Author’s response to reviews

Title: ADAM33 gene silencing by promoter hypermethylation as a molecular marker in breast invasive lobular carcinoma

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Version: 2 Date: 15 December 2008

Author’s response to reviews: see over
To the BMC Cancer Editorial Office

Dear Editors,

Please accept our enclosed manuscript entitled "ADAM33 gene silencing by promoter methylation as a molecular marker in breast invasive lobular carcinoma" that I am resubmitting for consideration for publication in the BMC Cancer Journal. We include the reviewer’s answers, and the proposed modifications are explained together with the page and line number in the revised paper.

The reviewers suggested language corrections. We have sent the paper to American Journal Experts for English revision. We received an editorial certification (included in this document).

We look forward to hearing from you regarding your opinion of the suitability of the revised manuscript for publication in the BMC Cancer Journal.

Sincerely yours,

Giseli Klassen, PhD
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ADAM33 as a marker in breast lobular carcinoma
The reviewer’s answers

Reviewer
Gulnur Guler

1- The small spelling errors.
We agree and have corrected the words. (page 2, line 6 and line 29)

2-The terms ILC and IDC
We have written the complete names as suggested. (page 2, lines 7 and 8)

3-Methods
About the first question
We have detailed the clinicopathological data and included information about samples that were frozen tissues (page 2, line 44).

We did not review the clinical data. The clinical features were collected from pathological laboratory analyses that were carried out by a medical service from Nossa Senhora das Graças Hospital.

About suggestions
We have detailed the clinicopathological patient features, as can be seen in page 3, lines 1 to 7.

4-The reviewer asked about immunohistochemistry data.
We have included a detailed description as suggested. (page 3, lines 8 to 13)

5-Results
The reviewer requested a discussion about the mechanisms of epigenetic changes.
We agree and have discussed mechanistic possibilities on page 6, lines 36 to 44.

Reviewer
Khalid Sossey- Alaoui

The reviewer suggested some changes.
1-Remove Figure 2a
We agree and have removed the figure and renumbered the remaining figures. We have included in the text detailed information about CpG island localisation. (page 4, lines 36 to 45)

2- Figure 2c should be part of Figure 3
We agree and have renamed the figure 2c for 3a.

3- The reviewer asked about a second CpG island
We agree with this observation. We have included the description of this island and an explanation for why we excluded it from our study. (page 6, lines 8 to 13).

ADAM33 as a marker in breast lobular carcinoma
Reviewer
William Coleman

The reviewer has minor comments

1- Methods.
We have included the clinicopathological data. We have included all data solicited and the information about the samples. (page 2 lines 44 to 47 and page 3 lines 1 to 14)
All breast cancers were primary tumours collected in the Nossa Senhora das Graças Hospital, Curitiba, Paraná, and were immediately frozen at -80 °C until DNA extraction. No paraffin-embedded samples were used in our study.

2- The second comment was about breast cancer cell line origins
We agree about the text and have modified it to include the correct information (page 3 lines 17 to 28). For the purpose to analyse ADAM33 gene expression we used total mRNA provided by Dr Michael O’Hare from the Ludwig Institute in London. The Ludwig Institute from São Paulo, Brazil, helped us by providing breast cancer cell lines for culture, DNA extraction, protein extraction and 5-aza-dCR treatment.

3- The treatment with 5-azadCR question
The cell line tolerance was seven days. We did not observe significant cell death during this period. (page 4, lines 27 to 29)

4- The western blot results
The reviewer suggests an additional positive control. Nevertheless, the reviewer agrees that the western blot results detected a signal in normal immortalised breast cell lines (Figure 1b), which correlated with RT-PCR data (Figure 1a). The experimental objective was to correlate the absence of ADAM33 expression with protein down-regulation. We propose that the ADAM33 down-regulation involves DNA methylation, not post-transcriptional regulation.

5- The labels in Figure 3a
The Figure 3a has been corrected.
ADAM33 as a marker in breast lobular carcinoma