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Original Article

Upregulation of the signal transducers and activators of transcription 3 (STAT3) pathway in lymphatic metastases of papillary thyroid cancer

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Abstract: Papillary thyroid cancer (PTC) has an impressive propensity for lymphatic spread. Signal transducers and activators of transcription 3 (STAT3), constitutively activated in many different cancers, may play a role in PTC lymphatic metastases. We examined 49 patients with PTC, 22 with and 27 without lymphatic metastases. All patients had a total thyroidectomy with lymph node dissection to document true node negative cases. The level of STAT3 expression in benign, non-neoplastic thyroid tissue is barely detectable by immunohistochemistry. Only 11 of the 35 (31%) specimens exhibited weak immunostaining for STAT3 and pSTAT3 was found weakly positive in 3 of 35 (9%) benign specimens. Expression of STAT3 in all PTC primary tumors was 98% (40/41) and thus significantly higher than corresponding benign thyroid tissue (p=0.0001). pSTAT3 was found in 37% of primary tumors (15/41) and this was significantly higher than pSTAT3 expression in benign tissue (p=0.006). Comparing node-positive and node-negative primary tumors, there was no difference in staining intensity for STAT3 where strong (2+) staining was seen 12/19 node-positive tumors and 13/22 node-negative tumors (p=1). Regarding pSTAT3 expression in primary PTC tumors, node negative cases (n=22) exhibited significantly less staining compared to node positive cases (n=19). Only 4 of 22 (18%) cases in the node-negative group were weakly (1+) positive for pSTAT3 while 12 of 19 (58%) cases in the node-positive group were positive (p=0.011) with 45% of these specimens exhibiting strong (2+) staining. Lymphatic metastases were highly positive (>93%) for both STAT3 and pSTAT3. The STAT3 pathway is ubiquitous in PTC and activated pSTAT3 is significantly upregulated in PTC tumors with metastatic disease. This study is the first to suggest a potential role for activated pSTAT3 in lymphatic metastases in thyroid cancer.

Keywords: STAT3, immunohistochemistry, metastases, papillary thyroid cancer

Introduction

Papillary thyroid cancer (PTC) has an impressive propensity for lymphatic spread. Up to 1 in 2 patients will demonstrate metastases within cervical lymph nodes and it is now accepted that lymphatic involvement, especially in elderly patients, is associated with increased morbidity and mortality [1-3]. Lymphatic spread in papillary thyroid cancer, either at the initial diagnosis or as recurrent disease, is treated by surgical extirpation and radioiodine ablation [4-6]. The current surgical approach at first presentation, in addition to thyroidectomy, is to remove the central compartment nodes if clinically involved as determined by radiologic or clinical assessment [7]. However, occult micrometastases may be seen in up to 80% of papillary thyroid carcinomas and recurrent disease after thyroidectomy has been documented in up to 25% of patients over 10 years [1,6,8]. Recurrent lymphatic metastases are difficult to extirpate and surgical complications are increased in the reoperative setting [9-10]. Patients may also be subject to multiple attempts at radioiodine ablation with associated cost and morbidity [10-11]. The use of prophylactic level 6 dissection is thus debated pending more accurate markers of

lymphatic spread in papillary thyroid carcinoma [7,12].

At present no marker has been proposed to identify patients at risk of metastatic thyroid carcinoma. Biomarkers such as Galectin-3, HMBE and CK-19 have been examined to discriminate between malignant and benign nodules but expression of these markers is not unique in metastatic disease [13,14]. There is evidence for important differences between tumors with and without lymphatic spread. Increased expression of cell cycle regulators such as cyclin D1 as well as growth factors VEGF-C and bFGF have been found in metastatic papillary thyroid tumors [15,16]. Markers predictive for metastases, in addition to clinical assessment, could assist in treatment planning. In particular, patients at risk of aggressive disease may be selected for more aggressive surgery including prophylactic lymph node dissection as well as intensive adjuvant therapy with radioactive iodine ablation [10-12]. Thus more advanced immunohistochemical assessments may help stratify risk of PTC metastases and assist clinicians in selecting patients best suited for aggressive surgical and medical therapy.

STAT3 is a signal transduction pathway implicated in malignancy. Constitutive activation of STAT3 contributes to cell growth and malignant cellular transformation in many types of human cancers and there is evidence supporting STAT3 as an oncogene [17]. STAT3 up regulates cell-cycle facilitators including cyclin D1 and c- *myc*, as well as anti-apoptotic proteins such as survivin, Bcl-xL and Mcl-1 [17]. STAT3 has been studied in a number of different malignancies and it is clear that it is an important pathway in lymphatic metastases [17-19]. In PTC the STAT3 pathway has not been examined. However a number of different downstream proteins involved in the STAT3 pathway, including cyclin D1, survivin, VEGF and BcL have been shown to play a role in malignant progression in thyroid carcinoma [20-23]. Based on this information, we hypothesized that STAT3 activation plays a role in the metastasis of thyroid carcinoma.

In this study we examined the expression of total STAT3 and the phosphorylated, activated form pSTAT3 in benign, non-neoplastic thyroid tissue and in PTC tumors with and without lymphatic metastases. Our aim is to test our hypothesis that STAT3 is a predictive marker for lymphatic spread in thyroid cancer.

Materials and methods

Patients

Ethics approval was obtained through the Royal North Shore Hospital Ethics Committee and the University of Alberta Heath Research Ethics Board. A total of 49 patients were selected during the period of 2004-2008 with papillary thyroid carcinoma, 22 with and 27 without lymphatic metastases. In all cases patients had a total thyroidectomy with a level 6 lymph node dissection such that histopathology could be used to document the true node negative cases that complemented our clinical assessment via ultrasound. The pathology assessments confirmed the diagnosis of papillary thyroid carcinoma and the absence of special high grade pathologic variants such as insular or tall cell papillary thyroid cancer. The classification of thyroid tumors was based on WHO criteria (2004). Two pathologists separately assessed the specimens to document primary tissue diagnosis as well as the presence of lymphatic metastases in nodes sectioned.

Immunohistochemistry

Immunohistochemical staining for STAT3 and pSTAT3 was performed using standard techniques as previously described [24,25]. Formalin-fixed, paraffin-embedded tissue sections of 4 um thickness were deparaffinized and hydrated. Heat-induced epitope retrieval was performed using Tris buffer (pH 9.9; DAKO, Mississauga, ON, Canada) and a rapid microwave histoprocessor [RHS; Milestone, Bergamo, Italy]. After incubation at 100°C for 10 minutes, slides were washed in running tap water for 5 minutes, followed by a wash with PBS (pH 7.2) for 5 minutes. Tissue sections were then incubated with anti-pSTAT3 antibody (1:60) or anti-STAT antibody (1:50) in a humidified chamber at 4°C. After three washes with PBS, tissue sections were incubated with anti-rabbit IgG and peroxidase (EnVision, DAKO) for 30 minutes at room temperature. The tissue sections were incubated with 3,3-diaminobenzidine/H_2O_2 for color development. Immunohistochemical staining was examined by two pathologists blinded to the array composition.

Scoring

All tumors were prepared by microdissection to exclude necrosis. Measurements were done in

Table 1. Patient demographics

	Age (mean)	Female (male)	Tumor size (cm)	Nodes assessed (mean)
Node negative	51	22 (5)	1.0	6.5
Node positive	48	18 (4)	2.3	15.3

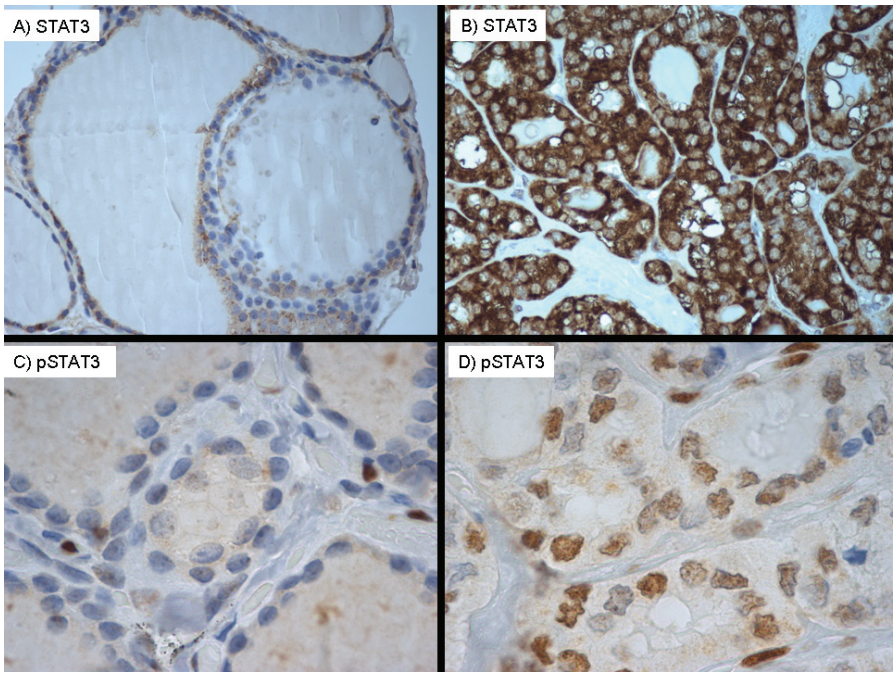

Figure 1. Representative non-neoplastic benign thyroid tissue at high power (200X) stained for STAT3 (**A**) and representative primary tumor (**B**). Corresponding pSTAT3 stains are shown in figures (**C**) and (**D**). STAT3 staining was seen predominately in the cytoplasm with pSTAT3 most often seen within the nucleus.

triplicate for three separate sections of the tumor and averaged to minimize heterogeneity within the tumors. Negative staining was defined as the absence of cytoplasmic stain and staining of <10% of the nuclei. Cytoplasmic staining was scored on a 3-point scale based on intensity: negative (0), weakly positive (1+), and strongly positive (2+). When staining was present but not found in the cytoplasm, nuclear staining was judged positive if >10% of nuclei were positive. For pSTAT3, the presence of any areas with >10% tumor cells showing definitive nuclear staining were scored positive. Endothelial cells, which are often positive for pSTAT3, served as internal positive controls whereas reactive lymphoid cells in benign tonsils served as negative controls. If tumor sections were fragmented or too small to permit measurements in three separate sections, they were not included in the analysis.

Statistical analysis

The association between pSTAT3, STAT3 and lymphatic involvement was evaluated by X2 test and Fisher's exact test. Statistical analyses were carried out by SPSS for Windows 11.0 [SPSS Inc; Chicago, Ill].

Results

The patient demographics and tumor characteristics are shown in **Table 1**. The lymph node metastases are seen in tumors of varying sizes and the extent of nodal involvement varies from solitary nodes within level 6 to extensive central and lateral metastases. There is no statistically significant variation in lymph node metastases with respect to patient age or gender.

STAT3 expression: benign thyroid tissue

Shown in **Figure 1** are representative sections of STAT3 and pSTAT3 immunohistochemical stains for benign, non-neoplastic as well as malignant thyroid tissue. STAT3 and pSTAT3 levels in benign thyroid tissue derived from samples distant from thyroid tumors were assessed and

Table 2. STAT3 and pSTAT3 staining in PTC

Score		0	1+	2+
Benign thyroid tissue (n=35)	STAT3	24	11	0
	pSTAT3	32	3	0
Node negative primary (n=22)	STAT3	0	9	13
	pSTAT3	18	4	0
Node positive primary (n=19)	STAT3	1	6	12
	pSTAT3	8	6	5
Lymph node specimens (n=13)	STAT3	1	5	7
	pSTAT3	3	8	2

quantified in **Table 2** (n= 35). The level of STAT3 expression was not or barely detectable in all cases. Specifically, only 11 of the 35 (31%) specimens exhibited immunostaining for STAT3, and all of these positive cases showed only weak positivity (1+) (see **Table 2**). Immunoreactivity for pSTAT3 was detectable in only 3 of 35 benign specimens; all of these positive cases were only weakly positive (1+).

STAT3 expression in primary tumors

STAT3 and pSTAT3 levels in papillary thyroid carcinomas, for both primary tumors with and without lymphatic metastases, were then assessed. The immunostaining for both markers were heterogeneous. For the expression of STAT3 in PTC primary tumors, 40 of 41 (98%) cases of primary PTC tumors were positive for this marker and this is significantly higher than that seen for benign tissue (p=0.0001) (**Table 2**). For pSTAT3, 15 of 41 (37%) of the primary PTC tumors were positive and this difference is statistically significant compared to benign tissue (p=0.006) (**Table 2**).

STAT3 expression in primary tumors correlates with the presence/absence of lymph node metastasis

We examined STAT3 and pSTAT3 expression in primary tumors with, or without, nodal metastases. Node negative cases (n=22) significantly differed from node positive cases (n=19) regarding pSTAT3 expression in primary tumors. Only 4 of 22 (18%) cases in the node-negative group were positive for pSTAT3 but 11 of 19 (58%) cases in the node-positive group were positive and this difference is statistically significant (p=0.011) (**Table 2**). In addition, while none of the 22 node-negative cases were strongly positive for pSTAT3 (2+), 5 of 19 (26%) in the node-positive group were strongly positive for this marker (p=0.016). Expression of STAT3 was seen equally in the two groups with 100% positivity in node-positive primary tumors (19/19) and 98% positivity in node-negative primary tumors (21/22) (**Table 2**). There was no difference in staining intensity for STAT3 where strong (2+) staining was seen 12/19 node-positive tumors and 13/22 node-negative tumors (p=1). In the lymphatic metastatic deposits 12 of 13 cases were STAT3 positive and 11 of 13 cases expressed pSTAT3 (**Table 2**). There was no significant difference in staining positivity or intensity between STAT3 and pSTAT3 in the nodal deposits.

STAT3 expression does not correlate with tumor size

The size of the primary tumors in our cohort varied from less than 1 centimeter to over 10 centimeters. To ensure that size of the nodule did not confound our analysis, we examined the staining intensity for both total STAT3 and pSTAT3 as a function of nodule size as shown in **Figure 2**. For both total STAT3 (p=0.08) and pSTAT3 (p=0.10) there does not appear to be any relationship between module size and the intensity of staining.

STAT3/pSTAT3 as predictive markers

We assessed the utility of STAT3 and pSTAT3 in predicting malignancy and lymph node metastases. STAT3, positive in virtually all primary tumors, demonstrated similar expression for tumors with and without nodal metastases. Thus as a marker it is neither specific nor sensitive for nodal metastases. However, pSTAT3 was more specific, but not sensitive, in discriminating metastatic primary thyroid carcinoma from local disease (see **Table 3**).

Table 3. Predictive value of pSTAT3 and STAT3 for metastatic papillary thyroid carcinoma

Marker	Accuracy (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
STAT3	66	63	41	48	56
pSTAT3	71	58	82	73	69

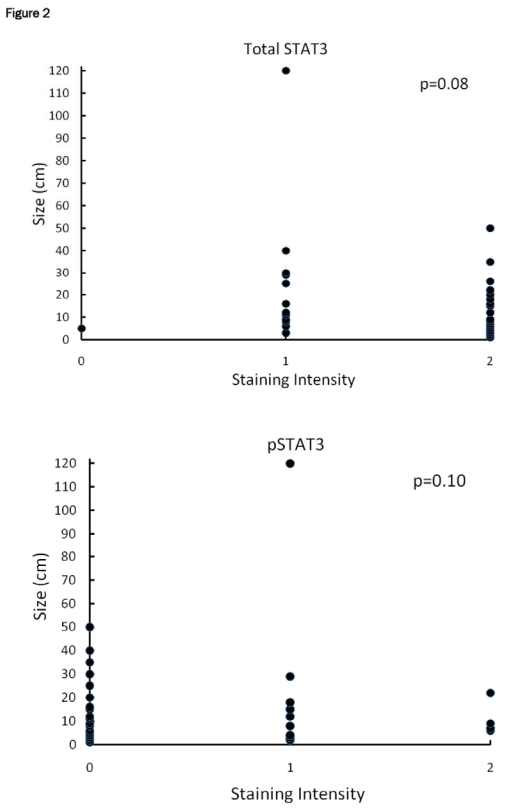

Figure 2. Scatter plot of STAT3 (a) and pSTAT3 (b) expression levels as a function of primary tumor size in papillary thyroid carcinoma for tumors with and without metastases.

Discussion

To our knowledge, this is the first study that documents the possible role of STAT3 signaling in lymphatic metastases in thyroid cancer. The increase in expression of STAT3 and the activated, phosphorylated form pSTAT3 in metastatic PTC supports a role for STAT3 signaling in lymph node metastases in thyroid cancer. Our results in PTC are similar to that seen in other solid tumors where STAT3 activation is linked to prognosis and lymph node metastases [17-20]. Thus far only one study in the literature investigates the utility of immunohistochemistry in predicting lymph node metastases in thyroid carcinoma [16]. Our study is unique in that we demonstrate differences in STAT3 and pSTAT3 expression within the primary tumors that, if validated on a larger cohort, could be used as an adjunct to cytologic analysis may be used to help plan therapy. This is an important clinical problem as patients with aggressive PTC will often experience recurrent disease. Biomarkers predicting lymphatic spread could be utilized to select patients for aggressive surgery with lymph node dissection and radioactive iodine ablation at initial presentation with a view to mitigating the chance for disease recurrence and future morbidity.

STAT3 clearly has an important role in the development and progression of many different human tumors [19]. Most of the genetic mutations studied in thyroid cancer, namely B-RAF, RAS and RET/PTC are understood to activate the MAPK/ERK kinase which activates the extracellular signal regulated kinase [ERK]. Thus the MAPK/ERK pathway represents the most studied pathway in thyroid carcinoma but the role of STAT3 in thyroid carcinoma is relatively unknown. However the STAT3 pathway is also activated in RET/PTC rearrangements as well [26-28]. Of interest to our current work, RET/PTC mutations typically occur at a younger age, have a classic histology and very commonly spread to lymph nodes [27,28]. Constitutive activation of STAT3 leads to altered regulation of a number of genes involved in cell cycle and angiogenesis and thus may also be important in neoplastic transformation and metastatic spread [26,29]. In addition B-RAF mutations, linked with lymphatic metastases, also was recently documented to activate the STAT3 pathway in other malignancies [30]. Based on our data it appears that the STAT3 pathway, in addition to the MAPK/ERK pathway, has an important role in lymphatic metastases of papillary thyroid carcinoma.

Altered proliferative, apoptotic and cell cycle

markers have been linked to lymphatic metastases in thyroid cancer [17]. Most often noted is the increased expression of cyclin D1 in both primary papillary thyroid carcinomas and lymph node metastases [15]. Other cell cycle proteins including cyclin E, p27kip1, p57kip2 have also been surveyed in thyroid carcinoma and metastatic specimens but the diagnostic or prognostic role for cell cycle regulators in thyroid carcinoma is debated [14]. Studies of thyroid carcinoma have also demonstrated increased expression of MMP-2 in metastatic disease [16]. VEGF-C and -D have also been identified in PTC lymphatic metastases and VEGF-C and bFGF were shown to exhibit high levels of expression in lymphatic metastases [16]. The STAT3 pathway is known to transcriptionally regulate cyclin D1 and decreased levels of phosphorylated, activated STAT3 are linked to lower levels of MMP-2 and VEGF in other malignancies [31]. Thus the STAT3 pathway may influence expression of these markers however other signaling systems, including the PI3K/AKT and MAPK/ERK pathways, may also play a role in the phenotypic differences in protein expression in thyroid carcinoma. Additional experimentation is required to outline the relative contribution of these different pathways to lymphatic spread and their possible utility as diagnostic markers. It is more likely that a large panel of markers, including pSTAT3 and VEGF, would be of better diagnostic and predictive utility.

To conclude, we identify for the first time the role of the STAT3 pathway in papillary thyroid metastases. Outlining the role of STAT3 and pSTAT3 in papillary thyroid carcinoma will aid the development of biomarkers that will allow clinicians to identify patients at risk for lymphatic metastases. Using immunohistochemistry and biomarkers to improve FNA cytological analysis will allow surgeons to perform early lymph node dissections and clinicians to utilize radioiodine ablation to treat aggressive disease or to avoid unnecessary overtreatment in tumors with a low risk for metastatic spread. Future studies will examine the utility of biomarkers from other signaling pathways (MAPK/ERK, Akt), when combined with pSTAT3, to identify patients with PTC metastases.

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References

[1] Pai SI, Tufano RP. Reoperation for recurrent/persistent well-differentiated thyroid cancer. Otolaryngol Clin North Am. 2010; 43:353-63

[2] Lundgren CI, Hall P, Dickman PW, Zedenius J. Clinically significant prognostic factors for differentiated thyroid carcinoma: a population-based, nested case-control study. Cancer 2006; 106:524–531

[3] Hughes CJ, Shaha AR, Shah JP, Loree TR. Impact of lymph node metastasis in differentiated carcinoma of the thyroid: a matched-pair analysis. Head Neck 1996; 18:127–132

[4] Machens A, Hinze R, Thomusch O, Dralle H. Pattern of nodal metastasis for primary and reoperative thyroid cancer, World J Surg 2002;26:22–28.

[5] Coburn M, Teates D, Wanebo JH. Recurrent thyroid cancer. Role of surgery versus radioactive iodine (I131), Ann Surg 1994; 219:587–593.

[6] Rotstein L. The role of lymphadenectomy in the management of papillary carcinoma of the thyroid. J Surg Oncol 2009; 99:186-188.

[7] Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Thyroid 2009; 19:1167-1214.

[8] Udelsman R. Treatment of persistent or recurrent papillary carcinoma of the thyroid–the good, the bad, and the unknown. J Clin Endocrinol Metab 2010; 95:2061-2063

[9] Machens A, Hauptmann S, Dralle H. Lymph node dissection in the lateral neck for completion in central node-positive papillary thyroid cancer. Surgery 2009; 145:176-181.

[10] Dralle H, Machens A. Surgical approaches in thyroid cancer and lymph-node metastases. Best Pract Res Clin Endocrinol Metab 2008; 22:971-987

[11] Carling T, Long WD 3rd, Udelsman R. Controversy surrounding the role for routine central lymph node dissection for differentiated thyroid cancer. Curr Opin Oncol 2010; 22:30-34.

[12] Sywak M, Cornford L, Roach P, Stalberg P,

Sidhu S, Delbridge L. Routine ipsilateral level VI lymphadenectomy reduces postoperative thyroglobulin levels in papillary thyroid cancer. Surgery 2006; 140:1000-1005

[13] Chiu CG, Strugnell SS, Griffith OL, Jones SJ, Gown AM, Walker B, Nabi IR, Wiseman SM. Diagnostic utility of galectin-3 in thyroid cancer. Am J Pathol 2010; 176:2067-2081.

[14] Griffith OL, Chiu CG, Gown AM Jones SJ, Wiseman SM. Biomarker panel diagnosis of thyroid cancer: a critical review. Expert Rev Anticancer Ther 2008;8:1399-1413.

[15] Lee SH, Lee JK, Jin SM Lee KC, Sohn JH, Chae SW, Kim DH. Expression of cell-cycle regulators (cyclin D1, cyclin E, p27kip1, p57kip2) in papillary thyroid carcinoma. Otolaryngol Head Neck Surg 2010;142:332-337.

[16] Liang H, Zhong Y, Luo Z, Huang Y, Lin H, Luo M, Zhan S, Xie K, Ma Y, Li QQ. Assessment of biomarkers for clinical diagnosis of papillary thyroid carcinoma with distant metastasis. Int J Biol Markers 2010; 25:38-45.

[17] Huang S. Regulation of metastases by signal transducer and activator of transcription 3 signaling pathway: clinical implications. Clin Cancer Res 2007;13:1362-1366.

[18] Barbieri I, Pensa S, Pannellini T, Quaglino E, Maritano D, Demaria M, Voster A, Turkson J, Cavallo F, Watson CJ, Provero P, Musiani P, Poli V. Constitutively active Stat3 enhances neu-mediated migration and metastasis in mammary tumors via upregulation of Cten. Cancer Res 2010; 70:2558-2567.

[19] Devarajan E, Huang S. STAT3 as a central regulator of tumor metastases. Curr Mol Med 2009; 9:626-633.

[20] Mitsiades CS, Hayden P, Kotoula V, McMillin DW, McMullan C, Negri J, Delmore JE, Poulaki V, Mitsiades N. Bcl-2 overexpression in thyroid carcinoma cells increases sensitivity to Bcl-2 homology 3 domain inhibition. J Clin Endocrinol Metab 2007; 92:4845-4852.

[21] Antonaci A, Consorti F, Mardente S, Natalizi S, Giovannone G, Della Rocca C. Survivin and cyclin D1 are jointly expressed in thyroid papillary carcinoma and microcarcinoma. Oncol Rep 2008;20:63-67.

[22] Londero SC, Godballe C, Krogdahl A, Bastholt L, Specht L, Sørensen CH, Pedersen HB, Pedersen U, Christiansen P. Papillary microcarcinoma of the thyroid gland: is the immunohistochemical expression of cyclin D1 or galectin-3 in primary tumour an indicator of metastatic disease? Acta Oncol 2008;47:451-457.

[23] Zhang HY, Meng X, Du ZX, Fang CQ, Liu GL, Wang HQ, Deng WW. Significance of survivin, caspase-3, and VEGF expression in thyroid carcinoma. Clin Exp Med 2009;9:207-213.

[24] Rosen DG, Mercado-Uribe I, Yang G, Bast RC Jr, Amin HM, Lai R, Liu J. The role of constitutively active signal transducer and activator of transcription 3 in ovarian tumorigenesis and prognosis. Cancer 2006; 107:2730-2740

[25] Dien J, Amin HM, Chiu N, Wong W, Frantz C, Chiu B, Mackey JR, Lai R. Signal transducers and activators of transcription-3 up-regulates tissue inhibitor of metalloproteinase-1 expression and decreases invasiveness of breast cancer. Am J Pathol 2006; 169:633-642.

[26] Hwang JH, Kim DW, Suh JM, Kim H, Song JH, Hwang ES, Hwang IY, Park KC, Chung HK, Kim JM, Park J, Hemmings BA, Shong M. Activation of signal transducer and activator of transcription 3 by oncogenic RET/PTC (rearranged in transformation/ papillary thyroid carcinoma) tyrosine kinase: roles in specific gene regulation and cellular transformation. Mol Endocrinol 2003; 17:1155-1166

[27] Kim YR, Byun HS, Won M, Park KA, Kim JM, Choi BL, Lee H, Hong JH, Park J, Seok JH, Kim DW, Shong M, Park SK, Hur GM. Modulatory role of phospholipase D in the activation of signal transducer and activator of transcription (STAT)-3 by thyroid oncogenic kinase RET/PTC. BMC Cancer 2008; 8:144-152

[28] DeLellis RA. Pathology and genetics of thyroid carcinoma. J Surg Oncol. 2006;94:662-669.

[29] Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol 2008;21:37-43.

[30] Lang SA, Schachtschneider P, Moser C, Mori A, Hackl C, Gaumann A, Batt D, Schlitt HJ, Geissler EK, Stoeltzing O. Dual targeting of Raf and VEGF receptor 2 reduces growth and metastasis of pancreatic cancer through direct effects on tumor cells, endothelial cells, and pericytes. Mol Cancer Ther 2008;7:3509-3518.

[31] Wang XH, Liu BR, Qu B, Xing H, Gao SL Yin JM, Wang XF, Cheng YQ. Silencing STAT3 may inhibit cell growth through regulating signaling pathway, telomerase, cell cycle, apoptosis and angiogenesis in hepatocellular carcinoma: potential uses for gene therapy. Neoplasma 2011;58:158-171.