Reviewer's report

Title: Differential Expression of Cancer Associated Proteins in Breast Milk Based on Age at First Full Term Pregnancy and Length of Nursing

Version: 1 Date: 30 November 2011

Reviewer: Georgios Pampalakis

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An interesting study describing the alterations of certain protein levels in milk that may associate with breast cancer risk. The study is well conducted, technically sound, and potential limitations are mentioned.

Minor comments/suggestions to improve the manuscript

1. What was the source of recombinant KLKs, and what was the source of their antibodies used in the ELISA?
2. Regarding the method for the KLK specific ELISA used in this study, was it developed in their laboratory, based on what protocol (lanthanide based-time resolved fluorescence?), proper citations should be given.
3. The article describing KLK6 methylation by Pampalakis et al. 2009 (Cancer Res.) shows that indeed the normal mammary epithelial cells are partially methylated which indicates that either there is a mixed population of cells or within the cell there are methylated and unmethylated alleles, therefore the 57% of methylation that the authors describe is logical. Furthermore, since the CpG sites are limited in number and no CpG island is located at the KLK6 proximal promoter, it may be possible for minor changes in the methylation pattern to induce the KLK6 expression. And the authors find lower methylation in the samples obtained after 2 months (although not statistically significant). I suggest including a figure with their findings on this topic.
4. The change in protein concentration in milk that the authors describe does it correlate with the total volume of milk? For example higher volumes of milk have lower concentration of protein but overall the total protein content is the same.
5. On introduction last sentence page 3 it is stated “We have identified cancer associated proteins in breast nipple aspirate fluid, fluid from the milk ducts of nonlactating women, including kallikrein related peptidases (KLKs), basic fibroblast growth factor (bFGF) [9], and YKL-40 [10].” Please give proper citation for the KLK identification/quantification in milk ducts.
6. What are the types of cells found in the cell pellet of milk samples? The authors do not analyze this part in more detail, they only mention in the discussion section that the samples contain epithelial cells and leucocytes. I suggest moving this part to the results section. What were the results for KLK6 methylation in the epithelial enriched cell fraction?
7. Why the authors did not study the methylation status of the KLK10 gene as
Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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