Variations in the *PDCD6* Gene Are Associated with Increased Uterine Leiomyoma Risk in the Chinese

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Programmed cell death 6 (*PDCD6*) participates in T cell receptor, Fas, and glucocorticoid—induced programmed cell death. To test the relationship between *PDCD6* polymorphisms and uterine leiomyomas (UL) risk, we investigated the association of two SNPs (rs4957014 and rs3756712) in *PDCD6* with UL risk in a case–control study of 295 unrelated premenopausal UL patients and 436 healthy postmenopausal control subjects in a population of China. Genotypes of the two SNPs were determined with the use of PCR-restriction fragment length polymorphism assay. Significantly increased UL risks were found to be associated with the T allele of rs4957014 and the T allele of rs3756712 ($p=0.016$, odds ratio [OR]=1.325, 95% confidence intervals [CI]=1.053–1.668 for rs4957014; $p<0.0001$, OR=1.898, 95% CI=1.457–2.474 for rs3756712, respectively). Increased UL risks were associated with them in different genetic models. The present study provided evidence that rs4957014 and rs3756712 are associated with UL risk, the results indicated that genetic polymorphisms in *PDCD6* may contribute to the development of UL.

Introduction

UTERINE LEIOMYOMA (UL) is a benign tumor that occurs in 20%–40% of women in their reproductive years (Day Baird *et al.*, 2003; Duhan, 2011). Various clinical problems such as pelvic pain, abnormal uterine bleeding, urinary frequency, infertility, and recurrent pregnancy loss are attributed to this disease (Vollenhoven *et al.*, 1990; Hart *et al.*, 2001). Approximately 7% of ULs report moderate to severe abdominal pain (Lippman *et al.*, 2003). The prevalence of UL during pregnancy is estimated to be 0.3%–2.6%, of which 10% result in pregnancy complications (Suwandinata *et al.*, 2008). For the above reasons, ULs are the most commonly cited indication for hysterectomy (Duhan, 2011). Predisposing factors for UL include environmental and genetic factors, such as early menarche, nullparity, obesity, African–American ethnicity, tamoxifen use (Ross *et al.*, 1986; Samadi *et al.*, 1996; Marshall *et al.*, 1997; Templeman *et al.*, 2009), and polymorphism of the *ORC5L*, *LHFPL3*, and *PCOLCE* genes (Hodge *et al.*, 2009). However, the UL risk associated with programmed cell death 6 (*PDCD6*) has not been reported.

PDCD6 (a gene product of *PDCD6*) is a 22-kD protein containing five repetitive helix E-loop-helix F topology (EF-hand motifs) called penta-EF-hand (PEF) domain (Maki *et al.*,

2002). *PDCD6* is a Ca^{2+} -binding protein, and has been found to interact with Alix/AIP1 and their orthologs (Wu *et al.*, 2002), ASK1 (Hwang *et al.*, 2002), and Sec31A (Shibata *et al.*, 2007), so as to participate in the T cell receptor, Fas, and glucocorticoid-induced programmed cell death (Vito *et al.*, 1996). Previous research showed that *PDCD6*-deficient mice develop normally without obvious abnormalities in the immune system (Jang *et al.*, 2002). Nonetheless, potential physiological roles of *PDCD6* in the regulation of ER stress-induced apoptosis (Rao *et al.*, 2004), neuronal cell death during development (Mahul-Mellier *et al.*, 2006), and cancers (la Cour *et al.*, 2003; Mollerup *et al.*, 2003; Subramanian and Polans, 2004; Aviel-Ronen *et al.*, 2008; la Cour *et al.*, 2008; Yamada *et al.*, 2008; Hoj *et al.*, 2009) have been reported.

The *PDCD6* gene, also designated as *Apoptosis-linked gene 2* (*ALG-2*), is located at 5p 15.33. We selected two tag SNPs (rs4957014 and rs3756712) of the *PDCD6* gene from the International HapMap project (<http://hapmap.ncbi.nlm.nih.gov/>). A tag SNP is a representative SNP in a region of the genome with high linkage disequilibrium (the nonrandom association of alleles at two or more loci). These two tag SNPs are located in the intron region of the *PDCD6* gene. Studies have found that introns may have evolved to function as network control molecules in the higher organisms, freeing such organisms from the

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constraints of a simple single-output protein-based genetic operating system, and act as controls influencing the activity of other genes (Mattick and Gagen, 2001).

To date, the association between these two SNPs and diseases has not been reported. Therefore, in the present study, we investigated the association of these two SNPs with UL risk in a case-control study of 295 unrelated premenopausal UL patients and 436 healthy postmenopausal control subjects in a Chinese population.

Material and Methods

Subjects

This study was performed with the approval of the ethics committee of the Second University Hospital of Sichuan University and all the participants provided written informed consent. A hospital based case-control study was conducted, including 295 unrelated premenopausal women with UL ranging in age from 23 to 49 (mean $\pm$ SD, 41.87 ± 6.94) between July 2009 and December 2011 at the Second University Hospital of Sichuan University. The inclusion criteria were a diagnosis of UL (confirmed by histopathology) and indication for surgical treatment. A group of control subjects consisted of 436 healthy postmenopausal women ranging in age from 48 to 71 years (mean $\pm$ SD, 54.79 ± 5.25) was selected randomly from a routine health survey in the same hospital. The inclusion criteria for the control group were postmenopausal period and absence of UL after clinical and ultrasonographic evaluation. Otherwise, we excluded from the control group, women with abnormal uterine bleeding and from the case group, those who did not undergo surgical treatment. All subjects were Han women living in the Sichuan Province of southwest China.

Genotyping

Genomic DNA of each individual was extracted from 200 μ L of EDTA-anticoagulated peripheral blood samples by a DNA isolation kit from Biotek (Peking, China). The procedure was performed according to the instruction manual. Primers were established with the PIRA PCR designer (http://cedar.genetics.sot-on.ac.uk/public_html/primer2.html). The primers used for amplification of the rs4957014 were 5'-TGGTGTTCATACCATTGACACTTGC-3' and 5'-CTCAGAACCAAGCAGGTTCTTCA-3', the primers used for amplification of the rs3756712 were 5'-TACAGTGGCAAAGGACCACA-3' and 5'-CACATTCCAGCACTCACCAC-3'.

These two selected SNPs were genotyped by using the PCR-restriction fragment length polymorphism assay in UL

patients and control subjects to explore the association between them and UL risk. PCRs were performed in a total volume of 25 μ L, including 2.5 μ L 10 $\times$ PCR buffer, 1.5 mM MgCl₂, 0.15 mM dNTPs, 0.5 μ M of each primer, 100 ng of genomic DNA, and 1U of Taq DNA polymerase. The PCR conditions for these two SNPs were 94°C for 4 min, followed by 32 cycles of 30 s at 94°C, 30 s at 62°C, and 30 s at 72°C, with a final elongation at 72°C for 10 min. PCR products were digested overnight with specific restriction enzymes and the digested PCR products were separated by a 6% polyacrylamide gel and staining with 1.0 g/L of argent nitrate: *Hph*I for rs4957014 (Supplementary Fig. S2; Supplementary Data are available online at www.liebertpub.com/gtmb), allele G is cuttable, yielding two fragments of 13 and 100 bp, allele T is uncuttable and the fragment is still 113 bp; *Rsa*I for rs3756712 (Supplementary Fig. S1), allele G is cuttable, yielding two fragments of 99 and 66 bp, allele T is uncuttable and the fragment is still 165 bp. The genotypes were confirmed by DNA sequencing analysis (BigDye[®] Terminator v3.1 Cycle Sequencing Kits; Applied Biosystems, Foster City, CA). About 10% of the samples were randomly selected to perform repeated assays and the results were 100% concordant (Supplementary Fig. S1 and Supplementary Fig. S2).

Statistical analysis

All data analyses were carried out by SPSS 13.0 statistical software (SPSS, Inc., Chicago, IL). Genotype frequencies of rs4957014 and rs3756712 were obtained by directed counting and the Hardy-Weinberg equilibrium was evaluated by the chi-square test. Genotypic association tests in a case-control pattern assuming codominant, dominant, recessive, overdominant, or log-additive genetic models were performed using SNPstats (Sole *et al.*, 2006). Odds ratio (OR) and respective 95% confidence intervals (CI) were reported to evaluate the effects of any difference between alleles and genotypes. A $p < 0.05$ was regarded as statistically significant in UL patients compared to healthy controls.

Results

Both rs4957014 and rs3756712 were successfully genotyped in 295 UL patients and 436 control subjects. Genotype distributions of these two polymorphisms in our cases and control subjects were consistent with the Hardy-Weinberg equilibrium. Allele frequencies of these two polymorphisms for 295 UL patients and 436 control subjects are shown in Table 1. As shown in Table 1, significantly increased UL risks were found to be associated with the T allele of rs4957014 and the T allele of rs3756712 ($p = 0.016$, OR = 1.325, 95% CI = 1.053–1.668 for

TABLE 1. ALLELE FREQUENCIES OF SELECTED POLYMORPHISMS IN PDCD6 AMONG UTERINE LEIOMYOMAS AND CONTROLS AND THEIR ASSOCIATION WITH UL RISK

Polymorphisms	Allele	UL patients N = 295 (%)	Controls N = 436 (%)	OR (95% CI)	p-Value
rs4957014	G	160 (27.1)	288 (33.0)	1.325 (1.053–1.668)	0.016
	T	430 (72.9)	584 (67.0)		
rs3756712	G	96 (16.3)	235 (26.9)	1.898 (1.457–2.474)	<0.0001
	T	494 (83.7)	637 (73.1)		

N corresponds to the number of individuals.

Boldfaced values indicate a significant difference at 5%.

PDCD6, programmed cell death 6; UL, uterine leiomyoma; OR, odds ratio; CI, confidence intervals.

TABLE 2. GENOTYPE FREQUENCIES OF SELECTED POLYMORPHISMS IN PDCD6 AMONG UL AND CONTROLS AND THEIR ASSOCIATION WITH UL RISK

Genetic model	Genotype	UL	Controls	Logistic regression ^a	
		N = 295 (%)	N = 436 (%)	OR (95% CI)	p-Value
<i>rs4957014 G/T</i>					
Codominant	T/T	149 (50.5%)	193 (44.3%)	1.00 (reference)	0.013
	G/T	132 (44.8%)	198 (45.4%)	1.16 (0.85–1.57)	
	G/G	14 (4.8%)	45 (10.3%)	2.48 (1.31–4.69)	
Dominant	T/T	149 (50.5%)	193 (44.3%)	1.00 (reference)	0.097
	G/T-G/G	146 (49.5%)	243 (55.7%)	1.28 (0.96–1.73)	
Recessive	T/T-G/T	281 (95.2%)	391 (89.7%)	1.00 (reference)	0.005
	G/G	14 (4.8%)	45 (10.3%)	2.31 (1.24–4.29)	
Overdominant	T/T-G/G	163 (55.2%)	238 (54.6%)	1.00 (reference)	0.86
	G/T	132 (44.8%)	198 (45.4%)	1.03 (0.76–1.38)	
Log-additive				1.35 (1.06–1.72)	0.013
<i>rs3756712 G/T</i>					
Codominant	T/T	211 (71.5%)	241 (55.3%)	1.00 (reference)	<0.0001
	G/T	72 (24.4%)	155 (35.5%)	1.88 (1.35–2.64)	
	G/G	12 (4.1%)	40 (9.2%)	2.92 (1.49–5.71)	
Dominant	T/T	211 (71.5%)	241 (55.3%)	1.00 (reference)	<0.0001
	G/T-G/G	84 (28.5%)	195 (44.7%)	2.03 (1.48–2.79)	
Recessive	T/T-G/T	283 (95.9%)	396 (90.8%)	1.00 (reference)	0.0064
	G/G	12 (4.1%)	40 (9.2%)	2.38 (1.23–4.62)	
Overdominant	T/T-G/G	223 (75.6%)	281 (64.5%)	1.00 (reference)	0.0013
	G/T	72 (24.4%)	155 (35.5%)	1.71 (1.23–2.38)	
Log-additive				1.80 (1.39–2.32)	<0.0001

N corresponds to the number of individuals.

^aCrude analysis.

Boldfaced values indicate a significant difference at the 0.05.

rs4957014; $p < 0.0001$, OR = 1.898, 95% CI = 1.457–2.474 for rs3756712, respectively).

The results for genotypic association analysis are shown in Table 2, as shown in Table 2, significant associations were observed for these two SNPs. For SNP rs4957014, significantly increased UL risk was found to be associated with the TT genotype in a codominant model, compared with the GG genotype ($p = 0.013$, OR = 2.48, 95% CI = 1.31–4.69); and compared with the GG genotype, significantly increased UL risk was associated with T allele carriers (TT/TG genotypes) in a recessive model of the G allele ($p = 0.005$, OR = 2.31, 95% CI = 1.24–4.29). For SNP rs3756712, significantly increased UL risks were found to be associated with the TT genotype and GT genotype in a codominant model, compared with the GG genotype ($p < 0.0001$, OR = 2.92, 95% CI = 1.49–5.71 for the TT genotype and OR = 1.88, 95% CI = 1.35–2.64 for the GT genotype, respectively). Compared with GT/GG genotypes, the TT genotype had a 2.03-fold increased UL risk ($p < 0.0001$, 95% CI = 1.48–2.79) in a dominant model of the G allele. Compared with the GG genotype, TT/TG genotypes had a 2.38-fold increased UL risk ($p = 0.0064$, 95% CI = 1.23–4.62) in a recessive model of the G allele. Compared with the GT genotype, TT/GG genotypes had a 1.71-fold increased UL risk ($p = 0.0013$, 95% CI = 1.23–2.38) in an overdominant model. Moreover, significantly elevated UL risks were found to be associated with both SNPs by using log-additive analyses.

Discussion

In the present study, for the first time, we identified the associations between SNPs in *PDCD6* and UL. Our findings

suggested that the T allele of rs4957014 and the T allele of rs3756712 may increase UL risk. In addition, rs4957014 was associated with elevated UL risk in the codominant model and recessive model; rs3756712 was associated with increased UL risk in the codominant, dominant model, and recessive models. Moreover, significantly elevated UL risks were found to be associated with both SNPs by using log-additive analyses.

PDCD6 is a Ca^{2+} -binding protein having five repetitive EF-hand motifs called PEF domain (Maki *et al.*, 2002). This calcium-binding protein has been found to interact with Alix/AIP1 and their orthologs (Wu *et al.*, 2002), ASK1 (Hwang *et al.*, 2002), and Sec31A (Shibata *et al.*, 2007), so as to participate in T cell receptor, Fas, and glucocorticoid-induced programmed cell death (Vito *et al.*, 1996). *PDCD6*-interacting proteins commonly contain proline-rich regions, and *PDCD6* recognizes at least two distinct Pro-containing motifs: PPYP(X)nYP (X, variable; $n = 4$ in Alix and PLSCR3) and PXPFG (represented by Sec31A) (Maki *et al.*, 2011).

PDCD6 functions as a Ca^{2+} -dependent adaptor that bridges the *PDCD6* interacting protein X (Alix) and Tumor Susceptibility Gene 101 (TSG101) (Okumura *et al.*, 2009). Alix/AIP1 is a cytoplasmic protein of 98 kDa, containing a proline-rich C-terminal region encompassing several Src homology domain 3 (SH3)-binding motifs. Alix/AIP1 has also been implicated in multivesicular sorting mediated by the interactions of the N-terminal region of the protein with the chromatin-modifying protein 4b (CHMP4b) (Katoh *et al.*, 2003), while the C-terminal region is associated with *PDCD6* interactions. It has been proposed that Alix/AIP1 could form a molecular link between endosomal sorting and cell death pathways (Trioulier *et al.*, 2004; Mahul-Mellier *et al.*, 2006; Sadoul, 2006). The Alix/

PDCD6 complex also acts as components of the TNF-R1 pathway through allowing the recruitment of pro-caspase-8 onto endosomes containing TNF-R1, a step thought to be necessary for activation of the apical caspase. In line with this, expression of Alix deleted of its PDCD6-binding site (Alix-DeltaPDCD6) significantly reduced TNF-R1-induced cell death, without affecting endocytosis of the receptor. In a more physiological setting, TNF-R1 regulated programmed cell death of motoneurons, which were inhibited by Alix-DeltaPDCD6 (Raoul *et al.*, 2000; Mahul-Mellier *et al.*, 2008). Furthermore, PDCD6 could regulate the subcellular localization and the c-Jun N-terminal kinase (JNK) activity modulation of ASK1 by a direct interaction (Hwang *et al.*, 2002).

The role of PDCD6 in cancer development has been explored gradually, but the potential mechanism is still unknown. Previous studies found that PDCD6 is overexpressed in a variety of human tumors (la Cour *et al.*, 2003; Mollerup *et al.*, 2003; Aviel-Ronen *et al.*, 2008; la Cour *et al.*, 2008). However, Mel290 cells derived from a human tumor were found to express lower levels of PDCD6 mRNA and protein than normal melanocytes (Yamada *et al.*, 2008). PDCD6 downregulation mediated by siRNA led to a significant reduction in viability of HeLa cells indicating that PDCD6 may contribute to tumor development and expansion (la Cour *et al.*, 2008; Hoj *et al.*, 2009). The downregulation of PDCD6 suggests one way by which these cells may achieve a selective advantage, by interfering with Ca^{2+} -mediated apoptotic signals and thereby enhancing cell survival (Subramanian *et al.*, 2004). Although the role is not fully understood in cancer development, PDCD6 was identified as a prognostic biomarker in patients with unresected gastric cancer (Yamada *et al.*, 2008).

The current study might have some limitations. Our study was limited by a relatively small sample size, which weakened our ability to solidify the statistic associations. Further studies in different ethnic groups and with a larger sample size could help to confirm the true significance of the association between these polymorphisms and the susceptibility to UL.

In conclusion, for the first time, this study demonstrated that rs4957014 and rs3756712 are associated with increased UL risk. However, further studies are needed to reveal the association between the two selected SNPs and the status of the PDCD6 expression.

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Author Disclosure Statement

No competing financial interests exist.

References

Aviel-Ronen S, Coe BP, Lau SK, *et al.* (2008) Genomic markers for malignant progression in pulmonary adenocarcinoma with bronchioloalveolar features. *Proc Natl Acad Sci U S A* 105: 10155–10160.

Day Baird D, Dunson DB, Hill MC, *et al.* (2003) High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol* 188:100–107.

Duhan N (2011) Current and emerging treatments for uterine myoma—an update. *Int J Womens Health* 3:231–241.

Hart R, Khalaf Y, Yeong CT, *et al.* (2001) A prospective controlled study of the effect of intramural uterine fibroids on the outcome of assisted conception. *Hum Reprod* 16:2411–2417.

Hodge JC, Cuenco KT, Huyck KL, *et al.* (2009) Uterine leiomyomata and decreased height: a common HMG2 predisposition allele. *Hum Genet* 125:257–263.

Hoj BR, la Cour JM, Mollerup J, Berchtold MW (2009) ALG-2 knockdown in HeLa cells results in G2/M cell cycle phase accumulation and cell death. *Biochem Biophys Res Commun* 378:145–148.

Hwang IS, Jung YS, Kim E (2002) Interaction of ALG-2 with ASK1 influences ASK1 localization and subsequent JNK activation. *FEBS Lett* 529:183–187.

Jang IK, Hu R, Lacana E, *et al.* (2002) Apoptosis-linked gene 2-deficient mice exhibit normal T-cell development and function. *Mol Cell Biol* 22:4094–4100.

Katoh K, Shibata H, Suzuki H, *et al.* (2003) The ALG-2-interacting protein Alix associates with CHMP4b, a human homologue of yeast Snf7 that is involved in multivesicular body sorting. *J Biol Chem* 278:39104–39113.

la Cour JM, Hoj BR, Mollerup J, *et al.* (2008) The apoptosis linked gene ALG-2 is dysregulated in tumors of various origin and contributes to cancer cell viability. *Mol Oncol* 1:431–439.

la Cour JM, Mollerup J, Winding P, *et al.* (2003) Up-regulation of ALG-2 in hepatomas and lung cancer tissue. *Am J Pathol* 163:81–89.

Lippman SA, Warner M, Samuels S, *et al.* (2003) Uterine fibroids and gynecologic pain symptoms in a population-based study. *Fertil Steril* 80:1488–1494.

Mahul-Mellier AL, Hemming FJ, Blot B, *et al.* (2006) Alix, making a link between apoptosis-linked gene-2, the endosomal sorting complexes required for transport, and neuronal death *in vivo*. *J Neurosci* 26:542–549.

Mahul-Mellier AL, Strappazzon F, Petiot A, *et al.* (2008) Alix and ALG-2 are involved in tumor necrosis factor receptor 1-induced cell death. *J Biol Chem* 283:34954–34965.

Maki M, Kitaura Y, Satoh H, *et al.* (2002) Structures, functions and molecular evolution of the penta-EF-hand Ca^{2+} -binding proteins. *Biochim Biophys Acta* 1600:51–60.

Maki M, Suzuki H, Shibata H (2011) Structure and function of ALG-2, a penta-EF-hand calcium-dependent adaptor protein. *Sci China Life Sci* 54:770–779.

Marshall LM, Spiegelman D, Barbieri RL, *et al.* (1997) Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. *Obstet Gynecol* 90:967–973.

Mattick JS, Gagen MJ (2001) The evolution of controlled multi-tasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. *Mol Biol Evol* 18:1611–1630.

Mollerup J, Krogh TN, Nielsen PF, Berchtold MW (2003) Properties of the co-chaperone protein p23 erroneously attributed to ALG-2 (apoptosis-linked gene 2). *FEBS Lett* 555:478–482.

Okumura M, Ichioka F, Kobayashi R, *et al.* (2009) Penta-EF-hand protein ALG-2 functions as a Ca^{2+} -dependent adaptor that bridges Alix and TSG101. *Biochem Biophys Res Commun* 386:237–241.

Rao RV, Poksay KS, Castro-Obregon S, *et al.* (2004) Molecular components of a cell death pathway activated by endoplasmic reticulum stress. *J Biol Chem* 279:177–187.

- Raoul C, Pettmann B, Henderson CE (2000) Active killing of neurons during development and following stress: a role for p75(NTR) and Fas? *Curr Opin Neurobiol* 10:111–117.
- Ross RK, Pike MC, Vessey MP, *et al.* (1986) Risk factors for uterine fibroids: reduced risk associated with oral contraceptives. *Br Med J (Clin Res Ed)* 293:359–362.
- Sadoul R (2006) Do Alix and ALG-2 really control endosomes for better or for worse? *Biol Cell* 98:69–77.
- Samadi AR, Lee NC, Flanders WD, *et al.* (1996) Risk factors for self-reported uterine fibroids: a case-control study. *Am J Public Health* 86:858–862.
- Shibata H, Suzuki H, Yoshida H, Maki M (2007) ALG-2 directly binds Sec31A and localizes at endoplasmic reticulum exit sites in a Ca²⁺-dependent manner. *Biochem Biophys Res Commun* 353:756–763.
- Sole X, Guino E, Valls J, *et al.* (2006) SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 22:1928–1929.
- Subramanian L, Crabb JW, Cox J, *et al.* (2004) Ca²⁺ binding to EF hands 1 and 3 is essential for the interaction of apoptosis-linked gene-2 with Alix/AIP1 in ocular melanoma. *Biochemistry* 43:11175–11186.
- Subramanian L, Polans AS (2004) Cancer-related diseases of the eye: the role of calcium and calcium-binding proteins. *Biochem Biophys Res Commun* 322:1153–1165.
- Suwandinata FS, Gruessner SE, Omwandho CO, Tinneberg HR (2008) Pregnancy-preserving myomectomy: preliminary report on a new surgical technique. *Eur J Contracept Reprod Health Care* 13:323–326.
- Templeman C, Marshall SF, Clarke CA, *et al.* (2009) Risk factors for surgically removed fibroids in a large cohort of teachers. *Fertil Steril* 92:1436–1446.
- Trioulier Y, Torch S, Blot B, *et al.* (2004) Alix, a protein regulating endosomal trafficking, is involved in neuronal death. *J Biol Chem* 279:2046–2052.
- Vito P, Lacana E, D'Adamio L (1996) Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* 271:521–525.
- Vollenhoven BJ, Lawrence AS, Healy DL (1990) Uterine fibroids: a clinical review. *Br J Obstet Gynaecol* 97:285–298.
- Wu Y, Pan S, Luo W, *et al.* (2002) Hp95 promotes anoikis and inhibits tumorigenicity of HeLa cells. *Oncogene* 21:6801–6808.
- Yamada Y, Arao T, Gotoda T, *et al.* (2008) Identification of prognostic biomarkers in gastric cancer using endoscopic biopsy samples. *Cancer Sci* 99:2193–2199.

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