

Short Communication

WWP2 and its association with PTEN in endometrial cancer

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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, $\geq 50\%$ myometrial invasion, grade, stage or recurrence. WWP2 expression was not different statistically between tumors and normal controls ($p = \text{NS}$). 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.

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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 (PIP₃) to phosphatidylinositol (4,5)-bisphosphate (PIP₂). 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 proteasome (Maddika et al., 2011; Ahmed et al., 2012). Degradation by the proteasome 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 (Athanasidou et al., 2007; Terakawa et al., 2003; Inaba et al., 2005). If ubiquitination by WWP2 and degradation by the proteasome plays a role, even in a few cases of endometrial cancer, this process could be a potential target for therapeutic intervention (Chantray, 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

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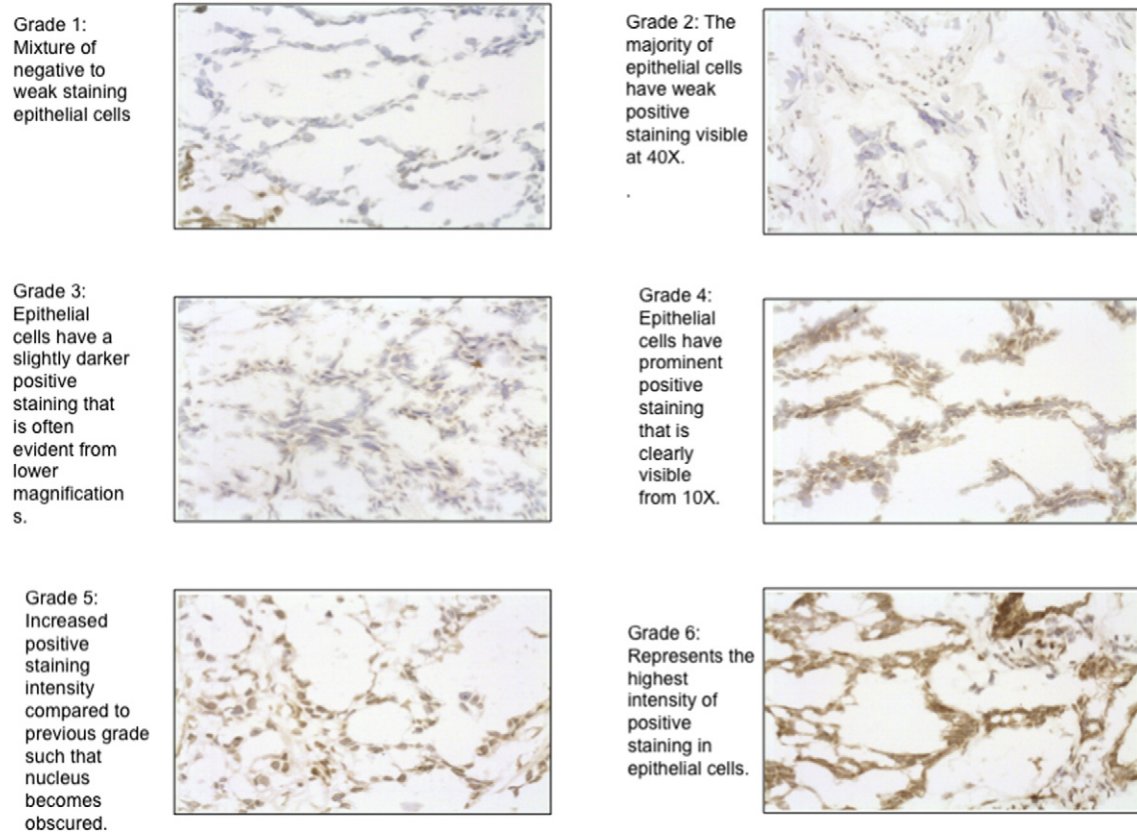


Fig. 1. Grading system for PTEN Immunohistochemistry.

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)

WWP2 expression in tumors in control (normal or high PTEN staining) and experimental (low PTEN on IHC, normal or high gene expression) group

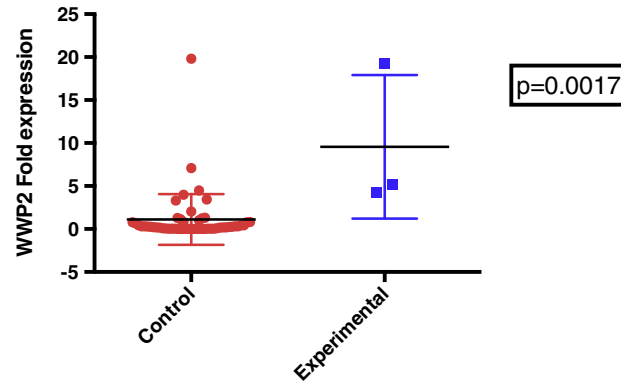


Fig. 2. WWP2 expression in tumors in control (normal or high PTEN staining) and experimental (low PTEN on IHC, normal or high gene expression) groups.

available in Table 1. This was a largely postmenopausal population with early stage and low-grade disease, with a recurrence rate of 6%.

Out of the fifty-one tumors, three (6%) had PTEN mRNA at least one-fold but low PTEN by IHC (<2). The median fold expression of WWP2 in this group was 5.235 (4.278–19.2) compared to 0.2576 (0.000035–19.8) in tumors with normal or high staining of PTEN (Mann–Whitney test $p = 0.0017$) (Fig. 2).

We examined the association of WWP2 expression and clinical outcomes and prognostic variables including LVSI, MI, grade, stage and recurrence. We did not find any statistically significant differences between tumors with elevated expression of WWP2 (≥ 2 fold expression) compared to those tumors with low expression (<2 fold expression) (Table 2).

The 51 tumor samples were then compared to the five available normal controls. There were no statistically significant differences between the tumor set and the normal set in WWP2 expression (Fig. 3).

Table 2
Comparison of clinical characteristics between low and high WWP2 expression tumors (all $p = NS$).

	Low WWP2 expression (<2) $n = 41$	High WWP2 expression (≥ 2) $n = 10$
LVSI	10 (24%)	1 (10%)
MI > 50%	13 (32%)	0 (0%)
Grade > 1	14 (34%)	5 (50%)
Stage > I	8 (19%)	2 (20%)
Recurrence	3 (7%)	0 (0%)

WWP2 expression in tumors compared to normal controls

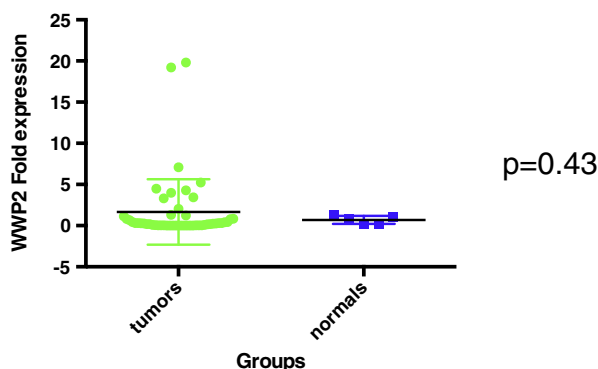


Fig. 3. WWP2 expression in tumors compared to normal controls.

4. Discussion

This paper investigates the association of WWP2 and PTEN in endometrial cancers. WWP2 gene expression was significantly elevated in three tumors in this cohort that had low PTEN staining despite normal or high gene expression of PTEN.

PTEN protein ubiquitination and degradation in endometrial cancer has been demonstrated previously. In work by Cheung, et al., mutations in proteins within the PI3K pathway result in PTEN ubiquitination and degradation through the proteasome (Cheung et al., 2011). However, WWP2 has not been investigated in endometrial cancer previously. The proteasome could prove to be a novel target in endometrial cancer. Proteasome inhibitors such as bortezomib and carfilzomib are already FDA approved for multiple myeloma and could be investigated in other cancers such as endometrial cancer (Aghajanian, 2004).

Strengths of this study include that it investigates a novel mechanism of PTEN loss in endometrial cancer and shows that there is a potential association with increased transcriptional levels of WWP2 and PTEN protein loss. The weakness of this study is that it includes a very small number of tumors and controls. With such small numbers, if a difference existed between clinical characteristics we may not have been able to detect it.

In summary, WWP2 expression was not different between tumors and normal controls, but WWP2 gene expression was significantly elevated in three tumors in this cohort that had low PTEN staining despite normal or high gene expression of PTEN. WWP2 expression by RT PCR was not associated with clinical prognostic variables in this small cohort.

Conflict of interest

The authors have no conflicts of interest to disclose.

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