Author's response to reviews

Title: Expression of the RON receptor tyrosine kinase and its association with tumourigenic phenotypes in gastric carcinoma

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Author's response to reviews: see over
Dear Nicola Jones

MS: 1492538134207325

Expression of the RON receptor tyrosine kinase and its association with tumourigenic phenotypes in gastric carcinoma

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BMC Cancer

Thank you for the opportunity to revise our manuscript. The comments raised by the reviewers are very pertinent and instructive, and we have addressed these concerns as detailed below.

Additionally, we have modified the text of the Methods section to include the following additional information: “Gastric samples were used with the consent of the patients and permission of the Ethics Committees of Hospitals”.

Additionally, we ensure that BMC Cancer is the only journal currently considering the manuscript!

Best regards!
Zhou dong- hui!
September 12, 2008

Reviewer: Maria Flavia Di Renzo

The Authors did not mention the name of the anti-RON mouse monoclonal antibody used for IHC. However, the two mouse monoclonal antibodies available from Santa Cruz are both raised against the extracellular domain of the receptor. Although some intracellular staining might be expected in over expressing cells, membrane staining is expected. Unfortunately, membrane staining is not visible in their figures. In addition, labeling of paraffin-embedded material is not anticipated.

Comment1: Demonstrate the ability of this monoclonal Ab to label paraffin embedded, RON positive, cultured cells.
Response: Regarding the need for more specific antibody information, we have modified the text of the Methods section to include: “Anti-RON rabbit monoclonal antibody Ron β (C-20) (Santa Cruz Biotechnologies, USA) was used at a dilution of 1:400.” Additional specifics regarding antigen retrieval were also included in the Immunohistochemistry section of the Methods.

Regarding the suggestion to demonstrate antibody labeling of RON positive cultured cells, we would submit that although anti-RON rabbit monoclonal antibody Ron β is used to label the extracellular domain of the receptor, intracellular staining might be expected since the specimens were selected from an archival series of gastric cancers. We predict that membrane staining would be predominant if the stained specimens derived from samples previously frozen with liquid nitrogen, or alternatively, if the samples derived from an established cell line.

Comment 2: Show that another antibody, for which peptide used for immunization is available, labels similarly the same specimens and that staining could be displaced by the above peptide;

Response: We thank the reviewer for these suggestions. We did not perform staining studies of cultured cells, however, we repeated the labeling experiments using another RON-specific antibody (a gift from Prof. Yao and Prof. Wang) that was described in the following publication: “Altered expression of the RON receptor tyrosine kinase in various epithelial cancers and its contribution to tumourigenic phenotypes in thyroid cancer cells. J Pathol 2007, 213: 402-411). We found our results to be consistent with these published data.

Comment 3: if possible, show a similar ratio between the intensity of labeling of each specimen with IHC and the amount of protein detected by WB

Response: Sorry not to perform RON staining in the samples previously frozen with liquid nitrogen by IHC.
Reviewer: Ernest Camp

Comment 1: In the methods, the method of determining RON staining should be more clearly defined such as who performed the analysis or was it a blinded evaluation, at what magnification, and how many images per tumor were evaluated.
Response: We agree with the reviewer’s suggestions and have modified the text to include the following additional information: “The assessment of the grade of staining was determined using a blinded evaluation procedure by experienced pathologists. High-power fields (400×) using standard light microscopy were divided into ten sections which were randomly scored.”

Comment 2: The authors referenced paraneoplastic tissue throughout the paper as part of their evaluation. The significance of this evaluation needs to be made more clear. In the methods, paraneoplastic tissue should be better defined as well as in the results. It was unclear to me as to the significance of this portion of the analysis as compared to the RON staining of the neoplastic tissue.
Response: The paraneoplastic tissues analyzed in this study included 29 samples that were taken from the gastric mucous layer 0.5 cm away from various paraffin-embedded gastric carcinoma specimens. Gastric cancer tissue is associated with the epithelial dysplasia of the stomach, which due to its proximity to the gastric carcinoma, is considered to represent a region of possible precancerous lesions. Therefore, by reporting the expression of RON in paraneoplastic tissue, we are providing data to support our hypothesis that RON plays an important role in promoting the transformation of normal cells to a malignant phenotype. We have revised the first paragraph of the Results section and in the second paragraph of the Discussion section to include these points of clarification regarding the use of paraneoplastic tissues in this study.

Comment 3: The observation of the RON splice variants is very interesting
although the authors did not report the significance in terms of the relationship to clinicopathologic factors. Further analysis would be very interesting and clinical correlation with the splice variants would be important to understand.

Response: We agree with the reviewer’s suggestion, and unfortunately were unable to report additional clinicopathologic significance of our RON splice variant data due to the limited number of frozen tissues available for Western blotting analysis. However, the results from the Western blotting analysis did show that RON splice variant, RON-165, was present and we hypothesize that detection of RON protein expression in our 98 cases of gastric cancer may also include expression of RON splice variants, and further testing would be needed to confirm this.

Comment 4: In the discussion, the statement discussing the significance of RON staining“furthermore, the results of the study…..” is unclear and need rewording. Since no correlation was observed with overall prognosis and long-term survival, this summary statement needs to more reflect the findings in the results section.

Response: We agree with the reviewer’s suggestion that this statement was unclear, and upon revisiting this portion of the text for revision, we found that was an unnecessary statement. We modified the subsequent sentence to more clearly explain our comparison between RON expression data from other tissue models with the RON expression data presented in this study of gastric cancer that indicates that expression of RON may be a valuable marker in detecting malignant tissues.

Comment 5: In the title, the use of tumourigenic phenotypes is unclear and should be revised.

Response: We agree with the reviewer’s suggestion and have revised the title to read: “Expression of the RON receptor tyrosine kinase and its association with gastric carcinoma versus normal gastric tissues”.