Reviewer's report

Title: Identification of Apoptosis-related Biomarkers of Ductal Carcinoma in situ with Microinvasion of the Breast

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Reviewer: FS Al-Joudi

Reviewer's report:

Manuscript title: Identification of apoptosis-related biomarkers of DCIS with microinvasion of the breast.

A first remark to the authors: an un-acceptable note by the authors saying that they have referred to a language expert: this means that no one is supposed to test this matter anymore!! However, there are still many obvious language errors. Shall I overlook those since they have been made by an expert!!!

Altogether, the manuscript is a description of changes detectable in DCIS-Mi: it is a simple yet rewarding and useful piece of work they have performed, but the authors are still going round in the same circle of the first version of their manuscript.

Running title: biomarkers of the breast DCIS-Mi (NOT apoptosis: see below please).

Title: New title: contents are alright, but it may require major language corrections: it sounds like the DCIS may have originated outside the breast and that has gone to the breast in the form of a micro-invasion. It may be better as such: .....biomarkers of breast DCIS with microinvasion. I think that it should read: Identification of biomarkers IN breast DCIS with microinvasion or Identification of biomarkers IN DCIS of breast with microinvasion (without the word “apoptosis”); and this partly is explained later in the comments on the discussion.

Abstract:

Regarding mammography mentioning, the thought is that it MUST be removed, because the progression into DCIS-Mi, whether detected or not, it is there along with the molecular changes that accompany it, as anticipated by the authors. Furthermore, when it is not detected, then it is likely to be detected clinically later when it grows into IDC. By all means, the facts sought here are those that involve the accompanying molecular changes. However, you can mention the usefulness of mammography in detecting such cases in the discussion section. The writing of this article needs thoughts of a pathologist before that of a surgeon or a radiologist, especially to avoid distracting the reader into the thought that “it is mammography that made detection of molecular changes possible, without which no changes would have taken place!!!! Whereas mammography eventually
altered the clinical detection rates, IT DID NOT PUT ANY influence or change on the molecular mechanisms of invasion!!!! In brief, and as I already explained extensively in the previous review, regardless how the “Mi” has been detected or confirmed, the mechanisms underlying its formation remain the same.

You can mention TUNEL as well as IHC in the methods.

Also paragraph 1, sentence 3: It reads Identification of any differences……...: I suggest it becomes: Identification of biological differences between DCIS and DCIS-Mi may aid in understanding the nature and causes of the progression of DCIS into invasiveness.

In paragraph 3, the authors have defined biological factors as being estrogen R, progesterone R, and h-EGFR type-2. However, in the last paragraph, survivin is described as a biological feature, which may create a bit of confusion to the reader: I suggest that the last paragraph reads as such: Furthermore, the differential expression of survivin may serve in deciding the response to therapy and may have some prognostic significance.

Introduction: It is not necessary to interpret (as found in brackets) the meaning of nuclear grade.

Last paragraph: very weak, language and science: it requires re-writing, preferably without using “we” (as for example: Tumour progression may be induced or associated by alterations in the proliferative capacity and the apoptosis potential, and the findings may find some basic and applied interpretations especially useful for confirming a diagnosis, and in building up prognostic criteria). This MUST be done properly, as it was a mere wild statement which has neither support, nor proper place at this stage of the manuscript.

Materials and Methods.

The previous comment on the contradiction of ideas between the method of confirming the diagnosis of DCIS-MI by histopathology (or rather by mammography) still stands: this, once again proves that the mammography talk is not necessary: it merely suggests the presence of the case, and confirmation is by HP. I do not understand why the authors are stuck to their idea, although it is not a tidy way of expressing themselves in an article: once again, this is pathology talk and a molecular talk mainly, not a surgical talk.

Very little and still incomplete corrections have been done with the flow of information in the methodology section. Some language errors (especially with plural and singulars) are also there: maybe the authors are required to refer once again to their medical editor, whose first language is English.

Adhesive-coated slides: what adhesive are the authors talking about: it is certainly not glue, nor some form of super glue!! I suggest that the authors refer back to the technician who performed the mounting of slides to inform them what the “ADHESIVE” material is (it may be poly lysine).
The section on “evaluation of IHC should go up immediately after the section describing IHC before the section on “apoptosis”.

Discussion:
The first paragraph, written in blue color: this is far from clear: I could not understand what the authors are trying to say!! This may have been imported from another section without the required adjustment. However, reading through clarifies that this paragraph is completely repeated in the second paragraph!!!!!

In that second paragraph the authors are saying: the invasive tissue is extremely small and therefore it is often difficult to perform immunohistochemical analysis!!! What does this statement mean? Does it imply that the work presented in this manuscript detected only a fraction of the truly expressed antigens (markers under study), and often they were not detected? Please either explain or re-write: this is very important to revise.

In that same paragraph: the survivin gene is not part of the IAP family of proteins: it is the survivin molecule itself (please make the distinction clear). In this place is required also a molecular explanation of the increased apoptosis which accompanies the increase survivin expression.

The next paragraph contain an erroneous statement on the splice variants of survivin as a reason for not being detected in the cytoplasm: in fact, it may be the same variant detected in both the cytoplasm and the nucleus: this depends on the state of cellular division and transformation, and is explained in the literature. Furthermore, I thought that you would know the limitations and specificities of the primary anti-survivin antibodies used: these usually can recognize most variants, especially that the specificity of the antibody has not been given by the manufacturer (DAKO): hence the explanation given is very weak.

Some repeated statements appear later: repetitions are to be deleted. Also some contradictory statements appear in the later paragraphs: in two or more places, survivin has been described by the authors as “an apoptotic factor”: this is not correct since it is clearly defined as an anti-apoptotic factor. The authors may kindly look at that matter.

Altogether, the discussion still requires a bit of consolidating: it is weak, with repetitions, and has no fundamental gain from the results obtained.