

Peer Review File

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Review Comments

In this manuscript, Wang et al. demonstrate that numb expressed is decreased in cervical cancer and its depletion in SiHa cervical cancer cells promotes proliferation, migration and invasion. Whilst potentially interesting, all of the data is over-interpreted by the authors and several statements are not supported by the data.

- All of the mechanistic data concerning the role of numb in cervical cancer pathogenesis is done in SiHa cells. Therefore the manuscript must be changed from 'Numb suppression promoted the progression of squamous carcinoma of the cervix by stimulating Notch and Hedgehog signaling pathways' to 'Numb suppression promoted the pathogenesis of SiHa cells by stimulating Notch and Hedgehog signaling pathways'

Reply:

Thanks for the reviewer's advice and we have modified our text as advised (see Page 1, line 1-2).

- line 57 - '...genetic alterations play the key role for tumor genesis and progression.(9-11) Reference 10 is concerning numb and hedgehog signalling and is not appropriate here. A review on HPV mediated oncogenesis would be better (such as Scarth et al., J Gen Virol, 2021)

Reply:

Thanks for the reviewer's advice and we have changed the reference10 to 'James A Scarth, Molly R Patterson, Ethan L Morgan, Andrew Macdonald. The human papillomavirus oncoproteins: a review of the host pathways targeted on the road to transformation. The Journal of general virology. 2021;102(3):001540.' (see Page 14, line 344-346).

- Figure 1B - the IF image of SiHa cells is out of focus - please replace image with a better quality, in focus image

Reply:

Thanks for the reviewer's advice and we have replaced the image (see Fig 1).

- line 181 - 'It is suggested that Numb may be involved in the process of cervical cancer, and the main role may be in the nucleus.' None of the data in Figure 1A-B suggest this - therefore, this statement should be removed

Reply:

Thanks for the reviewer's advice and we have deleted this sentence (see Page 9, line 176).

- Figure 1C - the b-actin blot is not acceptable (bands have merged) - please replace image with a better quality image

Reply:

Thanks for the reviewer's advice and we have replaced the image (see Fig 1).

- The 'cell proliferation' assay in Figure 1D is insufficiently described in both the methods section and the figure legend. How is the percentage defined? EdU incorporation assays detected cells in S phase and is not a direct readout of cell proliferation. This must be described in much greater detail

Reply:

Thanks for the reviewer's advice and the 'cell proliferation' was described in methods section (see Page 7, line 143-144).

- line 205 - 'Collectively, our observations indicated that suppression of Numb in cervical squamous carcinoma cells dramatically accelerated...' must be replaced with 'Collectively, our observations indicated that suppression of Numb in SiHa cervical squamous carcinoma cells dramatically accelerated...'

Reply:

Thanks for the reviewer's advice and we have modified our text as advised (see Page 10, line 198-200).

- Figure 3 - all of the b-actin blots are not acceptable (bands have merged) - please replace images with a better quality images

Reply:

Thanks for the reviewer's advice and we have replaced the image (see Fig 3).

- line 214 - 'The upregulation of protein levels suggested Numb suppression stimulated Notch and Hedgehog-dependent malignant transformation.' The data does not show this - it shows that numb regulates the expression of Notch1, Hes1, Shh and Smo, not that they regulate the increased malignant phenotypes observed in numb-knockdown cells

Reply:

Thanks for the reviewer's advice and we have deleted this sentence (see Page 10, line 208).

- Figure 4A - blot is very low quality, please provide a better blot

Reply:

Thanks for the reviewer's advice and we have replaced the image (see Fig 4).

- Figure 4B - there are clear nuclei staining for numb but not the nuclear stain, suggesting the normal

nucleus staining is out of focus. Please provide a better image

Reply:

Thanks for the reviewer's advice and we have replaced the image (see Fig 4).

- Figure 4C-E - how many samples were included in each sub-type? Please put this in the figure legend. It seems unlikely that the difference between CC I-IIa and CC IIb-IV is significant. Similarly, it doesn't appear that the difference between no mets and mets would be significant

Reply:

Thanks for the reviewer's advice and we have replaced the image (see Fig 4).