Reviewer's report

Title: Up-regulated Expression of l-Caldesmon associated with Malignancy of Colorectal Cancer

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Reviewer: Amanda B Hummon

Reviewer's report:

The manuscript describes the determination a splice variant of Caldesmon is associated with more aggressive grade colon cancers. The authors provide both primary tissue evidence and then perform follow-up in vitro studies using commonly available cell lines. Overall, it is an interesting study and describes a potentially valuable target in colorectal cancer. However, there are some aspects of the experimental design and analysis that need to be addressed before it can be published in BMC Cancer.

2 Major Compulsory Revisions:

1) The RNA interference study is performed with only one siRNA targeting Caldesmon in a single cell line. This does not provide sufficient proof to conclude that knockdown of Caldesmon is causing changes in the MTT response. At the very least, the authors need to repeat the experiment with a second siRNA to rule out the possibility of off-target effects. With more than one siRNA, the authors can make the conclusion that reduction of Caldesmon is causing the change. Also, there is no evidence provided that Caldesmon is actually reduced. Either an RTPCR or Western showing either transcript or protein knockdown is needed.

2) In the discussion, the MTT results are used to conclude that knockdown of Caldesmon causes changes in invasiveness in cells. This is an enormous jump in logic and the phenotypic assays do not support this conclusion. MTT is an assay designed to measure the viability of cells, not invasive capacity. There are many in vitro assays that can be used to assess invasive capability, but MTT is not one of them.

Discretionary Revisions:

3) A table of patients characteristics would be helpful.

4) One cell line that was not included and would make an interesting comparison is SW480. SW620, the patient-matched metastatic line, is included, so it would be valuable to analyze it as well if possible.

5) More experimental detail about the MTT assay would be helpful. (# cells/well, # biological replicates, etc)

6) I would be interested in seeing the individual cell line data that was used to put together Figure 4b. I think that might be more compelling.
Given the current data, this manuscript needs revision prior to publication.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.