Reviewer’s report

Title: Decreased expression of RBM3 correlates with tumour progression and poor prognosis in urothelial bladder cancer

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Reviewer: David DeGraff

Reviewer’s report:

This manuscript by Boman and colleagues examines the loss of RBM3 in human bladder cancer with a special emphasis on pTa and pT1 tumor stages. Alterations in RBM3 expression have been associated with other malignancies. The patient cohort was rather large (n=344 patients). The authors report that loss of RBM3 is associated with advanced tumor stage and grade, disease specific survival, and 5 year overall survival. Kaplan-Meier analysis indicates significantly shorter progression free survival and five year overall survival. The authors are asking the right questions by focusing on pTa (high recurrences and very low rate of progression to invasive disease-not much understood about the biology) and pT1 (high grade T1 is a “clinical conundrum” and precursor to muscle invasive disease). Unfortunately, there was no association between RBM3 and recurrence rate.

Minor essential revisions:
1. Representative staining from each tumor stage should be shown.
2. Why was the combined nuclear score used, as opposed to binary positive/negative? What is the rationale for this approach?
3. The authors state that there was no obvious heterogeneity between duplicate cores for RBM3 staining. What was the % heterogeneity observed?
4. On the bottom of page 8, the authors state “There was a borderline significant association between RBM3 expression and CSS in both univariable analysis….” What is CSS? Cancer specific survival? This is the first time this is used, and is confusing. Did the authors mean DSS?
5. In the discussion, the authors state that they examined a larger “prospective cohort.” This is a retrospective analysis of archival tissue.

Major essential revisions:
1. Although this should be less of an issue in pTa disease, examination of different histological subtypes (pure squamous, adeno, small cell, micropapillary, etc) would be informative. Perhaps the strong association between RMB3 loss and advanced tumor stage is because advanced tumors exhibit anywhere from 20-40% mixed squamous differentiation?
2. Examination of lymph nodes dissected from patients undergoing cystectomy for RMB3 expression would be informative (preferably from matched patient
samples used in the TMA; ~20 or so randomly selected.), especially since there
may be an association with invasion.

3. In addition to the observed correlations between RBM3 expression and clinical
outcome, the authors should consider screening commonly used human cell
lines for RMB3 expression. This would inform other investigators interested in the
biological role of RBM3 in bladder cancer.

Discretionary revisions:

1. The potentially organ-specific influence of RBM3 on cell proliferation and
invasion is interesting, because it provides an opportunity for the design of a
simple experiment. The authors should identify bladder cancer cell lines to knock
down and overexpress RBM3. This would enable them to test how RBM3
expression impacts IC50 in cell lines following cisplatin exposure, the main stay
of chemotherapeutic treatment for advanced bladder cancer.

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests.