Genetic Alterations in the Phosphatidylinositol-3 Kinase/Akt Pathway in Thyroid Cancer

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Background: Aberrant activation of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway plays a fundamental role in thyroid tumorigenesis, particularly in follicular thyroid cancer (FTC) and aggressive thyroid cancer, such as anaplastic thyroid cancer (ATC). As the drivers of this process, many genetic alterations activating the PI3K/Akt pathway have been identified in thyroid cancer in recent years.

Summary: This review summarizes the current knowledge on major genetic alterations in the PI3K/Akt pathway. These include *PIK3CA* mutations and genomic amplification/copy gain, *Ras* mutations, *PTEN* mutations, *RET/PTC* and *PPAR γ /Pax8* rearrangements, as well as amplification/copy gain of *PIK3CB*, *PDK1*, *Akt*, and various receptor tyrosine kinase genes. Most of these genetic alterations are particularly common in FTC and many of them are even more common in ATC; they are generally less common in papillary thyroid cancer (PTC), in which the MAP kinase (MAPK) pathway activated by the *BRAF* mutation instead plays a major role. Methylation and, thus, epigenetic silencing of *PTEN*, a major negative regulator of the PI3K/Akt pathway, occurs in close association with activating genetic alterations of the PI3K/Akt pathway, constituting a unique self-enhancement mechanism for this pathway. Many of these genetic alterations are mutually exclusive in differentiated thyroid tumors, but with increasing concurrence from benign tumors to FTC to ATC. *RET/PTC*, *Ras*, and receptor tyrosine kinase could dually activate the PI3K/Akt and MAPK pathways. Most cases of ATC harbor genetic alterations in these genes or other genetic combinations that can activate both pathways. It is proposed that genetic alterations in the PI3K/Akt pathway promote thyroid cell transformation to FTC and that genetic alterations in the MAPK pathway promote cell transformation to PTC; accumulation of multiple genetic alterations that can activate both pathways promotes thyroid cancer aggressiveness and progression to ATC.

Conclusions: Genetic alterations are common in the PI3K/Akt pathway in thyroid cancer and play a fundamental role in the tumorigenesis and progression of this cancer. This provides a strong basis for the emerging development of novel genetic-based diagnostic, prognostic, and therapeutic strategies for thyroid cancer.

Introduction

AS IN MANY OTHER human cancers, genetic alterations are the driving force for the tumorigenesis and pathogenesis of thyroid cancer, particularly those that occur in genes encoding for key players of major signaling pathways. Follicular epithelial cell-derived thyroid cancer accounts for >95% of all thyroid malignancies and can be divided histologically into papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and anaplastic thyroid cancer (ATC). PTC can be further divided into several subtypes: conventional variant PTC (CPTC), follicular variant PTC (FVPTC), tall cell PTC

(TCPTC), and a few other rare types. There is also poorly differentiated thyroid cancer (PDTC) that has a prognostic risk between DTC (including PTC and FTC) and ATC. The genetic background varies with these different types of thyroid cancers, and certain molecular signaling pathways are consequently preferentially altered in them. A prominent example of such genetic alterations is the oncogenic *BRAF* mutation in the *Ras* → *Raf* → *MEK* → *ERK*/MAP kinase pathway (MAPK pathway), which occurs preferentially and commonly in PTC, particularly CPTC and TCPTC, and some ATC (1,2). Other classical oncogenic genetic alterations in the MAPK pathway in thyroid tumorigenesis include *RET/PTC*

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and *Ras* mutations (3–6). In recent years, the importance of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway in thyroid tumorigenesis has been also widely recognized (7). Recent studies have particularly explored the genetic backgrounds for this role of the PI3K/Akt pathway. As a result, many important genetic alterations have been identified in this pathway in recent years, providing insights into the molecular mechanisms in thyroid tumorigenesis and bases for the development of novel genetic-based strategies for the management of thyroid cancer.

Signaling of the PI3K/Akt Pathway

The PI3K/Akt signaling pathway has long been known to play a fundamental role in the regulation of various cellular and molecular functions, including glucose uptake, cell growth and proliferation, cell motility, and survival. In the last 15 years, the role of this signaling pathway in human tumorigenesis and its value as a therapeutic target in human cancers have been extensively investigated (8–11). There are several classes of PI3Ks, among which class I is the best characterized and most important in human tumorigenesis. It consists of heterodimers of a regulatory subunit, commonly the p85 unit, and one of p110 catalytic subunits. Among the p110 subunits, the α -type (PIK3CA) and β -type (PIK3CB) are widely expressed in different tissues and play a particularly important role in human cancers. PIK3CA and PIK3CB belong to class IA that is activated by receptor tyrosine kinases (RTK) as well as some G-protein-coupled receptors. These p110 catalytic subunits contain a binding site for the regulatory subunit, through which signals can be integrated from membrane receptors and activate the catalytic subunits. Ras is a classical player in the MAPK pathway, but it also plays an important role in the signaling of the PI3K/Akt pathway. One mechanism for this function of Ras is through its interaction with the Ras-binding site in the p110 catalytic subunits of PI3K. Thus, Ras can dually activate the MAPK and PI3K/Akt pathways. As illustrated in Figure 1, a typical starting point of the PI3K/Akt pathway signaling is the various membrane growth factor receptors that contain RTK, including vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), c-KIT, and c-MET. Activation of RTK by extracellular signals leads to the activation of the PI3K with resultant activated p110 catalytic subunit (e.g., PIK3CA), which in turn phosphorylates phosphatidylinositol-4,5-bisphosphate to produce phosphatidylinositol-3,4,5-trisphosphate (PIP₃). The function of PIP₃ in this signaling relay is to localize Akt to the cell membrane where Akt becomes phosphorylated and activated by the phosphoinositide-dependent kinases (PDK), most prominently PDK1. Akt is a Ser/Thr kinase and three types of its isoforms are found in human tissues—Akt-1, Akt-2, and Akt-3 (12). Activated Akt phosphorylates downstream protein effectors, among which is the mammalian target of rapamycin (mTOR), which has a well-established role in human cancers and, like Akt, is a prominent therapeutic target in today's cancer therapy designing (13). The amplified signaling cascade regulates a wide range of molecular and cellular functions, promotes cell proliferation, and inhibits cell apoptosis. A prominent negative regulator of the PI3K/Akt pathway signaling is the

FIG. 1. Schematic illustration of the highly simplified PI3K/Akt and MAP kinase pathways in thyroid cancer. Shown are the major elements in the two pathways. RTK, activated by growth factors, RET/PTC, and Ras can be coupled to both pathways. The remaining components regulate their respective pathways as described in the text. PTEN is a negative regulator of the PI3K/Akt pathway signaling. PI3K, phosphatidylinositol-3 kinase; RTK, receptor tyrosine kinase.

protein product of the gene for phosphatase and tensin homolog deleted on chromosome ten (PTEN). PTEN is a phosphatase that dephosphorylates PIP₃ and thus terminates the signaling of the PI3K/Akt pathway (14,15). Given the antagonizing function of PTEN against the PI3K/Akt signaling, PTEN is a major human tumor suppressor and its genetic alterations, like those of other genes in the PI3K/Akt pathway, have serious consequences. Shown in Figure 1 are the key players in the molecular interplay in the PI3K/Akt pathway signaling that have been best characterized for their genetic alterations in thyroid cancer as will be each discussed in this review.

Mutations and Genomic Amplifications/Copy Gain of the *PIK3CA* Gene in Thyroid Cancer

Genetic alterations of the *PIK3CA* gene have been widely reported in human cancer. Activating mutations in the *PIK3CA* gene were initially found in colorectal, gastric, breast, and ovarian cancers, and high-grade brain tumors, supporting the important oncogenic role of this gene in human tumorigenesis (16). *PIK3CA* mutation was subsequently explored in various differentiated thyroid tumors but found

to be uncommon in them (17). In a subsequent extended study, *PIK3CA* mutations in exons 9 and 20 were found to occur in 23% of ATC (18). *PIK3CA* mutations were also found in several more recent studies in FTC and ATC with lower frequencies, around 10%–15% (19–23). Similarly to the occurrence of *BRAF* mutation in PTC-derived metastases (24,25), *PIK3CA* mutation can occur in some cases of metastases derived from aggressive cancers, such as PDTC and ATC (23), consistent with the general distribution patterns of the two mutations in primary thyroid tumors. Overall, *PIK3CA* mutation can occur, particularly in PDTC and ATC and their recurrent/metastatic disease, but does not seem to be a major genetic event in thyroid cancer in general.

Amplification/genomic copy gain of the *PIK3CA* gene as an important oncogenic event is common in human cancers, including ovarian cancer in which *PIK3CA* amplification was initially discovered (26). Lack of common *PIK3CA* mutations in thyroid cancer, particularly DTC, promoted search for genetic amplification or copy gain of this gene in thyroid cancer. This led to the initial discovery of genetic copy gain of the *PIK3CA* gene with a high prevalence in thyroid cancer, particularly FTC and ATC, being 24% and 42% in the former and the latter, respectively (17,27). This common occurrence of *PIK3CA* copy gain in thyroid cancer was confirmed in several subsequent studies in different ethnic populations (19–22,28). Fluorescent *in situ* hybridization studies demonstrated that this *PIK3CA* copy gain in thyroid cancer represented mainly genomic amplification of the *PIK3CA* gene (17,20). Several previous studies showed that the *PIK3CA* amplification was associated with overexpression of the PIK3CA protein (19) and phosphorylation of Akt (17,22), suggesting that this genetic alteration of the *PIK3CA* gene is functional and represents a relevant mechanism for the activation of the PI3K/Akt pathway in thyroid cancer. Association of *PIK3CA* amplification with increased PIK3CA protein expression and kinase activity or Akt phosphorylation was documented in many other human cancers, such as ovarian and uterine cervical cancers (26,29,30). In the Asian and American populations, *PIK3CA* amplification in thyroid cancer occurred mainly in FTC and ATC and uncommonly in PTC (17,19, 21,22,27,28), which is consistent with the notion that the PI3K/Akt pathway plays a major role in the tumorigenesis of FTC and ATC, as opposed to the MAPK pathway that plays a major role in the tumorigenesis of PTC (5). In a Middle East population, *PIK3CA* amplification was found to be common also in PTC by both quantitative real-time polymerase chain reaction and fluorescent *in situ* hybridization analyses (20). The reason for this difference is not clear. *PIK3CA* amplifications could occur in benign thyroid adenoma (BTA), although with a low prevalence (17,19,28), suggesting a possible early role of this genetic alteration in thyroid tumorigenesis. Aberrant genomic copy number has long been known to be associated with transition from precancerous epithelial lesions to cancers (31). *PIK3CA* amplification represents such a genomic copy gain, as exemplified by its association with progression of dysplasia into invasive squamous cell carcinoma (32). A recent study showed that *PIK3CA* amplification was strongly associated with distant metastasis, lymph node metastasis, advanced tumor stage of nasopharyngeal carcinoma, and decreased patient survival (33). The occurrence of *PIK3CA* amplification in some cases of BTA raises the question whether these benign thyroid tumors could progress into

malignancy if given sufficient time. There was a clear and progressive increase in the prevalence of *PIK3CA* amplifications from BTA to FTC and to ATC (19,28), which is consistent with a role of this genetic alteration in promoting the aggressiveness and progression of thyroid cancer.

Common *Ras* Mutations in Follicular Thyroid Tumors

Mutations in the three types of *Ras* genes—*H-Ras*, *K-Ras*, and *N-Ras*—are commonly seen in human cancers with a frequency, on average, of 30% (34,35). *Ras* mutations are also common in thyroid tumors, particularly in FVPTC (36), FTC (19,22,28,37,38), and PDTC (23,39), with a prevalence around 20%–40% in most series. *Ras* mutations are reported in PTC with a varying and generally low prevalence (40), reflecting varying compositions of FVPTC, CPTC, and TCPTC, with the latter two rarely harboring *Ras* mutations. *Ras* mutations are also frequently seen in BTA, with a collective prevalence of about 20% in a meta-analysis (37) and 26% in a recent large study (38). It is unclear whether *Ras* mutations with physiological expression in BTA are oncogenic and can convert the benign tumor into thyroid malignancy. In fact, when introduced into normal thyroid epithelial cells, an H-Ras mutant induced only well-demarcated and differentiated colonies with phenotype consistent with follicular adenoma (41) and cell proliferation without loss of differentiation (42). In a recent transgenic mouse study, conditional physiological expression of a K-Ras mutant alone in the thyroid gland failed to transform thyroid cells to thyroid cancer even after 1 year (43). It appears that additional genetic alterations are required to transform thyroid cells but *Ras* mutation alone, with physiological expression, cannot. Indeed, concurrent mutated *Ras* gene expression and *PTEN* deletion resulted in rapid development of aggressive FTC and animal death within weeks in transgenic mouse studies (43).

Ras is widely known for its classical role in the MAPK pathway signaling through interacting with the Raf kinase. As discussed above, *Ras* can also regulate the PI3K/Akt pathway by directly interacting with the Ras-binding site of p110 catalytic subunits of PI3K (44,45). *Ras* mutants could activate the PI3K/Akt pathway, which also required direct interaction with the Ras-binding site on the p110 catalytic subunits of PI3K, such as PIK3CA (46). Interestingly, in colon cancer, K-Ras mutant preferentially activated the MAPK pathway, whereas N-Ras mutant did not (47). It is not clear whether different types of *Ras* mutations exert different oncogenic roles by preferentially activating different signaling pathways in thyroid tumorigenesis. The most common type of *Ras* mutations in thyroid cancer is the *N-Ras* mutation in codon 61, with *H-* and *K-Ras* mutations, in either codon 12/13 or codon 61, being much less common. This is the pattern observed by many groups (19,22,23,28,36–39), except for ones that showed that *K-Ras* mutation was the most common (48,49). The fact that *Ras* mutations occur particularly in FTC and FVPTC in which aberrant PI3K/Akt signaling is a major signaling abnormality whereas they rarely occur in CPTC and TCPTC in which the *BRAF* mutation-activated MAPK signaling dominates (1,2) is consistent with the expectation that *Ras* mutations are major activators of the PI3K/Akt pathway in thyroid tumors, particularly follicular tumors. Some studies demonstrated a good association of *Ras* mutations with the phosphorylation of Akt in thyroid cancer (20,22), consistent

with an important role of *Ras* mutations in the activation of the PI3K/Akt pathway in this cancer.

Genetic and Epigenetic Alterations of the *PTEN* Gene in Thyroid Cancer

PTEN mutations are relatively uncommon in sporadic human cancers. Inactivating germline mutations of this gene are the main cause of Cowden syndrome (50), which is characterized with a propensity of patients to develop tumors, including thyroid tumors, particularly follicular thyroid tumors, including FTC. As discussed above, as a lipid phosphatase that degrades PIP_3 , *PTEN* normally antagonizes and terminates the signaling of the PI3K/Akt pathway. Given the importance of this negative regulation of the PI3K/Akt pathway by *PTEN*, Cowden syndrome provides a unique human model illustrating the oncogenic power of aberrant PI3K/Akt signaling in the tumorigenesis of follicular thyroid tumors. Somatic *PTEN* mutations also occur in thyroid cancers, particularly in FTC and ATC, although with a relatively low prevalence (19,22,28). Loss of heterozygosity or deletion of the *PTEN* gene is relatively common in thyroid tumors (51,52). The real extent of genetic abnormalities of the *PTEN* gene in sporadic thyroid cancer likely exceeds what is currently seen as multiple alteration sites of the gene exist, some of which may be missed in relatively limited genetic analyses in most studies.

An epigenetic inactivating mechanism through aberrant methylation of the *PTEN* gene also exists in various thyroid tumors, particularly FTC and ATC (53–55). When quantitatively analyzed using quantitative real-time methylation polymerase chain reaction, *PTEN* methylation was found to be progressively and steadily increased from BTA to FTC and to ATC (55). Methylation of a gene is usually associated with its silencing (56). Therefore, methylation of *PTEN* in thyroid tumors, particularly in FTC and ATC, which showed the highest extent of this methylation (55), is consistent with the previously reported decreased or even absent *PTEN* expression in a subset of these cancers (57,58). Interestingly, *PTEN* methylation in thyroid tumors was found to be closely associated with classical activating genetic alterations in the PI3K/Akt pathway, including *Ras* mutation, *PIK3CA* mutation, *PIK3CA* amplification, and mutations of the *PTEN* gene itself in thyroid tumors (55). This is consistent with a previously proposed mechanistic model for the regulation of the PI3K/Akt pathway in thyroid tumors (55), as illustrated in Figure 2. In this model, activation of the PI3K/Akt pathway by its genetic alterations may activate the methylating system of the cell, which, in turn, causes hypermethylation and hence epigenetic silencing of the *PTEN* gene, effectively removing the terminating mechanism of the PI3K/Akt signaling. This represents a unique self-enhancing mechanism of the PI3K/Akt pathway. This mechanism may be particularly relevant in ATC as genetic alterations of the PI3K/Akt pathway and, correspondingly, *PTEN* methylation were shown to be particularly common in this aggressive cancer (55). The potentially and conceivably malicious nature of such a self-enhancing mechanism for the activation of the PI3K/Akt pathway signaling may contribute to the pathogenesis and aggressiveness of ATC. Thus, the oncogenic power of loss of *PTEN* seems to lie in its cooperation with other genetic alterations of the PI3K/Akt pathway in the tumorigenesis of thyroid cancer. This mechanism is consistent with the recent findings in a transgenic mouse model in which deletion of *PTEN*

FIG. 2. Diagrammatic illustration of a self-enhancement mechanism of the activation of PI3K/Akt pathway through *PTEN* methylation in thyroid cancer. In this model, activating genetic alterations, including gene copy gain or mutations in the PI3K/Akt pathway activate the pathway. This in turn leads to *PTEN* methylation, resulting in silencing of the *PTEN* gene. Loss of *PTEN* removes the inhibitory pressure of the pathway, forming a malicious self-activation cycle for the PI3K/Akt pathway signaling. This is seen most commonly in aggressive thyroid cancers, such as anaplastic thyroid cancer (ATC), as discussed in the text.

or physiological expression of a *Ras* mutant each alone did not cause thyroid cancer, but simultaneous introduction of the two genetic events caused aggressive thyroid cancer (43).

RET/PTC as an Activator of the PI3K/Akt Pathway in Thyroid Tumors

RET/PTC represents a recombinant protein product from a chromosomal rearrangement with the combination of the 3' portion of the *RET* gene and the 5' portion of a partner gene (3,59). This recombination results in constitutive activation of the tyrosine kinase in *RET*. Among the more than 10 types of *RET/PTC*, which are mainly found in thyroid cancer, the most common and important types are *RET/PTC1* and *RET/PTC3*. *RET/PTC* is an established oncogenic activator of the MAPK pathway. Several studies have suggested that *RET/PTC* is also involved in the signaling of the PI3K/Akt pathway. For example, transfection of thyroid cells with *RET/PTC3* could increase Akt phosphorylation and PI3K/Akt signaling (60,61). *RET* tyrosine kinase could activate the PI3K/Akt pathway through binding of several proteins, including the p85 regulatory subunit of PI3K, to tyrosine 1062 of *RET* (62). The transforming ability of a mutant *RET* of MEN2A, which, like *RET/PTC*, had constitutive activation of the *RET* tyrosine kinase, required binding of the p85 regulatory subunit of PI3K to tyrosine 1062 of *RET* to activate PI3K (63). It is conceivable that *RET/PTC* activates the PI3K/Akt pathway through a similar mechanism. Other mechanisms for the involvement of *RET/PTC* in the signaling of the PI3K/Akt pathway may also involve direct phosphorylation and activation of PDK1 (64) and Akt (65) by *RET/PTC*. Therefore, *RET/PTC* is an important player in the deranged signaling of the PI3K/Akt pathway in thyroid tumorigenesis. This may be a particularly important mechanism in PTC of pediatric patients as most of these patients harbor *RET/PTC* (1,3,59). *RET/PTC* occurs in about 15%–20% of adult PTC patients and, presumably, also plays a role in the activation of the PI3K/Akt pathway in these patients. *RET/PTC* is generally found to be mutually exclusive with *BRAF* mutation in PTC (1). This is thought to be evidence

supporting the notion that either of the two genetic alterations can sufficiently and oncogenically activate the MAPK pathway. However, some studies did also show coexistence of *RET/PTC* and *BRAF* mutation in PTC and ATC (22,66–68). Coexistence of *RET/PTC* and *BRAF* mutation was found to be more common in recurrent PTC, suggesting that coactivation of the MAPK and PI3K/Akt pathway by coexistence of the two genetic alterations may have a special role in thyroid cancer aggressiveness as will be further discussed in the following section. Many studies also showed that *RET/PTC* could occur, with high prevalences, in benign thyroid tumors (69–71) and Hashimoto's thyroiditis (72–74). Hyalinizing trabecular tumors of the thyroid gland also frequently harbored *RET/PTC* (75), which is largely a benign thyroid tumor (76). Therefore, it appears that *RET/PTC* alone, under physiological expression, may not be sufficiently oncogenic and additional coexisting genetic alterations may be required for thyroid cell transformation.

Genetic Alterations in the RTK Genes and Other Genes in the PI3K/Akt Pathway in Thyroid Cancer

In addition to the several genes in the PI3K/Akt pathway discussed above, there are many other genes that are involved in the initiation or transduction of the signaling of the PI3K/Akt pathway. Genetic alterations of these genes have been reported with varying rates in human cancers (77,78). A recent study extensively investigated mutation and genomic copy gain of many of these genes in thyroid cancer, particularly FTC and ATC (22). These included RTK genes *EGFR*, *PDGFR α* and β , *VEGFR1* and 2, *c-MET*, and *c-KIT*, as well as *PDK1*, *Akt-1*, *Akt-2*, and *PIK3CB*. Interestingly, although mutations were not common in these genes examined on selected exons, genomic copy gain, likely representing genomic amplifications, was common in most of these genes in thyroid cancer, particularly in ATC, up to 40%–45% for *EGFR*, *PDGFR β* , and *VEGFR1*. Consistent with these results are the previous findings that many of these genes were overexpressed in thyroid cancer (79). Like the highly prevalent genetic copy gain of the *PIK3CA* found in this study and as discussed above, *PIK3CB* copy gain was also found to be common, with a prevalence of around 40% in both FTC and ATC. A relatively high prevalence of *PTEN* mutation of 17% was found in ATC. This study also analyzed *Ras* mutations, *RET/PTC*, and *BRAF* mutation and showed prevalences comparable to previous reports. Overall, virtually every case of FTC or ATC harbored at least one genetic alteration, and coexistence of two or more was seen in the majority of cases of ATC. Specifically, 93% of FTC and 96% of ATC harbored at least one genetic alteration, and coexistence of two or more was seen in 77% of ATC. Many of these genetic alterations were correlated with phosphorylation of Akt, suggesting that they functioned as activators of the PI3K/Akt pathway. This study provided a strong genetic basis for a fundamental and wide role of the aberrant PI3K/Akt pathway signaling in thyroid cancer, particularly FTC and ATC.

There are several other genetic alterations that can activate the PI3K/Akt pathway in thyroid tumors. One is the *PPAR γ /Pax8* rearrangement. This genetic event was initially identified in FTC (80) and latter also found in BTA (81). Through a dominant-negative inhibition mechanism, the protein product *PPAR γ /Pax8* can negatively affect Pax8. As the latter is

normally an upregulator of *PTEN* expression (82), *PPAR γ /Pax8* can thus indirectly suppress expression of *PTEN*, leading to the activation of the PI3K/Akt signaling. The preponderance of *PPAR γ /Pax8* in follicular tumors, in which the PI3K/Akt pathway signaling dominates as discussed above, is consistent with the role of this recombinant protein in PI3K/Akt signaling. A recent study showed that *Akt-1* mutation could also occur in thyroid cancer, but only in some cases of recurrent/metastatic cancers, suggesting that this is a relatively late genetic event during thyroid cancer progression (23). Interestingly, in a mouse model with a knock-in mutant *TR β* gene, the *TR β* mutant could directly interact with the p85 regulatory subunit of PI3K and activate the PI3K/Akt signaling in FTC that developed in these transgenic mice (83). This represents a novel mechanism for the activation of the PI3K/Akt pathway. It remains to be investigated whether this mechanism may occur in human thyroid cancer.

Accumulation of Genetic Alterations in the PI3K/Akt Pathway and Their Concurrence with Genetic Alterations in the MAPK Pathway in the Progression from Low-Grade to Aggressive Thyroid Tumors

Genetic alterations in the PI3K/Akt pathway have an interesting and oncogenically important distribution pattern in different thyroid cancer. Two studies on different ethnic populations demonstrated that the classical genetic alterations, including *PIK3CA* mutation and genetic copy gain, *Ras* mutations, and *PTEN* mutations, were mutually exclusive in DTCs, suggesting that any of these genetic alterations was a sufficient oncogenic event for the activation of the PI3K/Akt pathway in these thyroid cancers (19,28). These genetic alterations were far more common in FTC than in PTC, perfectly echoing hyperphosphorylation of Akt and over-activation of the PI3K/Akt pathway preferentially occurring in FTC over PTC (84). Coexistence of these genetic alterations was occasionally seen in differentiated thyroid tumors but occurred commonly in ATC (19,22). There is a clear rise in the prevalence of these genetic alterations, either individually or concurrently, from BTA to DTC and to ATC, reaching an extremely high prevalence in ATC (19,22). These genetic data strongly support a role of aberrantly activated PI3K/Akt pathway in the development of aggressiveness of thyroid cancer, consistent with the finding of increased Akt phosphorylation particularly in invasive areas of thyroid cancer (85).

Many of the genetic alterations found in ATC could potentially activate both the MAPK and PI3K/Akt pathways, including RTK gene copy gain, *RET/PTC*, and *Ras* mutations. *BRAF* mutation that can activate the MAPK pathway commonly coexisted with genetic alterations that could activate the PI3K/Akt pathway (e.g., the *PIK3CA* copy gain) in ATC. Genetic alterations or their combinations that could activate both the MAPK and PI3K/Akt pathways were found in up to 81% cases of ATC (22). Common coexistence of *BRAF* mutation with *RET/PTC*, *Ras* mutations, *PIK3CA* mutation and amplification, *Akt-1* mutation, and other genetic alterations that can activate the PI3K/Akt pathway have been recently also reported in other studies, mostly in aggressive cancers such as recurrent/metastatic cancers, PDTC, and ATC (20,23,49,68). In general, the PI3K/Akt pathway is commonly activated in FTC, whereas the MAPK pathway is commonly activated in PTC by their corresponding common genetic

alterations, and the two pathways are commonly dually activated in aggressive thyroid cancer, such as metastatic/recurrent cancers and ATC. This is consistent with a recently proposed model in which genetic alterations in the PI3K/Akt pathway and MAPK pathway promote transformation of follicular thyroid cells to FTC and PTC, respectively, and accumulation of these genetic alterations and their concurrence in both pathways drive the aggressiveness and progression of DTC into aggressive thyroid cancer, such as ATC (19). This model is illustrated in Figure 3.

Interestingly, this model seems to also be applicable to other human cancers, such as melanoma in which simultaneous activation of the PI3K/Akt pathway by silencing of PTEN and the MAPK pathway by expression of a BRAF mutant led to aggressive progression and metastasis of the tumor (86).

Clinical Implications

The genetic studies discussed above on the PI3K/Akt pathway in thyroid cancer have important diagnostic, prognostic, and therapeutic implications. Recent studies tested the diagnostic value of combinational use of several genetic alterations, including *Ras* mutation, *RET/PTC*, *PPAR γ /Pax8*, and *BRAF* mutation in the diagnostic evaluation of thyroid

FIG. 3. Schematic illustration of the PI3K/Akt and MAP kinase pathways driven by the increasing accumulation of genetic alterations in the progression from low-grade to high-grade thyroid cancers. In this model, primary genetic alterations in the MAP kinase pathway, such as the *BRAF* mutation, transform the thyroid cell into PTC. As the genetic alterations accumulate, particularly with those that can also activate the PI3K/Akt pathway, the cancer progresses into aggressive type, such as ATC. If primary genetic alterations, such as genetic or epigenetic silencing of *PTEN*, occur in the PI3K/Akt pathway, the thyroid cell is transformed into follicular thyroid tumors. As genetic alterations accumulate in this pathway, a follicular adenoma can progress into FTC when tumor invasion occurs, which can progress further into ATC when genetic alterations accumulate further, particularly if there are genetic alterations that can dually activate the PI3K/Akt and MAP kinase pathways, such as *Ras* mutations or genomic amplifications of the receptor tyrosine kinase genes. The increasing intensity of the gray tint in the circles represents increasing genetic alterations and the signaling pressure of the pathways in the progression from thyroid cells to benign thyroid tumors to differentiated thyroid cancers and to ATC. The figure is adapted from reference 19 with permission. FTC, follicular thyroid cancer.

nodules (38,87,88). This approach could increase the sensitivity of conventionally used diagnostic modalities for thyroid nodules and was thought to be particularly useful for thyroid nodules with indeterminate cytology. However, unlike *BRAF* mutation, which occurs only in thyroid cancer, *Ras* mutation, *RET/PTC*, and *PPAR γ /Pax8* can all also occur in BTA and even Hashimoto's thyroiditis with relatively high prevalences, although lower than cancer, as discussed in the previous sections. Consequently, the specificity of this diagnostic approach could be potentially problematic, and further studies are required to determine how this approach can be practically applied diagnostically. This is different than the prognostic molecular marker, *BRAF* mutation, which is associated with aggressiveness of PTC and can therefore be practically useful in guiding the risk stratification of PTC (1,2). In this sense, the combinational use of the mutation markers discussed above might be prognostically useful since *Ras* mutation, *RET/PTC*, and likely *PPAR γ /Pax8* predict a less aggressive course of thyroid cancer than the *BRAF* mutation (1,2).

Some of the genetic alterations in the PI3K/Akt pathway may also be prognostically useful in certain clinical settings. For example, *N-Ras* mutations were recently shown to be prevalent in PDTTC and predict an increased mortality of this cancer (39). As discussed above, *BRAF* mutation has been well known to be associated with aggressiveness of PTC (1,2). A recent study demonstrated that coexistence of *PIK3CA* amplification with *BRAF* mutation conferred PTC a particularly high risk for aggressiveness, including large tumor size, metastasis, and recurrence of PTC (20), raising the possibility that the combination pattern of the two genetic markers could identify a subgroup of *BRAF*-mutation-positive PTC with a particularly high risk for aggressiveness.

The current knowledge of genetic alterations in the PI3K/Akt pathway has also opened the possibility for development of specific genetic-based therapeutic strategies targeting the PI3K/Akt pathway in thyroid cancer. *BRAF*-mutation-dependent response of thyroid cancer cells to MEK and *BRAF* mutant inhibitors has been well-documented and represents a good example of genetic-based targeting of the MAPK pathway in thyroid cancer (89,90). Similar dependence of thyroid cancer cells on genetic alterations in the PI3K/Akt pathway in response to Akt and mTOR inhibitors has been demonstrated recently (91), providing the first evidence that effective genetic-based targeting of the PI3K/Akt pathway is possible with certain drugs for thyroid cancer. As dual involvement of the MAPK and PI3K/Akt pathways driven by their respective and concurrently existing or shared genetic alterations is a fundamental mechanism in the tumorigenesis of aggressive thyroid cancers, such as ATC, targeting both pathways may be needed for effective treatment of these cancers. The two recent studies demonstrating synergism of MEK and mTOR inhibitors in the inhibition of thyroid cancer cells support this idea (92,93). In fact, the presence of genetic alterations in the two pathways seemed to predict an even better synergism in simultaneously targeting the two pathways to treat thyroid cancer (93).

Concluding Remarks

Genetic alterations are common in the PI3K/Akt pathway, which is the basis for aberrant signaling of this pathway in

thyroid cancer. The recent identification of many activating genetic alterations, including mutations and genomic amplification/copy gain, in many key genes in this pathway represents a remarkable progress in our understanding of the oncogenic role of this pathway in thyroid tumorigenesis. Knowledge of the genotype of the PI3K/Akt pathway as well as that of the MAPK pathway will, and in fact, have already started to have an important impact on the development of novel diagnostic, prognostic, and therapeutic strategies for patients with thyroid cancer.

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Disclosure Statement

The author declares that no competing financial interests exist.

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