Value of H-TERT and CD10 in differentiating endometrial carcinoma from atypical endometrial hyperplasia: an immunohistochemical study

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Context
The histopathological differentiation between atypical endometrial hyperplasia and well-differentiated conventional endometrial carcinoma is sometimes tricky, particularly in endometrial dilatation and curettage specimens, to the extent that a differentiating marker is sought.
Aim
This study was devoted to evaluate the immunohistochemical expression of CD10 and human telomerase reverse transcriptase (H-TERT) in atypical endometrial hyperplasia and endometrial carcinoma to determine their role in differentiating both lesions.
Patients and methods
Thirty paraffin blocks of endometrial biopsy distributed as 15 cases of atypical endometrial hyperplasia and 15 cases of conventional endometrial carcinoma were studied immunohistochemically using antibodies against CD10 and H-TERT. Data were represented as mean, SD, and percentage. The Fisher exact test was used to compare immunoexpression between atypical endometrial hyperplasia and endometrial carcinoma. The Mann–Whitney *U*-test and the Kruskal–Wallis test were used to compare between the two marker expressions in both lesions. The one-way analysis of variance test was used to determine whether the difference was significant. A *P*-value of less than 0.05 was considered significant.
Results
A statistically significant difference was observed between CD10 and H-TERT expression in both lesions, but only H-TERT significantly correlated with international federation of gynecology and obstetrics (FIGO) tumor grades in endometrial carcinoma cases. Although H-TERT labeling index upregulates with CD10 weaker expression, the relation between the two markers was not significant.
Conclusion
Both CD10 and H-TERT may be involved in the progression from the atypical endometrial hyperplasia to endometrial carcinoma as well as to differentiate between the two lesions. However, only H-TERT may be associated with the prognosis of endometrial carcinoma.

Keywords:
CD10, endometrial carcinoma, H-TERT, hyperplasia

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Introduction

Carcinoma of the endometrium is the sixth most common cancer among women worldwide. It is the fourth most common cancer among women in the UK. In Egypt it accounts for 2.6–3.5% of all cancer incidences [1].

Endometrial carcinoma is a primary malignant tumor arising from the endometrium, which has the ability to invade the myometrium and to spread to distant sites. The 5-year survival rate is 96% if the cancer is diagnosed at an early stage, but markedly decreases to 17% if diagnosed at an advanced stage [2]. Therefore, awareness of the biomarkers that could be relevant to malignant change of the endometrium is appreciated.

Endometrial hyperplasia is defined as abnormal growth of endometrial glands, which is considered as a precursor for endometrial carcinoma, especially if associated with atypia. Besides the neoplastic transformation of epithelial cells lining the endometrial glands, there is also stromal transformation from endometrial to desmoplastic type [3]. The histopathological differentiation between atypical endometrial hyperplasia and well-differentiated conventional endometrial carcinoma is sometimes tricky, particularly in endometrial dilatation and curettage biopsy

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specimens, to the extent that a differentiating marker is sought [4].

The aim of this study was to evaluate the expression of CD10 and human telomerase reverse transcriptase (H-TERT) in atypical endometrial hyperplasia and endometrial carcinoma using immunohistochemistry in order to evaluate their effective role in differentiating between the two lesions.

CD10 is a zinc-dependent cell membrane metalloproteinase, also known as common acute lymphoblastic leukemia antigen, which is involved in several biological activities through the regulation of signal transduction of bioactive neuropeptides as well as vasoactive peptides. Many studies reported that CD10 expression in cancer stromal cells is associated with tumor progression and biological aggressiveness. In gynecologic diseases, CD10 is a sensitive marker used to identify normal and neoplastic endometrial stromal cells to the extent that it can be used perfectly to differentiate between endometrial stromal tumors and uterine smooth muscle tumors [5].

H-TERT is a reverse transcriptase enzyme that carries its own RNA molecule. It is a ribonucleotide, which is used as a template to elongate telomeres by adding the polynucleotide 'TTAGGG' to its end [6].

A telomere is a region of repetitive sequences found at each end of a chromatid; its function is to protect the end of the chromosome from deterioration or fusion with neighboring ones [7]. Telomerase reverses telomere shortening by replacing short bits of telomeres, which are, under normal circumstances, shortened during cell division by mitosis, thus allowing the cell to divide in an endless manner, which is a feature of cancerous growth [6].

Despite their effective role, the evaluation of the relationship between stromal expression of CD10 and epithelial expression of H-TERT in tumor progression is generally deficient, not to mention that the association between these two molecules in endometrial carcinoma has not been previously clarified. That is why this study has been conducted.

Patients and method

This study was conducted on 30 paraffin blocks of endometrial biopsy, distributed as 15 cases diagnosed as atypical endometrial hyperplasia and 15 cases of conventional endometrial carcinoma. All cases were collected from the Department of Pathology, Faculty of Medicine, Cairo University, as well as private laboratories during the period from September 2012 to December 2017. Cases of endometrial carcinoma were graded into grades 1, 2, and 3 according to the international federation of gynecology and obstetrics (FIGO) system:

> FIGO 1: predominance of glandular growth with patent lumina, preserved polarity of the epithelial cells and absent-to-mild epithelial stratification together with less than 5% nonsquamous solid component.
> FIGO 2: 6–50% nonsquamous solid component.
> FIGO 3: more than 50% nonsquamous solid component.

This architectural grading is upgraded by one if there is severe nuclear atypia (pleomorphism, nuclear enlargement, and prominent nucleoli) [8].

Each paraffin block was recut using rotary microtome at 4 μm thickness and then mounted on glass slides to be stained with hematoxylin and eosin for routine histopathological examination and on charged slides for immunostaining using standard immunoperoxidase method.

The primary antibodies used were as follows:

(1) CD10: CD10/CALLA, Ab-2, mouse monoclonal antibody (clone 56C6, cat. no. sc-58939, dilution 1 : 50; Santa Cruz Biotechnology Inc., Santa Cruz, California, USA). Positive control used was a section of tonsil.
(2) H-TERT: H-TERT, goat anti-human monoclonal antibody (clone sc-7215, code: c20, dilution 1 : 50; Santa Cruz Biotechnology Inc.). Positive control used was a case of squamous cell carcinoma.

Immunohistochemical evaluation:

(1) CD10: CD10 immunostaining was scored as follows:
    (a) Negative: no stromal staining.
    (b) Weak: either diffuse weak staining or weak or strong focal staining in less than 30% of stromal cells.
    (c) Strong: strong staining in 30% or more of stromal cells [9].
(2) H-TERT: the H-TERT immunostaining was evaluated using labeling index (LI), which was defined as the number of positive nuclei/total number of nuclei ×100. Only diffuse nuclear with or without nucleolar accentuation was considered as positive. About 500–1000 cells were counted in different areas of tissue sections [10].

Statistical analysis

Data were collected, coded, and analyzed using SPSS software, version 9 (IBM, chicago, USA), under Windows XP. Data were represented in the form of mean, SD, and percentage. The Fisher exact test was used to compare immunoexpression between atypical endometrial hyperplasia and endometrial carcinoma. The Mann–Whitney *U*-test and the Kruskal–Wallis test were used to compare between H-TERT and CD10 expression in atypical endometrial hyperplasia and endometrial carcinoma. The one-way analysis of variance test was used to determine whether the difference was significant. Significance was established at *P*-value less than 0.05.

Results

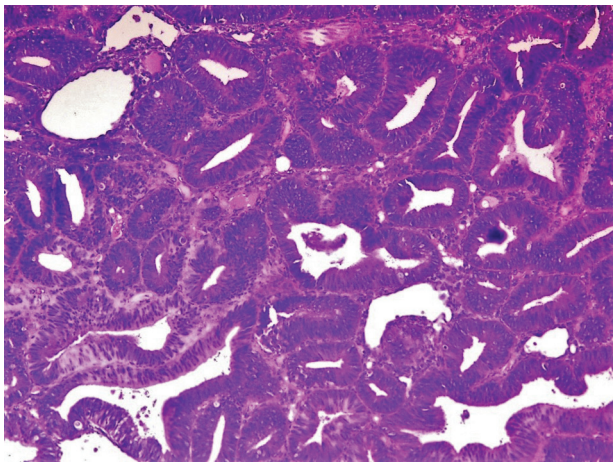
The present study consisted of 30 paraffin blocks of endometrial biopsy; 15 cases were diagnosed as atypical endometrial hyperplasia (Fig. 1) and the remaining 15 cases were diagnosed as endometrial carcinoma (Fig. 2), conventional type.

The age of the patients with atypical endometrial hyperplasia ranged from 43 to 55 years with a mean age of 48.3±3.5 years. However, in endometrial carcinoma, the age of patients ranged from 55 to 66 years with a mean age of 59.5±4.1 years. The FIGO grading system in the studied endometrial carcinoma cases revealed predominance of grade 2 as it was seen in 11 (73.4%) cases, in contrast to FIGO grades 1 and 3, which were seen in two cases for each (13.3 and 13.3%, respectively).

CD10 immunohistochemical expression

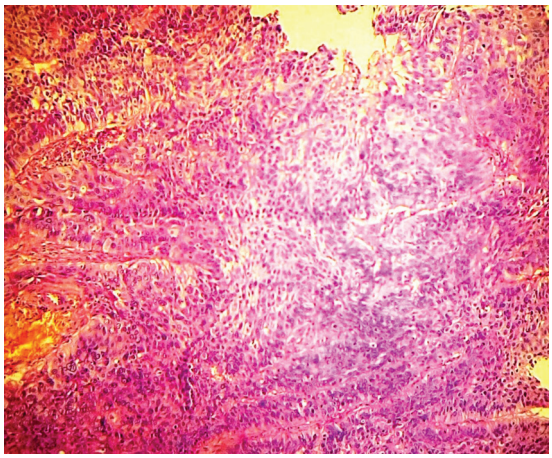
CD10 was expressed in all cases of atypical endometrial hyperplasia. Strong intensity was seen in 86.7% of cases (Fig. 3), whereas weak intensity was observed in 13.3% of cases only (Fig. 4). In cases of endometrial carcinoma, CD10 was positively expressed in 10 (10/15) cases

Figure 1



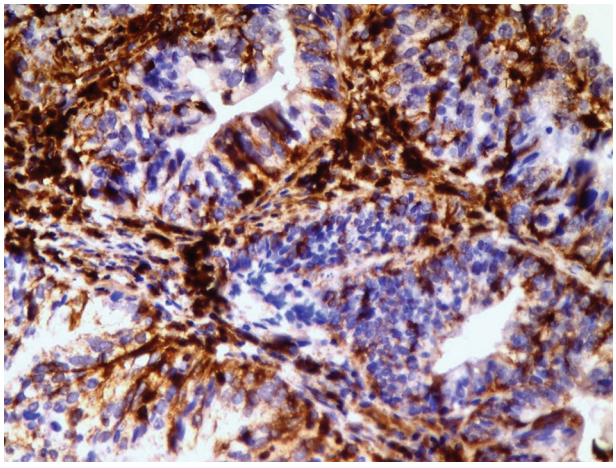
A case of atypical endometrial hyperplasia. Hematoxylin and eosin, ×200

Figure 2



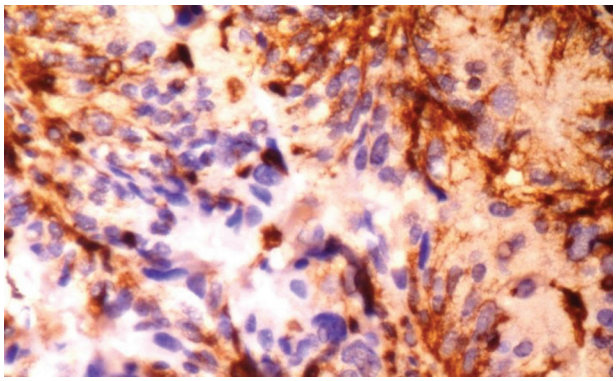
A case of endometrial carcinoma, conventional type (hematoxylin and eosin, ×200)

Figure 3



A case of atypical endometrial hyperplasia showing strong stromal CD10 immunoexpression (CD10, ×400)

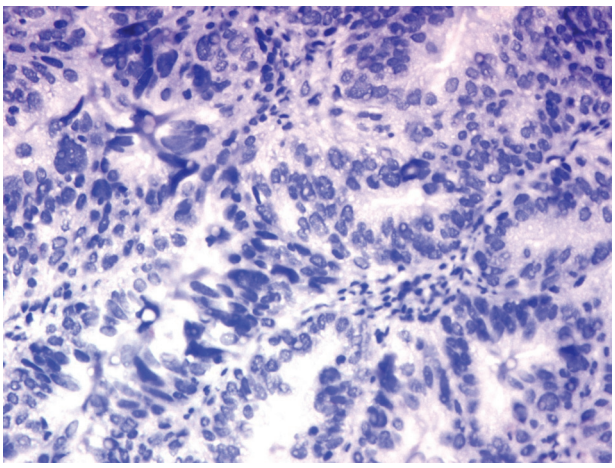
Figure 4



A case of atypical endometrial hyperplasia showing weak stromal CD10 immunoexpression (CD10, ×400)

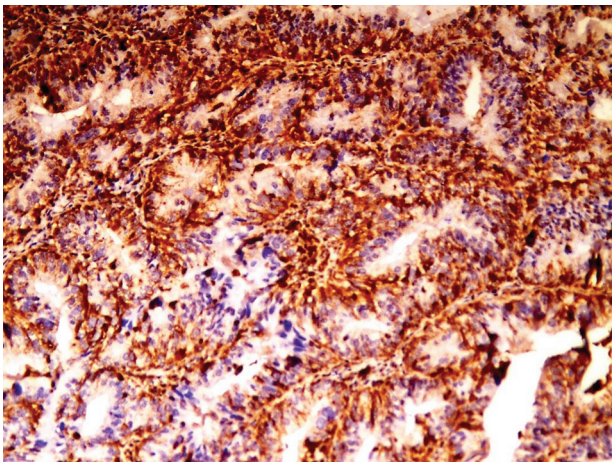
compared with five cases that were negative to CD10 (Fig. 5). Strong intensity was seen in 20% of positive cases (Fig. 6), whereas weak intensity was seen in 80% of positive cases (Fig. 7). On comparing CD10 expression in atypical endometrial hyperplasia and endometrial carcinoma (Table 1), strong intensity was more prevalent in cases of atypical endometrial hyperplasia (86.7%) compared with cases of endometrial carcinoma, which showed predominance of weak intensity (53.4% of total cases). These results were statistically highly significant. Moreover, on comparing CD10 expression in different FIGO grades of endometrial carcinoma cases, CD10 showed weak expression in slightly more than half of FIGO 1 cases (54.5%), whereas FIGO 2 cases were equally divided between negative and weak expression (50% each). As for FIGO 3, cases were equally divided between weak and strong expression (50% each). These results were statistically nonsignificant.

Figure 5



A case of conventional endometrial carcinoma showing negative stromal immunoexpression for CD10 (CD10, ×400)

Figure 6



A case of conventional endometrial carcinoma showing strong stromal CD10 immunoexpression (CD10, ×200)

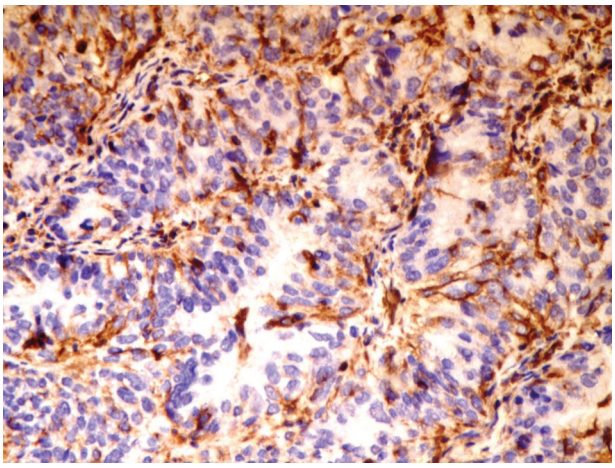
H-TERT immunohistochemical expression

H-TERT was expressed in all cases of atypical endometrial hyperplasia as well as endometrial carcinoma. In cases diagnosed as atypical endometrial hyperplasia H-TERT LI ranged from 1 to 5 with a mean LI of 2.42±1.1 (Fig. 8). However, H-TERT LI in cases of endometrial carcinoma ranged from 20 to 90 with a mean LI of 48.7±22.6 (Fig. 9). However, on comparing H-TERT expression between atypical endometrial hyperplasia and endometrial carcinoma cases (Table 2), H-TERT mean LI was much higher in endometrial carcinoma compared with atypical endometrial hyperplasia cases (48.7±22.6 and 2.42±1.1, respectively). These results were statistically significant. As for FIGO grades in endometrial carcinoma cases, the lowest mean LI was seen in FIGO 2 (44.5), whereas the highest mean LI was seen in FIGO 3 (60). These results were statistically significant.

Relation between CD10 and H-TERT expression

In atypical endometrial hyperplasia as well as in endometrial carcinoma cases, the mean LI of H-TERT was found to be lower in strong CD10 immunopositive cases and upregulated as CD10

Figure 7



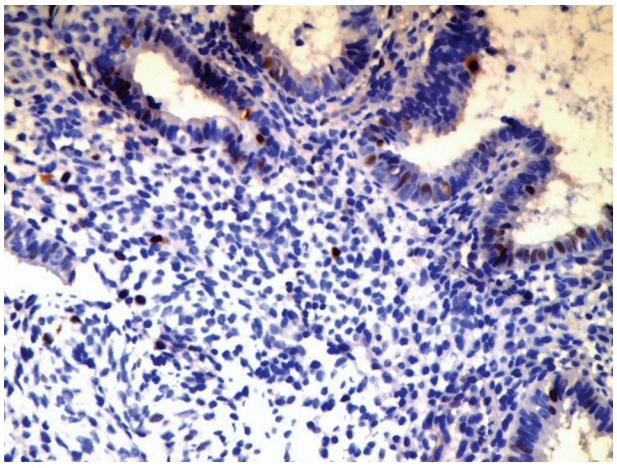
A case of conventional endometrial carcinoma showing weak stromal CD10 immunoexpression (CD10, ×400)

Table 1 Comparison between immunohistochemical expression of CD10 in atypical endometrial hyperplasia and endometrial carcinoma cases

CD10	Atypical hyperplasia [*N* (%)]	Endometrial carcinoma [*N* (%)]
Negative	0 (0)	5 (33.3)
Weak	2 (13.3)	8 (53.4)
Strong	13 (86.7)	2 (13.3)
Total	15 (100)	15 (100)

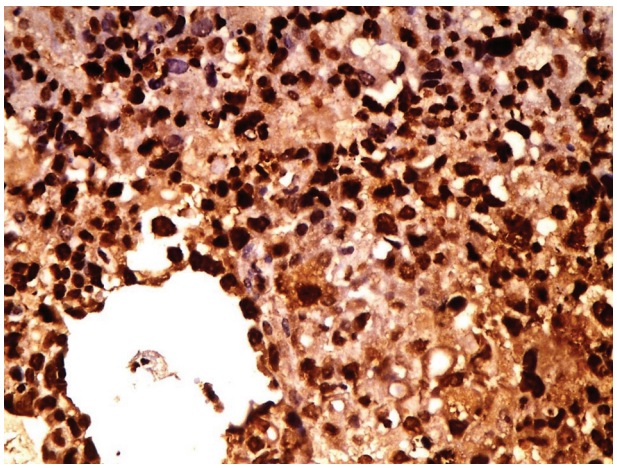
$P<0.0001$.

Figure 8



A case of atypical endometrial hyperplasia showing positive H-TERT immunoexpression in few malignant epithelial cells representing the lowest H-TERT labeling index (H-TERT, ×400)

Figure 9



A case of conventional endometrial carcinoma showing positive H-TERT immunoexpression in almost all malignant epithelial cells representing the highest H-TERT labeling index (H-TERT, ×400)

Table 2 Comparison between immunohistochemical expression of H-TERT in atypical endometrial hyperplasia and endometrial carcinoma cases

H-TERT LI	Atypical hyperplasia	Endometrial carcinoma
Minimum	1	20
Maximum	5	90
Mean	2.42	48.7
SD	1.1	22.6

LI, labeling index. $P<0.05$.

expression became weak; however, the results were not statistically significant.

Discussion

Endometrial hyperplasia, particularly with atypia, is considered as a precursor for endometrial carcinoma. As the management of endometrial carcinoma is more aggressive and the prognosis is far worse compared with atypical endometrial hyperplasia, differential diagnosis between the two lesions is very important. The differentiation between atypical endometrial hyperplasia and endometrial carcinoma is not always an easy task as it seems, and thus emerges the importance of using different ancillary tools, of which the most practical is immunohistochemistry [3].

In our study we tried to evaluate the role of two markers in differentiating atypical endometrial hyperplasia from endometrial carcinoma. One is a stromal marker, which is CD10, a sensitive marker to identify normal and neoplastic endometrial stromal cells [4], whereas the other is an epithelial marker, H-TERT, which replaces the lost bits of DNA during cell division allowing the cell to divide eternally [11].

Our study was conducted on 30 paraffin blocks of endometrial biopsy, distributed as 15 cases diagnosed as atypical endometrial hyperplasia and 15 cases of conventional endometrial carcinoma, conventional type. As universally known, both lesions mostly occur in the perimenopausal years of the patients' life. Not surprisingly, our study revealed that the mean age of endometrial carcinoma was higher than that of atypical endometrial hyperplasia (59.5±4.1 and 48.3±3.5, respectively).

All cases of atypical endometrial hyperplasia positively expressed CD10 with predominance of strong intensity (86.7% of cases). As for cases of endometrial carcinoma, CD10 was positively expressed in only 66.7% of cases with predominance of weak intensity (80% of positive cases). Thus, on comparison, a highly statistically significant difference was observed between CD10 expression in atypical endometrial hyperplasia and endometrial carcinoma, which implies that CD10 could be used as a reliable marker to differentiate between these two lesions. In endometrial carcinoma cases, we noticed that there was no relation between CD10 expression and FIGO tumor grades as weak expression was seen in no less than 50% of cases in all grades. These findings are in agreement with those stated by Ahmed and Muhammad [4], who concluded a downregulation of CD10 expression as the tumor progresses from atypical endometrial hyperplasia to endometrial carcinoma; however, their results were statistically nonsignificant. Moreover, they could not observe any significant relation between CD10 expression and FIGO tumor grades. Similarly, Suzuki *et al.* [12] reported a strong stromal CD10 expression in normal endometrium, which was

downregulated after carcinomatous transition. However, unlike our study they observed that such expression was markedly reduced in high FIGO tumor grades.

These results are actually rational because CD10 is a sensitive marker to identify endometrial stromal cells to the extent that it can be used to differentiate endometrial stromal nodule and low-grade endometrial stromal sarcoma from cellular leiomyoma and low-grade leiomyosarcoma [13]. Thus, basically it should be more expressed in atypical endometrial hyperplasia, where the glands are surrounded by endometrial stroma, than in endometrial carcinoma in which the endometrial stroma is replaced by desmoplastic one [12].

It cannot be denied that the stroma surrounding any tumor plays a very important role in its progression. Moreover, the interaction between cancer cells and surrounding stromal cells allows for the progression of the tumor [14]. Nevertheless, there are other factors not less important than the stroma that could affect the tumor progression. One of these factors is the telomerase, detected by H-TERT immunohistochemical marker. It is a reverse transcriptase enzyme, which is used as a template to elongate telomeres in order to allow the cells to divide in an endless way, which is a feature of cancerous growth [11].

In our study, H-TERT was expressed in all cases of atypical endometrial hyperplasia as well as endometrial carcinoma. However, the mean LI of H-TERT was markedly higher in endometrial carcinoma cases compared with atypical endometrial hyperplasia cases (48.7±22.6 and 2.42±1.1, respectively). Therefore, on comparison, a statistically significant difference was observed between H-TERT expression in atypical endometrial hyperplasia and endometrial carcinoma, suggesting that H-TERT could be used to solve the dilemma in differentiating between the two lesions. Of course if a cutoff value for H-TERT mean LI was reached to definitely differentiate between the two lesions it would be far better, but this needs a large number of cases to be studied. Other researchers are encouraged to complete in this field. As for FIGO tumor grades in endometrial carcinoma cases, H-TERT significantly upregulates with higher FIGO tumor grades. Our results did not differ from those obtained by Dong *et al.* [15,16], and Brustmann [17].

The possible relation between CD10 expression and H-TERT in atypical endometrial hyperplasia and endometrial carcinoma cases was studied in our research. Although there was a steady increase in H-TERT LI as the intensity of CD10 immunoexpression decreases, this relationship was not statistically significant. To our knowledge, the relation between these two markers in endometrial lesions has not been previously studied.

Conclusion

From this humble work we conclude that both CD10 and H-TERT may be involved in the progression from the atypical endometrial hyperplasia to endometrial carcinoma, as well as to differentiate between the two lesions. However, only H-TERT may be implicated in the prognosis of endometrial carcinoma. Our findings may be an important observation that could have diagnostic and prognostic implications as well as promising potential target for development of novel therapies, a subject for further investigations.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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