Reviewer's report

Title: 3-Phosphoinositide-dependent Protein Kinase-1 (PDK1) Promotes Invasion and Activation of Matrix Metalloproteinases

Version: 1 Date: 15 February 2006

Reviewer: James Woodgett

Reviewer's report:

General

This paper describes the properties of a cell line that has been engineered to over-express PDK1, an important protein kinase involved in transmitting PI3K pathway signals and activating several AGC kinase family members. The authors performed a gene expression experiment on the cells to identify differentially regulated genes and assessed the invasiveness of the cell line using established methods including cleared mammary fat pad isografts. The authors detected expression of the kinase using a phospho-specific antibody to PDK1 in a significant fraction of breast cancers.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. It isn't clear that the over-expressed PDK1 is actually active since this protein kinase depends on 3' phosphorylated lipids for activation via translocation to the membrane. There are no data on the activation state of downstream PDK1 targets (these data may be present in a previous paper, but the authors should include some data here to demonstrate it is active). For example, in figure 4B it isn't clear whether Akt is activated. Why does LY294002 treatment cause loss of the total Akt protein signal? What is the level of S473 phosphorylation compared to addition of a known agonist?

2. I think it is too much of a stretch to make a meaningful comparison of the two Comma cell lines with MCF10A and MCF7 since these lines have multiple genetic changes. Indeed, I was surprised that there were so few transcriptional changes in the PDK1/Comma cells given the major phenotypic differences between the PDK1 expressors and the parental line, as described. Are these phenotypes reversible with PI3K inhibitors (it is not clear enough from the data presented).

3. The tissue microarray data are problematic. As far as I am aware, measurement of phospho-serine 241 is not necessarily a measure of the kinase activity of PDK1. This phosphorylation site appears to be constitutive. There is also a need for appropriate controls to show that the correct phospho-epitope is being detected (e.g. immunoblot of fresh frozen samples, removal of the signal with a phosphatase). Several reports indicate that the PI3K pathway is activated in breast cancer as measured by S473 of Akt. The data in this manuscript suggest that PDK1 is activated in 90% of these tumours. In this case, why is phospho-Akt only detected in ~60-65%? Is there any evidence of evidence of gene amplification or specific activation of PDK1 in breast cancer?

4. Is there a correlation between PDK1 protein levels or activity with other components in breast cancer? It is the task of the authors to prove that PDK1 plays a driving role in human breast cancer given the other known components that have been shown to be mutationally activated or amplified.
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Towards the end of the results section (no page numbers) the authors refer to S241 as S142.

Discretionary Revisions (which the author can choose to ignore)

None

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.