Author's response to reviews

Title: Hypoxia-inducible factor-1 alpha, in association with inflammation, angiogenesis and MYC, is a critical prognostic factor in patients with HCC after surgery

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Author's response to reviews: see over
Dear Prof. Sabina Alam,

Thank you very much for your kind letter of “MS: 3931181912856725” on September 7, 2009. We do appreciate the opportunity to resubmit our manuscript and are grateful for the reviewers’ and Editor’s constructive comments. Your valuable advices would be most helpful in optimizing our manuscript. Based on your comments and requests, we have made some modifications and explanations on the original manuscript. Here, we attached the revised manuscript for your further evaluation.

A document answering every question from the reviewers and changes in the manuscript, together with a file documenting some additional changes, was summarized and enclosed. Also, a revised manuscript with the correction sections red marked was attached as the supplemental material and for easy check/editing purpose. Should you have any questions, please contact us without hesitation.

We hope you and the referees will find our revised manuscript acceptable and the paper meets the editorial requirements. It’s also our great pleasure to share our experience with other scientists. We look forward to your expert advice and that of your editorial staff.

With great regards,

Yours Sincerely,
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Responses to the reviewers

- **Response to Reviewer: Prof. YUTAKA YAMAMOTO**

1. The authors should change from tumor hypoxia to HIF-1 alpha in Title.

   **Response:** We are greatly appreciative of the reviewer’s favorable comments. We accept that “hypoxic condition in tumor is not identical the expression levels of HIF-1alpha although HIF-1alpha is a master regulator of essential adaptive responses to hypoxia”. The changed title following the suggestion is more accurate to reflect our present research.

   **Related changes in the revised manuscript:**

   We have changed from “tumor hypoxia” to “Hypoxia-inducible factor-1alpha” in Title.

2. The authors should change or delete the word “hypoxia” in line 3, 16 and 18 in Abstract, last line in Background session, line 7, 22, 59, 60 and 61 in Discussion session and line 3 and 4 in Conclusions session.

   **Response:** We are grateful to the reviewer for the carefulness. This suggestion made our manuscript become focused on the role of HIF-1alpha.

   **Related changes in the revised manuscript:**

   We have changed the words “hypoxia” to “HIF-1 alpha” in lines 3, 16 and 18 in the Abstract. In last line in the Background, we also changed the word “hypoxia” to “the crucial factor” representing “HIF-1 alpha” mentioned in the same sentence. We deleted the sentence “In other words, tumor hypoxia is one of the most critical factors for prognostication of HCC” in line 7 in the Discussion. The words “hypoxia” in lines 22, 59, 60 and 62 in the Discussion of the original manuscript have been changed to “HIF-1 alpha”. For the same reason, we also delete the sentence “Although the exact mechanisms remain to be fully understood, the expression of HIF-1α could be a putative and specific index to
represent, as well as the molecular target of, the tumor hypoxia and to study the correlation with other pathophysiological processes.” in line 28 in the Background of the original manuscript.

- **Response to Reviewer : Prof. Goshi Shiota**

*Question 1:* Why are the molecules including Cox-2, MMP7,9, VEGF, etc selected? These molecules are associated with HCC, however its significance and importance do not seem to have impacts.

*Response:* Thank you for reminding us. We have described it in the Background of the revised manuscript. These factors were selected for at least two of the three aspects: being crucial in inflammation or angiogenesis; being active in tumor metastasis or invasion; being mechanistically related to hypoxia. We do know that a lot of factors were related to inflammation or angiogenesis or hypoxia, but we can only selected few of them which were important and representative that we believed based on literatures. The significance and importance of these molecules in HCC have been thoroughly described in lines 41-45 in the Discussion.

**Related changes in the revised manuscript:**

1. The sentence “These factors were selected for at least two of the three aspects: being crucial in inflammation or angiogenesis; being active in tumor metastasis or invasion; being mechanistically related to hypoxia.” was added in the Background.

2. The sentences “COX-2 may regulate HCC growth by COX-2-derived PG signaling pathway. PDGFRA may regulate tumor angiogenesis by PDGFRA-p70S6K pathway which is related to the function of fibroblast growth factor (FGF) and expression of VEGF and hepatocytes growth factor (HGF). MMPs increase HCC invasion and growth through the degradation of extracellular matrix ” were added in lines 41-45 in the Discussion.

*Questions 2:* Why did the authors pick up HIF-1 alpha? The explanation is not
enough.

**Response:** Hypoxic condition in tumor is not identical the expression levels of HIF-1alpha although HIF-1alpha is a master regulator of essential adaptive responses to hypoxia. According to the requirements of another reviewer, we have changed the title and some unsuitable words in manuscript so that it can accurately reflect that our research is focused on HIF-1 alpha instead of hypoxia.

**Related changes in the revised manuscript:**

We have changed “tumor hypoxia” to “Hypoxia-inducible factor-1alpha” in the Title. We have also changed the words “hypoxia” to “HIF-1 alpha” in lines 3, 16 and 18 in the Abstract. In last line in the Background, we changed the word “hypoxia” to “the crucial factor” representing “HIF-1 alpha” mentioned in the same sentence. We deleted the sentence “In other words, tumor hypoxia is one of the most critical factors for prognostication of HCC” in line 7 in the Discussion. The words “hypoxia” in line 22, 59, 60 and 62 in the Discussion of the original manuscript has been changed to “HIF-1 alpha”. We also deleted the sentence “Although the exact mechanisms remain to be fully understood, the expression of HIF-1α could be a putative and specific index to represent, as well as the molecular target of, the tumor hypoxia and to study the correlation with other pathophysiological processes.” in line 28 in the Background of the original manuscript.

**Questions 3:** Are there array data in manuscript?

**Response:** The gene expression data in the current study contained no array based data like cDNA microarray and oligonucleotide microarray. All the relative expression data were generated using real time quantitative reverse transcriptase PCR analysis using SYBR green I dye.

**Questions 4:** English is poor.

**Response:** Thank you for your creative comments. We tried our best to improve
the English including getting help from our native English speaking friends.

**Related changes in the revised manuscript:**

Some additional language flaws were detected and carefully corrected accordingly. For example, factor should be factors in line 4 of the Conclusions. We deleted the sentence “where tumor recurrence is largely due to intrahepatic dissemination” in line 10 in Background which is little correlated to our topic. We changed the word “were” to “was” in line 13 in “Prognosis” of the Results, and “play” to “plays”, “cooperate” to “cooperates”, “have” to “has” in the Discussion. All the changes were addressed in our revised manuscript.
Additional changes in our manuscript

1. For the completion of our data, a new additional file (Additional file 2: Additional table 1 for degree of HIF-1 alpha immunohistochemical staining) was added and marked in *Tissue microarray and Immunohistochemistry* of Methods. The orders of other additional files were also changed in paper.

2. Two new references were added in lines 41-45 in the Discussion to explain the importance and significance of COX-2 and MMPs according to the comments of the reviewer. Consequently, the orders of other references were also changed in paper.

3. We list the information of Additional files in a separate section of our revised manuscript while the original manuscript not.