Original Paper

Snail Immunoexpression in Endometrioid Endometrial Carcinomas

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ABSTRACT: Endometrial carcinoma is one of the most prevalent cancers affecting women with epidemiological placing it as the sixth most common cancer in women. One of the factors implicated in EMT (epithelial-mesenchymal transition), Snail is regarded as having a pivotal role. We selected a number of 30 endometrial carcinomas, in a 2-year period (2020-2022). Snail immunoexpression was identified in the tumor cells for 70% of the endometroid carcinoma cases studied. Tumor cells showed both nuclear and cytoplasmic expression but only nuclear signals were quantified. The average percent of marked tumor cells was 38.6±24.9, corresponding to well differentiated carcinomas. Our analysis also showed a significant association between higher tumor grade and snail expression (p=0.000). Alteration of the epithelial-mesenchymal phenotype in endometrial carcinomas by Snail overexpression in high-grade and advanced-stage lesions constitutes mechanisms involved in the process of tumor progression.

KEYWORDS: *Endometroid endometrial carcinoma, epithelial-mesenchymal transition, Snail, immunohistochemistry*

Introduction

Endometrial carcinoma is one of the most prevalent cancers affecting women with epidemiological placing it as the sixth most common cancer in women [1].

Of its subtypes, by far the most prevalent is the endometrioid variant.

Although the role of estrogenic stimulation is well established in the tumorigenesis of this entity, the mechanics of disease progression are not fully resolved.

One such mechanism of interest with implication towards development of aggressive phenotypes is the epithelial-mesenchymal transition pathway.

The central feature of cells undergoing this transition is decreased E-cadherin expression with consecutive loss of cell-cell adhesion and cell motility [2,3].

One of the factors implicated in EMT, Snail is regarded as having a pivotal role.

It is also regarded as the primary repressor of E-cadherin transcription [4,7,8,9].

Materials and Methods

We selected a number of 30 endometrial carcinomas, in a 2-year period (2020-2022).

The case study analyzed came from patients hospitalized and operated on in the Gynecologyand Surgery clinics of the Craiova County Emergency Clinical Hospital.

The hysterectomy pieces were fixed in 10% buffered formalin, processed by the usual paraffin embedding technique, followed by sectioning at 3-5µm and standard staining with Hemalaum-Eosin (Bio-Optica kit) in the Laboratory of Pathological Anatomy in the same hospital.

Later, the histopathologically investigated cases were subjected to immunohistochemical examination in the Morphopathology Discipline Laboratory of UMF Craiova.

The immunohistochemical study was of the type with enzyme detection used as a LSAB (Labelled Streptavidin-Biotin2 System) technical work piece.

For the statistical analysis we used comparison tests ($\chi 2$ test, One-Way ANOVA) within the SPSS 10 software (Statistical Package for the Social Sciences), the *p values* <0.05 being considered significant.

The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, the written informed consent being obtained from the patients.

Table 1. The antibodies we used in the study.

Antibody	Host, Clone, Manufacturer	Dilution	Pretreatment	External positive control
Snail	Mouse anti-human AB180714 Dako	1:150	Microwaving in citrate buffer, pH 6	Afterbirth

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Results

The immunohistochemical analysis comprised a total of 30 endometrioid carcinoma cases.

The results obtained were statistically interpreted according to clinical and morphopathological parameters.

Of the total of 30 cases, analysis of the morphological parameters yielded an average age of diagnosis of 60 years.

Most of the assessed lesions were well and moderately differentiated (at 14 and 9 cases respectively), with invasion of the inner half of the myometrium (18 cases) and no lymph node metastasis (28 cases).

Most of the cases were assigned Stage I according to the pTNM classification (18 cases) (Table 2).

This study showed a concordance between tumor extension (T category) and tumor stage.

The immunohistochemical markers used for the analysis of the selected cases of endometrioid carcinoma are known to be involved in EMT with quantified impact on disease progression of various malignant entities.

Table 2. Distribution of studied cases in accordance with clinical and morphological parameters.

Utilized parameters	Variables	No. of cases
Age	<50	3
	>50	27
Grade	G1	14
	G2	9
	G3	5
Positive lymph nodes	N0	28
	N1	2
Stage	I	18
	II	8
	III	4

In our estimation, data concerning correlates between epithelial mesenchymal transition profiles and tumor behavior of this entity is incomplete.

For this purpose, we utilized a marker for the SNAIL transcription factor and analyzed the expression in concordance with clinical and morphological parameters.

Snail immunoexpression was identified in the tumor cells for 70% of the endometroid carcinoma cases studied.

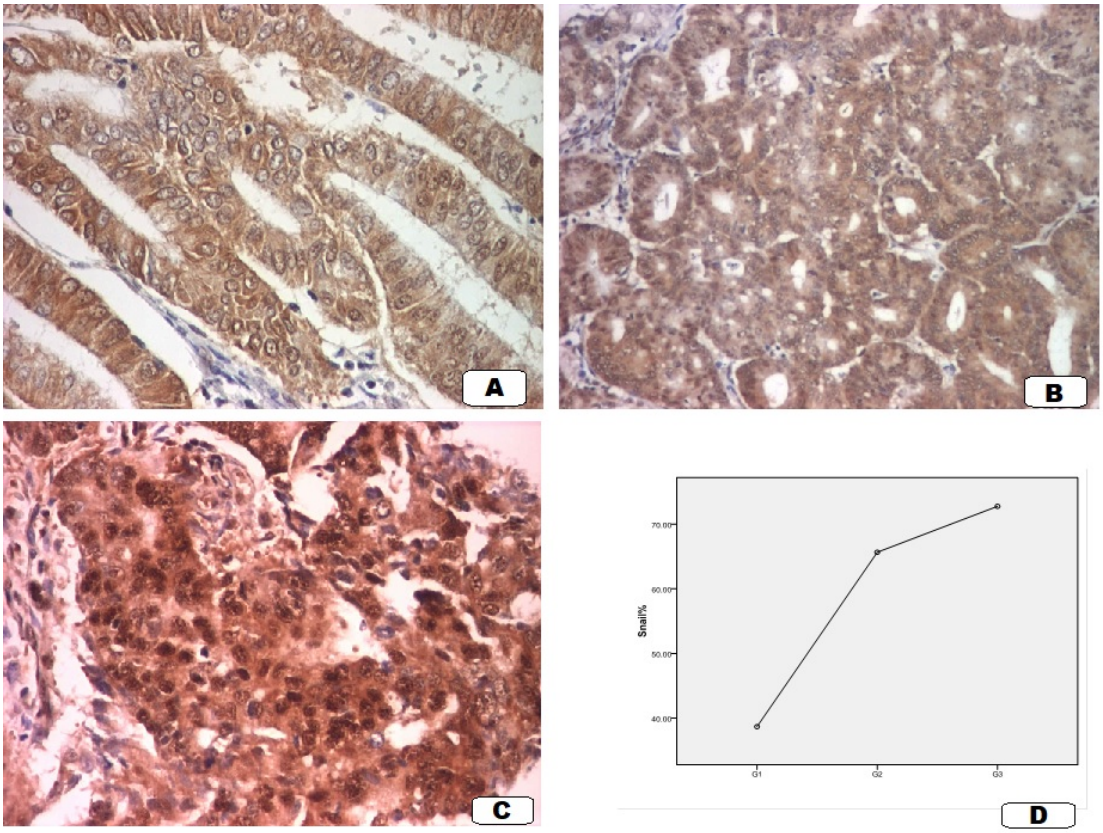

Figure 1. Endometrioid endometrial carcinoma, Snail (A, B, C) immunostaining, 20x; (A,) WD-well differentiated, (B) MD-moderate differentiated and (C) PD-poorly differentiated, (D)ANOVA graphic representation of Snail statistical differences regarding the tumor differentiation degree.

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Table 3. Snail immunoexpression in concordance with clinical and morphological parameters.

Parameters		No. of cases	Snail		p
			% cells+	SMI	
Age	<50	3	38.3±6.3	2.3	0.265
	>50	27	55.7±25	7.1	
Grade	G1	14	38.6±24.9	3.8	**0.000**
	G2	9	65.6±36.1	8.6	
	G3	5	72.7±15	10.6	
N category	N0	28	51.4±25.8	6.3	0.357
	N	2	80 ±14.1	10	
T category/Stage	T1/ I	18	51.1±28.9	5.8	0.631
	T2/ II	8	54.8±23.5	7.6	
	T3/ III	4	65±16.9	9.8	

Tumor cells showed both nuclear and cytoplasmic expression but only nuclear signals were quantified.

The average percent of marked tumor cells was 38.6±24.9, corresponding to well differentiated carcinomas.

Reaction intensity was poor/moderate with an average score of 3.8 (Figure 1, Table 3).

In contrast, moderately and poorly differentiated carcinomas showed average percent-values of 65.6±18.1 and 72.7±15, respectively.

This population showed a moderate/great intensity of expression, with average scores of 8.6 and 10.6, respectively (Figure 1, Table 3).

Regarding extension (T category) and stage, the average percent of marked cells was greater in more advanced disease (pT3/Stage III) with 65±16.9 of cells being marked.

Staining intensity was variable, with an average score of 9.8.

Stage I/pT1 and Stage II/pT2 cases showed an average population of positive tumor cells of 51.1±28.9 and 54.8±23.5, respectively.

Reaction intensity was also variable, with an average score of 5.8 and 7.6, respectively (Table 3).

Our analysis also showed a significant association between higher tumor grade and snail expression (p=0.000) (Figure 1).

Discussion

Recent studies show the involvement of a multitude of transcription factors with a key role in EMT.

The most established are members of the SNAI family (Snail, Slug and Smuc), the δEF1 family (δEF1/ZEB1 and SIP1/ZEB2) and the Twist factors.

With the exception of Twist, all the mentioned transcription factors are E-cadherin repressors [5,6].

A reduction of E-cadherin expression can be explained through multiple mechanisms.

Gene hypermethylation, post-translational changes and transcription repression have been implicated.

Snail is one of the most important transcription repressors of E-Cadherin [4,7,8,9].

As a transcription factor, Snail is also established to be central to EMT, which as a process is pivotal in developing invasion and metastases of endometrial carcinomas [10,11,12,13].

This study quantified Snail expression and revealed a positive reaction in tumor cell nuclei for 70% of the cases.

Through stratified analysis of the cases we were able to investigate Snail immunoexpression in relation to tumor grade.

We were able to find a positive and statistically significant association between Snail expression and tumor grade.

The obtained results are in accord with the consensus regarding the association between Snail expression and tumor grade.

In a study by Abouhashem et. Al, analysis of E-Cadherin, Snail and Hif-1α expression, proved that low expression of E-Cadherin and increased nuclear expression of Snail and HIF-1α were significantly associated to tumor grade, myometrial invasion and number of positive lymph nodes [10].

Through similar methods, Tanaka et. al analysed 354 endometrial carcinoma cases for correlates between Snail immunoexpression and histopathological parameters.

They showed the same pattern of increasing tumor grade in a positive relation to Snail expression.

Authors concluded that Snail expression can be significantly associated to histological type, FIGO grade, miometrial invasion, presence of ascites, lymph node metastasis and low survival time of patients ($p<0,01$) [14].

Furthermore, a statistically significant positive association of Snail expression and tumor grade is also supported by German authors.

Their study included 87 cases of primary endometrioid carcinomas and 26 metastases of the same entity.

Snail expression was observed in 53.8% of metastases and 26.8% of primary tumors correlating to increasing tumor grade ($p=0,003$) and abnormal E-Cadherin expression ($p=0,003$).

Thusly, these authors also support the role of snail in disease progression for this entity [15].

A more recent study quantified Snail and Slug expression for poorly differentiated endometrioid endometrial carcinomas, serous and clear cell endometrial carcinomas and their association to prognosis and clinico-morpho-pathological characteristics.

The study finds Snail overexpression in all the 52 cases of poorly differentiated endometrial tumors, with an overexpression of Slug in 25% of the cases, with Slug being significantly associated to local recurrence [16].

Regarding local extension and stage, the study established that these two markers do not significantly associate with Snail expression ($p=0.631$) or age of patients ($p=0.265$) with results confirming the consensus [17].

Involvement of EMT in disease progression of endometrioid endometrial tumors is also supported by the generally increased expression of Snail in endometrial carcinomas compared to normally functioning endometrium [18].

A number of studies also show the importance of the Snail family of transcription factors in inducing EMT in cervical carcinomas, endometrial carcinomas of other histological types, ovarian and vulvar carcinomas [19,20,21,22,23].

Snail and slug are significantly associated with lymph node metastases [24].

Snail is overexpressed in primary and metastatic tumors and consistently reduces E-Cadherin expression [22].

A metanalysis published by Becker et. al. studied 2000 cases of nine different tumor types (breast, gastric, colonic, hepatic, ovarian, esophageal, head and neck and endometrial carcinomas) reported in 21 studies.

Their findings suggest an important role of Snail expression in hormone-dependent carcinomas with a less important impact for digestive carcinomas in maintaining an invasive phenotype [25,26,27].

Conclusions

Alteration of the epithelial-mesenchymal phenotype in endometrial carcinomas by Snail overexpression in high-grade and advanced-stage lesions constitutes mechanisms involved in the process of tumor progression.

The investigation of this mechanism involved in epithelial-mesenchymal transition in this type of carcinomas supports the additional study of both protective (E-cadherin) and aggressiveness (N-cadherin, P-cadherin and Snail) markers for the stratification of high-risk patients and at the same time they can constitute potential therapeutic targets.

Conflict of interests

No conflict of interest to declare

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