Reviewer's report

Title: PRAF3 induces apoptosis and inhibits migration and invasion in human esophageal squamous cell carcinoma

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Reviewer: Ben J Colleypriest

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Comment
The authors investigated the expression of PRAF3 in oesophageal squamous cell carcinoma and the role of overexpression in oesophageal squamous cell carcinoma cell lines specifically concerning apoptosis and migration. An interesting area for which there is minimal data for squamous oesophageal cancer. The authors use immunohistochemistry, qRT-PCR, western blotting to observe PRAF3 expression in histological specimens from treatment naïve surgical specimens. Over expression of PRAF3 was achieved using infection of replication deficient adenovirus and demonstrated at the mRNA and protein level. Functional effects of PRAF3 overexpression are assessed with MMP-2 and MMP-9 activity, annexin apoptosis assay, caspase-8 and 9 activity, wound healing and cell migration observations. The methods are appropriate and well described.

Minor Essential Revisions
1 - Observational data that expression of PRAF3 (immunohistochemically) correlated with pathological grade, tumour stage and lymph node metastasis is very interesting. Did both pathologists agree on the staging / pathological grading of all tumours? Which part of the tumour was used to asses PRAF3 expression in these experiments?

2 - PRAF3 overexpression in cell lines induces apoptosis through caspase-8 and caspase-9 dependent mechanisms. What percentage of cells were infected to provoke these results?

3 - The scale bars are missing from the legend in figure 4.

4 - There is a reference missing in the final paragraph {ref}

Major Compulsory Revisions
1 - The immunohistochemistry in figure 1 suggests that the expression of PRAF3 in normal squamous tissue is expressed as a decreasing gradient from the differentiating squames compared to the transit amplifying and stem cell compartment. PRAF3 has been shown to induce differentiation in certain cell lines and would provide an alternative explanation to the authors conclusion for the observation in figure 1 (1).
In subsequent cell line experiments neither cell line is characterised to their initial cell phenotype and differentiation status. Given that the majority of data relates to cell line experiments I think the cell lines need to be characterised at least prior to experimentation.

How was the activity of ad-cmv-null virus tested? Given that a proportion of the results relate to cell death and migration it is important that the ad-cmv-null virus is validated.

The limitation of applying functional data from cell lines to in vivo is not discussed. Are the results unique to cancer cell lines and what is the effect of PRAF3 overexpression on normal oesophageal cell? If PRAF3 is a lead molecule for the development of ESCC, as suggested by the authors, its role in normal oesophageal cells should be understood.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests