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Original Article

Overexpression of HIF-1α, metallothionein and SLUG is associated with high TNM stage and lymph node metastasis in papillary thyroid carcinoma

Ni Wang[1*], Chao-Ran Dong[1*], Rong Jiang[2], Cui Tang[1], Lei Yang[1], Qi-Feng Jiang[1], George G Chen[3], Zhi-Min Liu[1]

[1]Department of Biochemistry and Molecular Biology, Molecular Medicine and Cancer Research Center, Chongqing Medical University, Chongqing, China; [2]Department of Pathology, Molecular Medicine and Cancer Research Center, Chongqing Medical University, Chongqing, China; [3]Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong, China. [*]Equal contributors.

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Abstract: Hypoxia inducible factor-1α (HIF-1α) is upregulated by hypoxia, and involved in tumor growth and metastasis in many malignant tumors including papillary thyroid carcinoma (PTC). Metallothionein (MT) is a group of small molecular weight cysteine-rich proteins with a broad variety of functions. SLUG is a member of SNAIL superfamily of zinc finger transcriptional factors implicated in epithelial-mesenchymal transition (EMT). The purpose of this study was to examine HIF-1α, MT and SLUG expression in PTC and assess association of their expression with clinicopathological indicators. HIF-1α, MT and SLUG protein expression in 129 PTCs, 61 nodular hyperplasia and 118 normal thyroid tissue specimens were analyzed using immunohistochemistry. The protein expression levels of these three molecules were up-regulated in PTCs. High protein expression of HIF-1α, MT and SLUG was significantly correlated with high TNM stage (P=0.003, 0.002, 0.024, respectively) and lymph node metastasis (LNM) (P<0.001 for all three molecules). Furthermore, HIF-1α, MT and SLUG protein expression were correlated with one another. Concomitant high expression of any two of these three molecules had stronger correlation with high TNM stage (P≤0.001 for HIF-1α/MT, MT/SLUG and HIF-1α/SLUG) and LNM (P=0.008, 0.002, 0.019 for HIF-1α/MT, MT/SLUG and HIF-1α/SLUG, respectively) than did each alone, and concomitant high expression of all these three molecules is significantly associated with high TNM stage and LNM as compared with cases not showing such expression (P<0.001). These results demonstrated that the evaluation of HIF-1α, MT and SLUG expression in PTC may be useful in predicting the risk of LNM and high TNM stage.

Keywords: Hypoxia inducible factor-1α, metallothionein, SLUG, papillary thyroid carcinoma

Introduction

Papillary thyroid carcinoma (PTC) accounts for 80% of thyroid malignancy and is characterized by slow growth and an excellent prognosis. However, some cases exhibit aggressive behavior with hallmarks of lymph node metastasis (LNM), distant metastasis, treatment resistance, and mortality [1]. It is important to identify the characteristics of PTC that have a high risk for invasion, metastasis and progression.

Intratumoral hypoxia is an independent indicator of poor prognosis, and contributes to a more aggressive tumor phenotype [2, 3]. The response of tumor cells to hypoxia is mainly mediated through the hypoxia-inducible factors (HIFs), including HIF-1α, HIF-2α and HIF-3α [4, 5]. Among them, HIF-1α is best characterized as being responsible for the regulation of many hypoxia-inducible genes involved in tumor growth, metabolism, angiogenesis, and metastasis. Increased HIF-1α level in human tumors was associated with poor prognosis and resistance to therapy in ovarian cancer, oesophageal cancer, head and neck cancer [6]. So far, only few studies dealt with HIF-1α expression [7] and its correlation with clinicopathological features of PTC.

Metallothionein (MT) is a group of small molecular weight cysteine-rich proteins with a broad variety of functions [8-10]. The mammalian MT family consists of two ubiquitous isoforms (MT-I

and MT-II) and two tissue-specific isoforms (MT-III and MT-IV). The major physiological roles of MT include essential metal homeostasis, heavy metal detoxification, and redox capabilities [9]. However, studies have suggested that MT is pluripotent, contributing to a number of other fundamental processes, including proliferation, survival, metabolism, invasion and metastasis [8, 10]. Overexpression of MT has been shown to be related to tumor development and progression in a variety of tumors, including those of breast, prostate, bladder and oral epithelium [11]. Recent research suggested a close relationship of HIF-1α and MT. HIF-1α has been shown to function as a coactivator of MT gene transcription by interacting with metal-responsive transcription factor (MTF) during hypoxia [12, 13]. HIF-1α may be capable of inducing MT expression and thereby increases the biological aggressiveness of cancer cells.

SLUG is a member of SNAIL superfamily of zinc finger transcriptional factors implicated in epithelial-mesenchymal transition (EMT), and was first identified as a developmental protein critical for neural crest formation in chick embryos [14]. SLUG expression is correlated with reduced cell adhesion, increased cell migration and invasion, and is associated with biological aggressiveness in several tumor types [15]. High SLUG expression in breast tumors associates with the aggressive phenotype [16]. Recent research showed that SLUG can be activated by hypoxia or HIF-1α, facilitating invasion and metastasis in human cancers [17, 18].

Given their associations with biological aggressiveness of several human cancers in hypoxic condition, the aim of the present study was to examine the HIF-1α, MT and SLUG expression and to assess the association of HIF-1α, MT and SLUG expression with several clinicopathological indicators, and to evaluate potential usefulness of the three molecules in prediction for invasion, metastasis and progression of PTC.

Materials and methods

Case selection and tissue sample preparation

Tumor specimens for immunohistochemistry were obtained from 129 PTC patients who underwent thyroidectomy in the Department of Surgery, the First Affiliated Hospital, Chongqing Medical University, between Jan 2010 and Jan 2013. There were 32 men and 97 women with a median age of 45 years. According to histopathologic diagnosis, there were 80 classic PTC, 21 follicular variant of PTC, 15 tall cell variant of PTC and 13 oncocytic variant of PTC. The size of primary tumor ranged from 0.3 to 6.0 cm (2.28 ± 1.38). According to AJCC classification [19], there were 69 patients with stage I and stage II, 60 with stage III and stage IV. Sixty-eight patients were confirmed to have LNM. Besides, benign thyroid disease specimens were obtained from 61 patients with nodular hyperplasia. 118 normal thyroid tissues were taken from the contralateral lobe of PTC specimens, which exhibit apparently normal morphology as a control. The study protocol was approved by the Research Ethics Committee of Chongqing Medical University and informed consent was obtained from all patients.

Tissue microarray

Formalin-fixed, paraffin-embedded blocks were routinely prepared from surgical specimens of PTC, nodular hyperplasia and normal thyroid tissue. Representative areas containing tumor, nodular hyperplasia or normal thyroid tissue were identified by a pathologist. Duplicate tissue cores with a diameter of 0.6 mm were taken from each specimen (Beecher Instruments, Silver Springs, USA) and arrayed on a recipient paraffin block, using standard procedures [20]. Serial 5-μm-thick sections were cut with a Leica microtome (Leica Microsystems; Wetzlar, Germany) and mounted onto polylysine-coated slides.

Immunohistochemical staining

Sections from TMA blocks were dewaxed in xylene and gradually hydrated. Antigen retrieval was achieved by microwaving in 0.01 M citrate buffer (pH 6.0) for 10 min. After microwave treatment, the slides were treated with 3% hydrogen peroxide in methanol at room temperature for 30 min to block the endogenous peroxidase and followed by blocking with 10% normal goat serum (50062Z, Invitrogen, USA) in PBS at room temperature for 1 h. The slides were then incubated overnight at 4°C in the primary rabbit anti-HIF-1α (BS3514, 1:50, Bioworld Technology, USA), mouse anti-MT (ab12228, 1:50, Abcam, USA), and rabbit anti-SLUG (BS1853, 1:50, Bioworld Technology, USA)

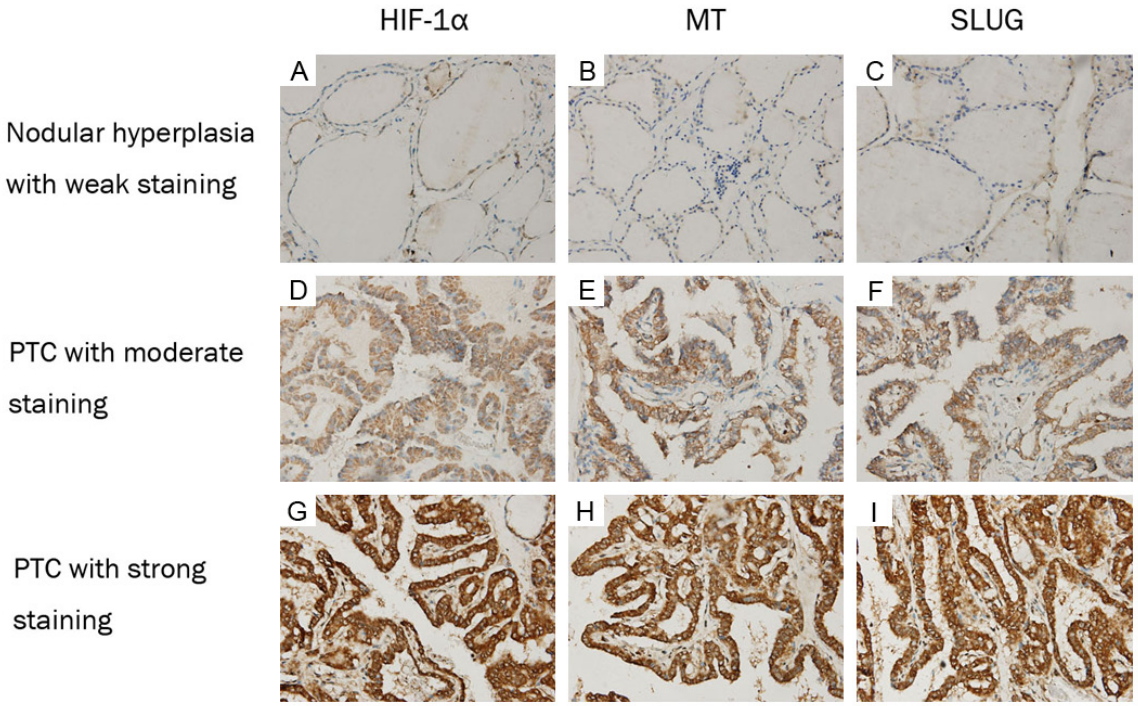

Figure 1. Immunohistochemical staining for HIF-1α, MT and SLUG. Columns correspond to immunostaining for HIF-1α, MT and SLUG, respectively. The first row exhibits weak staining of nodular hyperplasia tissues with the indicated antibody (A-C); the succeeding rows show moderate staining (D-F) and strong staining (G-I) of HIF-1α, MT and SLUG in PTCs. All the pictures are in high-power fields (× 400).

Table 1. Immunohistochemical analysis of HIF-1α, MT and SLUG expression in 129 PTCs, 61 nodular hyperplasia and 118 normal thyroid tissue specimens according to the scoring system

Score	HIF-1α			MT			SLUG		
	Normal thyroid tissue (n)	Nodular hyperplasia (n)	PTC (n)	Normal thyroid tissue (n)	Nodular hyperplasia (n)	PTC (n)	Normal thyroid tissue (n)	Nodular hyperplasia (n)	PTC (n)
0									
Negative	91	37	2	96	39	4	94	36	3
+									
1	23	16	6	20	17	13	21	17	11
2	4	6	10	2	5	15	3	7	13
3	0	2	15	0	0	18	0	1	16
4	0	0	17	0	0	20	0	0	18
++									
6	0	0	21	0	0	21	0	0	22
8	0	0	25	0	0	16	0	0	20
+++									
9	0	0	20	0	0	14	0	0	16
12	0	0	13	0	0	8	0	0	10

The immunohistochemical scores in PTCs, nodular hyperplasia and normal thyroid tissue specimens were determined as the multiplication of proportion score and intensity score.

diluted with blocking solution. For negative isotype controls, the sections were incubated in mouse immunoglobulin G (NI03-100UG, 1:1500, Merck Millipore, Germany) or rabbit immunoglobulin G (NI01-100UG, 1:1000, Merck Millipore, Germany) diluted with blocking solution. After defrosting at 37°C for 30 min, the slides were washed with PBS and incubat-

ed with a secondary biotinylated goat-anti-rabbit antibody (ZB-2010, Zhongshan Golden Bridge Biotechnology, China), or goat-anti-mouse antibody (ZB-2305, Zhongshan Golden Bridge Biotechnology, China) for 30 minutes, peroxidase-labeled streptavidin (ZB-2404, Zhongshan Golden Bridge Biotechnology, China) for 20 minutes and diaminobenzidine chromogen substrate (Sigma, USA) for 5 minutes. Slides were counterstained with hematoxylin, dehydrated in a graded alcohol solution, and mounted.

Immunohistochemical scoring

A semiquantitative assessment of immunohistochemical (IHC) scoring was performed by two observers blinded to the diagnosis. The IHC score was assigned based on staining intensity and percentage of positive cells. The intensity score was assigned as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The proportion score was assigned as 0 (<5% positive cells), 1 (6-25% positive cells), 2 (26-50% positive cells), 3 (51-75% positive cells), and 4 (>75% positive cells). Multiplication of the intensity and percentage scores gave rise to the final staining score: 0 (negative), + (1-4), ++ (5-8), and +++ (9-12). For statistical analysis, a final staining score of negative or + was combined into the low expression group, and a final staining score of ++ or +++ was combined into the high expression group.

Statistical analysis

Statistical analysis was performed using SPSS 18.0 statistical software. Significance was assessed using Chi-square and Spearman rank as appropriate, to compare the groups. $P<0.05$ was considered statistically significant.

Results

Immunohistochemical expression of HIF-1α, MT and SLUG in PTCs, nodular hyperplasia and normal thyroid tissues

HIF-1α, MT and SLUG protein expression were examined by immunohistochemistry and illustrated in **Figure 1**. The immunoreactivities of HIF-1α, MT and SLUG were detected in the cytoplasm and nuclei. In nodular hyperplasia tissues, there were only a few follicular cells with weak staining for HIF-1α (A), MT (B) and SLUG (C). However, in PTCs, some cases had quite a few tumor cells with moderate staining for these three molecules (D-F), and some cases had a lot of tumor cells with strong staining for the three molecules (G-I). As shown in **Tables 1** and **2**, like the normal thyroid tissues, the majority of nodular hyperplasia tissues have negative or 1 IHC score, no cases showed high expression (≥5) of these three molecules. However, in PTCs, the majority of cases have ≥3 IHC score, high expression (≥5) was present in 79 (61.2%), 59 (45.7%) and 68 (52.7%) of 129 cases for HIF-1α, MT and SLUG, respectively. The differences in HIF-1α, MT and SLUG protein expression levels between PTCs and normal thyroid tissues as well nodular hyperplasia tissues were statistically significant ($P<0.001$).

Correlation of HIF-1α, MT and SLUG protein expression with clinicopathological features in PTCs

The correlation of HIF-1α, MT and SLUG protein expression with clinicopathological data was assessed by Chi-square test and summarized in **Table 2**. There were no significant differences in HIF-1α, MT and SLUG protein expression between patients with different histologic subtype of PTC ($P=0.768$, $P=0.673$, $P=0.867$ respectively), between older (>45) and younger (≤45) patients ($P=0.175$, $P=0.581$, $P=0.824$, respectively), between male and female patients ($P=0.504$, $P=0.577$, $P=0.957$, respectively), between patients with larger (>2.3) and smaller (≤2.3) tumor size ($P=0.232$, $P=0.469$, $P=0.927$, respectively). However, HIF-1α, MT and SLUG protein expression were significantly correlated with TNM stage and LNM, PTC patients with high TNM stage (III-IV) had higher protein expression of these three molecules than those with low TNM stage ($P=0.003$, $P=0.002$, $P=0.024$, respectively), and PTC patients with LNM showed higher protein expression of these three molecules than those without LNM ($P<0.001$ for all the three molecules).

Correlation of HIF-1α, MT and SLUG protein expression with one another in PTCs

The correlation of HIF-1α, MT and SLUG protein expression with one another was assessed by Spearman rank test. As shown in **Table 3**, 45/129 PTCs showed high expression and

Table 2. Correlation of HIF-1α, MT and SLUG protein expression with clinicopathological parameters in 129 PTCs

Characteristics	Case (n)	HIF-1α			MT			SLUG		
		Low	High	*P*-value	Low	High	*P*-value	Low	High	*P*-value
Tissue type										
Normal thyroid tissue	118	118	0		118	0		118	0	
Nodular hyperplasia	61	61	0	-	61	0	-	61	0	-
PTC	129	50	79	<0.001[a] <0.001[b]	70	59	<0.001[a] <0.001[b]	61	68	<0.001[a] <0.001[b]
Classic PTC	80	33	47	0.768	41	39	0.673	40	40	0.867
Follicular variant of PTC	21	6	15		12	9		9	12	
Tall cell variant of PTC	15	6	9		8	7		6	9	
Oncocytic variant of PTC	13	5	8		9	4		6	7	
Age (years)										
≤45	69	23	46	0.175	39	30	0.581	32	37	0.824
>45	60	27	33		31	29		29	31	
Gender										
Male	32	14	18	0.504	16	16	0.577	15	17	0.957
Female	97	36	61		54	43		46	51	
Tumor size (cm)										
≤2.3	83	29	54	0.232	47	36	0.469	39	44	0.927
>2.3	46	21	25		23	23		22	24	
TNM stage										
I-II	69	35	34	0.003	46	23	0.002	39	30	0.024
III-IV	60	15	45		24	36		22	38	
Lymph node metastasis										
Absent	61	35	26	<0.001	52	9	<0.001	45	16	<0.001
Present	68	15	53		18	50		16	52	

P-values derived using Chi-square test to compare the expression of HIF-1α, MT and SLUG between subgroups defined by each clinicopathological parameter; [a]stands for significant difference between PTCs and normal thyroid tissues; [b]stands for significant difference between PTCs and nodular hyperplasia. P<0.05 stands for significant difference.

Table 3. Correlation of HIF-1α, MT and SLUG protein expression with one another in 129 PTCs

Proteins	HIF-1α				SLUG			
	Low	High	r_s	*P*-value	Low	High	r_s	*P*-value
MT								
Low	36	34	0.283	0.001	45	25	0.371	<0.001
High	14	45			16	43		
SLUG								
Low	32	29	0.266	0.002				
High	18	50						

P-values for Spearman rank test; HIF-1α, MT and SLUG were tested pairwise; P<0.05 stands for significant difference.

36/129 displayed low expression for both HIF-1α and MT. The correlation between HIF-1α and MT expression was statistically significant (r_s=0.283, P=0.001). Similarly, there was a statistically significant correlation between expression of HIF-1α and SLUG (r_s=0.266, P=0.002). For both HIF-1α and SLUG, 50/129 PTCs showed high expression. In addition, high expression for both MT and SLUG was present in 43/129 PTCs. A significantly positive correlation (r_s=0.371, P<0.001) was also present between expression of MT and SLUG.

Association of concomitant HIF-1α, MT and SLUG high expression with TNM stage and LNM in PTCs

Given that HIF-1α, MT and SLUG protein expression were correlated with one another, and statistical analysis showed that PTC patients with high TNM stage and LNM have higher protein expression of these three molecules than those

Table 4. Correlation of concomitant expression of HIF-1α, MT and SLUG with TNM stage

	TNM stage		
	I-II, n (%)	III-IV, n (%)	*P*-value
HIF-1α/MT			<0.001[a]
(1) Both HIF-1α/MT low expression	32 (88.9)	4 (11.1)	
(2) One of HIF-1α/MT high expression	27 (56.2)	21 (43.8)	0.001[b]
(3) Both HIF-1α/MT high expression	10 (22.2)	35 (77.8)	
MT/SLUG			<0.001[a]
(1) Both MT/SLUG low expression	39 (86.7)	6 (13.3)	
(2) One of MT/SLUG high expression	22 (53.7)	19 (46.3)	0.001[b]
(3) Both MT/SLUG high expression	8 (18.6)	35 (81.4)	
HIF-1α/SLUG			<0.001[a]
(1) Both HIF-1α/SLUG low expression	27 (84.4)	5 (15.6)	
(2) One of HIF-1α/SLUG high expression	30 (63.8)	17 (36.2)	<0.001[b]
(3) Both HIF-1α/SLUG high expression	12 (24.0)	38 (76.0)	
HIF-1α/MT/SLUG			
Not all of HIF-1α/MT/SLUG high expression	67 (69.8)	29 (30.2)	<0.001[c]
All of HIF-1α/MT/SLUG high expression	2 (6.1)	31 (93.9)	

Correlation of concomitant expression of HIF-1α, MT and SLUG with TNM stage was measured by Chi-square test; [a]stands for significant difference among the three groups; [b]stands for significant difference between group (2) and group (3); [c]stands for significant difference between groups with and without concomitant expression of all three molecules.

Table 5. Correlation of concomitant expression of HIF-1α, MT and SLUG with LNM

	LNM		
	Absent n (%)	Present n (%)	*P*-value
HIF-1α/MT			<0.001[a]
(4) Both HIF-1α/MT low expression	30 (83.3)	6 (16.7)	
(5) One of HIF-1α/MT high expression	22 (45.8)	26 (54.2)	0.008[b]
(6) Both HIF-1α/MT high expression	9 (20.0)	36 (80.0)	
MT/SLUG			<0.001[a]
(4) Both MT/SLUG low expression	37 (82.2)	8 (17.8)	
(5) One of MT/SLUG high expression	18 (43.9)	23 (56.1)	0.002 [b]
(6) Both MT/SLUG high expression	6 (14.0)	37 (86.0)	
HIF-1α/SLUG			<0.001[a]
(4) Both HIF-1α/SLUG low expression	27 (84.4)	5 (15.6)	
(5) One of HIF-1α/SLUG high expression	22 (46.8)	25 (53.2)	0.019[b]
(6) Both HIF-1α/SLUG high expression	12 (24.0)	38 (76.0)	
HIF-1α/MT/SLUG			
Not all of HIF-1α/MT/SLUG high expression	60 (62.5)	36 (37.5)	<0.001[c]
All of HIF-1α/MT/SLUG high expression	1 (3.0)	32 (97.0)	

Correlation of concomitant expression of HIF-1α, MT and SLUG with LNM was measured by Chi-square test; [a]stands for significant difference among the three groups; [b]stands for significant difference between group (2) and group (3); [c]stands for significant difference between groups with and without concomitant expression of all three molecules.

with low TNM stage and without LNM, we further evaluated the correlation of TNM stage and LNM with concomitant expression of HIF-1α/MT, MT/SLUG or HIF-1α/SLUG. As shown in **Tables 4** and **5**, the incidences of high TNM stage and LNM were significantly higher in patients with high expression of HIF-1α/MT (77.8% and 80.0%, respectively) than in those patients with high expression of only one of these two molecules (43.8% and 54.2%, respectively), or in those patients without high expression for either of these two molecules (11.1% and 16.7%, respectively). Similar results were observed in PTCs with high expression of MT/SLUG and HIF-1α/SLUG. There were statistically signifi-cant differences in the incidence of high TNM stage and LNM between patients with high expression of only one and any two of the three molecules (P≤0.001 for HIF-1α/MT, MT/SLUG and HIF-1α/SLUG in TNM stage, P=0.008, 0.002, 0.019 for HIF-1α/MT, MT/SLUG and HIF-1α/SLUG in LNM, respectively). In addition, statistical analysis showed that concomitant high expression of all the three molecules is signi-ficantly associated with high TNM stage and LNM as compared with cases not showing such expression (P<0.001). As demonstrated in **Figure 2**, A-C is a representative of PTC with TNM stage I and without LNM showing low expres-

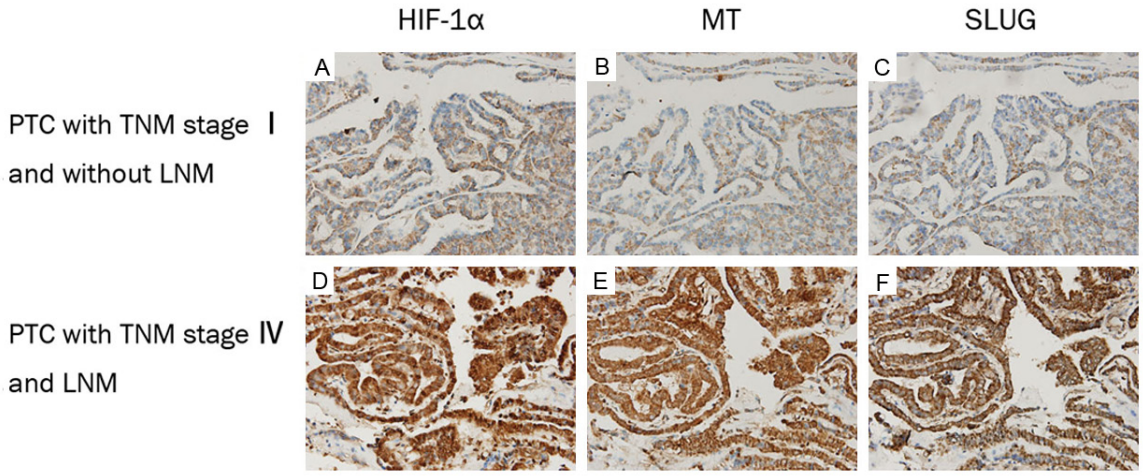

Figure 2. Association of concomitant HIF-1α, MT and SLUG high expression with high TNM stage and LNM in PTCs. Columns correspond to immunostaining for HIF-1α, MT and SLUG, respectively. The first row is the immunostaining of a representative of PTC with TNM stage and without LNM showing low expression of HIF-1α (A), MT (B) and SLUG (C); the second row is the immunostaining of a representative of PTC with TNM stage IV and LNM showing high expression of HIF-1α (D), MT (E) and SLUG (F). All the pictures are in high-power fields (× 400).

sion of HIF-1α, MT and SLUG; D-E is a representative of PTC with TNM stage IV and LNM showing high expression of all the three molecules, HIF-1α, MT and SLUG, respectively.

Discussion

Hypoxia is a common condition found in a wide range of solid tumors and has been increasingly recognized to play a central role in different stages of tumor progression [2, 6]. Tumor adaptation to hypoxia is predominantly regulated by HIF-1α. HIF-1α promotes invasion and metastasis through regulation of critical factors controlling metastatic potential of tumor cells, such as MT [12, 13] and SLUG [17, 18]. To date, studies have shown that HIF-1α, MT and SLUG are overexpressed in several human tumors, including those of ovarian, oesophageal, head and neck [6], breast, prostate, bladder, and oral epithelium [11, 15, 16]. Moreover, there have been several studies which respectively showed that HIF-1α [5], MT [21, 22] or SLUG [23] is upregulated in thyroid carcinoma compared with normal thyroid tissues or benign lesion. However, no study examined simultaneously the expression of HIF-1α, MT and SLUG, and assessed systematically correlation of their expression with clinicopathological features in PTC. In our present study, we examined HIF-1α, MT and SLUG protein expression in 129 PTCs, 61 nodular hyperplasia and 118 normal thyroid tissues using immunohistochemistry. The results demonstrated that no cases of normal thyroid tissue and nodular hyperplasia show high protein expression of HIF-1α, MT and SLUG. However, in PTCs, high protein expression was present in 61.2%, 45.7% and 52.7% of cases for HIF-1α, MT and SLUG, respectively. The differences in HIF-1α, MT and SLUG protein expression between PTCs and normal thyroid tissues as well nodular hyperplasia were statistically significant ($P<0.001$). Then we assessed the correlation of HIF-1α, MT and SLUG protein expression with several clinicopathological indicators. We found that HIF-1α, MT and SLUG protein expression were not associated with histologic subtype, gender, age, tumor size. However, there was a significant correlation of HIF-1α, MT and SLUG protein expression in relative to TNM stage and LNM. High protein expression of HIF-1α, MT and SLUG was associated with high TNM stage and LNM. These results suggested that HIF-1α, MT and SLUG may play an important role in invasion, metastasis and progression of PTC.

Additionally, our study, for the first time, demonstrated a significantly positive correlation between HIF-1α, MT and SLUG expression in PTCs. HIF-1α expression is positively correlated with MT expression ($r_s=0.283$, $P=0.001$) and SLUG expression ($r_s=0.266$, $P=0.002$). This finding could be supported by several recent studies. It has been reported that HIF-1α can act as a transcriptional coactivator to upregu-

late MT expression in hypoxic condition [12, 13]. MT has been shown to be a target gene of HIF-1α. Conversely, MT can increase HIF-1α transcriptional activity by suppressing ROS accumulation or activating ERK/mTOR pathway [24, 25]. There exists mutual promotion between HIF-1α and MT. Meanwhile, HIF-1α can also serve as a transcriptional activator to induce SLUG expression in hypoxia [17, 18]. Moreover, a significantly positive correlation (r_s=0.371, P<0.001) was present between expression of MT and SLUG. This result would be explained by that MT may upregulate SLUG expression through increasing HIF-1α transcriptional activity.

Given that HIF-1α, MT and SLUG protein expression were positively correlated with one another, and the expression of these single molecules was related to TNM stage and LNM, we subsequently evaluated the association of concomitant expression of HIF-1α, MT and SLUG with TNM stage and LNM in PTCs. The results showed that concomitant high expression of any two of these three molecules had stronger correlation with high TNM stage and LNM than did each alone. Concomitant high expression of all three molecules strongly correlates with high TNM stage and LNM.

In summary, our results, for the first time, demonstrated a positive correlation of HIF-1α, MT and SLUG expression in PTCs. High expression of HIF-1α, MT and SLUG was associated with high TNM stage and LNM. Concomitant high expression of any two or all of the three molecules had stronger correlation with high TNM stage and LNM than did each alone. Consequently, our results provide a possible basis for prediction of LNM and progression in PTC. Future studies in larger sets of patients will be necessary to determine the utility of these molecules as biomarkers of tumor diagnosis and prognosis in PTC.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhi-Min Liu, Department of Biochemistry and Molecular Biology, Molecular Medicine and Cancer Research Center, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China. Tel: 86-23-68485868; Fax: 86-23-68485868; E-mail: liuzm9999@aliyun.com

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