Original Paper

Snail and E-Cadherin Immunoexpression in Clear Cell Renal Cell Carcinoma

BIANCA-CĂTĂLINA ANDREIANA[1], ALEX-EMILIAN STEPAN[2], CLAUDIU MĂRGĂRITESCU[2], OANA TĂISESCU[3], ANDREI OSMAN[3], CRISTIANA EUGENIA SIMIONESCU[2]

[1]*PhD Student, Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[2]*Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[3]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*

ABSTRACT: Renal cell clear cell carcinomas (ccRCC) represent 75% of the malignant renal neoplasias. A critical molecular characteristic in the epithelial-mesenchymal transition (EMT) is the loss of E-cadherin expression, as well as the epithelial-mesenchymal transcription factors, one of the most important of which is Snail. In the current study, we analyzed the immunoexpression of the two markers in 46 cases of clear cell renal cell carcinomas, with reference to the most important prognosis histopathological factors. Our results indicated significant associations between the overexpression of Snail, under expression of E-cadherin and high Fuhrman grade. E-cadherin immunoexpression was high in incipient tumoral stages, and Snail was overexpressed in advanced tumoral stages of ccRCC. Our study supports the importance of this antibody panel in the prognosis of ccRCC.

KEYWORDS: *Clear cell renal cell carcinoma, Snail, E-cadherin*

Introduction

Clear cell renal cell carcinoma (ccRCC) represent the most frequent malignant neoplasias of the kidney, and about 4% of the malignant neoplasms in adults [1].

Epithelial-mesenchymal transition (EMT) is a biological process in which epithelial cells lose polarity and cell-cell contact and acquire a mesenchymal phenotype [2]. The process is characterized by the loss of epithelial markers and the overexpression of mesenchymal markers, playing a key role in the process of invasion and metastasis [3].

E-cadherin is part of the cadherin family and it is a transmembrane protein which acts as a tumoral suppressor that inhibits invasion and metastasis [4], and its expression is reduced in EMT [6,7].

Snail is a zinc-finger transcription factor, overexpressed in some cancers [8,9], directly involved in the repression of E-cadherin, which favors the acquisition of the mesenchymal phenotype [10,11].

Materials and methods

The study was done on 46 cases of ccRCC diagnosed in the Pathology Laboratory of the County Clinical Emergency Hospital in Craiova between 2015 and 2017. The biologic material consisted of nephrectomy specimens from patients admitted in the Urology Clinic of the same hospital and were processed through the classic method of fixation in buffered formalin 10%, paraffin embedding and Hematoxilin-Eosyn (HE) staining.

We studied clinical parameters (age, gender, tumor size) and histopathology (Furhman grade, invasion of fat tissue, vascular invasion, tumor stage, presence of necrosis). The cases were divided into two categories according to the nuclear grade, the ones with Fuhrman 1 and 2 were classified in low Fuhrman grade group, and the ones with grade 3 and 4 in the high Fuhrman grade group.

In order to immunostaining, the sections were prepared for incubation with primary antibodies (dewaxing in xylene, rehydrating in alcohol, endogenous enzyme and unspecific blocking, microwaving for antibody retrieval), at 4°C, overnight. The working system was represented by Labelled Streptavidin-Biotin 2 (LSAB2) system (Dako, Redox, Romania, code K0675), and we used 3,3'-diaminobenzidine tetrahydrochloride (Dako, Redox, Romania, code K3468) as chromogen.

The quantification of the reactions was done using a final composite score (CS), that included the intensity of the reactions and the percent of marked cells. Therefore, according to the number of marked tumor cells, the studied cases were split in the following categories: 0 (no marked cells), 1 (<25%), 2 (25-50%), 3 (51-75%) and 4 (>75%). The intensity of the embedding was also classified in 4 categories:

0 (absent), 1 (low), 2(moderate), 3(high). CS was considered negative for values of 0, low positive for values between 1-4 and high positive for values between 6-12. For the statistical analysis, we used Pearson and chi-square (χ^2) tests in the SPSS20 software.

Our aim was to analyze the immunoexpression of Snail and E-cadherin compared to histopathological (Fuhrman grade, fat tissue invasion, vascular invasion, tumor stage, the presence of necrosis) and clinical (age, sex, tumor size) parameters to identify the cases that lose the epithelial phenotype and develop a mesenchymal phenotype.

The study was approved by the local ethics committee (no.41/27.03.2018).

Results

The clinical-epidemiological study was done on 46 cases of ccRCC, in relation to Snail and E-cadherin expression that are shown in Table 1.

Table 1. SNAIL, E-cadherin immunoexpression and their relation with the investigated clinicopathological parameters

Clinicopathological parameters		No. cases	SNAIL	E-cadherin
		p-chi square		
Gender	Males	30	p=0.881	p=0.135
	Females	16		
Age (years)	<=60	21	p=0.793	p=0.063
	>60	25		
Fuhrman grade	low	35	p=0.006	p=0.000
	high	11		
Tumor sizes (cm)	<=4	11	p=0.362	p=0,121
	>4 and <=7	16		
	>7 and <=10	14		
	>10	5		
Fat tissue invasion	present	30	p=0.041	p=0.283
	absent	16		
Vascular invasion	present	9	p=0.149	p=0.031
	absent	37		
pT/tumoral stage	I	12	p=0.101	p=0.56
	II	4		
	III	30		
	IV	0		
Necrosis	present	24	p=0.429	p=0.687
	absent	22		

In this study, most of the ccRCC cases were identified in male patients (65.21%), with an average diagnostic age of 60.18±10 years (Table 1). Tumors had sizes between 1.5-16cm, with an average of 7.05cm. Most cases were determined to be low Fuhrman grade (76%) (Fig.1.A; Fig.1.B) and tumor stage III (65.21%). Fat tissue invasion was present in 65.21% cases (Fig.1.C), vascular invasion in 19.56% of cases and necrosis was present in 52.17% of cases (Table 1). In this study, the tumor extension (pT) matched tumor stage.

Snail immunoreaction was identified in 93.47% if the 46 ccRCC cases that were analyzed, with cytoplasmic localization. The Snail immunoexpression varied according to Fuhrman grade both in percentage of marked cells and intensity. Low Fuhrman grade ccRCC had an average percentage of marked cells of 19.05±11.77, with the intensity of the reactions low and moderate and an average CS value of 1.75 (Fig.1.D). For high Fuhrman grade ccRCC the intensity of the staining reactions was moderate and high, with average percentage values of positive cells of 33.05±25.70 and a CS value of 3.92 (Fig.1.E). In advanced stage ccRCCs (pT3/stage III), the average percentage of marked cells was 27.41±18.61, the intensity of the reactions was moderate or absent and CS value was 2.87.

Through statistical analysis of the analyzed parameters, we have shown significant associations between high CS of Snail and high Fuhrman grade (p=0.007) (Fig.1.E) and fat tissue invasion (p=0.03).

The E-cadherin immunoreaction was noticed in 86.95% of the 46 analyzed cases, with cytoplasmic and membrane localization. The

 10.12865/CHSJ.45.02.09

immunoexpression varied according to Fuhrman grade both in percentage and intensity. Low Fuhrman grade ccRCCs had an average percent of marked cells of 46±22.97, the intensity of the reactions was moderate and average CS value was 4.97 (Fig.1.F). For high Fuhrman grade ccRCC, average percentage of marked cells was 4.6±4.3 and the intensity of the staining reactions was mostly low, with average CS value of 0.69 (Fig.1.G). In advanced stage ccRCCs (pT3/stage III), the average percent of marked tumor cells was 31.29±25.85, the intensity of reactions was mostly moderate and average CS value was 3.48. The statistical analysis indicated significant associations between high CS, E-cadherin and low Fuhrman grade (p=0.000) (Fig.1.H) and the absence of vascular invasion (p=0.031).

The analysis of the investigated markers indicated a statistically significant negative correlation between E-cadherin and SNAIL (p <0.05, Pearson) (Fig.2).

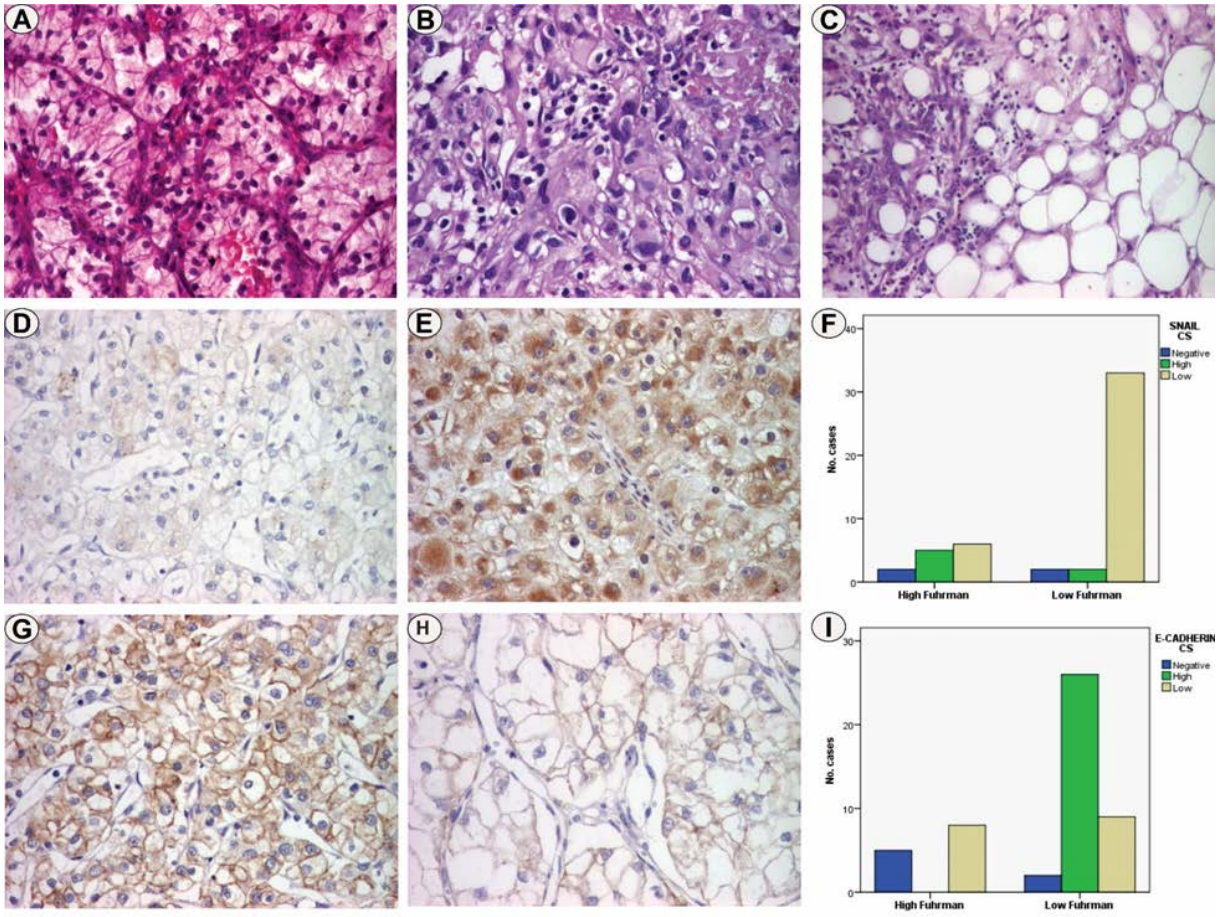

Fig. 1. ccRCC: (A) Low grade, Fuhrman 1, HE, x100; (B) High grade, Fuhrman 3, HE, x100; (C) Fat tissue invasion; (D)Low grade, SNAIL immunostaining, ×100; (E) High grade, SNAIL immunostaining, ×100; (F) Cases distribution depending on Fuhrman grade and SNAIL CS; (G)Low grade, E-cadherin immunostaining, ×100; (H) High grade, E-cadherin immunostaining, ×100; (I) Cases distribution depending on Fuhrman grade and E-cadherin CS.

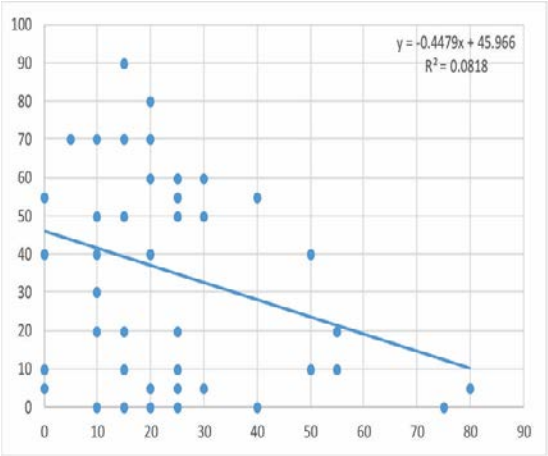

Fig.2. Values distribution of the labeled cells for the investigated markers

Discussion

Epithelial-mesenchymal transition (EMT) is characterized by the loss of expression of epithelial markers and the acquisition of mesenchymal markers [12]. One of the most important roles in this process is played by the Snail transcription factor [13]. An important characteristic of EMT is the loss of E-cadherin expression [14,15], which is expressed in epithelial cells and its expression is reduced during EMT [6,7].

Snail is a zinc-finger transcription factor and an important repressor of E-cadherin [16]. The Snail expression was observed in several malignant tumors where it was correlated with the tumor invasion and metastasis [16,17].

In this study we quantified the immunostaining of Snail and noticed that the immunoreaction was identified in the cytoplasm of tumor cells in 92% of the studied cases. For E-cadherin, the immunostining was identified in 70% of the cases, with cytoplasmic and membrane pattern.

Through the analysis of the cases we investigated the Snail immunomarking and discovered statistically significant associations between the increase of Snail expression and high Fuhrman grade. Statistical analysis also revealed significant associations between Snail immunoexpression and the presence of fat tissue invasion. We have also determined a statistically significant association between E-cadherin expression and low Fuhrman grade and the absence of vascular invasion.

Reports in the literature confirm the results of our research on overexpression of Snail in high Fuhrman grade ccRCC. Mikami et al. evaluated the expression of Snail in ccRCC and reported positive associations between Snail expression and tumor stage and Fuhrman grade [18]. They noticed that ccRCC with low Snail expression show relatively high percentage of E-cadherin marked tumor cells compared to tumors with high Snail immunoexpression, but the difference was not statistically significant [18].

Similar results were reported in other studies [13,19,20]. Liu et al. also analyzed the association between the intensity of Snail immunostaining of tumor cells on cytoplasmic and nuclear levels and clinical-pathological parameters of ccRCC patients and concluded that those cases with high cytoplasmic Snail expression matched high Fuhrman grade ccRCC [21]. Cytoplasmic expression of Snail did not present a statistically significant association with any other clinical-pathological parameter [21]. Similar to our results, Messai et al. reported that the Snail expression is lower in low grade carcinomas than high grade ones [22].

Cai et al. have also reported that the expression of E-cadherin and Snail in ccRCC was significantly correlated with the tumor differentiation grade. Unlike our study, they also determined significant associations between the Snail expression and clinical stage, local invasion and distant metastasis, and a negative correlation between the expressions of Snail and E-cadherin [20].

There are other studies in which the low expression of E-cadherin was significantly associated with unfavorable prognosis in renal carcinomas [23, 24]. Similar to our study, Xinqi et al. did not determine a significant association between the expression of E-cadherin and tumor stage, necrosis, age and sex but, unlike our results, they also didn't determine an association with Fuhrman grade [25].

Conclusions

In this study, high-grade ccRCCs have associated high Snail and reduced E-cadherin expression, suggesting the loss of epithelial phenotype and the acquisition of a mesenchymal one.

The results indicate that these markers could be useful to evaluate the aggressiveness of lesions and to determine a better targeted therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

10.12865/CHSJ.45.02.09

References

1. Gupta K, Miller JD, Li JZ, Russell MW, Charbonneau C. Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev, 2008, 34(3): 193-205.
2. 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.
3. Son H, Moon A. Epithelial-mesenchymal transition and cell invasion. Toxicol Res, 2010, 26(4):245-252.
4. Larue L, Antos C, Butz S, Huber O, Delmas V, Dominis M, Kemler R. A role for cadherins in tissue formation, Development, 1996, 122(10):3185-3194.
5. Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene, 2005, 24(50):7443-54.
6. Hay ED. An Overview of Epithelio-Mesenchymal Transformation. Acta Anat (Basel), 1995, 154(1):8-20.
7. Hay ED, Zuk A. Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced. Am J. Kidney Dis, 1995, 26(4):678-90.
8. Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer, 2007, 7(6):415-428.
9. Evans AJ, Russell RC, Roche O, Burry TN, Fish JE, Chow VW, Kim WY, Saravanan A, Maynard MA, Gervais ML, Sufan RI, Roberts AM, Wilson LA, Betten M, Vandewalle C, Berx G, Marsden PA, Irwin MS, Teh BT, Jewett MA, Ohh M. VHL promotes E2 box-dependent E-cadherin transcription by HIF-mediated regulation of SIP1 and snail. Mol Cell Biol, 2007, 27(1):157-169.
10. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest, 2009, 119(6):1429-1437.
11. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res, 2014, 33:62.
12. Son H, Moon A. Epithelial-mesenchymal Transition and Cell Invasion. Toxicol Res, 2010, 26(4):245-52.
13. Meng FD, Li Y, Tian X, Ma P, Sui CG, Fu LY, Jiang YH. Synergistic effects of snail and quercetin on renal cell carcinoma Caki-2 by altering AKT/mTOR/ERK1/2 signaling pathways. Int J Clin Exp Pathol, 2015, 8(6):6157-6168.
14. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol, 2005, 17(5):548-558.
15. Hulpiau P, van Roy F. Molecular evolution of the cadherin superfamily. Int J Biochem Cell Biol, 2009, 41(2):349-369.
16. Muqbil I, Wu J, Aboukameel A, Mohammad RM, Azmi AS. Snail nuclear transport: the gateways regulating epithelial-to-mesenchymal transition? Semin Cancer Biol, 2014, 27:39-45.
17. Wu Y, Zhou BP. Snail: more than EMT. Cell Adh Mig, 2010, 4(2):199-203.
18. Mikami S, Katsube KI, Oya M, Ishida M, Kosaka T, Mizuno R, Mukai M, Okada Y. Expression of Snail and Slug in renal cell carcinoma: E-cadherin repressor Snail is associated with cancer invasion and prognosis. Lab Invest, 2011, 91(10):1443-1458.
19. O'Mahony FC, Faratian D, Varley J, Nanda J, Theodoulou M, Riddick AC, Harrison DJ, Stewart GD. The use of automated quantitative analysis to evaluate epithelial-to-mesenchymal transition associated proteins in clear cell renal cell carcinoma. PLoS one, 2012, 7(2):e31557.
20. Cai J. Roles of transcriptional factor Snail and adhesion factor E-cadherin in clear cell renal cell carcinoma. Exp Ther Med, 2013, 6(6):1489-1493.
21. Liu W, Liu Y, Liu H, Zhang W, An H, Xu J. Snail predicts recurrence and survival of patients with localized clear cell renal cell carcinoma after surgical resection. Urol Oncol, 2015, 33(2): 69.e 1-10.
22. Messai Y, Noman MZ, Derouiche A, Kourda N, Akalay I, Hasmim M, Stasik I, Ben Jilani S, Chebil M, Caignard A, Azzarone B, Gati A, Ben Ammar Elgaaied A, Chouaib S. Cytokeratin 18 expression pattern correlates with renal cell carcinoma progression: relationship with Snail. Int J Oncol, 2010, 36(5):1145-1154.
23. Fang Y, Wei J, Cao J, Zhao H, Liao B, Qiu S, Wang D, Luo J, Chen Wet. Protein expression of ZEB2 in renal cell carcinoma and its prognostic significance in patient survival. PloS One, 2013, 8(5):e62558.
24. Yamasaki T, Seki N, Yamada Y, et al: Tumor suppressive microRNA-138 contributes to cell migration and invasion through its targeting of vimentin in renal cell carcinoma. Int J Oncol, 2012, 41: 805-817.
25. Xinqi Zhang, Mingxi Yang, Hua Shi, Jianxin Hu, Yuanlin Wang, Zhaolin Sun, and Shuxiong Xu. Reduced E-cadherin facilitates renal cell carcinoma progression by WNT/β-catenin signaling activation. Oncotarget, 2017, 8(12): 19566-19576.

Corresponding Author: Alex Emilian Stepan, Department of Pathology, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Romania, e-mail: astepan76@yahoo.com