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Original Article

CD147 and HPV16 oncoprotein expression in cervical squamous cell carcinoma and the clinical implications

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Abstract: CD147 participates in tumour invasion and metastasis in various human malignancies. Human papillomavirus 16 (HPV16) oncoproteins HPV16E6 and HPV16E7 are frequently observed in cervical squamous cell carcinoma (CSCC). HPV16E6 and HPV16E7 induce carcinogenesis by degrading tumour suppressor proteins p53 and pRB, respectively. In this study, we investigated the expression levels of CD147, HPV16E6, HPV16E7, p53 and pRB by immunohistochemical analysis of CSCC tissues from 57 first-visit patients. The positive rates of CD147, HPV16E6, HPV16E7 and p53 expression were significantly higher in the cancer tissues than in the para-cancer tissues ($P<0.001$, $P=0.021$, $P<0.001$, $P<0.001$, respectively). In particular, CD147 was expressed in 84.2% of cancer tissues and 5.3% of para-cancer tissues. CD147 expression and its co-expression with HPV16E6 (CD147-HPV16E6 co-expression) both correlated with regional lymph node metastasis (N stage, $P=0.013$, $P=0.014$), FIGO stage ($P=0.001$, $P=0.001$), and tumour size ($P=0.006$, $P=0.049$). Furthermore, CD147 expression was positively associated with HPV16E6 ($P=0.047$), p53 ($P=0.028$) and pRB ($P=0.022$) expression. CD147-HPV16E6 co-expression was also associated with p53 expression ($P=0.022$). In conclusion, CD147 was specifically expressed in CSCC tissues and could be a promising CSCC oncotarget. Both CD147 expression and CD147-HPV16E6 co-expression correlated with tumour malignancy and poor prognosis, making them both potential cancer-related biomarkers in CSCC therapy. In addition, the interaction among CD147, HPV16E6 and p53 might be involved in CSCC progression.

Keywords: CD147, HPV16 oncoprotein, clinical implication, statistical association, cervical squamous cell carcinoma

Introduction

Cervical cancer is one of the most common gynaecologic malignant tumours with a combined worldwide incidence of nearly 500,000 new cases every year [1]. It was responsible for approximately 260,000 deaths, which accounted for 7.5% of all female cancer deaths in 2012 according to World Health Organization (WHO) estimates [2]. The 5-year survival rate for localized and early-stage cervical cancer is 91.5%, while approximately 13% of cervical cancer patients are diagnosed with metastatic cervical cancer, which has a 5-year survival rate of 16.5% and a median survival time of only 8 to 13 months [3, 4]. Cervical squamous cell carcinoma (CSCC) accounts for approximately 85% to 90% of all cervical cancers. There have not been any radical treatments developed for cervical cancer patients to date, and the general detection method of potential precancerous lesions is to receive regular early screening tests, such as human papillomavirus (HPV) testing and Pap smears.

It is well known that persistent infection with high-risk HPV is the essential factor for the formation of cervical intraepithelial neoplasia [5]. To date, more than 100 types of HPV have been identified, and approximately 30 of them contribute to cervical malignancy progression [6, 7]. Of all the HPV types, HPV16 and 18 are the most frequently observed high-risk HPV types. They are responsible for the development of approximately 80% of all cervical cancer cases [6, 8], and HPV16 alone has been observed in 60% of cervical cancer cases [9]. HPV16E6 and HPV16E7, two nonstructural HPV16 proteins, play essential roles in inducing infected epithelial cells to become dysplastic and form warts

or even tumours by exploiting the host cells to synthesize HPV structural proteins [10]. An important carcinogenesis mechanism involves the binding of HPV16E6 to p53 to promote its degradation and mutation and the inactivation of retinoblastoma protein (pRB) by HPV16E7, which together disrupt cell cycle regulation and induce cell proliferation, genome instability, block apoptosis, and escape the innate immune system [7, 9]. p53 and pRB are tumour suppressor proteins encoded by the *Tp53* and *RB1* genes, respectively [11]. p53 functions as a transcription factor that binds to specific DNA sequences to promote cell cycle arrest, apoptosis and senescence [12]. Mutations in the *Tp53* gene are present in more than 50% of patients with malignant tumours, and p53 is rarely expressed in healthy people. Mutant p53 proteins have half-lives of several hours compared to 20 minutes for wild-type p53. Therefore, antibodies against p53 are dependable markers for mutant p53 [13]. pRB acts as a transcriptional regulator in DNA synthesis and cell-cycle control. It plays a key role in regulating the ability of cells to enter S phase, which has been linked to its ability to regulate transcription [14].

Extracellular matrix metalloproteinase inducer (EMMPRIN), also known as basigin or CD147, is a glycosylated transmembrane protein that belongs to the immunoglobulin superfamily. It has several distinct functions, including spermatogenesis, the inflammatory response and tumour invasion [15, 16]. CD147 expression by tumour cells stimulates peritumoural fibroblasts to produce matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM) [17]. ECM degradation can induce epithelial tumour cell invasion and metastasis [18]. Therefore, abundant CD147 surface expression in several tumour types facilitates tumour metastasis, modulating cell substrate and adhesion processes [19]. According to Feng et al., CD147 expression was significantly increased in poorly differentiated tissues in colon cancer, cervical cancer, oesophageal cancer and lung cancer [20]. CD147 expression is also associated with breast carcinoma risk factors and significantly correlates with tumour grading and tumour-node-metastasis stages in hepatocellular carcinomas [21]. It is now recognized as an effective therapeutic target for hepatocellular carcinoma [22].

In this research, we first aimed to demonstrate the clinical impact of CD147 and HPV16 oncoprotein (HPV16E6 and HPV16E7) expression in first-visit CSCC patients. Furthermore, we explored their possible relationship with p53 and pRB, which are modulated by HPV16E6 and HPV16E7, respectively, in CSCC progression. The positive correlation could further suggest a mechanism that underlies CSCC progression through the CD147-HPV16-related pathway.

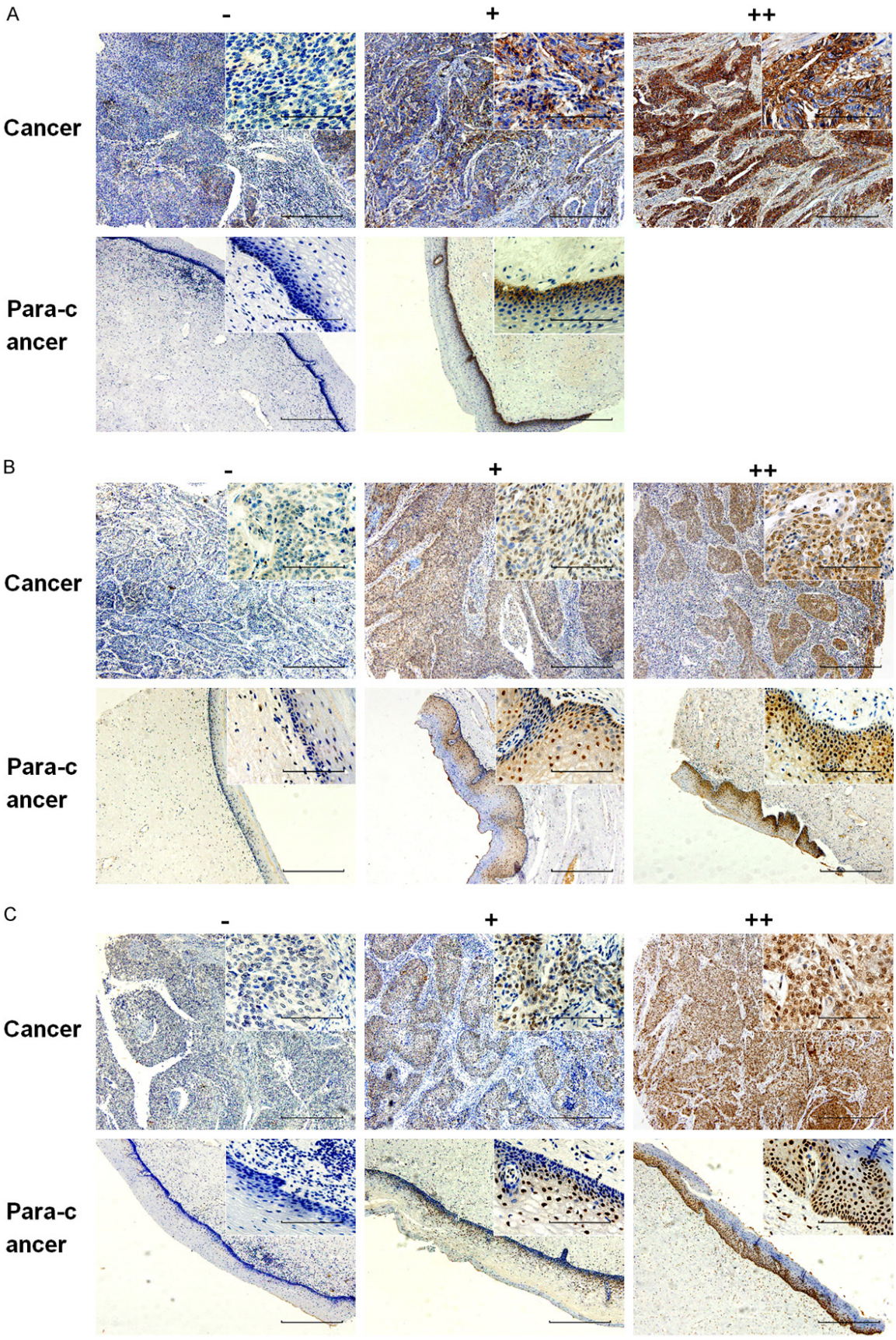
Materials and methods

Patients and tissue chips

Tissue chips contained cancer and para-cancer tissues from 57 female patients who were histologically confirmed as squamous cell carcinoma during their preliminary diagnosis (Shanghai Outdo Biotech Company, Shanghai, China). Detailed pathological and clinical data, including age (18 to 72), pathology grade (I, II, III), tumour-node-metastasis stage (TNM), FIGO stage (I-III; International Federation of Gynecology and Obstetrics), tumour size (0.5× Length × Width^2) [23] and lymph node invasion were obtained from each patient's medical records.

Immunohistochemistry

Four micron serial sections from formalin-fixed, paraffin-embedded tissues were cut onto glass slides. After deparaffinization with xylene and dehydration in a graded ethanol series (100%, 95%, 90%, 85% and 75%), antigen retrieval was performed in an autoclave in boiling 10 mM citrate buffer (pH 6.0) for 2 minutes. After allowing the slides to cool down to room temperature, sections were treated with hydrogen peroxide in methanol (30% H_2O_2 solution: H_2O: methanol =1:1:9) for 15 minutes at room temperature to block endogenous peroxidase activity, then washed in phosphate buffered saline (PBS, pH 7.35). Sections were then treated with nonspecific staining blockers (ZSGB-Bio, Beijing, China), followed by incubation in anti-CD147 antibody (1:300; Cell Engineering Research Center, Fourth Military Medical University, Xi'an, China), anti-HPV16E6 antibody (1:200; Biorbyt LLC, San Francisco, California, United States), anti-HPV16E7 antibody (1:250; Biorbyt LLC., San Francisco, California, United States), anti-p53 antibody (1:400, Antibody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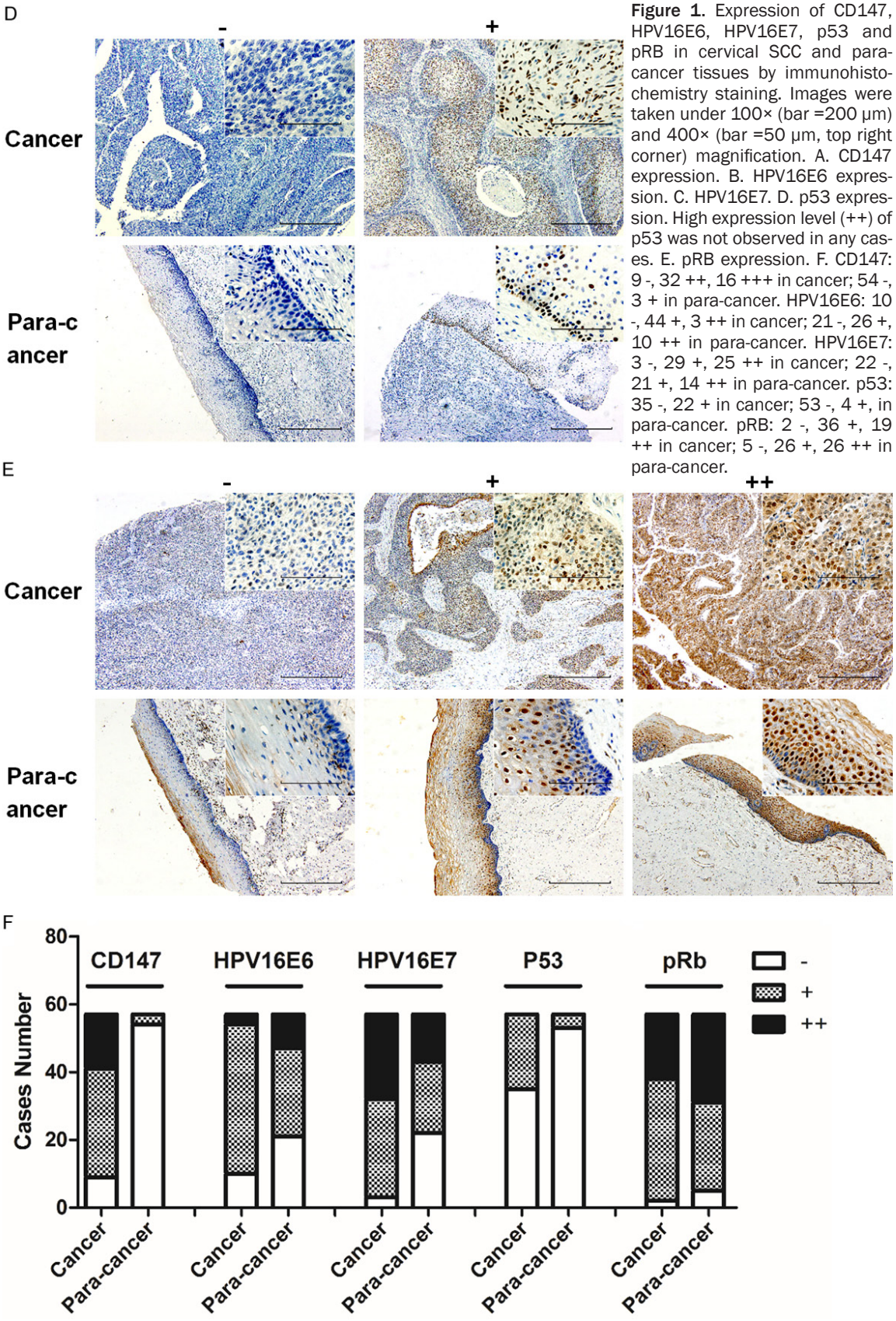

Figure 1. Expression of CD147, HPV16E6, HPV16E7, p53 and pRB in cervical SCC and para-cancer tissues by immunohistochemistry staining. Images were taken under 100× (bar =200 μm) and 400× (bar =50 μm, top right corner) magnification. A. CD147 expression. B. HPV16E6 expression. C. HPV16E7. D. p53 expression. High expression level (++) of p53 was not observed in any cases. E. pRB expression. F. CD147: 9 -, 32 ++, 16 +++ in cancer; 54 -, 3 + in para-cancer. HPV16E6: 10 -, 44 +, 3 ++ in cancer; 21 -, 26 +, 10 ++ in para-cancer. HPV16E7: 3 -, 29 +, 25 ++ in cancer; 22 -, 21 +, 14 ++ in para-cancer. p53: 35 -, 22 + in cancer; 53 -, 4 +, in para-cancer. pRB: 2 -, 36 +, 19 ++ in cancer; 5 -, 26 +, 26 ++ in para-cancer.

Table 1. Expression and significant differences of CD147, HPV16E6, HPV16E7, p53 and pRB in CSCC cancer or para-cancer tissues (n=57)

	CD147		HPV16E6		HPV16E7		p53		pRB	
	-	+, ++	-	+, ++	-	+, ++	-	+, ++	-	+, ++
Cancer	9 (15.8%)	48 (84.2%)	10 (17.5%)	47 (82.5%)	3 (5.3%)	54 (94.7%)	35 (61.4%)	22 (38.6%)	2 (3.5%)	55 (96.5%)
Para-cancer	54 (94.7%)	3 (5.3%)	21 (36.8%)	36 (63.2%)	22 (38.6%)	35 (61.4%)	53 (93.0%)	4 (7.0%)	5 (8.8%)	52 (91.2%)
P	**0.000**[1]		**0.021**[1]		**0.000**[1]		**0.000**[1]		0.438[2]	

Note: Number of positive and negative cases was calculated based on immunohistochemistry staining results (**Figure 1A-F**). [1]*P* value was estimated by Pearson χ^2 test; [2]*P* value was estimated by Fisher's exact test. Font Bold when *P*<0.05.

Revolution Inc., San Diego, California, United States) and anti-pRB antibody (1:50; Biorbyt LLC., San Francisco, California, United States) overnight at 4°C, followed by PBS washes (3×5 min). Subsequently, sections were incubated in goat anti-mouse/rabbit IgG-Biotin (ZSGB-Bio, Beijing, China) for 15 minutes at room temperature and washed in PBS (3×5 min). Sections were then incubated with streptavidin-HRP (ZSGB-Bio, Beijing, China) for 15 minutes at room temperature and washed with PBS (3×5 min). Finally, each chip was treated with 50 μl diaminobenzidine (DAB) working solution (ZSGB-Bio, Beijing, China) at room temperature for 3-5 minutes and washed with PBS. All tissue sections were counterstained with haematoxylin, dehydrated with xylene and ethanol and mounted with resinene. Images were acquired with an inverted microscope (CKX41; Olympus) equipped with a digital camera under 100× and 400× magnification.

Evaluation of protein expression

The expression of CD147, HPV16E6, HPV16E7, p53 and pRB were semi-quantitatively evaluated by two independent blinded pathologists. The immunoreactivity of the five proteins was graded according to the percentage of positive cells and the staining intensity. The scoring for the percentage of positive cells was as follows: 0-5%=0; 5%-50%=1; 50%-100%=2. Scoring for staining intensity was as follows: negative =0; weak =1, strong =2. The two scores were multiplied, and the final immunoreactivity grade was determined as follow: 0 for negative expression (-); 1-2 for low expression level (+); 4 for high expression level (++).

The co-expression (for example, the co-expression of CD147-HPV16E6, CD147-HPV16-E6-p53, etc.) was defined as follows: any case in which two or all three investigated proteins were positively stained was judged as co-expressed (+); otherwise, it was judged as not co-expressed (-).

Statistical analysis

The Pearson χ^2 test was used to analyse significant differences in protein expression between cancer and para-cancer tissues. The clinical impact of CD147, HPV16E6, HPV16E7, p53, pRB and their co-expression and relevance was analysed using linear-by-linear association or Fisher's exact test according to the appropriate conditions. Differences were considered to be statistically significant when 2-sided P<0.05. All statistical analyses were performed using the SPSS Statistics v22.0 software.

Results

CD147, HPV16E6, HPV16E7, p53 and pRB protein expression in CSCC and para-cancer tissues

To systematically identify the clinical significance of CD147 expression and its relationship with CD147, HPV16E6, HPV16E7, p53 and pRB expression, we first investigated the expression of CD147 HPV16E6, HPV16E7, p53 and pRB by immunohistochemistry (**Figure 1**) and the changes in their expression in CSCC tissues and para-cancer tissues using a Pearson χ^2 test (**Table 1**). CD147 expression was notably higher in CSCC cases (48 of 57, 84.2%) than in para-cancer cases (3 of 57, 5.3%), and this difference was statistically significant (*P*<0.001, **Table 1**), suggesting that CD147 is a potential therapeutic target due to its specificity in CSCC tissues (**Figure 1A** and **1F**).

HPV16E6 and HPV16E7 expression was high in both cancer and para-cancer tissues (47 of 57, 82.5% in cancer and 36 of 57, 63.2% in para-cancer for HPV16E6; 54 of 57, 94.7% in cancer and 35 of 57, 61.4% in para-cancer for HPV-16E7; **Table 1**) the difference was statistically significant between CSCC and para-cancer tissues (*P*=0.021 for HPV16E6; *P*<0.001 for HPV16E7; **Figure 1B**, **1C** and **1F**). p53 expression was low in both CSCC (22 of 57, 38.6%)

Table 2. Correlation of CD147, HPV16E6, HPV16E7, p53 and pRB expression with clinical parameters (n=57)

	CD147				HPV16E6				HPV16E7				p53			pRB			
	- (n=9)	+ (n=32)	++ (n=16)	*P*	- (n=10)	+ (n=44)	++ (n=3)	*P*	- (n=3)	+ (n=29)	++ (n=25)	*P*	- (n=35)	+ (n=22)	*P*	- (n=2)	+ (n=36)	++ (n=19)	*P*
Age																			
≤46 (n=31)	5	22	4	0.067[2]	8	23	1	0.091[2]	2	15	14	1.000[2]	19	12	0.985[1]	0	22	9	1.000[2]
>46 (n=26)	4	10	12		2	21	2		1	14	11		16	10		2	14	10	
Pathology grade																			
I-II (n=46)	7	25	14	0.611[2]	8	36	2	1.000[2]	2	23	21	0.571[2]	28	18	1.000[2]	2	29	15	0.756[2]
III (n=11)	2	7	2		2	8	1		1	6	4		7	4		0	7	4	
T stage																			
T1 (n=45)	9	24	12	0.228[2]	9	33	3	1.000[2]	3	23	19	0.584[2]	28	17	1.000[2]	2	26	17	0.375[2]
T2-T3 (n=12)	0	8	4		1	11	0		0	6	6		7	5		0	10	2	
N stage																			
N0 (n=34)	8	20	6	**0.013[2]**	7	27	0	0.146[2]	0	18	16	0.252[2]	21	13	0.946[1]	1	22	11	1.000[2]
N1 (n=23)	1	12	10		3	17	3		3	11	9		14	9		1	14	8	
FIGO stage																			
I (n=24)	8	13	3	**0.001[2]**	6	18	0	**0.033[2]**	0	13	11	0.270[2]	15	9	0.909[1]	1	13	10	0.685[2]
II (n=10)	0	7	3		1	9	0		0	5	5		6	4		0	9	1	
III (n=23)	1	12	10		2	18	3		3	11	9		14	9		1	14	8	
Tumor size																			
≤12 cm^3 (n=21)	5	15	1	**0.006[1]**	6	14	1	0.236[2]	0	10	11	0.244[2]	12	9	0.617[1]	0	11	10	0.071[2]
>12 cm^3 (n=36)	4	17	15		4	30	2		3	19	14		23	13		2	25	9	
Lymph nodes invasion																			
Not found (n=33)	6	16	11	0.699[1]	3	29	1	0.263[2]	3	16	14	0.499[2]	19	14	0.490[1]	2	24	7	**0.021[2]**
Yes (n=24)	3	16	5		7	15	2		0	13	11		16	8		0	12	12	

Note: [1]*P* value was estimated by linear-by-linear association; [2]*P* value was estimated by Fisher's exact test; Font Bold when $P<0.05$.

Table 3. Correlation of CD147-HPV16E6, CD147-p53, CD147-HPV16E7, CD147-pRB, CD147-HPV16E6-p53 and CD147-HPV16E7-pRB co-expression with clinical parameters (n=57)

	CD147-HPV16E6			CD147-p53			CD147-HPV16E7			CD147-pRB			CD147-HPV16E6-p53			CD147-HPV16E7-pRB		
	- (n=18)	+ (n=39)	*P*	- (n=35)	+ (n=22)	*P*	- (n=11)	+ (n=46)	*P*	- (n=9)	+ (n=32)	*P*	- (n=9)	+ (n=32)	*P*	- (n=9)	+ (n=32)	*P*
Age																		
≤46 (n=31)	12	19	0.210[1]	19	12	0.985[1]	6	25	0.991[1]	5	26	0.512[1]	21	10	0.852[1]	6	25	0.501[1]
>46 (n=26)	6	20		16	10		5	21		6	20		17	9		7	19	
Pathology grade																		
I-II (n=46)	14	32	0.728[2]	28	18	1.000[2]	9	37	1.000[2]	9	37	1.000[2]	30	16	0.735[2]	11	35	0.725[2]
III (n=11)	4	7		7	4		2	9		2	9		8	3		2	9	
T stage																		
T1 (n=45)	17	28	0.080[2]	28	17	1.000[2]	11	34	0.097[2]	11	34	0.097[2]	30	15	1.000[2]	13	32	0.050[2]
T2-T3 (n=12)	1	11		7	5		0	12		0	12		8	4		0	12	
N stage																		
N0 (n=34)	15	19	**0.014[2]**	21	13	0.946[1]	8	26	0.497[2]	9	25	0.170[2]	24	10	0.449[1]	9	25	0.427[1]
N1 (n=23)	3	20		14	9		3	20		2	21		14	9		4	19	
FIGO stage																		
I (n=24)	14	10	**0.001[1]**	15	9	0.909[1]	8	16	0.099[2]	9	15	**0.015[2]**	17	7	0.474[1]	9	15	0.121[1]
II (n=10)	1	9		6	4		0	10		0	10		7	3		0	10	
III (n=23)	3	20		14	9		3	20		2	21		14	9		4	19	
Tumor size																		
≤12 cm (n=21)	10	11	**0.049[1]**	12	9	0.617[1]	5	16	0.729[2]	5	16	0.729[2]	14	7	1.000[2]	5	16	1.000[2]
>12 cm (n=36)	8	28		23	13		6	30		6	30		24	12		8	28	
Lymph nodes invasion																		
Not found (n=33)	9	24	0.416[1]	19	14	0.490[1]	8	25	0.326[2]	8	25	0.326[2]	20	13	0.259[1]	10	23	0.117[1]
Yes (n=24)	9	15		16	8		3	21		3	21		18	6		3	21	

Note: [1]*P* value was estimated by linear-by-linear association; [2]*P* value was estimated by Fisher's exact test. Font Bold when $P<0.05$.

Table 4. Association of CD147 expression with HPV16E6, HPV16E7, p53 and pRB expression in CSCC

	HPV16E6				p53			HPV16E7				pRB			
	- (n=10)	+ (n=44)	++ (n=3)	*P*	- (n=35)	+ (n=22)	*P*	- (n=3)	+ (n=29)	++ (n=25)	*P*	- (n=2)	+ (n=36)	++ (n=19)	*P*
CD147															
- (n=9)	1	8	0	**0.047**	9	0	**0.028**	1	4	4	1.000	0	4	5	**0.022**
+ (n=32)	9	23	0		18	14		2	15	15		0	21	11	
++ (n=16)	0	13	3		8	8		0	10	6		2	11	3	

Note: *P* value was estimated by Fisher exact test. Font Bold when *P*<0.05.

Table 5. Association of CD147-HPV16E6 co-expression with p53 expression and CD147-HPV16E7 co-expression with pRB expression in CSCC

	p53				pRB			
	- (n=35)	+ (n=22)	*P*		- (n=2)	+ (n=36)	++ (n=19)	*P*
CD147-HPV16E6				CD147-HPV16E7				
- (n=18)	15	3	**0.022**[1]	- (n=11)	0	6	5	0.349[2]
+ (n=39)	20	19		+ (n=46)	2	30	14	

Note: [1]*P* value was estimated by linear-by-linear association. [2]*P* value was estimated by Fisher's exact test. Font Bold when *P*<0.05.

and para-cancer tissues (4 of 57, 7.0%, *P*< 0.001, **Table 1**; **Figure 1D** and **1F**). Finally, pRB expression was similar in CSCC tissues (55 of 57, 96.5%) and para-cancer tissues (52 of 57, 91.2%; *P*>0.05; **Table 1**; **Figure 1E** and **1F**).

In summary, CD147, HPV16E6, HPV16E7 and p53 expression levels were significantly different in CSCC and para-cancer tissues, suggesting that these four molecules were specifically expressed in CSCC.

The clinical relevance of CD147, HPV16E6, HPV16E7, p53, and pRB expression and their co-expression

Based on immunohistochemical staining and the statistical analyses above, we investigated the correlation of CD147, HPV16E6, HPV16E7, p53, and pRB expression levels with clinical parameters, including age, pathology grade, T stage (primary tumour malignancy), N stage (regional lymph node metastasis), FIGO stage, tumour size and lymph node invasion of 57 patients (**Table 2**). CD147 expression was more frequently observed in cases with higher N stage (*P*=0.013), FIGO stage (*P*=0.001) and larger tumour size (P=0.006) but was not correlated with age, pathology grade, T stage or lymph node invasion. In addition, HPV16E6 expression was positively associated with FIGO stage (*P*=0.033). HPV16E7 and p53 expression did not show significant associations with any clinical parameters. pRB significantly correlated with lymph node invasion (*P*=0.021).

To further investigate the potential co-action of CD147 with HPV16E6, HPV16E7, p53 and pRB that could be related to clinical parameters, we examined the co-expression levels of CD147-HPV16E6, CD147-p53, CD147-HPV16E7, CD-147-pRB, CD147-HPV16E6-p53 and CD147-HPV16E7-pRB (**Table 3**). CD147-HPV16E6 co-expression positively correlated with N stage (*P*=0.014), FIGO stage (*P*=0.001) and lymph node invasion (*P*=0.049). CD147-pRB co-expression was associated with FIGO stage too.

Association analysis of CD147, HPV16E6, HPV16E7, p53 and pRB expression levels in CSCC

As mentioned above, p53 is modulated by HPV16E6 and pRB is modulated by HPV16E7. We attempted to investigate the relationship between CD147 with HPV16 oncoproteins (HPV16E6 and HPV16E7) and their downstream targets p53 and pRB, respectively, by examining linear trends (**Tables 4** and **5**). Considering the signed rho of the Spearman rank test of CD147, HPV16E6, HPV16E7, p53 and pRB pairwise comparisons (data not shown), CD147 expression positively associated with HPV16E6 (*P*=0.047) and p53 (*P*=0.028) and negatively associated with pRB (*P*=0.022, **Table 4**), suggesting that CD147 expression

increased as HPV16E6 and p53 expression increased, but decreased as pRB levels increased in CSCC patients. We observed a relationship between CD147-HPV16E6 co-expression and p53 (P=0.022, **Table 5**), suggesting that there were certain interactions among CD147, HPV16E6 and p53 during CSCC development.

Discussion

In recent years, research on CD147 in oncology and oncotherapy has increased. Our research centre has exerted significant effort to understand the crucial role of CD147 in carcinogenesis and tumour formation. Furthermore, we have already developed antibody-based drugs targeting CD147 to treat hepatic carcinoma and lung cancer [15, 24]. However, the role of CD147 in CSCC remains to be fully elucidated. CD147 has been reported to be a prognostic factor for cervical cancer radiotherapy [25]. Huang XQ et al. have reported that CD147 expression was associated with Glucose transporter 1 (GLUT-1), and their co-expression indicated radiation resistance and poor CSCC prognosis [26]. Furthermore, the co-expression of CD147-matrix metallopeptidase 9 (MMP9), CD147-monocarboxylate transporters 1 and 4 (MCT1 and MCT4), CD147-epidermal deficiency of a disintegrin and metalloproteinase 17 (ADAM17) are considered to indicate the clinical significance of cervical cancer [27-29]. Our study aimed to identify the clinical significance of CD147 expression and CD147-HPV16 oncoprotein (HPV16E6 and HPV16E7) co-expression in first-visit CSCC patients. We then examined the relevance of CD147 and HPV16 oncoprotein co-expression, along with their interacting molecules (p53 and pRB), which may suggest a function for the CD147-HPV16 interaction in CSCC tumourigenesis and progression. We first confirmed that the expression of CD147, HPV16E6, HPV16E7 and p53 was significantly different in CSCC and para-cancer tissues. In particular, CD147 expression showed high specificity for cancer tissues (84.2% positive in cancer and 5.3% positive in para-cancer cases), suggesting that CD147 was a promising biomarker and safe oncotarget for CSCC treatment. We then studied the relationship between CD147 and several clinical parameters and found that CD147 expression and CD147-HPV16E6 co-expression statistically associated with regional lymph node metastasis (N stage), FIGO stage and tumour size. Since these three clinical parameters could represent tumour metastatic capability, overall malignancy and tumour growing ability, respectively, we suggest that CD147 expression levels are indicative of the malignancy and prognosis of CSCC patients. According to the guidelines of the National Academy of Clinical Biochemistry (NACB), squamous cell carcinoma antigen (SCC-AG), cancer antigen 125 (CA-125), carcino-embryonic antigen (CEA), and Cyfra21-1, among others [30], are acknowledged indicators of cervical cancer and are under clinical application. It has been reported that CA-125 and Cyfra21-1 are strongly related to tumour size, FIGO stage and Lymph node status [31]. Altogether, we considered CD147 and CD147-HPV16E6 co-expression as two potential indicators of CSCC prognosis. Although pRB expression was also related to some clinical parameters, the correlation was not as strong because pRB expression was not significantly different between CSCC and para-cancer tissues.

The mechanisms of CD147 in CSCC tumourigenesis, progression and invasion remain to be elucidated. Previous studies have demonstrated that CD147 promotes tumour cell movement and metastasis. It has been reported that the interaction of CD147 with Annexin A2 and the DOCK3-β-catenin-WAVE2 signalling pathway [32] and its involvement in TGF-β-induced epithelial-mesenchymal transition helps induce hepatocellular carcinoma invasion [33]. In our study, the clinical implication of CD147-HPV16E6 co-expression suggested that the CD147-HPV16 interaction might be involved in CSCC progression. Therefore, we investigated p53 and pRB expression, each of which is directly affected by HPV16E6 and HPV16E7, respectively. In our statistical studies, we found that p53 expression is statistically associated with CD147 and CD147-HPV16E6. Therefore, we hypothesize that CD147, HPV16E6 and p53 exist in a signalling pathway to promote CSCC tumourigenesis and progression. This hypothesis requires further studies on the exact mechanism of CD147, HPV16E6 and p53 interaction using cytobiological and molecular biological methods.

In conclusion, our study investigated 57 cases of first-visit CSCC patients. We found that

CD147 expression was specific to CSCC. CD147 expression and CD147-HPV16E6 co-expression levels were significantly associated with regional lymph node metastasis, FIGO stage and tumour size. In CSCC tissues, CD147 expression was positively linearly correlated with both HPV16E6 and its affected target p53, and CD147-HPV16 co-expression was also related to p53. Based on these findings, we believe that CD147 is a potential target for cervical oncotherapy. Furthermore, our data suggest that CD147 expression and CD147-HPV16E6 co-expression are two indicators of malignancy and prognosis in CSCC patients, and an interaction between CD147, HPV16E6 and p53 may exist in regulating CSCC progression.

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Disclosure of conflict of interest

None.

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