Reviewer Report

Title: Genome-wide determination of on-target and off-target characteristics for RNA-guided DNA Methylation by dCas9 methyltransferases

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Reviewer Comments to Author:

In this study "Genome wide determination of on-target and off-target characteristics for RNA-guided DNA Methylation by dCas9 methyltransferases (CRISPRme)" Lin et al. generated CRISPR fusion proteins with DNMT3A/B proteins and tested them for their on/off target effects. As applications for CRISPR proteins are being presented on a weekly basis, also with potential clinical use, it is important to assess the specificity of these approaches, even if the results might be to a certain extent redundant and replicated in other studies.

While there is potentially interesting data presented by the authors, I found several aspects of the study inconclusive. My general and specific concerns are as follows:

1) In general, this study should be presented in a shorter format. The authors have undoubtedly created a lot of data and invested time and money in the study, however, the manuscript is difficult to read and is not very cohesive. For example, the CRISPRme1 and CRISPRme2 should probably be compared side by side.

2) I don't understand the argument that hypomethylated regions are "likely stochastic DMRs resulted from in vitro cell cultivation and manipulations", while hypermethylated regions have to be the consequence of CRISPRme off-targeting. Hypomethylated regions should be used as a metric for noise in the experiments and to access false positive rates. I am very worried that the number of hypomethylated DMRs is in the same range as the number of hypermethylated DNA (group 1: hypermethylated DMR (hyper-DMR) = 16169, hypomethylated DMR (hypo-DMR) = 11172; group 3: hyper-DMR = 12500, hypo-DMR = 11996). To me this suggests that the off target effects the authors see are merely an expression of noise in the system. Unless the authors can rectify this relation, I am afraid their study remains inconclusive or underpowered for a majority of the claims.

3) The authors find significant de novo methylation in of the uPA promoter with scrambled gRNAs, although to a slightly lower extent than the uPA targeting gRNA. I am surprised that these off target effects the authors describe (there was another one on GAPDH, I think) sampling so few loci do not translate into genome-wide elevations of methylation levels.

In conclusion, I think the authors have done a significant amount of work, but I am wondering whether they are presenting the data in the best way possible and whether they are drawing the right conclusions. Maybe it would be best to concentrate on some core messages (inhibition not being methylation dependent, for example). I am especially worried about the off target effect conclusions, which in my opinion are not supported by the data.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? No

Conclusions

Are the conclusions adequately supported by the data shown? No

Reporting Standards

Does the manuscript adhere to the journal's guidelines on minimum standards of reporting? Yes

Statistics

Are you able to assess all statistics in the manuscript, including the appropriateness of statistical tests used? Yes, and I have assessed the statistics in my report.

Quality of Written English

Please indicate the quality of language in the manuscript: Acceptable

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