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Field methylation silencing of the protocadherin 10 gene in cervical carcinogenesis as a potential specific diagnostic test from cervical scrapings

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PCDH10 is a member of the protocadherin cell adhesion molecule family, which are frequently downregulated in cancers. This study aimed to characterize the methylation silencing of the *PCDH10* gene in the full spectrum of cervical carcinogenesis and to clarify if a field effect of methylation might be a target for a diagnostic test from cervical scrapings. Methylation silencing of *PCDH10* was found in four of five cervical cancers and one of two cervical precancerous cell lines, which could be reversed by demethylation treatment. The same methylation was detected in 85.7% (24/28) of invasive cancer tissues, 36.4% (4/11) of high-grade squamous intraepithelial lesions, 20% (1/5) of low-grade squamous intraepithelial lesions, and none (0/17) of the normal cervical tissues from non-cancer subjects. In addition, methylation was also frequently found in histologically 'normal' cervical tissues adjacent to cancer lesions (7/13, 53.8%) and, less frequently, in vaginal and endometrial tissues (1/8, 12.5%). Further investigation of cervical scrapings revealed cancer-specific methylation of *PCDH10* with a methylation rate of 71% (22/31) in invasive cancer, 27.9% (12/43) in carcinoma *in situ*, and none in high-grade squamous intraepithelial lesions excluding carcinoma *in situ* ($n = 12$), low-grade squamous intraepithelial lesions ($n = 27$), and normal controls ($n = 66$) ($P < 10^{-16}$). Compared to the high-risk human papilloma virus test, *PCDH10* methylation testing of cervical scrapings was more specific (92 vs 60%) but less sensitive (71 vs 96%) in detecting invasive cervical cancer. This study demonstrated field methylation of the *PCDH10* gene specifically in the invasion stage of cervical carcinogenesis, which might be used to develop a highly specific diagnostic test for cervical scrapings. (*Cancer Sci* 2009; 100: 2175–2180)

Cervical cancer is one of the most common malignancies among women worldwide.[1] High-risk human papilloma virus (HPV) was detected in virtually all cervical cancer tissues and regarded as a necessary etiological cause of cervical cancer.[2] However, HPV infection is clearly not a sufficient factor in cervical carcinogenesis as most HPV infections are transient and benign. Other genetic and/or epigenetic alterations are also critical in cervical carcinogenesis.

Members of the cadherin family function as tumor suppressors through maintenance of cell–cell adhesion and/or inhibition of the Wnt pathway by interaction with cytoplasmic β-catenin.[3–5] Tumor suppressor genes are frequently downregulated by DNA methylation in tumorigenesis, including cervical carcinogenesis.[6–8] Genes of the cadherin family have been found to be downregulated in human malignancies, resulting in tumor progression, invasion, and metastasis.[3,9] Protocadherin 10 (PCDH10), also known as OL-protocadherin, is a newly identified protocadherin of the cadherin family and contains six extracellular repeats.[10] PCDH10 is essential for brain development, especially in elongating striatal axons and guiding thalamocortical projections.[11] The gene locus of *PCDH10* at 4q28.3 is frequently deleted in hepatoma, colorectal cancer, prostate cancer, pancreatic cancer, and breast cancer.[12–16] A recent study demonstrated a tumor suppressor role of PCDH10 with inhibition of the growth, migration, and invasion of nasopharyngeal cancer cells upon overexpression.[17] Hypermethylation of the *PCDH10* gene was found in nasopharyngeal, haematological, breast, and gastric cancers as well as in several other cancer cell lines.[17–20]

To clarify the clinical scenario (the specific step in carcinogenesis in which the *PCDH10* gene is silenced by methylation), the present study surveyed the status of *PCDH10* methylation in the full spectrum of cervical carcinogenesis, including low-grade squamous intra-epithelial lesion (LSIL or mild dysplasia) and high-grade squamous intra-epithelial lesion (HSIL) excluding carcinoma *in situ* (CIS) (moderate dysplasia and severe dysplasia), CIS, squamous cell carcinoma (SCC), and adenocarcinoma (AdenoCA). To know the extent of the methylation effect on the neoplastic cervix, we also tested the normal counterparts of the uterine cervix in three groups: (1) normal cervical tissue from the non-cancer patient (normal); (2) normal tissues remote to the cervical cancer lesion (NCx); and (3) normal tissue adjacent to the cervical cancer lesion (NNC). The results showed highly specific field methylation of *PCDH10* at the *in situ*-to-invasion stage of cervical carcinogenesis.

Materials and Methods

Cell culture and DNA demethylation. Sixteen cell lines including five cervical cancer cells (SiHa, CaSki, HeLa, HeLaS3, C33A), two HPV16- and HPV18-immortalized cervical epithelial cells displaying features of CIS (Z172 and Z183, respectively),[21] one non-HPV-immortalized keratinocyte (HaCaT), two breast cancer cell lines (MCF7 and MDA-MB 231), three lung cancer cell liness (A549, CL1-0, and CL1-5), two ovarian cancer cell lines (OVCAR3 and SKOV3), and one melanoma cell line (MDA-MB 435) were studied. HeLa, HeLaS3, HaCaT, Z172, Z183, MDA-MB 435, and breast cancer cells were cultured in DMEM medium. SiHa, CaSki, OVCAR3, SKOV3, and lung cancer cells were cultured in RPMI medium. C33A cells were cultured in MEM medium. Z172 and Z183 cells were supplemented with 10% Nu serum and 1% hydrocortisone, OVCAR3 cells with 20% FCS, and the rest of the cells with 10% FCS. For demethylation, cells were treated with 10 μM 5′-aza-2′-deoxycytidine. The media containing 5′-aza-2′-deoxycytidine was changed every 24 h for 4 days, and cells were harvested at day 5.

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Study patients and controls. A hospital-based cohort of cervical carcinogenesis was established at the Buddhist Tzu-Chi General Hospital, Hualien, Taiwan from December 2005 to April 2009. Women with newly found abnormalities in Pap smears were invited. Exclusion criteria were acute or chronic non-HPV infections, immune-compromised status, pregnancy, history of previous cervical neoplasia, genital warts, other cancers, and previous surgeries of the uterine cervix. A standard guideline issued by National Health Research Institute, Taiwan, for screening, diagnosis, and management of cervical neoplasia (http://www.nhri.org.tw/NHRI_ADM/userfiles/file/tcog/gog_b.pdf) was followed in all subjects. At enrollment, all subjects were subjected to cervical scrapings, colposcopy with necessary biopsies, and venous blood collection. Two normal cervix-control groups were recruited. Healthy women received a regular Pap smear with a 'negative for cervical neoplasia' result, and patients underwent hysterectomy on account of benign tumors of the uterine corpus with histologically proven normal cervix being categorized into 'normal cervix from non-cancer women (normal)'. Patients with gynecological cancers of a non-cervical origin and a histologically proven normal cervix served as controls of 'normal cervix from cancer patient'.

Clinical specimens. Surgical tissues were carefully procured with respect to their anatomical locations and the nature of the tumor lesions to prevent cross-contamination. Primary and metastatic cervical tumors with different severities were procured during the operation. Grossly normal cervical tissues from gynecological malignancies of non-cervix sites (NC) were also procured. Normal tissues with a different topological relationship to the cervical cancer lesions were procured. They included NNC and NCx, such as those from the vagina and endometrium. Tissue specimens were collected in multiple tubes by snap-freezing in liquid nitrogen. The mean time of exposure of these specimens at room temperature was 19.9 ± 2 min. Cervical scrapings were collected with a cytobrush before colposcopy, cervical biopsy, or other cervical procedures. Typically, the same swab of cervix was collected after applying the Papanicolaou smear. Residual material on the swab was transferred into a universal tube containing 3 mL PBS. Following thorough agitation, it was then dispensed, snap-frozen, and stored at –80°C. Overall, the study materials included: (1) cervical swabs of 27 SCC, four AdenoCA, 57 HSIL, 27 LSIL, and 66 normal controls; and (2) tumor tissues of 22 SCC, six AdenoCA, 11 HSIL, five LSIL, five metastatic lesions, and normal control tissues of 11 NN, 16 NNC, six NC, and eight NCx. The disease nature of the swabs and tissues were proved by cytology and histology, respectively. All specimens were procured under an Institutional Review Board-approved project of Research Tissue Bank of Buddhist Tzu-Chi General Hospital. This study was also approved by the Institutional Review Board with informed consent obtained from all subjects.

RT-PCR. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was reverse-transcribed to cDNA using the First Strand cDNA Synthesis kit (Roche, Mannheim, Germany). PCR was carried out with primers located at exon 1 (5′-ACTGCTATCAGGTATGCCTG-3′) and exon 2 (5′-GTCTGT CAACTAGATAGCTG-3′) of the *PCDH10* gene.[17] The β-actin gene was amplified as an internal control, with primers 5′-AACTCCATCATGAAGTGTGACG-3′ and 5′-GATCCACATCT GCTGGAAGG-3′. PCR was carried out with initial denaturing at 95°C for 10 min, followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min.

Methylation-specific PCR and bisulfite genomic sequencing. Genomic DNA was extracted from cervical scrapings and tissues using the DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA was modified with sodium bisulfite using the DNA Methylation Gold Kit (Zymo Research, Orange, CA, USA), which converted unmethylated cytosines to uracil while leaving methylated cytosines unchanged. Methylation-specific PCR (MS-PCR) designed by Ying *et al*.[17] was used to detect CpG methylation of the *PCDH10* gene, with methylation-specific primers of 5′-TCGTTAAATAG ATACGTTACGC-3′ and 5′-TAAAAACTAAAAACTTTCCGCG-3′, and unmethylation-specific primers of 5′-GTTGTTAAATAGAT ATGTTATGT-3′ and 5′-CTAAAAACTAAAAACTTTCCACA-3′. White blood cell (WBC) DNA treated with *Sss*I (New England Biolab) was used as the methylated control, and untreated WBC DNA was used as the unmethylated control. All of the single MS-PCR contained 10 ng bisulfate-converted DNA template. The MS-PCR products were visualized on a 2% agarose gel containing ethidium bromide and illuminated under UV light. For bisulfite genomic sequencing (BGS), the promoter region from –328 to +18 containing 27 CpG sites was PCR amplified with primers of 5′-GTTGATGTAAATAGGGGAATT-3′ and 5′-CTTCAACCTCTAAACCTATAA-3′. The PCR products of the bisulfate-converted DNA were cloned and sequences of five randomly picked clones were determined.

HPV detection and typing. The presence of HPV DNA and the genotypes were determined by a highly sensitive assay of consensus PCR and reverse blot hybridization as previously described.[22,23] Briefly, the MY11 (5′-GCMC AGGGWC AT AAYAATGG-3′) and biotinylated GP6+(5′-GAAAAAT AAACTGT AAATC AT ATTC-3′) consensus primers were used to amplify a fragment of approximately 192 bp in the L1 open reading frame of the HPV genome for 40 cycles. The PCR products were then hybridized with an Easychip HPV Blot (King Car, I-Lan, Taiwan), which included oligonucleotide probes of 39 different types of HPV (types 6, 11, 16, 18, 26, 31, 32, 33, 35, 37, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 72, 74, 82, CP8061, CP8304, L1AE5, MM4, MM7, and MM8), and were visualized with biotinylated antibodies and alkaline phosphatase conjugation. The overall sensitivity of HPV detection of this assay was 1–50 copies of HPV genome equivalents, with no cross-reactivity with amplicons of other HPV genotypes.[24]

Statistic analysis. Fisher's exact test was carried out using SPSS 15.0 (SPSS, Chicago, IL, USA).

Results

***PCDH10* gene was methylation silenced in most cervical cancer cell lines.** Among five cervical cancer cell lines (SiHa, CaSki, HeLa, HeLaS3, and C33A) and one immortalized keratinocyte (HaCaT) examined by RT-PCR, only C33A expressed PCDH10. In two HPV-immortalized human cervical epithelial cells displaying features of CIS (Z172 and Z183), only Z183 weakly expressed PCDH10. Also, PCDH10 seemed to be less expressed in the more invasive (MDA-MB 231) than the less invasive (MCF7) breast cancer cells. It was also non-expressed or weakly expressed in one of the three lung cancer cells, two ovarian cancer cells, and melanoma cells studied (Fig. 1a). After demethylation with 10 μM of 5′-aza-2′-deoxycytidine, all four cervical cancer cell lines resumed expression of *PCDH10* (Fig. 1b), with the methylation status confirmed by MS-PCR (Fig. 1c). These results showed that *PCDH10* was silenced by methylation in cervical cancer cells.

Methylation of *PCDH10* specifically occurred in cervical cancer but not normal cervix. The methylation status of *PCDH10* in tissue specimens including normal cervix, cervical dysplasia, CIS, and invasive carcinoma was examined. Methylation of *PCDH10* was found in 85.7% (24/28) of invasive cervical cancers including 22 of 23 SCC and two of five AdenoCA, 80% (4/5) of metastatic cervical lesions, 36.4% (4/11) of CIS or moderate/severe dysplasia (including weakly positive in each of the moderate dysplasia

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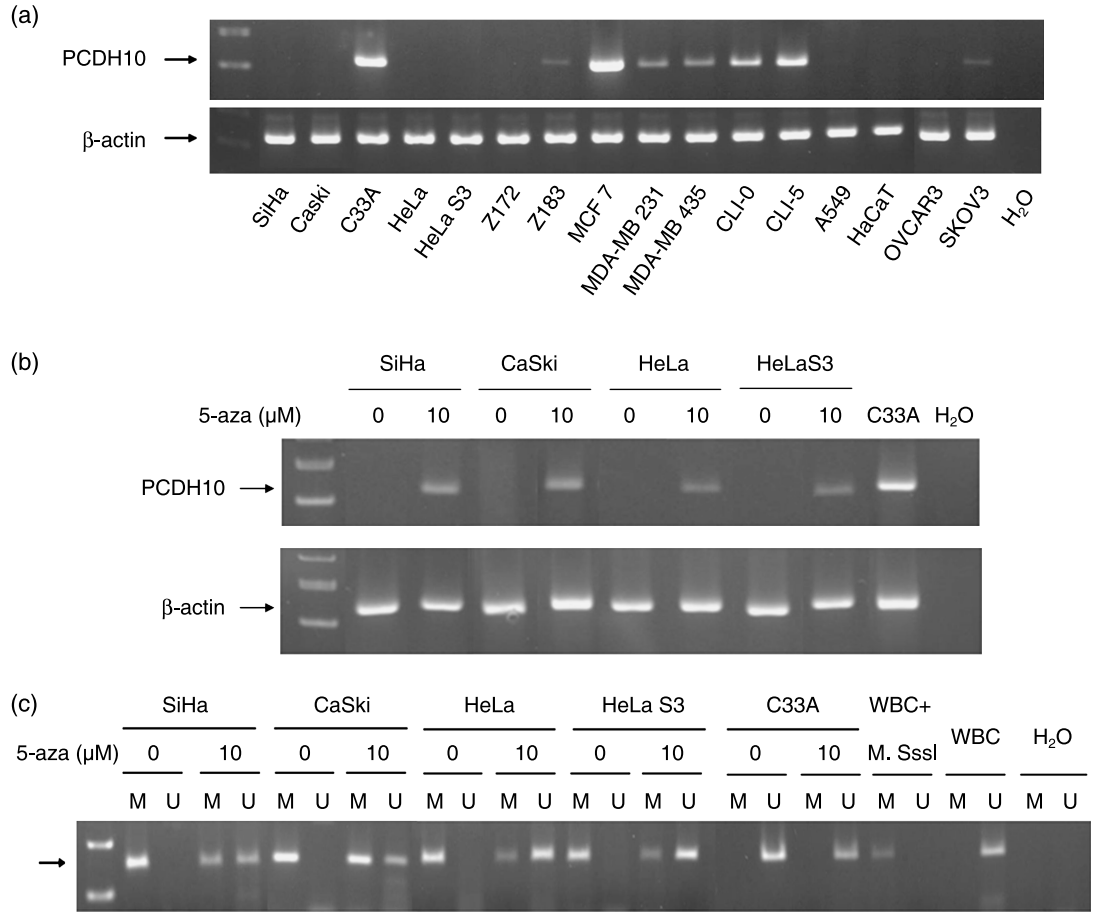

Fig. 1. *PCDH10* was silenced by hypermethylation in cervical cancer cells. (a) Among five cervical cancer cell lines (SiHa, CaSki, HeLa, HeLa3, and C33A), two human papillomavirus (HPV)-immortalized human cervical epithelial cell lines (Z172 and Z183), and one immortalized keratinocyte (HaCaT) were examined by RT-PCR. Only C33A expressed a significant amount of PCDH10. PCDH10 was weakly expressed or non-expressed in the more invasive breast cancer cell line (MDA-MB 231), melanoma cells (MDA-MB 435), one of the three lung cancer cell lines (A549), and two ovarian cancer cell lines (OVCAR3 and SKOV3). (b) After treatment with 10 μM 5-aza-dC, *PCDH10* was re-expressed in SiHa, Caski, HeLa, and HeLaS3 cells. (c) Methylation-specific-PCR (MS-PCR) confirmed the demethylation treatment. In contrast to little unmethylated (U) *PCDH10* before treatment, both methylated (M) and U forms were found in these four cancer cells after demethylation treatment. Untreated white blood cell (WBC) DNA and H_2O were used as negative controls. The arrow signified the expected location of the PCR product.

and severe dysplasia and strong positive in 2 of 9 CIS), and 20% (1/5) of mild dysplasia, but not in 17 normal cervical tissues from normal control subjects (Fig. 2a; Table 1).

Field effect of *PCDH10* methylation in cervical cancer. Various topological controls of normal tissues from cervical or non-cervical cancers were tested for the extent of field methylation and its specificity. Methylation of *PCDH10* was found in 53.8% (7/13) of NNC but in only 12.5% (1/8) of NCx (Table 2), indicating the limit of the field methylation. Methylation of *PCDH10* was not found in six NC (Table 2).

Methylation of *PCDH10* in cervical scrapings was more specific than high-risk HPV DNA test. The field effect of *PCDH10* methylation was anticipated to develop a feasible test for cervical carcinogenesis in cervical scraping specimens. Indeed, methylation of the *PCDH10* gene was noted in cervical swabs of 71% (22/31) of SCC or AdenoCA (including 20 of 27 SCC, and two of four AdenoCA), 27.9% (12/43) of CIS, and 0 of 12 CIN2 or 3, 27 LSIL, and 66 normal (Table 3). The specific methylation of *PCDH10* in cervical cancer was further confirmed by BGS, where the methylation status of the 27 CpG sites around the promoter was determined in representative specimens. Extensive methylation of almost all CpG sites were noted in SCC and AdenoCA, but not seen in CIS, LSIL, and normal (Fig. 2b). On the other hand, high-risk HPV DNA was detected in 21 of 22 (95.4%) SCC or AdenoCA, 31 of 31 (100%) HSIL, 9 of 12 (75%) LSIL, and 3 of 65 (4.6%) normal (Fig. 2a; Table 3). The sensitivity, specificity, positive predictive value, and negative predictive value of *PCDH10* methylation in the diagnosis of SCC or AdenoCA of the cervix in this hospital-based cohort was 71, 91.9, 64.7, and 93.8% respectively. On the other hand, the performances of the HPV test were 95.5, 60.2, 32.8, and 98.5%, respectively (Table 4).

Table 1. Methylation of *PCDH10* in tissue specimens

	Age ± SE (years)	Methylation (%)	$P^{\dagger}$
Normal	50.8 ± 3.3	0/17 (0)	
NN		0/11 (0)	
NC		0/6 (0)	
NNC	52.3 ± 3.3	7/13 (53.8)	
LSIL	38.8 ± 8.0	1/5 (20)	
HSIL	49.5 ± 4.1	4/11 (36.4)	
SCC/AdenoCA	56.7 ± 2.3	24/28 (85.7)	
Metastatic CA	53.4 ± 8.8	4/5 (80)	1.48E-08

†Fisher's exact test. AdenoCA, adenocarcinoma; CA, carcinoma; HSIL, high-grade squamous intra-epithelial lesion; LSIL, low-grade squamous intra-epithelial lesion; NC, normal tissue from non-cancer organ of cancer patient; NN, normal tissue from non-cancer patient; NNC, normal tissue next to cervical cancer; SCC, squamous cell carcinoma.

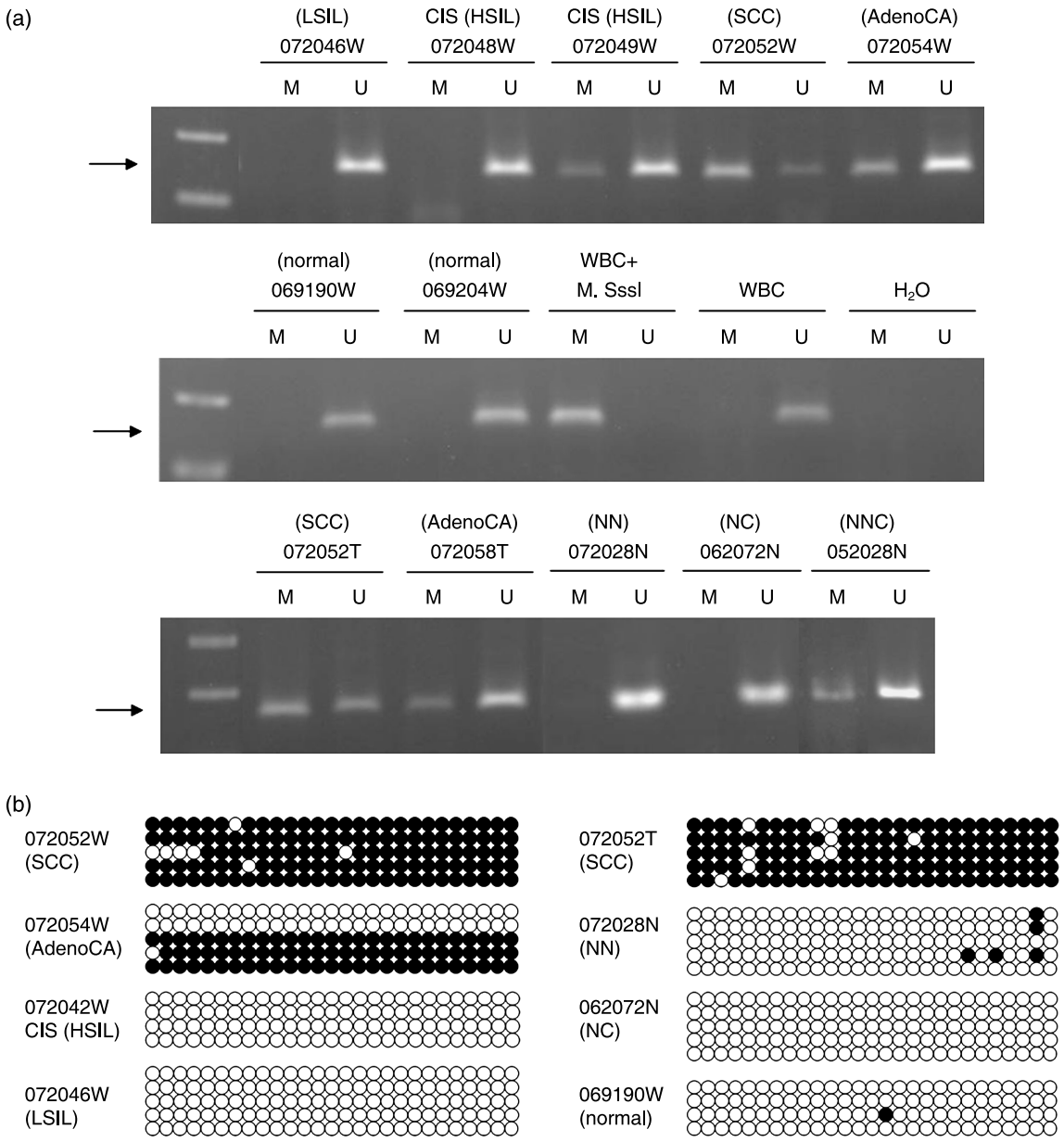

Fig. 2. Methylation of *PCDH10* promoter was closely related to cervical carcinogenesis. (a) Representative methylation-specific-PCR (MS-PCR) results are shown indicating that only unmethylated (U) *PCDH10* was noted in cervical scrapings from the normal control and patients with low-grade squamous intra-epithelial lesion (LSIL), but both U and methylated (M) *PCDH10* were noted from patients with carcinoma *in situ* (CIS) (high-grade squamous intra-epithelial lesion; HSIL), squamous cell carcinoma (SCC), and adenocarcinoma (AdenoCA). White blood cell (WBC) treated with *Sss*I methylase served as the methylation control, and untreated WBC served as the U control. (b) The detailed methylation status of the 27 CpG sites (indicated by circles) located at the promoter of the *PCDH10* gene were determined in representative samples. Both cervical scrapings (W) and tissues (T) of SCC and AdenoCA showed extensive methylation of almost all CpG sites, with the proportion of methylation higher in SCC than AdenoCA. M and U CpG sites are shown by closed and open circles respectively. The arrow signified the expected location of the PCR product. NC, normal tissue from non-cancer organ of cancer patient; NN, normal tissue from non-cancer patient; NNC, normal tissue next to cervical cancer.

Discussion

PCDH10 was shown to be an important tumor suppressor gene, inhibiting the growth, migration, and invasion of nasopharyngeal cancer cells.[17] Promoter methylation and transcription silencing of *PCDH10* were frequently found in nasopharyngeal, esophageal, breast, colorectal, cervical, lung, and hepatocellular carcinoma cell lines.[17] Our study confirmed the methylation silencing of *PCDH10* in cervical cancer cell lines, and disclosed frequent methylation of *PCDH10* in cervical cancer lesions but not the normal controls. Although the chromosomal locus 4q28.3, where *PCDH10* resides, was reported to be frequently deleted in many cancers,[12–16] we did not find alleleic deletion in microsatellite markers adjacent to the *PCDH10* gene (Supporting information Fig. 1).

The present study surveyed clinical specimens across the full spectrum of cervical carcinogenesis, from normal cervix, LSIL, HSIL-CIN2 or 3, HSIL-CIS to invasive carcinoma. Methylation of *PCDH10* was only found in *in situ* and invasive carcinoma (Fig. 2; Table 1), indicating that methylation silencing occurs predominantly at the CIS stage of carcinogenesis. In addition to PCDH10, E-cadherin was downregulated at early invasion of breast and prostate cancers.[24,25] These results suggest that cancer cells may evolve agglomerative silencing of cadherin

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Table 2. Topological controls used to test the field effect of *PCDH10* methylation

ID	Primary cancer			Topological control specimens		
	Diagnosis	Site	Methylation	Procured site	Type	Methylation
052028	SCC of cervix, stage 2a	Cervix	Positive	Vagina	MC	Negative
062037	SCC of cervix, stage 4b	Cervix	Positive†	Vagina	MC	Positive
062020	Adenocarcinoma of cervix, stage 2a	Cervix	Positive	Pelvic LN	MC	Positive
062043	SCC of cervix, stage 3a at least	Cervix	Negative†	Vagina	MC	Positive
062049	Recurrent SCC of cervix, with lymph node metastasis	Cervix	N/A	Common iliac LN	MC	Positive
052014	SCC of cervix, stage 2a	Cervix	Positive	Cervix	NNC	Positive
052028	SCC of cervix, stage 2a	Cervix	Positive	Cervix	NNC	Positive
				Vagina	NNC	Positive
062011	SCC of cervix, stage 2a	Cervix	Positive	Cervix	NNC	Positive
062045	SCC of cervix, stage 2a	Cervix	Positive	Cervix	NNC	Positive
				Vagina	NNC	Positive
072052	SCC of cervix, stage 1b	Cervix	Positive	Cervix	NNC	Positive
072094	SCC of cervix, stage 1b2	Cervix	Positive	Cervix	NNC	Positive
				Stroma under SCC	NNC	Positive
062048	SCC of cervix, stage 2a	Cervix	Positive	Cervix	NNC	Negative
072058	Adenocarcinoma of cervix, stage 1b	Cervix	Positive	Cervix	NNC	Negative
082012	Villoglandular adenocarcinoma of endocervix	Cervix	Negative†	Cervix	NNC	Negative
072081	CIS of cervix	Cervix	Negative†	Cervix	NNC	Positive
082016	CIS of cervix	Cervix	Negative†	Cervix	NNC	Negative
082071	Poorly differentiated adenosquamous Ca of cervix, stage 1b1	Cervix	Negative	Vagina	NNC	Negative
082085	SCC of cervix, stage 1a	Cervix	Negative	Cervix	NNC	Negative
062063	Adenocarcinoma of endometrium, stage 3c	Endometrium	Positive	Cervix	NC	Negative
062072	Adenocarcinoma of endometrium, stage 1b	Endometrium	Negative	Cervix	NC	Negative
062091	Endometroid Ca of oviduct, stage 2	Fallopian tube	Negative	Cervix	NC	Negative
072098	Adenocarcinoma of endometrium, stage 1a	Endometrium	Negative	Cervix	NC	Negative
082031	Adenocarcinoma of endometrium, stage 1a	Endometrium	Negative	Cervix	NC	Negative
082069	Serous cyst adenocarcinoma of ovary, stabe 3c	Ovary	Negative	Cervix	NC	Negative
062048	SCC of cervix, stage 2a	Cervix	Positive	Endometrium	NCx	Positive
072094	SCC of cervix, stage 1b2	Cervix	Positive	Ovary	NCx	Negative
				Pelvic LN	NCx	Negative
082001	Adenocarcinoma of cervix, stage 1b2	Cervix	Negative	Common iliac LN	NCx	Negative
082012	Villoglandular adenocarcinoma of endocervix	Cervix	Negative†	Endometrium	NCx	Negative
082016	CIS of cervix	Cervix	Negative†	Endometrium	NCx	Negative
082065	Neuroendocrine differentiated carcinoma of cervix, stage 4b	Cervix	Negative	Vagina	NCx	Negative
				Uterus	NCx	Negative

†Data were collected from swab specimens. Ca, carcinoma; LN, lymph node; MC, metastatic cancer; N/A, not available; NC, normal cervix from cancer patient; NCx, non-cervical tissue from cervical cancer patient; NNC, normal tissue next to cancer; SCC, squamous cell carcinoma.

Table 3. Methylation of *PCDH10* and human papilloma virus (HPV) typing in cervical swab specimens

	Age ± SE (years)	Methylation (%)	$P^{\dagger}$	Age ± SE (years)	High-risk HPV (%)	$P^{\dagger}$
Normal	53.2 ± 1.8	0/66 (0)		53.2 ± 1.8	3/65 (4.6)	
LSIL	46.8 ± 2.1	0/27 (0)		49.2 ± 2.2	9/12 (75)	
HSIL exclude CIS	46.6 ± 5.4	0/12 (0)		52.9 ± 6.7	8/8 (100)	
CIS	49.4 ± 2.4	12/43 (27.9)		52.4 ± 3.6	23/23 (100)	
SCC/AdenoCA	58.9 ± 2.4	22/31 (71.0)	2.31E-17	57.1 ± 2.9	21/22 (95.5)	6.02E-30

†Fisher's exact test. AdenoCA, adenocarcinoma; CIS, carcinoma *in situ*; HSIL, high-grade squamous intra-epithelial lesion; LSIL, low-grade squamous intra-epithelial lesion; SCC, squamous cell carcinoma.

genes by methylation[26] to acquire plasticity and mobility before invasion.

Methylation of *PCDH10* was found in the cancer tissues as well as the adjacent, histologically normal cervical tissue, but not in remote tissues in the vagina or endometrium. This suggests a field effect of hypermethylation occurring before the invasion of cervical cancer. It takes at least 10 years for the HSIL to become invasive cervical cancer. Long-term epigenetic modification of the *in situ* cancer may be required for cancer cells to acquire the plasticity or a state of epithelial–mesenchymal transition for invasion, presumably by dedifferentiation into a more stemness state.[27] Our study indicates that this epigenetic effect seems not to be confined to cancer cells, rather they may act in a field, most probably through interaction with the underlying stroma.[28]

The field hypermethylation of *PCDH10* was made feasible to develop a sensitive and specific test using cervical scrapings to detect cervical cancer. In the present study, a single PCR test of cervical scraping for *PCDH10* methylation achieved a sensitivity

Table 4. Comparison of sensitivity, specificity, positive, and negative predictive value in a hospital-based study

	Methylation of *PCDH10*				High-risk HPV			
	Sensitivity %	Specificity %	PPV %	NPV %	Sensitivity %	Specificity %	PPV %	NPV %
Normal/LSIL HSIL/SCC/AdenoCA	39.5	100	100	64.1	98.1	84.4	81.3	98.5
Normal/SIL exclude CIS CIS/SCC/AdenoCA	45.9	100	100	72.4	97.8	76.5	68.8	98.5
Normal/SIL SCC/AdenoCA	71	91.9	64.7	93.8	95.5	60.2	32.8	98.5

AdenoCA, adenocarcinoma; CIS, carcinoma *in situ*; HPV, human papilloma virus; HSIL, high-grade squamous intra-epithelial lesion; LSIL, low-grade squamous intra-epithelial lesion; NPV, negative predictive value; PPV, positive predictive value; SCC, squamous cell carcinoma; SIL, squamous intraepithelial lesion.

of 71% and specificity of 91.9% in identifying invasive cervical cancer, which was better than any current diagnostic test in practice, including Pap smear and HPV test. In a head-to-head comparison with a highly performed high-risk HPV DNA chip test on the same cervical scrapings, the methylation test was much more specific than the HPV test. Due to its high prevalence in normal and LSIL women, testing of high-risk HPV has a low (32.8%) positive predictive value in diagnosing cervical cancer, which can be even lower when conducted in a general population. In addition, our study also showed that cancers of adjacent organs, that is, the endometrium, rarely reveal field methylation of *PCDH10* in normal-appearing uterine cervix, thus adding to the specificity of the test for the origin of cancer. This novel methylation marker may thus be used in screening for cervical cancer and the triage of ambiguous or low-grade smear results, with more certainty of positivity than the HPV test.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Absence of loss of heterozygosity (LOH) of microsatellite marker adjacent to the *PCDH10* gene.

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