Reviewer’s report

Title: Role of LPAR3, PKC and EGFR in LPA-induced cell migration in oral squamous carcinoma cells

Version: 2
Date: 2 January 2014
Reviewer: Hiroaki Niiro

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< Major Compulsory Revisions >
Elucidation of molecular mechanisms of LPA-mediated invasion of cancer cells would provide a key to the development of novel anti-cancer drugs. In this study the authors suggest a critical role of LPAR3, PKC and EGFR in LPA-induced migration of oral squamous carcinoma cells. Data currently provided, however, are not sufficient to warrant publication for the following reasons.

(1) Importance of LPAR3 in LPA-mediated cell migration would be a novel aspect in this paper. The conclusions were, however, mainly drawn from the results using the chemical inhibitor (i.e. Ki16425). Given the data showing that this inhibitor also partially blocks EGF effects (Fig. 4), another technique (e.g. silencing of LPAR3) should be employed to show more direct evidence for this conclusion.

(2) Although the authors previously identified critical signaling pathways of EGF/HGF-mediated migration of these cells, this issue remains elusive in the context of LPA-mediated migration. Which MAP kinase or PI3 kinase is more crucial? The finding that blockade of EGFR transactivation almost completely abrogates cell migration in E10 and SCC-9 cells would be a bit disappointing. Molecular dissection of EGFR-dependent and –independent pathways would strengthen the paper.

(3) Statistical analysis should be carried out to help interpret the results.

< Minor Essential Revisions >
(1) Explanation of the discrepancy of LPAR expression at mRNA and protein levels would be addressed: levels of LPAR2 protein are much higher in E10 cells than SCC-9 cells (Fig. 2).

(2) Previous papers showed that in addition to EGFR, HER2 is strongly tyrosine phosphorylated upon LPA stimulation in SCC-6 cells and gastric cancer cells (Cancer Res, 2002; BBRC, 2005). Is this not the case in this study?

(3) The detection of production of endogenous EGF-like proteins (e.g. amphiregulin, HB-EGF, TGF#) might help confirm the requirement of EGFR transactivation in SCC-9 cells.

< Discretionary Revisions >
(1) In Fig.1A, the data of E10 cells at 23 h are shown. The first paragraph in
Results sections, however, describes the optimal time point of 24 h. Which time point is correct?

(2) GF109203X blocks activation of both conventional and novel PKCs (Fig. 6). More selective PKC inhibitor would help uncover a critical pathway for cell migration.

(3) Akt is apparently constitutively phosphorylated in D2 cells compared with E10 and SCC-9 cells (Fig. 7 & 8). Is this a part of reason for spontaneous migration of D2 cells?

**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:**

I declare that I have no competing interests.