Relationships between *SLC26A7* expressions and extra-thyroid metastasis of papillary thyroid carcinoma

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Papillary thyroid carcinoma (PTC) is the most common type (accounting for over 80%) of thyroid cancer.[1] Although PTC is a well-differentiated endocrine neoplasm, unfortunately, certain PTCs show malignant characteristics such as aggressiveness and metastasis; these characteristics contribute to a poor 5-year survival rate.[1] However, the fundamental molecular mechanism of extra-thyroid metastasis remains unclear.

The solute carrier family 26 member 7 (*SLC26A7*) gene, an anion transporter, previously was reported to function as a chloride-bicarbonate exchanger in the kidney and stomach. The latest research has indicated *SLC26A7* functions as a novel iodide transporter in the thyroid; *SLC26A7* dysfunction can affect thyroid hormonogenesis and cause congenital goitrous hypothyroidism.[2] Importantly, low *SLC26A7* expression might contribute to carcinogenesis in anaplastic thyroid carcinoma, and significantly correlated with patients' poor prognosis.[3] However, the regulation of *SLC26A7* and the underlying mechanism of how its expression affects extra-thyroid metastasis of PTC have not been completely elucidated. Therefore, the present study aimed to explore the effect of *SLC26A7* expression on clinicopathological characteristics of PTCs, and further reveal the possible trigging factors.

Three microarrays datasets, GSE129562, GSE60542, and GSE6004, were obtained from the Gene Expression Omnibus (GEO) repository. GEO2R was used to screen differentially expressed genes (DEGs) between PTC tumors and normal thyroid. Filtered cut-off criteria were established according to adjusted *P* value <0.05 and $|\log(\text{fold change})| > 1$, visualized in a volcano plot. All the included tumors had metastasized. Overlapped DEGs were defined as intersections genes, visualized in a Venn plot. Gene Ontology (GO) terms annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were employed to functionally enrich DEGs using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8, as visualized in a bubble plot. GO comprises biological processes, cellular components, and molecular functions. The Cancer Genome Atlas (TCGA) clinical cohort database was downloaded from cBioPortal for Cancer Genomics (http://www.cbioportal.org/) online platform and used to conduct survival analysis. Gene expression profiling interactive analysis (GEPIA) was used for expression and staging analysis, visualized in boxplot and violin plot.

To validate the reliability of the public database mining, a hospital-based cross-sectional study was performed. This study was approved by the Research Ethics Committee of the Second Hospital of Shandong University (No. KYLL-2019(KJ)P-0084) and was performed in accordance with the ethical standards of the *Declaration of Helsinki*. The inclusion and exclusion criteria are the same as those of our previous study.[4] Informed consent was obtained from all patients with PTC who underwent thyroidectomy at the Department of Thyroid Surgery, the Second Hospital of Shandong University (Jinan, China), from July 2019 to December 2019. The pathology was determined postoperatively by the pathologist from the Department of Pathology. After excluding medullary, follicular, and other thyroid carcinomas, 35 PTCs were identified.

Tissue RNA isolation and real-time polymerase chain reaction (RT-PCR) assay used a reagent kit (Servicebio, Wuhan, Hubei, China, No. G3013) and followed the manufacturer's instructions. The primer sequences were shown in Supplementary Table 1, http://links.lww.com/

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CM9/A701. The quantitative analysis calculated (according to the $2^{-\Delta\Delta Ct}$ method) and compared the ratio of the mRNA expression in tumor with that in normal thyroids. A ratio of >1.00 was defined as up-regulated; <1.00 was defined as down-regulated. The methods of urinary iodine concentration (UIC) detection, water iodine assessment, and clinicopathological characteristics extraction were the same as those in our previous study.[4]

Continuous data were presented as mean ± standard deviation and categorical data were expressed as numbers (percentages). Student's *t* test and Fisher exact test were used for comparative analysis using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Survival curves were generated via the Kaplan–Meier method in R 3.5.1 (The University of Auckland, Auckland, New Zealand), and the significance was analyzed using a log-rank test. Logistic regression analysis was performed using SPSS 22.0. Photoshop 13.0.1 (San Jose, California, USA) and "ggplot2" in R 3.5.1 were employed for the visualization of experimental data. $P < 0.05$ was regarded as statistically significant.

A total of 190 overlapped DEGs (102 up-regulated and 88 down-regulated) were identified among the three microarray datasets: 438 up-regulated and 335 down-regulated (total 773) in GSE129562, 526 up-regulated and 578 down-regulated (total 1104) in GSE60542, and 178 up-regulated and 145 down-regulated (total 323) in GSE6004 [Supplementary Figure 1 and Supplementary Table 2, http://links.lww.com/CM9/A701]. GO and KEGG analyses showed that up-regulated DEGs were mainly enriched in "pathways in cancer", "wound healing", "extracellular exosome", and "protein homodimerization activity" [Supplementary Figure 2, http://links.lww.com/CM9/A701]; whereas, down-regulated DEGs were mainly enriched in "mineral absorption," "cellular response to zinc ion," "extracellular space," and "calcium ion binding" [Supplementary Figure 3, http://links.lww.com/CM9/A701].

We chose *SLC26A7* from 190 overlapped DEGs for further analysis, because it was found to function as a novel iodide transporter in the thyroid in 2019 and its dysfunction might contribute to carcinogenesis,[2] but the effect of *SLC26A7* expression on clinicopathological characteristics in PTC is still unclear. TCGA analysis showed lower expression of *SLC26A7* correlated with shorter disease-free survival (DFS)/progression-free survival (PFS) and worse staging; the expression of *SLC26A7* in the tumor was down-regulated compared with that in normal thyroid [Supplementary Figure 4, http://links.lww.com/CM9/A701].

To verify the reliability of the public database mining and evaluate the effect of *SLC26A7* expression levels on clinicopathological characteristics, 35 PTC (10 men and 25 women; age: mean ± standard deviation, 44.3 ± 12.6 years; range, 24–72 years) specimens with their adjacent normal thyroid tissues were collected to detect the *SLC26A7* mRNA expression, using RT-PCR. About one-third of the analyzed specimens [34.3% (12/35)] exhibited low *SLC26A7* expression in tumors compared with those in the adjacent normal thyroids. The proportion of extra-thyroid metastasis in patients with low *SLC26A7* expression was significantly higher than that in high *SLC26A7* expression [83.3% (10/12) *vs.* 43.5% (10/23), $P = 0.026$]. No notable association was observed between *SLC26A7* expressions with other clinicopathological characteristics [Table 1]. Logistic regression analysis showed that low *SLC26A7* was identified as an independent risk factor for extra-thyroid metastasis (multivariate analysis: odds ratio [OR] = 7.108; 95% confidence interval [CI]: 1.048–48.218, $P = 0.045$] [Supplementary Table 3, http://links.lww.com/CM9/A701]. Suppressing *SLC26A7* may create global hypomethylation of the genome and hypermethylation of the CpG islands leading to thyroid carcinogenesis and metastasis.[3] Therefore, suppressing *SLC26A7* might disturb methylation to promote extra-thyroid metastasis of PTCs.

To determine the trigging factors of *SLC26A7* down-regulation, we assessed the relationship between iodine nutrition status and *SLC26A7* expression. More than 90% of ingested iodine is excreted in urine, so we used UIC (a widely accepted indicator) to indicate the iodine nutrition status. A high UIC was significantly associated with low *SLC26A7* expression [17.6% (3/17) for non-high UIC *vs.* 50.0% (9/18) for high UIC, $P = 0.047$] and extra-thyroid metastasis [35.3% (6/17) for non-high UIC *vs.* 77.8% (14/18) for high UIC, $P = 0.013$] [Supplementary Table 4, http://links.lww.com/CM9/A701]. High UIC was identified as an independent predictor for low *SLC26A7* (multivariate analysis: OR = 5.980, 95% CI: 1.067–33.515, $P = 0.042$) and extra-thyroid metastasis (multivariate analysis: OR = 6.846; 95% CI: 11.352–34.664, $P = 0.020$) [Supplementary Tables 5 and 6, http://links.lww.com/CM9/A701]. It should be noted that treatment with high doses of iodine (7.3 mg/L) can dramatically reduce the expression levels of iodine transporters in Wistar rat thyroid tissues compared with that of controls.[5] Down-regulated iodine transporters may promote local invasion and distant metastasis seen in thyroid carcinoma patients.[3] Therefore, we speculated that high iodine might function as the trigging factor for down-regulation of *SLC26A7* and promote extra-thyroid metastasis of PTCs.

Our study has some limitations. First, there was no sufficient evidence to infer that *SLC26A7* plays the most significant role in extra-thyroid metastasis; other regulatory pathways might be involved. Second, this is only a correlation evaluation performed based on a cross-sectional study with a small sample size, and lacks the results supported by a clinical cohort with a larger sample. These findings need to be verified in experimental animals to clarify the upstream and the downstream regulatory relationships and identify the most significant pathway.

This study suggests down-regulation of *SLC26A7*, which may be trigged by high iodine, might play a role in promoting extra-thyroid metastasis of PTCs. Further animal experiments are needed to explore this regulatory relationship.

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Table 1: Clinicopathological characteristics of PTC patients with different *SLC26A7* expression levels ($n = 35$).

Characteristics	Total ($n = 35$)	*SLC26A7* expression		Statistics	*P*
		Low ($n = 12$)	High ($n = 23$)		
Age					
≤45 years	16 (45.7)	7 (58.3)	9 (39.1)	–	0.311
>45 years	19 (54.3)	5 (41.7)	14 (60.9)		
Gender				–	0.434
Female	25 (71.4)	10 (83.3)	15 (65.2)		
Male	10 (28.6)	2 (16.7)	8 (34.8)		
BMI				–	0.583
<24.0 kg/m^2	14 (40.0)	5 (41.7)	9 (39.1)		
≥24.0 kg/m^2	21 (60.0)	7 (58.3)	14 (60.9)		
Blood pressure (mm/Hg)					
Systolic	79.1 ± 8.7	79.5 ± 9.0	78.8 ± 8.8	0.214*	0.832
Diastolic	130.2 ± 12.9	133.0 ± 15.1	128.8 ± 11.8	0.912*	0.369
Ultrasound					
TIRADS grade				–	0.077
≥4c	19 (54.3)	9 (75.0)	10 (43.5)		
≤4b	16 (45.7)	3 (25.0)	13 (56.5)		
Multifocality				–	0.285
Yes	11 (31.4)	5 (41.7)	6 (26.1)		
No	24 (68.6)	7 (58.3)	17 (73.9)		
Pathology					
Primary tumor size				–	0.408
>1.0 cm	18 (51.4)	7 (58.3)	11 (47.8)		
≤1.0 cm	17 (48.6)	5 (41.7)	12 (52.2)		
Capsular invasion				–	0.569
Yes	30 (85.7)	10 (83.3)	20 (86.9)		
No	5 (14.3)	2 (16.7)	3 (13.1)		
Location				–	0.603
Bilaterally	15 (42.8)	5 (41.7)	10 (43.5)		
Unilaterality	20 (57.2)	7 (58.3)	13 (56.5)		
Extra-thyroid metastasis				–	0.026
Yes	20 (57.1)	10 (83.3)	10 (43.5)		
No	15 (42.9)	2 (16.7)	13 (56.5)		
Complication				–	0.483
Yes	22 (62.9)	7 (58.3)	15 (65.2)		
No	13 (37.1)	5 (41.7)	8 (34.8)		
Lymph nodes district				–	0.257
Central	27 (77.1)	8 (66.7)	19 (82.6)		
Non-central	8 (22.9)	4 (33.3)	4 (17.4)		
Central lymph metastasis				–	0.062
Yes	16 (59.3)	7 (87.5)	9 (47.4)		
No	11 (40.7)	1 (12.5)	10 (52.6)		

Data were shown as mean ± standard deviation or *n* (%). *t values. BMI: Body mass index; PTC: Papillary thyroid carcinoma; TIRADS: Thyroid Imaging, Reporting and Data System; –: Not applicable.

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Conflicts of interest

None.

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