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ORIGINAL ARTICLE

Correlation between sodium-iodide symporter expression and circulating tumor cell positivity in differentiated thyroid carcinoma*

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Abstract

Objective We investigated the correlation between the expression of the sodium-iodide symporter (NIS) and the detection of circulating tumor cells (CTCs) in differentiated thyroid carcinoma (DTC).

Methods NIS expression in differentiated thyroid and the positive rate of CTCs in the peripheral blood were determined by immunohistochemistry S-P and flow cytometry from the records of 172 cases of differentiated thyroid carcinoma.

Results Seventy-six cases (44.2%) expressed NIS in the differentiated thyroid and 63 cases (36.6%) were positive for CTCs in the peripheral blood. There was a significant difference between N0 and N1 in the expression of NIS (χ^2 = 6.015, P = 0.014) and the positive rate of CTCs (χ^2 = 14.035, P = 0.001). N0 and N1 also differed significantly in the expression of NIS (r = -0.383, -0.610, P = 0.002, < 0.001). The differences in the NIS expression, but not in the positive rate of CTCs, were significant among the different pathological subtypes (χ^2 = 7.897, P = 0.005; χ^2 = 1.455, P = 0.228, respectively). There was a significant negative correlation between the highly differentiated type and intermediate differentiation type both in the expression of NIS and positive rate of CTCs (r = -0.591, -0.443, P < 0.001, P = 0.002).

Conclusion There was a significant negative correlation between the expression of tissue NIS and positive rate of CTCs in the peripheral blood in DTC. The malignancy level and lymph node metastasis in differentiated thyroid carcinoma were negatively correlated with NIS expression and positively correlated with the positive rate of CTC.

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Keyword: differentiated thyroid cancer (DTC); sodium-iodide symporter (NIS); circulating tumor cell flow cytometry

In recent years, the sodium-iodide symporter (NIS) and circulating tumor cells (CTCs) have been studied in thyroid carcinoma. These factors have gradually become a reference index for cancer diagnosis, therapy evaluation, and prognosis [1-3]. However, whether CTCs can be used to indicate individualized treatment of tumors has not been widely examined. In this study, we evaluated the correlation between NIS expression and the positive rate of CTCs in differentiated thyroid carcinoma. We have also discussed herein the radioiodide treatment of CTCs in differentiated thyroid carcinoma.

Materials and methods

Subjects

From February 2008 to October 2013, 172 cases of differentiated thyroid carcinoma were enrolled in Gansu Provincial Tumor Hospital, China. There were 38 males and 134 females, age 14–73 years, with a median age of 36.7 years. All patients were pathologically diagnosed according to uniform standards for diagnosis and treatment [4–5]. There were separated into 4 groups based on tumor size: 28 cases of T1, 67 cases of T2, 46 cases of T3, and 31 cases of T4. Sixty-four cases were lymph node stage N0 and 108

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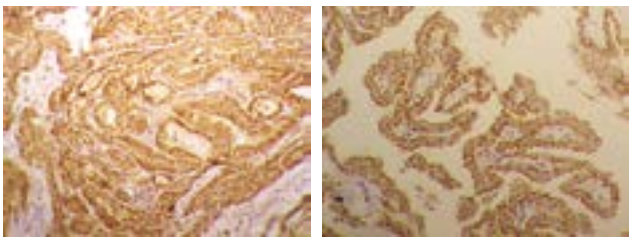

Fig. 1 Immunohistochemical detection of NIS-positive expression in thyroid cancer (× 400)

cases were lymph node stage N1. Well-differentiated tumors were diagnosed in 124 cases and intermediated tumors were diagnosed in 48 cases. Before obtaining blood samples, all patients provided written informed consent. Patients did not receive chemotherapy, radiation therapy, or radionuclide therapy before blood sample collection.

Equipment and reagents

The FACS Calibur flow cytometer was from BD Biosciences (USA). Anti-CK19/FITC were purchased from eBioscience (USA). The Muc1/CD227 antibody was from Abcam (UK). Breaking agent (No. 641776) and FACS hemolysin (No. 349202) were purchased from BD Biosciences. NIS mouse anti-human monoclonal antibody (MAB3562) was from Millipore (USA). The S-P kit was from Boster Bioengineering Co., Ltd. (China).

Immunohistochemical analysis

All slides were stained with immunohistochemistry S-P by immunohistochemistry (IHC). Analysis was conducted by one pathologist (AB) who was blinded to the related clinical information. NIS expression was used as positive control and primary antibody substituted for phosphate-buffered saline was used as a negative control. NIS-positive expression was mainly observed on the cell membrane, and positive expression was claybank or brown, but the negative result showed no color. IHC results were analyzed by two pathologists, defined in a semiquantitative manner, using the I × E product method[6]. Each slice was randomly selected from five visual fields under a microscope (× 400), and each field had an average of 200 cells. The I-Grading scale was as follows: 0, same as the background or weak staining; 1+, pale yellow staining; 2+, yellow or claybank staining; 3+, brown staining. The E-Grading scale was as follows: 0, ≤ 10%; 1+, 10–25%; 2+, 26–50%; 3+, ≥ 51%. I × E integral evaluation: ≤ 1, negative (-); 1–4, weakly positive (+); ≥6, strongly positive (+ +) (Fig. 1).

Detection of CTC in peripheral blood

Five milliliters of venous blood were collected 1 week after surgery, 10% EDTA-Na_2 was used for anticoagulation, and marked respectively by anti-CK19 and MUC1/CD227. A FACS Calibur flow cytometer was used, setting forward scatter and side scatter to eliminate the various fragments and granulum from the sample. The results excluded single positive cases of cytokeratin 19 (CK19) or polymorphic epithelial mucin1 (MUC1). Both CK19 and MUC1 were expressed as CTC-positive cases in the peripheral blood.

Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (USA), and data from two groups were compared using Fisher's exact test or χ^2 analysis. Statistical analysis was performed by using the Pearson method, to test the significance, the risk level was set to 0.05.

Results

NIS and CTC-positive expression (Table 1)

In 172 cases of differentiated thyroid carcinoma patients, 76 cases were NIS-positive in thyroid cancer tissue (44.2%), while 63 cases were CTC-positive in the peripheral blood (36.6%).

Differences in gender, age (≤ 45 years old, > 45 years old), NIS-positive rates, and CTC-positive rates in thyroid cancer patients were not significant.

The NIS-positive rates were significantly different between lymph node stage N0 and N1 (χ^2 = 6.015, P = 0.014), and the CTC-positive rates were significantly different (χ^2 = 14.035, P = 0.001). The NIS-positive rates were significantly different among the pathological subtypes, while CTC-positive rates were significantly different (χ^2 = 1.455, P = 0.228).

Correlation between NIS-positivity and CTC-positivity

There was a significant negative correlation in lymph node stage N0 between the NIS-positive rates and CTC-positive rates (r = -0.383, P = 0.002), and in lymph node stage N1 (r = -0.610, P < 0.001). There was also a significant negative correlation in highly differentiated between cases the NIS-positive rates and CTC-positive rates (r = -0.591, P < 0.001), and in intermediate cases (r = -0.443, P = 0.002) (Table 2).

Discussion

NIS is a class of membrane proteins on the basement membrane of thyroid follicular epithelial cells, which mediates active transport of iodine in the thyroid. Their main role is to promote the reverse concentration

Table 1 NIS and CTC expression in differentiated thyroid carcinoma

	n	NIS expression		χ^2	*P*	CTC expression		χ^2	*P*
		(+)	(-)			(+)	(-)		
Sex									
Males	38	15 (39.5%)	23 (60.5%)	0.49	0.508	15 (39.5%)	23 (60.5%)	0.170	0.680
Females	134	61 (45.5%)	73 (54.5%)			48 (35.8%)	86 (64.2%)		
Age (years)									
≤ 45	98	45 (45.9%)	53 (54.1%)	0.277	0.599	34 (34.7%)	64 (65.3%)	0.367	0.545
> 45	74	31 (41.9%)	53 (54.1%)			29 (39.2%)	45 (60.8%)		
Lymph node stage									
N0	64	36 (56.3%)	28 (43.7%)	6.015	0.014	12 (18.75%)	52 (81.25%)	14.035	< 0.001
N1	108	40 (37.0%)	68 (63.0%)			51 (47.22%)	57 (52.78%)		
Pathologically									
Highly differentiated	124	63 (50.8%)	61 (49.2%)	7.897	0.005	42 (33.87%)	82 (66.13%)	1.455	0.228
Intermediate	48	13 (27.1%)	35 (72.9%)			21 (43.75%)	27 (56.25%)		

Table 2 Comprehensive analysis of NIS-expression and CTC-positive results in DTC

	n	Conditions				*P*	*r*
		CTC(-) NIS(+)	CTC(-) NIS(-)	CTC(+) NIS(-)	CTC(+) NIS(+)		
Lymph node stage							
N0	64	34	18	10	2	0.002	-0.383
N1	108	37	20	48	3	< 0.001	-0.610
Pathologically							
Highly-differentiated	124	59	23	38	4	< 0.001	-0.591
Intermediate	48	12	15	20	1	0.002	-0.443

gradient of thyroid to transport inorganic iodine and participate in the biosynthesis of thyroid hormone. Iodide uptake in thyroid follicular cells mainly depends on NIS function and structural integrity. DTC has some functions of normal thyroid cells and maintains their iodide uptake ability. NIS expression is the basis of diagnosis and radioiodide therapy for DTC. Diagnosis and treatment can be determined by detecting the ability of NIS to take up iodide for DTC. However, approximately 30% of patients exhibit "dedifferentiation" in tumor cells because of tumor recurrence, metastasis, chemotherapy, radiotherapy, and ^{131}I therapy. Dedifferentiated thyroid cancer shows a loss of NIS-expression, which is the loss of function of the "iodine pump", resulting in the failure of ^{131}I therapy [7–8]. CTCs are tumor cells that enter the blood circulation through a primary lesion or metastasis, and may enter the circulating blood before forming a solid tumor lesion [9]. Circulation blood is the only method of causing distant metastasis of tumor cells; CTC and hematogenous metastasis of tumors are directly related, and thus the detection of CTCs facilitates the early diagnosis of tumor metastasis, monitoring of postoperative recurrence and metastasis of the tumor, and the choice of individualized treatment strategies [10–11]. According to the results of large sample multivariate analysis, CTCs can be used as independent prognostic factors for tumor treatment as well as dynamic monitoring of CTC changes to predict tumor curative effects and tumor progress earlier in patients with tumors in the peripheral blood of CTC. Surgery alone cannot achieve effect a radical cure, and thus systemic adjuvant therapy is required. Therefore, in-depth studies of CTC can improve the understanding of the mechanisms of tumor metastasis and provide a new basis for early treatment of anti-tumor metastasis [12–13] .

This study showed that patients with thyroid cancer lymph node metastasis N0 and N1, tumor tissue NIS showed significantly positive expression and peripheral blood showed significantly CTC-positive differences (P = 0.041, < 0.001). NIS expression in patients in the N0 group was significantly negatively correlated with the CTC-positive rate (r = 0.383, P = 0.383), while NIS expression in the N1 group showed a significantly negative correlation with the CTC-positive rate (r = 0.610, P < 0.001). These results tentatively suggest that thyroid cancer cells with a loss of NIS expression have actively growing tumors; as the size and internal pressure of the tumor increases, lymph node metastasis occurs, and tumor cells may be removed from the tumor and enter the peripheral circulation. From another perspective, after malignant tumors show metastatic lymph node metastasis, tumor cell proliferation and metabolism are accelerated in the lymphoid tissue. Tumor cells not only metastasize to another lymph node through the lymph, but also enter the blood circulation, resulting in CTC multiplication in the peripheral blood [14]. These thyroid tumor tissues in which NIS showed loss of expression lost the iodine function of NIS, and 131I showed poor efficacy and poor prognosis. The NIS expression-positive rate between various pathological subtype differences were significant (P = 0.005), but there was no significant difference in the rate of positive of

CTCs (P = 0.228). NIS expression in highly differentiated and intermediate tumors was significantly negatively correlated with the CTC-positive rate (r = 0.591, 0.443, P < 0.001, P = 0.002). In the pathological subtype of differentiated thyroid carcinoma, the development of high differentiation was slow, malignant grade, and showed a good prognosis, with a 10-year survival rate of over 85% [15]. Highly differentiated thyroid cancer cells expressed NIS, and radionuclide irradiation therapy was positive and the prognosis was good for NIS-positive expression, but the CTC-positive rate was low in these patients. The deteriorated and lymph node metastasis of thyroid cancer was positively correlated with the peripheral blood CTC-positive rate and negatively correlated with tumor tissue NIS expression. When peripheral blood CTC was positive, tumor tissues lost NIS expression.

This study showed that detection of CTCs in the peripheral blood can be used to preliminarily evaluate NIS expression and predict efficacy, which is very important for radioiodide therapy for DTC. CTCs are promising new circulating markers for DTC, which have excellent practical value of efficacy assessment and prognosis.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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