Reviewer's report

Title: High expression level and nuclear localization of Sam68 are associated with progression and poor prognosis in colorectal cancer

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Reviewer: Claudio Sette

Reviewer's report:

In this work Liao and colleagues investigate the clinical relevance of the expression of Sam68, an RNA binding protein frequently upregulated in human cancers, in colorectal cancer. The authors describe a positive correlation between high levels of Sam68 expression and its nuclear localization with colorectal cancer progression and poor outcome. The work relies on standard techniques, such as Western Blot, RT-PCR and immunohistochemistry and it is not particularly original, as the authors have reported very similar analyses of Sam68 and of its upregulation in renal, breast, cervical and oral tongue carcinomas in 4 previous publications from October 2009 to now. Nevertheless, the large number of patient specimens analysed by immunohistochemistry and the possible prognostic value of the expression and subcellular localization of Sam68 represent solid aspects of the study. The manuscript is not very accurate in the description of what is known about Sam68 and is flawed by an excessive weight given to the work of the authors with respect to that of other scientists in the field. This negligence may negatively affect the information that the general reader receives on the role(s) of this specific protein in human cancer.

Major Points:

Introduction: the literature cited in regard to the function of Sam68 is very old and largely incomplete, especially regarding the potential role of this protein in cancer. The authors mainly refer to studies focused on the signal transduction function of Sam68 in the cytoplasm. However, Sam68 is mainly localized in the nucleus of most cancer cells and articles in the last 10 years have clearly demonstrated that its activity in transcriptional (Babic et al., Oncogene 2006; Rajan et al J Pathol 2008) and post-transcriptional (Matter et al., Nature 2002; Paronetto et al., J Cell Biol 2007; Paronetto et al., Cancer Res 2010; Valacca et al., J Cell Biol 2010) regulation of gene expression is relevant to human cancer. Moreover, reference 15 suggested that ablation of Sam68 promoted neoplastic transformation, thus suggesting that Sam68 functions as a tumor suppressor, not a pro-oncogene as stated by the authors in reference to this work. Since this manuscript is mainly descriptive, the description of the literature should be accurate and complete;

Page 6: The first evidence that Sam68 was upregulated in human cancers was shown in prostate carcinomas (Busà et al., Oncogene 2007; Rajan et al., J Pathol 2008). These articles preceded the many articles published by the authors
on the upregulation of Sam68 in several other human cancers and should be correctly mentioned in the text when upregulation of Sam68 in human cancers is first described;

Page 8-9: please specify what antibody (catalogue number) was used for the immunohistochemistry; please, provide also the details of the methods used for the evaluation and classification of Sam68 staining in tissues. The authors refer to two of their articles (ref 21 and 23) that deal with different proteins (Centromere protein H and AEG-1), not with Sam68. Moreover, reference 21 and reference 24 refer to the same article. This Referee does not see any reason to cite these two articles, which refer to different proteins analysed in different types of cancers and should be deleted from the reference list. All is needed here is to specify in detail the methods used to analyze Sam68 expression in colorectal cancer tissues. Again, this manuscript is a descriptive study and the description of the results should be accurate and easy to understand for the reader.

Results: page 12, in line 3 the authors state: “nuclear localization of Sam68 correlated significantly with tumor differentiation (P = 0.002) and advanced Dukes stage (P = 0.013) and T stage (P = 0.021), but not with N stage or distant metastasis.”, whereas in line 12 they state “However, no significant associations were observed between the subcellular localization of Sam68 and any other clinicopathological feature including age, gender or tumor histology.” Do the authors mean with tumor histology only the type of epithelium (i.e. mucinous, columnar etc.) and not the differentiation status of the cells? Please specify better in the text.

Results: what is the relevance of the upregulation of Sam68 in CRC cells? Since the authors have tested Sam68 overexpression in several CRC cell line models, they could investigate the molecular contribution of this protein in the process of tumoral transformation, as author have already performed for other cancer types (e.g. breast cancer, renal cancer etc.).

Discussion: the first page repeats the same concepts just examined in the Results section and could be deleted or largely shortened.

Page 15 lines 5-9: Taylor et al., (2004) (ref 5 in the text) demonstrated that up-regulation of Sam68 in fibroblasts, not its down-regulation, caused cell cycle arrest and apoptosis; reference 7 reports the phenotype of Sam68 knockout mice, and does not deal with the role of Sam68 in oncogenesis as stated by the authors; as mentioned already for the Introduction, the authors refer to the literature incorrectly and inappropriately;

Page 15, lines 13-16: Busà et al 2007 and Rajan et al., 2008 (refs 20 and 26 in the text) did not report a significant correlation with poorer prognosis in prostate carcinomas, as incorrectly stated by the authors;
Page 16, lines 3-7: the authors state “Interestingly, patients with cytoplasmic Sam68 localization had a better clinical outcome than patients with Sam68 nuclear localization. In agreement with these findings, cytoplasmic localization of Sam68 correlated significantly with known risk factors for progression and poor prognosis in human renal cell carcinoma and breast cancer [17, 19].” How can they state that the two lines of evidence are in agreement? In one case, the present work, cytoplasmic localization is a favourable prognostic value; in the other two studies it was presented as a negative prognostic value. The authors should seriously consider the differences in the two studies and discuss here possible scenarios in which Sam68 promotes oncogenesis more when it is localized in the nucleus (CRC) or in the cytoplasm (breast and renal carcinomas) depending on the cell type or tissue of the lesion.

For the same reasons a more detailed description of Sam68 nuclear functions in the Introduction and in the Discussion is necessary. The authors conclude this paragraph stating that “The role of nuclear Sam68 in tumorigenesis is largely unknown…”. This is quite a peculiar statement, as the majority of the articles published in high profile journals on Sam68 in the last 10 years have focused on its nuclear functions, such as transcription, alternative splicing and nuclear export of target RNAs. The fact that the authors have not addressed the nuclear function of Sam68 in their previous papers does not mean that nothing is known.

Minor Points:

page 3, third last line, page 11, line 8 and in many other parts of the manuscript: Sma68 should read Sam68

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests