# Phosphoinositide 3-kinase alterations in the thyroid gland – a review study

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**Abstract**

The phosphoinositide 3-kinase (PI3K) pathway has become a subject of great interest of researchers in biomedicine worldwide. Recent studies have shown that PI3K plays a crucial role in various cellular functions: cell cycle progression, cell survival, adhesion and motility, angiogenesis, glucose homeostasis, cell size and organ size control. The phosphoinositide 3-kinase pathway alterations (*PIK3CA* gene mutations and/or amplification) have been observed in various human tumours. In the majority of diagnosed cases, mutations are localized in one of the three "hot spots" in the gene, responsible for coding catalytic subunit $\alpha$ of class I PI3K (*PIK3CA*). Mutations in *PIK3CA* were confirmed in breast, colon and endometrial cancers, while mutations and amplification of the *PI3KCA* gene are characteristic for thyroid cancer, as well. It results in increased activity of serine-threonine kinase (Akt), followed by excessive activation of cellular functions, uncontrolled proliferation and carcinogenesis.

**Key words:** *PIK3CA*, mutation, amplification, thyroid cancer, thyroiditis.

## Phosphoinositide 3-kinase overview

Phosphoinositide 3-kinase (PI3K) belongs to a large family of lipid-phosphoinositide kinases (PIKs) – responsible for phosphorylation of lipids, in particular of the so-called phosphoinositides (PtdIns). Phosphoinositide kinases have been classified into three families, depending on the site of phosphorylation on carbohydrate: PI3K, phosphoinositide 4-kinase (PI4K), and phosphoinositide 5-kinase (PI5K).

The PI3K family is composed of three classes. Class I is responsible for the conversion of phospahtydylinositol-4,5-biphosphatate (PtdIns-4,5-$P_2$) to phospahtydylinositol-3,4,5-triphosphatate (PtdIns-3,4,5-$P_3$). This class consists of two subclasses: Ia – dimeric components, composed of catalytic subunits p110$\alpha$, p110$\beta$, p110$\delta$, associated with p85 regulatory subunit; and subclass Ib, which is a heterodimer consisting of a p110$\gamma$ catalytic subunit, connected with a p101 regulatory subunit. All four isoforms of class I possess not only lipid but also protein kinase activity. Subclass Ia is responsible for transmission signals from receptor tyrosine kinases (RTK) such as EGFR (epidermal growth factor receptor), IGFR (insulin-like growth factor receptor) and PDGFR (platelet-derived growth factor receptor), and subclass Ib is activated by G-protein coupled receptors – GPCR (RAS-GTP). These four isoforms have different locations in tissue and reveal some determined and significant

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functions in processes such as development, inflammation, autoimmunity and malignancy [i.e. PIK3CA (α-type) regulates insulin signalling [1], PIK3CB (β-type) regulates shear platelet activation [2], PIK3CD (δ-type) regulates T- and B-cell activation [3], PIK3CG (γ-type) regulates the activation, as well as migration of neutrophils and macrophages [4]]. A study performed on gene-targeting mice has revealed that PIK3CA and PIK3CB are indispensable for early embryonic development, whereas PIK3CD and PIK3CG deficiency results in some compromise of the immune system (Figure 1) [5-7].

Among the isoforms of the catalytic subunits, only the α-type has been shown to harbour oncogenic mutations or amplification in its gene (*PIK3CA*) in human cancer [8, 9].

Class II kinases are membrane bound, activated by membrane receptors; class III is responsible for producing cellular PtdIns-3-P.

Serine-threonine kinase (Akt) is a downstream effector of PI3K. There are three enzymatic isoforms of Akt (also known as protein kinase B, PKB): *Akt1* (*locus* 14q32), *Akt2* (*locus* 19q13), *Akt3* (*locus* 1q43).

Phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*) is a tumour suppressor gene, localized at chromosome 10q23, which encodes protein-lipid phosphatase, terminating the signalling of the PI3K pathway by dephosphorylation of PtdIns-3,4,5-P$_3$. Phosphatase and tensin homologue deleted on chromosome 10 is very often mutated in human cancers and LOH (loss of heterozygosity) at the *PTEN locus* occurs frequently in both primary and metastatic tumours. In addition, germ line mutations of *PTEN* are associated with hereditary cancer predisposition syndromes, such as Cowden's disease (CD), characterized by the development of benign hamartomas in multiple organs, as well as by an increased risk of development of breast and thyroid cancer, most frequently follicular thyroid cancer [10].

When the PI3K complex is activated by signals from RTK, then, upon activation, PIK3CA phosphorylates PtdIns-4,5-P$_2$, forming the secondary messenger PtdIns-3,4,5-P$_3$. A reverse reaction is catalyzed by PTEN. PtdIns-3,4,5-P$_3$ acts as an anchor which, *via* Plekstrin Homology domain (PH), binds Akt and localizes this enzyme closely to the cell membrane, where it becomes activated by the phosphoinositide-dependent kinase (PDK1). The activated Akt phosphorylates downstream protein effectors and amplifies the signalling cascade, enhancing cell proliferation and survival (Figure 1).

## Phosphoinositide 3-kinase and human cancer

The process of carcinogenesis is permanently one of the most interesting and significant issues for researchers in different fields of medicine. The conservative PI3K pathway has been found mutated or deregulated in a broad variety of human cancers. The mechanism of pathological activation of the PI3K pathway includes amplification and mutations of the gene encoding the catalytic subunit of *PIK3CA* and activating mutations in
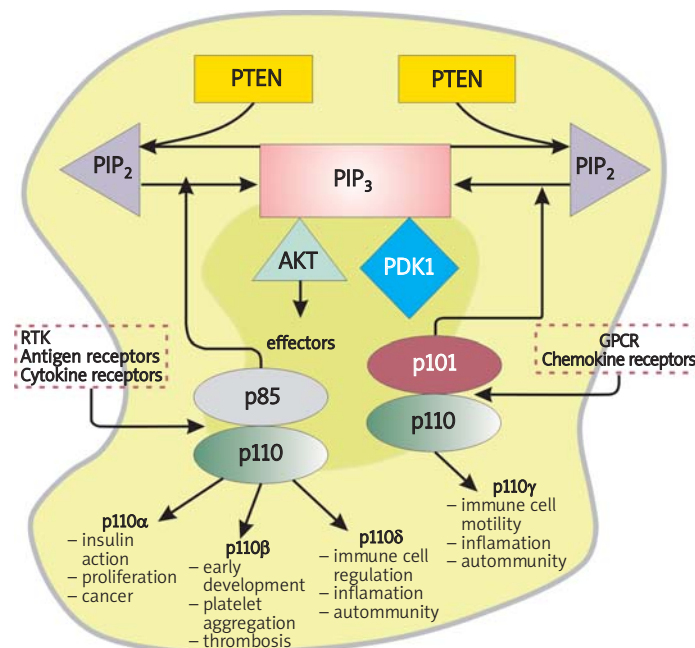


**Figure 1.** Model of PI3K activation. Explanations of abbreviations – see text of the paper

the regulatory subunit of class Ia PI3K. Gene *PIK3CA* is located on chromosome 3q26.3 and consists of 20 exons, encoding 1068 amino acids and yielding a 124 kDa size protein [9].

Eighty percent of the mutations in the *PIK3CA* gene are located in three regions within the helical (exon 9) and catalytic (exon 20) and p85 binding (exon 1 and 2) domains [11]. Furthermore, with the exception of three (3) deletions, two (2) insertions and one (1) complex in-frame deletion, almost all the mutations in *PIK3CA* are missense mutations, resulting from the change of a single nucleotide and leading to amino acid substitution in *PIK3CA* protein (http://www.sanger.ac.uk/genetics/CGP/cosmic/). The location of the mutations induces one of several distinct mechanisms [12], enhancing *PIK3CA* kinase activity and phosphorylation of Akt.

The highest percentage of activating somatic mutations of the *PIK3CA* gene have been identified particularly in breast cancer [13], colorectal cancers [11] and endometrium cancer [14], and also in other human neoplasms such as: glioblastoma [11], gastric cancer [11, 15], hepatocellular cancer [15], ovarian cancer [16] and lung cancer [11]. The incidence rates of these mutations indicate that *PIK3CA* is one of the two most commonly mutated genes identified in human cancers (the other being *KRAS*).

The *PIK3CA* gene is also frequently amplified in several human cancers, for instance in ovarian [17] and cervical cancers [18]. *PIK3CA* amplification has also been shown in non-small cell lung cancer [19], squamous cell carcinoma [20], oesophageal adenocarcinoma [21] and gastric carcinoma [22]. Increased copy number is associated with increased cell growth and decreased apoptosis. *PIK3CA* copy gain was correlated with increased Akt phosphorylation and activity and with tumour progression in the lung [19].

Somatic mutations in the p85 regulatory subunit, leading to constitutive activation of the catalytic subunit, have been observed in ovarian and colon tumours [23].

The above-mentioned results of numerous studies have established the role of *PIK3CA* as an oncogene in human cancers. PI3K is also thought to be implicated in the metastatic phenotype and promote carcinoma invasion. This phenotype appears to be independent of kinase Akt activation.

The evidence for the role of isoforms other than PIK3CA in carcinogenesis is less compelling. The PIK3CB isoform is overexpressed in colon and bladder carcinoma [24]. Some authors have reported that PI3KCG may have some role in the development and progression of chronic myeloid leukaemia [25] and elevated expression of PIK3CD has been noticed in glioblastoma [26] and acute myeloid leukaemia [27].

## Phosphoinositide 3-kinase and thyroid gland

Thyroid carcinoma is the most frequently observed neoplasm of the 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 thyroid carcinoma (DTC) of papillary or follicular type (PTC – papillary thyroid carcinoma, FTC – follicular thyroid carcinoma). Growth factor receptors with tyrosine kinase activity, such as the receptors for IGF-I (insulin growth factor-I) and TGF-$\alpha$ (transforming growth factor-$\alpha$) are often expressed at increased levels in thyroid neoplasms; in addition, autoendocrine production of IGF-1 and TGF-$\alpha$ is found, suggesting that these growth factors are associated with tumour progression. Increased signalling through these receptors plays a crucial role in thyroid cancer development. One of the major downstream mediators of signalling, initiated by the aforesaid receptors, is the PI3K pathway. These facts could explain the increased activation of the PI3K pathway in thyroid malignancies. Therefore, we have attempted to review – with particular attention – the contribution of the PI3K pathway in neoplastic transformation in the thyroid gland.

Previous studies have shown increased activities of the PI3K pathway in sporadic thyroid cancer [28-32] (Table I). Similarly to other human cancers, the majority of *PIK3CA* gene mutations in thyroid

**Table I.** PIK3CA alterations in thyroid neoplasms

| | FTA | | FTC | | PTC | | ATC | |
|---|---|---|---|---|---|---|---|---|
| | Copy gain [%] | Mutations [%] | Copy gain [%] | Mutations [%] | Copy gain [%] | Mutations [%] | Copy gain [%] | Mutations [%] |
| Garcia-Rostan *et al.* [28] | – | – | – | 8 | – | 2 | – | 23 |
| Wu *et al.* [29] | 12 | – | 24 | – | 5 | – | – | – |
| Hou *et al.* [30] | 17 | 6 | 28 | 6 | 12 | 3 | 42 | 12 |
| Wang *et al.* [31] | 8 | 0 | 29 | 13 | 14 | 1 | – | – |
| Abubaker *et al.* [32] | – | – | – | – | 53.1 | 1.9 | – | – |

*Copy gain has been defined as ≥ 4 copies*

neoplasm have been reported in exon 9 (helical domain) and 20 (kinase domain) [30]. Mutations in *PIK3CA* have been more commonly observed in FTC and ATC (anaplastic thyroid carcinoma) while being rather uncommon in PTC [28, 30, 31].

There is also a high prevalence of *PIK3CA* copy gain, especially in ATC (42%) and FTC (28%), which may be an explanation of the progression of FTC to ATC [30]. Additionally, *PIK3CA* mutations have been observed in undifferentiated parts of tumour tissue of insular carcinoma [28].

*PIK3CA* amplification has also been reported in some FTAs (follicular thyroid adenomas) as well as in thyroid cell lines [29, 30]. These findings are of potential practical interest and might highlight the important role of *PIK3CA* amplification also as a tumour initiating event in the development of thyroid tumours [29, 30].

Very interesting are the results of the recent study by Abubaker *et al.* [32], in which the authors conclude that ethnic differences probably underlie the high incidence of *PIK3CA* amplification in PTC among the Middle East population. *PIK3CA* amplification was seen in 53.1% of analyzed PTC cases and mutation (exon 9 and 20) of the *PIK3CA* gene in almost 2% of PTC cases [32]. Abubaker *et al.* [32] showed that Akt kinase was activated even more frequently in PTC, regardless of the presence of *PIK3CA* alterations. That observation suggests that other common oncogenes, including *RAS* [33], *cMET* [34] and mutations [35] or rearrangements [36] of the *RET* (rearranged during transfection) gene (*RET*/PTC oncogenes), may be engaged in Akt activation.

An early study demonstrated Akt activation in Cowden's syndrome *via* inactivation of *PTEN*, characterized by benign and malignant thyroid tumours, breast cancer and colon cancer [10]. More recently, increased activation of Akt has also been observed in sporadic thyroid carcinoma, especially in FTC [37], but in PTC as well [38]. Other studies have demonstrated activation of Akt in 10/10 FTC, 26/26 PTC and 2/10 follicular variants of PTC but only in 4/66 normal tissue samples and 2/10 FTA [39]. The presence of Akt in normal thyroid tissue and in FTA has been rather rare; however, in some cases, Akt activation could be observed in the nuclear compartment of the cells in some regions of FTA and even in normal thyroid tissue, similarly to that in FTC. This fact suggests that the distribution of Akt could be an indicator for pre-malignant cells. Furthermore, the high incidence of Akt activation observed in PTC may be related not only to activation of the PI3K pathway through PI3K aberrations, but also to other genetic alterations, such as *BRAF*, *RAS*, *MET* mutations, and *RET/PTC* rearrangements, which are more often observed in PTC.

The *PIK3CA* copy gain was uncommonly over-lapped with *PIK3CA* gene mutations (with some single exceptions of FTA, FTC and ATC, where coexistence of the two alterations was observed) [30, 31]. This provides genetic evidence that *PIK3CA* copy gain possesses similar oncogenic function as a classical gene mutation in the pathway in question.

In addition, mutual exclusivity among *PIK3CA* alterations and *RAS* mutations, and *PTEN* mutations was seen in FTA, FTC and PTC, thus suggesting an independent role of each of them in carcinogenesis [30]. However, in ATC, coexistence of those genetic alterations was observed, which indicates that the alterations all together play an important role in the transformation to ATC [30].

Conversely, the coexistence of alterations of the PI3K pathway and *BRAF* mutation was rather frequent in PTC and ATC, suggesting that the RAS/BRAF/MAPK pathway and PI3K pathway play jointly an important role in thyroid carcinogenesis [40]. Numerous *BRAF*-mutated PTC cases have shown coexisting *PIK3CA* amplification. Interestingly enough, in some cases, the authors have also observed coexistence of *RAS* mutation and *PIK3CA* amplification [32], which was not observed in previous studies [30, 31].

Wu *et al.* [29] found a strong association of *PIK3CA* copy gain with high-risk clinico-pathological features in thyroid cancer and suggested a possible role of this genetic alteration in thyroid tumour progression. Furthermore, oncogenic activation of *PIK3CA* in thyroid cancer has been primarily associated with ATC, which represents the end point of tumour progression in the multistage genetic model of thyroid carcino-genesis [28].

Additionally, PI3K involvement in the regulation of cell motility [41] and tumour cell invasion has been documented. PI3K activation has shown an important role in the spread of thyroid cancer cells, both locally and distantly. Accordingly, it has recently been shown that the activation of Akt is more intensive in the invasive regions of thyroid carcinomas and in the corresponding lymph nodes or distant metastases [39]. Vasko *et al.* [39] have also reported a difference in intracellular distribution of Akt activation between FTC and PTC. In FTC, phosphorylated Akt is localized primarily in the nucleus; conversely, in PTC, it has been localized in the cytoplasm, except for invasive and metastatic regions, where it is expressed in both compartments [39]. The results of the present study may indicate that activation of the PI3K pathway can enhance the invasion of cancer cells. This may also be an explanation for the differences in metastatic phenotypes between PTC and FTC.

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## Phosphoinositide 3-kinase and autoimmune disease of the thyroid gland

There is more and more evidence for the essential role of class I PI3K in the regulation of the immune system. It has been observed that PIK3CD inactivation leads to impairment of antigen receptor signalling in B and T cells and to attenuation of immune responses [3]. Furthermore, it has been shown that PIK3CG deficiency in neutrophils leads to defects in migration and respiratory burst in response to GPCR agonist and chemotactic agents. Additionally, PIK3CG, through GPCR stimulation, and following Akt activation, regulates thymocyte development, T cell activation, neutrophil migration and the oxidative burst [4]. PIK3CG is also employed in the chemo-attractant-induced response and in the production of T-cell-independent antigen-specific antibodies, composed of immunoglobulin λ light chain (TI-Igλ1) [42]. Summing up, the contribution of these two isoforms of class Ia PI3K in the inflammatory process is undeniable.

The most frequent inflammation in the thyroid gland is Hashimoto's thyroiditis (HT), which is an autoimmune disease characterized by infiltration of thyroid tissues by lymphocytes which replace its parenchyma *via* lymphoid tissue, leading to hypothyroidism. The recent study by Larson *et al.* [43] showed increased activity of phosphorylated Akt1 and Akt2 (as markers of PI3K activity) in HT and its connection with increased incidence of well-differentiated thyroid cancer in HT patients. These results indicate that deregulation of the PI3K pathway in HT could predispose to neoplastic transformation in the thyroid gland [44]. However, the PI3K pathway also has an important role in the inflammatory response, and the high activity of Akt1 and Akt2 can be related to this process, as well. Also it is worth mentioning that there have been no studies examining the expression of genes of the PI3K pathway in patients with HT so far, and the high expression of Akt in PTC and in HT may be related to the activation not only by PI3K aberrations but also by other genetic alterations such as *BRAF*, *RAS*, *MET* mutation and *RET/PTC* rearrangements. Therefore, further studies should be attempted, searching for gene expression of *PIK3CA* in HT to confirm this observation.

## Phosphoinositide 3-kinase and target therapy

PI3K may be considered to be a potential drug target in therapy of cancer and inflammatory diseases because progression of these conditions comprises similar mechanisms: migration, survival, proliferation and differentiation.

The mutant PI3K cancer target appears to be an important goal for molecular biologists and medical doctors. *PIK3CA* mutations enhance its function, which is much easier to control, compared to function loss. The biggest barrier to obtaining mutant specificity to PIK3CA is the unsatisfactory knowledge of the molecular pathomechanism which brings about an increase in its activity. Furthermore, class I PI3K is composed of hetero-dimers, which are very important in various normal physiological processes; therefore, a broad spectrum inhibitor of PI3K can be accompanied by a lot of side effects and poor tolerance. Thus, this task requires further scientific and technical investigations, according to the results of clinical studies [45].

## Conclusions

The PI3K pathway is a significant regulator of cellular growth, transformation, adhesion, survival and motility. Amplifications and mutations in the *PIK3CA* gene have been reported in many human cancer types, including thyroid cancer. Alterations of *PIK3CA* have proven to increase kinase activity of PIK3CA, contributing to cellular transformation.

Furthermore, the participation of PIK3CA alterations in development and progression of thyroid tumours has been observed. In addition, recent data suggest that the PI3K pathway also regulates cell motility and tumour cell invasion in thyroid cancer. Therefore, better understanding of pathomechanisms of these events, together with progress in PI3K pathway inhibitor research and development, might bring benefits in diagnosis and treatment of thyroid tumours.

Additionally, increased Akt expression in HT and in HT within thyroid cancer may indicate a pathogenic relationship between these two conditions. Further research on the molecular background will clarify whether there is any association between HT and thyroid cancer.

## Acknowledgments

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