Reviewer's report

Title: Heterogeneity of mammary lesions represents molecular differences

Version: 1 Date: 25 August 2006

Reviewer: Kornelia Polyak

Reviewer's report:

General
The goal of this manuscript was to characterize genetic and gene expression changes that occur during the progression MIN-O mouse model of breast cancer. Using this approach they determined that different MIN-O lines with different ER expression and metastatic potential are molecularly heterogeneous with characteristic changes present in each line. Almost all these changes were already present in MIN-O premalignant tumors and only a few additional alterations were detected as these progressed to invasive carcinomas. The general conclusions, although not the specific molecular events, appear to be similar to what was previously described for human breast tumor progression. Thus, based on this the authors suggest that this is a good model of human breast cancer.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. The authors used prelactating (pregnant?) mammary gland as control for their gene expression studies. While it is true that the proliferation index of this tissue may be closer to that of tumors, it is likely that pregnancy induced dramatic differentiation and associated changes in gene expression profiles. Thus, it would be better if the authors also used a virgin, non-pregnant mammary gland as an additional control for their expression studies.

2. It would be also useful to state how “clean” the tumors were (e.g. fraction of tumor cells within the tumor) and preferentially use purified epithelial cells for the gene expression and aCGH study to ensure that lack of tumor cell purity is not a confounding factor. In fact, the finding that several of the genes downregulated in tumors compared to MIN-O premalignant lesions correspond to ECM (stroma?) related genes suggests that the tumors and preinvasive lesions have different % of stromal cells. The similarity in keratin 2-8 expression levels is not sufficient to determine this especially if this was done solely using arrays (keratin 2-8 is an abundant gene, thus, the array spot may be saturated and hybridization signal may not be an accurate measure of mRNA levels). The authors should provide histologic confirmation of the purity of the various tissue samples and also test the expression of selected genes in the different samples using alternative methods (immunohistochemistry or mRNA in situ hybridization).

3. It would be best if the authors would confirm at least a few selected aCGH results by a more quantitative method for example quantitative PCR. Especially for deletions this would be necessary.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
The authors did not mention if the expression of known ER targets was also higher in the ER positive estrogen dependent lines than in the ER negative ones. This would be important to know, since this more likely reflects the activity of the ER signaling pathway than the expression of enzymes etc. involved in estrogen metabolism.

Discretionary Revisions (which the author can choose to ignore)

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 importance in its field
Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests