Answering Reviewers (*00503539*)

Manuscript Number	5780
Manuscript Title	Meta-analysis of HIF-1a Immunohistochemical Expression as a Prognostic Role in Gastric Cancer

Specific comments:

1. Abstract: The authors described that "Further studies are required to confirm these results." in Abstract. There is, however, no further description about this problem. The concrete proposal about this problem should be provided in Discussion.

Because of some limitations and heterogeneity, we hope that further studies are required. For example, a large size and multi center study. We can revise it as "Further studies of large sample and multi center are required to confirm these results" if reviewers feel necessary.

Thanks for reviewer's suggestion.

2. Introduction: The authors referred "low-quality" and "high-quality" studies. Is this description appropriate? If appropriate, what are the criteria of both qualities?

The criteria for nonrandomised studies, including case-control and cohort studies, is the Newcastle-Ottawa quality assessment scale (NOS); and "high-quality" studies was graded as level (6 to 9 scores). Actually, one articles *[Hypoxia-inducible factor-1alpha expression and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncol Rep. 2003 Jul-Aug; 10(4):797-802.]* **included by the published meta-analysis reported HIF-1a for gastric cancer was inappropriate, because this article reported GIST, not gastric cancer. If reviewer considered "low-quality" and "high-quality" studies improper, we can delete it.**

Thanks for reviewer's suggestion.

3. Methods: The criteria of HIF-1a positive case are not clear. What the proportion of positive cancer cells per total cancer cells in HIF-1a positive cases?

4. Methods: It may be better that tumor location and tumor size could be included in clinicopathological features.

The criteria of HIF-1a positive case and the proportion of positive cancer cells per total cancer cells in HIF-1a positive cases, was not uniform for some selected studies. The tumor location and tumor size was not included in all clinicopathological features. It was impossible to match the criteria of HIF-1a positive case and patient characteristics in all of the studies, so we have applied a random effects model to take between-study variation into consideration to limit influence. This status was also one of several limitations for the results of this meta-analysis. And further studies of large sample and multi center are required.

Thanks for reviewer's suggestion.

5. Discussion: "Gastric cancers do not usually express HIF-1a, and 548 (50%) of 1,103 gastric cancer patients had HIF-1a positive expression in this meta-analysis." Is this sentence right? It seems that the 50% may be unusual. "However, once gastric cancer cells acquire HIF-1a expression, ….." What is the mechanism of acquisition of HIF-1a expression by gastric cancer cells?

This is our negligence, and this sentence had some logical problem. Thanks for reviewer's suggestion.

Revision:

"**Not all of gastric cancers express HIF-1a and 548 (50%) of 1103**

gastric cancer patients had HIF-1a positive expression in this meta-analysis. However, once gastric cancer cells acquire HIF-1a expression, they transform to more aggressive and metastatic behavior."

What is the mechanism of acquisition of HIF-1a expression by gastric cancer cells?

Hypoxia stress is a common phenomenon in human solid tumors and plays a critical role in tumor progression. Under hypoxia condition, HIF-1 is induced and activates many kinds of hypoxia-induced genes. HIF-1 consists of HIF-1a and HIF-1b subunits, HIF-1b is constitutively expressed, while HIF-1a is the functional subunit and is regulated by oxygen. Under normoxic conditions, HIF-1α is rapidly degraded, while under hypoxic conditions, HIF-1α heterodimerizes with the sustaining expression and increases the transcription of target genes, which include vascular endothelial growth factor, erythropoietin, glucose transporters, glycolytic enzymes, insulin-like growth factor II, and hexokinase-II, which was involved in many aspects of cancer biology including cell survival, glucose metabolism, cell invasion, and angiogenesis.

Thanks for reviewer's suggestion.

Manuscript Number	5780
Manuscript Title	Meta-analysis of HIF-1a Immunohistochemical Expression as a Prognostic Role in Gastric Cancer
Review Time	2013-10-07 23:38

META-ANALYSIS OF HIF-1A IMMUNOHISTOCHEMICAL EXPRESSION AS A PROGNOSTIC ROLE IN GASTRIC CANCER

Ⅰ. In this paper the authors tried to perform a meta-analysis about the role of HIF-1A in gastric cancer, based on only 9 from about 100 studies published in the literature.

We have performed this meta-analysis based on 9 from about 221 studies published in the literature, not 9 from 100 studies.

Thanks for reviewer's suggestion.

Ⅱ Although the idea of paper is valuable, it is quite similar with a recent paper published by Zhang ZG et al. in 2013, Asian Pac J Cancer Prev (Title 7 from Reference list).

There are some differences between our meta-analysis and the recent paper published by Zhang ZG et al. in 2013, Asian Pac J Cancer Prev.

The published meta-analysis has some defects. First, one articles *[Hypoxia-inducible factor-1alpha expression and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncol Rep. 2003 Jul-Aug; 10(4):797-802.]* **included by the published meta-analysis reported HIF-1a for gastric cancer was inappropriate, because this article reported GIST, not gastric cancer. Second, the method, for example, the Parmar method, was not mentioned how they extracted data about overall survival (OS) and disease free survival (DFS) when some studies did not have direct information. Third, there are**

three "high-quality" studies in our meta-analysis published this year. (Title 8-10 from Reference list). We think these articles are important to make a comprehensive meta-analysis. Finally, we analyzed the relationship between the expression of HIF-1a and clinicopathologic features of gastric cancer, which is very useful to provide information for decision-making in gastric cancer clinically. But the published meta-analysis has not mentioned it.

Thanks for reviewer's suggestion.

Ⅲ. There are several aspects that do not allow publication of the paper in the present format. However, the paper can be modified and submitted to other journal, according to the following suggestions:

We feel very sorry for your evaluation. We think our article is very strict and valuable, and it is very suitable for publication in this journal, because World Journal of Gastroenterology is a high impact papers.

Thanks for reviewer's suggestion.

1. TITLE PAGE Title – I suggest replacing the meta-analysis with Review because meta-analysis should involve a higher number of studies, nine of them being enough for such statistical study Authors – although the paper reports a pathological aspect of gastric cancer, the seven authors are surgeons. I suggest reducing the number of authors – two many for nine studies – and to ask for a pathologist point of vision and include him in the list of authors.

As is known to all, there is a difference between meta-analysis and review. Meta-analysis, or quantitative synthesis, is the technique of statistically combining the results of different studies, done on

different samples that have each examined and presented findings on a similar relationship. But review is usually a qualitative study, with a strong subjective consciousness, no strict practices. This study is meta-analysis, not review.

We do not think it is necessary to include a pathologist in the list of authors, although we have received some interesting suggestions from a pathologist. Authorship credit should be in accordance with the standard proposed by the International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Thanks for reviewer's suggestion.

2. ABSTRACT - Aim: A meta-analysis should include more than 24 titles, as in this paper. - Results: This part of the abstract is partially incorrect. One hand, because the authors mentioned that HIF1a expression correlated with worse tumor differentiation…..what about the other types of gastric tumors excluding the adenocarcinomas??? – what about mucinos, poorly cohesive, neuroendocrine, lymphomas….if it is a metaanalysis of gastric CANCER, it should include all tumors form WHO classification. If not, the title and study design should be changed. On the other hand, the nine studies included in this analysis were published before and after 2010 – this is a very important aspect that disqualify the study for publication – a pathologist would be contacted for a restaging of the tumors – according with the AJCC classification VIth edition or VIIth edition used from 2010…..WITHOUT THIS RESTAGING THIS STUDY IS TOTALLY UNADEQUATED. More than, because HIF1a is involved in angiogenesis, a correlation with serum and histological aspects of VEGF is mandatory to be included….

As we know, a meta-analysis does not require that "aim" in the

abstract should include more than 24 titles.

The terms used for the search were: "HIF-1a ", "hypoxia-inducible factor-1a" and "Gastric Cancer", " Gastric Neoplasm", "Stomach Neoplasm". We think those terms is enough for our meta-analysis.

It was impossible to restage of the tumors in all of the studies, so we have applied a random effects model to take between-study variation into consideration to limit influence. This status was also one of several limitations for the results of this meta-analysis.

Thanks for reviewer's suggestion.

3. INTRODUCTION - first paragraph – second phrase – both histological differentiation (tumor differentiation is an improper term) and tumor type should be taken into account; fifth phrase – wrong spelling - second paragraph – second phrase – last part (from '....which was known...' should be removed – the authors already mentioned this aspect in first paragraph); same for last phrase

4. METHODS - Study selection ? First paragraph – 'gastric carcinoma'should also be included as key term - Outcomes of interest ? First paragraph – both histological differentiation (tumor differentiation is an improper term) and tumor type should be taken into account and TNM staging system should be mentioned – ideally, it is to restaging the tumors according to the VIIth edition of AJCC ? Second paragraph – to exclude papers wrote by same team all authors should be extracted, not only first one

5. RESULTS - Selection of trials ? It is not a proper selection – few studies (9) - only one study from Europe, with several genetic differences compared with Asian patients – this is the reason why the study from Turkey had results discordants to the other studies (see the graphics) - papers published before and after 2010, with different staging systems - after consulting of results, I can see that the studies are inhomogenous, reason why, in graphics, an improper reporting of results occured, only 4-5 studies being included for specific parameters… ? I suggest to reduce this paragraph and to resume it in tables – there are too many numbers and data, very difficult to understand them

We feel very sorry that we can not accept you suggestions.

Thanks for reviewer's suggestion.

6. DISCUSSION - First paragraph ? first phrase should be reworded, being difficult to be understood - second phrase – this is not a comprehensive study or a detailed meta-analysis…..It is a review –.

We insist that our manuscript is a meta-analysis, not a review.

Thanks for reviewer's suggestion.