Int J Clin Exp Pathol 2014;7(3):1060-1068
www.ijcep.com /ISSN:1936-2625/IJCEP1401016

Original Article

High expression of CRAM correlates with poor prognosis in patients with cervical carcinoma

Teng Hou[1*], Dongxia Liang[2*], Dong Yang[3], Jiehua He[1], Yongwen Huang[1], Yanna Zhang[1]

[1]*State Key Laboratory of Oncology in South China, Sun Yat-Sen University Cancer Center, Collaborative Innovation Center for Cancer Medicine, Guangzhou, GD 510060, China;* [2]*Department of Gynecology, Henan Cancer Hospital, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, HN 450008, China;* [3]*Department of Urology, Henan Cancer Hospital, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, HN 450008, China.* [*]*Equal contributors.*

Received January 7, 2014; Accepted February 5, 2014; Epub February 15, 2014; Published March 1, 2014

Abstract: Aims: Atypical chemokine receptors (ACRs) have been reported to scavenge or alter the localization of their chemokine ligands. However, CRAM, a newly identified ACR member, is lack of ligand scavenging properties. The present study was to investigate the clinical significance of CRAM in cervical carcinoma. Methods: The expression of CRAM in primary cervical cancer and paired normal tissues from adjacent regions was examined using Real time PCR. Moreover, CRAM protein expression was analyzed in 272 cervical specimens including 50 normal cervical tissues, 40 cases of carcinoma in situ of cervix (CIS), and 182 cases of cervical cancer by immunohistochemistry. Results: Real time PCR showed that the expression level of CRAM was markedly higher in cervical cancer than that in normal cervical tissues. The expression rate of CRAM in normal cervical tissues, CIS, and cervical cancer increased gradually ($p < 0.01$). In addition, the expression level of CCL19 was positively associated with that of CRAM ($p < 0.05$). Moreover, high expression level of CRAM was correlated with lymph node metastasis and histological subtype. In multivariate Cox regression analysis, high expression level of CRAM was a negative indicator for both overall ($p = 0.028$) and recurrence-free survival ($p = 0.010$). Conclusion: The present study suggested that CRAM could be a clinical prognostic marker for patients with cervical cancer and might be a potential therapeutic target for cervical cancer. Our data extended previous research on the predictive value of ACRs.

Keywords: Atypical chemokine receptor, CRAM, cervical cancer, prognosis

Introduction

Chemokine receptors are cell-surface (G-protein coupled) receptors (GPCR) containing seven transmembrane domains that are found on the surface of certain cells. They work by triggering an intracellular signalling cascade mediated by G-protein activation [1]. Chemokine receptors together with their respective ligands control nearly all classes of leukocytes trafficking in the immune system [2]. In addition, they are involved in different stages of tumor progression, inducing leukocyte infiltration into tumors, angiogenesis, tumor cell proliferation and migration. Recently, much attention has been focused on the so-called atypical chemokine receptors (ACRs). ACRs belong to the GPCR family characterized by a seven transmembrane domain structure. However, ACRs are unable to induce the full spectrum of classical GPCR signaling because they are lack of highly conserved DRYLAIV motif within the second intracellular loop [3]. Initially, the ACR family comprises three receptors, Duffy antigen receptor for chemokines (DARC), D6, and Chemocentryx chemokine receptor (CCX-CKR), which can bind a wide range of chemokine ligands [4]. In general, these ACRs regulate the complex chemotactic network by scavenging or altering the localization of their chemokine ligands [5].

One exception is CRAM, which is the most recently identified member of the ACR family [6]. Encoded by the CCRL2 gene, it exhibits high homology to CC chemokine receptors. The CCRL2 gene is located on chromosome 3p21 in close proximity to the chemokine receptor gene

Table 1. Distribution of CRAM expression in cervical cancer patients according to clinicopathologic characteristics

Characteristic	No.	CRAM		*P*
		+	-	
Age (y)				0.298
≤ 40	85	42	43	
> 40	97	40	57	
FIGO Stage				0.925
IB	136	61	75	
> IB	46	21	25	
Histological subtype				0.030
Squamous cell carcinoma	162	68	94	
Adenocarcinoma	20	14	6	
Differentiation				0.764
Grade 1/2	81	35	46	
Grade 3	101	47	54	
Timor Size				0.870
≤ 4 cm	129	59	70	
> 4 cm	53	23	30	
LN Metastasis				0.043
-	152	63	89	
+	30	19	11	
Recurrence				0.017
-	157	65	92	
+	25	17	8	
Total NO. of patients	182	82	100	

cluster region where CCR1 to CCR5, CCR8 to CCR10, XCR1 and CX3CR1 located [7]. CRAM was initially identified as a receptor for the homeostatic chemokine CCL19 in radioactive labelling studies. This binding is not followed by degradation of CCL19, which is quite different from classical chemokine scavenging by other ACR members [8]. Besides, CRAM binds unrelated chemotactic protein adipokine chemerin, while chemerin binding does not trigger ligand internalization [9]. The CRAM expression has been found on both lymphoid and non-lymphoid organs, including spleen, lymph node, fetal liver, bone marrow, heart, and lung [10]. More recently, CRAM expression has been detected in astrocytes and microglia, and the expression was up-regulated by LPS stimulation [11]. In vitro study has also shown that CRAM expression was elevated in glioma cell lines [12]. However, the clinical and functional significance of CRAM expression in human cancers remains unknown.

Our previous study has shown that ACR members, DARC, D6, and CCX-CKR, could be used as prognostic markers for patients with cervical squamous cell cancer [13]. As a new member of ACR family, the internalizing ability of CRAM is quite different from other ACR members. CRAM could bind chemotactic proteins, while it is devoid of ligand scavenging property, suggesting that clinical and prognostic value of CRAM may be different from other ACRs. In the present study, we for the first time characterized the CRAM expression in human cervical carcinoma. We found that the expression level of CRAM was correlated with lymph node status and histological subtype. Moreover, high expression level of CRAM was strongly associated with reduced overall and recurrence-free survival time. Both univariate and multivariate analysis suggested that CRAM expression was an independent prognostic marker for cervical cancer patients. Our study extended previous research on the prognostic value ACRs by showing that CRAM could be a novel clinical marker of poor prognosis for patients with cervical cancer.

Materials and methods

Patients and tissue specimens

A total of 272 paraffin-embedded specimens including 50 normal cervical tissues, 40 CIS, and 182 cervical cancers were obtained from petients, who were histopathologically and clinically diagnosed at Sun Yat-Sen University Cancer Center (SYSUCC) from 2002 to 2007. For the use of these clinical materials for research purposes, prior patient's consents and approval from the Institute Research Ethics Committee were obtained. Clinicopathologic staging was determined according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines for clinical staging [14]: 105 were allocated to stage IB1, 31 to stage IB2, 28 to stage IIA1, 10 to stage IIA2, and 8 to stage IIB. Clinical information of the samples is summarized in **Table 1**. In addition, 10 pairs of freshly prepared cervical tumor and matched normal tissues from adjacent regions were collected at SYSUCC in 2011.

RNA extraction, reverse transcription (RT) and real-time PCR

Total RNA from fresh surgical cervical cancer tissues was extracted using the Trizol Reagent (Invitrogen), according to the manufacturer's

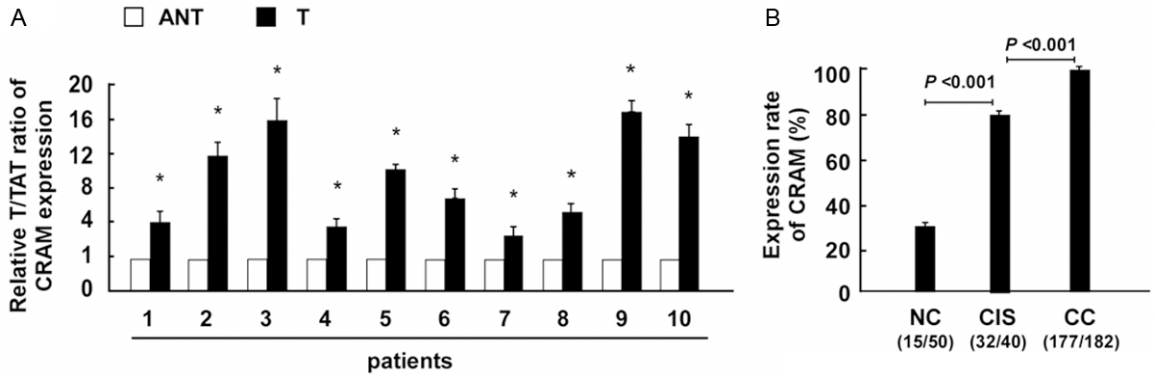

Figure 1. Elevated CRAM expression in cervical cancer. A: Real-time PCR analysis of CRAM expression in 10 paired cervical tumor tissues (T) and their adjacent normal tissues (ANT). GADPH was used as loading control. B: Distribution of CRAM in normal cervix (NC), carcinoma in situ of cervix (CIS), and cervical cancer (CC) by immunohistochemistry.

instructions. The RNA was pretreated with RNase-free DNase (Promega), and 2 ug RNA was used for cDNA synthesis primed with random hexamers. Real-time PCR was performed using the Applied Biosystems 7500 Sequence Detection system. Sequences of the primers are: CRAM forward primer 5'-ACAAGTATGACGCCCAGGCACT-3', reverse primer 5'-CCAGGATAAGCACAACCAGGAGA-3', GAPDH forward primer 5'-GAATCTACTGGCGTCTTCACC-3', reverse primer 5'-GTCATGAGCCCTTCCACGATGC-3'. Expression data were normalized to the housekeeping gene GAPDH as a loading control.

Immunohistochemistry

For immunohistochemical analysis, formalin-fixed, paraffin-embedded specimens were cut into 4 μm sections and mounted onto poly-L-lysine-coated slides. The sections were deparaffinized, rehydrated, and then boiled for 10 min in 10 μmol/L citrate buffer solution (pH 6.0) using a microwave oven. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 30 min, and non-specific staining was blocked by treating the slides with 1% fish skin gelatin for 30 min at room temperature. Subsequently, slides were incubated overnight with primary antibodies against CRAM (Abcam, 1:100), CCL19 (Abcam, 1:100), and LCA (Abcam, 1:500). After washing with PBS, the slides were incubated with prediluted secondary antibody (Abcam), followed by further incubation with diaminobenzidine (DAB). Finally, the sections were counterstained with hematoxylin and mounted.

The degree of immunostaining of the sections was separately evaluated by two pathologists. For CRAM, 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 CRAM and CCL19 was defined as negative for score 0, and positive for score > 0 (low expression for score 1-4 and as high expression for scores of 6-12). For LCA, scoring was undertaken using a Chalkley point array [18]. 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.

Statistical analysis

All statistical analyses were carried out using SPSS 16.0 statistical software package.

Survival of patients was estimated by Kaplan-Meier analysis and the differences were compared by the log rank test. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the predictive value of the

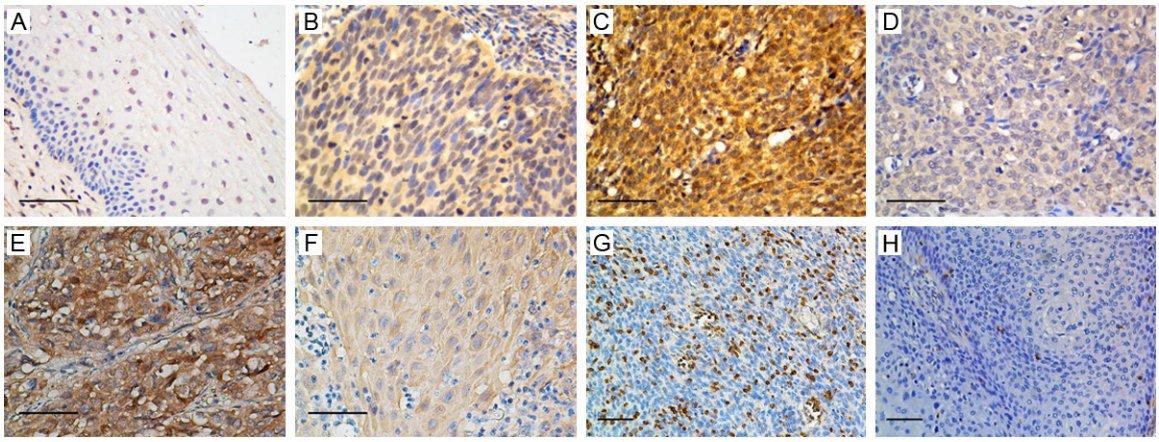

Figure 2. Immunostaining of cervical cancers. Representative immunostaining of CRAM staining in normal cervical tissues (A) and carcinoma in situ of cervix (B). Representative examples of high (C) and low (D) CRAM staining, high (E) and low (F) CCL19 staining, and positive (G) and negative (H) LCA staining in cervical cancer tissues. Scale bar, 100 μm.

CRAM expression and clinical features. Cox proportional hazards multivariate regression model was used to select independently significant prognostic factors for cervical cancer. The χ^2 test or Fisher's exact test were used to analyze the correlation between CRAM expression and clinicopathologic features. Spearman's rank was used to evaluate the correlation between CRAM with CCL19 and LCA staining. In all cases, $P < 0.05$ was considered statistically significant.

Results

Increased expression of CRAM in cervical cancer

To determine whether CRAM is associated with the progression of cervical cancer, we determined the expression of CRAM in 10 pairs of matched cervical cancer tissue and adjacent nontumorous tissue using RT-PCR analyses. As shown in **Figure 1A**, CRAM expression was upregulated in all 10 cervical cancer samples compared with their paired adjacent nontumorous tissue. In addition, we examined the expression of CRAM in 182 cervical cancer, 40 CIS, and 50 normal cervical tissues. Normal cervical tissue showed positive CRAM in 15 (30.0%), CIS presents 32 (80.0%), and cervical cancer positively stained 177 (97.3%) cases (**Figure 1B**). The representative immunostaining of CRAM in normal cervix, CIS, and cervical cancer was shown in **Figure 2A-D**.

Correlation of CRAM expression with CCL19, and LCA

As CCL19 has been reported to play a crucial role in regulating lymphocyte infiltration, we analyzed the correlation of CCL19 expression with the expression of CRAM, which is an identified chemokine receptor of CCL19. Moreover, we examined the correlation of CRAM expression with lymphocyte infiltration by evaluating the expression of LCA, a marker of leukocytes. The expression level of CCL19 was positively associated with that of CRAM (Spearman's rho (ρ) = 0.167, p = 0.024). The expression of LCA was negatively correlated with CRAM expression level, while this finding did not reach statistical significance (ρ = -0.120, p = 0.108) (**Table 4**). The representative immunostaining of CCL19 and LCA was shown in **Figure 2E-H**.

Association between CRAM expression and the clinical features of cervical cancer

To further investigate the roles CRAM in cervical tumorigenesis, we analyzed the IHC data of CRAM to determine their relationship with the clinical features of cervical cancer. Our data showed that CRAM expression level was strongly correlated with histological subtype (P = 0.030), lymph node metastasis (P = 0.043), and tumor recurrence (P = 0.017) of patients with cervical cancer, while it was not correlated with age, FIGO stage, differentiation, or tumor size. Moreover, Kaplan-Meier analysis was also

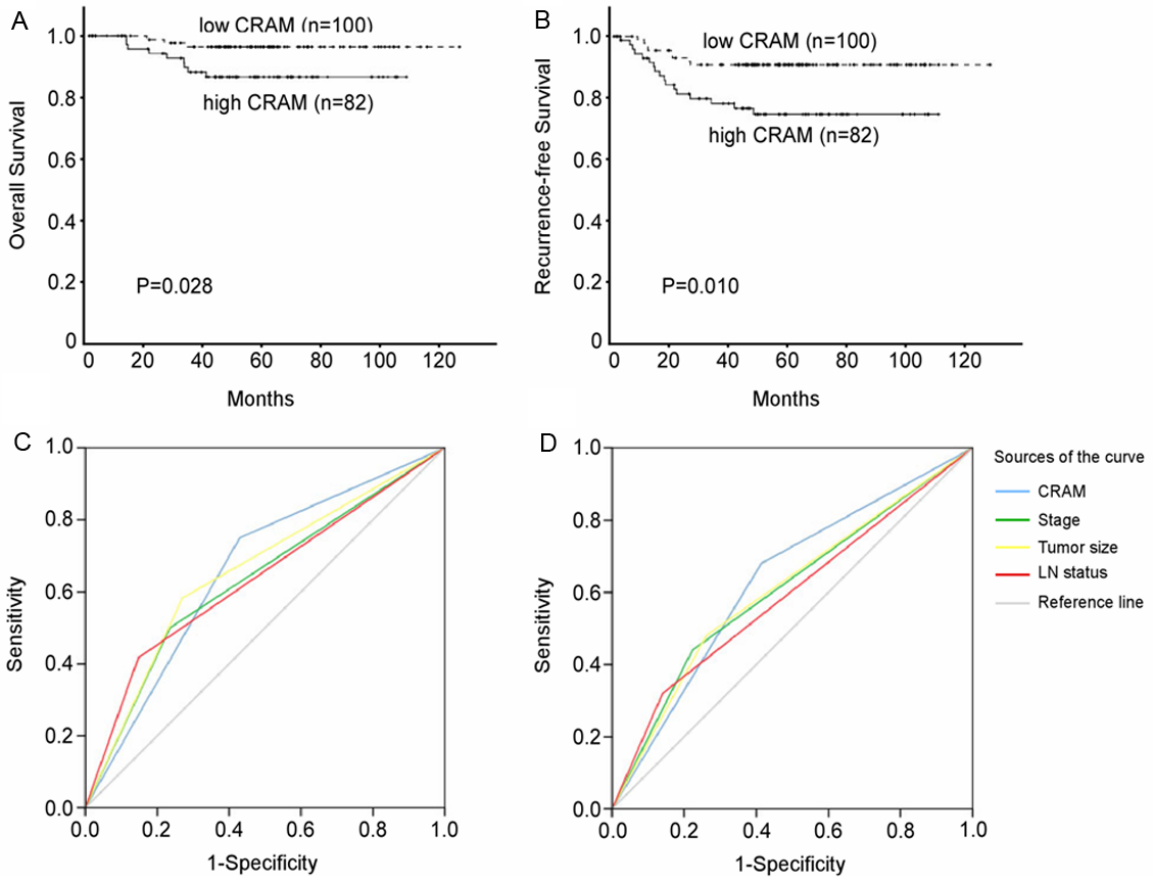

Figure 3. Kaplan-Meier and ROC curve analyses for CRAM expression. A, B: Kaplan-Meier analysis of overall survival and recurrence-free survival in relation to CRAM expression level in 182 cervical cancer patients. C, D: ROC curve analysis for CRAM expression and different clinicopathological features were performed to evaluate the survival status. C: CRAM expression (AUC = 0.660; P = 0.064), stage (AUC = 0.632; P = 0.126), tumor size (AUC = 0.656; P = 0.071), and lymph node status (AUC = 0.635; P = 0.119) implied statistical associations with overall survival. D: CRAM expression (AUC = 0.633; P = 0.033), stage (AUC = 0.609; P = 0.082), tumor size (AUC = 0. 609; P = 0.079), and lymph node status (AUC = 0.590; P = 0.149) were used to test the recurrence-free survival.

Table 2. Univariate Cox regression analysis of overall survival (OS) and Recurrence-free survival (RFS) in patients with cervical cancer

Prognostic variables	OS		RFS	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age (> 40 vs ≤ 40)	1.153 (0.372-3.576)	0.805	1.504 (0.683-3.314)	0.311
FIGO Stage (> IB vs IB)	3.120 (1.006-9.674)	0.049	2.593 (1.177-5.714)	0.018
Differentiation (Grade 3 vs 1/2)	2.539 (0.765-8.433)	0.128	1.017 (0.462-2.240)	0.967
Timor Size (> 4 cm vs ≤ 4 cm)	3.707 (1.176-11.686)	0.025	2.429 (1.108-5.327)	0.027
LN Metastasis (+ vs -)	4.104 (1.301-12.940)	0.016	2.709 (1.168-6.280)	0.020
CRAM expression (H vs L)	3.881 (1.051-14.337)	0.042	2.861 (1.235-6.632)	0.014
CCL19 expression (H vs L)	1.783 (0.566-5.617)	0.324	1.448 (0.640-3.227)	0.374
LCA expression (+ vs -)	0.287 (0.093-0.892)	0.031	0.432 (0.194-0.961)	0.040

applied to calculate the effect of CRAM expression and clinical outcome of cervical cancer. The log-rank test showed that high expression level of CRAM was correlated with poor overall survival and recurrence-free survival (**Figure 3A** and **3B**). Furthermore, we analyzed the sen-

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

Prognostic variables	OS		RFS	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age (> 40 vs ≤ 40)	1.702 (0.433-6.693)	0.446	1.591 (0.635-3.985)	0.322
FIGO Stage (> IB vs IB)	3.105 (0.997-9.673)	0.051	2.470 (1.108-5.509)	0.027
Differentiation (Grade 3 vs 1/2)	1.518 (0.383-6.009)	0.552	1.412 (0.576-3.465)	0.451
Timor Size (> 4 cm vs ≤ 4 cm)	2.272 (0.645-7.999)	0.201	2.215 (0.999-4.909)	0.050
LN Metastasis (+ vs -)	3.475 (1.093-11.046)	0.035	1.638 (0.673-3.984)	0.277
CRAM expression (H vs L)	3.540 (0.939-13.346)	0.062	3.102 (1.334-7.212)	0.009
CCL19 expression (H vs L)	1.852 (0.513-6.687)	0.347	1.483 (0.604-3.640)	0.195
LCA expression (+ vs -)	0.296 (0.095-0.926)	0.036	0.435 (0.183-1.031)	0.110

sitivity and specificity of CRAM based on the IHC data. ROC curve analysis confirmed the predictive value of CRAM expression regarding clinical prognosis (**Figure 3C** and **3D**).

Univariate and multivariate Cox regression analyses for prognosis of patients with cervical cancer

To determine whether CRAM expression level is an independent prognostic factor of patient outcomes, univariate and multivariate Cox regression analyses were used. As **Table 2** shows, FIGO stage (p = 0.049 and p = 0.018, respectively), tumor size (p = 0.025 and p = 0.027, respectively), lymph node metastasis (p = 0.016 and p = 0.020, respectively), CRAM expression level (p = 0.042 and p = 0.014, respectively), and LCA expression (p = 0.031 and p b 0.040, respectively) were significantly correlated with both overall and recurrence-free survival. In multivariate Cox regression analysis shown in **Table 3**, lymph node metastasis (p = 0.035) and LCA expression (p = 0.036) were recognized as independent prognostic factors for overall survival, while FIGO stage (p = 0.027) and CRAM expression (p = 0.009) were recognized as independent predictors for recurrence-free survival.

Discussion

The key finding of the current study is that elevated CRAM expression is associated with poor clinical prognosis and lymph node metastasis of patients with cervical cancer. In this study, we showed that CRAM was up-regulated in cervical cancer tissues as compared with that in normal cervical tissues. We further found that the CCL19 expression was inversely associated with CRAM expression. Moreover, we demonstrated that the expression rate of CRAM increases as cervical cancer progresses to more advanced stages. Furthermore, we found that the high expression level of CRAM protein is significantly correlated with the lymph node metastasis and reduced survival time of patients with cervical cancer. Taken together, our study suggests that CRAM may play a crucial role in cervical carcinogenesis and might represent a novel indicator for the prognosis of cervical cancer.

Cervical cancer is one of a leading lethal malignancy in female reproductive system, with an estimated 500,000 new cases and 270,000 deaths reported worldwide each year [15]. The mortality in developing countries is 10 times higher than in developed countries [16]. Although there have been major improvements in the management of cervical cancer, treatment of lymph node metastasis and locally advanced tumors remains a major challenge [17]. As a result, more than 70% of patients with lymph node metastasis will develop recurrent disease, which was observed only in about 10-20% of the patients without advanced cervical cancer [18]. Therefore, any insight into biological markers that help to predict lymph nodes metastasis and development of this malignancy may provide potential therapeutic targets for the treatment of cervical cancer.

CRAM is a heptahelical serpentine receptor that was originally identified in murine macrophage cell line RAW 264.7 [19]. The human CCRL2 gene produces two transcript variants known as CRAM-A and CRAM-B, the sequences of which differ by the presence of 12 additional amino acids at the N-terminus [7]. CRAM

Table 4. Spearman's correlation coefficients (ρ) when CRAM expression was analyzed for possible correlation with CCL19 and LCA

Variables		CCL19	LCA
CRAM	ρ	0.167*	-0.120
	p value	0.024	0.108

Note: *Correlation is significant at the 0.05 level (2-tailed).

expression has been detected on almost all hematopoietic cells, including circulating T cells, macrophages, neutrophils, monocytes, CD34+ BM precursors, and natural killer cells [20]. In non hematopoietic cells, CRAM expression has been described in barrier cells such as bronchial epithelium and endothelial cells [20]. Recently, efforts have been made to illustrate the biological functions of CRAM. Using the CRAM knockout mice model, Zabel et al. found that CRAM is required to promote IgE-dependent tissue inflammation and leukocyte infiltration [9]. Otero et al. also suggested a non-redundant role of CRAM in lung DC trafficking to regional lymph nodes and in the induction of Th2- driven allergic airway inflammation [21]. More recently, evidence has linked CRAM to cancer development and progression. Catusse et al. proposed that CRAM is involved in the control of CCR7/CCL19 mediated responses and cell migration in B-cell chronic lymphocytic leukemia [6]. Yin et al. revealed that elevated CRAM promotes glioblastoma cell migration and invasion in vitro [12]. However, thus far, there has been no report on the clinical implications and prognostic roles of CRAM. In this study, we showed that CRAM is up-regulated in cervical cancer as compared with normal cervical tissues. Additionally, the expression rate of CRAM in normal cervix, carcinoma in situ of cervix, and cervical cancer increased gradually in ascending order, indicating an oncogenic role of CRAM in cervical carcinoma.

In the past few years, it has been proposed that ACRs might function as tumour suppressors by negative regulation of chemokine availability [22]. Nibbs reported that D6 transgenic keratinocytes have increased considerable protection from tumor formation, while D6-deficient mice have enhanced susceptibility to cutaneous carcinoma formation in response to chemical carcinogens [23]. Shen et al. demonstrated that DARC serves to clear angiogenic chemokines from the prostate tumor microenvironment, and that lack of DARC contributes to increased tumor growth [24]. Feng et al. unraveled that overexpression of CCX-CKR significantly inhibit lung cancer growth both in vivo and in vitro [25]. Moreover, ACRs expression has been associated with clinical prognosis for cancer patients. Zeng et al. showed that DARC, D6, and CCX-CKR and their coexpression were significantly correlated with higher overall and recurrence-free survival [26]. Our recent study also indicated that DARC, D6, and CCX-CKR expression may be considered as positive prognostic indicators in patients with cervical squamous cell carcinoma [13]. Interestingly, the present study, in contrast to previous studies, showed that CRAM expression is positively correlated with the lymph node status and overall and recurrence-free survival in patients with cervical cancer. Besides, CRAM expression was positively correlated with the expression of its chemokine ligand CCL19. The finding is probably due to the lack of ability of CRAM to scavenge chemokine ligands. It's possible that the increased intra-tumor concentrations of chemokines may promote cancer growth and tumor cell dissemination. This hypothesis is supported by Zabel and colleagues [9], who demonstrated that CRAM could bind chemerin and increase the local concentration of the chemoattractant. When examining the correlation of CRAM expression with LCA expression, the finding is no longer significant, probably due to lower sample size. Furthermore, CRAM expression was higher in squamous cell carcinomas than that in adenocarcinoma, which is in agreement with previous studies showing that adenocarcinoma of the cervix carries worse prognosis than squamous cell carcinoma of the cervix [27, 28].

In conclusion, this is the first study evaluating the possibility of using CRAM as a ACR to predict poor clinical outcome in cervical cancer. Our present results extended the limited findings in our previous reports and pointed to increasing levels of heterogeneity among the atypical chemokine receptor family. Further studies are needed to clarify the precise mechanism that CRAM might promote cervical cancer development and progression.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yanna Zhang, State Key Laboratory of Oncology in South China, Sun Yat-Sen University Cancer Center, Collaborative Innovation Center for Cancer Medicine, Guangzhou, GD 510060, China. Tel: 8600-150-1192-9630; Fax: 8600-20-8734-3014; E-mail: zhangyannapds@gmail.com

References

[1] Mantovani A, Bonecchi R and Locati M. Tuning inflammation and immunity by chemokine sequestration: decoys and more. Nat Rev Immunol 2006; 6: 907-918.

[2] Mantovani A, Savino B, Locati M, Zammataro L, Allavena P and Bonecchi R. The chemokine system in cancer biology and therapy. Cytokine Growth Factor Rev 2010; 21: 27-39.

[3] Cancellieri C, Vacchini A, Locati M, Bonecchi R and Borroni EM. Atypical chemokine receptors: from silence to sound. Biochem Soc Trans 2013; 41: 231-236.

[4] Hansell CA, Simpson CV and Nibbs RJ. Chemokine sequestration by atypical chemokine receptors. Biochem Soc Trans 2006; 34: 1009-1013.

[5] Segerer S, Jedlicka J and Wuthrich RP. Atypical chemokine receptors in renal inflammation. Nephron Exp Nephrol 2010; 115: e89-95.

[6] Catusse J, Leick M, Groch M, Clark DJ, Buchner MV, Zirlik K and Burger M. Role of the atypical chemoattractant receptor CRAM in regulating CCL19 induced CCR7 responses in B-cell chronic lymphocytic leukemia. Mol Cancer 2010; 9: 297.

[7] Hartmann TN, Leick M, Ewers S, Diefenbacher A, Schraufstatter I, Honczarenko M and Burger M. Human B cells express the orphan chemokine receptor CRAM-A/B in a maturation-stage-dependent and CCL5-modulated manner. Immunology 2008; 125: 252-262.

[8] Leick M, Catusse J, Follo M, Nibbs RJ, Hartmann TN, Veelken H and Burger M. CCL19 is a specific ligand of the constitutively recycling atypical human chemokine receptor CRAM-B. Immunology 2010; 129: 536-546.

[9] Zabel BA, Nakae S, Zuniga L, Kim JY, Ohyama T, Alt C, Pan J, Suto H, Soler D, Allen SJ, Handel TM, Song CH, Galli SJ and Butcher EC. Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis. J Exp Med 2008; 205: 2207-2220.

[10] Fan P, Kyaw H, Su K, Zeng Z, Augustus M, Carter KC and Li Y. Cloning and characterization of a novel human chemokine receptor. Biochem Biophys Res Commun 1998; 243: 264-268.

[11] Zuurman MW, Heeroma J, Brouwer N, Boddeke HW and Biber K. LPS-induced expression of a novel chemokine receptor (L-CCR) in mouse glial cells in vitro and in vivo. Glia 2003; 41: 327-336.

[12] Yin F, Xu Z, Wang Z, Yao H, Shen Z, Yu F, Tang Y, Fu D, Lin S, Lu G, Kung HF, Poon WS, Huang Y and Lin MC. Elevated chemokine CC-motif receptor-like 2 (CCRL2) promotes cell migration and invasion in glioblastoma. Biochem Biophys Res Commun 2012; 429: 168-172.

[13] Hou T, Liang D, Xu L, Huang X, Huang Y and Zhang Y. Atypical chemokine receptors predict lymph node metastasis and prognosis in patients with cervical squamous cell cancer. Gynecol Oncol 2013; 130: 181-187.

[14] Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet 2009; 105: 103-104.

[15] Haie-Meder C, Morice P and Castiglione M. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21 Suppl 5: v37-40.

[16] Colombo N, Carinelli S, Colombo A, Marini C, Rollo D and Sessa C. Cervical cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7: vii27-32.

[17] Barbera L and Thomas G. Management of early and locally advanced cervical cancer. Semin Oncol 2009; 36: 155-169.

[18] Friedlander M and Grogan M. Guidelines for the treatment of recurrent and metastatic cervical cancer. Oncologist 2002; 7: 342-347.

[19] Shimada T, Matsumoto M, Tatsumi Y, Kanamaru A and Akira S. A novel lipopolysaccharide inducible C-C chemokine receptor related gene in murine macrophages. FEBS Lett 1998; 425: 490-494.

[20] Migeotte I, Franssen JD, Goriely S, Willems F and Parmentier M. Distribution and regulation of expression of the putative human chemokine receptor HCR in leukocyte populations. Eur J Immunol 2002; 32: 494-501.

[21] Otero K, Vecchi A, Hirsch E, Kearley J, Vermi W, Del Prete A, Gonzalvo-Feo S, Garlanda C, Azzolino O, Salogni L, Lloyd CM, Facchetti F, Mantovani A and Sozzani S. Nonredundant role of CCRL2 in lung dendritic cell trafficking. Blood 2010; 116: 2942-2949.

[22] Ulvmar MH, Hub E and Rot A. Atypical chemokine receptors. Exp Cell Res 2011; 317: 556-568.

[23] Nibbs RJ, Gilchrist DS, King V, Ferra A, Forrow S, Hunter KD and Graham GJ. The atypical chemokine receptor D6 suppresses the development of chemically induced skin tumors. J Clin Invest 2007; 117: 1884-1892.

[24] Shen H, Schuster R, Stringer KF, Waltz SE and Lentsch AB. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. FASEB J 2006; 20: 59-64.

[25] Feng LY, Ou ZL, Wu FY, Shen ZZ and Shao ZM. Involvement of a novel chemokine decoy receptor CCX-CKR in breast cancer growth, metastasis and patient survival. Clin Cancer Res 2009; 15: 2962-2970.

[26] Zeng XH, Ou ZL, Yu KD, Feng LY, Yin WJ, Li J, Shen ZZ and Shao ZM. Coexpression of atypical chemokine binders (ACBs) in breast cancer predicts better outcomes. Breast Cancer Res Treat 2011; 125: 715-727.

[27] Lee KB, Lee JM, Park CY, Cho HY and Ha SY. What is the difference between squamous cell carcinoma and adenocarcinoma of the cervix? A matched case-control study. Int J Gynecol Cancer 2006; 16: 1569-1573.

[28] Kleine W, Rau K, Schwoerer D and Pfleiderer A. Prognosis of the adenocarcinoma of the cervix uteri: a comparative study. Gynecol Oncol 1989; 35: 145-149.