Reviewer's report

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

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Reviewer: Dieter Niederacher

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In the paper by Pamela S Larson et al. entitled CDKN1C/p57kip2 is a candidate tumor suppressor gene in human breast cancer the authors hypothesize that decreased CDKN1C, a cyclin-dependent kinase inhibitor, may be involved in human breast carcinogenesis.

LOH of CDKN1C and in the 11p15.5 region were determined in 82 breast cancers, CDKN1C mRNA level was examined in 10 cancers by qRT-PCR and CDKN1C protein expression in 20 cancers by immunohistochemistry. Rare LOH (19%) of the intragenic marker was seen in the FFPE set, CDKN1C expression was reduced (at least 40%) in 9/10 cases. Comparing CDKN1C protein expression in luminal epithelial and myoepithelial cells of normal breast epithelium, CIS and IC lesions of breast cancer specimens a striking loss of CDKN1C protein expression was seen in CIS myoepithelial cells whereas no significant difference was seen in luminal epithelial cells of the different lesions. There were no associations with clinico-pathologic parameters.

Despite several limitations due to small numbers of samples analysed and the very small number of cases with LOH, RNA and IHC results the most interesting observation is the complete loss of protein expression in myoepithelioial cells of CIS lesions. Unfortunately these results were mixed with the results of the luminal epithelial cells where this difference was not seen, consequently the interpretations of the combined results are not differentiated and several conclusions are not properly consistent with the data.

"Major Compulsory Revisions":

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 little difference it is in contrast to the hypothesis postulating a loss of CDKN1C tumor suppressor gene (TSG) function in tumor cells: there are more cases of CIS / IC with higher percentages of positiv cells than in normal luminal cells (data presented in Table 2 and Figure 3).

2) Due to the dramatic increase in the ratio of luminal to myoepithelial cells in CIS and IC it has to be assumed that results of DNA (LOH) and RNA (RT-PCR) analysis reflect AI/LOH and CDKN1C expression of luminal located cancer cells and therefore can not be correlated to loss of CDKN1C protein expression seen in myoepithelial cells of CIS. Together with the fact that there is no difference of
CDKN1C protein expression in normal and CIS/IC luminal cancer cells the overall conclusion that CDKN1C protein expression decreases, 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.

3) I wonder why the authors combine the data of both different cell types (luminal and myoepithelial cells) instead of focusing the discussion on the interesting observation of the complete loss of CDKN1C protein expression in the myoepithelial cell layer of CIS. It is generally accepted that breast cancer arises mainly in the luminal epithelial compartment whereas recent studies indicate that myoepithelial cells may function as a cellular tumor suppressor maintaining tissue polarity and tissue integrity in the normal breast. Moreover, recent work has shown that myoepithelial cells isolated from CIS are drastically altered and it has been suggested that myoepithelial cells which generally continue to surround preinvasive in-situ carcinomas might play a key role in breast tumor progression by regulating the in situ to invasive carcinoma transition (for reviews see the special issue of the J. Mammary Gland Biol Neoplasia; 10(3) 2005).

Within this context it would be very interesting to discuss wether the observed loss of CDKN1C protein expression in myoepithelial cell layers of CIS may be part of a dedifferentiation process of these cells leading to reversal of their tumor suppressive abilities. To clarify the putative inactivation mechanism and/or downregulation of the CDNK1C (e.g. epigenetic inactivation) in myoepithelial cells of CIS would be very interesting.

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**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'