Author's response to reviews

Title: Transcription factor AP-1 in esophageal squamous cell carcinoma: Alterations in activity and expression during Human Papillomavirus infection

Authors:

Showket Hussain (shussain1712@yahoo.co.in)
Alok C. Bharti (alokcharti@icmr.org.in)
Irfana Salam (irfanasalam@gmail.com)
Mohammad A. Bhat (drmakbarbhat@yahoo.co.uk)
Mohammad M. Mir (mirmuzaffar11@gmail.com)
Suresh Hedau (suresh_hedau@hotmail.com)
Mushtaq A. Siddiqi (siddiqimushtaq@yahoo.co.in)
Seemi F. Basir (seemifb@gmail.com)
Bhudev C. Das (dasbc@acbr.du.ac.in)

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Author's response to reviews: see over
Prof. Bhudev C. Das, Ph.D, FNASc, FASc, FAMS, FNA
(Prof. Gurbaksh Singh Chair)  J.C. Bose National Fellow

To,

Prof. Diana Marshall
Scientific Editor
BMC Cancer

Dear Prof. Marshall,

This has reference to our manuscript no. 1821723027296826 entitled “Transcription factor AP-1 in esophageal squamous cell carcinoma: Alterations in activity and expression during Human Papillomavirus infection”. Thank you very much for your mail and kindly giving us an opportunity to revise our manuscript for publication in your esteemed journal-“BMC-Cancer”. We are thankful to both the Referees and the Associate Editor for their helpful comments. We have now revised our manuscript taking their each and every comment into consideration and have done the English corrections to the best of our ability and understanding. The corrections are highlighted in red in the text of the manuscript and the point-wise answers to Referee’s comments are as follows:

Referee No.1

Q1. In Figure 2, free probe lanes from normal tissues appear to be less intense in comparison to those from tumor tissues. The authors have to show the full gel or explain how equal loading in each lane was ensured.

Ans: We have now given the full gel picture of the band shift assay as advised by the referee. It is also to confirm that an equal amount of protein is always taken for both band shift assay and western blotting.

Q2. In western blotting experiments, it is not clear if beta actin control was done for each of the proteins analyzed and if the authors did any quantitation.

Ans: In western blotting experiments β-actin was done for each of the protein tested by stripping and reprobing the blot. Quantitation of the bands was performed and the description of methodology used has now been elaborated in method section page 8-9.

Q3. c-Jun appears to be more in tumor tissue (Figure 5) compared to normal tissue. It is not clear as to why this does not participate in AP-1 formation even after it is upregulated. Since authors analyzed the total protein and not nuclear fraction the interpretation is difficult for their results with western blotting.

Ans: Many thanks to the referee for pointing out this aspect and we agree with him, but it is well known that sometime even if the protein is highly expressed (as revealed by western blotting), it does not participate in DNA binding activity. Furthermore, in the present study as already indicated by the referee that our western blotting was done on total protein and not on nuclear fraction, hence it is possible to observe over expression of c-jun in total proteins. Alternately, Jun-B may compete out c-jun in AP-1 complex formation. This aspect has been discussed appropriately in the text with relevant references in page 15 Para 2.

Referee No.2

We are thankful to referee 2 for his constructive comments indicating some grammatical and typing errors which have been taken care of.

Answers to Comments of Associate Editor

1. Description of results in Abstract has been modified as suggested.
2. The final conclusion both in Abstract as well as in Discussion has been rewritten and modified as advised.
3. Author’s contributions have been modified as per the guidelines of the Journal.

I hope, you will now find the manuscript in order and it would be acceptable to you. We look forward to your early favorable decision on it.

With warm regards,

Yours sincerely,

(B.C. Das)