Rearrangements of *NTRK1* oncogene in papillary thyroid carcinoma

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Abstract

Papillary thyroid carcinoma (PTC) represents an example of tumour with high incidence of oncogenic sequences, such as *RET*/PTC and *Trk*. Both of them arise from the fusion of 3' terminal sequences of TK domain of *RET* or *NTRK1* gene, respectively, with 5' terminal sequences of their activating genes. In case of *NTRK1* oncogene, several rearrangement types are observed, characteristic for PTC: *Trk* (TMP3), *Trk*-T1, *Trk*-T2, *Trk*-T3 and *Trk*-2h, observed in human breast cancer cell line. Studies from different geographical regions, revealed significant population differences in the incidence of *Trk* rearrangements (0-50%), while within the same population, the frequency of *Trk* in spontaneous and radiation-associated PTCs is similar. The results of studies, focused on the correlation between tumour genotype and the histopathological type of tumour, involving cases of both *RET*/PTC and *Trk* rearrangements in PTC, are not unequivocal. In many studies, no correlation was observed between the presence of *RET* and/or *NTRK1* rearrangement and such parameters, as patient's age at diagnosis, gender, histopathological type of tumour or clinical stage (TNM stage grouping), although the earliest clinical symptoms and the worst disease outcomes were observed for *RET/NTRK1* rearrangement positive tumours. Differences in the rearrangement incidence between male and female patients were associated with the latency period of radiation-associated tumours, being significantly lower in women. In general, it is assumed that oncogenic *Trk* sequences are typical for the spontaneous type of PTC .

INVITED REVIEW

INTRODUCTION

The role of genetic background in the pathogenesis of thyroid neoplasms, including papillary thyroid carcinoma (PTC), has been carefully reviewed [13,15,23,29,34]. Differences, observed in the expression of mRNA for the enzymes, participating in purine and pyrimidine pathways [31], as well as in gene expression, are associated with tissue hyperplasia development, in the range from benign toxic adenomas (Gsp and TSH receptor genes) to either follicular (*Ras* oncogene) or papillary (*RET*/PTC, *NTRK1*, *met* oncogenes) carcinomas. It is assumed that mutations in these oncogenes may be associated with an early stage of neoplastic transformation of thyroid cells [46, 15]. Molecular changes in suppressor genes (e.g., in *P53* gene or *Rb* gene) concern more aggressive and less differentiated forms of thyroid carcinomas, confirming the thesis on their participation in later neoplastic stages. It seems to be interesting that the high percent of genetic changes, which are responsible for thyroid cancer, begins from gene rearrangement and chromosome translocations (e.g., *RET*/PTC, *Trk* oncogenic sequences, Pax-8/PPARgamma) [46]. The early stage of development of this type of tumours may then be a consequence of "*de novo*" activation or expression of a few protooncogenes, such as: *Ras, RET, TSHR, NTRK1, met* or *Gsp* or receptors of the growth factors [46,66]. It is assumed that in PTC, chromosomal rearrangements, activating tyrosine kinase receptors, constitute a common feature, while the expression of different chimeric forms of *NTRK1* mRNA may have prognostic significance and may be associated with a worse outcome [4,46]. On the other hand, as observed by Gimm *et al.* [14], the role of *NTRK1* chimeric forms, as a prognostic marker, is rather ambiguous. The expression of NTRK1 receptor in an early stage of sporadic medullary thyroid carcinoma (MTC) is variable, while in later stages of MTC, only NTRK2 and NTRK3 expressions are detected [14].

Among thyroid carcinomas, PTC is an example of the frequently observed occurrence of chimeric oncogenes. Molecular studies have shown specific genetic changes of the rearrangement type in well differentiated, spontaneous PTCs [48,49,52,53]. Cytogenetic analysis of these tumours has revealed chromosome rearrangements, leading to the occurrence of two oncogenic sequences: *RET*/PTC and *Trk* family. Both sequences are derived from the fusion of 3'-terminal sequences of tyrosine kinase domain of *RET* and *NTRK1* genes with 5'-terminal sequences of other genes. In result, intrachromosomal inversions of chromosome 10 or 1 are observed [53]. The expression of chimeric forms of *RET*/PTC varies from 10% to 40% in spontaneous PTC [6,41,48,54,69] but, in some populations (French, Taiwan Chinese), it is much lower, ranging from 0% to 17% [11,35]. It is known that the incidence of *NTRK1* oncogene rearrangements is variable too, ranging from 0% to 50%, depending on the population [4,5,6,48,67]. Especially frequent (50%) activation of *NTRK1* gene in PTC was observed in the Italian population [4,5].Other studies, performed in the Japanese population, revealed that the incidence of gene activation was lower than 10% [67], while no cases of *Trk* rearrangement were observed in the Chinese population [33].

As compared with the high prevalence, approximately 60% of *RET* rearrangements, reported for PTC after radiation [32], *NTRK1* rearrangements appear rarely [1]. On the other hand, it has been shown that not only is the oncogenic sequence of *NTRK*1 most often observed but is also most characteristic for spontaneous PTC, the occurrence of which is not associated with previous radiation [53].

It has been found that oncogenic sequences from the *Trk* family are activated by recombination with, at least, three genes: *tropomyosin gene (TPM), translocated promoter region gene (TPR)* or *TRK-fused gene (TFG),* referred to as *TAG* (*TRK-activating gene*) [7,17,18,19,20]. Because of the fact that various, structurally different activating genes participate in intrachromosomal inversions of chromosome 1, the rearrangements have been divided into specific types (Table 1). Since the year 1992, the products of *NTRK1* oncogene recombination have been defined as the *transforming receptor kinase – thyroid oncogene (Trk*-T*),* where subsequent numbers: *Trk* (-T1, -T2, -T3) indicate the identification sequence [20].

Table 1. Types of oncogenic sequences of *Trk* family.

Type of rearrangement	Activating gene	*Locus* of the activating gene	The authors and the year of discovery
Trk (TPM3) (otherwise *TPM3/NTRK*1, *Trk*)	*TPM* (gene for tropomyosin, a nonmuscle isoform)	1q22-23	Martin-Zanca *et al.,* 1986 [38]
Trk-T1	*TPR* (translocated promoter region gene)	1q25	Greco *et al.,* 1992 [20]
Trk-T2	*TPR* (translocated promoter region gene)	1q25	Greco *et al.,* 1992 [20]
Trk-T3	*TFG* (*TRK-fused gene*)	3q11-12	Greco *et al.,* 1995 [17]
Trk-2h *(in vitro)**	*L7a* (a gene for ribosomal protein L7a)	9q33-34	Ziemiecki *et al.,* 1990 [71]

*observed in breast carcinoma

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Molecular and biochemical characterization of *NTRK1* protooncogene and *Trk* protein product

NTRK1 protooncogene, encoding the high-affinity receptor tyrosine kinases for neurotrophins of the nerve growth factor (NGF) family, has been mapped to chromosome 1(1q21-q22) [68], although there have been divergent hypotheses, concerning the gene localization. In a combined study of somatic cell hybrids and in situ hybridization assay, two *loci* of the gene have been revealed: 1q32-q41 [42] or more proximal, i.e., 1q23-1q24 [47]. The neurotrophic tyrosine kinase receptor (NTKR) is a membrane-bound receptor that, upon neurotrophin binding, phosphorylates itself and the members of the MAPK pathway. The presence of this kinase leads to cell differentiation and may play some role in specifying sensory neuron subtypes.

Molecular analysis of human *NTRK1* oncogene, a transforming gene, isolated from the colon carcinoma biopsy, encodes for a 140.000 Da glycoprotein, designated as gp140$^{proto\text{-}trk}$ [38]. However, its primary translational product is a 110.000 Da glycoprotein, which becomes immediately glycosylated, presumably during its translocation into endoplasmic reticulum. This molecule, designated as gp110$^{proto\text{-}trk}$, is further glycosylated to the mature form of gp140$^{proto\text{-}trk}$. Both gp110$^{proto\text{-}trk}$ and gp140$^{proto\text{-}trk}$ proteins possess *in vitro* kinase activity, specific for the tyrosine residues [39].

Low levels of *NTRK1* protooncogene transcripts have been found in certain human tumour cell lines of haematopoietic and mesenchymal origin. It is possible that *Trk* protooncogene products, when expressed in its physiological environment, may have a very short half-life, making its detection difficult [39].

It has been demonstrated that *NTRK1* gene, 25 Kb long, is organized in 17 exons, out of which, exon 9 is alternatively spliced [22]. The length of the gene encoding regions ranges from 18 bp to 394 bp; the regions are separated by introns of the total length of 170 bp. It has been demonstrated that the 5' terminal sequence is not susceptible to translation; neither does it contain any TATA sequences (TATA box), which are known to indicate an increased susceptibility to unzipping and denaturation. It has been observed, however, that regions, rich in GC pairs, occur at this part of the gene, together with a few locations of transcription binding factors: Sp1, AP1, AP2, AP3, ATF, GCF. It is assumed that it is a regulatory region of this gene [22,24].

It has generally been known that neurotrophins and their receptors play an important role in the control of both the central and the peripheral nervous system. The study, performed on mice, demonstrated that NTRK1 receptor is a primary mediator of the trophic actions of NGF *in vivo* and that this signalling pathway plays the main role in the development of the nervous system [2, 65]. The ability of neurotrophins to bind with higher affinity requires association of their monomer receptors with receptors of higher molecular mass and of lower affinity, i.e., the receptors of tyrosine kinase (TRK). Among these receptors, tyrosine kinase receptor A, designated as NTRK1 (TrkA) and specific for neurotrophin (the nerve growth factor – NGF) is the most important one [22,28]. The nerve growth factor stimulates TRK protein phosphorylation in nerve cell lines, which demonstrates participation of this protein in the functional transfer of NGF-induced signal. It has been detected that different cascades of cellular signalling events involved several adapter proteins (i.e., Shc protein) and enzymes (i.e., phospholipase C) which ultimately promote the signalling pathway [70].

There is a strong evidence that the protein product of *NTRK1* protooncogene (gp140$^{proto\text{-}trk}$) is necessary for the functional transfer of either NGF- or neurotrophin 3 (NT-3)-induced signal to enter the cells into S phase. These mitogenic effects of NGF or NT-3 depend on the expression of gp140$^{proto\text{-}trk}$ and its phosphorylation [9, 30]. The study, conduced by Ehrhard *et al.* [12], has shown that *NTRK1* undergoes expression in monocytes. It is where mRNA synthesis increases during the differentiation of monocytes into macrophages, suggesting that NGF – for which NTRK1 is a receptor – beside its oncogenic function – is also an immunoregulatory cytokine, exerting its effects on monocytes, while the expression of *NTRK1* protooncogene depends on the maturation process.

Figure 1 presents a diagram of the structure of unchanged ("wild-type") NTRK1 receptor and of known chimeric fusion products.

The mechanism of human *NTRK1* oncogene activation

The genes of tyrosine kinase receptors often play the role of targets for oncogenic activation by chromosomal rearrangements, linking the domain of tyrosine kinase (TK) with the amino-terminal residues of various "activating" genes. These rearrangements lead to the generation of chimeric mRNA and, in consequence, of a chimeric form of proteins, which have the activity of tyrosine kinase and are subject of constitutive expression in somatic cells. This event initiates an increased activity of TK domain in tissues, in which no receptor expression occurs in physiological conditions [7,20].

For the first time *NTRK1* gene rearrangements were discovered in DNA from colorectal carcinoma [39]. The oncogenic variants of *NTRK1: Trk(TPM*3*), Trk*-T1*, Trk*-T2*, Trk*-T3, observed in PTC, are localized in those DNA regions which include breakpoint clusters and are at the base of deletion of the N-terminal domain of tyrosine kinase (TK) and fusion of the remaining part of the domain with the 5'- terminal sequences of, at least, three activating genes: *TPM*3*, TPR* or *TFG.* This type of fusion leads - in consequence - to the intrachromosomal inversion of chromosome 1 or translocations between nonhomologous 1 and 3 chromosomes t(1;3) (Table 1).

It has also been shown in many cases that the phenomennon of illegitimate recombination, occurring between nonhomologous sequences, predisposes *NTRK1* gene to fusions with other genes. The possibility to illegitimate recombination may probably be caused by the sequences present in breakpoint regions, which are susceptible to recombination, as well as by palindrome sequences, direct and reversed repetitions and sequences of the Alu family [7].

Trk(*TPM3*) oncogenic sequence

Initially, this type of rearrangement was observed in colorectal carcinoma [38]. The *Trk*(*TPM3*) oncogenic sequence results from the replacement of extracellular domain of normal tyrosine kinase receptor by sequences, encoding 221 amino-terminal residues of *nonmuscle tropomyosin gene* (*TPM3*). The replacement of this putative extracellular domain by nonmuscle tropomyosin sequences is sufficient to activate its transforming potential. As a result of this illegitimate recombination, a protein of 70 kDa is produced [7,44].

The performed studies indicate that, in all the PTC cases with *Trk*(*TPM*3) rearrangement, the recombination involved the 611 bp fragment of *NTRK1* intron, localized upstream to the transmembrane domain of the gene and the *TPM*3 gene intron, located between exons 7 and 8 [7].

It has been found that the sequences of tropomyosin gene and those of tyrosine kinase are necessary for proper transforming activity. It has been demonstrated that the replacement of tropomyosin sequence in *NTRK*1 oncogene by sequences of other genes of cytoskeletal proteins, such as β-actin or β-globin, leads to total loss of transforming activity [10]. These results illustrate the important role, played by tropomyosin sequence, in gaining transforming features by tyrosine kinase.

It has been assumed that the sequences of tropomyosin gene probably contribute to the activation of *NTRK1* oncogene not by facilitating TK domain interactions with specific structures of the cytoskeleton but by enabling its folding into a constitutively active configuration [10].

The ability of *NTRK1* and *TPM3* gene sequences to recombination was also confirmed by the simultaneous presence of reciprocal products, e.g., *NTRK1*/*TPM*3 and *TPM3*/*NTRK1* sequences in the cells [19,20]. In addition, using two-colour fluorescence in situ hybridization and two-dimensional microscopy, it was found that two *loci* in thyrocyte nuclei display a distance, reduced with respect to peripheral blood lymphocytes, thus supporting the assumption that spatial proximity of translocation prone gene loci may favour gene rearrangements [58]. The studies, revealing a simultaneous presence of reverse transcripts, confirm the hypothesis that *Trk*(*TPM3*) rearrangements result from reciprocal, balanced, intrachromosomal inversion of chromosome 1 [7].

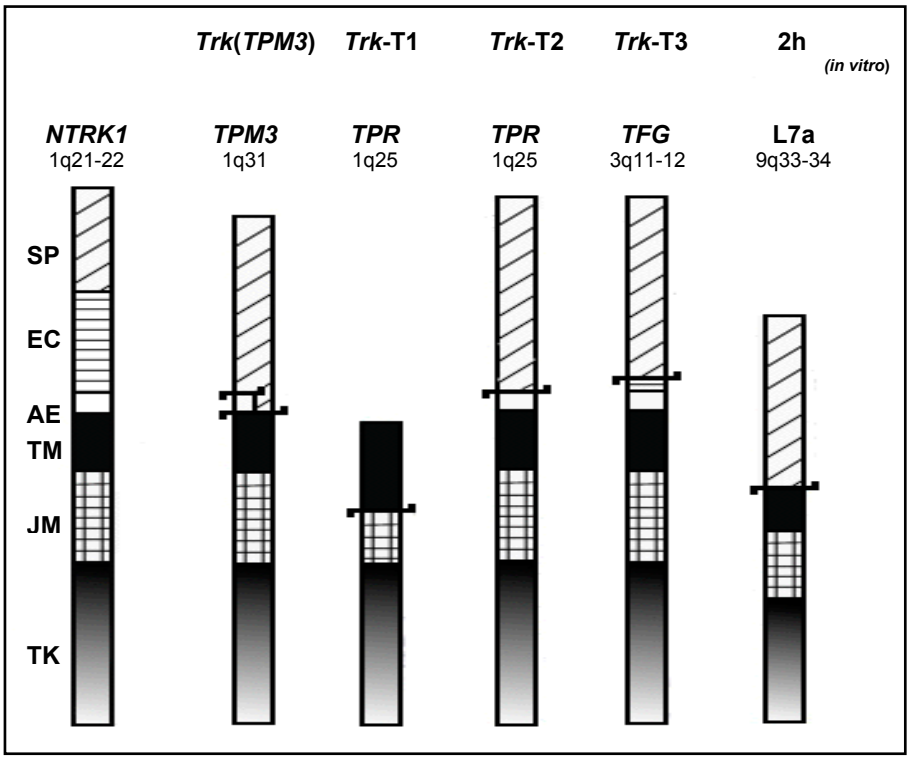

Figure 1. "Wild-type" NTRK1 receptor and products of *NTRK1* oncogene fusion with different activating genes, together with their chromosome localization. The horizontal lines indicate the points of fusion. SP – signal peptide, EC – extracellular domain, AE – alternative exon, TM – transmembrane domain, JM – perimembrane domain, TK – tyrosine kinase domain (after: Musholt *et al.*, 2000 [48], modified).

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Trk-T1 and *Trk*-T2 oncogenic sequences

It has been proposed in several reports that the special proximity of gene *loci* may favour gene rearrangements, where the spatial genome localization is an influencing factor in the formation of specific chromosomal translocation in cancer cells. The study of Roccato *et al.* [58,60,61], concerning *Trk* oncogenes (mainly *Trk*-T3), confirmed the concept of "the spatial contiguity model", recently proposed for some of *RET* rearrangements (*H4/RET* sequences) [50]. It has been found that *Trk*-T1 and *Trk*-T2 sequences, which contribute to the constitutive expression of *NTRK1*, are created by balanced rearrangements, the mechanisms of which include deletions, inversions and reciprocal translocations between the two homologues of chromosome 1 [19,48]. Sequence analysis of the break-point sites within *NTRK1* and *TPR* genes revealed the presence of only little homology. This evidence indicates that a non-homologous recombination event in thyroid tumours would create sequences sensitive to DNA damage.

Trk-T1 displays a structure different from that of *Trk*-T2, although formed by the same genes - *TPR* and *NTRK1*. They both result from fusion of 3'-terminal sequences of tyrosine kinase domain of *NTRK1* oncogene with 5'-terminal sequences of *TPR* (*translocated promoter region*) gene but, in a different portion, supported by *TPR* [19]. It is known that both *TPR* and *NTRK1* genes are located on chromosome 1 (*TPR* gene is localized in 1q25 region, while *NTRK1* in 1q21-22, respectively) and are separated by 30 Mbp. *TRP* gene encodes a protein, which – most probably – is a component of the cytoskeleton [20,43,62].

The hybrid *Trk*-T1 mRNA contains 598 nucleotides of *TPR* gene and 1148 nucleotides of *Trk* protooncogene [20]. In case of *Trk*-T2, this chimeric form involves the longer part of *TPR* gene. This part of sequences encodes the protein, longer of 737 amino acids, in comparison with *Trk*-T1 sequence [20]. It has been found that both *TPR* regions, involved in the *Trk*-T1 and *Trk*-T2 rearrangements, show a high content of AT sequences, d(GT)n region and significant homology to the Alu sequences which can predispose to recombination [19].

Some authors identified reciprocal *NTRK1/TPR* and *TPR/NTRK1* forms at the level of mRNA transcription in spontaneous PTC and also in PTC associated with radiation. This fact confirms the ability of chromosome 1 to intrachromosomal inversion [1]. On the other hand, the lack of reverse transcripts, described in some reports, may be caused by instability of mRNA *Trk* protooncogene transcripts, which may have a very short half-life, making its detection difficult [39]. To summarize, the presence in the cancer DNA of the reciprocal products of the rearrangements indicate that inversion of chromosome 1 is the molecular mechanism producing *Trk*-T1 and *Trk*-T2 oncogenes [21].

The results of an *in vivo* study, performed on transgenic mice, have proven that the expression of *Trk*-T1 oncogene causes thyroid carcinoma, originating from follicular cells of the epithelium. It confirms the thesis that *Trk*-T1 oncogene is involved in the neoplastic transformation of thyroid cells [62].

Oncogenic sequence of *Trk*-T3

Trk-T3 is an example of an oncogene from *Trk* family, resulting from a rearrangement between genes, localized on various chromosomes. *Trk*-T3 oncogene is a product of the reciprocally balanced chromosomal translocation t(1;3) between *TFG* gene (*Trk-fused gene*), also called *TAG* (*Trk-activating gene*), localized on chromosome 3 (3q11-12) [40], and exon 8 of *NTRK*1 gene with locus on chromosome 1 [17]. It has been determined that *Trk*-T3 oncogene encodes a 68 kDa cytoplasmic protein [17].

An analysis of cDNA sequence of *Trk*-T3 oncogene, isolated from a transformed cell line, has proven that *Trk*-T3 consists of 1 412 nucleotides, derived from *NTRK1* gene and of 598 nucleotides, belonging to *TFG* gene. The *Trk*-T3 sequence contains a coiled-coil domain within *TFG* gene, which may provide the resultant oncoprotein with an ability to produce protein complexes, most probably of trimer or tetramer structures [16,17,51]. Studies have confirmed that both the coiled-coil domain of *TFG* gene and the N-terminal region are necessary for the activation and full transforming activity of TRK-T3 oncoprotein, although their roles are revealed at various stages of the transformation process [16]. Recent biochemical studies have indicated that the transforming activity of Trk-T3 oncoprotein depends also on the sequences, localized outside the coiled-coil domain, such as BP1 domain, especially the SH2-binding motif [59,61]. Recently, it has been demonstrated that fibroblast growth factor receptor substrates, such as FRS2 and FRS3 are influenced and activated by TRK-T1 and TRK-T3 oncoproteins in *Trk* oncogens' signal transduction. It has been investigated that FRS2 and FRS3, after phosphorylation during co-expression with TRK oncoproteins, contribute to the mitogenic signal, triggered by Trk-T1 and Trk-T3. Therefore, it has been suggested that FRS2/FRS3 proteins are involved in the thyroid carcinogenesis, mediated by TRK oncoproteins [57].

Oncogenic sequence of *Trk*-2h

It has been confirmed that this sequence is a fusion of *NTRK1* protooncogene and the gene, encoding L7a ribosomal protein. This sequence was isolated from human cell line of breast carcinoma [71].

Correlation between genotype and phenotype

As it has been proven, there are significant differences in the incidence of *Trk* rearrangements among the populations from different geographic areas. There is no association between the presence of *NTRK1* oncogene

rearrangement and such parameters, as patient's age, gender, histopathological variant of PTC and tumour assignment to particular stages in different clinical staging systems [1,6,55].

However, some authors have reported, that the incidence of *NTRK1* oncogene activation is higher in the group of patients below 30 years of life, and a significant association was found between the presence of *Trk* rearrangements and the advanced stage of tumour and less favourable disease outcome [3,4,48].

It has been noted that some of the studied tumours – which the presence of *Trk* rearrangement – demonstrated a high proliferation index, indicating quick tumour development [1].

The studies, concerning the correlation between tumour genotype and its phenotype, did not bring any unequivocal results. It is thought, however, that the occurrence of metastases to lymph nodes is associated with the presence of *NTRK1* dimeric forms. It is also postulated that the type of activation itself, together with the involved oncogene, is less important from the fact that the domain of tyrosine kinase undergoes constitutive expression, providing cells with the ability of constitutive autophosphorylation and proliferation [1].

The phenotype of tumours with *RET/NTRK1* rearrangement is not different from the general histopathological pattern of PTC, regarding the degree of differentiation or tumour development, although, certain studies have revealed that the phenotype in question correlates with worse clinical outcome of the disease [4,55]. The difference between male and female patients was distinctive, when the time of latency of radiation induced tumour was taken into account. Unlike men, did female patients demonstrate a significantly lower incidence of rearrangements in carcinomas, developing in later periods (after 10 years) than in tumours more quickly revealing their presence (the latency period below 10 years). The studies did not, however, enable to draw any relationship between the presence of *Trk* rearrangement and the disease progression [5], although an earlier occurrence of symptoms was observed in cases of *RET/NTRK1* positive tumours. This fact leads to a question, if the time of latency is shorter in this class of tumours or, on the other hand, whether the occurrence of *RET*/PTC and *Trk* rearrangements are, in any way, connected with patient's age.

Rearrangements of *NTRK1* oncogene and their relationship with radiation

Little is known about the molecular mechanisms, leading to radiation-induced tumours of the thyroid gland [5]. Radiation induces breaks and deletions in DNA chain and stimulates aberration changes, initiating chromosomal translocations and intrachromosomal rearrangements [55].

The thyroid gland is extremely sensitive to radiation in its early period of development. Papillary thyroid carcinoma was diagnosed in children, following radioiodine therapy or in those, living in areas, contaminated after the Chernobyl disaster [5,32,55,56]. A considerable increase of *NTRK1* oncogene activation has been proven in young persons (4–30 years), both in exposed and unexposed to radiation, while the general incidence of *NTRK1* oncogene rearrangements in PTCs is similar between spontaneous and radiation-induced tumours, amounting to 12% [5]. At the same time, other studies revealed that in radiation induced PTCs, *NTRK1* oncogene rearrangements are less frequent (3.3%) than *RET* rearrangements [1,55]. It has been postulated that *RET*/PTC rearrangements are the main genetic changes, associated with ionizing radiation induced thyroid tumours [5,8,56] and with patient's age, being highly significant [3,4].

The studied cases of thyroid follicular adenomas, occurring in result of radiation, have turned out to be negative with regards to the presence of *NTRK1* oncogene rearrangements [5].

In to-date studies, an analysis of various types of *Trk* rearrangement has also been performed, correlating the results with patient age, the dose of radioactive iodine or tumour latency period. It was demonstrated that, in younger patients, after the Chernobyl disaster, *NTRK1* rearrangements were rare (3.3%) [55]. Except of the few cases of *RET* gene rearrangement, two cases of *NTRK1* oncogene activation were identified, including *TPM3/NTRK1* and *Trk*-T2 rearrangement. The performed studies clearly suggest that, in radiation induced carcinomas, oncogenic activation via *RET* gene rearrangement prevails, with a higher incidence of *RET*/PTC rearrangement in tumours with shorter latency periods (below 10 years) [55].

It has been assumed that *Trk* oncogenic sequence is characteristic for spontaneous PTCs, i.e., those, developed without any association with previous radiation. Following the reports of many investigators, the rearrangements of *NTRK1* protooncogene in spontaneous forms of PTC are found with the frequency from 0% to 50%, this value varying in different populations [3,5,6,33,52,67].

Other pathological syndromes associated with molecular changes of *NTRK1* protooncogene

Mutations of *NTRK1* gene have been found in patients with congenital insensitivity to pain with anhidrosis (CIPA). The pioneer studies of Smeyne *et al.* [65], which revealed severe sensory and sympathetic neuropathies in mice, lacking *NTRK1* gene, prompted other researchers to studies on the human homologue of this gene and on its association with CIPA [25,27,36,37,64].

*NTRK*1 gene mutations, such as deletions, splice site aberrations, frameshift mutations or missense mutations within the domain of tyrosine kinase of this gene, leading to amino acid substitution in conservative sites of protein, were defined as the genetic background for CIPA development [25,27,37,45]. It has been proven that mutations

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of the oncogene occurred in TK extracellular domain, involved in binding of the nerve growth factor (NGF), and in the intracellular signal transduction domain [37]. The results of those studies suggest that the NGF-TRK system plays an important role in the development and functioning of the nociceptive reception system, as well as in thermoregulation balance via sweating in people [25].

In case of CIPA-affected families, rare double or triple mutations have also been observed in the *NTRK1* gene, concerning the same chromosome and consisting of Arg58Ser, His598Tyr and Gly607Val replacements. To-date, such mutations have been described in *HEXA* gene, leading to Tay-Sachs disease, or in *LDLR* gene, causing familial hypercholesterolemia [37].

Molecular studies in patients with CIPA create an opportunity to explain the role of the NGF-TRK receptor system and the missense mutations in *NTRK1*, leading to the loss of gene functions, and provide information on the relationship between the structure of the tyrosine kinase receptor and the gene function [26,36].

In case of sporadic medullary thyroid carcinoma (MTC), immunohistochemical studies have revealed consistent expression of tyrosine kinase receptor type 2 (*NTRK2; NTRKB*) and the variable expression of *NTRK1* (*TRKA*) and *NTRK3* (*TRKC*), especially in the first phase of tumour development. In later stages of MTC development, *NTRK2* expression is decreasing, while *NTRK3* expression is increasing [14]. Moreover, it has been found that receptors from *Trk* family undergo expression only in a subset of normal C cells of the thyroid gland [14].

An analysis of mutations in cells of sporadic MTC did not reveal any changes in *NTRK2* sequence, while it did reveal sequence changes in *NTRK3* oncogene in 573C/T (exon 5), in 678T/C (exon 6) and in 1488C/G (exon 12). It turned out that those mutations had also occurred in the germinal line, sequence changes were also observed in control samples and differences in their incidence (between carcinoma and control) were not statistically significant. The authors suggest that the observed sequence variants in *NTRK2* and *NTRK3* oncogenes, are not responsible for the differences in protein level, however, a very low effect of gene penetration cannot be excluded either [14]. Thus, the rearrangements of *NTRK1* oncogene seem to be characteristic exclusively for PTC, although the differences in their incidence, as observed by researchers, may be associated either with various geographic factors or by selection of different study techniques [5].

CONCLUSIONS

In papers, concerning molecular background of PTC, the authors often analyse the oncogenic rearrangements of *RET/NTRK1* as the main genetic factor of prognostic significance [48]. Also important is the fact that the common, shared features of the activated oncogenes include their ubiquitous expression, the ability for dimerization, translocation to cytoplasm, resulting in interactions with various substrates. This type of tyrosine kinase (TK) activation provides the cells with a constitutive autophosphorylation potential, independent of the ligand, and proliferative prevalence, that probably leading to tissue hyperplasia. It has also been proven that, in the oncogenic activity of *RET* or *NTRK1* (*Trk*-T3) gene rearrangements, also protein factors, such as adaptor protein Shc [63, 60], are involved. The results of many studies also allow a hypothesis to be proposed about various factors, initiating thyroid tumour development from the epithelial tissue, such as *Gsp*, *TSHR*, *Ras, RET* or *NTRK1* genes, seemingly the most important factors. The obtained data suggest a significant role of these particular genes in the initiation of either spontaneous or radiation induced thyroid tumours but the interactions among the above mentioned genes cannot be excluded [66].

On the other hand, there are reports in which no rearrangements of *NTRK1* protooncogene were demonstrated in PTCs [33] or reports, where large differences in their incidence (0-50%) were observed in the studied populations. This fact suggests that the activation of TK receptor of *NTRK1* oncogene seems to be less significant in the molecular pathogenesis of PTC than *RET* [33]. It is assumed, however, that both oncogenes (*RET* and *NTRK1*) are submitted to chromosome translocations, unique in their specificity, which are responsible for the presence of oncogenic fusion proteins, initiating neoplastic hyperplasia of the thyroid epithelium [62].

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