Reviewer’s report

Title: MicroRNA profile in very young women with Breast Cancer

Version: 1
Date: 2 February 2014

Reviewer: Patricia Casbas-Hernanez

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The manuscript ‘MicroRNA profile in very young women with Breast Cancer’ seeks to distinguish breast cancer that arises in very young patients (less than 35 years old) from those that arise in older patients (older than 65 years old) using microRNA profiling. It is a well written document with a clearly stated question; it combines the emerging field of microRNA with an important clinical dilemma. The findings may have implications for how breast cancers in young women are managed. However there are certain aspects of the methods, analysis and limitations of the study that should be reconsidered:

- Major Compulsory Revisions

1- In the description of the study population it would be relevant to know if the biopsies used for the study were from primary breast cancer or if it was a recurrence. Also, if women that participated in this study had a family history of breast cancer and their BMI.

2- The majority of papers the authors reference are studying ethnically diverse populations. African American women are known to have higher rates of cancer at young age (and more aggressive). The authors should state the ethnic makeup of their population, how their population is different/similar to the previously published populations, as well as explain how their study is more/less appropriate to answer their question and what limitations or strengths does their population present when trying to extrapolate their findings to other populations.

3- The authors did not provide any information regarding their 3 ‘normal’ patients. It is important to know the characteristics of these patients (age, BMI, etc).

4- There are no methods for explaining how histologic staining was made or how markers were measured. Please provide description of how the subtypes were defined (Luminal A, Luminal B...).

5- The authors state that all tissue samples contained more than 30% of tumor material; the variation from 30% to 100% is a big range of epithelial content and may explain some of the differences observed among young and old tumors. This is a variable that should be included in table 1 and adjusted for in the analysis. Breast density decreases 1-2% every year in normal circumstances; this change in breast density implies a change in the epithelial and stromal contents of the breast. Therefore, young breasts have more epithelium than older breast which have more stromal content. These changes in breast composition are accompanied by changes in gene expression patterns when whole tissue gene expression studies are performed (Relationship of mammographic density...).
and gene expression: analysis of normal breast tissue surrounding breast cancer. Sun et al. Clin Cancer Res. 2013 Sep 15;19(18):4972-82.). Hence, microRNAs expression in whole tissue may also be influenced by the amount of stroma and epithelium within a tumor.

6- When assessing potential confounders for the microRNA profiles in the discovery set, the authors study the influence of tumor size, nodal status, ki67% and histological grade. However, they should also study the influence of tumor subtype in this discovery data set (the same way they adjust later in the manuscript for the validation test set).

- Minor Essential Revisions
7- Figure 3 is missing axis labels, please add labels for classification.
8- Dates and timelines for tissue acquisition and RNA processing are important elements to add to the methods.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.