Reviewer's report

Title: Gene signatures ESC, MYC and ERG-fusion are early markers of a potentially dangerous subtype of prostate cancer

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Reviewer: Dan Mercola

Reviewer's report:

The authors set out to use independent expression data for fresh frozen prostate cancer cases to validate profiles previously developed by Markert et al. with four classifications of outcome of prostate cancer. Simple validation of the four groups was not observed but using estimated gene expression values of the original study two groups were noted of potential significant clinical properties: a group characterized by ESC, ERG-fusion and MYC+ rich signatures in patients diagnosed with low Gleason score but with poor clinical prognosis and a second group with good prognosis. Agreement was improved by correction for the known amount tumor and non tumor tissue thereby being one of the few studies to account for the tissue type heterogeneity of prostate cancer. The authors also assess whether genetic heterogeneity was a factor. These are potentially important findings. Several caveats remain.

The major drawback is that 54 patients from the authors' cohort of 116 patients were assigned to gene profiles associated with bad outcome but the actual outcome for most of these patients are not known so a major check is not available. Similarly there is no independent statistic for the significance of the composition of a cluster. Alternatively the false discovery rate (FDR) for the classification (clustering) should be determined. This might be done by using a random selection of values from Figure 1A not including the chosen profile or even use random values within the range of values shown in Figure 1A and repeating the clustering process and counting the percent of the 54 patients clustered together where a patient is a member with a value above or below a suitable threshold based on the current cluster. There is a facility in R among other packages for this process where it can be repeated a large number of times, say 100, to provide an average for the number of the 54 patients that are clustered together as one estimate of FDR. Several alternatives can be imagined. More rigorous would be to a random selection of the genes of Markert et al. to cluster their cases and use exact values in place of the visual estimates. An estimate of the FDR is essential.

Did the authors demonstrate that the new subgroup can cluster ‘Unassigned’ samples in Figure 1B into Bad or Good Prognosis groups?

Correlation is one way to help with the classification. Were other methods used such as Euclidean distance with consistent results?
It is not clear how “normal” tissue was defined and chosen which should be described in detail especially any distance separation from tumor.

The term ‘diagnosis’ and ‘prognosis’ are occasionally used as equivalent, e.g. line 41 which should be corrected throughout as the focus in prognosis only.

Minor points are:
Line 86: ‘poly clonal composition’ vs. ‘multi-nodality’.
Line 357: ‘expectedly’ vs. ‘As expected’.ad the first

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

None