Author's response to reviews

Title: Aberrant methylation of the M-type phospholipase A2 receptor gene in leukemic cells

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Editor
Prof. Dr. Paolo Bruzzi
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Dear Prof. Dr. P. Bruzzi,

Thank you for your consideration of our MS. We would also like to thank the Referee for the critiques, all of which we found very helpful.

According to your request, we included the following.
Point-by-point description of the changes made to the revised MS according to the suggestions follows.

Response to Reviewer#2:
Comments to authors:
In the study entitled "Aberrant methylation of the M-type phospholipase A2 receptor gene in leukemic cells", the authors are interested in discovering whether the DNA methylation of PLA2R1 promoter is altered in leukemic cells. The authors display for the first time an increase in PLA2R1 promoter methylation in peripheral blood samples and in bone marrow aspirates from leukemic patients when compared to healthy ones. Hypermethylation of the promoter is also observed in some cancer cell lines, and a treatment by a demethylating agent in 2 cancer cell lines provokes PLA2R1 mRNA re-expression. They also displayed that PLA2R1 methylation status correlates with IPSS classification and thus may be useful as an additional biomarker.

Specific comments:
COMMENT #1
The first figure support a link between PLA2R1 DNA methylayion and its mRNA level in 2 cancer cell lines. Unfortunately, in patient samples there is no demonstration of this link between the increase in PLA2R1 promoter methylation observed in leukemia samples compared to the healthy ones and a decrease levels of PLA2R1 mRNA. I think this point have to be tackle.

We agree with the reviewer that the question of whether a link between PLA2R1 gene methylation and PLA2R1-mRNA expression exist in blood samples of leukemic patients is of special interest. However, it contrast to the 100% PLA2R1 methylation in U937 and Jurkat leukemic cell lines (Fig. 1), we did not find such a complete PLA2R1 methylation in the DNA isolated from blood leukocytes of leukemic patients using HRM analyses (Fig. 3). Moreover, melt curve analyses demonstrated that in all studied leukemic blood samples at least two leukocyte subfraction were present characterized by hypermethylated or unmethylated PLA2R1 sequences (for example Fig. 6). The latter cell subfraction is likely characterized by unaffected normal blood leukocytes which may express PLA2R1-mRNA. Therefore, a simple isolation of RNA and DNA from blood leukocytes and following HRM and RT-PCR analyses will not be able to answer the question of whether the identified hypermethylation correlates with PLA2R1 expression. Therefore, it will be necessary first to identify the cell subfraction which is responsible for the identified PLA2R1 hypermethylation in peripheral blood samples of leukemic patients and second, to isolate this cell subfraction from peripheral blood mononuclear cells for RNA preparation and RT-PCR analyses. These analyses will be the objective of a further study for which adequate amounts of blood cells are necessary for bisulfite modification and HRM analyses (at least 500 ng DNA are needed for bisulfite modification) as well as RT-PCR analyses. Such recruitment of blood samples is now under
prearrangement and the obtained results will be published separately.

For this reason, in the revised version of the manuscript it is written (page 17, lanes 17-20):

“Therefore, in future studies it will be interesting to define cell types, in which the PLA2R1 gene is hypermethylated and whether in these cells the hypermethylation is connected with PLA2R1 silencing.”

COMMENT #2
I do not understand the interest of the figure 3 that is just a zoom (if I understand properly) of the results presented for the patients in the Figure 2B. If presented, it should be as Figure 2C.

We agree with the reviewer and removed figure 3 in the revised version of the manuscript.

COMMENT #3
In figure 2, I guess instead of "methylierung" should be "methylation".

It is correct and we used "methylation" instead of "methylierung" in Figure 2 of the revised version of the manuscript.

COMMENT #4
In conclusion, I recommend publication only if the authors are able to show a correlation between PLA2R1 DNA methylation and mRNA expression in leukemia samples.

This aspect we already discussed in the COMMENT #1.

In conclusion, we would like to thank the reviewer for the constructive critiques, which ultimately helped us to significantly improve the MS and to work out the strategy of further studies in this field.

We hope you may find the revised version of our manuscript suitable for publication in BMC Cancer. Please rest assured that this work is original and not under consideration elsewhere.

Sincerely yours,

Mario Menschikowski, PhD
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