Reviewer's report

Title: Novel markers for differentiation of lobular and ductal invasive breast carcinomas by laser microdissection and microarray analysis

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Reviewer: Edgar Dahl

Reviewer's report:

General

This study by Turashvili et al. used a combination of laser-microdissection and DNA array based expression profiling to identify genes that are specifically expressed in lobular and ductal invasive cancer. Furthermore, the authors compared expression between normal ductal and normal lobular cells and third, gene expression between breast tumor and normal breast tissue in general.

This is an interesting piece of work. Though the number of tumors (10) and samples (30) analyzed is rather small the data appear useful since quite a number of well known breast tumor markers were confirmed in this study. The study is suitable for publication in BMC Cancer if the following items are clarified.

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

The study somewhat suffers from having included too many comparisons and too many descriptions of deregulated genes. The authors should first focus on the comparison of lobular invasive tumor and ductal invasive tumor, which has not been carefully analyzed (if at all) by other groups. Secondly, the comparison of normal lobular and normal ductal tissues is interesting and should be discussed, however one should keep in mind that these "normal breast tissues" were derived from postmenopausal women, which breast tissues are rather inactive in metabolism.

Abstract

The abstract should give a clear understanding on the number and types of analyzed tissues (10+10+5+5). Results should name the major finding (those differential expressed genes, that were validated by in situ hybridization or immunohistochemistry).

Background

The author mention that IDC and ILC are similar in many respects, but then refer to references. The state of the art knowledge on this important point should presented in more detail. On the other hand, the study of Korkola et al. is discussed in detail with gene names in the Background/Introduction. These concrete data should rather be discussed in the actual discussion in comparison to the genes found by Turashvili et al.

METHODS:

The first sentence: Should mean: Altogether ten surgical specimens with either invasive ductal carcinoma (n=5) or invasive lobular carcinoma (n=5) were investigated.

What does the lysis buffer for the microdissected cells from Qiagen contain?

RNA amplification: The authors should state how many cycles (21-33) they actually did for their experiments. They should provide a reference that such high level amplification of Target RNA will not let to a reduction in RNA complexity (=loss of non-abundantly expressed genes). Why did they not use linear amplification e.g. by two round of in vitro transcription?

RESULTS:

Genes differentially expressed between normal ductal and lobular cells: In the text USP25, TMPRSS3, ACACB ..... until MAP4K5 are described as upregulated in normal lobular cells (please mention ! compared to normal ductal cells) however in Table 2 this genes have a arrow down, meaning according to the legend of Table 2: downregulated in lobular cells. What is correct?
The authors should verify once more all descriptions on up- and downregulated genes on consistency.

Table 3 starts with Tumor vs Normal cells and continues with IDC vs normal before ILC vs Normal is shown. In the text first ILC vs Normal is discussed. It would be nicer to have a continuous order (with a focus on the two most important comparisons mentioned above).

The SFRP1 gene is mentioned as downregulated in the paragraph "comparison between lobular carcinoma vs. normal cells", however, in Table 3 it is only found in the list tumor vs. Normal. In the same context DVL1 is mentioned as being differentially expressed between lobular and ductal carcinoma. This makes things complicated. Within the results sections the author should precisely stick to the genes of the actual comparison.
In the discussion one could mention that Wnt pathway genes have been found in several comparisons.

DISCUSSION:
The discussion is written quite nicely. However, I cannot follow the conclusion that Wnt signalling is not activated in ductal carcinoma cells because SFRP1 and MMP7 are downregulated. SFRP1 is a potent inhibitor of Wnt signalling (e.g. Suzuki et al.; Nat Genet. 2004 36:417-22), its downregulation in breast cancer is associated with poor prognosis (Veeck et al.; Oncogene. 2006 25:3479-88) that may be caused by aberrant Wnt signalling via β-catenin (Lin et al., PNAS, 97:4262-6.). As the authors state MMP7 expression in different tissue may be regulated by different pathways, this may hold true even for normal and malignant breast tissue.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Page 13
was downregulated in our tumor cells
rather: was downregulated in the tumor specimens analyzed in this study
Page 15
p=0.000000 is rather uncommon
better use p<0.0001

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: Needs some language corrections before being published

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