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Short Communication

WWP2 and its association with PTEN in endometrial cancer

Aine E. Clements [a,*], Veronica Bravo [b], Christopher Koivisto [b], David E. Cohn [a], Gustavo Leone [b]

[a] Division of Gynecology, The Ohio State University College of Medicine, United States
[b] Human Cancer Genetics, The Ohio State University College of Medicine, United States

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ABSTRACT

We wished to determine if WWP2 gene expression and PTEN protein levels inversely correlate in human endometrial cancer tissues. Fifty-one endometrioid endometrial tumors and five normal endometrial controls were available for analysis. PTEN protein levels were assessed by immunohistochemistry (IHC). WWP2 and PTEN gene expression were quantitated by RT PCR. Clinical and pathologic information was collected by chart review. We found that in tumors with low PTEN protein but normal mRNA expression there were significantly higher levels of WWP2 expression ($p = 0.0017$). Increased WWP2 expression was not associated with clinical prognostic factors including lymphovascular space invasion, ≥50% myometrial invasion, grade, stage or recurrence. WWP2 expression was not different statistically between tumors and normal controls (p = NS). Therefore, in this cohort, tumors with low PTEN protein but normal mRNA expression had elevated levels of WWP2 expression. This suggests that WWP2 may be playing a role in PTEN degradation in endometrial cancer.

1. Introduction

Endometrial cancer is the most common gynecologic malignancy in the United States, with 52,630 new cases predicted to be diagnosed in 2014 and 8590 dying of the disease (Cancer.gov, n.d.). The incidence of this disease has increased 21% since 2008, and the death rate per 100,000 has increased more than 100% in the past 20 years. This is despite overall death rates from cancer having decreased by 1.6% per year in women (Sorosky, 2012). Understanding the etiology of this disease as well as discovering new therapies is therefore becoming increasingly important.

PTEN is the most commonly mutated gene in endometrial cancer and its pathway is an important therapeutic target. Endometrioid histology is the most common subtype in endometrial cancer and also has the highest frequency of PTEN mutations (Di Cristofano and Ellenson, 2007). PTEN is the major tumor suppressor of the PI3K pathway. PTEN dephosphorylates phosphatidylinositol (3,4,5)-triphosphate (PIP_3) to phosphatidylinositol (4,5)-bisphosphate (PIP_2). PIP3 can bind to AKT causing a conformational change allowing phosphorylation at two amino acid residues (Hollander et al., 2011). Phosphorylated AKT can then go on to promote cellular growth, proliferation, angiogenesis, and prevent apoptosis. Loss of the tumor suppressive function of PTEN is thought to lead to carcinogenesis through constitutive activation of AKT.

PTEN protein levels can be altered upstream at the genetic level through mutation, but also at the transcriptional level or post-translational level. Post-translational modifications such as phosphorylation, acetylation, oxidation and ubiquitination are known to effect PTEN protein levels (Hollander et al., 2011). The frequency of such post-translational modifications is unknown in endometrial cancer. WWP2 is an E3 ubiquitin ligase that has been described to directly interact with PTEN and leads to its degradation by the proteosome (Maddika et al., 2011; Ahmed et al., 2012). Degradation by the proteosome leads to absence of protein, but normal levels of mRNA remain. If post-translational modification is the only method of alteration of the protein, normal mRNA levels should be present. This would signal not only normal transcription, but also likely no significant genetic alterations. PTEN protein levels can be determined by many methods, including immunohistochemistry (IHC). In fact, several investigators have found that low PTEN levels by IHC are associated with worse prognosis (Athanassiadou et al., 2007; Terakawa et al., 2003; Inaba et al., 2005). If ubiquitination by WWP2 and degradation by the proteosome plays a role, even in a few cases of endometrial cancer, this process could be a potential target for therapeutic intervention (Chantry, 2011).

The objective of this study was to determine if WWP2 gene expression and PTEN protein levels inversely correlate in human primary endometrial cancer tissues. Specifically, if WWP2 expression was elevated in tumors with low PTEN by IHC but normal or high levels of gene expression. Secondary objectives included determining whether *WWP2* gene expression was associated with clinical prognostic

* Corresponding author at: 500 Thomas Lane, Columbus, OH, 43214, United States.
E-mail address: aine.clements@ohiohealth.com (A.E. Clements).

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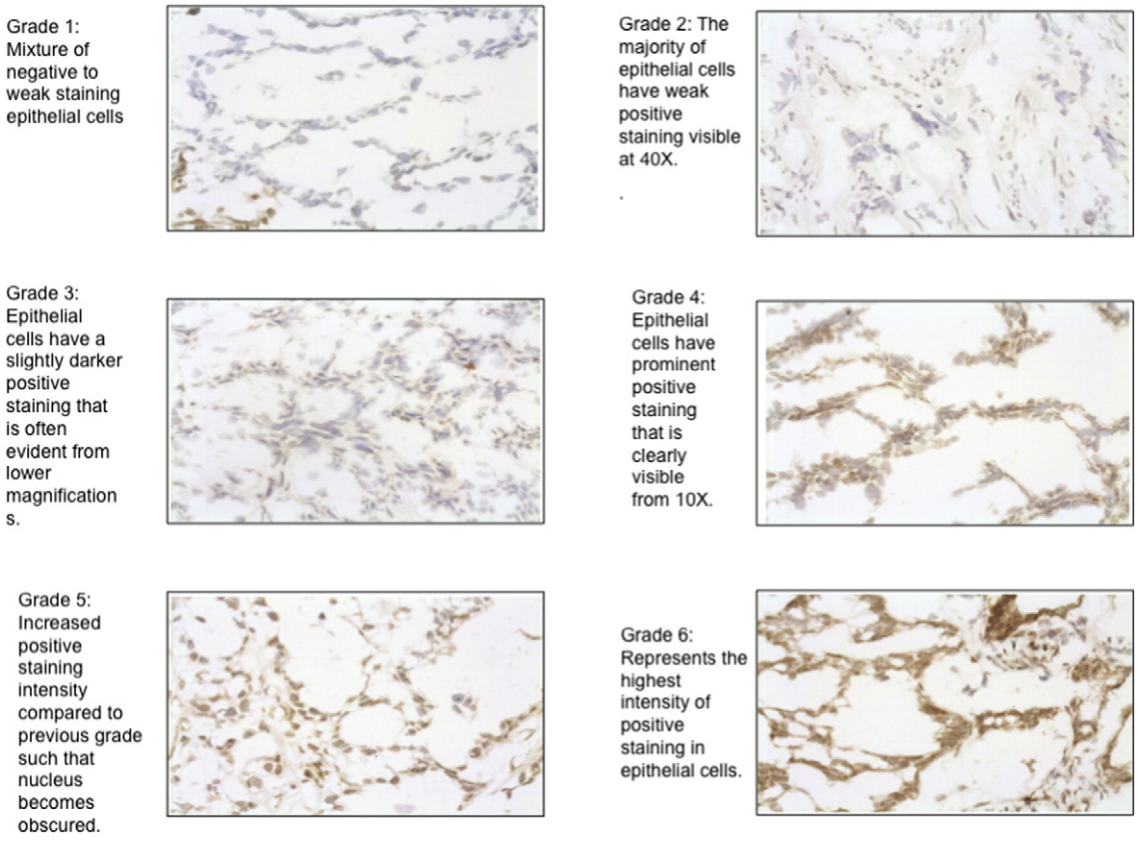

Fig. 1. Grading system for PTEN Immunohistochemistry.

variables and whether *WWP2* levels were increased in endometrial cancers compared to normal endometrium.

2. Materials and methods

Institutional review board approval was first obtained at our institution. Fifty-one endometrioid endometrial tumors from 2007 to 2010 collected at hysterectomy had fresh frozen tissue available for analysis, and five normal endometrial controls were also available for analysis. Slides from the tissues were created and were stained with a monoclonal antibody specific for the region surrounding amino acid 390 of human PTEN. IHC was then evaluated by two reviewers and scored using a system of 1–6 (Fig. 1). The reviewers were blinded to the results of the real time PCR. A normal cutoff for PTEN staining was designated as any score of 2, and any score of less than 2 defined as low PTEN staining, based on the median value.

Frozen tissue was then used for RNA extraction using TRIzol and Chloroform techniques, and cDNA was made using reverse transcription. Real time PCR (RT PCR) was then performed for *PTEN*, *WWP2*, and *GAPDH* as the control gene. The normal control with the median value was set to be the reference, and fold expression of the genes were then determined from this value using cycle thresholds (Ct) and by calculating delta–delta Ct. A fold expression of 1 was therefore designated to be normal expression of *PTEN* and *WWP2*. Greater than 1 was designated as high, less than 1 designated as low.

Tumors that had normal or high amounts of *PTEN* mRNA (at least 1 fold) but low PTEN by IHC (<2) were compared to tumors with ≥2 PTEN staining. Tumors that had low amounts of PTEN on staining but normal or high amounts of PTEN mRNA were thought to have loss of PTEN through mechanisms other than genetic mutation or transcriptional changes. Expression levels of *WWP2* in these tumors were compared using the Mann–Whitney test. We then evaluated whether high *WWP2* expression by RT PCR was associated with the poor prognostic factors of lymphovascular space invasion (LVSI), 50% or greater myometrial invasion (MI), grade, advanced disease, and recurrence by Fisher's exact test after retrospective chart review. We also compared *WWP2* expression levels in the tumors compared to the normal controls using the Mann–Whitney test.

3. Results

The median IHC score in the tumor cohort was 2 and in the normal cohort was 2.5 (p = NS). The details of the stage, myometrial invasion, grade, LVSI, positive nodes, recurrence and age of the patients are

Table 1
Clinical characteristics of cancer patients.

Clinical variable	Number (percent)
Stage	
IA	34 (67)
IB	7 (14)
II	1 (2)
IIIA	2 (3)
IIIB	0 (0)
IIIC1	1 (2)
IIIC2	5 (10)
IVA	0
IVB	1 (2)
Greater than 50% myometrial invasion	13 (25)
Grade	
1	32 (63)
2	12 (23)
3	7 (14)
Lymphovascular space invasion	11 (21)
Positive lymph nodes	8 (16)
Recurrence	3 (6)
Median age (range)	61 (46–87)