June 20, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11437-review.doc).

Title: Clinicopathological and prognostic relevance of ARID1A protein loss in colorectal cancer

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewed by 0505466, question 1: The manuscript may be improved by linguistic review. Please find attached the manuscript with highlighted some linguistic corrections.

Thank you for your kind revision. We have had our manuscript reviewed by a professional English language editing company mentioned in "The Revision Policies of BPG for Article". The linguistic errors have been checked and corrected all through the manuscript.

(2) Reviewed by 0505466, question 2: There is definitely a selection bias in the included stage IV patients. Patients with extended metastatic disease or/and poor performance status would probably not be operated on. According to international guidelines, patients with stage IV disease will receive initially systemic chemotherapy, except in the case of bowel obstruction or hemorrhage. This selection bias should be discussed.

Thank you very much for your attentive revision. There is a selection bias in stage IV patients included in our study. Considering our intent to test the status of ARID1A protein expression in the primary colorectal cancer tissues, we included only patients who received resection of their tumors in colon and rectum. For the 71 stage IV patients, there were 50 cases with bowel obstruction or hemorrhage before surgery. In the rest 21 patients, we identified 12 cases to be with resectable liver and/or lung metastases. The other 9 patients were with un-resectable metastases. None of the 71 patients were found to have extremely heavy tumor burden. Thus stage IV patients in our study didn't represent the general stage IV population. This issue was now discussed in the manuscript. Please see the fifth paragraph in DISCUSSION.

(3) Reviewed by 0505466, question 3: The authors state that all patients received chemotherapy according to NCCN guidelines. Does that mean that stage IV patients received different drug regimen than other patients in the adjuvant setting? This should be discussed.

All the patients received standard chemotherapy, which was established according to the NCCN guidelines of the time. The patients included in our study were diagnosed from 2001 to 2009. During this period, some important clinical trials contributed to the revision of NCCN guidelines and changed the clinical practice. There were some differences of drug regimens between stage IV and early stage patients. For example, there were more patients receiving single drug of fluorouracil. However, this didn't have much influence on the conclusions of our study. This issue was added into DISCUSSION, please see the fifth paragraph.

(4) Reviewed by 0505466, question 4: The authors note that none of the patients received

radiotherapy. That is remarkable since nowadays in the majority of cases with rectal cancer radiotherapy is preoperatively or less often postoperatively. Is the statement correct that none of the patients received radiotherapy? If actually radiotherapy has been given to patients, did radiotherapy have any influence on the results in the study?

Patients with preoperative radiotherapy were not included in this study, because tumor characteristics and ARID1A expression might be influenced by preoperative radiation. We had reviewed the medical records of patients with rectal cancer carefully. Some cases without metastatic rectal cancer received postoperative radiotherapy. However, only 3 cases received postoperative radiotherapy in stage IV patients with rectal cancer. These findings had little influence on the results in this study that ARID1A was associated with late TNM stage, distant metastasis and poor histological differentiation, as well as better prognosis in stage IV patients. And, we apologize for the inappropriate statement in our manuscript. Corrections have been made in the manuscript and we also talk about it in the fifth paragraph in DISSCUTION.

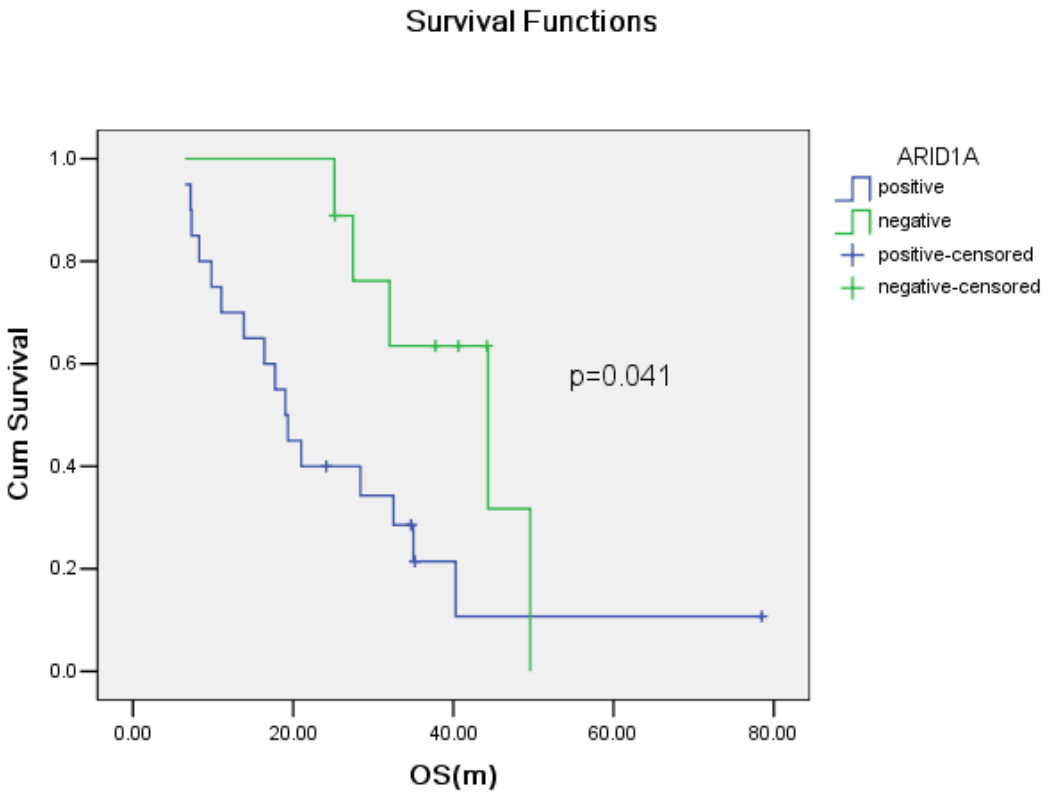
(5) Reviewed by 0505466, question 5: Colon and rectum cancer may not be the same disease. A comparison between the results of colon and rectum cancer patients may be interesting.

The expression of ARID1A protein was compared between colon and rectum cancer patients. There was no difference of ARID1A expression between colon and rectal cancer patients. Please see Table 2 in the manuscript for detailed information. In addition, we did further analyses for the correlation between ARID1A expression and the clinicopathological characteristics as well as overall survival in colon and rectal cancer patients respectively. The results were listed as follows:

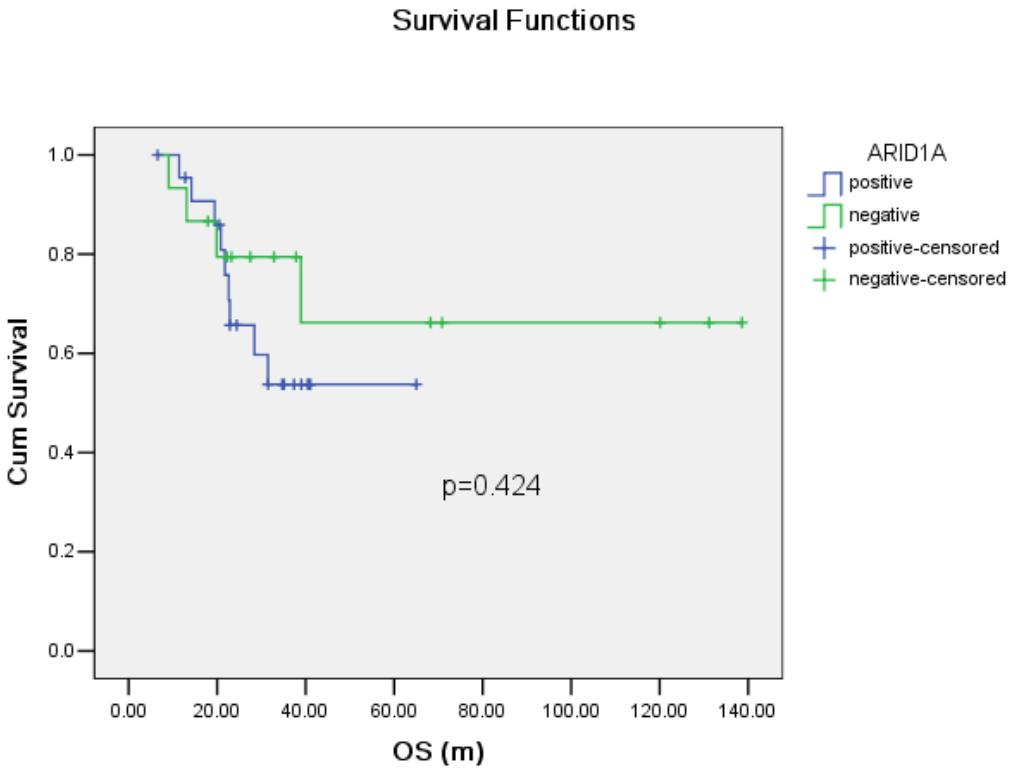
Supplementary Table 1 Correlation between ARID1A expression and the clinicopathological characteristics of patients with colon cancer and rectal cancer.

Characteristics	Colon cancer (n = 111)			Rectal cancer (n = 98)		
	ARID1a		*p* value	ARID1a		*p* value
	negative	positive		negative	positive	
Gender			0.600			**0.004**
Male	28 (87.5)	66 (83.5)		14 (63.6)	68 (89.5)	
Female	4 (12.5)	13 (16.5)		8 (36.4)	8 (10.5)	
Age			0.952			0.216
≦55	16 (50.0)	39 (49.4)		14 (63.6)	37 (48.7)	
> 55	16 (50.0)	40 (50.6)		8 (36.4)	39 (51.3)	
TNM stage (AJCC)			0.257			**0.009**
I	4 (12.5)	16 (20.3)		0 (0.0)	15 (19.7)	
II	8 (25.0)	23 (29.1)		5 (22.7)	12 (15.8)	
III	10 (31.3)	20 (25.3)		2 (9.1)	23 (30.3)	
IV	10 (31.3)	20 (25.3)		15 (68.2)	26 (34.2)	
T stage			0.208			0.084
T1	1 (3.1)	5 (6.3)		0 (0.0)	4 (5.3)	
T2	5 (15.6)	12 (15.2)		1 (4.5)	17 (22.4)	
T3	11 (34.4)	10 (12.7)		9 (40.9)	23 (30.3)	
T4	15 (46.9)	52 (65.8)		12 (54.5)	32 (42.1)	
N stage			0.827			0.573
N0	16 (50.0)	42 (53.2)		7 (31.8)	33 (43.4)	
N1	7 (21.9)	15 (19.0)		10 (45.5)	24 (31.6)	
N2	9 (28.1)	22 (27.8)		5 (22.7)	19 (25.0)	
M stage			0.524			**0.004**
M0	22 (68.8)	59 (74.7)		7 (31.8)	50 (65.8)	
M1	10 (31.3)	20 (25.3)		15 (68.2)	26 (34.2)	

Pathologic differentiation			0.938			**0.001**
Poor	4 (12.5)	14 (17.7)		10 (45.5)	8 (10.5)	
Moderate	25 (78.1)	54 (68.4)		11 (50.0)	58 (76.3)	
Well	3 (9.4)	11 (13.9)		1 (4.5)	10 (13.2)	
Tumor size (cm)			0.344			0.348
≦5	18 (56.3)	52 (65.8)		13 (59.1)	53 (69.7)	
> 5	14 (43.8)	27 (34.2)		9 (40.9)	23 (30.3)	



Supplementary Figure 1 The Kaplan-Meier curves for stage IV colon cancer patients with negative ARID1A expression versus positive ARID1A expression.



Supplementary Figure 2 The Kaplan-Meier curves for stage IV rectal cancer patients with negative ARID1A expression versus positive ARID1A expression.

The associations of ARID1A expression with clinicopathological characteristic and prognosis in colon cancer and rectal cancer were different in our study. ARID1A protein loss was associated with late TNM stage, distant metastasis, and poor pathologic differentiation in rectal cancer patients. However, no association was found in colon cancer patients (Supplementary Table 1). In contrary, the prognostic value of ARID1A expression was more apparent in patients with colon cancer compared with rectal cancer (Supplementary Figure 1 and 2). Multivariate survival analyses showed that ARID1A protein loss had a trend to be associated with better prognosis in patients with colon cancer [*HR* (95% *CI*): 2.71 (0.98-7.53)].

These results were concluded from subgroup analyses, and the sample sizes for colon cancer and rectal cancer were both small. The results were not inclusive. We didn't add them in the manuscript.

(6) Reviewed by 00343118, question 1: ARID1A is implicated in several cancers and till now poorly studied in CRC by imunohistochemistry. However, since data shown have an apparent inconsistency, regarding loss ARID1A between worst clinical feature and best OS in stages IV, it is require some molecular approches and discussion of the resulting data. Thus, mutations in ARID1A gene to be correlate with ARID1A expression, and as well as molecolar caracterizzaziotn of MSH1 alteration. Expression of ARID1A in teh cytoplasm could be also reported in a separated point.

Our study was based on previous reports about the association between ARID1A and cancers. The mutation frequency of ARID1A was higher in MSI patients in endometrial carcinomas [Mod Pathol. 2014 Feb;27(2):255-61;]. There were several studies demonstrating ARID1A gene mutation in colorectal cancer, and the mutation rate seemed higher in patients with MSI colorectal cancer [Nature, 2012, 487(7407): 330-337; Int J Cancer. 2014 Aug 1;135(3):611-23]. There had been reports that MSI was associated with poor differentiation and better survival in colorectal cancer. Thus the inconsistency in the associations of ARID1A protein loss with worst clinical feature and best OS might be partially

explained by its association with MSI. However, microsatellite instability was not routinely tested in our cancer center. We couldn't get information of MSI, thus we failed to analyze their associations in this study. It's a very interesting subject that deserves our exploration in future study.

ARID1A is a member of the SWI/SNF complexes, which function as ATP-dependent chromatin remodelers. These complexes remodel nucleosomes and to modulate transcription. Thus, effective ARID1A protein is located in nucleus. Previous studies of ARID1A by IHC also considered nuclear immunoreactivity as positive expression [Hum Pathol, 2013, 44(7): 1365-1374; J Pathol, 2011, 224(3): 328-333]. Thus, we didn't consider immunoreactivity of ARID1A protein in cytoplasm in this study.

(7) Reviewed by 00343118, question 2: Minor points: IHC and scoring section: PBST could be written in full. Table 1 it is lack a %.

Thank you for your attentive revision. We have written PBST in full and added the missing % in Table 1.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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