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Dysregulated Expression of Tensin 2 and Components of the PI3 Kinase/Akt Signaling Pathway in Human Thyroid Carcinoma

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Abstract

Background: The phosphatidylinositol 3-kinase/Akt signaling pathway is recognized as a key driver of cancer cell survival and proliferation, and is often contingent upon an impairment of expression/function of the PTEN tumor suppressor, a negative regulator of this pathway. In addition, the cytoskeletal signaling protein Tensin 2 has also been implicated as a negative regulator of this pathway. However, the PI3K pathway remains to be fully characterized in clinical thyroid carcinomas. The aim of this study is to determine the expression of components of the PI3K pathway in neoplastic and normal tissue sections obtained from patients with thyroid carcinoma.

Methods: Tissues from 58 cases with thyroid carcinoma underwent immunohistochemistry for activated Akt (phosphorylated Akt, pAkt), Tensin 2 and PTEN.

Results: A total of 100% of thyroid cancerous tissues were positive for pAkt staining compared to 67.9% of normal tissues. In contrast, 46.8% of cancer tissues were positive for Tensin 2 compared to 61.7% of normal tissues. For PTEN, 82.8% of cancerous tissues and 67.2% of normal tissues stained positive for this protein. There were no associations between the expression levels of the molecules with the patients' clinicopathological characteristics.

Conclusion: We have found evidence for an enhanced activation of the PI3K/Akt signaling pathway in clinical thyroid carcinoma tissues. This can be coupled with concomitant downregulation of Tensin 2. Further work is required to determine the relative significance of PTEN expression versus its activity in thyroid carcinoma in order to determine its role in the observed increased Akt activity.

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Introduction

Akt, also known as protein kinase B (PKB) or Akt1, is a protein kinase involved in cell proliferation and survival.[1] Akt is a key member of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway, which is well established in regulating cellular processes such as survival, growth and migration. In order to be phosphorylated (pAkt) and subsequently activated, Akt needs to be anchored to the cell membrane through interaction, via its pleckstrin homology (PH) domain, with phosphoinositide-3, 4, 5-triphosphate (PIP3) lipids in the inner membrane leaflet. PIP3 is produced by activated PI3K following phosphorylation of phosphoinositide-4, 5-biphosphate (PIP2).[1] The tumor suppressor gene PTEN is a phosphatase that acts in the opposite manner to PI3K by removing the phosphate in the D3 position of the inositol ring of PIP3, thereby inhibiting the PI3K/Akt signaling pathway. PTEN plays a significant role in inducing cell cycle arrest and apoptosis, as well as in other aspects of cell physiology.[2,3] Decreased PTEN expression results in poor control of G1 arrest, apoptosis, and/or cell-cell adhesion.[4-6] Loss of PTEN expression coupled with Akt activation/phosphorylation has been reported in many malignancies, including its association with poor prognosis in endometrial cancer.[7,8] Therefore, immunohistochemical evaluation of PTEN expression has shown value as a method of pathological screening.[9-12]

Tensin 2, also known as C1-TEN,[13] is an intracellular protein that belongs to the tensin family of focal adhesion proteins believed to link transmembrane proteins such as integrins to the cytoskeleton.[14,15] Tensin 2 has been shown to phenotypically influence cells in the same manner as PTEN, through inhibiting the Akt signaling pathway but without influencing the mitogen-activated protein kinase (MAPK/ERK) signaling pathway.[13] Tensin 2 overexpression has been shown to suppress Akt signaling and inhibit cell survival, proliferation, and migration;[13] this may be mediated through a putative PTEN-like phosphatase activity in Tensin 2.[16]

pAkt is a key positive player in PI3K/Akt cell growth and survival signaling, whereas PTEN and Tensin 2 appear to antagonize this process. The present study aims to investigate the expression of these three molecules in tumor tissues from patients with thyroid carcinoma and compare the results with the surrounding normal resected tissues. Expressions of pAkt, PTEN and Tensin 2 have been assessed for correlation with the patients' clinicopathological parameters.

Materials and Methods

Thyroid cancer samples

The samples utilized in this study included paraffin-embedded, formalin-fixed tissues from 58 cases with papillary (n=45), medullary (n=9), and

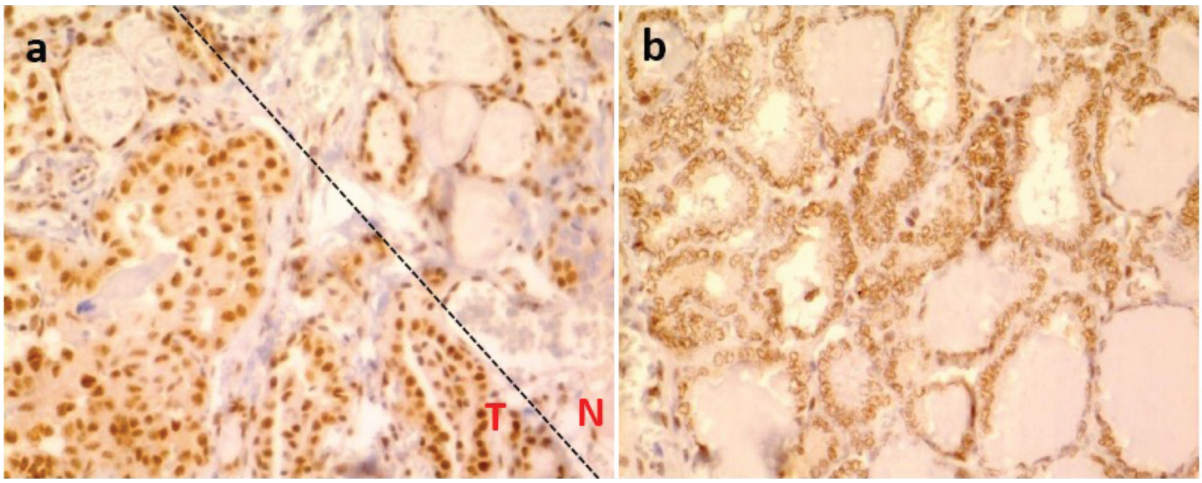

Figure 1. Immunohistochemical staining of pAkt in non-neoplastic and neoplastic thyroid tissues. A) Papillary carcinoma and adjacent normal thyroid tissue. The dotted line roughly indicates the border between the tumor (T) and normal (N) tissue. B) Follicular variant of papillary thyroid carcinoma (100×).

other types of thyroid carcinoma (n=4) collected from the Tissue Bank of the Pathology Department at Shiraz University of Medical Sciences. The samples were taken from 43 women and 15 men with an age range of 14-87 years (median: 42 years). Out of 51 tumors that were measured for size, 9 were less than 2 cm in diameter, 32 were between 2 and 5 cm, and 10 were larger than 5 cm. According to the American Joint Committee on Cancer Stage TNM classification, 30 tumors were designated to be at the 1st, 11 at the 2nd, and 11 at the 3rd stage of the disease. The stages of the others were unknown.

Immunohistochemical staining

Tissue sections of 5 µm thickness were cut, mounted on adhesive–coated slides, and stored in a dry environment until use. We used the following antibodies for immunohistochemical staining: rabbit polyclonal antibody to pAkt (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:1000 dilution; monoclonal antibody 6H2.1 against human PTEN (Dako, Glostrup, Denmark) at 1:450 dilution; and an in-house rabbit polyclonal antibody against human Tensin 2[13] at 1:350 dilution. The sections were deparaffinized and hydrated by passing through xylene and a graded series of ethanol concentrations. Antigen retrieval was performed at 95-100°C in tris-EDTA buffer, pH 9.0, in a water bath for 40 min. For blocking endogenous peroxidase activity, the sections were incubated in 0.3% hydrogen peroxide for 60 min. After blocking for 30 min in normal goat serum (dilution 1:10), the sections were incubated overnight with the primary antibodies at 4°C. The next day, slides were rinsed in PBS and incubated for 30 min with peroxidase-conjugated EnVision™ + Dual Link reagents (EnVision/HRP, Dako). The chromogenic reaction was performed with 3,3′-diaminobenzidine chromogen solution for 5 min, which resulted in the expected brown-colored signal. Finally, after rinsing with deionized water, the slides were counterstained with hematoxylin, dehydrated, mounted with xylene-based mounting medium and covered with a coverslip. Intensity of staining was classified separately for the nucleus and the cytoplasm and graded as strong (+++), moderate (++), weak (+), or negative (-). Figures 1, 2 and 3 illustrate, respectively, the immunohistochemical staining of pAKT, PTEN and Tensin 2 in non-neoplastic and neoplastic thyroid tissues.

Statistical analysis

The SPSS software package version 13 (SPSS, Chicago, IL, USA) was used to analyze the data. Pearson chi-square and Fisher's exact tests were used for a comparison of categorical variables and to examine the association between pAkt, PTEN and Tensin 2 expressions with various clinicopathological parameters that included tumor size, type and stage. A *P*-value of less than 0.05 was considered to be statistically significant.

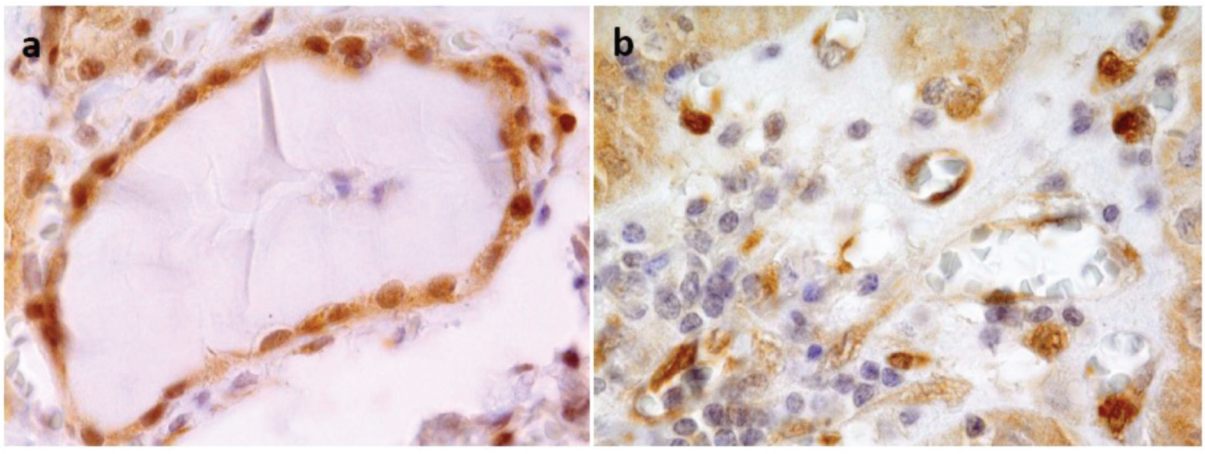

Figure 2. Immunohistochemical staining of PTEN in non-neoplastic and neoplastic thyroid tissues. A) Normal thyroid tissue. B) Papillary thyroid carcinoma (400×).

Table 1. Immunohistochemical expression grades of PTEN, pAkt and Tensin 2 in tumor and accompanying normal tissues from thyroid carcinoma patients.

Tissue:	Normal			Tumor		
	Negative/weak	+1	+2	Negative/weak	+1	+2
PTEN (58 normal, 58 tumor)	19	38	1	10	37	11
Tensin 2 (47 normal, 47 tumor)	18	29	-	25	22	-
pAkt (56 normal, 55 tumor)	18	33	5	-	50	5

Results

We assessed expressions of pAkt, PTEN and Tensin 2 through immunohistochemical analysis of 58 paraffin-embedded sections from both tumor and accompanying normal tissues. The results are shown in Table 1. Of the normal tissues, 29/47 (61.7%) were positive for Tensin 2, whereas 22/47 (46.8%) of cancer tissue sections were positive for Tensin 2, which showed a lower proportion of cancer tissues that expressed Tensin 2. The reverse pattern was observed for both PTEN and pAkt expression, with 39/58 (67.2%) of normal tissues versus 48/58 (82.8%) of tumor tissues that stained positive for PTEN. There were 38/56 (67.9%) normal versus 55/55 (100%) tumor tissues that stained positive for pAkt. The remainder of the tissues showed either weak or negative staining.

The relationship between the expression patterns of PTEN, pAkt and Tensin 2 with the clinicopathological parameters of the patients is presented in Table 2. Statistical analysis indicated no significant association between either pAkt, PTEN, and Tensin 2 expression to gender, tumor type, tumor size, and stage.

Discussion

The PI3K/Akt pathway is believed to play a key role in the tumorigenic development of various neoplasms. The phosphorylation and subsequent activation of Akt following PIP3-mediated membrane anchoring triggers a signaling pathway that promotes tumor cell survival and subsequent processes involved in oncogenesis.[1] Furthermore, both PTEN and Tensin 2 inhibit the activation of the Akt pathway, and therefore may play critical roles in controlling cancer cell growth, proliferation, and survival. In this study, by immunohistochemical staining, we have evaluated the expression levels of pAkt, PTEN and Tensin 2 in normal and neoplastic tissue sections resected from 58 cases with thyroid carcinoma. The presence and levels of expression were compared between the neoplastic and normal parts of the tissues, and subsequently evaluated for association with the patients' clinicopathological parameters.

The results indicated that pAkt more highly and prevalently expressed in cancerous tissue compared to normal thyroid tissues. While all of the different types of thyroid cancer tissues were

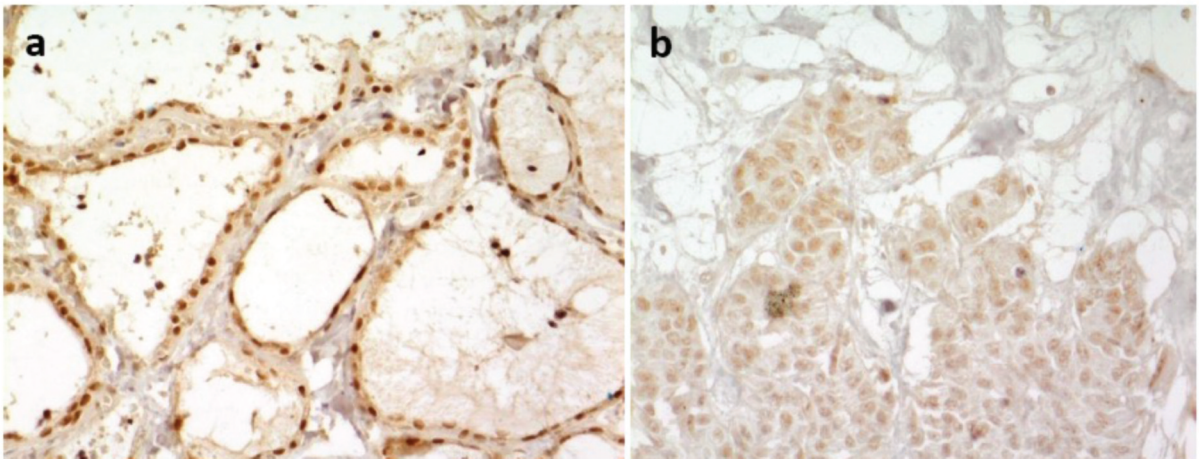

Figure 3. Immunohistochemical staining of Tensin 2 in non-neoplastic and neoplastic thyroid tissues. A) Normal thyroid tissue. B) Papillary thyroid carcinoma (100×).

Table 2. The association between expression patterns of PTEN, pAkt and Tensin 2 in tumor tissues to clinicopathological parameters in thyroid carcinoma patients. There were no significant differences between any of the parameters.

	PTEN			Tensin 2		pAkt	
Characteristics:	**Negative/weak**	**+1**	**+2**	**Negative/weak**	**+1**	**+1**	**+2**
Gender							
Male	2	8	5	9	4	14	0
Female	7	29	6	16	17	36	4
Tumor type							
Medullary	1	3	6	6	3	8	1
Papillary	7	33	4	17	18	38	4
Other	2	1	1	2	1	4	0
Tumor size (Cm)							
<2	1	7	1	4	3	7	0
≥2, <5	7	18	6	12	12	28	3
≥5	1	6	3	3	5	8	1
Tumor stage							
1	3	20	6	13	9	25	3
2	4	6	1	2	6	9	1
3	2	6	3	5	5	10	0

positive for pAkt, approximately two-thirds of the normal tissues were positive. Consistent with our results, Vasko et al. have observed Akt activation in the majority of thyroid cancer patients by immunohistochemistry.[17] Activated Akt is associated with tumorigenesis through phosphorylating numerous targets[1,18,19] including mammalian target of rapamycin complex 1 (mTORC1) which activates the cellular protein synthesis machinery.[20] pAkt also inactivates glycogen synthase kinase-3 (GSK3)[21] and causes an increase in activation of Myc and cyclin D1, all of which are related to increased cell proliferation.[22,23]

In addition to the observed increased expression of pAkt in thyroid tumor samples, this was the first study to have reported on concomitant expression of Tensin 2 in clinical thyroid cancer samples. Tensin 2, which might possess PTEN-like phosphatase activity and thereby inhibit the Akt signaling pathway,[13] was expressed in 46.8% of tumor tissues compared to 61.7% of normal tissues. Therefore, this downregulation of Tensin 2 has suggested that it might possess tumor or metastasis suppressive properties, either through a similar mechanism to PTEN or through its own signaling interactions inside the cell that regulate cell growth and migration.[13] Clearly, these results pave the way for further investigations into the mechanisms of action of Tensin 2 and its role in thyroid cancer development and spread.

In contrast to a reduced Tensin 2 expression in thyroid cancer tissues, we have observed more frequent expression of PTEN phosphatase in tumor tissues (82.8% tumor vs. 67.2% normal). PTEN is a tumor suppressor believed to play an important role in modulating cell cycle progression and/or apoptosis.[2,3] Therefore, our findings appear to contradict those of similar studies, which have shown decreased PTEN expression in follicular carcinomas compared with normal thyroid tissue.[24,25] In other cancer types, the percentage of PTEN positive endometrial tissues was reported to gradually decrease from normal proliferative endometrium to benign architectural changes of unopposed estrogen, to endometrial intraepithelial neoplasia.[12,26] Significantly lower expression of nuclear PTEN, but not cytoplasmic PTEN, was reported in colorectal carcinoma and adenoma compared to normal mucosa samples.[27] Reduction and loss of PTEN protein expression has also been noted in soft tissue sarcomas[28] as well as non-small-cell lung cancer[29,30]. Nevertheless, in a large immunohistochemical study on clear cell adenocarcinoma samples, a total of 62.5% of tissues were graded as single or double positive

for PTEN.[31] Differences in results could be simply explained by differences in methodology, especially immunohistochemical grading. However, Yamada et al. have suggested that these differences are not mere technical controversies, but instead demonstrate that regulation of PTEN function is highly sensitive to the cellular environment.[32] It is important to recognize that PTEN expression level alone may not entirely account for its regulatory role in affecting the status of Akt/PI3K signaling in thyroid tumors. For example, as an alternative, individual mutations in PTEN may affect its enzymatic activity as a phosphatase, thereby altering its ability to antagonize PI3K action. PTEN mutations that produce this effect and facilitate cancer progression have also been frequently detected in human cancers.[33,34] Therefore, the level of PTEN phosphatase activity may be a more accurate representative of the outcome of the PTEN pathway rather than its absolute expression level at least in some cancers and in some ethnic backgrounds.

Statistical analyses indicated no associations between the expression levels of pAkt, PTEN, and Tensin 2 with the patients' clinicopathological characteristics of gender, tumor type, tumor size, and stage. Therefore, collectively, the data might suggest roles for PTEN, pAkt and Tensin 2 in the initiation of thyroid carcinoma rather than its progression.

In conclusion, the results of the present study have revealed overexpression of pAkt and concomitant downregulation of Tensin 2 in neoplastic tissues from thyroid cancer. The results imply that these molecules, and possibly their interplay, may participate in carcinogenesis processes that lead to thyroid carcinoma. The increased expression in cancer tissues of the other pAkt signaling inhibitor, PTEN, warrants further investigation into its activity status in these cancers.

Acknowledgments

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Conflict of Interest

No conflict of interest is declared.

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