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Atypical chemokine receptors predict lymph node metastasis and prognosis in patients with cervical squamous cell cancer

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HIGHLIGHTS

- We described the characterization of ACR (DARC, D6, and CCX-CKR) expression with clinicopathological and immunohistochemical features.
- We found that ACR expression was correlated with lymph node status and prognosis of cervical squamous cell carcinoma.
- We found that the three ACRs could be used as prognostic markers in cervical cancer patients.

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ABSTRACT

Objective. Atypical chemokine receptors (ACRs), including CCX-CKR, DARC, and D6, have been reported to be involved in cancer invasion and metastasis. The objective of this study was to investigate the prognostic importance of ACRs in patients with cervical squamous cell carcinoma (CSCC).

Methods. The expression of three ACRs was investigated by immunohistochemical (IHC) examination in a total of 317 cervical specimens including 40 normal cervical tissues, 50 cases of carcinoma in situ of cervix (CIS), and 227 cases of CSCC by immunohistochemistry.

Results. The expression rate of DARC and CCX-CKR in CSCC, CIS, and normal cervix increased gradually ($p < 0.01$). D6 expression is decreased in CSCC compared to either in CIS or in normal cervix ($p < 0.05$). In addition, the expression of CCL2 and CCL19 was inversely associated with ACR expression ($p < 0.05$), while that of LCA was positively correlated with ACR expression ($p < 0.05$). Moreover, DARC expression, CCX-CKR expression, and ACR coexpression were negatively correlated with lymph node metastasis ($P < 0.01$). D6 expression and ACR coexpression were negatively related to tumor size ($p = 0.018$) and recurrence ($p = 0.028$). In multivariate Cox regression analysis, CCX-CKR expression was a positive indicator for overall survival ($p = 0.008$), and D6 expression was an independent predictor of both overall and recurrence-free survival ($p = 0.041$) in CSCC.

Conclusions. Our results suggest that the loss of ACRs may play important roles in the tumorigenesis and migration of cervical cancer. ACR expression may be considered as prognostic markers in patients with CSCC.

Introduction

Cervical cancer is the second most common malignancy of female reproductive system [1]. One of the most important prognostic parameter in patients with cervical cancer is regional lymph node metastasis [2]. About one-half of the cervical cancer patients with pelvic lymph node metastasis will have recurrence of disease, with most of them dying of uncontrolled disease [3]. The reported 5-year survival rate for patients who undergo relapse ranges from 3 to 13% [4,5], while there is the lack of predictive markers for lymph node metastasis in patients with cervical cancer.

Chemokines and their receptors, such as CXCL12/CXCR4, CCL19 (CCL21)/CCR7, and CXCL13/CXCR5, have been implicated mostly in cell migration [6]. Recently, increasing attention has been drawn to atypical chemokine receptors (ACRs), which comprise a group of 7 transmembrane domain proteins structurally similar to G protein-coupled receptors [7]. However, atypical chemokine receptors do not induce classical signaling via the typical G protein-mediated pathways [8]. This may be due to the lack of canonical DRYLAIV motif within the second intracellular loop, which normally enables G protein coupling and induces the G protein-mediated signaling [9]. The ACR family, including Duffy antigen receptor for chemokines (DARC), D6, and Chemocentryx chemokine receptor (CCX-CKR), has the potential to

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modify the bioavailability of chemokines [10]. DARC was initially discovered as the Duffy red blood group antigen, and it mediates the internalization of inflammatory chemokines of the CC (cysteines next to each other) and CXC (cysteines separated by a single amino acid) groups [11]. D6 binds multiple inflammatory CC chemokines, while it does not recognize homeostatic CC chemokines [12]. CCX-CKR was recently discovered and was found to localize predominantly in the epithelial cells of heart and lung [13]. Previous studies have shown that ACRs are involved in the process of tumor progression and metastasis [14,15]. As chemokines contribute to immune suppression, tumor infiltration, angiogenesis, and migration [16], ACRs inhibit tumor progression by competitively binding to chemokines and in turn decreasing their bioactivities [17]. However, there is no published report of whether the expression of ACRs correlates with progression or clinical outcomes of cervical cancer.

In this study, we report for the first time the characterization of DARC, D6, and CCX-CKR expression in human cervical squamous cell cancer. We found that the expression of DARC and CCX-CKR was conversely correlated with lymph node status. Multivariate analysis suggested that D6 and CCX-CKR expression were independent prognostic markers for CSCC patients. Our results strongly suggest that ACRs might play an anticancer role in cervical cancer development and might be valuable prognostic and metastatic markers for CSCC patients.

Material and methods

Patients and tissue specimens

A total of 317 paraffin-embedded samples were obtained from CSCC patients, who underwent radical hysterectomy at Sun Yat-Sen University Cancer Center between Jan 2001 and June 2006. The samples were composed of 40 normal cervical tissues, 50 CIS, and 227 CSCC specimens. For the use of these clinical materials for scientific purposes, prior patient's consent and approval from the Institute Research Ethics Committee were obtained. None of the patients had received chemotherapy or radiotherapy before surgery. After surgery, patients were treated with adjuvant radiotherapy or concurrent chemoradiation therapy, depending on the lymph node status, the stage of the disease, the parametrial status, and tumor differentiation, according to the national guidelines. Postoperative radiotherapy was given to 65 patients. Postoperative chemotherapy was administered to 110 patients. Patients with lymph node metastasis received both chemotherapy and radiotherapy postoperatively. The clinical stage of the patients was classified according to the International Federation of Gynecology and Obstetrics criteria as follows: 130 were allocated to stage IB1, 38 to stage IB2, 40 to stage IIA1, 13 to stage IIA2, and 8 to stage IIB. The median age of the patients was 42.7 years (range 25–68 years).

Immunohistochemistry

IHC analysis was performed to examine protein expression of DARC, D6, CCX-CKR, chemokine (C–C motif) ligand 2 (CCL2), chemokine (C–C motif) ligand 19 (CCL19), and leukocyte common antigen (LCA). Briefly, freshly cut 4 μm sections were deparaffinized and rehydrated in declining grades of ethanol. Antigen retrieval was performed by submerging the sections into a 10 μmol/L citrate buffer solution (pH 6.0) for 10 min in a microwave oven. The slides were then treated with 3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity, followed by incubation with 1% fish skin gelatin to block the nonspecific staining. Tissue sections were incubated overnight with antibodies against DARC (Abcam, 1:250), D6 (Abcam, 1:250), CCX-CKR (Abcam, 1:250), LCA (Abcam, 1:500), CCL2 (Abcam, 1:100), and CCL19 (Abcam, 1:100). After washing, the sections were incubated with prediluted secondary antibody (Abcam), followed by further incubation with 3,3-diaminobenzidine tetrahydrochloride (DAB). Finally, the slides were counterstained with hematoxylin and mounted in an aqueous mounting medium.

All stained slides were separately evaluated by two pathologists. For DARC, D6, CCX-CKR, CCL2, and CCL19, the IHC score was defined by multiplying the percentage of cytoplasmic positive cells by the intensity. The intensity of stained cells was graded semi-quantitatively into four levels: 0 (no staining); 1 (weak staining = light yellow); 2 (moderate staining = yellow brown) and 3 (strong staining = brown); and the percentage was scored as: 0, negative; 1, 10% or less; 2, 11% to 50%; 3, 51% to 80%; or 4, 80% or more positive cells. The scoring system for DARC, D6, and CCX-CKR was defined as negative for score 0 and as positive for scores of 1–12, whereas that for CCL2 and CCL19 was defined as negative for scores of 0–3, and as positive for scores of 4–12. Two or three coexpression of ACRs was regarded as the coexpression of ACRs. For LCA, scoring was undertaken using a Chalkley point array [18]. In brief, three hot spots with the highest density of positive cells were selected per tumor. A 25 cross hair grid was used to score each hot spot at a magnification of ×200. Positive immune cells that touched or overlapped with tumor epithelial compartments were counted as stained cells. A region was considered positive if there were more than five stained cells per unit area, and was considered negative if there were 0–5 stained cells per unit area. The cutoff values were chosen on the basis of a measure of heterogeneity with the log-rank test statistical analysis with respect to overall survival and recurrence-free survival.

Follow-up and statistical analysis

All statistical analyses were carried out using SPSS (version 16.0, Chicago, USA) statistical software. Follow-up was available for all patients with a median time of 60.4 months (range 0.5–131.6 months). The overall survival and recurrence-free survival were calculated as the time from the date of the primary surgery to the date of death or first recurrence. Survival of patients was estimated by Kaplan–Meier analysis and the differences were compared by the log rank test. Cox proportional hazards multivariate regression model was used to select independently significant prognostic factors for CSCC. The correlation between ACR expression and clinicopathologic features was assessed using the χ^2 test or Fisher's exact test, while the correlation between ACRs with CCL2, CCL19, and LCA staining was evaluated using the Spearman's rank. $P < 0.05$ in all cases was considered statistically significant.

Results

Expression of ACRs in CSCC, CIS, and normal cervical tissues

To investigate the potential roles of ACRs in the development and progression of cervical cancer, we determined the expression of DARC, D6, and CCX-CKR in 227 CSCC, 50 CIS, and 40 normal cervical tissues. The three ACR proteins were mainly located in the cytoplasm of tumor cells, but rarely in the nucleus. The representative immunostaining of DARC, D6, and CCX-CKR in normal cervical tissues (Fig. 1, A–C), CIS (Fig. 1, D–F), and CSCC (Fig. 1, G–L) was shown in Figure 1. Normal cervical tissue showed positive DARC in 39 (97.5%), D6 in 32 (80.0%), and CCX-CKR in 40 (100%) cases, CIS presents 42 (84.0%), 44 (88.0%), and 43 (86.0%), and CSCC positively stained 168 (74.0%), 162 (71.4%), and 179 (78.9%), respectively (Table 1).

Correlation of ACR expression with CCL2, CCL19, and LCA

Since chemokines play a crucial role in modulating immune response, we assessed the correlation of ACR expression with the expression of their chemokine ligands (CCL2 for DARC and D6, CCL19 for CCX-CKR). We also analyzed the correlation of ACR expression with lymphocyte infiltration by evaluating the expression of LCA, a marker of leukocytes. The

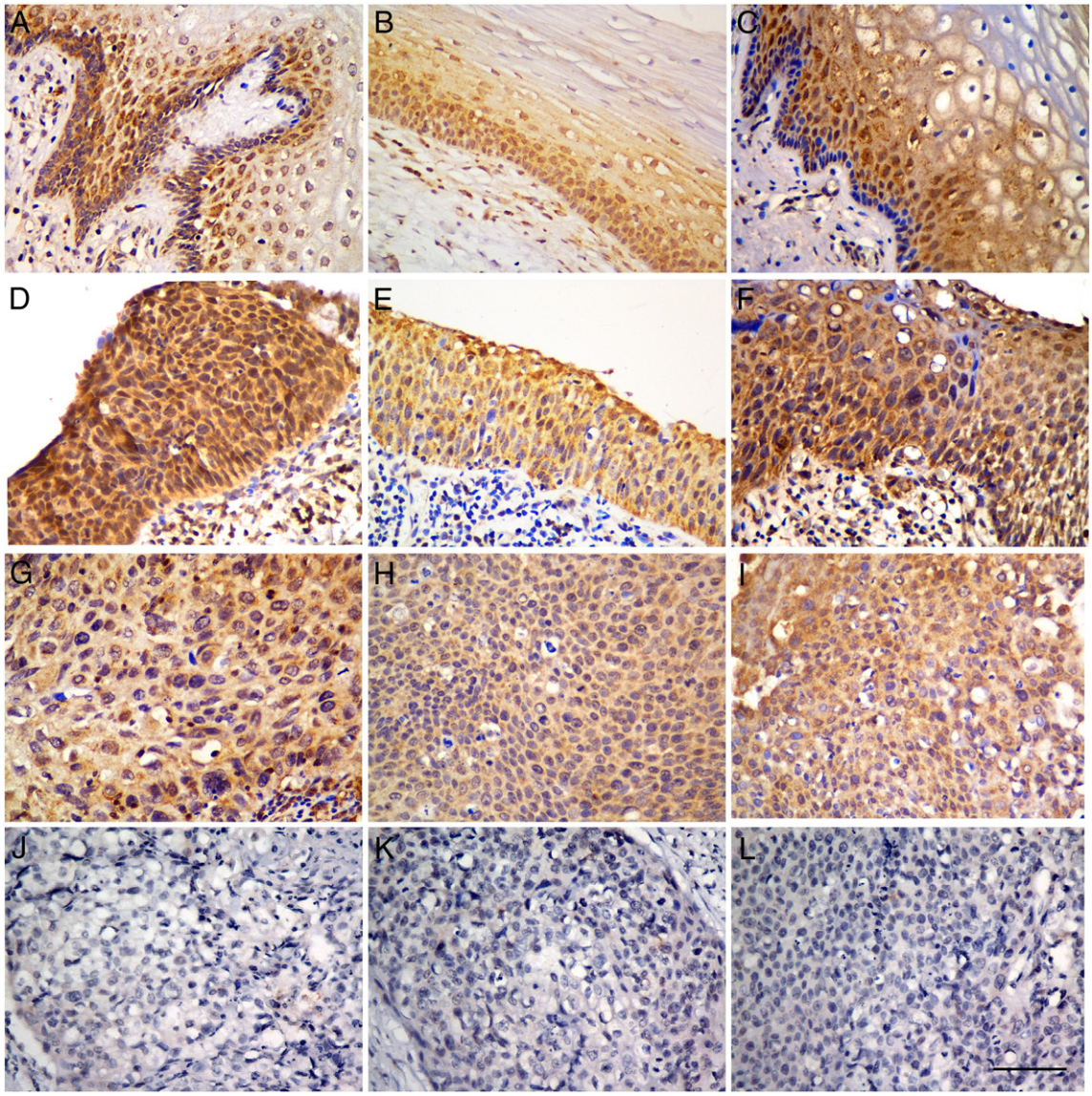

Fig. 1. Representative immunostaining of DARC, D6, and CCX-CKR in normal cervical tissues (A–C), carcinoma in situ of cervix (D–F), and cervical squamous cell cancer (G–I, positive; J–L, negative). Scale bar, 100 μm.

representative IHC data was shown in Figure S1. The expression of CCL2 was inversely associated with DARC (Spearman's rho (ρ) = −0.146, p = 0.028) expression, D6 (ρ = −0.159, p = 0.017) expression, and ACR coexpression (ρ = −0.216, p = 0.001). The expression of CCL19 was inversely associated with CCX-CKR (ρ = −0.167, p = 0.012) expression and ACR coexpression (ρ = −0.141, p = 0.033). The expression of LCA was positively correlated with DARC expression (ρ = 0.203, p = 0.002), D6 expression (ρ = 0.146, p = 0.028), and ACR coexpression (ρ = 0.136, p = 0.040) (Table S1).

Expression of ACRs is associated with CSCC clinical features

To further investigate the roles ACRs in the tumorigenesis and development of CSCC, we examined the correlation between ACRs and the clinical characteristics in 227 CSCC cases. Our data showed that DARC expression was significantly correlated with lymph node involvement (P < 0.001), that D6 expression was strongly correlated with tumor size (p = 0.018) and tumor recurrence (p = 0.028), and that CCX-CKR expression was associated with tumor stage

Table 1
Distribution of DARC, D6, and CCXCKR in CSCC, carcinoma in situ of cervix, and normal cervix.

Characteristic	DARC			D6			CCX-CKR			Coexpression		
	+ n(%)	− n(%)	*P*	+ n(%)	− n(%)	*P*	+ n(%)	− n(%)	*P*	+ n(%)	− n(%)	*P*
Invasive cancer	168(74.0)	59(26.0)	0.002	162(71.4)	57(28.6)	0.033	179(78.9)	48(21.1)	0.005	97(49.5)	99(50.5)	0.601
Carcinoma in situ	42(84.0)	8(16.0)		44(88.0)	6(12.0)		43(86.0)	7(14.0)		27(54.0)	23(46.0)	
Normal cervix	39(97.5)	1(2.5)		32(80.0)	8(20.0)		40(100)	0(0)		23(57.5)	17(42.5)	

($p = 0.033$), tumor size ($p = 0.026$), and lymph node status ($p = 0.003$) (Table 2). In addition, the coexpression of the three ACRs was significantly correlated with tumor stage ($p = 0.032$), tumor size ($p = 0.003$), tumor recurrence ($p < 0.001$), and lymph node status ($p = 0.006$) (Table 2). There was no significant relationship between ACR expression and patient age or tumor differentiation.

Patient survival analysis indicated that the D6 and CCX-CKR expression in CSCC significantly linked to patients' survival time, signifying that D6 positive expression was correlated with favorable overall survival and recurrence-free survival (Fig. 2, A–B), and that CCX-CKR positive expression was correlated with longer overall survival time (Fig. 2, C–D). However, there was no significant difference between DARC positive and negative groups with respect to clinical survival time (Fig. 2, E–F). Moreover, patients with tumors exhibiting the coexpression of ACRs had significantly longer overall and recurrence-free survival compared with tumors without ACR coexpression (Fig. 2, G–H). These results suggest that ACRs may be associated with disease development in CSCC.

Univariate and multivariate Cox regression analysis for prognosis of patients with CSCC

In the univariate Cox proportional hazard regression model analysis shown in Table 3, tumor size ($p = 0.043$ and $p = 0.045$, respectively), lymph node metastasis ($p = 0.033$ and $p = 0.037$, respectively), LCA expression ($p = 0.023$ and $p = 0.046$, respectively), D6 expression ($p = 0.003$ and $p = 0.028$, respectively), and ACR coexpression ($p = 0.003$ and $p < 0.001$, respectively) were significantly correlated with both overall and recurrence-free survival. The CCX-CKR expression ($p < 0.001$) and FIGO stage ($p = 0.024$) were significantly associated with overall survival. In a multivariate Cox regression analysis shown in Table 4, CCX-CKR expression showed significant association with overall survival ($p = 0.004$). D6 expression was an independent predictor of both overall and recurrence-free survival ($p = 0.027$ and $p = 0.028$, respectively).

Discussion

In the current study, we observed that the expression rate of DARC and CCX-CKR decreases as CSCC progresses to more advanced stages. We also found that the CCL2 expression was inversely associated with DARC expression, D6 expression, and ACR coexpression, that the CCL19 expression was inversely correlated with CCX-CKR expression and ACR coexpression, and that the LCA was positively correlated with DARC expression, D6 expression, and ACR coexpression. Moreover, we demonstrated that D6 expression and ACR coexpression could predict favorable overall and recurrence-free survival, while CCX-CKR expression was positively associated with overall survival, but not with recurrence-free survival in CSCC patients. Furthermore, we proposed that DARC and CCX-CKR expression were negatively correlated with lymph node metastasis in patients with CSCC. Our study suggests that ACRs not only represent valuable biomarkers for the prediction of CSCC prognosis, but also play crucial roles in cervical cancer progression.

It is becoming clear that ACRs might act as decoy receptors to deplete extracellular chemokines, and hence inhibit angiogenesis in the primary tumor and reduce metastasis [19,20]. DARC is a transcytosis receptor that binds with ELR + (glutamine–leucine–arginine motif in N-terminus) CXC and some CC chemokines and causes ligand internalization without lysosomal degradation [21]. Accumulating evidence suggests that the enhanced expression of DARC attenuates cancer growth and metastasis [22], while the lack of DARC expression might lead to enhanced tumor growth [23]. D6 acts as a scavenger for inflammatory CC chemokines. It binds and clears the chemokines CCL2 and CCL5, which are important components of cancer-related inflammation [21]. Similarly to D6, CCX-CKR binds with high affinity to chemokines CCL19, CCL21, CCL25, and CXCL13. These chemokines participate in CCR7/CCL19 (CCL21), CCR9/CCL25, and CXCR5/CXCL13 axes which are involved in cancer cell migration [24]. All these studies have indicated that ACRs may contribute to the initiation and progression of cancer.

To investigate whether ACRs are correlated to the progression of CSCC, we characterize the expression of DARC, D6, and CCX-CKR in normal cervical tissues, CIS, and CSCC specimens. Our findings showed that the expression rate of ACRs in CSCC, CIS, and normal cervix increased in gradual ascending order. An exception to this is the finding of expression peak of D6 in CIS. This finding may be related to the function of inflammatory molecules such as TGF-β, which has been reported to be increased in CIN [25], and which could upregulate the expression level of D6 [26]. Statistical analysis revealed that DARC expression significantly correlates with lymph node status, that D6 expression correlates with tumor size and recurrence, and that CCX-CKR expression correlates with FIGO stage, tumor size, and lymph node status. Our observations are in agreement with previous studies demonstrating that the expression of ACRs was associated with tumor stage [27]. The correlations of ACR expression with lower tumor stage suggested that ACRs may play an anticancer role in the tumorigenesis and development of CSCC.

Considering the roles of chemokines in driving lymphocyte infiltration, we examined the correlation of ACR expression with the expression of their chemokine ligands and LCA. We found that the

Table 2
Distribution of ACR expression in CSCC patients according to clinicopathologic characteristics.

Characteristic	No.	DARC (+) N (%)	*P*	D6 (+) N (%)	*P*	CCX-CKR (+) N (%)	*P*	Coexpression (+) N (%)	*P*
Age (y)			0.762		0.659		0.255		0.112
≤40	105	79 (75.2)		73 (69.5)		79 (75.2)		48 (45.7)	
>40	122	89 (73.0)		89 (73.0)		100 (82.0)		69 (56.5)	
FIGO stage			0.246		0.121		**0.033**		**0.032**
IB1	130	100 (76.9)		98 (75.4)		109 (83.8)		75 (57.6)	
>IB1	97	68 (70.1)		64 (66.0)		70 (72.1)		42 (43.3)	
Differentiation			0.566		0.066		0.936		0.693
Grade 1/2	84	64 (76.2)		66 (78.6)		66 (78.6)		45 (53.6)	
Grade 3	143	104 (72.7)		96 (67.1)		113 (79.0)		72 (50.3)	
Timor size			0.131		0.018		0.026		**0.003**
≤4 cm	174	133 (76.4)		131 (73.6)		143 (82.2)		100 (56.9)	
>4 cm	53	35 (66.0)		31 (64.2)		36 (67.9)		17 (34.0)	
LN metastasis			<**0.001**		0.288		**0.003**		**0.006**
−	194	154 (79.4)		141 (72.7)		159 (82.0)		108 (55.6)	
+	33	14 (41.2)		21 (63.6)		20 (60.6)		9 (27.3)	
Recurrence			0.392		**0.028**		0.103		<**0.001**
−	196	147 (75.0)		145 (74.0)		158 (80.6)		111 (56.6)	
+	31	21 (67.7)		17 (54.8)		21 (67.7)		6 (19.4)	

Note: ACRs including DARC, D6, and CCX-CKR.

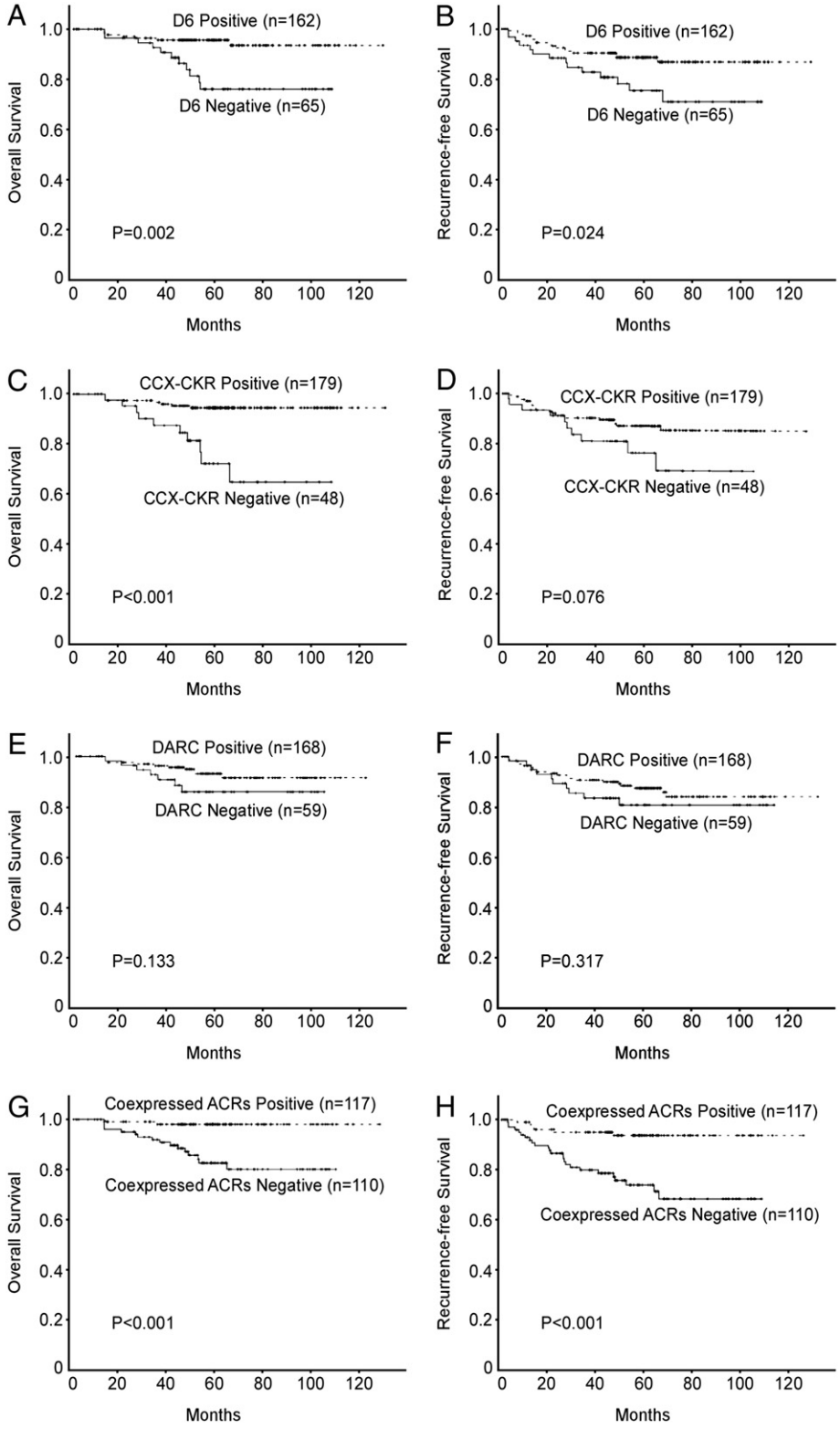

Fig. 2. Kaplan–Meier analysis of overall survival and recurrence-free survival in relation to D6 (A–B), CCX-CKR (C–D), and DARC (E–F) expression, and ACR coexpression (G–H) in 227 cervical squamous cell cancer (CSCC) patients.

expression of CCL2 and CCL19 was inversely correlated with ACR expression, which agrees with previous studies that DARC and D6 mediate effective scavenging of CCL19, and that CCX-CKR scavenges CCL2 [19,24,28]. Interestingly, the expression of LCA was positively correlated with DARC expression, D6 expression, and ACR coexpression, which does not go along with their described anti-chemotaxis property. It is possible that ACR expression may represent a mechanism to activate immune response. This hypothesis is supported by McKimmie and colleagues [26], who proposed that D6 may be critically involved in the initiation of immune responses. It is also possible that, beyond their roles in scavenging chemokines, ACRs may activate intracellular signaling pathways involved in lymphocyte chemotaxis.

Table 3
Univariate Cox regression analysis of overall survival (OS) and recurrence-free survival (RFS) in patients with CSCC.

Prognostic variables	OS		RFS	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age (>40 vs ≤40)	2.415 (0.906–6.438)	0.078	1.989 (0.965–4.099)	0.062
FIGO stage (>IB1 vs IB1)	3.280 (1.169–9205)	**0.024**	2.054 (0.997–4.234)	0.050
Differentiation (Grade 3 vs 1/2)	1.590 (0.631–4.007)	0.325	1.148 (0.550–2.396)	0.714
Timor size (>4 cm vs ≤4 cm)	2.618 (1.033–6.639)	**0.043**	2.099 (1.018–4.327)	**0.045**
LN metastasis (+ vs −)	2.910 (1.091–7.761)	**0.033**	2.280 (1.049–4.957)	**0.037**
DARC expression (+ vs −)	0.490 (0.190–1.266)	0.141	0.682 (0.321–1.449)	0.320
D6 expression (+ vs −)	0.230 (0.094–0.628)	**0.003**	0.451 (0.222–0.916)	**0.028**
CCX-CKR expression (+ vs −)	0.189 (0.074–0.479)	<**0.001**	0.512 (0.241–1.088)	0.082
ACR coexpression (+ vs −)	0.110 (0.025–0.477)	**0.003**	0.205 (0.084–0.500)	<**0.001**
CCL2 expression (+ vs −)	1.782 (0.707–4.490)	0.220	1.109 (0.538–2.285)	0.779
CCL19 expression (+ vs −)	1.276 (0.455–3.580)	0.643	1.387 (0.639–3.014)	0.408
LCA expression (+ vs −)	0.182 (0.042–0.793)	0.023	0.424 (0.182–0.984)	**0.046**

In favor of this possibility is the finding that CCX-CKR could recruit putative signaling scaffold β-arrestin in response to chemokines [29].

We further investigate the relationship between ACR expression and prognosis in CSCC patients. We found that patients without D6 expression had shorter overall and recurrence-free survival time. In addition, we observed that CCX-CKR expression is positively correlated with overall survival in CSCC. When analyzing the prognostic value of DARC expression in CSCC patients, the finding is no longer significant. This result might be due to different efficiency of the three ACRs in chemokine internalization and subsequent lysosomal degradation. An association between ACR expression and clinical outcome has been reported in several studies. Feng et al. demonstrated that lack or low CCX-CKR expression indicated a poor survival rate in breast cancer patients [30]. Sun et al. reported that high DARC expression correlated with a higher survival rate in laryngeal squamous cell carcinoma [31]. Wu et al. discovered that D6 expression was positively correlated to disease-free survival in breast cancer [18], which is consistent to our observation. Furthermore, we found that coexpression of the three ACRs is inversely associated with overall and recurrence-free survival. In accordance with our finding, Zeng et al. suggested that the coexpression of ACRs was associated with better overall survival and recurrence-free survival in breast cancer [27]. These results imply a combined role of these decoy receptors. The combined ACR expression could enhance the prognostic value of single ACR alone in patients with CSCC. A possible criticism of our findings could be that the FIGO stage is a prognostic factor of overall but not of recurrence-free survival. The finding is probably due to variation and small sample size. Moreover, the administration of postoperative adjuvant therapy may also affect patients' recurrence.

Table 4
Multivariate Cox regression analysis of overall survival (OS) and recurrence-free survival (RFS) in patients with CSCC.

Prognostic variables	OS		RFS	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age (>40 vs ≤40)	2.564 (0.922–7.129)	0.071	2.276 (1.075–4.821)	0.055
FIGO Stage (>IB1 vs IB1)	2.811 (0.929–8.501)	0.067	2.047 (0.955–4.383)	0.065
Differentiation (Grade 3 vs 1/2)	1.482 (0.512–4.292)	0.468	1250 (0.562–2.780)	0.584
Timor Size (>4 cm vs ≤4 cm)	1.213 (0.431–3.410)	0.714	1.027 (0.48–2.354)	0.950
LN Metastasis (+ vs −)	1.238 (0.379–4.051)	0.724	1.055 (0.398–2.794)	0.914
DARC expression (+ vs −)	0.703 (0.217–2.280)	0.557	0.977 (0.406–2.354)	0.959
D6 expression (+ vs −)	0.333 (0.125–0.885)	**0.027**	0.451 (0.222–0.916)	**0.028**
CCX-CKR expression (+ vs −)	0.247 (0.094–0.647)	**0.004**	0.750 (0.330–1.709)	0.494
CCL2 expression (+ vs −)	1.073 (0.374–3.082)	0.895	1.081 (0.495–2.363)	0.844
CCL19 expression (+ vs −)	1.346 (0.442–4.095)	0.601	1.248 (0.547–2.845)	0.599
LCA expression (+ vs −)	0.366 (0.096–1.393)	0.140	0.490 (0.209–1.147)	0.100

It is noteworthy that DARC and CCX-CKR have been found, in our study, to be inversely correlated with lymph node status, which concurs with previous studies in that ACR expression was associated with tumor progression and metastasis. Similarly to our study, Bandyopadhyay et al. demonstrated that interaction between DARC and Kangai 1 (KAI1) significantly suppressed tumor cell proliferation and metastasis by modulating the expression of senescence controlling genes, T-box transcription factor 2 (TBX2) and cyclin-dependent kinase inhibitor 1 (p21) [32]. Addison et al. described that tumors derived from DARC-expressing cells had significantly decreased tumor associated vasculature and metastatic potential as compared to tumors derived from DARC-negative cells [33]. Cheng et al. suggested that CCX-CKR could inhibit the growth and metastasis of breast cancer by attracting and sequestrating chemokines [34]. Zeng et al. reported that the expression of the ACRs was inversely associated with lymph node metastasis [27]. Taken together, these observations suggest that DARC and CCX-CKR may serve as a potentially metastatic indicator and an important growth regulator for CSCC.

To our knowledge, the present study is the first to analyze the prognostic values of ACRs in CSCC. In conclusion, we have found that the three ACRs could be used as prognostic markers in CSCC patients. More importantly, we have found that CSCC patients without DARC or CCX-CKR expression are more prone to have lymph node metastasis, and that without D6 expression are more likely to have recurrent disease. Further studies are needed to elucidate the roles of ACRs during the development of CSCC, and it may lead to new therapeutic target for cervical cancer.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2013.04.015.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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