

Response to reviews

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Title: CD24 genetic variants contribute to overall survival in patients with gastric cancer

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This study enrolled a relative larger cohort, with good design and statistical analysis.

We sincerely thank you for your kind reviewing and advice.

However, there are several questions about this manuscript:

1. What is the influence of these SNPs on CD24 protein expression and function, low expression, decreased activity or alternative RNA splicing, and should be fully discussed.

Answer: The P-534C/A locates in the promoter region of CD24 and it may affect the transcriptional activity. P170C/T is a missense variant and it may alter the quantity and quality of CD24 protein. We have modified in our revised manuscript according to your advice.

2. From the results, these SNPs are not correlated with any clinicopathological characteristics of gastric cancer, while associated with patients' survival time. How to explain the role of CD24 in gastric development and progression by this result? Is it a driver gene or only passenger?

Answer: A large fraction of our gastric cancer patients came from the rural area near the city of our hospital. They had highly various duration of clinical

manifestation of tumor because of several factors such as the capability to pay or convenient accessibility of high-quality medical services. In this sense, CD24 may be not correlated with the clinicopathological characteristics of gastric cancer. However, when the tumors were eradically resected, all the patients were at the same starting line and we could evaluate the role of CD24 in the prognosis of gastric cancer after operation. Therefore, it is possible that CD24 may be associated with the prognosis of gastric cancer although CD24 was not associated the status of the tumors when the patients were diagnosed.

Our research did not study the underlying exact mechanism of the role of CD24 in the development, progression and prognosis of gastric cancer, thus we cannot determine whether CD24 was the driver gene of gastric cancer or just a passenger.

3. In introduction, the correlation between CD24 expression and gastric disorders after *Helicobacter felis* was mentioned. What about the relationship between CD24 SNPs and gastric cancer susceptibility in HP infected patients?

Answer: CD24 may play a role in HP-related inflammation as suggested in animal. However, we did not observe that CD24 was associated with HP-related gastric cancer as showed by the table below even adjusting for the possible influences of age and sex. And we did not include this table in our manuscript.

Stratified analysis of the association between SNPs of CD24 and gastric cancer

	HP (+)			HP (—)		
	cancer	case	<i>P</i>	cancer	case	<i>P</i>
P534						
A/A	92 (20.3)	108 (22.6)	0.4868	46 (21.4)	102 (21.1)	0.9494
A/C	242 (53.4)	237 (49.6)		111 (51.6)	245 (50.7)	
C/C	119 (26.3)	133 (27.8)		58 (27.0)	136 (28.2)	
P170						
C/C	193 (42.6)	219 (45.8)	0.6097	98 (45.6)	191 (39.5)	0.2786
C/T	209 (46.1)	207 (43.3)		94 (43.7)	227 (47.0)	
T/T	51 (11.3)	52 (10.9)		23 (10.7)	65 (13.5)	
P1527						
TG/TG	373 (82.3)	397 (83.1)	0.7733	177 (82.3)	414 (85.7)	0.2513
TG/del	80 (17.7)	81 (17.0)		38 (17.7)	69 (14.3)	

* *P* values were calculated by Chi-square test.

4. Patients with HP infection or gastric atrophy were diagnosed by ELISA test. The pathological diagnosis about HP infection or gastric atrophy should better be provided.

Answer: Indeed, diagnosis by pathological method is more accurate than ELISA. However, it is impractical as the control and the gastric atrophy groups are examinees from the health examination cancer. We can never require them to receive the invasive gastroscopy and biopsy if they do not have related symptoms. Therefore, we use the relatively low-invasive serum test by ELISA as suggested by Cao et al (Screening of atrophic gastritis and gastric cancer by serum pepsinogen, gastrin-17 and Helicobacter pylori immunoglobulin G antibodies. Journal of digestive diseases 2007; 8(1):15-22).

5. In result 1, the clinicopathological characteristics of gastric cancer should be provided. For those gastric cancer patients, what kinds of surgery were performed, radical or palliative? What about the post-operative treatment?

Answer: We have added the clinicopathological characteristics of gastric cancer in the table 1 and depicted them in the first part of the Results section.

Only gastric cancer patients receiving radical surgery were included in our study. Radiotherapy post operation is very rare that we did not analyze it. Post-operative chemotherapy is defined as the patients received the therapy for at least 3 cycles after surgery. One third of the patients received this type of therapy. By and large, chemotherapy was classified into three regimens: FOLFOX-4 regimen (combination with 5-fluorouracil, leucovorin and oxaliplatin); XELOX regimen (capecitabine and oxaliplatin) and other such as capecitabine or 5-fluorouracil alone. We analyzed the effect of chemotherapy on prognosis in our revised manuscript and found that post-operative chemotherapy protected patients from early death (Table 5).

6. In result 4, association of CD24 SNPs with disease free survival of gastric cancer patients should better be analyzed. The detail cox regression result should be provided.

Answer: Thank you for your advice. We agree with you that it is may be more

appropriate to evaluate the association of CD24 SNPs with the disease free survival of gastric cancer. However, we cannot obtain precise data on the disease free survival, as only a small fraction of our patients will do periodic physical examination because of the financial conditions and consciousness on health. Most of our patients will not go to hospital if they don't feel very bad. Therefore, for most subjects, we could only know that whether he/she is alive; if not, when he/she passed away and what is the main cause.

We detailed results of the multivariate COX regression model in Table 5 in the revised manuscript.

7. In result 5, the examples of IHC result with different SNP should be provided.

Answer: Thank you for your advice. We have added the relative results in our revised manuscript (Figure 3).