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Immunoexpression of transcription factors in urothelial bladder carcinomas

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Abstract

Transcription factors play a central role in the epithelial–mesenchymal transition (EMT), which is one of the biomolecular mechanisms involved in the progression of urothelial carcinomas of the bladder (UCB). In this study, we analyzed the immunoexpression of Twist 1, Snail, Slug and *β*-catenin in relation to histopathological prognostic parameters of UCB. The obtained results indicated the association of Snail and *β*-catenin expression with low grade and early stage of UCB, as well as the association of Twist 1 and Slug expression with high grade and advanced stage lesions. The specific or sequential action of transcription factors in the bladder tumoral EMT may be useful for identifying the aggressive lesions.

Keywords: urothelial carcinoma, Twist 1, Snail, Slug, *β*-catenin.

Introduction

Urothelial carcinomas represent over 90% of malignant tumors in the bladder [1]. The lesions ranked the ninth place worldwide, being responsible for about 165 000 deaths annually [2, 3].

Urothelial carcinoma of the bladder (UCB) raises particular socio-economic problems, being the most expensive malignant tumors per patient due to the recurrence, survival, need to monitor by repeated tumor transurethral resections (TURs) and sometimes by cystectomy [4]. In this regard, numerous biomolecular mechanisms involved in the initiation and progression of urothelial carcinomas have been investigated, which has led to the identification of divergent pathogenic pathways for non-invasive and invasive lesions, as well as to the initiation of clinical trials having different therapeutic targets [5, 6]. However, the current treatment protocols fail to increased the survival rate (less than 60% at five years), recurrence risk (over 50%) and quality of life which remains the lowest reported to all cancers [4, 7].

One of the biomolecular mechanisms involved in the progression of UCB is the epithelial–mesenchymal transition (EMT) [8, 9]. In this process, transcription factors occupy a central place, being involved in regulating the cadherin switch, loss of epithelial phenotype and the acquisition of the mesenchymal phenotype, overexpression of some growth factors [10–12]. The most studied transcription factors in the urinary bladder in relation with EMT are represented by Twist 1, Snail, Slug, and the Zeb factors family. Also by translocating the membrane expression in cytoplasm and nucleus, *β*-catenin can act as a transcriptional cofactor [11–13].

The involvement of transcription factors in tumor EMT is an attractive therapeutic target [14], which sustain for the analysis of the relationship of these factors with tumor aggressiveness parameters.

In this study, we analyzed the immunoexpression of Twist 1, Snail, Slug and *β*-catenin in relation to the histopathological prognostic parameters of UCB.

Materials and Methods

In this study, we analyzed a total of 42 cases of bladder urothelial carcinomas diagnosed in the Department of Pathology, Emergency County Hospital, Craiova, Romania, in the last five years, the patients being hospitalized and investigated in the Department of Urology. The biological material was represented by removed cystectomy specimens, fixed in 10% buffered neutral formalin, processed for paraffin embedding and Hematoxylin–Eosin (HE) staining. The histopathological assessment of the tumors was done according to the latest literature data [15]. The study included primary bladder urothelial carcinomas, without distant metastases or previous oncological therapy.

We investigated clinicopathological parameters (age, gender, differentiation degree, depth of invasion, lymph node involvement, stage) in relation to the immunoexpression of the transcription factors represented by Twist 1, Snai1, Snai2, *β*-catenin (Table 1).

In order to immunostaining, the sections were prepared for incubation with primary antibodies (dewaxing in xylene, rehydrating in alcohols, endogenous enzyme and unspecific blocking, microwaving for antibody retrieval) at 4^0C, overnight. The working system was represented by Labeled Streptavidin–Biotin (LSAB) 2 system (Dako, Redox, Romania, code K0675), and we used 3,3'-diaminobenzidine tetrahydrochloride (Dako, Redox, Romania,

code K3468) as chromogen. External positive controls and external negative controls were used for the immunostaining reactions.

Table 1 – ***The antibodies and immunostaining protocol***

Antibody	Clone/ Manufacturer	Dilution	Pretreatment	External positive control
Twist 1	Twist 1/ LSbio	1:1000	Microwaving in citrate buffer, pH 6	tonsil
Snai1 (Snail)	Polyclonal/ ABcam	1:50	Microwaving in citrate buffer, pH 6	placenta
Snai2 (Slug)	1A6/Novus Biologicals	1:50	Microwaving in Tris-EDTA, pH 9	tongue
β-catenin	*β*-catenin 1/ Dako	1:50	Microwaving in citrate buffer, pH 6	colon

EDTA: Ethylenediaminetetraacetic acid.

The assessment of reactions was done by using a score resulting by multiplying of labeled cells and intensity of reactions. Referring to intensity, the reactions were scored as 1 (mild), 2 (moderate) and 3 (strong) and depending on the number of labeled cells, the reactions were scored as 1 (<25% labeled cells), 2 (26–49% labeled cells), 3 (>50% labeled cells). The final scores were considered low for 1–4 and high for 6–9 values. We used χ^2 (*chi*)-square and Pearson tests within SPSS 10 software for the statistical analysis, for *p*-values less than 0.05 the results being considered significant. For the images acquisition, the Nikon Eclipse E600 microscope equipped with Lucia 5 software was used. The local ethical committee approved the study.

Results

The clinicopathological data analysis revealed in this study the predominance of UCB in male patients (80.9%), most with over 50 years (95.2%) with a mean age at diagnosis of 63.2±8.7 years. Most of the analyzed cases were high-grade carcinomas (52.3%), with the muscularis propria invasion (64.2%), without lymph node metastasis (92.8%) and in the tumor stage II (61.9%) (Table 2).

Table 2 – ***Clinicopathological parameters***

Clinicopathological parameters	No. of cases
Age [years]	<50: 2, >50: 40
Gender	Males: 34, Females: 8
Degree of differentiation	LG: 20, HG: 22
Depth of invasion (T)	T1: 9, T2: 27, T3: 3, T4:3
Lymph node status (N)	N0: 39, N1: 3
Stage	I: 9, II: 26, III: 3, IV: 4

LG: Low grade; HG: High grade.

The assessment of performed immunohistochemical reactions indicated differences in the expression of the transcription factors in relation to the histopathological analyzed parameters.

Twist 1 immunoexpression

Twist 1 immunoexpression was identified at the nuclear level in all analyzed cases. The reactions were present both in tumor cells and stromal elements represented by endothelial cells, fibroblasts, lymphocytes, plasma cells, some macrophages.

Twist 1 immunoreactions were superior in the case of high-grade carcinomas with deep invasion and advanced stages. Thus, for high-grade carcinomas, the number of marked tumor cells was 76.6±11.8, the intensity of the reactions being moderate or strong, and the mean final score being 8.3. By comparison, for low-grade carcinomas the number of labeled cells was 56.7±11.2, moderate or increased intensity and the final score 6, difference which was statistically significant (p=0.000, *chi*-square test) (Figure 1). The analysis of Twist 1 reactions in relation to the invasion depth indicated higher values for deep invasion carcinomas compared to invasive lesions in the lamina propria, but the aspects were statistically insignificant (p=0.580, *chi*-square test). Thus, the highest mean values of the final Twist 1 score were observed in pT2-T4 lesions, in which the number of labeled cells was comprised between 45–95% compared to pT1 lesions in which the value was 56.2±8.5, the reactions intensity being strong or moderate for both categories. The aspects were similar for the analysis of Twist 1 expression in relation to the tumor stage. Although the number of marked cells was higher for carcinomas that had lymph node metastases compared to those without metastases (90.0±5 *vs.* 65.4± 14.3), the intensity of the reactions was strong or moderate for both categories, and the statistical differences were insignificant (p=0.348, *chi*-square test) (Table 3).

Snail immunoreactions

Snail immunoreactions were observed at the nuclear and cytoplasmic level in 34 cases, respectively 80.9% of the analyzed cases. Similarly to the other transcription factors analyzed, the reactions were present in stromal mononuclear elements. We found differences in Snail expression relative to histological analyzed parameters. Thereby, in the case of low-grade carcinomas (48±8.6 labeled cells, moderate/strong intensity and average score 6.2), the immunoreactions were significantly superior to those of high grade (38.2±11.8 labeled cells, variable intensity and mean score 4.0) (Figure 1). Although the reactions were superior in the case of superficial tumors (pT1) without lymph node metastases (pN0) and initial stages (tumor stages I and II), the differences were statistically insignificant (p>0.05, *chi*-square test) (Table 3).

Table 3 – ***Transcription factors immunoexpression and their relation with histological parameters***

Histological parameters		Twist 1 score, *p** level	Snail score, *p** level	Slug score, *p** level	*β*-catenin score, *p** level
Differentiation degree	LG	6.0	6.2	1.8	6.7
	HG	8.3	4.0	4.7	4.8
		p=0.000	*p*=0.034	*p*=0.037	*p*=0.036

Histological parameters		Twist 1 score, *p** level	Snail score, *p** level	Slug score, *p** level	*β*-catenin score, *p** level
Depth of invasion (pT)/Stage	T1	6.5	6.0	2.4	6.7
	T2	7.0	5.5	3.0	5.8
	T3	9.0	3.0	6.3	4.6
	T4	9.0	2.0	8.0	5.3
		p=0.580	*p*=0.192	*p*=0.000	*p*=0.718
Lymph node (pN)	N0	7.0	5.4	3.5	6.0
	N1	9.0	2.6	6.7	4.6
		p=0.348	*p*=0.122	*p*=0.148	*p*=0.335
Tumor stage	I	6.5	6.0	2.4	6.7
	II	6.9	5.5	2.8	5.9
	III	9.0	3.0	6.3	4.6
	IV	9.0	2.6	7.5	5.0
		p=0.498	*p*=0.107	*p*=0.000	*p*=0.698

*p** level (*chi*-square test); LG: Low grade; HG: High grade.

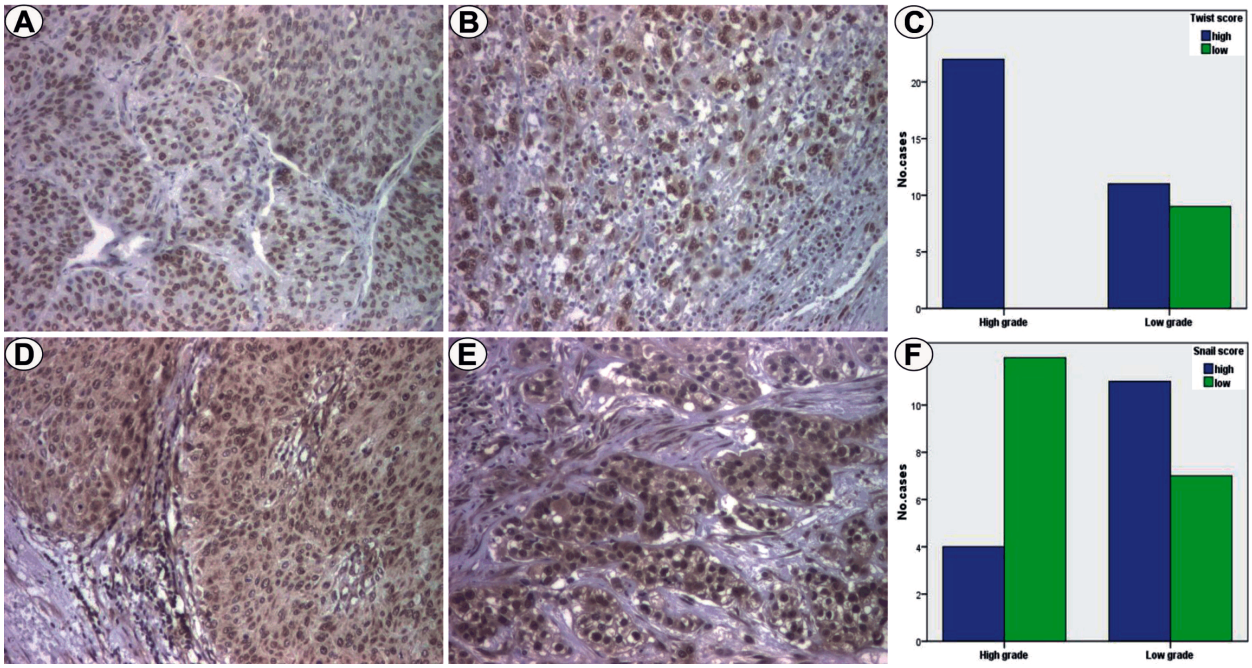

Figure 1 – ***Urothelial carcinoma of the bladder: (A) Low grade, Twist 1 immunostaining (×100); (B) High grade, Twist 1 immunostaining (×100); (C) Cases distribution depending on tumor grading and Twist 1 scores; (D) Low grade, Snail immunostaining (×100); (E) High grade, Snail immunostaining (×100); (F) Cases distribution depending on tumor grading and Snail scores.***

Slug immunoexpression

Slug immunoexpression was found in 30 cases, which represented 71.4%, the immunostaining being observed in nuclear and cytoplasmic level, both in tumor cells, as well as in the stromal elements.

The reactions were higher in high-grade carcinomas and in advanced stages. In the case of low-grade tumors, the number of labeled cells was 22.7±8.2, low or moderate intensity of reactions and an average score of 1.8, while for high-grade tumors the number of positive cells was 41.3±9.9, variable reactions intensity and mean Slug score of 4.7, which were statistically significant (*p*=0.037, *chi*-square test) (Figure 2).

In relation to the depth of invasion, the number of labeled cells was 24.1±5 in pT1 lesions, 33.2±11.9 in pT2, 50±5 for pT3 and 50±8.6 for pT4 cases. The intensity of the Slug reactions was low or moderate in pT1/T2 carcinomas, and strong or moderate in pT3/T4 lesions, the mean Slug scores being 2.4 in the case of pT1, 3.0 in pT2, 6.3 in pT3 and 8.0 in pT4 cases, aspects that were statistically significant (*p*=0.000, *chi*-square test). The aspects were similar in the case of the analysis of the reactions in relation to the tumor stage, the mean values of the Slug score being significantly higher in the advanced stages compared to the stages I and II (*p*=0.000, *chi*-square test). Although the number of marked cells was higher for metastatic carcinomas compared to those without metastases, respectively 48.3±7.6 and 32.9±12.5, the intensity of the reactions was variable and the statistical differences of the mean Slug scores (6.7 in pN1 and 3.5 in pN0 cases) were insignificant (*p*=0.148, *chi*-square test).

In this study, we did not find any differences in the localization of the Snail and Slug expression in relation to the histopathological parameters.

β-catenin immunoreaction

β-catenin immunoreaction was identified predominantly at the membranous and cytoplasmic level as well as in rare nuclei of tumor cells in 32 cases, which represented 76.1% of the investigated group. Also, the immunostaining was observed in peritumoral stromal elements. In this study, we found significantly higher levels of *β*-catenin expression in low-grade carcinomas (62.3±13 labeled cells, moderate/strong intensity and average score 6.7) compared to high-grade lesions (47±12 marked cells, moderate/strong intensity and average score 4.8) (Figure 2).

We have found the predominance of membrane *β*-catenin immunostaining in the case of low-grade carcinomas with superficial invasion (pT1) and cytoplasmic and nuclear in the case of high-grade carcinomas and invasion at least in muscularis propria (pT2-T4).

Also, the reactions were superior in case of lamina propria (pT1) invasive carcinomas, with no lymph node metastases (pN0) and in the initial stages (stages I and II), but the aspects were without statistical significance ($p>0.05$, *chi*-square test) (Table 3).

The analysis of the percentage values of the investigated markers indicated significant positive linear correlations between Twist 1 and Slug ($p=0.000$, Pearson's test), and between Snail and *β*-catenin ($p=0.001$, Pearson's test) (Figure 3).

Between the two groups, we found statistically insignificant negative linear relations ($p>0.05$, Pearson's test). In this study, we did not find other statistical associations of marker-analyzed immunoexpression with the age and gender of patients diagnosed with UCB.

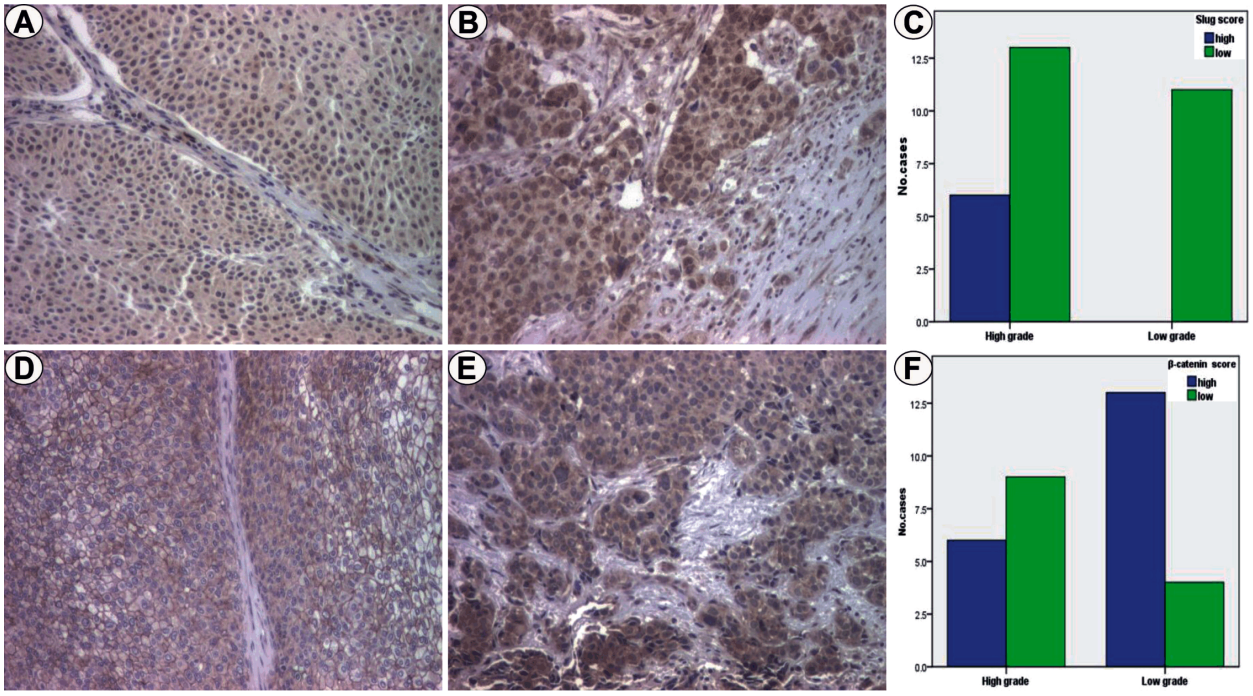

Figure 2 – ***Urothelial carcinoma of the bladder: (A) Low grade, Slug immunostaining (×100); (B) High grade, Slug immunostaining (×100); (C) Cases distribution depending on tumor grading and Slug scores; (D) Low grade, β-catenin immunostaining (×100); (E) High grade, β-catenin immunostaining (×100); (F) Cases distribution depending on tumor grading and β-catenin scores.***

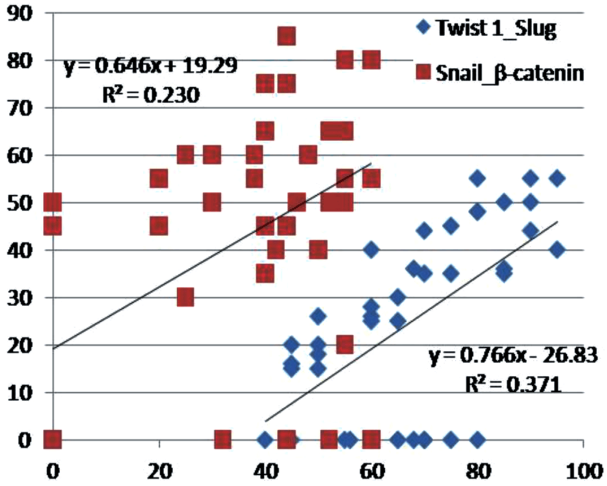

Figure 3 – ***Values distribution of the labeled cells for the investigated markers.***

Discussion

Transcription factors are part of the largest family of proteins that are involved in the transmission of genetic information from DNA sequences to messenger RNA using RNA polymerase [16, 17]. The transcription is a complex process in which there may be several factors that co-operate in order to regulate a genetic sequence, factors that in turn can have numerous regulating mechanisms for their expression [18, 19]. Targeting the transcription factors is an effective way of controlling the gene expression, an aspect that is an attractive target for anticancer therapy [14, 20, 21].

Transcription factors are involved in regulating the carcinomas EMT process, which is a complex biomolecular mechanism that plays a role in the progression of lesions, respectively in invasion and metastasis [8–10]. The process consists in acquiring a mesenchymal migratory

phenotype of cancer cells, and altering the epithelial phenotype and intercellular adhesion system, whose the main component is E-cadherin [10, 11]. At the same time, the process can be induced by numerous other molecular pathways, like as Wnt, TGF-β, Hedgehog, Notch, whose interconnection with tumor EMT has been demonstrated [11]. The studies for transcription factors in EMT bladder tumors are relatively few, and the process seems to be temporary and only in some tumor compartments [8]. This aspect, along with the large number of proteins involved in EMT and interaction with other biomolecular pathways of tumor progression, advocates the investigation of transcription factors as initiators and process regulators. In our study, we analyzed the immunoexpression in UCB of some factors involved in the tumor EMT, represented by Twist 1, Snail Slug and β-catenin.

Twist 1 is a protein that is part of the basic helix-loop-helix (bHLH) family and is involved in EMT by loss of epithelial profile expression and intercellular adhesion molecules, respectively of E-cadherin [8, 22]. It also regulates overexpression of N-cadherin and promotes the tumor motility and invasiveness [8, 22]. Twist also plays a role in promoting tumor angiogenesis and generating cancerous stem cells [8, 22, 23].

Twist 1 was analyzed in EMT of carcinomas with different locations such as colorectal, breast, prostate, pulmonary, hepatic, oral cavity, ovarian or esophageal, the high expression being associated with aggressive lesions [24–27].

In our study, the Twist 1 immunoreactions were present in all the analyzed cases at nuclear levels as well as in the stromal elements. There are studies that have identified the Twist expression in cytoplasm, with reactions seen in about 40% of the tumors analyzed [23, 28].

In our study, the Twist immunoexpression was significantly superior in high-grade UCB, and insignificantly superior in advanced stage lesions. For the bladder urothelium, some studies have indicated the overexpression of Twist 1 in high-grade, invasive, metastatic and advanced stage carcinomas, the marker reactions being correlated with negative prognosis of lesions and a low survival rate [9, 29, 30]. On the contrary, other studies indicated the absence of the relation of Twist 1 expression with the degree or stage of UCB, including in relation to other clinical parameters [23].

However, the most studies support the utility of Twist 1 as the therapeutic target, which additionally can increase susceptibility of UCB to conventional treatments, some studies suggesting that Twist 1 inhibition can restore E-cadherin expression, indicating the reversible aspect of the EMT process [31–33].

Zinc finger protein SNAI1 (Snail) and SNAI2 (Slug) represent a class of transcription factors involved in regulating the expression of intercellular adhesion molecules and especially in inhibiting E-cadherin expression, having a role in promoting the EMT process both in embryogenesis and carcinogenesis [34–37].

Snail and Slug are involved in promoting cellular motility, invasion and tumor metastasis, being markers associated with reduced survival, including urothelial carcinomas [8, 25]. Snail is involved in epithelial remodeling, cell cycle inhibition, apoptosis resistance and angiogenesis, aspects studied in carcinomas with different locations, such as colorectal, gastric, mammary and endometrial [8, 38]. Slug overexpression was also associated with the poor prognosis for colorectal, gastric, breast or lung carcinomas [39–41]. In prostate carcinoma, while some studies indicate the role of Slug in invasion of cancer cells [42], others support the factor involvement in inhibiting tumor proliferation by the negative regulation of cyclin D1 expression [43].

In our study, the immunostainings were cytoplasmic and nuclear for both markers, the reactions being identified for Snail in 80.9% and for Slug in 71.4% of cases. Snail reactions were superior in low-grade and early stage carcinomas, while for Slug the reactions were superior to high-grade and advanced stages lesions.

In the literature data about UCB, the Slug immunoexpression was associated with high-grade and advanced stage lesions, while Snail appears to correlate with the relapse rate of superficial carcinomas, being less expressed in metastasis compared to primitive tumors [8, 30, 44]. The aspects may be considered consistent with the results of this study if we take into account that the most relapsing lesions are the low-grade and superficial lesions, respectively, without invasion in muscularis propria.

Similarly with Twist, the inhibition of Snail expression led to reversal of EMT in experimental animal models [11], and in the case of inhibition of Slug expression in increasing tumor sensitivity to treatment [45], which designates these transcription factors as potential targets for therapy.

β-Catenin is located at the surface of cell membranes, being involved in the regulation of adhesion and cell growth, respectively in the stabilization of epithelia, through the structural and functional connections with E-cadherin [46, 47]. The diminution of β-catenin membrane expression has been observed in carcinomas with different locations, such as esophagus, stomach, colon, breast [47, 48]. It also plays an important role in differentiation and cellular motility, and as a mediator of the Wnt pathway plays a role in tumor initiation and progression [46]. In the case of normal epithelia, β-catenin is expressed in the membrane, and in the case of carcinomas, the protein predominates in the cytoplasm or nucleus [46]. The cytoplasmic expression of β-catenin, followed by the nuclear translocation, allows the protein to participate as a transcriptional cofactor, which is emphasized in the case of aggressive carcinomas with different locations [11, 46]. However, in some studies, β-catenin expression has been observed at the membranous level [49] and sometimes in the case of early stage carcinomas [46].

In our study, β-catenin reactions were observed in 76.1% of the cases, predominantly membranous for low grade UCBs. In the case of high-grade and in advanced stages lesions, the immunoreactions were inferior and located cytoplasmic/nuclear.

The studies that investigated β-catenin expression in UCB indicated controversial results. Thus, some studies indicate the association of β-catenin overexpression in aggressive urothelial carcinomas, with immunoreaction at cytoplasmic and nuclear levels [50]. In other studies,

diminished β-catenin membrane expression is indicated for high-grade and aggressive carcinomas [51].

In the literature, there have been studies that have shown a positive association of cytoplasmic and nuclear expression of transcription factors and the presence of activated stromal fibroblasts, association that appears to be involved in the invasive phenotype of UCB [37]. In this study, for all the analyzed factors, we found the presence of positive stromal elements associated with tumor cells, both in the fibroblasts, as well as in the inflammatory mononuclear and endothelial cells. The appearance may suggest the interrelation of the analyzed transcription factors with tumor micromedium and tumor paracrine mechanisms.

There are data in the literature that indicate different relations between transcription factors of EMT. Thus, Snail and Slug factors have synergistic effects, but experimental animal models indicate different roles at different stages of tumor progression [36]. Also, synergistic and even complex formation between β-catenin and Snail for Wnt pathway activation is indicated [52].

In our study, we found positive linear correlations of β-catenin–Snail and Slug–Twist expression, and negative linear relations between these two groups of factors, which supports the sequential involvement in urothelial carcinogenesis or their specific expression for different histological subtypes of UCB.

Conclusions

In this study, Snail and β-catenin immunoexpression was associated with low-grade and early stage UCB, while Slug and Twist 1 immunoexpression was associated with high-grade and advanced lesions. The obtained results support the specific or sequential action of the transcription factors in the bladder EMT. The markers analyzed in this study may be useful for identifying UCB with progression potential and for better stratification of patients for therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Pasin E, Josephson DY, Mitra AP, Cote RJ, Stein JP. Superficial bladder cancer: an update on etiology, molecular development, classification, and natural history. Rev Urol, 2008, 10(1):31–43.

[2] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol, 2017, 71(1):96–108.

[3] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin, 2015, 65(2):87–108.

[4] Tomlinson B, Lin TY, Dall'Era M, Pan CX. Nanotechnology in bladder cancer: current state of development and clinical practice. Nanomedicine (Lond), 2015, 10(7):1189–1201.

[5] Netto GJ. Molecular biomarkers in urothelial carcinoma of the bladder: are we there yet? Nat Rev Urol, 2011, 9(1):41–51.

[6] Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. Nat Rev Cancer, 2005, 5(9):713–725.

[7] Avis NE, Deimling GT. Cancer survivorship and aging. Cancer, 2008, 113(Suppl S12):3519–3529.

[8] Hrbácek J, Brisuda A, Babjuk M. Involvement of epithelial-mesenchymal transition in urinary bladder cancer progression. A review. Debates Bladder Cancer, 2011, 3:1.

[9] Paliwal P, Arora D, Mishra AK. Epithelial mesenchymal transition in urothelial carcinoma: twist in the tale. Indian J Pathol Microbiol, 2012, 55(4):443–449.

[10] Kiesslich T, Pichler M, Neureiter D. Epigenetic control of epithelial-mesenchymal-transition in human cancer. Mol Clin Oncol, 2013, 1(1):3–11.

[11] Voulgari A, Pintzas A. Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic. Biochim Biophys Acta, 2009, 1796(2):75–90.

[12] McConkey DJ, Choi W, Marquis L, Martin F, Williams MB, Shah J, Svatek R, Das A, Adam L, Kamat A, Siefker-Radtke A, Dinney C. Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer. Cancer Metastasis Rev, 2009, 28(3–4):335–344.

[13] Steinestel K, Eder S, Schrader AJ, Steinestel J. Clinical significance of epithelial-mesenchymal transition. Clin Transl Med, 2014, 3:17.

[14] Yeh JE, Toniolo PA, Frank DA. Targeting transcription factors: promising new strategies for cancer therapy. Curr Opin Oncol, 2013, 25(6):652–658.

[15] Moch H, Humphrey PA, Ulbright TM, Reuter VE (eds). World Health Organization (WHO) classification of tumours of the urinary system and male genital organs. Vol. 8, International Agency for Research on Cancer (IARC) Press, Lyon, 2016.

[16] Levine M, Tjian R. Transcription regulation and animal diversity. Nature, 2003, 424(6945):147–151.

[17] Karin M. Too many transcription factors: positive and negative interactions. New Biol, 1990, 2(2):126–131.

[18] Wang RS, Zhang XS, Chen L. Inferring transcriptional interactions and regulator activities from experimental data. Mol Cells, 2007, 24(3):307–315.

[19] Everett L, Hansen M, Hannenhalli S. Regulating the regulators: modulators of transcription factor activity. Methods Mol Biol, 2010, 674:297–312.

[20] Redell MS, Tweardy DJ. Targeting transcription factors for cancer therapy. Curr Pharm Des, 2005, 11(22):2873–2887.

[21] Bhagwat AS, Vakoc CR. Targeting transcription factors in cancer. Trends Cancer, 2015, 1(1):53–65.

[22] Puisieux A, Valsesia-Wittmann S, Ansieau S. A twist for survival and cancer progression. Br J Cancer, 2006, 94(1):13–17.

[23] Tang X, Xing J, Li W, Wu Z, Zhang K, Zheng J. Expression of transcription factor Twist1 in bladder urothelial carcinoma and its clinical significance. J BUON, 2013, 18(1):211–219.

[24] Watanabe O, Imamura H, Shimizu T, Kinoshita J, Okabe T, Hirano A, Yoshimatsu K, Konno S, Aiba M, Ogawa K. Expression of twist and wnt in human breast cancer. Anti-cancer Res, 2004, 24(6):3851–3856.

[25] Fan XJ, Wan XB, Yang ZL, Fu XH, Huang Y, Chen DK, Song SX, Liu Q, Xiao HY, Wang L, Wang JP. Snail promotes lymph node metastasis and Twist enhances tumor deposit formation through epithelial-mesenchymal transition in colorectal cancer. Hum Pathol, 2013, 44(2):173–180.

[26] Kwok WK, Ling MT, Lee TW, Lau TC, Zhou C, Zhang X, Chua CW, Chan KW, Chan FL, Glackin C, Wong YC, Wang X. Up-regulation of TWIST in prostate cancer and its implication as a therapeutic target. Cancer Res, 2005, 65(12):5153–5162.

[27] Wushou A, Hou J, Zhao YJ, Shao ZM. Twist-1 up-regulation in carcinoma correlates to poor survival. Int J Mol Sci, 2014, 15(12):21621–21630.

[28] Fondrevelle ME, Kantelip B, Reiter RE, Chopin DK, Thiery JP, Monnien F, Bittard H, Wallerand H. The expression of Twist has an impact on survival in human bladder cancer and is influenced by the smoking status. Urol Oncol, 2009, 27(3): 268–276.

[29] Zhang Z, Xie D, Li X, Wong YC, Xin D, Guan XY, Chua CW, Leung SC, Na Y, Wang X. Significance of TWIST expression and its association with E-cadherin in bladder cancer. Hum Pathol, 2007, 38(4):598–606.

[30] Yu Q, Zhang K, Wang X, Liu X, Zhang Z. Expression of transcription factors snail, slug, and twist in human bladder carcinoma. J Exp Clin Cancer Res, 2010, 29:119.

[31] 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(5):473–479.

[32] Shen CH, Wu JD, Jou YC, Cheng MC, Lin CT, Chen PC, Tseng YS, Shi CS, Chen SY, Chang DC, Lee YR. The correlation between TWIST, E-cadherin, and beta-catenin in human bladder cancer. J BUON, 2011, 16(4):733–737.
[33] Yun SJ, Kim WJ. Role of the epithelial-mesenchymal transition in bladder cancer: from prognosis to therapeutic target. Korean J Urol, 2013, 54(10):645–650.
[34] Paznekas WA, Okajima K, Schertzer M, Wood S, Jabs EW. Genomic organization, expression, and chromosome location of the human SNAIL gene (SNAI1) and a related processed pseudogene (SNAI1P). Genomics, 1999, 62(1):42–49.
[35] Zhao W, Zhou Y, Xu H, Cheng Y, Kong B. Snail family proteins in cervical squamous carcinoma: expression and significance. Clin Invest Med, 2013, 36(4):E223–E233.
[36] Dhasarathy A, Phadke D, Mav D, Shah RR, Wade PA. The transcription factors Snail and Slug activate the transforming growth factor-beta signaling pathway in breast cancer. PLoS One, 2011, 6(10):e26514.
[37] Schulte J, Weidig M, Balzer P, Richter P, Franz M, Junker K, Gajda M, Friedrich K, Wunderlich H, Östman A, Petersen I, Berndt A. Expression of the E-cadherin repressors Snail, Slug and Zeb1 in urothelial carcinoma of the urinary bladder: relation to stromal fibroblast activation and invasive behaviour of carcinoma cells. Histochem Cell Biol, 2012, 138(6):847–860.
[38] Nieto MA. Epithelial-mesenchymal transitions in development and disease: old views and new perspectives. Int J Dev Biol, 2009, 53(8–10):1541–1547.
[39] Uchikado Y, Okumura H, Ishigami S, Setoyama T, Matsumoto M, Owaki T, Kita Y, Natsugoe S. Increased Slug and decreased E-cadherin expression is related to poor prognosis in patients with gastric cancer. Gastric Cancer, 2011, 14(1):41–49.
[40] Shih JY, Yang PC. The EMT regulator slug and lung carcinogenesis. Carcinogenesis, 2011, 32(9):1299–1304.
[41] Shioiri M, Shida T, Koda K, Oda K, Seike K, Nishimura M, Takano S, Miyazaki M. Slug expression is an independent prognostic parameter for poor survival in colorectal carcinoma patients. Br J Cancer, 2006, 94(12):1816–1822.
[42] Uygur B, Wu WS. SLUG promotes prostate cancer cell migration and invasion *via* CXCR4/CXCL12 axis. Mol Cancer, 2011, 10:139.
[43] Liu J, Uygur B, Zhang Z, Shao L, Romero D, Vary C, Ding Q, Wu WS. Slug inhibits proliferation of human prostate cancer cells *via* downregulation of cyclin D1 expression. Prostate, 2010, 70(16):1768–1777.
[44] Bruyere F, Namdarian B, Corcoran NM, Pedersen J, Ockrim J, Voelzke BB, Mete U, Costello AJ, Hovens CM. Snail expression is an independent predictor of tumor recurrence in superficial bladder cancers. Urol Oncol, 2010, 28(6):591–596.
[45] Dong A, Jiao X, Chen D, Hao F, Zhang K. Targeting of slug sensitizes anaplastic thyroid carcinoma SW1736 cells to doxorubicin *via* PUMA upregulation. Int J Biochem Mol Biol, 2016, 7(3):48–55.
[46] Santoro A, Pannone G, Papagerakis S, McGuff HS, Cafarelli B, Lepore S, De Maria S, Rubini C, Mattoni M, Staibano S, Mezza E, De Rosa G, Aquino G, Losito S, Loreto C, Crimi S, Bufo P, Lo Muzio L. Beta-catenin and epithelial tumors: a study based on 374 oropharyngeal cancers. Biomed Res Int, 2014, 2014:948264.
[47] Takayama T, Shiozaki H, Shibamoto S, Oka H, Kimura Y, Tamura S, Inoue M, Monden T, Ito F, Monden M. Beta-catenin expression in human cancers. Am J Pathol, 1996, 148(1):39–46.
[48] Dolled-Filhart M, McCabe A, Giltnane J, Cregger M, Camp RL, Rimm DL. Quantitative *in situ* analysis of beta-catenin expression in breast cancer shows decreased expression is associated with poor outcome. Cancer Res, 2006, 66(10): 5487–5494.
[49] Balasundaram P, Singh MK, Dinda AK, Thakar A, Yadav R. Study of *β*-catenin, E-cadherin and vimentin in oral squamous cell carcinoma with and without lymph node metastases. Diagn Pathol, 2014, 9:145.
[50] Senol S, Yildirim A, Ceyran B, Uruc F, Zemheri E, Ozkanli S, Akalin I, Ulus I, Caskurlu T, Aydin A. Prognostic significance of survivin, *β*-catenin and p53 expression in urothelial carcinoma. Bosn J Basic Med Sci, 2015, 15(4):7–14.
[51] Clairotte A, Lascombe I, Fauconnet S, Mauny F, Félix S, Algros MP, Bittard H, Kantelip B. Expression of E-cadherin and alpha-, beta-, gamma-catenins in patients with bladder cancer: identification of gamma-catenin as a new prognostic marker of neoplastic progression in T1 superficial urothelial tumors. Am J Clin Pathol, 2006, 125(1):119–126.
[52] Zucchini-Pascal N, Peyre L, Rahmani R. Crosstalk between beta-catenin and snail in the induction of epithelial to mesenchymal transition in hepatocarcinoma: role of the ERK1/2 pathway. Int J Mol Sci, 2013, 14(10):20768–20792.

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