Author's response to reviews

Title: CDKN1C/p57kip2 is a candidate tumor suppressor gene in human breast cancer

Authors:

Pamela S Larson (pamkent@bu.edu)
Benjamin L Schlechter (benjamin@schlechter.net)
Chia-Lin King (chialin.king@gmail.com)
Qiong Yang (qyang@bu.edu)
Chelsea N Glass (cng16@yahoo.com)
Charline Mack (charline.mack@bmc.org)
Robert Pistey (rrobbie1@aol.com)
Antonio de las Morenas (adlm@bu.edu)
Carol L Rosenberg (crosenbe@bu.edu)

Version: 3 Date: 17 January 2008

Author's response to reviews: see over
January 18 2008

Dr Lolu da-Silva  
Senior Assistant Editor  
BMC-series journals  
editorial@biomedcentral.com  
http://www.biomedcentral.com

Dear Dr da-Silva

We thank you and the referees for your interest in MS 1804078406159037 “CDKN1C/p57kip2 is a candidate tumor suppressor gene in human breast cancer”. We have revised the MS in response to the critiques and we are resubmitting it for further consideration. Additions and major modifications in the revised text are italicized.

Two new tables, Table 1 and Table 4, have been added. The original Table 2 has been simplified and is now Table 3. Figure 1 has been revised slightly. We have reviewed new publications on CDKN1C, re-accessed GEO to make sure no new CDKN1C expression data were deposited, and made a few minor changes in wording and formatting to increase clarity and accuracy. Below, we respond to each point raised by the referees.

Referee 1:

“1. Because they investigated very little number of the cases in this report, clinico-pathologic analysis is not enough.”
We have added a new table, Table 1, which summarizes clinical-pathologic features of all cases.

“2. Are there any relationship between the tumors with AI/LOH and clinicopathologic factors, such as ER, PgR, HER2 etc?”
We analyzed whether a relationship exists between the tumors with AI/LOH and histology, grade, and expression of ER, PR or HER2. We find no compelling or consistent evidence of any association (AI/LOH at single marker, THO1, was associated with a single parameter – ER/PR staining, p = 0.04). We note this in the revised MS (pg 12), but it is not clear how to interpret the observation.

“3. Table 2 is very complicated. The table shows negative data concerning relationship between clinicopathologic factors and mRNA or protein expression of CDKN1. So the author should simplify this table.”
We have simplified the original Table 2, which is now Table 3. This table now contains only protein expression data. The RNA expression values have been removed from this table and added to Figure 1, which depicts RNA expression changes.
Referee 2:

Referee 2’s comments are concerned with the need to distinguish events in luminal cells compared to myoepithelial cells. We agree fully that this is an important issue, and the IHC results address this. In contrast, our DNA and RNA studies detect genetic changes within all components of the cancer’s epithelium combined.

“1. The IHC results of the luminal epithelia cells clearly show that there is no significant difference in the frequencies or percentages of positive stained cells in normal epithelium, CIS and IC……If there is any…difference it is in contrast to the hypothesis postulating a loss of CDKN1C tumor suppressor gene function in tumor cells”.

We quantified the % positively staining cells in myoepithelium of normal and CIS samples and % positively stained cells in luminal epithelium of normal, CIS and IC samples. For myoepithelium, the difference between normal and CIS was highly significant (p = 0.0002). For luminal epithelium, the differences between normal and IC, and between CIS and IC were not significant (p = 0.3116 and p = 0.4143, respectively). The difference between normal and CIS was just barely significant (p = 0.0445).

These results indicate that in CIS there is a substantial decrease in the normally intense myoepithelial layer cell staining for CDKN1C. In contrast, luminal cell staining is less intense, less frequent and relatively uniform across histologies. We view IHC’s ability to distinguish between events in luminal and myoepithelial layer cells as enhancing our understanding of the data, and not as contradicting our conclusion that, overall, CDKN1C expression decreases in cancer epithelium.

“2. Due to the …increase in the ratio of luminal to myoepithelial cells in CIS….it has to be assumed that results of DNA…and RNA…analysis reflect…luminal… cancer cells and therefore cannot be correlated to loss of CDKN1C expression in myoepithelial cells of CIS. …the overall conclusion that CDKN1C protein expression decreased, at both the mRNA and protein level, in the large majority of breast cancers, which does not appear to be mediated by AI/LOH at the gene is not conclusive.”

The DNA and RNA in this study derive from microdissected lesions, which contain both luminal and myoepithelial cells in varying proportions. (There are relatively more myoepithelial cells in TDLUs than in CIS, and none in IC). Therefore, our LOH and gene expression results reflect events in both cell types. The IHC results tease out cell-type specific events at the protein level. While suggestive, mRNA and protein levels cannot always be directly correlated.

LOH is clonal, and is either present or absent [a dichotomized event] in a cell. For LOH to be detected, it must be present in a substantial subset of the cells examined. We can be fairly sure that there is no LOH of CDKN1C in luminal cells, although we cannot rule out LOH of CDKN1C in myoepithelial cells. To evaluate this, we would need to microdissect a population highly enriched for myoepithelial cells. This would be an
important project, but it is technically challenging and beyond the scope of the present report.

In contrast to LOH, gene expression measurements represent the average expression of all the cells in a population. Our data indicate a consistent, moderate decrease in CDKN1C expression in CIS compared to TDLU. We cannot distinguish whether this decrease is due to all cells having moderately decreased expression, or to a subpopulation [e.g., myoepithelial cells] having dramatically decreased expression, or to a decrease in the number of cells [e.g., myoepithelial cells] having constant high expression. Future experiments to address this question will be important. We include this point in the discussion (pg 14).

“3. ….the authors combine the data of both…cell types….instead of focusing the discussion on the interesting observation of the complete loss of CDKN1C protein expression in the myoepithelial layer….”. “To clarify the putative inactivation mechanism and/or downregulation of CDKN1C….would be very interesting.”

The loss of CDKN1C protein expression from the myoepithelial layer may be our most provocative finding. This finding is consistent with the hypothesis the referee suggests: that myoepithelial cells might function as tumor suppressors regulating progression from the in-situ to the invasive stage. Specifically, loss of CDKN1C expression in myoepithelial cells may permit progression of the neoplastic luminal cells. The mechanism(s) underlying loss of CDKN1C protein expression are unknown, as are the mechanisms by which myoepithelial cells exert their putative tumor suppressor function. Possible mechanisms include epigenetic changes resulting in diminished CDKN1C transcription, or post-transcriptional effects on the CDKN1C mRNA, such as via a miRNA. We have now added these points to the discussion, and we have included the reference in the bibliography (citation 46). We agree that clarifying the mechanism(s) leading to decreased CDKN1C RNA and/or protein would be interesting, but that is beyond the scope of this report.

We hope these responses address the important issues raised by the referees in a satisfactory manner. We believe the revisions have strengthened the paper. We look forward to your response.

Sincerely,

Carol L Rosenberg