The immunoexpression of p53 and Snail in endometrioid endometrial carcinomas

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Abstract

Endometrial cancer is one of the most common tumors in women worldwide. P53 has a well-known function as tumor suppressor, but it can also regulate the tissues metabolism, differentiation and development. Snail is a zinc-finger transcription factor, involved in the cell differentiation and survival. We analyzed the immunoexpression of p53 and Snail in 55 cases of endometrioid endometrial carcinoma (EEC), in relation with the histopathological prognosis parameters and tumoral compartments, respectively intratumoral and advancing edge areas. For both markers, we found a statistically significant association with histological grade, in relation with tumoral compartments. P53 and Snail can be used in developing EEC targeted treatment.

Keywords: endometrioid carcinoma, p53, Snail.

Introduction

Endometrial cancer is one of the most common cancers in women worldwide and represents the sixth cause of death by cancer in women [1]. From all endometrial cancer, 97% are carcinomas and only 3% are sarcomas [2]. Endometrial carcinogenesis includes two types of carcinomas, respectively the endometrioid type I, the most frequent, which is influenced by excessive estrogenic stimulation and develops on a previous endometrial hyperplasia and the non-endometrioid type II, which appears de novo and has a lower incidence and a poor prognosis [1].

P53 (tumor protein p53 – TP53) was first described as an oncogene [3]. Many studies had come and it is now known that p53 is a tumor suppressor gene [4]. It was described as “the guardian of the cell cycle”, due to its function in conserving stability by preventing genome mutation [5]. It can arrest growth by stopping the cell cycle in either the G1 or G2 phase [4]. It can activate DNA repair proteins and it can initiate the apoptosis if DNA damage proves to be irreparable [4].

Snail, a zinc-finger transcription factor, was described for the first time as a regulator of mesoderm formation [6]. Snail is important for tumor cells in obtaining resistance to apoptosis, therefore increasing tumor survival [7]. Also, Snail induces migratory properties, helping malignant cells to separate from the primary tumor and to be able to invade and generate metastasis [7, 8].

Aim

In this study, we analyzed the p53 and Snail immunoexpression in endometrial endometrial carcinoma (EEC) tumor compartments, in relation to the histopathological (HP) prognostic parameters of the lesions.

Materials and Methods

The study included a number of 55 patients diagnosed with EEC, in the Department of Pathology, Emergency County Hospital, Craiova, Romania. The specimens were received from the Clinics of Surgery and Gynecology of the same hospital. The classification of cases for grade and tumor stage was done according to World Health Organization (WHO) recommendations (2014) [9]. The parameters investigated were represented by age, histological grade, depth of myometrial invasion, lymph nodes involvement, International Federation of Gynecology and Obstetrics (Fédération Internationale de Gynécologie et d’Obstétrique – FIGO) stage. The Ethical Committee approved this study and each patient has signed a written informed consent.

We analyzed the cases using the classic HP technique consisting in paraffin embedding and Hematoxylin–Eosin (HE) staining. The immunohistochemical (IHC) reactions for p53 and Snail were performed using Polymer Detection Kit-MACH 4 Universal HRP-Polymer (Biocare, code M4U534L), processed according to the protocol indicated in datasheet. The 4-μm sections were cut, deparaffinized in xylene, hydrated in graded ethanol and immersed in 0.3% hydrogen peroxide, for 30 minutes, to block endogenous peroxidase activity. For the antigen retrieval, we used the Heat-Induced Epitope Retrieval (HIER) technique, the slides being microwaved 20 minutes in Tris-EDTA (ethylenediaminetetraacetic acid) buffer (pH 9) for p53 and in citrate buffer (pH 6) for Snail. The sections were incubated over night, at 4°C, with the primary antibody represented by mouse monoclonal anti-human p53 (clone DO-7, Dako, 1:50 dilution) and rabbit polyclonal anti-human Snail (clone ab180714, Abcam, 1:50 dilution).

To observe the signals, we used 3,3’-diaminobenzidine
tetrahydrochloride (DAB, Dako, code 3468) followed by counterstaining with Mayer’s Hematoxylin. In this study, we used negative controls, in which the phosphate-buffered saline (PBS) solution replaced the primary antibody. We analyzed the nuclear staining for p53 and Snail.

The IHC assessment was performed using a positivity index (PI), which resulted by reporting the number of labeled cells to the total number of tumor cells counted at 20× microscope objective. For each case, we counted 1000 cells. We assess the signals in both intratumoral and advancing edge compartments.

The advancing edge was established as the deepest rim of cancerous tissue grown into the myometrium, having a size of one full microscopic field, at 20× magnification. The acquisition of the images was done on a Nikon Eclipse E600 microscope equipped with a color charge-coupled device (CCD) camera and the Lucia 5 software package.

Statistical analysis was made using the Pearson’s test, Student’s t-test and one-way analysis of variance (ANOVA) test within Statistical Package for the Social Sciences (SPSS) 12 software. The p-value <0.05 was considered to be statistically significant.

### Results

The clinico-histopathological study was done on 55 cases of EEC. The average age of patients was 60±7.2 years old. The analyzed EEC were well differentiated (G1) in 36.3% of cases, moderately differentiated (G2) in 43.6% of cases and poorly differentiated (G3) in 20% of cases (Table 1).

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>p53</th>
<th>Snail</th>
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<tbody>
<tr>
<td>Age [years]</td>
<td></td>
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<tr>
<td>&lt;50</td>
<td>p*=0.559</td>
<td>p*=0.822</td>
</tr>
<tr>
<td>≥50</td>
<td>p*=0.74</td>
<td>p*=0.701</td>
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<tr>
<td>Histological grade</td>
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<tr>
<td>G1</td>
<td>p**=0.012</td>
<td>p**=0.001</td>
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<tr>
<td>G2</td>
<td>p**=0.001</td>
<td>p**=0.001</td>
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<tr>
<td>G3</td>
<td>p**=0.001</td>
<td>p**=0.001</td>
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<tr>
<td>Depth of invasion (pT)</td>
<td></td>
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<tr>
<td>&lt;1/2 myometrium</td>
<td>p*=0.087</td>
<td>p*=0.46</td>
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<tr>
<td>≥1/2 myometrium</td>
<td>p*=0.42</td>
<td>p*=0.42</td>
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<tr>
<td>Lymph node involvement (pN)</td>
<td></td>
<td></td>
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<tr>
<td>N0</td>
<td>p*=0.006</td>
<td>p*=0.545</td>
</tr>
<tr>
<td>N1</td>
<td>p*=0.841</td>
<td>p*&lt;0.05</td>
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<td>FIGO stage</td>
<td></td>
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<tr>
<td>I</td>
<td>p**&lt;0.001</td>
<td>p**=0.759</td>
</tr>
<tr>
<td>II</td>
<td>p**=0.834</td>
<td>p**=0.834</td>
</tr>
<tr>
<td>III</td>
<td>p*&lt;0.001</td>
<td>p*&lt;0.001</td>
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</table>

Most of EEC cases, respectively 54.5%, had more than half of myometrium invaded. The lymph node involvement was observed in 10.9% of all EEC cases. Regarding the tumoral stage based on FIGO classification, most of EECs were classified as stage I, respectively 65.4% of cases.

P53 immunoreaction was identified at the nuclear level of tumoral cells in all cases, but also in endometrial stromal cells, myometrial cells and fibroblasts (Figure 1).

The average value of p53 PI for the analyzed group was 38.6±18.7 for the intratumoral compartment and 51.3±19.5 for the advancing edge, the differences being statistically significant (p<0.001, Student’s t-test).

When considering the histological grade of the tumor, we observed that the mean PI values for p53 were higher in poorly differentiated EEC compared to moderately and well differentiated EEC; these differences being statistically significant both in the intratumoral compartment (p<0.05, ANOVA test) and at the advancing edge (p<0.05, ANOVA test).

In terms of depth of myometrial invasion, we found mean values of p53 PI higher for EEC cases with invasion in the external half of myometrium compared with those with invasion only in the internal half of myometrium, but the differences were not statistically significant for both compartments, respectively intratumoral (p>0.05, Student’s t-test) and the advancing edge (p>0.05, Student’s t-test). The analysis of p53 PI in relation to the presence of lymph node involvement indicated variable values, higher at the advancing edge compared with the intratumoral compartment (67±9 vs. 53.3±9.7) and the differences were statistically significant for both compartments (p<0.05, Student’s t-test). The assessment of p53 PI in relation with the tumoral stage showed that the values were higher for carcinomas with advanced stages, compared to I and II stages, and the differences were statistically significant both at the intratumoral compartment (p<0.05, ANOVA test) and at the advancing edge (p<0.05, ANOVA test).
Snail immunoexpression was identified at the nuclear level in malignant cells and also in the cytoplasm, but only the nuclear reaction was assessed. The expression of Snail was present in all EEC cases (Figure 2).

The average value of Snail PI in the analyzed group was 81.5±22 for the intratumoral compartment and 70±21.6 for the advancing edge, the differences being statistically significant ($p=0.006$, Student’s $t$-test).

The assessment of the histological grade of the tumor showed that the mean values of Snail PI were higher in poorly differentiated EEC compared with moderately and well differentiated EEC, differences that were statistically significant both at the intratumoral compartment ($p<0.05$, ANOVA test) and at the advancing edge ($p<0.05$, ANOVA test).

Analyzing the depth of myometrial invasion, we found mean values of Snail PI higher for EEC cases with invasion in the external half of myometrium compared with those
with invasion in the internal half of myometrium, but the differences were not statistically significant for the two compartments ($p>0.05$, Student’s $t$-test). Regarding the Snail PI in relation to lymph node involvement, the mean values were higher to the intratumoral compartment compared with the advancing edge, but not statistically significant ($83.8 \pm 10.5$ vs. $73.6 \pm 25.8$). When considering the tumoral stage, the analysis of Snail PI presented higher mean values for carcinomas with advanced stages, compared with I and II stages, but the differences were not statistically significant both at the intratumoral compartment ($p>0.05$, ANOVA test) and at the advancing edge ($p>0.05$, ANOVA test).

Pearson’s correlation index revealed a positive linear correlation between the p53 and Snail at the intratumoral compartment ($p=0.174$) and at the advancing edge ($p=0.200$) (Figure 3).

In this study, we did not find statistically significant differences between PI for p53 or Snail and the age of patients.

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Figure 2 – Endometrioid endometrial carcinoma (Snail immunostaining, ×100): (A) Well differentiated, advancing edge; (B) Well differentiated, intratumoral; (C) Moderate differentiated, advancing edge; (D) Moderate differentiated, intratumoral; (E) Poorly differentiated, advancing edge; (F) Poorly differentiated, intratumoral.
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Discussions

Endometrial cancer is the most frequent gynecologic malignancy in the Western World with more than 280,000 new cases per year worldwide in 2008 [10]. In Romania, the endometrial cancer had an incidence of 8.06/100,000 women in 2013 [10]. The most common type of carcinoma of the uterus is endometrioid type [1].

In our study, the mean age was 60 ± 7.2 years. The literature data indicates various averages of age, in the moment of EEC diagnosis, ranged between 55.4–72 years old [11–13].

In this study, most of the cases were moderately EEC (43.6%), with external half invasion of the myometrium (54.5%), without lymph node involvement (89%) and with FIGO stage I (65.4%).

Other studies found a predominance of moderately differentiated EEC (45.26%), with internal half of the myometrium invasion (68.42%) [11]. Statistically significant differences were found in the literature, between the gross depth of myometrial invasion and the tumor grade [14]. Furthermore, patients with more than 50% of myometrium invasion on gross visual intraoperative estimation were more likely to have poorly differentiated endometrial cancer [14]. Regarding the presence of lymphovascular invasion, literature data indicated 57.14% of cases without lymphovascular invasion [15], respectively 75.4% also with negative lymphovascular space invasion [16]. In a similar manner to our results, other studies suggest that the majority of patients (89.5%) in the moment of diagnosis had stage I or II of disease [11].

p53 is a protein that regulates the cell cycle and acts as a tumor suppressor [7]. 17–61% of p53 mutations appear in endometrioid cancers towards 93–100% in serous type [8]. Various authors showed a high frequency of p53 mutations in different human cancers [17]. Overexpression of p53 is seen in high percentage of colorectal carcinoma, and also in tumors of the thyroid, uterus, breast, urinary bladder and gallbladder [17, 18].

In this study, we found superior values of p53 PI in EEC associated with high histological grade, both at the advancing edge and respectively in the intratumoral compartment, with more than half of myometrium invasion. The p53 PI for the lymph node invasion was higher at the advancing edge compared the intratumoral compartment. Regarding the tumoral stage, we observed in both compartments higher values of p53 PI in cases with FIGO stage III.

Ozsaran et al. found a strong expression of p53 in relation with advanced stage, high histological grade and the presence of lymphovascular invasion for endometrial cancer [19]. Furthermore, the literature also recognizes that overexpression of p53 is associated with a high grade, more advanced stages an also a poor prognosis [20]. In another study was also observed that an increase in p53 expression was statistically significant associated with high pathological grade and lymph node metastasis [21].

While some authors have identified associations between this marker and the parameters of aggressivity, others have not found such associations. Ragni et al. did not found associations between histological characteristics or early (stage I) and advanced (stages II–IV) endometrial carcinomas regarding the p53 overexpression [22].

Snail (SNAI1) belongs to the Snail family proteins that can induce the epithelial–mesenchymal transition (EMT) needed in the normal embryonic development [23], neural differentiation, cell division and cell survival [24]. Those proteins are also regulating the EMT-like processes, needed for tumor cell invasion, migration and metastasis [23]. The transcription factor Snail, as well as SLUG, TWIST or ZEB1, is a negative regulator of metastasis suppressor genes such as E-cadherin [25].

Several studies have assessed the associations between Snail and clinico-morphological parameters in various diseases. For example, Snail and nodal involvement are independent prognostic factors in gastric cancer cases [26]. Patients with colorectal carcinoma have elevated expression of mesenchymal marker Snail [27]. The activation of Snail induces invasive behavior in malignant tumor cells of the breast [28]. Baritaki et al. proposed Snail and Raf kinase inhibitor protein (RKIP) expressions as potential prognostic biomarkers [24]. Snail induces migration in cancer cells like breast cancer, gastric cancer, hepatocellular carcinomas, ovarian carcinoma, oral squamous cell carcinoma or head and neck cancer [24].

In our study, we observed a high Snail PI both at the advancing edge and intratumoral in cases with high histological grade, more than half of myometrium invasion and advanced FIGO stage III.
There are only a few studies that have assessed the expression of Snail for endometrioid endometrial carcinoma. Tanaka et al. found a statistical significant association between nuclear Snail expression and histological grade, FIGO stage, myometrial invasion and also the presence of lymph node metastasis [29]. Reduced E-cadherin and increased nuclear expression of Snail were significantly associated with HP grade, myometrial invasion, clinical stage and lymph node metastasis for endometrial carcinoma type I [30]. Whereas increased E-cadherin expression is a good prognosis marker, increased Snail expression is associated with poor prognosis in endometrioid endometrial carcinoma [30]. E-cadherin repressors, like Snail (SNAI1), Slug (SNAI2), HMG A2, ZEB1, TWIST1, had higher expression in EEC especially with deep myometrial invasion than in normal endometrium [31]. Positive Snail immunoreactivity in EEC metastasis was statistically associated with higher tumor grade and abnormal E-cadherin expression [32]. On the other hand, Supernat et al. found a lower Snail expression in endometrial cases than in normal endometrium [33]. However, decreased Snail expression was statistically significant associated with post-menopausal status [33]. Also, there are studies indicating superior response to treatment in case of Snail inhibition, which supports the inclusion of this transcription factor as a therapeutic target [34].

Conclusions

In this study, we found higher values of p53 and Snail expression in EEC with high grade and advanced stage. In relation with tumor compartments, p53 immunoreexpression was higher at the advancing edge, while Snail was more expressed in intratumoral areas. The analysis of values associated with expressions of the two markers indicated a positive linear correlation in both tumor compartments. These aspects suggest the utility of p53 and Snail in assessing the EEC aggressivity and propose the inclusion of these markers in panels of possible therapeutic targets.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors read and approved the final manuscript.

References

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