Reviewer's report

Title: MicroRNA profile in very young women with Breast Cancer

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Reviewer: Jodie Fleming

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Major Compulsory Revisions:

1. The authors provide a general comparative evaluation of miRNAs differentially regulated in tumors from young women and elderly, post-menopausal women. This limited study describes a set of miRNA that were differentially regulated in women of various ages, and while the concept could be informative to those intimately related in the field, the authors fail to perform any functional studies to confirm the function of the miRNA highlighted and their possible role in breast cancer cell behavior. Other studies have shown miRNA directly regulating cell motility, chemo-sensitivity, methylation of stem-related genes, etc. Similar functional studies should be included. Generally, qRT-PCR analysis is not sufficient for validation of such a broad conclusion in such a widely-read and diverse audience BMC journal. Moreover, a significant number of important patient demographics were not accounted for in the analyses that should be addressed to gain more insight from the current data presented within the manuscript.

2. As the authors are comparing young women with significantly higher levels of circulating estrogens to the older, post-menopausal women, an analysis of the newly identified miRNAs and how they may relate to estrogen signaling is a must. It is currently unknown if the tumors evaluated in this study are simply documenting miRNA regulated by estrogens. If the authors were to perform such an analysis, this may be of interest. Perhaps reporter assays or other in vitro analyses? If the authors were to test a set of miRNA for response or regulation by estrogen the studies would be a welcome contribution. Indeed the authors highlight some of the previous studies that have linked miRNA to estrogen signaling (refs 27/28), but do not follow up on this note.

3. In addition to circulating hormone levels between pre- and post-menopausal women, the issue of parity must be related to the authors’ findings. Pregnancy, lactation and involution are known to significantly influence and even reprogram breast tissue. It is suggested that the age and number of pregnancies can affect breast cancer onset as well as influence the differentiation state or stem cell population. The current study does not account for the parity status of the women from which the tumors were obtained. The status of the number of children and age at first birth would shed some light into the difference observed in miRNA expression. Was the alteration in miRNA levels simply due to pregnancy?
4. The correlation between patient BMI and miRNA should be considered. As the current literature is continually highlighting the importance and influence of BMI, obesity and metabolic syndrome on breast cancer risk and behavior, it would be most helpful to know the BMI status of the patients analyzed. The possibility exists that the miRNAs identified are differently regulate due to metabolic status.

5. Used ki67, size, nodal status, histological grade but not intrinsic molecular subtypes. A more in-depth analysis of the molecular subtype may have shed some light into the observed differences in cell motility /invasion, adherens junctions and cell adhesion molecules. Indeed, the authors state that the younger women suffer from “more aggressive’ phenotypes and other studies have “revealed miRNA expression to be specific to breast cancer intrinsic subtype” but fail to evaluate or relate any of the current studies observations to molecular subtype. Did the previous studies relating intrinsic subtype account for age? What were the demographics in comparison to the current study? The authors need to express how their study enhances the current knowledge in the field and currently is not convincing as a comprehensive analysis comparing the limitations and results of the numerous other studies is lacking. If the intent was to strengthen the argument presented on page 13 that breast cancer in young women is “a distinct entity beyond the intrinsic breast cancer subtype” then more functional and in depth analyses are needed.

6. A very similar study was recently published, though the authors argue that their results “pointed to genes barely implicated in the pathways of found in our study”. It would be helpful for the authors to discuss their results in more depth in comparison to the highly similar study conducted by Colak and Cols: their current argument is that the inclusion of six samples under the age of 35 was the reason for such a large discrepancy in results between the two studies.

Minor Compulsory Revisions:
Shift in font size throughout

Level of interest:An article of limited interest

Quality of written English:Acceptable

Statistical review:Yes, but I do not feel adequately qualified to assess the statistics.

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