Reviewer’s report

Title: Comparative Analysis of 14-3-3 Isoform Expression and Epigenetic Alterations in Colorectal Cancer

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Reviewer: Chen Wang

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The manuscript summarized studies of mRNA expression differences of 14-3-3 family proteins between cancer and normal colon tissues, and found 14-3-3 sigma and eta expression down-regulations are associated with promoter methylation. Some functional work was also done for 14-3-3 eta to verify its impacts in inducing transformation of NIH3T3 cell-line. Authors did a lot of works to address these few isoforms, so my overall impression is positive. A few comments and suggestions for Minor Essential Revisions:

1. Did authors try using IHC to tell where are the differential signals coming from? It concerned me a little bit the tissue heterogeneity from the methods “All adenocarcinomas (56 Grade II and 15 Grade III) were confirmed by 94 pathological evaluation and contained an average density of 51% lesion/49% stroma.” Just want to have some assurance or at least some discussions that 14-3-3 differential signals are not coming from stroma. Is that expected the stroma component proportions in tissue are so high, nearly half? Average 49% means there must be some samples with even higher stroma %.

2. For eta and sigma methylation analysis, some methylation and expression scatterplots are appreciated, with reports of actual negative correlation values. Consider to put as sub-panel plots with Fig. 4? I’m also wondering if you could also confirm this from TCGA, since illumina 27k or 450k methylation platform is in every TCGA cancer type.

3. Since TCGA was brought up, authors may want to look at RNA-seq data of colon cancer as well. Bottom line, are these 14-3-3 changes also confirmed from RNA-seq levels, which should show much greater dynamic changes than microarrays. Optionally, RNA-seq also has transcript levels and exon levels that might be helpful for further confirming.

4. depressed => down-regulated? suppressed? “A parallel analysis of microarray data from the TCGA dataset confirmed that expression of sigma and eta were depressed in colon tumors.”

5. I suggest either removing “it comes as no surprise” or rewording it; it’s a little bit strange statement. “it comes as no surprise that dysregulation of these proteins has been linked to several human diseases and that 14-3-3s have even been proposed as…”

6. Dynamic => diverse? “When compared with tumor stage several 14-3-3 genes exhibited a dynamic change in expression.”
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.

Declaration of competing interests:
I declare that I have no competing interests.