Reviewer's report

Title: The High Frequency Aberrantly methylated Targets in Pancreatic Adenocarcinoma Revealed by A Global DNA Methylation Analysis Using MethylCap-seq

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Reviewer number:1

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It is now well established that changes in the epigenomic landscape, including of DNA methylation profiles are a characteristic of many cancer types. These often include hypermethylation of promoter regions, characterized by CpG islands, of many genes as well as reduced methylation of repeated DNA sequences and some individual genes. Hypomethylation of repeat sequences has also been associated with illegitimate recombination and chromosomal instability. A broad range of genes are commonly methylated in different cancers, including pancreatic cancer.

To understand the extensive reprogramming and dysregulation of DNA methylation in pancreatic cancer, the authors have mapped the differentially methylated regions (DMRs) in pooled samples from 10 PC tissues and from 10 adjacent non-tumor tissues using MethylCap-seq. The technique involves in vitro capture of methylated DNA with the high affinity methyl-CpG binding domain of human MBD2 protein and subsequent analysis of enriched fragments by massively parallel sequencing.

Pertaining to the technique used, I wish to know author’s specific views on two aspects:

First, unsuccessful or incomplete capture reactions might have resulted in the sequencing of non-methylated DNA fragments, leading to inconsistencies in or the absence of methylation enrichment in their sample.

Second, poor sequencing library complexity and CpG coverage often limits the statistical power to call differential methylation, affecting ultimately the reproducibility of the dataset. Explain.

How was the quality control experiment performed?

A schematic representation of work-flow adopted for primary and secondary data analysis must be included. This will help the reader to get an over-view of the exercises performed.

I suggest the authors to provide a clear-cut account on two sections of the manuscript:

First, the principal objective and aim of the study is ill defined in the introduction. I

strongly believe that inclusion of the same in the last paragraph of introduction will strengthen the rationale of the study.

Second, what is translational utility of the work? This has to go a step-beyond explaining the importance of using the methylational profile as biomarker. Specifics must be provided for a few clusters only. A couple of lines on which specific experimental maneuvers will be necessary before these can be utilized in a clinical setting, must be included in the end segment of discussion. This will help to significantly elevate the impact of the article.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.