Int J Clin Exp Pathol 2016;9(2):1829-1838
www.ijcep.com /ISSN:1936-2625/IJCEP0018909

Original Article

Expression of ARTD8 and ARTD14 in human colorectal adenocarcinomas

Wei Zhao*, Chang Liu*, Yi Tang, Ming Li, Ming Xiao, Ya-Lan Wang

*Department of Pathology, Molecular Medicine and Cancer Research Center, Chongqing Medical University, Chongqing 400016, P. R. China. *Equal contributors.*

Received October 31, 2015; Accepted January 1, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: ARTD8 and ARTD14 express in some human tumor tissues and participate in the progression of cancers. However, the expression of ARTD8 and ARTD14 in colorectal adenocarcinoma and their associations with clinicopathological characteristics are yet poorly characterized. We have investigated the expression of ARTD8 and ARTD14 in human colorectal adenocarcinoma and normal colorectal tissues using immunohistochemistry. Immunohistochemical staining showed that ARTD8 and ARTD14 expressed in the cytoplasm and cell nucleus of human colorectal adenocarcinoma but only in the cytoplasm of normal colorectal tissues. Enhanced expression of ARTD8 and ARTD14 were observed in the cytoplasm of colorectal adenocarcinoma. The ARTD8 was overexpressed in the cell nucleus of rectum adenocarcinoma. The cytoplasmic staining of ARTD14 was higher in poorly differentiated adenocarcinomas. These data suggest that ARTD8 and ARTD14 probably participate in the progression of colorectal adenocarcinoma and the functions of ARTD8 and ARTD14 in the cytoplasm and nucleus maybe not identical. Furthermore, there is a difference in ARTD8 and ARTD14 expression pattern between colonic adenocarcinoma and rectal adenocarcinoma.

Keywords: ARTD8, ARTD14, colorectal adenocarcinoma, colonic adenocarcinoma, rectal adenocarcinoma, immunohistochemistry

Introduction

Mono-ADP ribosylation is a post-translational modification of proteins and comprises the transfer of an ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD+) to a specific acceptor molecule (i.e., free amino acids, proteins, DNA) by ADP-ribosyltransferases (ARTs) and certain bacterial toxins [1]. According to -the difference of mono-ADP ribosylated amino acids receptors, mono-ADP ribosylation has been subdivided into diphthamide-, arginine-, asparagine- and cysteine-specific ADP-ribosylation.

Currently, there are 17 different ARTDs and 5 different ARTCs which had been found in human tissues [2]. All the ARTs can be classified as poly-ADP-ribosyltransferases (ARTD1-6), mono-ADP-ribosyltransferases (ARDT7-17, ARTC1, ARTC2, ARTC3, ARTC4, ARTC5) [3, 4]. ARTD8 is a mono-ADP-ribosyltransferases and homologous to human BAL2b. ARTD8 is expressed in lymphoid organs and lymphocyte cell lines and designated as a kind of B aggressive lymphoma protein [5]. Furthermore, ARTD8 also expresses in human hepatocellular carcinoma [6]. As an intracellular protein, ARTD8 predominantly localizes at the perinuclear region in human fibrosarcoma cells and slightly at the peripheral region of the cells [7]. The expression of ARTD8 in colorectal carcinoma and its functions have not yet been elucidated.

ARTD14 has homologous amino acid sequence with the catalytic domain of PARPs, a centrally located WWE (tryptophantryptophan-glutamate) domain and a single CCCH-type zinc-finger structure with RNA binding capabilities [8]. In human tissue, ARTD14 was first discovered in head-and-neck squamous cell by bioinformatics. The expression of exogenous ARTD14 is located at cell nuclei in SAS cells. Moreover, it overexpressed in head-and-neck squamous cell carcinoma and oral squamous cell carcinoma [9]. However, the expression of ARTD14 in ovarian cancer cells is much lower than it in nor-

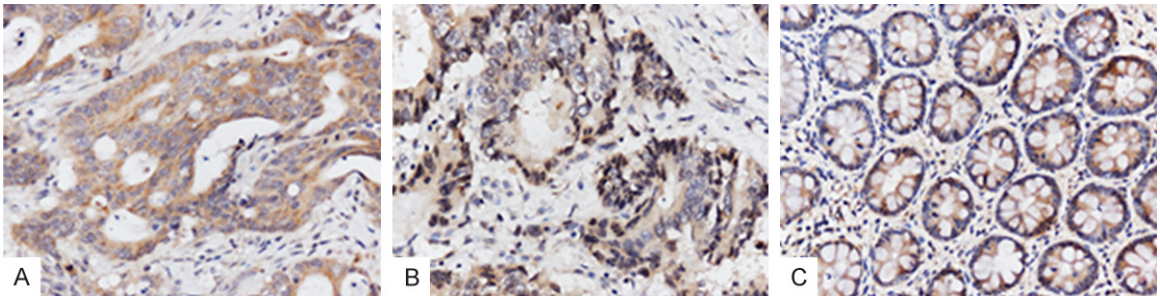

Figure 1. Expression of ARTD8 in normal colorectal tissue and colorectal adenocarcinoma. A. The positive staining of ARTD8 in the cytoplasm of normal tissues (×400). B. The positive staining of ARTD8 in the cytoplasm of colorectal adenocarcinoma (×400). C. The positive staining of ARTD8 in the cytoplasm and the cell nucleus of colorectal adenocarcinoma, simultaneously (×400).

Table 1. Expression of ARTD8 in colorectal adenocarcinoma and normal colorectal tissue

	-	+	++	+++	P*
Colorectal adenocarcinoma	0	25	29	11	0.021
Normal colorectal tissue	0	12	4	1	

P* (Wilcoxon test).

mal human ovarian epithelial cells [10]. However, the expression of ARTD14 in colorectal carcinoma and its functions have not yet been elucidated.

Previous studies have reported that ARTD8 can suppress the activity of JNK and β_-catenin-induced transcriptions are blocked by hyperactivation of JNK [11]. ARTD14 mediates β_-catenin by repressing of AHR transactivation [12, 13]. ARTD8 and ARTD14 both participate in the regulation of β_-catenin. But, it is not clear if there are some relationship between ARTD8 and ARTD14.

In this study, immunochemistry assay was used to investigate the expression of ARTD8 and ARTD14 in colorectal cancer. The aim is to make a preliminary study on the correlation of colorectal carcinoma and ARTD8, ARTD14.

Materials and methods

Patients and tissue samples

Cancer tissues and corresponding distal normal tissues were obtained from 65 patients diagnosed colorectal adenocarcinomas between 2012 and 2013 from the First Affiliated Hospital of Chongqing Medical University. The ages of patients were in the range of 31 to 85 years with a mean value of 61 years. 42 individuals were male and 23 individuals were female. There are 28 patients with colonic adenocarcinoma and 37 patients with rectal adenocarcinoma. All cases included 7 cases (11%) of well-differentiated adenocarcinomas, 46 cases (71%) of moderately differentiated adenocarcinomas, 12 cases (18%) of poorly differentiated adenocarcinomas and staged based on the tumor-node-metastasis (TNM) classification (I, n=11; II, n=25; III, n=21; IV, n=8). In all, 28 patients had lymph node metastasis, while the other 37 did not.

Immunohistochemistry

Tissues were fixed in formalin and embedded in paraffin. Consecutive -4 μm sections were cut from each Paraffin-embedded specimen. After dewaxing, the slides were immersed in different concentrations of ethanol for hydration and 0.3% peroxide in methanol to block endogenous peroxidase. Antigen retrieval was obtained by heating the sections in EDTA antigen retrieved buffer to 95°C-98°C in microwave oven. Then the slides were washed in phosphate-buffered saline for 3 times and blocked for 30 minutes by confining liquid (5% bovine serum) at 37°C. Antibodies for ARTD8 (Abcam, Cambridge, MA, USA)/ARTD14 (Abcam, Cambridge, MA, USA) were diluted to 1:500 and applied to tissue sections at 4°C overnight. The sections were incubated with anti-rabbit antibody and avidin-biotin peroxidase for 30 min at 37°C followed by the addition of SABC. Finally, specimens were stained by diaminobenzidine (DAB) and counterstained by hematoxylin. Immunostaining was scored by two independent experienced pathologists according to the standard presented in the study by Fromowitz et al. [14, 15]. The intensities of staining were divid-

Table 2A. The difference analysis of clinicopathological parameters and the expression of ARTD8

Clinicopathological features	No. of patients (n=65)	Positivity rate (%)	P* (cytoplasm)	P* (nucleus)
Age			0.638	0.357
<60	30	46.15%		
≥60	35	53.85%		
Sex			0.171	0.575
Males	42	64.62%		
Females	23	35.38%		
Site			0.446	0.044
Colon	28	43.08%		
Rectum	37	56.92%		
Lymph node metastasis			0.785	0.851
No	37	56.92%		
Yes	28	43.08%		
Invasive depth			0.246	0.155
Submucosa	2	3.08%		
Muscularis	11	16.92%		
Serosa and adventitia	52	80.00%		
Differentiation			0.205	0.302
Well	7	10.77%		
Moderate	46	70.77%		
Poor	12	18.46%		
TNM stage			0.180	0.465
I	11	16.92%		
II	25	38.46%		
III	21	32.31%		
IV	8	12.31%		

P* (Wilcoxon test).

ed into 4 conditions: 0 (no staining), 1 (weak staining, light yellow), 2 (brown), and 3 (strong, dark brown). 100 tumor cells were counted by random observations from 5 magnifications (10×20) per field; the proportion of positive cells of each slice was treated as the average proportion of positive cells in each field of vision. Positive rates were scored using the following scale: 0 for 0~5%; 1 for 6%~25%; 2 for 26%~50%; 3 for 51%~75%; 4 for 75% and above. The final scores were obtained by score of intensity plus positive proportion score: <2, negative (-); 2~3, slight positive (+); 4~5, moderately positive (++); 6~7, strongly positive (+++).

Statistical analysis

A chi-square test and the Wilcoxon test were applied to difference analysis. Correlation analysis was evaluated by using Spearman's correlation analysis. Results with P<0.05 were considered statistically significant different.

Results

Expression of ARTD8 in colorectal adenocarcinoma

Positive staining of ARTD8 was detected in both colorectal adenocarcinoma and normal colorectal tissue. In colorectal adenocarcinoma tissue, ARTD8 located in the cytoplasm (**Figure 1A**) and also located in the cell nucleus in some cases (9/56) simultaneously (**Figure 1B**). But, ARTD8 only expressed in the cytoplasm of normal tissues (**Figure 1C**). The positive staining of ARTD8 in the cytoplasm of tumors was higher than that of normal colorectal adenocarcinoma tissues (P<0.05) (**Table 1**). However, there was no difference for ARTD8 expression in the nucleus between tumors and normal colorectal tissues.

The association of ARTD8 expression and clinicopathological parameters was assessed in seven groups. The immunostaining of ARTD8 in the cell nucleus of rectal adenocarcinoma was higher than that in colon adenocarcinoma (P< 0.05). However, nucleus positive staining of ARTD8 in the cells had no statistically significant difference in different groups of age, sex, lymph node metastasis, TNM stage, invasive depth, tumor differentiation (P>0.05). Moreover, no statistical difference of the ARTD8 cytoplasmic positivity expression was shown in age, sex, tumor site, lymph node metastasis, TNM stage, invasive depth, tumor differentiation groups (P>0.05) (**Table 2A**, **2B**).

We also analyzed the expression of ARTD8 in the colon adenocarcinoma and rectal adenocarcinoma, separately. ARTD8 staining exhibited in both the cytoplasm and cell nucleus of colon adenocarcinoma and rectal adenocarcinoma. Furthermore, the positive ratios of ARTD8 in the cell nucleus and cytoplasm of colon adenocarcinoma were higher than that of normal tissues, even then the difference of the cell nucleus was not significant (P>0.05) (**Table**

Table 2B. The correlation analysis of clinicopathological parameters and the expression of ARTD8

Clinicopathological features	No. of patients (n=65)	Positivity rate (%)	P' (cytoplasm)	P' (nucleus)
Invasive depth			0.245	0.548
Submucosa	2	3.08%		
Muscularis	11	16.92%		
Serosa and adventitia	52	80.00%		
Differentiation			0.138	0.260
Well	7	10.77%		
Moderate	46	70.77%		
Poor	12	18.46%		
TNM stage			0.314	0.629
I	11	16.92%		
II	25	38.46%		
III	21	32.31%		
IV	8	12.31%		

P' (Spearman's correlation analysis).

Table 3A. Expression of ARTD8 in colonic adenocarcinoma and normal colorectal tissue

	Nucleus			Cytoplasm		
	-	+	P*	-	+	P*
Colonic adenocarcinoma	27	1	0.436	0	28	0.01
Normal colorectal tissue	17	0		0	17	

P* (Wilcoxon test).

Table 3B. Expression of ARTD8 in rectal adenocarcinoma and normal colorectal tissue

	Nucleus			Cytoplasm		
	-	+	P*	-	+	P*
Rectal adenocarcinoma	29	8	0.040	0	37	0.02
Normal colorectal tissue	17	0		0	17	

P* (Wilcoxon test).

3A). The positive ratios of ARTD8 in the cell nucleus and cytoplasm of rectum adenocarcinoma were higher than that in normal tissues (P<0.05) (**Table 3B**).

Expression of ARTD14 in colorectal adenocarcinoma

ARTD14 positivity was seen in both colorectal adenocarcinoma and normal colorectal tissues. In colorectal adenocarcinoma, positive staining of ARTD14 located in the cytoplasm (**Figure 2A**) and also appeared in the cell nucleus in some cases (10/55) (**Figure 2B**), concurrently. In normal colorectal tissue, positive staining of ARTD14 only located in the cytoplasm (**Figure 2C**). The positive ratio of ARTD14 staining in the cytoplasm of colorectal adenocarcinoma was significantly higher than those of normal colorectal tissue (P<0.05) (**Table 4**). However, ARTD14 expression of the nucleus were no significant difference between tumors and normal colorectal tissues (P>0.05).

The relationship between clinicopathological characteristics and ARTD14 expression was analyzed in seven groups. Our results revealed a positive correlation between the cytoplasmic staining of ARTD14 and tumor differentiation (P<0.05). However, there was no notable statistically significant correlation with age, sex, tumor site, lymph node metastasis, TNM stage, invasive depth and positive staining of ARTD14 in the cytoplasm (P> 0.05). While, the ARTD14 nuclear positivity was not significantly correlated with age, sex, tumor site, lymph node metastasis, TNM stage, invasive depth and tumor differentiation (P>0.05) (**Table 5A**, **5B**).

We also analyzed the expression of ARTD14 in the colon adenocarcinoma and rectal adenocarcinoma, separately. Positive staining of ARTD14 was seen in the cytoplasm and cell nucleus in both colonic adenocarcinoma and rectal adenocarcinoma. The expression of ARTD14 in colonic adenocarcinoma was higher than that in normal tissues in both cytoplasm and cell nucleus (P<0.05) (**Table 6A**). The ARTD14 which is expressed in the cytoplasm of rectal adenocarcinoma was higher than that in normal tissues with a statistically difference (P<0.05) (**Table 6B**).

Relationship between ARTD8 and ARTD14 in colorectal adenocarcinoma

In the colorectal adenocarcinoma, there was no correlation between the positive staining of ARTD8 and ARTD14 in both cytoplasm and the cell nucleus (P>0.05) (**Table 7**).

Discussion

ARTD8 has the PARP catalytic domain, which uses nicotinamide adenine dinucleotide (NAD+) as a substrate to transfer ADP-ribose onto itself

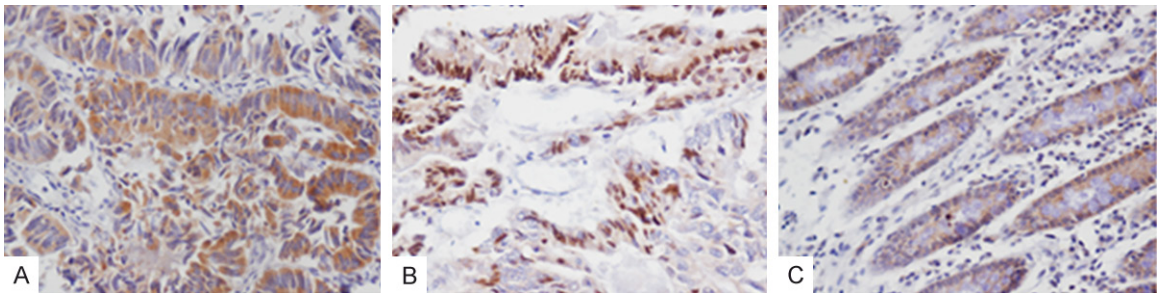

Figure 2. Expression of ARTD14 in normal colorectal tissue and colorectal adenocarcinoma. A. The positive staining of ARTD14 in the cytoplasm of normal tissues (×400). B. The positive staining of ARTD14 in the cytoplasm of colorectal adenocarcinoma (×400). C. The positive staining of ARTD14 in the cytoplasm and the cell nucleus of colorectal adenocarcinoma, simultaneously (×400).

Table 4. Expression of ARTD14 in colorectal adenocarcinoma and normal colorectal tissue

	-	+	++	+++	P	P*
Colorectal adenocarcinoma	9	25	23	8	0.011	0.025
Normal colorectal tissue	7	5	5	0		

P (chi-square test), P* (Wilcoxon test).

and p100, three copies of the macro domains that were first found at the C terminus of the macro-H2A histone protein and in the non-structural proteins of several types of ssRNA viruses [16]. The macro domain of mH2A is involved in the interaction of the nucleosome-remodeling complex SWI/SNF with chromatin, thus repress transcription [17]. The study previously shown that this enzymatic activity of ARTD8 was related to the Stat6-mediated transcription [18].

Previous publications have reported that the expression of ARTD8 has been detected in eosinophilic esophagitis and cirrhotic livers. The expression of ARTD8 has been seen in various tumor tissues including B-cell lymphomas, hepatocellular carcinoma, and multiple myeloma. As a member of the B aggressive lymphoma (BAL) family, ARTD8 is expressed in lymphoid organs and lymphocyte cell lines and it is linked to the aggressiveness of B-cell lymphomas in diffuse large B-cell lymphomas [19]. In human hepatocellular carcinoma, ARTD8 is overexpressed and has a connection with clinical prognosis. It is required for tumor growth in human hepatocellular carcinoma [6]. ARTD8 and its ADP-ribosyltransferase activity have crucial roles in the development of myeloma plasma. ARTD8 is highly expressed in myeloma plasma cells and there also has a correlation of ARTD8 expression with disease progression and poor survival in multiple myeloma. Furthermore, ARTD8 expression is stronger in the nucleus as compared with the cytoplasm in multiple myeloma. But the expression of ARTD8 in colorectal cancer is unclear. Our researches first showed that the ARTD8 expression of cytoplasm in colorectal adenocarcinoma was more than that in normal tissues. It suggested that ARTD8 probably participate in the progression of colorectal carcinoma.

JNK1 is a highly conserved serine/threonine protein kinas, which is primarily localized to the nucleus. JNK1 controls cell apoptosis and differentiation by integrating Wnt pathway and activating PKM2 by phosphorylation at Thr365 [6, 20]. It has been reported that ARTD8 knockdown markedly increased JNK1 activity in MM cell OPM-2 andARTD8 specifically bounding to JNK1p46 through its C-terminal portion. The overexpression of ARTD8 completely suppressed JNK1 activity. ARTD8 suppresses JNK1 result in the inhibition of the PKM2 activity. The inactivation of PKM2 which promotes the Warburg effect and tumor growth has been associated with the growth and survival of multiple cancer cells [21]. Besides, the inhibition of JNK leads to increased nuclear β_-catenin accumulation. The accumulation of nuclear β_-catenin functions as a transcriptional activator by getting together with the DNA binding protein TCF-4. When β_-catenin accumulates in the nuclear, the expression of c-myc is up-regulated [11]. In colorectal cancer cells, it is not clear if the ARTD8 expression increased could repress JNK1, resulting in the inactivation of PKM2 and β_-catenin accumulation, then leads to colorectal carcinoma. It is needed to be further studied.

Table 5A. The difference analysis of clinicopathological parameters and the expression of ARTD14

Clinicopathological features	No. of patients (n=65)	Positivity rate (%)	P* (cytoplasm)	P* (nucleus)
Age			0.583	0.299
<60	30	46.15%		
≥60	35	53.85%		
Sex			0.215	0.099
Males	42	64.62%		
Females	23	35.38%		
Site			0.076	0.25
Colon	28	43.08%		
Rectum	37	56.92%		
Lymph node metastasis			0.458	0.375
No	37	56.92%		
Yes	28	43.08%		
Invasive depth			0.236	0.236
Submucosa	2	3.08%		
Muscularis	11	16.92%		
Serosa and adventitia	52	80.00%		
Differentiation			0.070	0.688
Well	7	10.77%		
Moderate	46	70.77%		
Poor	12	18.46%		
TNM stage			0.752	0.141
I	11	16.92%		
II	25	38.46%		
III	21	32.31%		
IV	8	12.31%		

P* (Wilcoxon test).

In addition, ARTD8 can promote the binding of Stat6 to target genes, such as Fcer2a, it also associates with HDAC 2 and 3 [18]. By CHIP analysis, it has been shown that ARTD8 also has the ability to associate with DNA and associates with promoter rather than the coding sequence of the gene. It indicates that ARTD8 has its own function in the nucleus. In our researches, the results showed that ARTD8 was not only located in the cytoplasm and also in the nucleus in colorectal adenocarcinoma. This condition was similar to the expression of ARTD8 in multiple myeloma. However, in the normal colorectal tissue, ARTD8 was only seen in the cytoplasm. Despite the positive ratio of ARTD8 was no statisticaldifference between colorectal carcinoma and normal colorectal tissue, it is higher in colorectal carcinoma than that in normal colorectal tissue. It suggested that the functions of ARTD8 in the cytoplasm and nucleus maybe not identical in some cases.

ARTD14 is a TCDD-inducible poly (ADP-ribose) polymerase coded by TiPARP gene, which is located on chromosome 3 and includes 6 exons [22]. There are 43% amino acid sequence in ARTD14 and they are homologous to the catalytic domain of PARPs [23]. ARTD14 contains a shortened connecting loop and an isoleucine in place of the catalytic glutamate which is a critical residue within the HYE triad sequence. H532, Y564 and I631 of ARTD14 are tantamount to the catalytic HYE triad. Because of lacking the equivalent catalytic E988 residue of PARP-1, ARTD14 shares the activity of ARTs instead of the activity of Poly (ADP-ribose) polymerases [23]. It has confirmed ARTD14 could catalyze core histones as well as itself. The expression of ARTD14 has been detected in many murine tissues, such as liver, kidney, testis, spleen. The expression of ARTD14 is also seen in human tissues. ARTD14 had been observed in normal primary human ovarian surface epithelial cells and ovarian cancer cells, but the expression of ARTD14 in normal human ovarian surface epithelial cells was much higher than it in ovarian cancer cells. Researchers have found ARTD14 is primarily localized to the nucleus, although it has also been seen to exhibit cytosolic staining in HeLa cell [24]. In oral squamous cell carcinoma, ARTD14 have been proved overexpressed [9]. The expression of ARTD14 in colorectal cancer is unclear. Our study revealed that the positive staining of ARTD14 was observed in both colorectal adenocarcinoma and normal colorectal tissue. This condition is analogous to the expression of ARTD14 in ovary. However, we found the expression of ARTD14 in colorectal adenocarcinoma was higher than it in normal colorectal tissue. Meanwhile, our research also showed that the expression in poorly differentiated colorectal carcinoma was higher than it in well differentiated colorectal carcinoma. It was totally different from the expression of ARTD14 in ovary. The results suggested that ARTD14 probably participate in the progression of colorectal carcinoma. But the difference of ARTD14 expres-

Table 5B. The correlation analysis of clinicopathological parameters and the expression of ARTD14

Clinicopathological features	No.of patients (n=65)	Positivity rate (%)	P' (cytoplasm)	P' (nucleus)
Invasive depth			0.678	0.090
Submucosa	2	3.08%		
Muscularis	11	16.92%		
Serosa and adventitia	52	80.00%		
Differentiation			0.044	0.611
Well	7	10.77%		
Moderate	46	70.77%		
Poor	12	18.46%		
TNM stage			0.531	0.891
I	11	16.92%		
II	25	38.46%		
III	21	32.31%		
IV	8	12.31%		

P' (Spearman's correlation analysis).

Table 6A. Expression of ARTD14 in colonic adenocarcinoma and normal colorectal tissue

	Nucleus			Cytoplasm		
	-	+	P*	-	+	P*
Colonic adenocarcinoma	22	6	0.043	3	25	0.008
Normal colorectal tissue	17	0		7	10	

P* (Wilcoxon test).

Table 6B. Expression of ARTD14 in rectal adenocarcinoma and normal colorectal tissue

	Nucleus			Cytoplasm		
	-	+	P*			P
Rectal adenocarcinoma	33	4	0.163	6	31	0.046
Normal colorectal tissue	17	0		7	10	

P* (Wilcoxon test), P (chi-square test).

Table 7. Expression of ARTD8 and ARTD14 in the cell nucleus of colorectal adenocarcinoma

	ARTD8		
ARTD14	-	+	P'
-	8	47	0.707
+	1	9	

P' (Spearman's correlation analysis).

sion in different tissues and different tumors is no immediately clear.

Currently, little is known about the function of ARTD14. It has confirmed that ARTD14 could catalyze core histones as well as itself [13]. ATRD14 appears to influence transcript levels of Zfp42, Sox2, Pecam1 and Prdm14 [25]. The 3'end and the 5' end sequences of ARTD14 have high homology to RM1 and TIL, respectively. TIL is induced in T cells infiltrating progressing tumors, suggesting that ARTD14 might have a role in cancer progression [23]. The absence of ARTD14 not only causes steatohepatitis but also leads to dioxin-induced chronic toxicity, including metabolic disease and liver cancer [26]. Nevertheless, the loss of ARTD14 promotes development of ovarian cancer. ARTD14 also acts as a repressor of AHR transactivation. ARTD14 knockdown significantly increases constitutive AHR protein levels and its ability to repress AHR is dependent on its zinc-finger and catalytic domains. ARTD14 may regulate AHR by increasing the proteolytic degradation of AHR. The research has confirmed that AhR-_/-_ mice frequently developed colonic tumors, mostly in the cecum near the ileocecal junction. ARH plays an important role in promoting degradation of endogenous β_-catenin [12]. The accumulation of β_-catenin is an acknowledged mechanism of carcinogenesis and differentiation [27]. But if ARTD14 repress AHR, resulting in the accumulation of β_-catenin, then affects the differentiation of colorectal carcinoma was needed to be further studied.

In our researches, we also observed that the expressions of ARTD14 located in the cytoplasm and some of them located in the nucleus of colorectal adenocarcinoma concurrently. It is not similar to the expression of ARTD14 in HeLa cell. At the same time, the expression of ARTD14 was only observed in the cytoplasm in normal colorectal tissue. This shows that locations of ARTD14 may have a difference in normal tissues between in tumor tissues. And the function of ARTD14 in the nucleus of colorectal cancer cell requires a further exploration.

Accumulating evidence suggests that the mechanism of colorectal carcinoma conferred by various environmental and genetic factors is different for proximal and distal tumors. Colon and rectum have different embryological ori-

gins. The origins of the colon from embryonic mid gut and hind gut. This may account for the biological differences in colon and rectum tumors. Furthermore, the previous research has shown that tumors in the right/ascending colon are more likely to be hypermethylated with an elevated mutation rates than other colorectal carcinomas [28]. Microsatellite instability and the CpG island methylator phenotype (CIMP) are frequently associated with tumors in the right colon [29]. However, chromosomally unstable probably relates to the tumors in the distal bowel. Gender is likewise a kind of factor causing the difference between colon cancer and rectum cancer. Due to the differences in colon and rectum tumors, we also analyzed the expression of ARTD8 and ARTD14 in colon and rectum tumors, respectively. Our results showed that the expression of ARTD8 in the cell nucleus of rectal adenocarcinoma was higher than that in normal tissues. But there was not a notable statistically difference of the positive staining in the cell nucleus between colonic adenocarcinoma and normal tissues. The difference of ARTD8 staining in the cell nucleus between colonic adenocarcinoma and rectal adenocarcinoma suggested that the function of ARTD8 in the colonic adenocarcinoma and rectal adenocarcinoma is not indistinguishable.

Despite the higher expression in the cytoplasm in both colonic adenocarcinoma and rectal adenocarcinoma, there is only a difference of expression of ARTD14 in the cell nucleus between colonic adenocarcinoma and rectal adenocarcinoma. Researchers have shown that ARTD14 could catalyze core histones. Histones are the indispensable protein components of chromatin. Mono-ADP ribosylation of histones is thought to be linked to DNA repair and cell proliferation [30]. Ushiroyama and his coworkers show that mono-ADP-ribosylation of histone H1.3 on arginine residue R33 may reduce cyclic AMP-dependent phosphorylation of histone H1.3 on serine residue 36 [31]. In interphase, the phosphorylation of H3 correlates with chromatin relaxation. In mitosis, the phosphorylation of H3 correlates with chromosome condensation. Chromatin relaxation and chromosome condensation are indispensable for gene expression. On the basis of previous researches and our results of colonic adenocarcinoma and rectal adenocarcinoma, we presume that ARTD14 may be involved in the gene expression progress by catalyzing histone in colonic adenocarcinoma, which may not be as the same as the function of it in rectal adenocarcinoma. But the detail is needed to be further studied.

In our study, there was no further correlation of the positive staining of ARTD8 and ARTD14 in both cytoplasm and the cell nucleus. It suggested that ARTD8 and ARTD14 maybe have their own function in colonic adenocarcinoma and rectal adenocarcinoma. The molecular mechanism of ARTD8 and ARTD14 in colorectal carcinoma may not be identical. But the exact functions of ARTD8 and ARTD14 in colonic adenocarcinoma and rectal adenocarcinoma are still needed to be studied.

In brief, the current results first demonstrated that the expression of ARTD8 and ARTD14 in colorectal carcinoma was more than that in normal colonrectal tissues. The expression of ARTD8 and ARTD14 in colorectal carcinoma is not identical and there is a difference in their expression pattern between colonic adenocarcinoma and rectal adenocarcinoma. These results suggest that the expression of ARTD8 and ARTD14 may correlate with the progression of colorectal carcinoma. But the exact action of the expression of them is needed to be studied. Whether the expression of ARTD8 and ARTD14 may serve as a potential target for directed therapy in colorectal carcinoma remains unclear, also needed to be further studied.

Acknowledgements

This study was supported by the Research Training Fund (Natural Science) of Chongqing Medical University (Grant No. 201413) and the Basic and Advanced Research Project of the Science and Technology Commission of Yuzhong District of Chongqing (Grant No. 2014-0106).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ya-Lan Wang, Department of Pathology, Molecular Medicine and Cancer Research Center, Chongqing Medical University. 1 Medical School Road, Yu Zhong District, Chongqing 400016, P. R. China. E-mail: wangyalan074@126.com

References

[1] Ueda K and Hayaishi O. ADP-ribosylation. Ann Rev Biochem 1985; 54: 73-100.

[2] Amé JC, Spenlehauer C and de Murcia G. The PARP superfamily. Bioessays 2004; 26: 882-893.

[3] Citarelli M, Teotia S and Lamb RS. Evolutionary history of the poly (ADP-ribose) polymerase gene family in eukaryotes. BMC Evolut Biol 2010; 10: 308.

[4] Hakmé A, Wong HK, Dantzer F and Schreiber V. The expanding field of poly (ADP-ribosyl) ation reactions. EMBO Rep 2008; 9: 1094-1100.

[5] Cho SH, Goenka S, Henttinen T, Gudapati P, Reinikainen A, Eischen CM, Lahesmaa R and Boothby M. PARP-14, a member of the B aggressive lymphoma family, transduces survival signals in primary B cells. Blood 2009; 113: 2416-2425.

[6] Iansante V, Choy PM, Fung SW, Liu Y, Chai JG, Dyson J, Del Rio A, D'Santos C, Williams R and Chokshi S. PARP14 promotes the Warburg effect in hepatocellular carcinoma by inhibiting JNK1-dependent PKM2 phosphorylation and activation. Nat Commun 2015; 6: 7882.

[7] Yanagawa T, Funasaka T, Tsutsumi S, Hu H, Watanabe H and Raz A. Regulation of phosphoglucose isomerase/autocrine motility factor activities by the poly (ADP-ribose) polymerase family-14. Cancer Res 2007; 67: 8682-8689.

[8] Aravind L. The WWE domain: a common interaction module in protein ubiquitination and ADP ribosylation. Trends Biochem Sci 2001; 26: 273-275.

[9] Cha JD, Kim HJ and Cha IH. Genetic alterations in oral squamous cell carcinoma progression detected by combining array-based comparative genomic hybridization and multiplex ligation-dependent probe amplification. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 111: 594-607.

[10] Karras GI, Kustatscher G, Buhecha HR, Allen MD, Pugieux C, Sait F, Bycroft M and Ladurner AG. The macro domain is an ADP-ribose binding module. EMBO J 2005; 24: 1911-1920.

[11] Liao G, Tao Q, Kofron M, Chen JS, Schloemer A, Davis RJ, Hsieh JC, Wylie C, Heasman J and Kuan CY. Jun NH2-terminal kinase (JNK) prevents nuclear β-catenin accumulation and regulates axis formation in Xenopus embryos. Proc Natl Acad Sci 2006; 103: 16313-16318.

[12] Kawajiri K, Kobayashi Y, Ohtake F, Ikuta T, Matsushima Y, Mimura J, Pettersson S, Pollenz RS, Sakaki T and Hirokawa T. Aryl hydrocarbon receptor suppresses intestinal carcinogenesis in ApcMin/+ mice with natural ligands. Proc Natl Acad Scis 2009; 106: 13481-13486.

[13] MacPherson L, Tamblyn L, Rajendra S, Bralha F, McPherson JP and Matthews J. 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin poly (ADP-ribose) polymerase (TiPARP, ARTD14) is a mono-ADP-ribosyltransferase and repressor of aryl hydrocarbon receptor transactivation. Nucle Acids Res 2012; Gks1337.

[14] Fromowitz FB, Viola MV, Chao S, Oravez S, Mishriki Y, Finkel G, Grimson R and Lundy J. Ras p21 expression in the progression of breast cancer. Hum Pathol 1987; 18: 1268-1275.

[15] Qin JM, Fu XY, Li SJ, Liu SQ, Zeng JZ, Qiu XH, Wu MC and Wang HY. Gene and protein expressions of p28GANK in rat with liver regeneration. World J Gastroenterol 2003; 9: 2523-7.

[16] Goenka S, Cho SH and Boothby M. Collaborator of Stat6 (CoaSt6)-associated poly (ADP-ribose) polymerase activity modulates Stat6-dependent gene transcription. J Biol Chem 2007; 282: 18732-18739.

[17] Zolkiewska A and Moss J. Integrin alpha 7 as substrate for a glycosylphosphatidylinositol-anchored ADP-ribosyltransferase on the surface of skeletal muscle cells. J Biol Chem 1993; 268: 25273-25276.

[18] Mehrotra P, Riley JP, Patel R, Li F, Voss Le and Goenka S. PARP-14 functions as a transcriptional switch for Stat6-dependent gene activation. J Biol Chem 2011; 286: 1767-1776.

[19] Cho SH, Ahn AK, Bhargava P, Lee CH, Eischen CM, McGuinness O and Boothby M. Glycolytic rate and lymphomagenesis depend on PARP14, an ADP ribosyltransferase of the B aggressive lymphoma (BAL) family. Proc Natl Acad Sci 2011; 108: 15972-15977.

[20] Barbarulo A, Iansante V, Chaidos A, Naresh K, Rahemtulla A, Franzoso G, Karadimitris A, Haskard D, Papa S and Bubici C. Poly (ADP-ribose) polymerase family member 14 (PARP14) is a novel effector of the JNK2-dependent pro-survival signal in multiple myeloma. Oncogene 2013; 32: 4231-4242.

[21] Yang W, Xia Y, Hawke D, Li X, Liang J, Xing D, Aldape K, Hunter T, Yung WA and Lu Z. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell 2012; 150: 685-696.

[22] Katoh M and Katoh M. Identification and characterization of human TIPARP gene within the CCNL amplicon at human chromosome 3q25. 31. Int J Oncol 2003; 23: 541-547.

[23] Ma Q, Baldwin KT, Renzelli AJ, McDaniel A and Dong L. TCDD-inducible poly (ADP-ribose) polymerase: a novel response to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. Biochem Biophys Res Commun 2001; 289: 499-506.

[24] Vyas S, Chesarone-Cataldo M, Todorova T, Huang YH and Chang P. A systematic analysis of the PARP protein family identifies new func-

tions critical for cell physiology. Nat Commun 2013; 4: 2240.
[25] Roper SJ, Chrysanthou S, Senner CE, Sienerth A, Gnan S, Murray A, Masutani M, Latos P and Hemberger M. ADP-ribosyltransferases Parp1 and Parp7 safeguard pluripotency of ES cells. Nucle Acids Res 2014; 42: 8914-8927.
[26] Ahmed S, Bott D, Gomez A, Tamblyn L, Rasheed A, Cho T, MacPherson L, Sugamori KS, Yang Y, Grant DM3, Cummins CL, Matthews J. Loss of the Mono-ADP-Ribosyltransferase, TIPARP, Increases Sensitivity to Dioxin-Induced Steatohepatitis and Lethality. J Biol Chem 2015; 290: 16824-40.
[27] Chung GG, Provost E, Kielhorn EP, Charette LA, Smith BL and Rimm DL. Tissue microarray analysis of β-catenin in colorectal cancer shows nuclear phospho-β-catenin is associated with a better prognosis. Clin Cancer Res 2001; 7: 4013-4020.
[28] Network CG. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330-337.
[29] Iacopetta B. Are there two sides to colorectal cancer? Int J Cancer 2002; 101: 403-408.
[30] Boulikas T. Relation between carcinogenesis, chromatin structure and poly (ADP-ribosylation)(review). Anticancer Res 1990; 11: 489-527.
[31] Ushiroyama T, Tanigawa Y, Tsuchiya M, Matsuura R, Ueki M, Sugimoto O and Shimoyama M. Amino acid sequence of histone H1 at the ADP-ribose-accepting site and ADP-ribose· histone-H1 adduct as an inhibitor of cyclic-AMP-dependent phosphorylation. Eur J Biochem 1985; 151: 173-177.