

## The Expression of Twist in Differentiated Thyroid Carcinomas

CRISTINA MARIANA CALANGIU<sup>1</sup>, C.MĂRGĂRITESCU<sup>2</sup>,  
DANIELA CERNEA<sup>3</sup>, A.STEPAN<sup>2</sup>, CRISTIANA EUGENIA SIMIONESCU<sup>2</sup>

<sup>1</sup> Phd Student, Pathology Department, University of Medicine and Pharmacy of Craiova

<sup>2</sup> Pathology Department, University of Medicine and Pharmacy of Craiova

<sup>3</sup> Department of Care and Anesthesiology, University of Medicine and Pharmacy of Craiova

**ABSTRACT:** Differentiated thyroid carcinoma are lesions with a generally favorable prognosis, although there are patients with risk of recurrence and metastasis. In this study we analyzed 43 thyroid carcinomas referring to Twist expression in relation to clinicopathological parameters. The immunoreaction was identified in 79% of cases, the expression of twist being low or high, with no differences in relation to the tumors type or subtype. Twist immunoeexpression differ depending on tumor stage and presence of metastases, the immunostain being significantly higher in invasive tumors to adjacent structures and in cases of tumors with metastasized. In differentiated thyroid carcinomas overexpression of Twist is associated with an invasive and metastatic immunophenotype.

**KEYWORDS:** thyroid carcinomas, TWIST, immunoexpression

### Introduction

In the last two decades there have been progresses in understanding thyroid carcinoma histogenesis and biology. Papillary thyroid carcinomas (PTC) represents 80% of thyroid malignancies and is characterized by slow growth and an excellent prognosis [1]. Patients with PTC have the best survival rate of all types of thyroid cancer, with a ten-year rate to 95% [2], or by other studies 5-year survival is 96% and in 10 years 93 % [3]. Differentiated follicular thyroid carcinoma (FTC) constitute 10-17% of malignant thyroid pathology and also have a good long-term prognosis [4]. However, some cases have relatively early relapses, invasion of adjacent structures, lymph node metastases or distant metastases [5].

Thyroid carcinoma that invade local structures are associated with poor prognosis, but the invasion mechanisms are incompletely defined, limiting the development of new therapies. Understanding the molecular mechanisms involved in the progression of thyroid cancer may provide targets for more effective treatment in aggressive thyroid cancer.

Twist is considered to have oncogenic potential by promoting the proliferation and inhibition of apoptosis [6]. It acts in cooperation with Bmi1 inhibiting the expression of epithelial markers such as E-cadherin, and is associated with poor prognosis in a variety of cancers of the head and neck [7]. Loss of E-cadherin expression, in turn leads to loss of cell adhesion and polarity and acquisition of migratory and invasive phenotype of epithelial cells [8].

In this study we aimed the analysis of Twist expression in differentiated thyroid carcinomas and the correlation with clinical and morphological parameters.

### Material and Methods

The study included a total of 43 differentiated thyroid carcinomas selected in a period of nine years from patients hospitalized and operated in Surgery Clinics of Emergency County Hospital of Craiova. The surgical pieces were fixed in 10% buffered formalin, processed by the usual technique with paraffin embedding and Hematoxylin-Eosin stain. Classification of the tumors was made in accordance with literature data [1].

Subsequently the specimens were processed by immunohistochemistry, using citrate antigen recovery buffer pH 6 and a peroxidase-based polymer secondary detection system (Histofine polymer-HRP, Nichirei, Japan, ready to use, code 414151F). The visualization of reactions was done with DAB chromogen (code 3467, Dako), and to validate the results were used positive (tonsil) and negative (omitting the primary antibody) external controls. The tissues analyzed were incubated overnight at 4° C with monoclonal mouse antibody antihuman Twist, diluted 1/2000 (LSBio, code LS-C191858)

We quantified semiquantitatively the expression of Twist by a scoring system which has been assigned two independent specialists on the basis of staining intensity and percentage of positive cells [9]. The intensity score was noted with 0 (no staining), 1 (low intensity), 2

(moderate intensity), and 3 (high intensity). The score of the percentage stained cells was noted with 0 (<5% positive cells), 1 (6-25% positive cells), 2 (26-50% positive cells), 3 (51-75% positive cells), and 4 (> 75% positive cells). The multiplication of the intensity and percentage scores allowed the calculation of the composite final score: 0 (negative), + (1-4) + (5-8), and +++ (9-12). For the statistical analysis, a final staining score of negative or + was considered with low expression and a score of final staining ++ or +++ correspond to the group with high expression. The statistical analysis was performed using automatically SPSS10 software and chi-square and Fisher's exact tests.

### Results

Analysis of clinicopathological parameters of the 43 thyroid differentiated carcinomas indicated an upward trend since the third decade of life, the maximum incidence being after age 60 years, predominantly in females (table 1).

Histopathological analysis revealed that the most investigated PTC correspond to conventional type in 23 cases (59%), followed by follicular variant in 13 cases (33.3%), and tall cell variant in 3 cases (7.7%), while for FTC the conventional type was the most common, respectively in 3 cases (75%). The classification of lesions according to the TNM staging indicated 18 cases in stage I, 14 cases in stage II, 7 cases in stage III and 4 cases in stage IVA (Table 1).

**Table 1. The clinicopathological analyzed parameters**

Tumor type/ Parameter		Conventional PTC	Follicular PTC	Tall cell variant PTC	Conventional FTC	Clear cell variant FTC
Age	<45 years	10	4	1	1	0
	>45 years	13	9	2	2	1
Gender	female	20	12	3	3	1
	male	3	1	0	0	0
Stage	I	11	5	0	2	0
	II	7	5	0	1	1
	III	4	2	1	0	0
	IVA	1	1	2	0	0

In 10 cases of PTC, we found the presence of lymph node metastases, from which 5 cases conventional type, 3 cases of follicular variant and 2 cases tall cell variant.

In the study the analysis of TWIST expression revealed positivity in 34 of the cases, the signal being cytoplasmic and nuclear (Table 2). Nuclear or nuclear / cytoplasmic immunostain localization was present in 79% of cases, and negative in 21% of the cases. Because

TWIST is a nuclear transcription factor, for the immunohistochemical analysis we considering only the nuclear signal.

The evaluation of staining scores indicated for the majority of tumors values above 3. All variants of the PTC examined, except the tall cell variant, included cases with both low and high expression for TWIST (fig.1). To note that the three cases of PTC tall cell variant presented high expression for TWIST.

**Table 2. The incidence of thyroid carcinomas expression by TWIST staining scores**

Histologic type/ Immunoreaction	low expression		high expression	
	-	+	++	+++
Conventional PTC	5	8	6	4
Follicular PTC	3	5	4	1
Tall cell variant PTC	0	0	2	1
Conventional FTC	1	1	1	0
Clear cell variant FTC	0	1	0	0

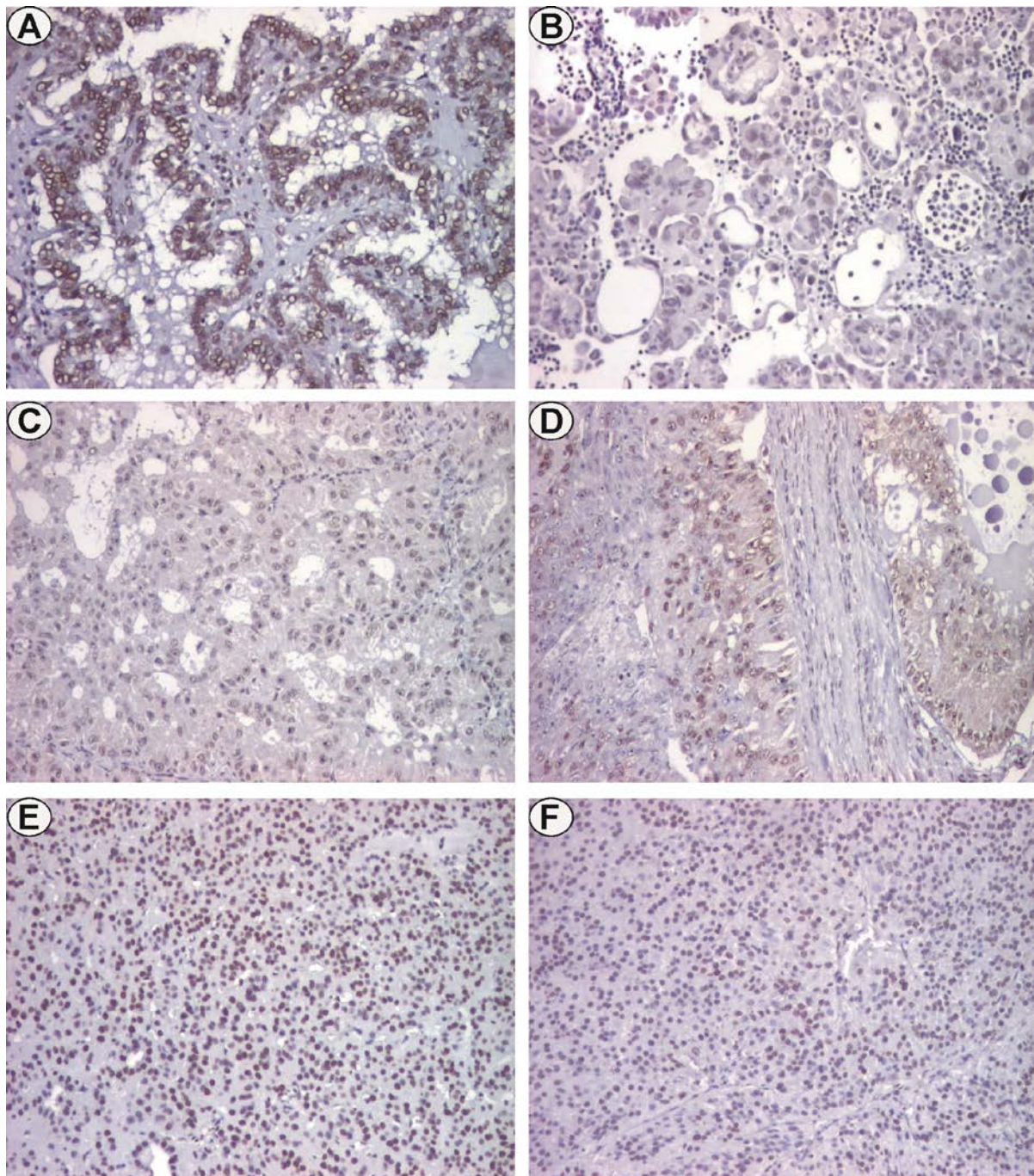
Thus, in the conventional papillary carcinoma, the average score of positivity was 5.83, with mostly moderate / high intensity of reactions and a percentage of the marked cells of

20-80% (fig.1A-B). Regarding follicular and tall cell variants of PTC, the positivity average score was 5.60, respectively 7.00, intensity mostly moderate / high and a percentage of labeled cells



between 20-80% and respectively 50-70 % (fig.1C-D). Comparative, FTC indicated a positivity score of 4.66, the reactions presenting

moderate / high intensity, with a percentage of labeled cells of 25-65% (fig.1E-F).

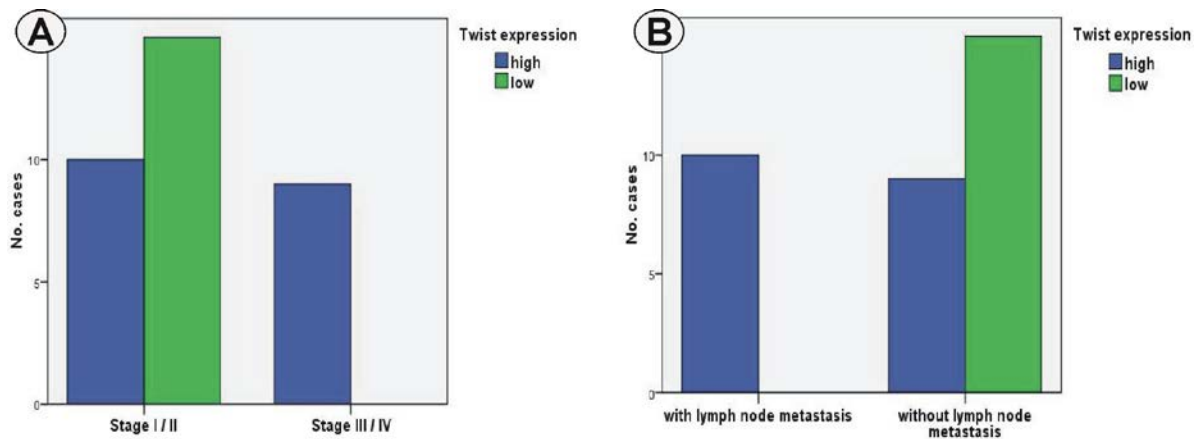


**Fig.1: Thyroid carcinoma, Twist expression, x100. A. Conventional PTC, high expression; B. Conventional PTC, low expression; C. Follicular PTC, low expression; D. Tall cell variant of PTC, high expression; E. Conventional FTC, high expression; F. Conventional FTC, low expression**

We found no differences in Twist expression depending on the type ( $p = 0.571$ , Fisher's Exact Test) or subtype of the tumor ( $p = 0.433$ , chi square test). Also, we found no statistical association of Twist

immunoexpression in relation to gender and age groups of patients.

The high grade Twist expression was associated with cases of thyroid carcinomas that presented invasion of adjacent structures ( $p=0.002$ , chi square test, fig.2A).



**Fig.2: Distribution of Twist positive cases in relation to the stage of tumors (A) and the presence of metastases (B)**

Also high grade Twist expression was associated with thyroid carcinomas that have associated lymph node metastases ( $p=0.01$ , chi square test, fig.2B)

## Discussions

Twist is a member of the class of transcription factors, one of the molecules involved in the regulation of epithelial-mesenchymal transition (EMT) via regulation of key proteins that maintain the characteristics of epithelial cells and which that gives a mesenchymal phenotype [10]. Twist is overexpressed in many tumor types, including breast, gastric, hepatocellular, prostate [11], lung [8], esophagus [12] and bladder [6]. In some studies Twist overexpression is associated with high-grade cancer aggressiveness and poor patient survival rate [13,14].

Epithelial-mesenchymal transition (EMT) is a dedifferentiation program that converts epithelial cells into mesenchymal phenotype and is involved in cancer progression [15]. More recent studies have indicated that EMT also plays a critical role not only in tumor metastasis, but also in tumor recurrence, processes that are considered to be closely related to the biology of cancer stem-like cells or initiation cancer cells [14-18]. Twist is a transcription factor that promotes EMT associated with tumor metastasis, cancer stem cells and drug resistance [19,20].

Twist role in tumor progression is associated with the metastatic process [10]. Twist overexpression increases the invasive and metastatic ability of cancer cells by downregulation of E-cadherin expression and

induction of EMT [10]. Twist plays an important role in some of the processes involved in the development of metastasis, such as angiogenesis, invasion and extravasation of chromosomal instability, and also protects cancer cells from apoptosis [21].

In our study we analyzed the Twist expression in 43 differentiated thyroid carcinomas. Both the variants of papillary and follicular thyroid carcinomas presented variable immunoreactions, with cases with low and high Twist expression with no statistically significant differences in relation to tumor type or subtype.

In a study conducted in 2013, Ni Wang et al. analyzed 121 papillary thyroid carcinomas and found low Twist expression in 56.6% of the cases, and high expression of the protein in 43.4% of the cases, without difference between tumor subtype, respectively, in conventional, follicular, tall cells and oncocytic variants; in the same study authors indicate non-significant differences of Twist expression in relation with pTNM stage, but a high protein expression in metastasizing papillary carcinomas [9]. In our study, low Twist expression was present in 53.8% of PTC and high expression in 46.2% of these lesions, most commonly, but not statistically significant in tall cell variant. Also, 75% of FTC cases present low expression and in 25% of cases we observed a high expression of Twist protein.

Twist expression is differently reported in the literature studies. Thus, D Buehler et al. in a study that investigated the expression of Twist 1 in 27 follicular carcinomas and 28 papillary thyroid carcinomas, including tall cell and follicular variants found no reactions in all



cases, the immunostaining being present only in anaplastic forms of tumors [22]. On the contrary, CN He et al. in 2008, on a lot of 50 papillary thyroid carcinomas indicate Twist positivity in all cases, the marker expression being associated tumor aggressiveness [23].

In our study we found differences of Twist expression in relation to tumor stage (stage I / II versus stage III / IV) and the presence of metastasis, the protein expression being significantly higher in invasive tumors and in cases of tumors that had metastasized. In this respect, the literature indicates Twist as a marker of tumor aggressiveness, and associated with forms of invasive and metastatic carcinomas, including thyroid tumors [9,13,14,22,23].

## Conclusions

In differentiated thyroid carcinomas, Twist overexpression is associated with an invasive and metastatic immunophenotype. Identifying of high grade Twist expression in various forms of differentiated papillary thyroid carcinomas supports for a personalized therapeutic attitude to increase patient survival.

## References

1. DeLellis RA, Lloyd RV, Heitz PU, Eng C. Pathology and genetics of tumours of endocrine organs. In: Kleihues P, Sobrin LH (eds), World health organization, Classification of Tumours, Lyon, IARC Press, 2004.
2. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. *Am J Med* 1994; 97:418-28.
3. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995. *Cancer* 1998; 83:2638-48.
4. Sobrinho-Simões M, Eloy C, Magalhães J, Lobo C, Amaro T. Follicular thyroid carcinoma. *Mod Pathol* 2011; 24:S10-8.
5. Hay ID, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, Maurer MS, McIver B, Mullan BP, Oberg AL, Powell CC, van Heerden JA, Goellner JR. Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. *World J Surg* 2002; 26:879-85.
6. Wallerand H, Robert G, Pasticier G, Ravaud A, Ballanger P, Reiter RE, Ferrière JM. The epithelial-mesenchymal transition-inducing factor TWIST is an attractive target in advanced and/or metastatic bladder and prostate cancers. *Urol Oncol* 2010; 28:473-9.
7. Cheng GZ, Chan J, Wang Q, Zhang W, Sun CD, Wang LH. Twist transcriptionally upregulates AKT2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. *Cancer Res* 2007; 67:1979-87.
8. Wang G, Dong W, Shen H, Mu X, Li Z, Lin X, Liu Y, Du J. A comparison of Twist and E-cadherin protein expression in primary non-small-cell lung carcinoma and corresponding metastases. *Eur J Cardiothorac Surg* 2011; 39:1028-32.
9. Wang N, Luo HJ, Yin GB, Dong CR, Xu M, Chen GG, Liu ZM. Overexpression of HIF-2 $\alpha$ , TWIST, and CXCR4 is associated with lymph node metastasis in papillary thyroid carcinoma. *Clin Dev Immunol* 2013; 2013:589423.
10. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; 117:927-39.
11. Yuen HF, Chua CW, Chan YP, Wong YC, Wang X, Chan KW. Significance of TWIST and E-cadherin expression in the metastatic progression of prostatic cancer. *Histopathology* 2007; 50:648-58.
12. Lee KW, Kim JH, Han S, Sung CO, Do IG, Ko YH, Um SH, Kim SH. Twist1 is an independent prognostic factor of esophageal squamous cell carcinoma and associated with its epithelial-mesenchymal transition. *Ann Surg Oncol* 2012; 19:326-35.
13. Qin Q, Xu Y, He T, Qin C, Xu J. Normal and disease-related biological functions of Twist1 and underlying molecular mechanisms. *Cell Res* 2012; 22:90-106.
14. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Briskin C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; 133:704-15.
15. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol* 2006; 7:131-42.
16. Raimondi C, Gianni W, Cortesi E, Gazzaniga P. Cancer stem cells and epithelial-mesenchymal transition: revisiting minimal residual disease. *Curr Cancer Drug Targets* 2010; 10:496-508.
17. Hollier BG, Evans K, Mani SA. The epithelial-to-mesenchymal transition and cancer stem cells: a coalition against cancer therapies. *J Mammary Gland Biol Neoplasia* 2009; 14:29-43.
18. Ansieau S, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S, Maestro R, Voeltzel T, Selmi A, Valsesia-Wittmann S, Caron de Fromental C, Puisieux A. Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell* 2008; 14:79-89.
19. Lee TK, Poon RT, Yuen AP, Ling MT, Kwok WK, Wang XH, Wong YC, Guan XY, Man K, Chau KL, Fan ST. Twist overexpression correlates with hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. *Clin Cancer Res* 2006; 12:5369-76.
20. Valdés-Mora F, Gómez del Pulgar T, Bandrés E, Cejas P, Ramírez de Molina A, Pérez-Palacios R, Gallego-Ortega D, García-Cabezas MA, Casado E, Larrauri J, Nistal M, González-Barón M, García-Foncillas J, Lacal JC. TWIST1 overexpression is associated with nodal invasion and male sex in primary colorectal cancer. *Ann Surg Oncol* 2009; 16:78-87.

21. Khan MA, Chen HC, Zhang D, Fu J. Twist: a molecular target in cancer therapeutics. *Tumour Biol* 2013; 34:2497-506.
22. Buehler D, Hardin H, Shan W, Montemayor-Garcia C, Rush PS, Asioli S, Chen H, Lloyd RV. Expression of epithelial-mesenchymal transition regulators SNAI2 and TWIST1 in thyroid carcinomas. *Mod Pathol* 2013; 26:54-61.
23. He CN, He L, Cheng JQ, Chen SC, Zhao HF, Zhai JP, Zhang J. Expression of Twist in papillary thyroid carcinomas and its roles in differential diagnosis. *Zhonghua Bing Li Xue Za Zhi* 2008; 37(1):35-9.

---

*Corresponding author: Alex Stepan, Assistant, MD, PhD, Department of Pathology, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Romania; Phone/Fax +40251-599 228, e-mail: [astepan76@yahoo.com](mailto:astepan76@yahoo.com)*