thibodeau SN, Ponder BAJ. Specific mutation of the *RET* proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nature Genet. 1994; **6**: 70–74.

122 Mulligan LM, Marsh DJ, Robinson BG, Schuffenecker I, Zedenius J, Lips CJM, Gagel RF, Takai S-I, Noll WW, Fink M, Raue F, Lacroix A, Thibodeau SN, Frilling A, Ponder BAJ, Eng C. Genotype-fenotype correlation in multiple endocrine neoplasia type 2: report of the international *RET* mutation consortium. J Intern Med. 1995; **228**: 343–346.

123 Mulligan LM, Ponder BAJ. Genetic basis of endocrine disease: multiple endocrine neoplasia type 2. J Clin Endocrinol Metab. 1995; **80**: 1989–1995.

124 Nakamura N, Carney JA, Jin L, Kajita S, Pallares J, Zhang H, Qian X, Sebo TJ, Erickson LA, Lloyd RV. RASSF1A and NORE1A methylation and BRAFV600E mutations in thyroid tumors. Lab Invest. 2005; **85**: 1065–1075.

125 Namba H, Gutman RA, Matsuo K, Alvarez A, Fagin JA. H-*ras* protooncogene mutations in human thyroid neoplasms. J Clin Endocrinol Metab. 1990; **71**: 223–229.

126 Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TL, Ohtsuru A, Saenko VA, Kanematsu T, Yamashita S. Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. J Clin Endocrinol Metab. 2003; **88:** 4393–4397.

127 Nemec J, Soumar J, Zamrazil V, Pohunkova D, Motlik K, Mirejowski P. Familial occurrence of differentiated (non-medullary) thyroid cancer. Oncology. 1975; **32**: 151–157.

128 Neumann S, Willgerodt H, Ackermann F, Reske A, Jung M, Reis A, Paschke R. Linkage of familial euthyroid goiter to the multinodular goiter-1 locus and exclusion of the candidate genes thyroglobulin, thyroperoxidase, and Na+/I- symporter. J Clin Endocrinol Metab. 1999; **84**: 3750–3756.

129 Nikiforov YE. Genetic alterations involved in the transition from well-differentiated to poorly differentiated and anaplastic thyroid carcinomas. Endocr Pathol. 2004; **15**: 319–327.

130 Nikiforov YE, Rowland JM, Bove KE, Monforte-Munos H, Fagin JA. Distinct pattern of *ret* oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res. 1997; **57**: 1690–1694.

131 Nikiforova MN, Ciampi R, Salvatore G, Santoro M, Gandhi M, Knauf JA, Thomas GA, Jeremiah S, Bogdanova TI, Tronko MD, Fagin JA, Nikiforov YE. Low prevalence of BRAF mutations in radiation-induced thyroid tumors in contrast to sporadic papillary carcinomas. Cancer Lett. 2004; **209**: 1–6.

132 Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, Santoro M, Fagin JA, Nikiforov JE. *BRAF* mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab. 2003; **88:** 5399–5404.

133 Oler G, Ebina KN, Kimura ET, Cerutti J. Investigation of BRAF mutation in a series of papillary thyroid carcinoma and matched-lymph node metastasis reveals a new mutation in metastasis. Clin Endocrinol. 2005; **4:** 509–511.

134 Paschke R. Constitutively activating TSH receptor mutations as the cause of toxic thyroid adenoma, multinodular toxic goiter and autosomal dominant non autoimmune hyperthyroidism. Exp Clin Endocrinol Diabet. 1996; **104** (suppl 4): 129–132.

135 Pickett CA, Agoff SN, Widman TJ, Bronner MP. Altered expression of cyclins and cell cycle inhibitors in papillary thyroid cancer: prognostic implications. Thyroid. 2005; **15**: 461–473.

136 Pierotti MA, Bongarzone I, Borrello MG, Greco A, Pilotti S, Sozzi G. Cytogenetics and molecular genetics of carcinomas arising from thyroid epithelial follicular cells. Genes Chrom Cancer. 1996; **16**: 1–14.
137 Ponder BAJ. Genetic screening for multiple endocrine neoplasia type 2. Exp Clin Endocrinol. 1993; **101**: 53–56.
138 Portella G, Vitagliano D, Borselli C, Melillo RM, Salvatore D, Rothstein JL, Vecchio G, Fusco A, Santoro M. Human N-ras, TRK-T1, and RET/PTC3 oncogenes, driven by a thyroglobulin promoter, differently affect the expression of differentiation markers and the proliferation of thyroid epithelial cells. Oncol Res. 1999; **11**: 421–427.
139 Powell Jr DJ, Russell JP, Li G, Kuo BA, Fidanza V, Huebner K, Rothstein JL. Altered gene expression in immunogenic poorly differentiated thyroid carcinomas from RET/PTC3p53–/– mice Oncogene. 2001; **20**: 3235–3246.
140 Pujol RM, Matias-Guiu X, Miralles J, Colomer A, de Moragas JM. Multiple idiopathic mucosal neuromas: a minor form of multiple endocrine neoplasia type 2B or a new entity? J Am Acad Dermatol. 1997; **37**(2 Pt 2): 349–352.
141 Puskas LG, Juhasz F, Zarva A, Hackler L Jr, Farid NR. Gene profiling identifies genes specific for well-differentiated epithelial thyroid tumors. Cell Mol Biol (Noisy-le-grand). 2005; **51**: 177–186.
142 Puxendu E, Moretti S, Elisei R, Romei C, Pascucci R, Martinelli M, Marino C, Avenia N, Rossi ED, Fadda G, Cavaliere A, Ribacchi R, Falorni A, Pontecorvi A, Pacini F, Pinchera A, Santeusanio F. BRAF V599E mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. J Clin Endocrinol Metab. 2004; **89**: 2414–2420.
143 Puzianowska-Kuźnicka M, Madej A, Krystyniak A, Stachlewska-Nasfeter E, Nauman A, Nauman J. Trijodotyronina i jej receptory jądrowe w procesie nowotworzenia [Triiodothyronine and its nuclear receptor in the process of neoplasia (In Polish)]. Post Biol Kom. 2002; **28**: 183–196.
144 Quesnel B, Fenaux P, Philippe N, Founier J, Bonneterre J, Preudhomme C, Peyrat JP. Analysis of p16 gene deletion and point mutation in breast carcinoma. Br J Cancer. 1995; **72**: 351–353.
145 Quiros RM, Ding HG, Prinz RA, Xu X. Evidence that one subset of anaplastic thyroid carcinomas are derived from papillary carcinomas due to BRAF and p53 mutations. Cancer. 2005; **11**: 2261–2268.
146 Romeo G, Ceccherini I, Celli J, Priolo M, Betsos N, Bonardi G, Seri M, Yin L, Lerone M, Jasonni V, Martuciello G. Association of multiple endocrine neoplasia type 2 and Hirschsprung disease. J Intern Med. 1998; **243**: 515–520.
147 Rouge L, Castedo S, Gomes P, Soares P, Clode A, Soares J. Cytogenetic findings in 18 follicular thyroid adenomas. Cancer Genet Cytogenet. 1993; **67**: 1–6.
148 Saitoh K, Takahashi H, Yamamoto M, Kishi H, Parsons PG. Expression of metastasis suppressor gene product, nm23 protein, is not inversely correlated with the tumour progression in human malignant melanomas. Histopathology. 1996; **29**: 497–505.
149 Sakai Y, Koizumi K, Sugitani I, Nakagawa K, Arai M, Utsunomiya J, Muto T, Fujita R, Kato Y. Familial adenomatous polyposis associated with multiple endocrine neoplasia type 1-related tumors and thyroid carcinoma: a case report with clinicopathologic and molecular analyses. Am J Surg Pathol. 2002; **2**6: 103–110.
150 Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol. 2004; **22**: 2954–2963.
151 Schagdarsurengin U, Gimm O, Hoang-Vu C, Dralle H, Pfeifer GP, Damman R. Frequent epigenetic silencing of the CpG island promoter of RASSF1A in thyroid carcinoma. Cancer Res. 2002; **62**: 3698–3701.
152 Schnabel P, Böhm M. Mutations of signal-transducing G proteins in human disease. J Mol Med.1995; **73**: 221–228.
153 Schutte M, Hruban RH, Geradts J, Maynard R, Hilgers W, Rabindran SK, Moskaluk CA, Hahn SA, Schwarte-Waldhoff I, Schmiegel W, Baylin SB, Kern SE, Herman JG. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Res. 1997; **57**: 3126–3130.
154 Sebolt-Leopold JS, Dudley DT, Herrera R, Van Becelaere K, Wiland A, Gowan RC, Tecle H, Barrett SD, Bridges A, Przybranowski S, Leopold WR, Saltiel AR. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. Nature Med. 1999; **5:** 810–816.
155 Sedliarou I, Saenko V, Lantsov D, Rogounovitch T, Namba H, Abrosimov A, Lushnikov E, Kumagai T, Nakashima M, Meirmanov S, Mine M, Hayashi T, Yamashita S. The BRAFT1796A transversion is a prevalent mutational event in human thyroid microcarcinoma. Int J Oncol. 2004; **6:** 1729–1735.
156 Shi YF, Zou MJ, Schmidt H, Juhasz F, Stensky V, Robb D, Farid NR. High rates of *ras* codon 61 mutation in thyroid tumors in an iodide-deficient area. Cancer Res. 1991; **51**: 2690–2693.
157 Soares P, Cameselle-Teijeiro J, Sobrinho-Simoes M. Immunohistochemical detection of p53 in differentiated, poorly differentiated und undifferentiated carcinomas of the thyroid. Histopathology. 1994; **24**: 205–210.
158 Soares P, Trovisco V, Rocha AS, Feijao T, Rebocho AP, Fonseca E, de Castro IV, Cameselle-Teijeiro J, Cardoso-Oliveira M, Sabrincho-Simoes M. BRAF mutations typical of papillary thyroid carcinoma are more frequently detected in undifferentiated than in insular and insular-like poorly differentiated carcinomas. Virchows Arch. 2004; **6:** 572–576.
159 Soares P, Trovisco V, Rocha AS, Limo J, Castro P, Preto A, Maximo F, Botelho T, Seruco R, Sobrinho – Simoes M. BRAF mutations and RET/PTC rearrangements are alternative events in etiopathogenesis of PTC. Oncogene. 2003; **17:** 4578–4580.
160 Soravia C, Sugg SL, Berk T, Mitri A, Cheng H, Gallinger S, Cohen Z, Asa SL, Bapat BV. Familial adenomatous polyposis-associated thyroid cancer – a clinical, pathological, and molecular genetics study. Am J Pathol. 1999; **154**: 127–135.
161 Suarez HG. Genetic alterations in human epithelial thyroid tumours. Clin Endocrinol. 1998; **48**: 531–546.
162 Suarez HG, Du Villard JA, Severino M, Caillou B, Schlumberger M, Tubiana M, Permentier C, Monier R. Presence of mutations in all three *ras* genes in human thyroid tumors. Oncogene. 1990; **5**: 565–570.
163 Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H. Cloning and expression of the ret protooncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene. 1988; **3**: 571–578.
164 Taruscio D, Carcangiu ML, Ried T, Ward DC. Numerical chromosomal aberrations in thyroid tumors detected by douuble fluorescence in situ hybridization. Genes Chrom Cancer 1994; **9**: 180–185.
165 Tennis M, Krishnan S, Bonner M, Ambrosone CB, Vena JE, Moysich K, Swede H, McCann S, Hall P, Shields PG, Freudenheim JL. p53 mutation analysis in breast tumors by a DNA microarray method. Cancer Epidemiol Biomarkers Prev. 2006; **15**: 80–85.
166 Teyssier JR, Liautaud-Roger F, Ferre D, Patey M, Dufer J. Chromosomal changes in thyroid tumors. Relation with DNA content, karyotypic features, and clinical data. Cancer Genet Cytogenet. 1990; **50**: 249–263.
167 Tsilchorozidou T, Vafiadou E, Yovos JG, Romeo G, McKay J, Lesueur F, Bonora E. A Greek family with a follicular variant of familial papillary thyroid carcinoma: TCO, MNG1, fPTC/PRN, and NMTC1 excluded as susceptibility loci. Thyroid. 2005; **15**: 1349–1354.
168 Tomoda C, Miyauchi A, Uruno T, Takamura Y, Ito Y, Miya A, Kobayashi K, Matsuzuka F, Kuma S, Kuma K, Kakudo K. Cribriformmorular variant of papillary thyroid carcinoma: clue to early detection of familial adenomatous polyposis-associated colon cancer. World J Surg. 2004; **28**: 886–889.
169 Trovisco V, de Castro IV, Soares P, Maximo V, Silva P, Magalhães J, Abrosimov A, Guiu XM, Sobrinho-Simoes M. BRAF mutations are associated with some histological types of papillary thyroid carcinoma. J Pathol. 2004; **202:** 247–251.
170 Trovisco V, Soares P, Preto A, de Castro IV, Lima J, Castro P, Maximo V, Botelho T, Moireira S, Meireles AM, Mgalhaes J, Abrosimov A, Cameselle-Teijeiro J, Sobrinho-Simoes M. Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. Virchows Arch. 2005; **446:** 589–595.

171 Trovisco V, Soares P, Soares R, Mgalhaes J, Sa-Couto P, Sobrinho-Simoes M. A new BRAF gene mutation detected in a case of a solid variant of papillary thyroid carcinoma. Hum Pathol. 2005; **36:** 694–697.
172 Van Aken E, De Wever O, Correia da Rocha AS, Mareel M. Defective E-cadherin/catenin complexes in human cancer. Virchows Arch. 2001; **439**: 725–751.
173 Vasko V, Hu Sh, Wu G, Xing JC, Larin A, Savchenko V, Trink B, Xing M. High prevalence and possible *de novo* formation of *BRAF* mutation in metastasized papillary thyroid cancer in lymph nodes. J Clin Endocrinol Metab. 2005; **90:** 5265–5269.
174 Vdovichenko KK, Markova SI, Belokhvostov AS. Mutant form of BRAF gene in blood plasma of cancer patients. Ann N Y Acad Sci. 2004; **1022:** 228–231.
175 Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Bartford D, Marais R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell.2004; **6:** 855–867.
176 Wasenius VM, Hemmer S, Karjalainen-Lindsberg ML, Nupponen NN, Franssila K, Joensuu H. MET receptor tyrosine kinase sequence alterations in differentiated thyroid carcinoma. Am J Surg Pathol. 2005; **29**: 544–549.
177 Weidner KM, Sachs M, Birchmeier W. The Met receptor tyrosine kinase transduces motility, proliferation, and morphogenic signals of scatter factor/hepatocyte growth factor in epithelial cells J Cell Biol. 1993; **121**: 145–154.
178 Weinberg RA. The retinoblastoma protein and cell cycle control. Cell.1995; **81**: 323–330.
179 Wiench M, Wloch J, Oczko M, Gubala E, Jarzab B. Rearrangement of *RET* gene in papillary thyroid carcinoma [Article in Polish] Wiad Lek. 2001; **54** (Suppl 1): 64–71.
180 Wozniak A, Wiench M, Olejniczak A, Wloch J, Lachinski A, Lange D, Olczyk T, Jarzab B, Limon J. Loss of heterozygosity in 73 human thyroid tumors. Neuroendocrinol Lett. 2005; **26**: 521–525.
181 Wreesmann VB, Ghossein RA, Patel SG, Harris CP, Schnaser EA, Shaha AR, Tuttle RM, Shah JP, Rao PH, Singh B. Genome-wide appraisal of thyroid cancer progression. Am J Pathol. 2002; **161**: 1549–1556.
182 Wyllie FS, Lemoine NR, Wiliams ED, Wynford-Thomas D. Structure and expression of nuclear oncogenes in multi-stage thyroid tumorgenesis. Br J Cancer. 1989; **60**: 592–598.
183 Xing M, Cohen Y, Mambo E, Tallini G, Udelsman R, Ladenson PW, Sidransky D Early occurrence of *RASSF1A* hypermethylation and its mutual exclusion with *BRAF* mutation in thyroid tumorigenesis. Cancer Res. 2004; **64**: 1664–1668.
184 Xing M, Tufano RP, Tufaro AP, Basaria S, Ewertz M, Rosenbaum E, Byrne PJ, Wang J, Sidransky D, Ladenson PW. Detection of BRAF mutation of fine needle aspiration biopsy specimens: a new diagnostic tool for papillary thyroid cancer. J Clin Endocrinol Metab. 2004; **89:** 2867–2872.
185 Xing M, Vasko V, Tallini G, Larin A, Wu G, Udelsman R, Ringel MD, Ladenson PW, Sidransky D. BRAF T1796A transversion mutation in various thyroid neoplasms. J Clin Endocrinol Metab. 2004; **89:** 1365–1368.
186 Xu X, Quiros RM, Gattuso P, Ain KB, Prinz RA. High prevalence of BRAF gene mutation in papillary thyroid carcinomas and thyroid tumor cell lines. Cancer Res. 2003; **63**: 4561–4567.
187 Xu B, Yoshimoto K, Miyauchi A, Kuma S, Mizusawa N, Hirokawa M, Sano T. Cribriform-morular variant of papillary thyroid carcinoma: a pathological and molecular genetic study with evidence of frequent somatic mutations in exon 3 of the beta-catenin gene. J Pathol. 2003; **199**: 58–67.
188 Yamamoto M, Hoshino H, Onizuka T, Ichikawa M, Kawakubo A, Hayakawa S. Thyroid papillary adenocarcinoma in a women with Peutz-Jeghers syndrome. Intern Med. 1992, **31**: 1117–1119
189 Ying H, Suzuki H, Zhao L, Willingham MC, Meltzer P, Cheng SY. Mutant thyroid hormone receptor beta represses the expression and transcriptional activity of peroxisome proliferator-activated receptor gamma during thyroid carcinogenesis. Cancer Res. 2003; **63**: 5274–5280.
190 Zafon C, Obiols G, Castellvi J, Tallada N, Galofre P, Gemar E, Mesa J, Simo R. nm23-H1 immunoreactivity as a prognostic factor in differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2001; **86**: 3975–3980.
191 Zou M, Al-Baradie RS, Al-Hindi H, Farid NR, Shi Y. S100A4 (Mts1) gene overexpression is associated with invasion and metastasis of papillary thyroid carcinoma. Br J Cancer. 2005; **93:** 1277–1284.
192 Zou M, Famulski KS, Parhar RS, Baitei E, Al-Mohanna FA, Farid NR, Shi Y. Microarray analysis of metastasis-associated gene expression profiling in a murine model of thyroid carcinoma pulmonary metastasis: identification of S100A4 (Mts1) gene overexpression as a poor prognostic marker for thyroid carcinoma. J Clin Endocrinol Metab. 2004; **89**: 6146–6154.
193 Zou M, Shi Y, al-Sedairy S, Farid NR. High levels of Nm23 gene expression in advanced stage of thyroid carcinomas. Br J Cancer. 1993; **68**: 385–388.