Reviewer’s report

Title: Rac3 induces a molecular pathway triggering breast cancer cell aggressiveness: Differences in MDA-MB-231 and MCF-7 breast cancer cell lines.

Version: 1 Date: 29 April 2012

Reviewer: Vimla Band

Reviewer’s report:

In this manuscript the authors investigated the role of RAC3 in breast cancer aggressiveness and TNF-induced apoptosis upon knockdown of RAC3 by siRNA. The authors observed that RAC3 is involved in the aggressiveness of cancer cells through ERK/NF-kB pathway. Although it is important to define pathways that regulate cancer invasion/migration, the conclusions drawn here are not convincing. Moreover, there is no mention of why the authors performed this study. Does RAC3 overexpressed or altered in breast cancers? Does its expression correlate with tumor grade, invasiveness or metastasis?

1. Fig. 4A--At 96h, the proliferation of RAC3 knockdown in MDA-MB-231 and MCF-7 cells significantly reduced. Does RAC3 knockdown causes cell cycle arrest or apoptosis? RAC3 may have a fundamental role for any cell proliferation or survival. The authors should include a normal cell line (such as MCF10A) to assess if RAC3 knockdown effect is tumor cell specific. Although, authors’ considered reduction in proliferation of MCF-7 upon RAC3 knockdown at 96 hr not significant, it seems significant decrease. Please explain.

2. The authors tried to link RAC3 knockdown effect on migration, adhesion and invasion, vasculogenic mimicry to MMP9, however there are some concerns regarding controls.

In Fig.4E, the authors showed that MMP9 mRNA decreased in RAC3 knockdown cells, but there is no RT-PCR internal control; also the authors did not use control siRNA and RAC3 siRNA side by side in the same gel, instead these seem two different gels. The authors also need to show MMP9 expression decreased in protein levels by western blotting.

3. The authors tried to connect the effect of RAC3 knockdown on TNFa induced apoptosis to ERK/NF-kB, again the evidence is not strong enough.

a. GAPDH is not a good control for cytoplasmic and nuclear protein. The authors need a specific cytoplasmic and nuclear protein as controls to show their cytoplasmic/nuclear fractionation are clean and nuclear NF-kB is down in RAC3 siRNA cells.

b. The authors concluded that RAC3 siRNA has no effect on apoptosis in MCF-7 cells and it is due to low expression of NF-kB, but they did not show NF-kB expression in both MDA-MB-231 and MCF-7 cells side by side for comparison.
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:
Reviewer declares no competing interest in this study.