

March 3, 2014



Dear Editor,

Please find enclosed the revised manuscript in Word format (file name: 7808-review.doc).

**Title:** THE ROLE OF PRMTs IN CANCER: COULD MINOR ISOFORMS BE LEAVING A MARK?

**Author:** R. Mitchell Baldwin, Alan Morettin and Jocelyn Côté

**Name of Journal:** *World Journal of Biological Chemistry*

**ESPS Manuscript NO:** 7808

The manuscript has been improved according to the suggestions of reviewers and we have addressed each specific point. Additionally, our changes to the review manuscript are highlighted throughout the text. Our responses to each of the specific points made by the reviewers is presented below.

Reviewer #1

*Reviewed by 00628961*

The review article submitted by Baldwin et al., entitled "THE ROLE OF PRMTs IN CANCER: COULD MINOR ISOFORMS BE LEAVING A MARK?" summarized the updated information on the role of PRMTs and their spliced isoforms on cancer development and progression. The authors provided a brief but comprehensive report on the expression and roles of PRMT1, PRMT2, CARM1 and PRMT7, in particular how each of them interacts with different cellular signaling pathways to uncover the underlying causes of disease. Although the potential mechanisms of PRMTs and their spliced isoforms in the cancer development and progression are not completely understood, this reviewer noticed that the article is informative and provides updated and important information, not only in the field of molecular biology, but also cancer research. There is no major compulsory revision needed. Essential revision is listed as follows: 1. The expression of PRMTs showed a tissue-specific manner, the authors should consider explaining why several PRMTs are involved in a particular cancer in more detail in the text. 2. Please provide the molecular weight of each PRMT in the Table 2.

**Specific criticisms:**

1. The expression of PRMTs showed a tissue-specific manner, the authors should consider explaining why several PRMTs are involved in a particular cancer in more detail in the text.
2. Please provide the molecular weight of each PRMT in the Table 2.

**Response:** There is no real trend in terms of expression and cancer type that can be determined from the reports that have been published thus far. Expression of these isoforms for the PRMTs has only been examined in a limited number of tumour types. Therefore, more research would need to be done to make a comment on the relationship between tissue expression and their association with specific cancer types. A new sentence addressing this point was added (Page 7). We have added the molecular weights of each PRMT isoform to Table 2.

Reviewer #2

Reviewed by 00064830

Baldwin et al., WJBC 2014 The current manuscript summarizes various literatures on protein arginine methyltransferases (PRMTs) that catalyze the methylation of a variety of protein substrates including histones and non-histone proteins. Based on the title, the emphasis should have been given on their isoforms and roles in cancer. However, they have only pointed out that the knowledge on this aspect is limited. No new information was included in the current review. Authors did good job on summarize the facts on different isoforms and how they were originated. Among nice members of PRMT family, only 4 of them including PRMT1, PRMT2, CARM1 and PRMT7 were reviewed. Reason why the other members including the major species PRMT5 were not reviewed was not given. Maybe no such isoform was found in PRMT yet, but it should be pointed out. The other important aspect that the current review has missed is the substrates and substrate specificity of the PRMTs. So far there are only about 150 non-histone proteins are methylated. This information was summarized in Guo et al., 2010, Nature Chemical Biology as a supplementary table. Do the isoforms have different substrate specificity? In addition, Figure 3 the unpublished data from the author's laboratory does not fit to the current review article and does not well support any of the authors' points. Delete it. Table 3 is not deserved as an independent table for a few reasons. 1. Because the information is only from a few publications. It seems to be misleading, making one to conclude that those isoforms only exist in breast and cervical cancers. 2. It was not well discussed in the text; 3. Some of the information can be folded into the Table 1. Additional discussion and correspondences between the illustrations and text are required in this review article.

#### **Specific criticisms:**

1. Baldwin et al., WJBC 2014 The current manuscript summarizes various literatures on protein arginine methyltransferases (PRMTs) that catalyze the methylation of a variety of protein substrates including histones and non-histone proteins. Based on the title, the emphasis should have been given on their isoforms and roles in cancer. However, they have only pointed out that the knowledge on this aspect is limited. No new information was included in the current review. Authors did good job on summarize the facts on different isoforms and how they were originated. Among nice members of PRMT family, only 4 of them including PRMT1, PRMT2, CARM1 and PRMT7 were reviewed. Reason why the other members including the major species PRMT5 were not reviewed was not given. Maybe no such isoform was found in PRMT yet, but it should be pointed out.

**Response:** We believe that this review does focus on the isoforms of PRMT family members. Expression of PRMT isoforms have been confirmed for only PRMT1, PRMT2, CARM1/PRMT4 and PRMT7. So this is the reason for only focusing on these four family members. There are predicted alternatively spliced isoforms for the other PRMT family members, however, these have not been confirmed nor studied at all. We have clarified the reason for only examining PRMT1, PRMT2, CARM1/PRMT4 and PRMT7 in the Introduction section (Page 5). There is limited knowledge with respect to the specific functions of all the PRMT isoforms that have been studied. There have been several recent reviews that have described the overall field of PRMTs and cancer, however, with the emergence of alternative PRMT isoforms we felt it was a new and exciting area of PRMT research that needed attention. We feel that the idea of these isoforms having potentially distinct functions and contributions to cancer is a new idea and this is the first review to highlight the literature describing these isoforms. The intent of this manuscript is to provide an opinion-style mini-review on a new emerging topic, and thus, not to include new results per se. We were invited by the WJBC to contribute this Review based on our recent work in alternatively spliced isoforms of PRMTs.

2. The other important aspect that the current review has missed is the substrates and substrate specificity of the PRMTs. So far there are only about 150 non-histone proteins are methylated. This information was summarized in Guo et al., 2010, Nature Chemical Biology as a supplementary table. Do the isoforms have different substrate specificity?


Response: We added to sections regarding the knowledge we have regarding enzymatic activity and substrate specificity of these PRMT isoforms, for PRMT1 (Page 8), PRMT2 (Page 18), CAMR1/PRMT4 (Page 24) and PRMT7 (Page 27). References to the arginine substrate repertoire of the PRMTs has also been added (Page 5). The supplementary table (in Guo et al. 2010) referred to by the reviewer includes both histone and non-histone.

3. In addition, Figure 3 the unpublished data from the author's laboratory does not fit to the current review article and does not well support any of the authors' points. Delete it. Table 3 is not deserved as an independent table for a few reasons. 1. Because the information is only from a few publications. It seems to be misleading, making one to conclude that those isoforms only exist in breast and cervical cancers. 2. It was not well discussed in the text; 3. Some of the information can be folded into the Table 1. Additional discussion and correspondences between the illustrations and text are required in this review article.

Response: Figure 3 represents unpublished data that we have generated in our laboratory. The graph represents mass spectrometry data from a SILAC immunopurification experiment where we isolated PRMT1v1 bound proteins and PRMT1v2 bound proteins independently. Using this method we are able to determine PRMT1v1-specific and PRMT1v2-specific interacting proteins/substrates. As well we can determine interacting proteins that are shared (ie. can bind to either PRMT1v1 or PRMT1v2). We have represented this data in a quadrant graph to highlight that these two alternatively spliced isoforms have their own set of binding partners/substrates, which emphasizes that while they share some functions they may also play distinct roles in cells. This exemplifies the differences between these two PRMT1 isoforms and we feel it is a key feature. We have added to the description of this data to clarify its importance (Page 8). We are currently preparing a manuscript for submission that identifies the proteins we found in this study. Additional references to the figures and tables have been added throughout to clarify ideas. We have condensed Table 3 into Table 2 to simplify the information on the isoforms. In the conclusion we have also added a clarifying point regarding the fact that all these isoforms have mainly been assessed in breast cancer cells. This is not because they are only in breast cancer cells, but due to the fact that they have not been studied at all in other tumour types (Page 27).

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Sincerely yours,



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