Reviewer's report

Title: DNA methylation changes in ovarian cancer are cumulative with disease progression and identify tumor stage.

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Reviewer: Yuanyuan Xiao

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The manuscript by Watts et al. investigates CpG methylation changes in ovarian cancer using a CpG island-based microarray. As my role of the statistical reviewer of this manuscript, I found it to be very well written and its publication will be of interest to a wide range of readers.

I have a few comments, however, which will only require a limited amount of extra efforts from the authors:

1) Using replicated hybridizations to estimate fold change FDR is a biased approach as the error structure in the real experiment concerns with biological variation not hybridization variation. So instead of examining pairwise replicate hybridizations, the authors should examine among the 9 independent ovarian samples. However, since the p-values derived from the ANOVA F-test is already multiple testing corrected by the Benjamini-Hochberg method, it seems unnecessary to estimate FDR separately for fold-changes and it is not uncommon for array studies to apply double criteria of significance and fold changes.

2) Typo: "Benjamini Hochburg False Discovery Rate" should be "Benjamini Hochberg..."

3) I have concerns about how cross validation is applied in building the classifiers and estimating prediction errors in this study. The authors select discriminative features at the outset instead of within each cross-validation fold, for instance for the SVM classification of normal/LMP and stage III cancer, the authors selected genes satisfying p-value cutoffs of 0.01, 0.005, 0.001 and 0.0005 using all samples and before classification is even applied. This results in data re-use and will result in an under-estimation of classification error, because the "validation" set is also used in feature selection and therefore influences classifier construction. For references on this "honest" cross validation issue, please consult (i) Dudoit S. et al, In Microarray Data Mining (G. Piatetsky-Shapiro and P. Tamayo (eds)), Special Issue of SIGKDD Explorations, 5: 56-68. and (ii) West M et al, Proc Nati Acad Sci 2001, 98(20):11462-7. I think this problem at least needs to be mentioned and discussed in the Discussion section.

4) I think an M vs A plot for an exemplary slide is needed in the Additional Data
section to give the readers a sense of data representation and quality.

**Level of interest:** An article of importance in its field

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