Reviewer's report

Title: SDF-1alpha concentration dependent modulation of RhoA and Rac1 modifies breast cancer and stromal cells interaction

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Reviewer: Xiangshan Zhao

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In this manuscript the authors investigated if SDF-1α can modulate breast cancer cell migration, invasion and adhesion. They found that SDF-1α can stimulate MDA-MB-231 cells migration, invasion and adhesion. They further found this stimulation effect is controlled by expression and activation of Rho GTPase.

The results presented in this manuscript provide some evidence that SDF-1α may have an influence on breast cancer migration, and adhesion. However, because all experiments were done in a one breast cancer cell line, it is not clear if the observation made in this manuscript can have broad application. Same study in myeloma cells has been previously published by other group (Anderson). Furthermore, some of the author’s results and hypothesis are inconvincible.

Major comments:

1. The authors use single cell lines (MDA-MB-231) for all the experiments, it is unclear if the results are specific to this cell line or also applied to other cell lines. In fact the authors showed that MCF7 do not express CXCR4 (S. Figure 1B) but still tightly attached to BMHC cells (Figure 1B), indicating that MCF7 adhesion is not regulated by SDF-1α/CXCR4.

2. The authors did not check the expression level or activity of different RhoGTPases, SDF-1α and CXCR4 in bone metastatic breast cancer samples, it is not clear if this study has any clinical relevance.

3. Same study in myeloma cells has been done by other group (Anderson). Although the authors in the present manuscript get some different results but the author’s results, interpretation/hypothesis are inconvincible. 1). The authors showed that knockdown of RhoA enhance the adhesion of cancer cell to BMHC (Figure 5E), but this result is contradictory to the results shown by Anderson’s group and is also contradictory to well established function of RhoA. Knockdown of RhoA should reduce adhesion. 2). In discussion (from line 349-351) the authors mentioned that SDF-1α’s effect involves inactivation of Rac1 but the authors have no data shown Rac1 is inactivated during SDF-1α treatment to support their conclusion. Actually RAC1 expression and activity are increased under treatment (Fig.4A, S.Fig.4B); 3). The conclusion or hypothesis stated from line 402 to line 406 is contradictory to the well-known functions of RhoA and Rac1. Vast amount of publications have shown that RhoA promote adhesion.
Inhibition of RhoA should reduce adhesion, not increase it. Similarly it well known that Rac1 regulates lamellipodia formation at the leading edge during migration. Rac1 has been shown to play important roles in cytoskeleton rearrangement, cancer cell migration, invasion and metastasis. Many studies have confirmed that inhibition of Rac1 reduce cancer cell migration, invasion and metastasis, not increase migratory properties as hypothesized by the authors. 4). In the abstract (line 45 - 46), the authors mentioned that “at high concentration Rac1 was promoting SDF-1# mediating-cell adhesion”. But in this manuscript the authors do not have any solid data to support this statement.

4. The description for matrigel culture (from line 290-292) is wrong, normal cells can also grow in matrigel. This tube formation assay is suitable to check cell to cell adhesion not to assess invasion. Due to cell to cell adhesion property of cells, if large number of cells are plated on top of matrigel, even normal cells will form branching/ductal structure within several hours. In order to check invasion, the authors should have used either matrigel coated trans-well chambers or embed small number of cells in matrigel. The results shown in Figure 3C is tube formation due to cell to cell adhesion, not invasion.

Minor comments:

1. In Figure 2, the authors should also check if inhibition of CXCR4 either by antibody or shRNA will block SDF-1# effect.

2. In Figure 4A, the authors should run all the samples in one gel.

3. The authors used three different SDF-1# concentrations for the experiments. What is the physiological SDF-1# concentration in human bone marrow microenvironment?

Level of interest: An article of limited interest

Quality of written English: Acceptable

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

Declaration of competing interests:

There is no competing interest