Reviewer's report

Title: Fibulin-1 is Epigenetically Down-regulated and Associated with Bladder Cancer Recurrence

Version: 2 Date: 4 July 2014

Reviewer: Mathilde Borg Houlberg Thomsen

Reviewer's report:

1. Is the question posed by the authors well defined? Yes
2. Are the methods appropriate and well described? Yes
3. Are the data sound? To some extend
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Not relevant
5. Are the discussion and conclusions well balanced and adequately supported by the data? Not adequately
6. Are limitations of the work clearly stated? Yes
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes
8. Do the title and abstract accurately convey what has been found? Yes
9. Is the writing acceptable? Yes, minor corrections could be made

Major Compulsory Revisions

Figure 1B: The authors conclude that the protein expression levels are significantly lower in the four selected bladder cancer cell lines compared to the normal bladder cell line – however levels are so low that this cannot be determined.

Figure 2B: The authors state that hypermethylation of promoter CpG islands is detected in all four bladder cancer cell lines which isn’t the case for 5637 and HT1376. This is problematic since all downstream experiments are performed using these two cell lines. Therefore the authors need to clarify whether the promoter region of FBLN1 is indeed methylated in these two cell lines in order to draw valid conclusion on the epigenetic influence.

Figure 3A: decreased viability is observed with pEGFP-FBLN1 compared to Mock and pEGFP in both cell lines, however it cannot be concluded that proliferation is reduced since the assay measures cell number. Also, the authors show that the pEGFP-FBLN1 undergoes apoptosis to a higher degree compared to Mock and pEGFP, thus it can only be concluded that FBLN1 induces reduced cell viability/apoptosis, but not a slower growth rate/proliferation.
Figure 3C+D: Cell viability should also be tested using the Lenti vector system in order to rule out, that the reduced colony formation is not due to transfected cells undergoing apoptosis and not due to reduced tumorigenicity.

Minor Essential Revisions

Figure 1D: Normal and tumor samples should be normalized to sample size in order to compare the two groups.

Figure 2C: Tumor samples should be normalized to their respective normal sample and when presenting relative expression, normals should be set to 100.

Figure 4E: The different conditions should be applied to the figure rather than letters a-d. Also, HUVEC could be indicated in E and F.

Discretionary Revisions

Please rephrase the sentence in the discussion: “Interestingly, the expression of fibulin-1 in cancer cell lines wasn’t fit well on the notion that found in tissues”.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.