Author's response to reviews

Title: Relationship between nm23-H1 Gene Product Expression and Lymphatic and Blood Vessel Invasion in Esophageal Squamous Cell Carcinoma

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PDF covering letter
Dear Sir:

I would like to resubmit the enclosed, revised manuscript entitled “Relationship between nm23-H1 Gene Product Expression and Lymphatic and Blood Vessel Invasion in Esophageal Squamous Cell Carcinoma” by M. Tomita et. al. for publication in ‘BMC Cancer’.

Although we initially submitted this manuscript to BMC Surgery, we submit this revised version to BMC Cancer in accordance with editor’s advice.

We have modified our manuscript in accordance with the reviewers' comments. We hope these changes will make our manuscript acceptable for ‘BMC Cancer’.

Please forward any correspondence regarding this manuscript to me.

Thank you very much for your kind consideration.

Sincerely yours,

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Response to prof. Referee 1

Thank you very much for your kind consideration.

The introduction seems to only discuss positive findings and not any negative research studies for nm23. This gives the wrong impression that this is a good marker that can predict prognosis. It would have been nice if they had given a more complete discussion of the role of nm23 expression in esophageal squamous cell carcinoma in the introduction.

According to the reviewer’s comment, we added the previous negative study of nm23 in lung cancer. However, the previous study of nm23 in esophageal squamous cell carcinoma is very few and we could not find negative studies about nm23 and esophageal squamous cell carcinoma.

The methods are fairly complete but had the authors tried to do any antigen retrieval to get better staining? It is also unclear how they came up with their definitions of positive vs negative results. This should be discussed.

We did not perform any antigen retrieval in this study because previous studies about nm23 in esophageal squamous cell carcinoma also did not perform any antigen retrieval. Our definition of positive vs. negative results was decided according to our previous study. In the majority of nm23 positive tumors, most of tumor cells contained a homologous cytoplasmic pattern for nm23. Therefore, staining results were clearly distinguished to positive and negative. We added these results in our revised text.

The results as they stand seemed justified. It would have been nice to have more clinical follow-up on these patients. What was the recurrence rate for these two groups? I would predict that there are too few patients to provide any good results.

In our revised text, we investigated the prognostic impact of nm23-H1 in this study. As shown in our revised text, we could not find a prognostic significance of nm23-H1 in our series. However, we found that reduced expression of nm23-H1 in esophageal squamous cell carcinoma is associated with a shorter overall survival rate in patients with lymph node involvement but not in patients without lymph node involvement.
The conclusion of the paper (p 8 line 12) seems to be correct. “nm23-H1 gene product expression may not be associated with distant metastasis”. I would encourage the authors to continue these studies with more patients and better follow-up. I would encourage them to define their expression of nm23- H1 into low, moderate and high levels rather then yes versus no. This might make the data better correlated.

We will continue these studies and we will investigate the relationship between nm23-H1 and distant metastasis.

As described above, in the present study, staining results were clearly distinguished to positive and negative. Therefore, we could not subdivide into low, moderate and high levels.
Response to prof. Sung Hoon Noh

Thank you very much for your kind consideration.

1) Authors described that “Lymph node metastasis in the ly0+ly1 and ly2+ly3 groups was 6/19 (31.6%) and 17/26 (65.4%) respectively. Although these numbers indicate a definite trend toward a correlation between lymphatic vessel invasion and lymph node metastasis the data did not reach statistical significance.” However, when I analyzed these figures with Chi-Square test the p-value was 0.025 (by 2-tailed Pearson Chi-Square).

So if the authors had analyzed these data as they analyzed blood vessel invasion, they should show detailed data of lymph node involvement according to ly0, ly1, ly2, and ly3, respectively.

Thank you very much for your kind advices and sorry for our mistakes. We analyzed again and found that the result of Chi-Square test was our mistake. We analyzed all datas again in our revised text. Once again, thank you very much.

2) Authors explained the reason of grouping the lymphatic invasion as small numbers of ly0 and ly3. However, lymphatic vessel invasion is the first and necessary step at the outset of the metastatic cascade, as authors mentioned in discussion. So, ly1 may have its impact on metastasis though the degree of lymphatic invasion is low. For clarifying the role of nm23-H1 in lymphatic spread, it would be helpful to analyze the relationship between expression of nm23-H1 and lymphatic invasion, not between expression of nm23-H1 and degree of lymphatic invasion.

In most of previous studies, lymphatic vessel invasion (LVI) was classified into (+) and (-). In Japan, LVI was graded as ly0: none; ly1: mild; ly2: moderate; and ly3: severe. Since the number of patients with ly0 in our series was only 1, patients with LVI(-) seems to be considered as only 1 case. I consulted to a pathologist, and his answer is as follows: The patient with obvious LVI was diagnosed as ly2 or ly3. However, when pathologists could not decide whether LVI(+) or (-), they diagnose as ly1. Therefore, LVI(-) can be considered as ly0+ly1 and LVI(+) is consistent with ly2+ly3. Therefore, our result is equal to an analysis of the relationship between expression of nm23-H1 and lymphatic invasion.
3) As authors mentioned in the discussion, Iizuka et al. reported that reduced expression of nm23-H1 in esophageal squamous cell carcinoma is associated with a shorter overall survival rate in patients with lymph node involvement but not in patients without lymph node involvement. In this manuscript, expression of nm23-H1 is closely associated with lymphatic invasion. Therefore, to illuminate the prognostic impact of nm23-H1 esophageal squamous cell carcinoma in this study, authors should analyze the survival with the patients enrolled in this study.

According to the reviewer’s advice, we investigated the prognostic impact of nm23-H1 in this study. As shown in our revised text, we could not find a prognostic significance of nm23-H1 in our series. However, we also find that reduced expression of nm23-H1 in esophageal squamous cell carcinoma is associated with a shorter overall survival rate in patients with lymph node involvement but not in patients without lymph node involvement.