EXPESSION AND SIGNIFICANCE OF ARL2 AND THE RELATIONSHIP WITH AXL AND STAT3 IN COLORECTAL CANCER

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ABSTRACT

Objective*: To study the expression and significance of ARL2 and the relationship with AXL and STAT3 in Colorectal Cancer (CRC).*

Methods: *Altogether 182 patients with CRC confirmed by histopathological examination admitted to the general surgery on January 1, 2014 to December 31, 2014 were chosen to study objects. Survival follow-up was completed by December 31, 2019 by telephone. The expression of ARL2, AXL and STAT3 in 182 CRC tissues and adjacent tissues were detected by the tissue microarray and immunohistochemistry. SPSS 20.0 software was used for statistical processing. Kaplan-Meier method used to drawn the survival curve, log-rank method used for survival univariate analysis, and Cox model used for the survival multiple factor analysis.*

Results: *The expression level of ARL2, AXL and STAT3 was increased in CRC tissues. ARL2 can be an independent prognostic factor for CRC. The expression level of ARL2 in phase I and II is higher than phase III and IV, while the expression of AXL in phase I andII is lower than phase III and IV (TNM staging) (P<0.05). The expression level of AXL and STAT3 is higher in the distant metastasis group (P<0.05). ARL2 positive expression was significantly increased in the survival group (P<0.05). The expression levels of ARL2 and AXL in CRC tissues were negatively correlated by Spearman rank correlation analysis(r=-0.375, p<0.001). ARL2 was positively related to STAT3 in CRC tissues (r=0.970, p<0.001). AXL was negatively related to STAT3 expression in CRC tissues (r=-0.332, p<0.001). The 5-year CRC survival rate of the ARL2 group with high expression level of different tumor sites, gender, TNM stage and lymph node metastasis group was all higher than that of the ARL2 group with low expression. Whereas, there was no difference in the 5-year survival rate of CRC with the expression level of ARL2 in the group under 65 years of age, and no difference in the expression level of ARL2 in the distant metastasis group.*

Conclusion: *It was a highly expression of ARL2 in CRC tissues, furthermore high expression of ARL2 improved 5-year survival in CRC. ARL2 maybe affect the expression of AXL negatively and is critical to the development and progress of CRC, and ARL2 played an vital part in the formation, invasion and metastasis of CRC.This study can provide a preliminary assessment of the prognosis of CRC, and ARL2 could be a curative target for CRC.*

Keywords: *ARL2, AXL, STAT3, colorectal cancer.*

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Introduction

Colorectal cancer (CRC) makes up similarly 10% of all cancers diagnosed annually and cancer contributes to death in the world. Recent years, it is the world's fourth most deadly cancer with almost 900000 deaths annually[1]. The overall prognosis of CRC was improved by early detection, adjuvant chemotherapy and the advance of resection[2, 3]. However, even in the early detected CRC, a great many evidence suggest that prognosis of CRC remains unsatisfactory[4, 5]. Then, develop a predicted marker related to its prognosis can help to choose a therapeutic method and cure CRC to some extent.

ADP ribosylation factor-like GTPase2 (ARL2), a highly conservative and widely expressed GTPase, can regulate differentiation, proliferation, vesicle transport in eukaryotes[6]. ARL2 can influence the

phosphorylation of p53 and result in microtubule isolation of p53 in MCF7 cells of breast cancer[7]. P53 is considered to be a tumor suppressor gene that inhibits tumorigenic development[8]. Some studies gave the suggestion that by regulating ARL2 expression can alter the course of many cancer, cell migration and invasion[9, 10]. ARL2 is increased in a plenty of tumors, for instance breast cancer, hepatocellular tumor and cervical cancer[9, 11, 12].

However, there are no exact and detailed studies about the relationship between prognosis of CRC and the expression level of ARL2. In CRC, AXL is an oncotarget in human[13]. With AXL overexpressed, CRC cell lines would be contributed to migration, invasion and epithelial mesenchymal transition[14]. Some studies have shown that AXL is a downstream signaling molecule of ARL2[15].

This signaling pathway may regulate STAT3 in the nucleus and then influence the further development of CRC[16]. In this study, we showed the distinct clinicopathologic patterns of ARL2, AXL and STAT3 expression in CRC by immunostaining. We confirmed ARL2 overexpression in CRC as an independent indicator of a favorable outcome for CRC among the Chinese.

Materials and methods

Source of case data and tissue samples

With the permission of the hospital ethics Committee, 182 CRC patients confirmed by histopathological examination and admitted to the general surgery department of our hospital on January 1, 2014 to December 31, 2014 were selected as study objects.

Male is 96 and female is 86; Their age distribution from 21 to 83, and the average age was 52. Fresh specimens were collected from primary CRC foci or paired colonic mucosa over 5 cm from the edge of the primary foci, which were verified by pathologist's tests. All patients refused preoperative radiotherapy and/or chemotherapy.

In all cases, knowing-agreeing was gained from the patient or maybe his family. Survival follow-up was completed by December 31, 2019. All patients were followed up by telephone or outpatient, with a follow-up rate of 93%.

Tissues microarrays and immunohistochemistry (IHC)

Tissue microchips (TMA) included 182 cases CRC tissues and its neighbouring normal colonic tissues, all of which were embedded in paraffin, then were derived from the Pathology Department of Affiliated Hospital of Xuzhou Medical University. Whole CRC patients were resected tumor tissues in the Affiliated Hospital of Xuzhou Medical University from January 1, 2014 to December 31, 2014. Patient clinical data was gained from the Health Information of Affifiliated Hospital of Xuzhou Medical University, for instance gender, age, CRC differentiation, CRC diameter, lymph node metastasis, TNM stage. In this 182 cases retrospective CRC group, there possessed 96 males and 86 females. The mean age was 63.18 years old (21 to 83). In terms of TNM stage, there possessed 98 patients of stage I and II, 84 patients of stage III and IV. In terms of the differentiation status, 27 patients were identified as poorly differentiated, 127 patients were identified as moderately differentiated, and 15 patients were identified as well differentiated. A majority of patients were regarded as adenocarcinoma for the pathologic type (169/182). A complete postoperative follow-up record was obtained for each patient. Survival time was subtracted the date of surgery from the date of death or the final followed visit. We gained the date of death from postoperative follow-up documents. The CRC TMAs was established at the Pathology Department of the Affifiliated Hospital of Xuzhou Medical University, using 1.5-mm diameter cores from paraffin blocks were punched out[17].

Immunohistochemical analysis of ARL2, AXL and STAT3 was executed on 182 CRC tissues and their control group. Firstly, CRC tissue samples were fixed in 10% formalin, following paraffin dehydrated and embedded. Secondly, the paraffin-embedded samples were cut into 3-5 mm successive slices.

Conventional dewaxing, microwave thermal extraction of antigens and quenching of endogenous peroxidase activity in slices. Primary antibodies (anti-ARL2 antibody, 1:100, CST 188322, Cambridge, MA, USA; anti-AXL antibody, 1:100, CST 8661, Cambridge, MA, USA; anti-STAT3 antibody, 1:500, CST 9139, Cambridge, MA, USA) were smeared to the slices at 4°C one whole night. On the second day, secondary antibodys were added to the slices for 1 hours at room temperature then following dyed by 3, 3'-diaminobenzidine (DAB). In the end, the slices were stained with 0.02% Hematoxylin for microscopic examination (OLYMPUS, IX73).

Immunohistochemical staining score

In the absence of clinicopathological information, the slides were scored by two pathologists and the results were unified after consultation.

Determination criteria of staining results:

• According to the staining intensity: 0 points, no any positive staining;

• 1 point, light yellow staining;

• 2 points, the dark yellow stain;

• 3 points, the brownish yellow stain.

Immunostaining intensity scores of ARL2, AXL and STAT3 immunostaining was scored as 0-3 (0, negative; 1, weak; 2, moderate; 3, strong); immunoreactivity cells percentage can be divided into percentage 1 (0-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%).

The final staining score for each section is the product of the staining score and the proportion of stained cells as the final staining score.

Statistical treatment

Using SPSS 20.0 software for statistical analysis. T test was used to compare the measurement data between the two groups. Paired Wilcoxon test was applied to assess the signifificance of ARL2, AXL and STAT3 staining in tumor and its adjacent tumor-free tissues. Using Chi-square test to detect the difference in gene expression between primary CRC lesions and adjacent tissues, and the correlation between protein expression in primary CRC lesions and the clinicopathologic features of CRC.

Spearman correlation analysis was applied to make a decision of the expression connection between the two proteins. If it was $p<0.05$, statistically significant would be considered. The survival curve was plotted by Kaplan-Meier method, the survival univariate analysis was performed by log-rank method. Univariate and multivariate Cox proportional hazards regression analyses were used to estimate the crude HRs, adjusted HRs.

Results

The expression level of ARL2 is conventionaly enhancived in CRC tissues, meanwhile upregulated ARL2 protein is positively relevant to clinicopathologic featuresbe

ARL2 expression in 182 CRC tissues and adjacent tumor-free tissues was tested by histopathological microarray and immunohistochemical methods. The expression percentage of ARL2 tissues was 79.67% in 182 CRC (145/182) (Table 1). And compared to the normal control group(NC), ARL2 expression was enhancived in CRC tissues (Figure 1a) ($p<0.001$). ARL2 was mainly distributed in the cytoplasm (Figure 1b). Paired Wilcoxon test for 182 CRC patients with adjacent tumor-free tissues showed that the expression of ARL2 protein was significantly up-regulated in the cancer tissues compared with the adjacent tumor-free tissues (Figure 1c) ($p<0.001$). Table 1 was listed the clinicopathologic features of the CRC patients. ARL2 expression was divided into low (immune reactivity score (IRS): 0-1) and high (IRS: 2-12) groups.

We found that the expression of ARL2 protein in CRC tissues was low (20.33%, 37/182) and high (79.67%, 145/182).High ARL2 protein expression was signifificantly positively correlated with TNM stage ($p<0.001$) and survival state ($p=0.003$). Whereas, there was no significant correlation between expression level of and age, gender, tumor diameter, lymph node metastasis, and distant metastasis or differentiation.

Variables	n	ARL2 expression		pa	AXL expression		pa	STAT3 expression		pa
		High (%)	Low (%)		High(%)	Low(%)		High(%)	Low(%)	
All patients	182	145 (79.67%)	37 (20.04%)		78 (42.86%)	104 (57.14%)		121 (66.48%)	61 (33.52%)	
Age										
≥60	123	94 (76.42%)	29 (23.58%)	0.117	52 (42.27%)	71 (57.73%)	0.820	105 (85.37%)	18 (14.63%)	0.132
<60	59	51 (86.44%)	8 (13.56%)		26 (%)	33 (%)		45 (76.27%)	14 (23.73%)	
Gender										
Male	96	71 (73.96%)	25 (26.04%)	0.497	43 (44.79%)	53 (55.21%)	0.578	78 (81.25%)	18 (18.75%)	0.663
Female	86	74 (86.05%)	12 (13.95%)		35 (40.70%)	51 (59.30%)		72 (83.72%)	14 (16.28%)	
Tumor diameter										
≥5cm	88	67 (76.14%)	21 (23.86%)	0.144	39 (44.32%)	49 (55.68%)		69 (78.41%)	19 (21.59%)	0.701
<5cm	94	78 (82.98%)	16 (17.02%)		39 (41.49%)	55 (58.51%)		81 (86.17%)	13 (13.83%)	
TNM stage										
I+II	99	80 (80.81%)	19 (19.19%)	<0.001	44 (44.44%)	55 (55.56%)	0.005	82 (82.83%)	17 (17.17%)	0.874
III+IV	83	65 (78.31%)	18 (21.69%)		40 (48.19%)	19 (51.81%)		68 (81.93%)	15 (18.07%)	
Lymph node metastasis										
N1/N2/N3	79	62 (78.48%)	17 (21.52%)	0.728	32 (40.51%)	47 (59.49%)	0.576	65 (82.28%)	14 (17.72%)	0.966
No	103	83 (76.85%)	20 (23.15%)		46 (44.66%)	57 (55.34%)		85 (82.52%)	18 (17.48%)	
Distant metastasis										
M1	16	12 (75.00%)	4 (25.00%)	0.628	6 (37.50%)	10 (62.50%)	0.016	10 (62.50%)	6 (37.5%)	0.029
M0	166	133 (80.12%)	33 (19.88%)		72 (43.37%)	94 (56.63%)		140 (84.34%)	26 (15.66%)	
Differentiationb										
Poor	27	20 (74.07%)	7 (25.93%)	0.413	10 (37.04%)	17 (62.96%)	0.525	12 (44.44%)	15 (55.56%)	0.004
Moderate/ high	142	115 (80.99%)	27 (19.01%)		62 (43.66%)	80 (54.34%)		103 (72.54%)	39 (27.46%)	
Survival state										
Survival	113	98 (86.73%)	15 (13.27%)	0.003	48 (42.48%)	65 (57.52%)	0.895	97 (85.84%)	16 (14.16%)	0.122
Dead	69	47 (68.12%)	22 (31.88%)		30 (43.48%)	39 (56.52%)		53 (76.81%)	16 (23.19%)	

Table 1: Relationship between expression of ARL2, AXL and STAT3 and clinicopathological features in CRC patients.

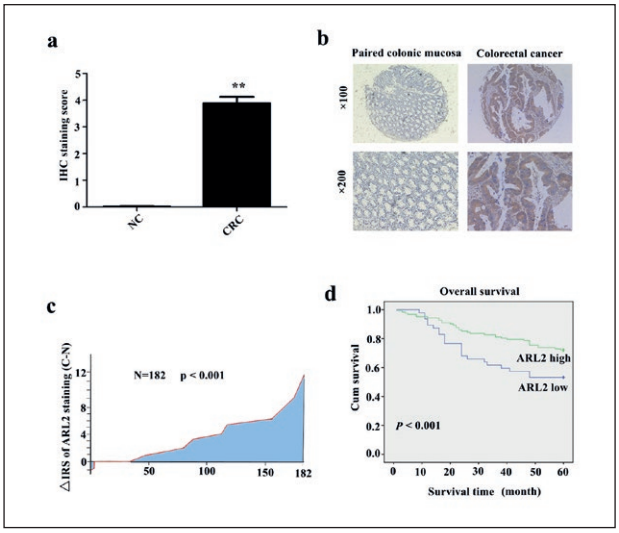

Figure 1: Increased ARL2 expression in CRC was positively correlated with overall survival rate. a IHC was used to detect the expression level of ARL2 in CRC tissues and adjacent tumo-free tissues (P<0.001). b ARL2 immunostaining for TMAs.

Note: top panel, magnification time was100; bottom panel, magnification time was 200. c The difference distribution of ARL2 staining intensity between CRC tissue and adjacent tumor-free tissue. d Higher ARL2 expression was associated with better overall cumulative survival in CRC patients (P<0.001, logrank test).

In 182 CRC tissues, the protein expressions of AXL and STAT3 were higher than those of adjacent tumor-free tissues, respectively, and the expressions of ARL2, AXL and STAT3 in CRC tissues were closely related

In 182 CRC tissues, the expression percentage of AXL and STAT3 was 42.86% (78/182) and 66.48% (121/182) (Table 1). And compared to the NC group, the expression of AXL and STAT3 was enhancived in CRC tissues (Fig. 2a & 2b) (p<0.001). AXL was found mainly in the cytoplasm (Fig. 2c), while STAT3 was found mainly in the cytoplasm and nucleus (Fig. 2d). It showed that the expressions of ARL2 and AXL in CRC tissues were negatively correlated in Spearman rank correlation analysis (r=-0.375, p<0.001). There was a positive correlation between ARL2 and STAT3 in CRC tissues (r=0.970, p<0.001). In CRC tissues, the expression of AXL was negatively correlated with the expression of STAT3 (r =-0.332, p<0.001).

For CRC, ARL2 is an independent prognostic factor

In order to determine whether ARL2 expression is related to 5-year overall survival (OS) disease-free cumulative survival (DFS) in CRC patients, Kaplan-Meier survival analysis and log-rank test were used. Our statisticaldata showed that CRC patients with elevatedARL2 protein expression trend to have better OS and DFS compared with CRC patients with decreased ARL2 expression (P<0.001 and P=0.003, respectively, Fig. 1d).

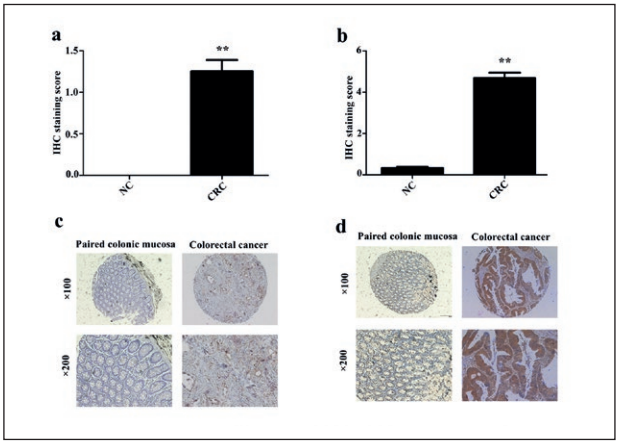

Figure 2: Expression of AXL and STAT3 was increased in CRC. a IHC was used to detect the expression level of AXL in CRC tissues and adjacent tumor-free tissues (P<0.001). b IHC was used to detect the expression level of STAT3 in CRC tissues and adjacent tumor-free tissues (P<0.001). c AXL immunostaining for TMAs.

Note: top panel, magnification time was 100; bottom panel, magnification time was 200. d STAT3 immunostaining for TMAs. Note: top panel, magnification time was 100; bottom panel, magnification time was 200.

Simultaneously, there was a high increase of the cumulative OS rate from low-ARL2 group (53.2%) to high-ARL2 group (72.1%).

Univariate and multivariate Cox regression models were used to verify the prognostic value of ARL2 expression in CRC. In univariate Cox regression analysis, Gender, Lymph node metastasis, Distant metastasis, TNM stage, ARL2 and STAT3 expression were the important prognostic factors affecting the CRC patients' OS and DFS (Table 2).

Variables[a]	Overall survival		Disease-specific survival	
	HR (95% CI)	P	HR (95% CI)	P
Gender	1.777 (1.028-3.071)	0.039	1.747 (1.008-3.027)	0.047
Age	1.006 (0.983-1.029)	0.635	1.005 (0.982-1.029)	0.655
Tumor diameter	1.128 (0.997-1.276)	0.055	1.131 (1-1.280)	0.05
LNM	3.579 (2.040-6.279)	<0.001	3.740 (2.108-6.634)	<0.001
Distant metastasis	7.564 (3.999-14.308)	<0.001	7.602 (4.013-14.402)	<0.001
TNM stage	2.738 (1.912-3.922)	<0.001	2.885 (1.998-4.166)	<0.001
ARL2	0.870 (0.792-0.956)	0.004	0.869 (0.790-0.955)	0.004
AXL	1.087 (0.957-1.235)	0.199	1.077 (0.943-1.230)	0.276
STAT3	0.910 (0.838-0.989)	0.026	0.911 (0.839-0.990)	0.029

Table 2: Univariate Cox regression analysis predicting survival of ARL2 expression and associated clinicopathological characteristics in CRC patients.

Note: CI: confidence interval, LNM: lymph node metastasis, aARL2: low vs high; age: ≤65 vs >65; gender: male vs female; LNM: N0 vs N1, N2, N3; distant metastasis: M0 vs M1; TNM stage was ranked as I-II vs III-IV; tumor diameter: ≤5 vs >5.

We further confirmed ARL2 data expression is still an independent sense of OS (P=0.03, 95% confidence interval (CI)=0.775-0.987) and DFS (P=0.029, 95% CI=0.773-0.986) prognosis biomarkers for the CRC patients after adjusting for TNM stage, differentiate type, distant metastasis, age, and gender in multivariate Cox regression model (Table 3).

On the whole, our results suggested that ARL2 expression can be a potential independent prognostic factor for OS and DFS in CRC patients.

Variables[a]	Overall survival		Disease-specific survival	
	HR (95% CI)	P	HR (95% CI)	P
Gender	1.714 (0.968-3.032)	0.064	1.679 (0.946-2.981)	0.077
Age	1.000 (0.977-1.024)	0.987	1.000 (0.976-1.024)	0.991
Tumor diameter	1.099 (0.957-1.262)	0.183	1.099 (0.957-1.263)	0.181
LNM	3.412 (1.199-9.711)	0.021	3.239 (1.135-9.241)	0.028
Distant metastasis	6.809 (2.375-19.519)	<0.001	6.115 (2.089-17.898)	0.001
TNM stage	0.960 (0.503-1.830)	0.901	1.026 (0.529-1.999)	0.941
ARL2	0.875 (0.775-0.987)	0.030	0.873 (0.773-0.986)	0.029
AXL	1.174 (1.033-1.335)	0.014	1.155 (1.011-1.320)	0.034
STAT3	0.960 (0.870-1.060)	0.422	0.965 (0.874-1.065)	0.476

Table 3: Multivariate Cox regression analysis models assessing the impact of covariates on overall and disease survival in CRC patients.
Note: CI: confidence interval, LNM: lymph node metastasis, aARL2: low vs high; age: ≤65 vs >65; gender: male vs female; LNM: N0 vs N1, N2, N3; distant metastasis: M0 vs M1; TNM stage was ranked as I–II vs III–IV; tumor diameter: ≤5 vs >5.

Relationship between the expression of ARL2, AXL and STAT3 in CRC and its clinicopathological features

ARL2 expression in phase I and II is higher than phase III and IV, while AXL protein expression in phase I and II is lower than phase III and IV (TNM staging) (P<0.05). The expression of AXL and STAT3 is higher in the distant metastasis group (P<0.05). High expression of ARL2 was noticeable growth in the survival group (P<0.05).

However, ARL2, AXL and STAT3 protein expression in CRC tissues was not correlated with age, gender, tumor diameter, lymphatic vessel invasion and other factors. As is shown in Table 1.

Subgroup analysis

Tumor site

In 92 colon cancer patients, 77 patients were with high ARL2 expression meanwhile 15 patients were with low ARL2 expression. It had a higher survival rate of the high ARL2 expression group compared with the low ARL2 expression group (P=0.001) (Figure 3a). Among 90 rectal cancer patients, 68 patients were with high ARL2 expression and 22 patients were with low ARL2 expression. It had a higher survival rate of the survival rate of high ARL2 expression group compared with low ARL2 expression (P=0.028) (Figure 3b).

Gender

Among 96 male CRC patients, 71 patients were with high ARL2 expression and 25 patients were with low ARL2 expression. It had a higher survival rate of the survival rate of high ARL2 expression group compared with the low ARL2 expression group (P=0.010) (Figure 3c). Among 86 female CRC patients, 74 patients were with high ARL2 expression and 12 patients were with low ARL2 expression. It had a higher survival rate of the survival rate of high ARL2 expression group compared with the low ARL2 expression group (P=0.048) (Figure 3d).

TNM staging

Among 84 patients with T1+T2 grade CRC, 66 patients were with high ARL2 expression and 18 patients were with low ARL2 expression. It had a higher survival rate than the low ARL2 expression group (P=0.001) (Figure 3e). Among the 98 T3+T4 grade CRC, 79 were with high ARL2 expression group and 19 were with low ARL2 expression group had a higher survival rate than the low ARL2 expression group (P=0.007) (Figure 3f).

Lymph node metastasis

Among 102 CRC patients without lymph node metastasis, 82 patients were with high ARL2 expression and 20 patients were with low ARL2 expression had a higher survival rate than those in the low ARL2 expression group (P=0.001) (Figure 3g). Among 80 CRC patients with lymph node metastasis, 63 patients were with high ARL2 expression and 17 patients were with low ARL2 expression had a higher survival rate than the low ARL2 expression group (P=0.014) (Figure 3h).

Age

Among 93 younger than 65 years CRC patients, 79 patients were with high ARL2 expression and 14 patients were with low ARL2 expression had no significant difference in overall survival (P=0.152) (Figure 3i). Among 89 aged 65 or older CRC patients, 66 patients were with high ARL2 expression and 23 patients were with low ARL2 expression had a higher survival rate than the low ARL2 expression group (P<0.001) (Figure 3j).

Distant metastases

Among 166 CRC patients without distant metastasis, 133 patients were with high ARL2 expression and 33 patients were with low ARL2 expression had a higher survival rate than the low ARL2 expression group (P<0.001) (Fig. 3k).

Among the 16 CRC patients with distant metastasis, 12 patients were with high expression and 4 patients were with low expression had no difference between the high expression group and the low expression group (P=0.683) (Figure 3l).

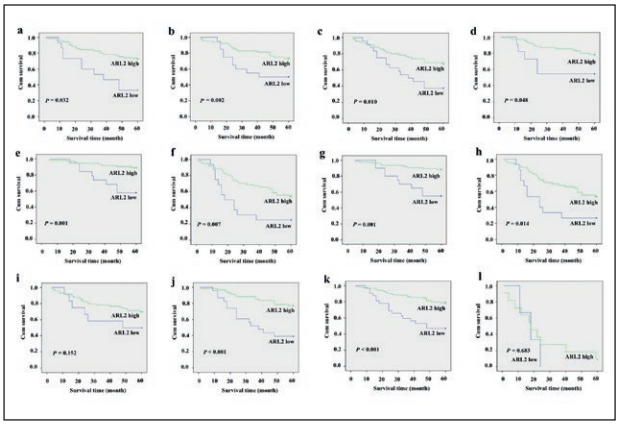

Figure 3: CRC subgroup analysis of overall cumulative survival. a Overall cumulative survival in the rectuma (P=0.032). b The Overall cumulative survival in the colon(P=0.002). c Overall cumulative survival in the male (P=0.010). d Overall cumulative survival in the female (P=0.048). e Overall cumulative survival in T1+T2 grade (P=0.001). f Overall cumulative survival in T3+T4 grade (P=0.007). g Overall cumulative survival in no lymph node metastasis (P=0.001). h Overall cumulative survival in lymph node metastasis (P=0.014). i Overall cumulative survival in younger than 65 years (P=0.152). j Overall cumulative survival in patients older than 65 years (P<0.001). k Overall cumulative survival in no distant metastasis (P<0.001). l Overall cumulative survival in distant metastasis (P=0.683).

Discussion

CRC is the most widespread malignant tumor worldwide, which 5-year survival rate remains low even with the development of new medical technologies in recent years[18]. The main reasons for poor prognosis are local invasion and distant metastasis[19]. Therefore, exploring the mechanism of CRC invasion and metastasis and searching for key genes can promote the radical cure of CRC.

ARL2 was strongly linked to the regulation of many kinds of tumors[10, 15]. The results of this study showed that it was higher of ARL2 protein expression in CRC tissues than that in adjacent tumor-free tissues, and was corelated with the TNM stage and survival status of CRC. If the expression of ARL2 is higher, much more CRC was in phase I and II, meanwhile it would with a higher 5-year survival rate. It indicated that ARL2 played a strong part in CRC, meanwhile, it may be a good indicator for the prognosis of CRC.

AXL is a oncogene in CRC. High expression of AXLcan be a marker of poor prognosis in CRC[14, 20]. In this study, We found that the expression of AXL in CRC tissues was higher than that in neighboring tumor-free tissues, and its high expression was related to TNM staging and distant metastasis. The AXL expression is higher, the more in TNM III and IV period in CRC and the higher the rate of distant metastasis.STAT3 is also a oncogene in CRC[21], and its regulatory pathways are complex[22]. In this study, we found that the expression of STAT3 in CRC tissues was higher than that in neighboring tumor-free tissues, and its high expression was related to distant metastasis. The higher expression of STAT3, the higher the incidence of distant metastasis was in CRC. In addition, AXL was negatively related to ARL2 in CRC by correlation analysis, while STAT3 was positively correlated with ARL2 and negatively correlated with AXL. COX multivariate survival analysis showed that positive expression of ARL2 and positive expression of AXL were independent risk factors for CRC survival. Therefore, we conclude that the expression of ARL2 maybe affect the expression of AXL negatively and plays a strong part in the development and progression of CRC.

In this paper, the 5-year CRC survival rate of the high ARL2 group with different tumor sites, gender, TNM stage and lymph node metastasis group was all higher than that of the low ARL2 group. The 5-year survival rate of CRC in the group under 65 years of age was not different from the expression level of ARL2, while there was no difference in the expression level of ARL2 in the distant metastasis group. Maybe it's because hereditary CRC syndromes are more common among younger patients[23]. This means that younger CRC patients have multiple gene mutations, as BRCA1 and BRCA2 Gene Mutations, MUTYH mutations, APC mutations, Mismatch repair (MMR) deficiency[24-27]. These genetic mutations can cause patients with advanced-stage (III or IV) disease, and CRC in younger patients appear histologically more aggressive[28, 29].

Young patients are often over treated in the adjuvant setting, with negligible survival benefit[30, 31], leading to an abnormal 5-year survival rate. How-

ever, older patients may be more likely to receive more reasonable treatment, leading to a more normal 5-year survival rate. In the distant metastasis group, the wide spread of the tumor made all organs more prone to failure, and the expression level of ARL2 had no influence on the 5-year survival rate.

In conclusion, there is high expression of ARL2 in CRC tissues, while its high expression improved 5-year survival in CRC. ARL2 maybe affect the expression of AXL negatively and is critical to the development and progress of CRC.

Therefore, ARL2 plays a strong part in the formation, invasion and metastasis of CRC. Detection of ARL2 may be a effective method for the prediction and metastasis of CRC. Therefore, studies on ARL2 expression in CRC are of great significance for the early diagnosis, treatment and prognosis of CRC. Interventions targeting ARL2 will provide new research directions and treatment approaches for CRC. This study can provide a preliminary assessment of the prognosis of CRC, and ARL2 may be a therapeutic target for CRC.

References

1) Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. The Lancet. 2019.394(10207): 1467-1480.
2) Uchiyama K, Yagi N, Mizushima K, et al. Serum metabolomics analysis for early detection of colorectal cancer. Journal of gastroenterology. 2016.52(6): 677-694.
3) Kosugi C, Koda K, Ishibashi K, et al. Safety of mFOLFOX6/XELOX as adjuvant chemotherapy after curative resection of stage III colon cancer: phase II clinical study (The FACOS study). International journal of colorectal disease. 2018.33(6): 809-817.
4) Wang J, Yan F, Zhao Q, et al. Circulating exosomal miR-125a-3p as a novel biomarker for early-stage colon cancer. Scientific Reports. 2017.7(1).
5) Buccafusca G, Proserpio I, Tralongo AC, et al. Early colorectal cancer: diagnosis, treatment and survivorship care. Critical reviews in oncology/hematology. 2019.136: 20-30.
6) Bailey LK, Campbell LJ, Evetts KA, et al. The Structure of Binder of Arl2 (BART) Reveals a Novel G Protein Binding Domain. Journal of Biological Chemistry. 2009.284(2): 992-999.
7) Béghin A, Matera EL, Brunet-Manquat S, et al. Expression of Arl2 is associated with p53 localization and chemosensitivity in a breast cancer cell line. Cell Cycle. 2008.7(19): 3074-3082.
8) Sun Z, Li A, Yu Z, et al. MicroRNA-497-5p Suppresses Tumor Cell Growth of Osteosarcoma by Targeting ADP Ribosylation Factor-Like Protein 2. Cancer biotherapy & radiopharmaceuticals. 2017.32(10): 371-378.
9) Peng R, Men J, Ma R, et al. MiR-214 down-regulates ARL2 and suppresses growth and invasion of cervical cancer cells. Biochemical and biophysical research communications. 2017.484(3): 623-630.
10) Li HJ, Sun XM, Li ZK, et al. LncRNA UCA1 Promotes Mitochondrial Function of Bladder Cancer via the MiR-195/ARL2 Signaling Pathway. Cellular Physiology and Biochemistry. 2017.43(6): 2548-2561.
11) Siddiqui FA, Alam C, Rosenqvist P, et al. PDE6D Inhibitors with a New Design Principle Selectively Block K-Ras Activity. ACS omega. 2020.5(1): 832-842.
12) Li K, Zhao B, Wei D, et al. Long non-coding RNA ANRIL enhances mitochondrial function of hepatocellular carcinoma by regulating the MiR-199a-5p/ARL2 axis. Environmental toxicology. 2020.35(3): 313-321.
13) Erika M, Giulia M, Claudia C, et al. AXL is an oncotarget in human colorectal cancer. Oncotarget. 2015.6(27): 23281-23296.
14) Uribe DJ, Mandell EK, Watson A, et al. The receptor tyrosine kinase AXL promotes migration and invasion in colorectal cancer. PloS one. 2017.12(7): e0179979.
15) Wang Y, Guan G, Cheng W, et al. ARL2 overexpression inhibits glioma proliferation and tumorigenicity via down-regulating AXL. BMC Cancer. 2018.18(1).
16) Aguilera TA, Giaccia AJ. Molecular Pathways: Oncologic Pathways and Their Role in T-cell Exclusion and Immune Evasion-A New Role for the AXL Receptor Tyrosine Kinase. Clinical Cancer Research. 2017.23(12): 2928-2933.
17) Hou PF, Jiang T, Chen F, et al. KIF4A facilitates cell proliferation via induction of p21-mediated cell cycle progression and promotes metastasis in colorectal cancer. Cell death & disease. 2018.9(5): 477.
18) The Lancet G, Hepatology. Colorectal cancer screening: is earlier better? The Lancet Gastroenterology & Hepatology. 2018.3(8): 519.
19) Resch A, Langner C. Risk Assessment in Early Colorectal Cancer: Histological and Molecular Markers. Digestive Diseases. 2015.33(1): 77-85.
20) Yang Lg, Cao Mz, Zhang J, et al. LncRNA XIST modulates HIF-1A/AXL signaling pathway by inhibiting miR-93-5p in colorectal cancer. Molecular Genetics & Genomic Medicine. 2020.8(4).
21) Schulz-Heddergott R, Stark N, Edmunds SJ, et al. Therapeutic Ablation of Gain-of-Function Mutant p53 in Colorectal Cancer Inhibits Stat3-Mediated Tumor Growth and Invasion. Cancer Cell. 2018.34(2): 298-314.
22) Ji K, Zhang M, Chu Q, et al. The Role of p-STAT3 as a Prognostic and Clinicopathological Marker in Colorectal Cancer: A Systematic Review and Meta-Analysis. PloS one. 2016.11(8): e0160125.
23) Strum WB, Boland CR. Clinical and Genetic Characteristics of Colorectal Cancer in Persons under 50 Years of Age: A Review. Digestive diseases and sciences. 2019.64(11): 3059-3065.
24) Oh M, McBride A, Yun S, Bhattacharjee S, Slack M, Martin JR, et al. BRCA1andBRCA2Gene Mutations and Colorectal Cancer Risk: Systematic Review and Meta-analysis. JNCI: Journal of the National Cancer Institute. 2018.110(11):1178-1189.

25) Yurgelun MB, Kulke MH, Fuchs CS, et al. Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer. Journal of Clinical Oncology. 2017.35(10): 1086-1095.
26) Aghabozorgi AS, Bahreyni A, Soleimani A, et al. Role of adenomatous polyposis coli (APC) gene mutations in the pathogenesis of colorectal cancer; current status and perspectives. Biochimie. 2019.157: 64-71.
27) Lee JJ, Chu E. Recent Advances in the Clinical Development of Immune Checkpoint Blockade Therapy for Mismatch Repair Proficient (pMMR)/non-MSI-H Metastatic Colorectal Cancer. Clinical Colorectal Cancer. 2018.17(4): 258-273.
28) Soyano AE, Baldeo C, Kasi PM. BRCA Mutation and Its Association With Colorectal Cancer. Clinical Colorectal Cancer. 2018.17(4): 647-650.
29) Boland PM, Yurgelun MB, Boland CR. Recent progress in Lynch syndrome and other familial colorectal cancer syndromes. CA: a cancer journal for clinicians. 2018.68(3): 217-231.
30) Patel SG, Ahnen DJ. Colorectal Cancer in the Young. Current gastroenterology reports. 2018.20(4):15.
31) Mauri G, Sartore-Bianchi A, Russo AG, et al. Early-onset colorectal cancer in young individuals. Molecular oncology. 2019.13(2): 109-131.

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