

Peer Review File

Article information: <http://dx.doi.org/10.21037/tcr-20-2252>

Review Comments:

Nuclear factor 90 (NF90), one of the Double-stranded RNA binding proteins, is involved in many cellular biological processes, includes cell proliferation, differentiation, angiogenesis, cell cycle and immunity. In the manuscript “Decreased expression of nuclear factor 90 correlates with worse outcomes in nasopharyngeal carcinoma”, authors uncovered the relationship between the NF90 expression by immunohistochemistry (IHC) and clinical outcomes in NPC patients.

Couple questions are required to be answered before it will be accepted.

(1) There was similar report (Mol Med Rep. 2019 Jul;20(1):125-134) about NF90 as a prognostic marker for gastric cancer in the PubMed. What is the novel idea in the paper? Please elaborate in the introduction.

Reply: The clinical significance of NF90 for nasopharyngeal carcinoma was uncovered for the first time, although other cancers have been reported, including gastric cancer. (Page 3, Line 63-68)

(2) The NF90 and NF45 was a complex (Theranostics. 2018 Nov 10;8(20):5676-5689). Why to only focus on NF90, not NF45 in the paper? Whether NF45 could also be a biomarker for nasopharyngeal carcinoma?

Reply: Thank you for your advice, we also observed this relationship between NF90 and NF 45. We selected NF90 in this study just because NF90 has been reported in other cancers, besides nasopharyngeal carcinoma, this implied NF90 may play more important role in carcinogenesis than NF45 to some extent. And the mechanism of occurrence of NF90 in nasopharyngeal carcinoma, especially the relationship with NF45, will be further revealed in the mechanism experiment.

(3) It is more convincing to validate the effects of NF90 in NPC cells by in vitro and in vivo experiments.

Reply: Yes, thanks for your good idea, this work have been in progress. We will report it in the next paper.

(4) The prognosis and survival of patients was correlated with treatment. In 216 enrolled patients, whether all patients received the same treatment program?

Reply: We agree with your viewpoint. In this study, all the 216 patients received the treatment program according to the AJCC staging system, which the same treatment program was administered by the same TNM stage. The mainly treatment program of NPC was based on radiotherapy, which may plus chemotherapy according to different

TNM stage. And in the statistical analysis we have taken the treatment as a variable into account, especially in univariate and multivariate survival analysis (Table 2,3).

(5) In the figure 2, it is better to provide more magnification of images. In the figure 2 legend, please supplement the illustration of scale bar.

Reply: The images in this study were very clear in the uploaded TIF format, but the transformation course of PDF may reduce resolution of these images. We will upload these images by attachment.

(6) IRS was a subjective assessment. I suggest to test the expression of NF90 by qRT-PCR and Western blot additionally.

Reply: Thanks for your suggestion. Firstly, IHC method is a semi-quantitative detection certain protein expression of routine work, so it is relatively objective. Secondly, we enrolled large samples to avoid subjectivity. Thirdly, this IRS assessment method was widely used in published papers. Lastly, we performed it by two independent pathologists and used ROC method to avoid the subjectivity (Page 4, Line-92-99). The mechanism of NF90 in NPC is in progress, and will uncover soon after.

(7) It was showed that NF90 expression was predominantly in the nucleus. But, the positive signals were not found in the nucleus. It is necessary to test the expression of NF90 by fluorescent IHC and co-staining with Hoechst or DAPI.

Reply: NF90 expression has been reported in the nucleus in other malignancies, and the same result was observed in nasopharyngeal carcinoma. The clear images will upload by attachment (Fig 2).

(8) The expression of NF90 was elevated in NPC tissues. In the results, why to say that over-expression of NF90 predicted favorable progression free survival (PFS) and overall survival (OS) in NPC? Please supplement in the discussion.

Reply: Thank you very much. In this study, we found that NF90 expression was elevated in NPC tissues, and elevated expression of NF90 was a protective factor or a suppressor from NPC progression by log-rank test and Cox regression analysis, so we say over-expression of NF90 predicted favorable PFS and OS in NPC. (Page7. Line175-183)

(9) How to identify the NF90 as an independent risk factor for NPC prognosis? Please supplement the illustration of independent risk factor in the discussion.

Reply: Thank you for your suggestion, in this study, we have found the NF90 was an independent risk factor for NPC patients by Cox regression method, please turn to the Page 7, Line 175-183 for our demonstration.