A minireview: the role of MAPK/ERK and PI3K/Akt pathways in thyroid follicular cell-derived neoplasm

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1. ABSTRACT

The MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway) and PI3K/Akt (lipid kinase phosphoinositide-3-kinase signaling pathway) play an important role in transmission of cell signals through transduction systems (ligands, transmembrane receptors and cytoplasmic secondary messengers) to cell nucleus, where they influence the expression of genes that regulate important cellular processes: cell growth, proliferation and apoptosis. The genes, coding the signaling cascade proteins (e.g., RET, RAS, BRAF, PI3K, PTEN, AKT), are mutated or aberrantly expressed in thyroid cancer derived from follicular thyroid cell. Genetic and epigenetic alternations, concerning MAPK/ERK and PI3K/Akt signaling pathways, contribute to their activation and interaction in consequence of malignant follicular cell transformation. This review is focused mainly on genetic alterations in genes, coding signaling pathway proteins. Moreover, it is additionally pointed out that genetic, as well as epigenetic DNA changing via aberrant methylation of several tumour suppressor and thyroid-specific genes are associated with tumour aggressiveness, being a jointly responsible mechanism for thyroid tumorigenesis. The understanding of this molecular mechanism provides access to novel molecular therapeutic strategies for inhibiting oncogenic activity of signaling pathways.

2. INTRODUCTION

Thyroid cancer accounts for the majority of the endocrine malignancy in the world and the incidence of this type of cancer is still increasing (1). Follicular cell-derived thyroid carcinoma – most common endocrine malignancy – includes several morphological subtypes which have traditionally been classified in independent groups: well-differentiated thyroid carcinoma (WDTC) represents more than 90% of all thyroid cancers, whereas poorly differentiated thyroid carcinoma (PDTC) and undifferentiated (anaplastic) thyroid carcinoma (UTC; ATC) accounts for approximately 2% to 10% (2-4) (Figure 1).

These subtypes of thyroid cancer are phenotypically distinct and display different potential degree of malignancy, from relatively indolent (PTC, papillary thyroid carcinoma) – with an excellent prognosis – to highly aggressive tumours (UTC) with a poor prognosis, due to lack of effective treatment. PTC, belonging to WDTC, is the most common type and accounts for 60-80% of all thyroid gland malignancies (2). It is confirmed that PDTCs displays intermediate biological and clinical features between WDTC and UTC. Indeed, clinical, epidemiologic, and genetic evidences, provided by many studies, support the hypothesis of gradual progression and dedifferentiation of thyroid cancer derived from
Figure 1. Classification of the follicular cell-derived thyroid carcinoma with emphasize to more aggressive variants of WDTCs.

Figure 2. Schematic activation of the MAPK/ERK pathway. Activated tyrosine kinases serve as binding sides for proteins with enzymatic activity (RAS, PI3K) and adapter proteins (Grb2-SOS, Shc).

Although the results, obtained in many studies, strongly support the progression concept in thyroid tumorigenesis, the knowledge about possible molecular mechanisms, underlying this process, is still insufficient. However, there have been studies, assessing the molecular factors that may play an essential role in dedifferentiation of WDTC (6,10,11). These factors affect tumour proliferation, growth, cellular survival, and may contribute to developing of particular thyroid cancer phenotype. An identification of molecular alterations, involved in thyroid carcinomas of follicular origin, may help implement novel therapeutic strategies beside surgery and adjuvant radioactive iodine treatment. Moreover, the recognized molecular factors may serve as potential therapeutic targets.

3. RAS/RAF/MEK/ERK SIGNALING PATHWAY

RAS/RAF/MEK/ERK (MAPK/ERK; mitogen-activated protein kinase/extracellular signal-regulated kinase) signaling pathway, called “MAPK pathway” is a classical conserved intracellular signaling pathway that transmits signals which regulate cell proliferation, differentiation, apoptosis and survival (12-14). This pathway functions as a family of numerous receptor, non-receptor kinases, GTP-binding proteins and transcription factors (c-Myc, Ets, CREB, c-Jun, c-Fos), which can be activated or inactivated by protein phosphorylation (14,15). Activation of this pathway conducts cells into mutator phenotype by increasing cell proliferation and dedifferentiation and inhibition of apoptosis (15,16). Transduction of oncogenic signals generated by cell surface receptors and cytoplasmic signaling components into the nucleus involving important protein-protein interaction (14) (Figure 2).

Physiologically, in cells which have not undergone any transformation, the activation of the signaling MAPK/ERK cascade starts at the cell membrane receptor upon its stimulation by extracellular mitogenic signals (e.g., growth factors, differentiation factors, tumour-promoting substances) (15). Most of these factors stimulate the activation of extracellular receptor tyrosine kinases (RTKs, e.g., RET, NTRK1) or various non-receptor cytoplasmic serine/threonine-specific kinases (Raf family, MAP, ERK), as well as small GTP-binding Ras proteins. The activation of Ras – a membrane-bound small G protein working upstream of MAPKs – occurs through an exchange of GDP for GTP, which converts Ras into its active form. Furthermore, Ras undergoes a conformational change and becomes active upon stimulation by coupling the Shc/Grb2/SOS (Shc adapter protein/growth factor receptor bound protein 2/son of sevenless) protein complex (see Figure 2). The process of Ras protein recruitment to the cell membrane is connected with post-translational modifications, preferentially in cysteine residue, through farnesylation mediated by farnesyl transferase (17). Ras protein upon activation, induces Raf proteins (A-Raf, B-Raf and C-Raf) which are translocated to the cellular membrane.

Normally the Raf family proteins are activated by series of modifications: dimerization (18), phosphorylation/dephosphorylation on different domains (19), disassociation of the Raf kinase inhibitory protein (RKIP) (20) and, finally, its activity is modulated by chaperone proteins, such as heat-shock protein 90 (Hsp90) (21). The activated Raf kinases phosphorylate and activate two mitogen-activated protein kinases: meiosis-specific serine/threonine protein kinases (MEKs), i.e., MEK1 and MEK2, as well as two immediately downstream extracellular-signal-regulated kinases (ERKs), i.e., ERK1 and ERK2. This cascade of phosphorylation of several serine/threonine-specific kinases leads to alterations in the expression of various genes in the nucleus, i.e., a set of transcription factors, including NF-k beta, AP-1, c-Myc, Ets-1 and c-Jun, which were initially identified as...
3.1. Aberrant signaling through the RAS/RAF/MEK/ERK pathway in thyroid tumorigenesis

Many recent studies, focused on molecular background of thyroid cancers, derived from follicular epithelial cell, have proven that aberrant signaling through the RAS/RAF/MEK/ERK cascade is a crucial factor for thyroid tumour initiation and development. Many genetic alterations concern the genes which are crucial in MAPKs signaling cascade (the genes encoding for membrane receptor tyrosine kinases and non-receptor tyrosine kinases) (Table 1).

3.1.1. The role of RET/PTC rearrangements

The essential role in the constitutive activation of RAS/RAF/MEK/ERK and initiation of thyroid tumorigenesis is played by recombinant RET proteins, consisting of the tyrosine kinase domain of the RET (membrane receptor tyrosine kinase for the glial cell-derived neurotrophic factor; GDNF), and a portion of an unrelated protein. The recombinant RET proteins derive from RET/PTC (rearranged in transformation/papillary thyroid carcinoma) rearrangements, that result from paracentric inversion or reciprocal chromosomal translocation of 17 and/or 10 chromosome.

Physiologically, RET expression is restricted to a subset of cells derived from embryonic neural crest cells. It is confirmed that, in the thyroid gland, a wild-type RET is expressed at high levels in the parafollicular C-cells, whereas in follicular cell activation of RET, the oncogene occurs by fusion of TK domain of RET gene with 5' terminal region of various genes (e.g., H4, RlalphaTKA, ELE1, GOLGA5). These gene fusions become a source of active promoters for the expression of TK domain of RET protein. In consequence of gene fusion, dimerization and ligand-independent constitutive RET protein activation take place. There are, at least, 15 different RET/PTC rearrangements, with RET/PTC1-3 sequences as the most common in PTC (25,30). RET/PTC oncogenic sequences occur in about 3-60% of sporadic cases of PTC, depending on population, thus, they are recognized as genetic markers of this type of cancer (6,25,30,32).

A molecular study, focused on the role of RET/PTC in cell activity, confirmed the diversity of biological effects of RET/PTC oncogenic activation, concerning the stimulation of cell proliferation, survival and extracellular matrix (ECM) invasion. The biological effects of RET/PTC/RAS/BRAF depend on the integrity of this signaling transduction and phosphotyrosine activation (35,36). In thyroid tumours with RET/PTC1 induction, the signal transduction mediated by phosphotyrosine 294, 404, or 451 has been shown to be critical for RET-induced transforming activity in vitro (36). Moreover, the presence of RET/PTC1 or RET/PTC3 in both mono- and polyclonal microscopic foci of thyroid nodules - not classified as PTC - may promote the PTC development (37). The special ability of RET/PTC oncogenic sequences to initiate the tumorigenesis was confirmed by in vitro experiments on thyroid follicular cell culture and in vivo studies on transgenic mouse model (36-39). Transgenic animals with confirmed expression of oncogenic sequences RET/PTC1 and RET/PTC3 were able to develop thyroid tumours (38,39). Regarding RET/PTC1, this oncogenic sequence is

Table 1. Protein kinase functions in MAPK/ERK signaling pathway and their genetic alterations in neoplastic transformation of thyroid follicular cell

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Protein and its function</th>
<th>Activation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET</td>
<td>10q11.2 1q23-24</td>
<td>Membrane receptor tyrosine kinases for extracellular growth factors (GDNF, NGF); cell proliferation and differentiation control.</td>
<td>Rearrangements RET/PTC, TRK</td>
<td>11,24-27</td>
</tr>
<tr>
<td>BRAF</td>
<td>7q34</td>
<td>non-receptor signaling transduction protein; cytoplasmic serine/threonine kinases; cell proliferation, differentiation, angiogenesis and adhesion control.</td>
<td>Point mutations, BRAF/ALK rearrangements</td>
<td>11,28,29</td>
</tr>
<tr>
<td>MPK1</td>
<td>MAP2K</td>
<td>Protein mitogen-activated serine/threonine kinases (EC 2.7.11), MAP; extracellular signal transduction proteins.</td>
<td>Gene mutations (?)</td>
<td>Any published</td>
</tr>
</tbody>
</table>

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Figure 3. The activation of MAPK/ERK pathway via Tyr1062 autophosphorylation and Shc protein binding.

protooncogenes involved in cell proliferation, growth, survival, and tumourigenesis (22,23).

### 3.1.1. The role of RET/PTC rearrangements

The essential role in the constitutive activation of RAS/RAF/MEK/ERK and initiation of thyroid tumorigenesis is played by recombinant RET proteins, consisting of the tyrosine kinase domain of the RET (membrane receptor tyrosine kinase for the glial cell-derived neurotrophic factor; GDNF), and a portion of an unrelated protein. The recombinant RET proteins derive from RET/PTC (rearranged in transformation/papillary thyroid carcinoma) rearrangements, that result from paracentric inversion or reciprocal chromosomal translocation of 17 and/or 10 chromosome.

Physiologically, RET expression is restricted to a subset of cells derived from embryonic neural crest cells. It is confirmed that, in the thyroid gland, a wild-type RET is expressed at high levels in the parafollicular C-cells, whereas in follicular cell activation of RET, the oncogene occurs by fusion of TK domain of RET gene with 5' terminal region of various genes (e.g., H4, RlalphaTKA, ELE1, GOLGA5). These gene fusions become a source of active promoters for the expression of TK domain of RET protein. In consequence of gene fusion, dimerization and ligand-independent constitutive RET protein activation take place. There are, at least, 15 different RET/PTC rearrangements, with RET/PTC1-3 sequences as the most common in PTC (25,30). RET/PTC oncogenic sequences occur in about 3-60% of sporadic cases of PTC, depending on population, thus, they are recognized as genetic markers of this type of cancer (6,25,30,32). In our study, conducted in Polish population, the most common rearrangements of RET oncogene (RET/PTC1-3) has been found in 21% of studied PTC cases (33). These differences between the prevalence of RET/PTC rearrangements are due to population variability, diverse methodology, nonclonal features of RET/PTC, as well as genetic heterogeneity of PTC (34). Similarly to many human cancers, autophosphorylation of tyrosine (Tyr) in 1062 position of RET protein was documented is a crucial molecular change during thyroid tumorigenesis (31). RET/PTC oncogenic sequence may bind the Shc adapter protein via Tyr1062 autophosphorylation that leads to MAPK/ERK pathway activation (35) (Figure 3).

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neoplastic transformation, appears that in beta-catenin transcriptional activity towards glycogen synthase kinase 3 beta (GSK3beta) associated with predisposition to PTC (46). It also should activate Akt protein, i.e., kinase transducing signals from thyrocytes, expressing aggressive behaviour, and is constitutively active in human is overexpressed in PTC (49, 50), associated with more transcriptional and signaling levels – with control crucial elements in tumour development. Growth factors and oncogenes to downstream targets, that study of Siraj et al. (50) documented that MET may activate Akt protein, i.e., kinase transducing signals from growth factors and oncogenes to downstream targets, that control crucial elements in tumour development.

3.1.2. The role of TRK rearrangements

An alternative to RET/PTC molecular changes, involved in the biological effects of constitutive activation of MAPK pathway, are oncogenic rearrangements of NTRK1 gene that encode the receptor for the high-affinity nerve growth factor (NGF) (51). Oncogenic rearrangements of NTRK1 (TRK sequences) – also found in PTC – represent less frequent genetic events. Studies performed in different populations revealed the occurrence of TRK rearrangements of 0-50% (reviewed in 52). In our study focused on Polish population, the main types of TRK sequences, i.e., TRK-T1 and TRK(TPM3), are recognised in 12% of studied PTC (33).

Oncogenic TRK sequences originate from the fusion of the TK domain of NTRK1 oncogene with 5’terminal sequence of, at least, three different genes (e.g., tropomyosin 3, TPM3; translocated promoter region, TPR; TRK fused gene, TFG) (53). The oncogenic activity of TRK sequence in thyroid follicular cells was confirmed in vivo in transgenic mice, where NTRK1chimeric protein (TRKT1) expression led to PTC development (54). Recently, it has been confirmed that NTRK1 cell signalling is modulated by the presence of p75 protein which is able to co-expression with NTRK1 receptor and contributes to enhanced neurotrophins binding. Moreover, p75 neurotrophin receptor and NTRK1 co-expression enhances the specificity of other TRKs for their preferred ligands (55,56). The detection of TRK rearrangements in PTC and neexpression of p75 provide some evidence for their participation in the etiopathogenesis of PTC (56). Additionally, it has been evidenced that TRK rearrangements may activate the MAPK pathway through a step upstream of Ras (51).

3.1.3. The role of Ras protein and RAS mutation

The activation of tyrosine kinase receptor (RET or NTRK1) via autophosphorylation of tyrosine residues in the intracellular domain, involving numerous adaptor proteins, leads to activation of membrane Ras proteins (G proteins) (15). Ras represents a family of small guanosine triphosphate (GTP)-binding proteins, which are recognized as essential downstream molecules of many cell receptors in the MAPK/ERK signaling pathway. Physiologically, Ras proteins, under stimulating signals from hormones, growth and differentiation factors, become activated via inducing the exchange of GDP with GTP, which converts Ras protein into active protein conformation (Figure 4). The Ras protein with bound GTP can activate a protein kinase cascade MAPK/ERK, which ultimately leads to phosphorylation of transcription factors in the nucleus, which, in turn, alter several gene expressions (22).

It has been confirmed that, after posttranslational modification of Ras proteins, they are recruited to the internal surface of cell membrane and serve as “molecular switching” (Ras-GTP/ Ras-GDP) in cell signal transfers. Moreover, Ras activation is connected with phosphorylation of Shc proteins and binding of adaptor Grb2/SOS (growth factor receptor-bound protein 2/son of sevenless) protein complex (57). The activated Ras protein functions as an adapter that binds to Raf kinases (A-Raf, B-
Raf, C-Raf) – i.e., intracellular MAPK effectors – and creates signaling regulation complex Ras-Raf. This active complex is transmitted to cell membrane where, via phosphorylation, MAPK/ERK cascade become activated. Finally, B-Raf activates the MEK and ERK kinases and initiates the induction of gene expression in cell nucleus (15) (see Figure 2).

The activation of Ras in thyroid tumours involves point mutations in the RAS gene. Typically, the activation of mutations occurs in certain codons of one of three Ras family genes (N-RAS, K-RAS and H-RAS) – mainly in codon 61 of N-RAS gene. This type of point mutation in “initiator” RAS gene is particularly prevalent (20-50%) in FTA (follicular thyroid adenoma) and FTC (follicular thyroid carcinoma), whereas, less frequently, it occurs in PTC (0-15%) (58,59). Our investigation, focused on the genetic background of PTC in the Polish population, confirmed the presence of point mutations in K-RAS at codons 31 and 61 with frequency of about 8% (60). Interestingly enough, in the follicular variant of PTC, remarkably frequent activating mutations in RAS oncogenes were observed which correlated with less prominent features of tumours (61,62). In relation to RAS mutation, it was suggested that the follicular variant of PTC might represent a similar to FTC molecular pathway of tumorigenesis.

Moreover, an in vitro study, conducted on thyrocyte cell line, has documented that K- or N- RAS protooncogene activation leads to a decreased expression of thyroid-specific genes (e.g., Tg, TSH) with a simultaneous increase of the proliferation index of follicular thyroid cells in the thyroid gland (63,64). Regarding the fact that RAS mutations have been recognized in benign, as well as in malignant thyroid neoplasms, it is approved that the activation of RAS oncogene appears in early steps of tumorigenesis in the thyroid gland (65,66). However, it has been documented that RAS activation – mainly via mutations at codon 61 – is involved in the progression and poor outcome of thyroid tumours (67). Moreover, it is considered that RAS oncogene activation is not a sufficient molecular event which leads to initiation of follicular cell transformation, as well as it has been approved to be an intermediate molecular event in cancer dedifferentiation and progression. In order to recapitulate the importance of Ras signalling molecules in thyroid cancer, it should be emphasised that the activated form of Ras protein (Ras-GTP) functions as an adapter protein that binds Raf kinases and causes their translocation to the cell membrane, where the next step of RAS/RAF/MEK/ERK pathway activation – i.e., Raf activation - takes place (68).

3.1.4. The role of B-Raf kinase and BRAF mutation

The active form of Ras (Ras-GTP) as an adapter protein may bind B-Raf with high affinity. B-Raf belongs to the family of Raf proteins (A-Raf, B-Raf, C-Raf), which are serine/threonine-specific kinases that integrate and regulate upstream flowing signals trough the MAP/ERK pathway (15). Among the three mammal Raf proteins, B-Raf kinase is an essential signalling protein with the highest kinase activity. It is known as the most important activator of MEK (22,68). Regarding A-Raf and C-Raf kinases, they play irrelevant role in molecular pathogenesis of thyroid gland, due to rare genetic alteration in genes coding these proteins (69).

The study of Davies et al (70) has documented the constitutive activation of B-Raf protein via point mutations of BRAF gene as a common genetic event in several types of human tumours, confirming the key role of this Raf kinase in tumorigenesis. Similarly, in vitro studies in benign thyroid cell models, have demonstrated a particularly important role for B-Raf kinase in the regulation of thyroid-specific differentiation and proliferative capacity (71). The activating mutations in BRAF gene – responsible for B-Raf kinase activity – are the most prevalent (36-69%), defined genetic abnormalities in thyroid cancers (72-75). Among all the mutations in BRAF gene, T1799A – causing a substitution of single glutamic acid for valine in 600 position of B-Raf protein (V600E) – has been recognized as the most important and frequently occurring (13-69%) in different populations (29,72-78). V600E mutation is characteristic mainly for PTC (about 45% cases), but also for PDTC and ATC derived from PTC (72,74-76,79,80). This fact confirms the hypothesis that some ATCs arise from PTCs and B-Raf kinases may be functionally important in thyroid dedifferentiation, although the mutations in BRAF may not be recognized as an independent factor inducing this process. The experiments on BRAF V600E positive transgenic mouse model have confirmed that this type of mutation possesses an oncogenic activity for thyroid cancer development. Transgenic mouse develops invasive and poorly differentiated thyroid cancer with progressive local invasion (81). Regarding human thyroid tumours, it should be stressed that the relationship between the BRAF V600E mutation and aggressive tumour behaviour, as well as the predictive nature of BRAF mutations, are controversial. In several studies, the presence of BRAF V600E mutation was associated with a more aggressive clinical course, an increase of tumour size and invasion via underexpression of matrix metalloproteinases (MMPs) (72,82-85). Moreover, the frequent occurrence of V600E mutation has been emphasised in more aggressive histopathological variants of PTC, i.e., tall cell or columnar cell, with the loss of responsiveness to radioiodine (84). On the other hand, this observation has not been confirmed by others in different populations (86-88). Finally, it has been proposed that a detection of BRAF V600E might be a useful marker for screening patients with unfavourable outcomes and in making decision to undertake a more aggressive initial therapy. This aspect is specially important for the early diagnosis of PTC in fine-needle aspiration samples. Another frequent mutation, recognized in BRAF gene and characteristic for thyroid cancer, is K601del (T1799-1801TGAdel), associated with the lymph node metastasis of PTC (79) and K601E (A1802G) recognized to be common in FTA (follicular thyroid adenoma) and in follicular variant of PTC (72,74,89). The mechanism of B-Raf protein activation has recently been investigated (90). It has been confirmed, that BRAF mutants acting as oncogenes and, particularly, as mutations in exon 15 of BRAF gene, leading to substitutions at 600 and 601 position.
Role of MAPK/ERK and PI3K/Akt pathways in thyroid cancers

Figure 5. The schematic structure of B-Raf protein with marked exemplary BRAF mutations, recognized as intermediate activators of kinase B-Raf activity, in P-loop and activation segments.

(V600E and K6001del/E) of B-Raf protein, have been recognized as the most constitutively activating mutations in thyroid cancer (72,74,79). It is suggested that V600E substitution, within the activation segment of the kinase domain, mimics the phosphorylation, due to the proximity of glutamine acid COOH residue, and leads to phosphorylation at 600 position in amino acid chain of B-Raf kinase domain (29). This new form of interaction keeps the B-Raf protein in a catalytically competent conformation, following continuous MEK phosphorylation (90). Biochemical analysis of V600E mutation, performed in many types of human cancers, has revealed that this molecular event disrupts the interaction between P-loop and activation segment of B-Raf kinase, thus disturbing their conformation (90,91). Moreover, a functional analysis of BRAF mutations – including V600E and K6001del/E – indicates that most of them are clustered in the activation segment of kinase domain, that significantly increases kinase activity and may be accepted as a strong kinase activator. After all, several BRAF mutations are recognised as intermediate activators of kinase activity but, probably, they are not essential activating mutations in BRAF gene (90) (Figure 5).

Recently, another important factor – BRAF/AKAP9 rearrangements, increasing the BRAF signalling – has been recognised in a small subset of PTCs as a novel mechanism of MAPK/ERK pathway activation (28). Moreover, the same research group has discovered an additional mechanism of BRAF kinase activation, involving numerous gains on chromosome 7 (there BRAF gene is located) or gene amplification in the classic variant as well as in the oncocytic variant of PTC (Hurtthle cells) (92).

Additionally, it has lately been documented that LKB1 - an upstream activator of AMPK (AMP-activated protein kinase) – is preferentially associated with B-RAF V600E activation via phosphorylation on multiple sites (mainly in Ser325 and Ser428) of LKB1 by ERK in melanoma cell (93). The activity of LKB1-AMPK protein kinase signaling pathway – involved in the regulation of cell proliferation in mammalian cancer – is negatively regulated by the oncogenic B-RAF V600E mutants, probably through its downstream MEK-ERK kinase signaling cascade (93). It may be possible that suppression of LKB1 function by B-RAF V600E plays an important role in B-RAF V600E-driven tumorigenesis in thyroid cancer, but this hypothesis should be confirmed in experimental studies.

3.2. RET/PTC, RAS, BRAF in RAS/RAF/MEK/ERK pathway and their influence on gene expression profiles in thyroid neoplasms

According to many studies, focused on the mechanisms of activation of MAPK/ERK pathway, it has been accepted that B-Raf (as well as other Raf kinases) is downstream signal transducing molecules for RET/PTC. Moreover, in human PTC activating mutations in BRAF and RAS genes, similarly as in TRK and RET/PTC rearrangements, are largely mutually exclusive. Therefore, it was suggested that the RAS/RAF/MEK/ERK pathway may represent a linear cascade whose activation promotes thyroid tumorigenesis (89,90). To test this hypothesis, many in vitro and in vivo studies have been conducted to determine the expression profile of MAPK/ERK cascade genes and the phenomenon of their overlapping as well as their role in the thyroid transformation (10,73,94). The results of these studies demonstrate that BRAF, RAS and RET/PTC alterations are mutually exclusive (73,89,95) and independently sufficient for cell signaling activation. On the other hand, it is suggested that RET/PTC and BRAF or RAS mutations can coexist, although very rarely, especially RET/PTC cooperates with BRAF alteration (96). It is possible that none of these molecular events individually could confer any advantage for clonal tumour proliferation and could thus become established in subsequent cell generations. However, one certain thing is that the presence of RET/PTC induces dedifferentiation of thyroid tumours. Regarding Ras, this protein influences on apoptosis of thyroid cell with RET/PTC alterations, as evidenced in a rat thyroid cell model (97).

Interestingly, the activation of Ras, Raf, and RET proteins – through the similar molecular mechanisms – can determine different biological features of thyroid tumours (11). It should be emphasised that PTCs with RET, RAS or BRAF alterations have been confirmed to determine the characteristic clinical and histopathological features of thyroid tumours. Thus, RAS mutation is involved mainly in the follicular variant of PTC and determines lung metastases (58,67), while the presence of BRAF mutation predisposes to extrathyroid invasion and dedifferentiation at more advantageous tumour stage (82,83).

Recently it has been recognized that, in human PTCs, the presence of RET/PTC, RAS, BRAF genotypes determines different gene expression profiles in thyroid tumours (10,85,94). In vivo, RET, TRK or RAS differently influence on dedifferentiation and thyroid-specific genes (e.g., Tg or TSH) activation. It was confirmed, that RET/PTC3 significantly reduced Tg mRNA level or active form of TSH, while TRK-T1 sequences, induced in vivo in follicular thyroid cell, influenced on Tg or TSH only at the minimal level (64). Moreover, Denning et al. (98) documented that RET/PTC1 oncogenic sequences altered
the immunoprofile of thyroid follicular cell and influenced on thyroid cancer development.

Gene expression analysis, based on DNA oligonucleotide microarrays data, has classified the tumours into three distinct groups, depending on their mutational genotype (94). Mesa et al. (85), based on transfected rat thyroid clonal cell population (PCCL3), established functional categories of genes that may help explain some differences and similarities in thyroid cancer features. Their study confirmed the existence of functional gene clusters – defined as a functional group of genes – that were preferentially activated by \( RET \) or \( BRAF \) expression. It is worth noting, that in thyroid cells with constitutively expressed oncogenic \( BRAF \), the expression of \( MMP \) – important in tumour invasion – is much greater than in cells harbouring \( RET/PTC3 \) oncogenic sequences. This fact may explain the predisposition of tumours with oncogenic \( BRAF \) expression to the more aggressive outcome and extrathyroid invasion and dedifferentiation from PTC to ATC (82, 83). Moreover, it has been confirmed that \( RET/PTC3, HRAS \) and \( BRAF \) oncogenes upregulate a large group of overlapping genes, involving CXC chemokines and their receptors, which are recognised as stimulators of mitogenic and invasive capacity of thyroid cancer cells (10). Finally, it has been documented that \( RET/PTC3 \) regulated genes are simultaneously dependent on \( BRAF \) gene expression. Additionally, a gene cluster coding for the mitochondrial electron transport chain pathway – influencing cell variability – was down-regulated in this group of cells with \( RET/PTC3 \) and \( BRAF \) expression (85).

4. PI3K/akt signaling pathway and its role in malignant transformation

PI3Ks (phosphatidylinositol 3-kinases) have been classified into three major subfamilies – classes IA and B, classes II and III – based on their structure and the substrate specificity (99). The most extensively investigated PI3Ks are class IA PI3Ks which are involved in a number of cellular functions, including cell growth, proliferation, and survival (100). It has been confirmed that only Class I (A and B) PI3Ks are involved in cancer development (101).

Class IA PI3Ks can be activated by receptor tyrosine kinases (RTKs), including: EGFR (epidermal growth factor receptor), PDGFR (platelet-derived growth factor receptor), FGFR (fibroblast growth factor receptor), IGF-1R (insulin-like growth factor 1 receptor), VEGFR (vascular endothelial growth factor receptor), interleukin receptors, interferon receptors, and integrin receptors. Additionally, intracellular proteins such as protein kinase C (PKC), SHP1, Rac, Rho, and Src can also activate PI3K (102).

Physiologically, after ligand-induced activation of specific receptors, PI3K can be activated through binding to the receptor p85 regulatory subunit of PI3K, followed by recruitment of the catalytic subunit of PI3K, p110, to this complex.

Alternatively, PI3K can be activated as a result of Ras activation (GTP- binding protein) which is able to induce the membrane translocation and activation of p110 subunit of PI3K. Upon activation, PI3K phosphorylates phosphatidylinositol-3,4-diphosphate (PIP2) into phosphatidylinositol-3,4,5-triphosphate (PIP3). The latter substrate, PIP3, acts as a second messenger, which binds PDK1 (phosphoinositide-dependent kinase 1) through pleckstrin homology (PH) domain and then activation of PDK1 occurs. The activated form of PDK1 phosphorylates Akt (cellular homolog of murine thymoma virus Akt8 oncoprotein) at Thr308 position activating its serine-threonine kinase activity. Akt is also recruited to the lipid-rich plasma membrane by its PH domain (99, 103) (Figure 6).

Akt (also known as protein kinase B, PKB) is the primary mediator of PI3K-initiated signaling. Akt has three isoforms: Akt1, Akt2, and Akt3 (PKBalpha, PKBbeta, and PKBgamma), which are encoded by three different genes. Upon activation, Akt phosphorylates a number of downstream targets for regulating various cellular functions, such as cell survival, cell cycle progression, migration, proliferation, metabolism, tumor growth, and angiogenesis (104, 105, 106).

It is worth noting that Akt kinase can cause inactivation of specific substrates or may activate other proteins. For instance, Akt can enhance cell survival through the inhibition of pro-apoptotic proteins such as forkhead (FOXO) family of transcription factors and Bcl2-antagonist of cell death (BAD) and can block the apoptosis through the induction of survival proteins such as Bcl2, IkappaB kinase (IKK), and human double minute 2 (HDM2) (101, 106).

It has been demonstrated that the PI3K/Akt signaling pathway play a key role in regulating cell cycle progression and proliferation. By blocking FOXO-mediated transcription of cell-cycle inhibitors, including p27Kip1, or directly by phosphorylating and thus inactivating p27Kip1, Akt promotes the G1/S phase transition. Akt induces cell proliferation by inhibiting the TSC1-TSC2 complex. Akt can also indirectly stabilize the cell-cycle protein e-Myc and cyclin D1 by inhibiting GSK3 (glycogen synthase kinase 3) (106, 107).

It has been shown that PI3K/Akt signaling is important for regulating HIF-1alpha and VEGF expression which are the confirmed mediators transmitting signals for tumor growth and angiogenesis. Moreover, it has been documented that Akt – the major target of PI3K – transmits oncogenic and angiogenic signals in malignant transformation (106).

The regulation of cell survival and cell cycle progression by the PI3K/Akt pathway implicates a crucial role of this pathway in carcinogenesis and cancer development. Indeed, the relationship between dysregulated PI3K activity and the onset of cancer is well-documented.

PI3K mutations – leading to increased PI3K signaling – have frequently been observed in many human cancers, including cancer of the thyroid gland.
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Figure 6. Schematic activation of the PI3K/Akt pathway.

(103,106,108-114). Those mutations involve genes encoding PI3K subunits, i.e., PIK3CA and PIK3CB (encoding the p110 catalytic subunit) and PIK3R1 (encoding p85 alpha regulatory subunit). PIK3CA alterations include activating somatic mutations, largely found in hot-spot regions of this gene, as well as frequent gene amplification, that lead to constitutively active form of the enzyme, similarly to the effect of somatic mutations in PIK3R1 gene (108, 115-117). AKT gene mutations and amplifications are also observed in a number of human cancers, including thyroid tumours (118-121).

The tumor suppressor gene, PTEN (phosphatase and tensin homologue deleted on chromosome 10), encodes the protein acting as the antagonist of PI3K. PTEN is a dual function lipid and protein phosphatase. Its lipid phosphatase function removes the 3′ phosphate of PIP3, leads to degradation of it and hence, it terminates the signaling downstream of activated PI3K. PTEN gene is frequently mutated or lost in many human cancers; the decreased PTEN expression is correlated with the progression of solid cancers (107, 122-124).

4.1. Aberrant signaling of PI3K/Akt pathway in thyroid tumorigenesis

There are clinical correlations and experimental in vitro and in vivo data that PI3K/AKT signaling pathway plays the key regulatory role, both in thyroid tumor formation and progression. It has been demonstrated that PI3K signaling pathway plays a central role in proliferation, survival, invasion and motility in thyroid tumorigenesis. Several recent studies have investigated the role of genetic alterations in this signaling pathway in thyroid cancers (114, 118, 125, 126). It has been shown that inhibition of PI3K and AKT isoforms reduces thyroid cancer cell cycle progression at G2/M phase transition and can induce apoptosis (127). Studies in animal models have indicated a role for AKT in thyroid metastasis process (118, 128).

The results of the study conducted by Hou et al. (125) indicate that 31% of benign follicular adenomas, 55% of FTCs, 58% of ATCs and 24% of PTCs harbored one of PI3K abnormalities, namely PIK3CA mutation or PIK3CA amplification or PTEN mutation. This supports a role for PI3K signaling in thyroid tumorigenesis, particularly for follicular neoplasias, and in thyroid cancer progression toward ATC.

In case of sporadic PTC, activation of the PI3K/Akt signaling pathway appears to be most prominent in tumor progression and activation of the RAS/RAF/MEK/ERK pathway is of primary importance in PTC development. In general, the data obtained from the studies on thyroid carcinomas suggest that genetically activated PI3K signaling represents a later-stage event in PTC, as enhanced activation of Akt dominates in the invasive forms of PTCs (105, 118). In sporadic follicular thyroid cancers, the mutually exclusive nature of loss of PTEN expression and activating mutations and amplifications of the PIK3CA gene supports the ability of constitutive PI3K signaling to cause FTC (103).
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Figure 7. Involvement of the MAPK/ERK and PI3K/Akt pathways in the follicle cell-derived thyroid tumorigenesis.

The coexistence of genetic alterations in the PI3K/Akt pathway, including PIK3CA amplification and mutations, PTEN mutations and methylation and RAS mutation is most dominant in ATC, being smaller in FTC, and the lowest in PTC and benign lesions (125). In general, the studies confirm that the increased activities of the PI3K/Akt pathway are most often seen in aggressive thyroid cancers, such as ATC, which represents the final step in the multistage genetic model of thyroid follicular cell-derived tumorigenesis (114,129-131). That clearly suggests a role for PI3K signaling in progression from differentiated to undifferentiated thyroid tumours.

A schematic model for the role of PI3K/Akt pathway in thyroid tumorigenesis and progression is presented in Figure 7, considering also the involvement of the MAPK/ERK pathway. It is accepted that oncogenic activation of the PI3K/Akt pathway drives the transformation of follicular thyroid adenoma (FTA) to FTC and then to ATC. Regarding PTC, it develops de novo after activation of the MAPK/ERK pathway - by such oncogenic alterations as RET/PTC rearrangements or BRAF mutation - and its transformation towards ATC may be facilitated by the PI3K/Akt pathway (125,132).

The role of molecular alterations of particular effectors in PI3K/Akt pathway in thyroid tumorigenesis is presented in the Table 2.

4.1.1. The role of PIK3CA alterations

PIK3CA gene – which encodes for the catalytic subunit of p110alpha of PI3K – has been found to be amplified or mutated (mainly in exon 9 and 20) in thyroid carcinomas (116,125,126,132). It should be stressed that mutations identified in this gene are relatively common in ATC (114,125), whereas they represent rather rare mechanism of PI3K/Akt activation in PTC (116,126).

Worth of noting is the fact of high frequency of PI3KCA gene amplification that predominate over mutations in this gene (114,131). PIK3CA gene amplification, has been correlated with increased Akt phosphorylation and its activity in thyroid tumour progression. Wang et al (132) have found a strong tendency toward association of PIK3CA amplification and some high-risk clinicopathological characters of thyroid carcinoma.

PIK3CA gene amplification is found in PTC, FTC and ATC. However, there are some differences in frequency of PIK3CA amplification in PTC, as reported by authors, ranging from 5-14% to more than 50%, depending, probably, on population (126,132). In most studies, however, a higher frequency of PIK3CA gain copy number (defined as 4 or more copies) is observed in FTC (24-28%) than in PTC (114,116,125,132).

Interestingly enough, PIK3CA amplification has also been reported in the benign adenomas, thus suggesting that this alteration could be an initiating event in thyroid tumorigenesis (116,125,132).

PIK3CB is another catalytic subunit of PI3K. Copy number gains of this gene have been found in FTC and ATC (131).
4.1.2. The role of AKT alterations

Akt protein activity, as mentioned earlier, is enhanced by increased PI3K activity or reduced PTEN activity. The other mechanisms include Akt overexpression or AKT mutations, genetic changes in kinases or phosphatases that regulate Akt action, mutations in Ras, as well as mutations and/or overexpression of a variety of tyrosine kinase receptors. Increased Akt phosphorylation was found to be correlated with thyroid tumour progression.

It has been demonstrated that the inhibition of Akt isoforms reduce thyroid cancer cell cycle progression at G2/M phase transition and can induce apoptosis (127). Studies on animal models have indicated some role for Akt also in thyroid metastasis process (118,128).

In human sporadic thyroid cancer – as well as in thyroid cancer cell lines – increased expression of AKT as well as increased levels of total Akt activity in comparison with normal tissue specimens has been found. Among the three isoforms of Akt, Akt 2 has appeared to be the predominant form in the malignant tissue (118). In some cases the malignant transformation has been related to AKT2 gene amplification, although generally the increase of Akt proteins has been more impressive. This fact suggests a more important role for post-transcriptional and post-translational alterations. Interestingly, Akt levels have been increased in all FTC and only in a subset of PTC samples (118).

4.1.3. The role of PTEN alterations

PTEN gene, which acts as a tumor suppressor gene through the action of its phosphatase protein product, is involved in thyroid tumorigenesis.

The role of PTEN in thyroid tumorigenesis was firstly evidenced in the Cowden disease, a dominant genetic syndrome whose characteristics include carcinomas of the thyroid gland, i.e., mostly FTC. Inactivating mutations of PTEN have been defined as the cause of this syndrome (133) and molecular alterations of PTEN, i.e., point mutations and somatic deletions, were recognized as an important step in the development of thyroid gland cancers (134).

The study, conducted on a mouse model, has shown that complete loss of Pten in the thyroid gland was sufficient to induce thyroid neoplasia with increased PI3K signaling (135). In human, several studies have been conducted assessing genetic changes in PTEN, i.e., mutations and deletions as well as aberrant expression level, in sporadic benign and malignant thyroid cancers (114,118,136-138). Although, it appeared that inactivating mutations and deletions in PTEN gene are relatively high in ATC (125) and they are infrequent in PTC (132).

Despite lack of mutations, decreased expression of PTEN, both at mRNA and protein level, is found in thyroid tumours. In addition to LOH (loss of heterozygosity) in one allele, an epigenetic inactivation of the other allele of PTEN via its promoter methylation can explain its silencing in thyroid cancer (137,139,140). Hou et al. (130) have found significant correlation between PTEN methylation and genetic alterations in the PI3K/Akt pathway – especially PIK3CA amplification and mutations, as well as RAS mutations.

5. INTERACTIONS BETWEEN RAS/RAF/MEK/ERK ALTERATIONS AND PI3K/AKT PATHWAY ACTIVATION

As described earlier in this review, increased mitogenic signaling through classical tyrosine kinase-activated pathways (RAS/RAF/MEK/ERK or MAPK/ERK) has proven to play a major role in thyroid cancer, especially in PTC initiation as well as in progression to ATC (141). The MAPK/ERK cascade is intimately linked with the PI3K/Akt signaling. The results of numerous studies indicate a cross-talk between the two pathways. Both PI3K/Akt and MAPK may result in the phosphorylation of many downstream targets and play a role in the regulation of cell survival and proliferation (103).

Mutations occurring in BRAF or RAS genes, as well as in RTK genes, can dually activate the PI3K/Akt and MAPK pathways. Several studies have been conducted looking for coexistence of genetic alterations involving the above mentioned genes and other important genes in the PI3K/Akt signaling pathway.

A strong association between RAS mutations and Akt activation has been confirmed and the obtained results suggest that PI3K/Akt activation by RAS mutations is an early genetic event in thyroid tumours development (126,129). Especially, that the concomitant occurrence of PIK3CA copy gains and RAS mutations was also found in benign thyroid carcinomas (125).
Furthermore, the results of the research work conducted by J. Abubaker et al. (126), showed that many PTC samples with BRAF mutations had coexisting PIK3CA amplifications, and the authors suggested the existence of a synergism between BRAF mutations and PIK3CA amplifications in PTC tumorigenesis. The overlapping of BRAF mutation and PI3K/Akt pathway–related genetic alterations was also found in ATC (114,125).

On the other hand, Raf activity has been found to be negatively regulated in some cell types (103) (see Figure 6).

The increasing accumulation of genetic alterations in the PI3K/Akt and MAPK suggests that both pathways play a key role in the tumorigenesis and aggressiveness of thyroid tumours (125,131). This has been further supported by Liu et al (131) who looked for genetic alterations in various RTKs genes – that could activate both the PI3K/Akt and MAPK pathways – in FTC and ATC. They found frequent copy gains of RTK genes (but no mutations), including EGFR, PDGFRalpha, PDGFRbeta, and VEGFR genes. RTK gene copy gains were more commonly associated with Akt phosphorylation than with ERK phosphorylation, suggesting that genetically altered RTKs preferentially use the PI3K/Akt pathway to promote ATC and FTC tumorigenesis and invasiveness (131).

The coexistence of genetic alteration in MAPK/ERK and PI3K/Akt effectors – relatively frequent in various thyroid tumors, particularly in FTC and ATC (125,132) – confirms the hypothesis of accumulation of molecular changes during dedifferentiation of the thyroid gland.

6. ACKNOWLEDGEMENTS

Both authors equally contributed to this article.

7. REFERENCES


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64. Portella, G., D. Vitagliano, C. Borselli, R.M. Melillo, D. Salvatore, J.L. Rothstein, G. Vecchio, A. Fusco & M. Santoro: Human N-ras, TRK-T1, and RET/PTC3 oncogenes, driven by a thyroglobulin promoter, differently affect the expression of differentiation markers and the


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**Abbreviations:** MAPK/ERK: mitogen - activated protein kinase/extracellular signal-regulated kinase signaling pathway; PI3K/Akt: lipid kinase phosphoinositide-3-kinase signaling pathway; WDTC: well-differentiated thyroid carcinoma; PDTC: poorly differentiated thyroid carcinoma; UTC: undifferentiated thyroid carcinoma; ATC: anaplastic thyroid carcinoma; FTC: follicular thyroid carcinoma; PDTC: poorly differentiated thyroid carcinoma; UTC: undifferentiated thyroid carcinoma; ATC: anaplastic thyroid carcinoma; FTC: follicular thyroid carcinoma; PTEN: phosphatase and tensin homologue; VEGF: vascular endothelial growth factor; IGF-1R: insulin-like growth factor 1 receptor tyrosine kinase; MPK1: MAP kinase 1 gene; MAP2K: mitogen-activated protein kinase/extracellular signal-regulated kinase; RTK: receptor tyrosine kinase; Shc/Grb2/SOS: Shc adapter protein/growth factor receptor bound protein 2/son of sevenless; RKIP: Raf kinase inhibitory protein; MEK: meiosis-specific serine/threonine protein kinase; ERK: extracellular-signal-regulated kinase; GDNF: glial cell-derived neurotrophic factor; MPK1: MAP kinase 1 gene; MAP2K: mitogen-activated protein kinase gene; RET/PTC: rearranged in transformation/papillary thyroid carcinoma; Tyr: tyrosine; ECM: extracellular matrix; STAT3: signal transducer and activator of transcription 3; GSK3beta: glycogen synthase kinase 3 beta; NGF: nerve growth factor; TPRM: tropomyosin 3; TPR: translocated promoter region; TFG: TRK fused gene; Grb2/SOS: growth factor receptor-bound protein 2/son of sevenless; FTA: follicular thyroid adenoma; FTC: follicular thyroid carcinoma; MMP: matrix metalloproteinase; AMPK: AMP-activated protein kinase; P3K: phosphatidylinositol 3-kinase; EGFR: epidermal growth factor receptor; PDGFR: platelet-derived growth factor receptor; FGF: fibroblast growth factor receptor; IGF-1R: insulin-like growth factor 1 receptor; VEGFR: vascular endothelial growth factor receptor; PKC: protein kinase C; PIP2: phosphatidylinositol-3,4-diphosphate; PIP3: phosphatidylinositol-3,4,5-triphosphate; PDK1: phosphoinositide-dependent kinase 1; PH domain: pleckstrin homology domain; Akt: cellular homolog of murine thymoma virus Akt8 oncoprotein; PKB: protein kinase B; FOXO: family of transcription factors; BAD: Bcl2-antagonist of cell death; IKK: IkappaB kinase; HDM2: human double minute 2; p27Kip1: cyclin-dependent kinase inhibitor; HIF-1alpha: hypoxia-inducible factor 1 alpha; VEGF: vascular endothelial growth factor; PTEN: phosphatase and tensin homologue deleted on chromosome 10; LOH: loss of heterozygosity;

**Key words:** Activating Mutations, Gene Amplification, Epigenetic Alteration, Signaling Pathways, MAPK/ERK, PI3K/Akt, thyroid Carcinoma, Review
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