Reviewer's report

Title: A stable explant culture of HER2/neu invasive carcinoma supported by alpha-SMA expressing stromal cells to evaluate therapeutic agents

Version: 2 Date: 3 December 2007

Reviewer: Mary Helen Barcellos-Hoff

Reviewer's report:

Major Compulsory Revisions

The identification of the two subpopulations as 'stromal' and 'tumor' is perhaps premature. The author states on page 15 that "we consider our stroma cells to be nonepithelial and our tumor cells to be a mixture of luminal and basal/myoepithelial phenotypes" however the 'stromal' cells are simply identified as such by virtue of morphology and alpha-smooth muscle actin staining. It is quite possible that the so-called stromal cells are indeed myofibroblasts from normal tissue. Two examples come to mind: the COMMA-1-D cell line described by Medina and colleagues (Exp Cell Res. 1988 Jul;177(1):109-21) maintains two subpopulations of cells despite evidence of shared mutations (suggesting clonality) and the publication by Terry VanDyke (Cell. 2005 Dec 16;123(6):1001-11) in which the highly proliferative mesenchyme of pRB tumors has undergone p53 loss. Thus it is quite possible that the 'myofibroblast' are also tumorigenic. It is therefore crucial to

1. genotype the different subpopulations to determine their relationship
2. to test the tumorigenicity of each population

The discussion of a putative stem cell component is inconclusive and should not be included in results without functional analysis of sorted cells.

The definition of forward scatter as 1N and 2N populations representing cycling and non-cycling cells is not consistent with standard cell cycle descriptive analysis and should be re-evaluated with BrdU incorporation vs DNA content profiles to determine the cell cycle distribution, the percent of cells in S-phase, and whether the large 2N population represents a cycling tetraploid population.

The assumption that MAM-1 represents a co-culture of tumor and stromal cells is also the basis for the microarray analysis which compare the "Bam1a cell line, a cloned mammary carcinoma cell line developed from a BALB-NeuT mouse and MAM-1 co-cultures". The author concludes that "two-fold difference is likely to represent the dilution of tumor cell RNA with stromal cell RNA in the MAM-1 co-culture" and that "Genes uniquely over-expressed by MAM-1 largely reflect the stromal signature of this breast cancer co-culture system". This analysis is premature in the absence of the identity of the 'stromal' genotype, and is imprecise given the author's
demonstration that the cell types could be easily separated using cell surface markers.

The conclusion that these "data suggest that Iressa preferentially targets signal transduction from the tumor cell HER2/neu leading to tumor cell death" are premature in that the although cell number may have decreased (relative to the controls) the author has not demonstrated cell death by any method as reduced cell number may result from decreased proliferation. PCNA staining is an indirect measure of cycle.

Minor Essential Revisions
The figures appear pixalated and may not reproduce well.

Discretionary Revisions
The itemized list in the introduction is non-standard and distracting.

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

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

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.