

RBM3: A Prognostic and Treatment Predictive Biomarker

Introduction

Cancer is continuously a major health problem throughout the world. In 2008, there were an estimated 12.7 million new cancer cases world-wide, and 7.6 million deaths that could be attributed to cancer¹. Due to a world population with an increased life-expectancy, there is no reason to expect a decline in cancer incidence in the near future².

Cancer, though often denoted as a singular disease, is truly a multitude of diseases. This understanding has evolved over the years, and though common knowledge today, many patients are still not receiving optimal treatment for their disease. For cancer patients to receive a more individualized treatment, there is still a need for new and better ways to stratify patients. The classical, pathology based, prognostic factors such as stage and grade of the tumor are insufficient for a correct estimation of patient prognosis. Additional information from immunopathology biomarkers promise to substantially improve this estimation, ultimately leading to a more individualized treatment, thus avoiding both under- and over treatment of patients.

RBM3 and the Human Protein Atlas

The RNA-binding motif protein 3 (RBM3) was recently identified via the Human Protein Atlas (HPA) as a potential oncology biomarker through the differential expression pattern present in several cancers investigated as part of the HPA project (proteinatlas.org)^{3,4}.

RBM3 is an RNA- and DNA-binding protein, whose function has not been fully elucidated. It has been shown that the protein is expressed as an early event in mild hypothermia, and also in other conditions relating to cellular stress, such as glucose deprivation and hypoxia⁵. During stress, RBM3 is thought to protect the cells by aiding in maintenance of protein synthesis needed for survival⁵. Recently,

it has also been shown that RBM3 attenuates stem cell-like properties in prostate cancer cells⁶.

RBM3 monoclonal antibody

The monoclonal Anti-RBM3 antibody AMAb90655, used in all cases presented here (available for purchase from Atlas Antibodies, Stockholm, Sweden, atlasantibodies.com), has shown excellent specificity in Western Blot analysis of human cell lines, and is routinely used for staining of formalin fixed paraffin embedded tissue in IHC. A representative image of immunohistochemical staining using the RBM3 monoclonal antibody AMAb90655 can be seen in Figure 1.

RBM3 as a prognostic biomarker

After identification of RBM3 as a potential prognostic biomarker, RBM3 protein expression has been analyzed using AMAb90655 in many different patient cohorts from various forms of cancer. Levels of RBM3 expression was found to have a significant connection to patient survival in breast⁷, colon⁸, ovarian^{9,10}, testicular, urothelial¹¹, and prostate¹² cancer as well as in malignant melanoma¹³.

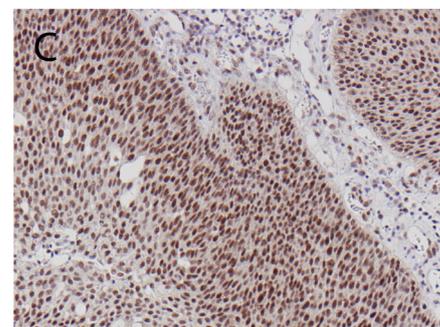
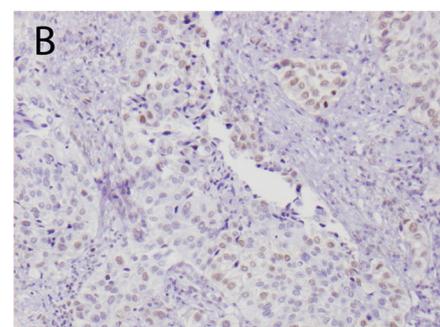
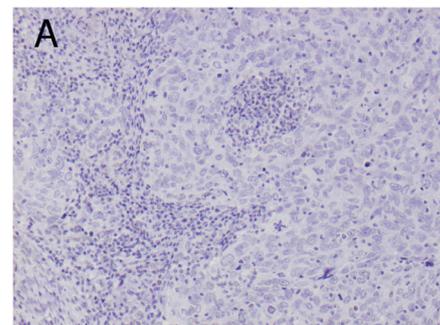


Figure 1

Figure 1 shows representative nuclear staining of RBM3 in urothelial bladder cancer, with no RBM3 expression (A), intermediate RBM3 expression (B) and high RBM3 expression (C) respectively.



Urothelial cancer

A major clinical problem in urothelial cancer is the identification of high-risk patients among those diagnosed with non-invasive disease. For patients with stage T1 disease (where the tumor has invaded connective tissue but not yet muscle), the prognosis is generally good, but nearly one third of these patients will still eventually require cystectomy when other therapies have failed. The identification of these patients at an early stage would be a great improvement for patients as well as society. Due to high recurrence rates, and need for invasive monitoring of the disease, the health care cost per patient is higher for urothelial cancer than for any other cancer in the USA¹¹. For patients with muscle-invasive disease, prognostic markers that could guide in the choice of treatment and/or treatment intensity would also mean an improvement in patient care.

RBM3 has been shown to be a prognostic marker in urothelial cancer, with a significantly improved survival for patients with high levels of RBM3 expression in their tumors¹¹ (see Figure 2). This means that analysis of RBM3 expression could be a prognostic factor for better stratification of patients, leading to a more individualized treatment of patients with urothelial cancer.

Figure 3 shows overall survival of the patients in the cohort with Ta (non invasive) and T1 tumors, and the same results can be seen as for the full cohort. For the subgroup of patients with T1 tumors, 5-year overall survival was significantly reduced for patients with tumors not expressing RBM3, indicating that IHC analysis of RBM3 expression may indeed be used to identify stage T1 patients in need of cystectomy. The prognostic significance of RBM3 in urothelial cancer has been confirmed in several independent cohorts.

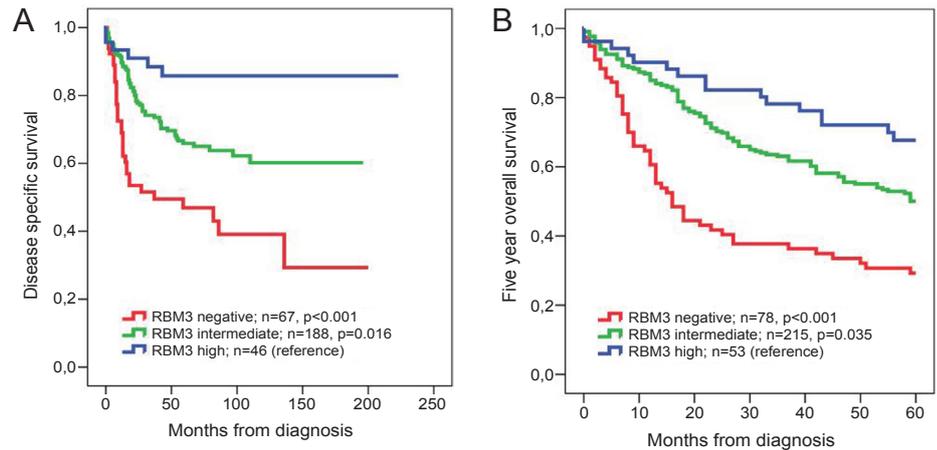


Figure 2

Figure 2 shows Kaplan-Meier analysis of cancer-specific (A) and 5-year overall (B) survival of 343 bladder cancer patients stratified according to RBM3 expression.

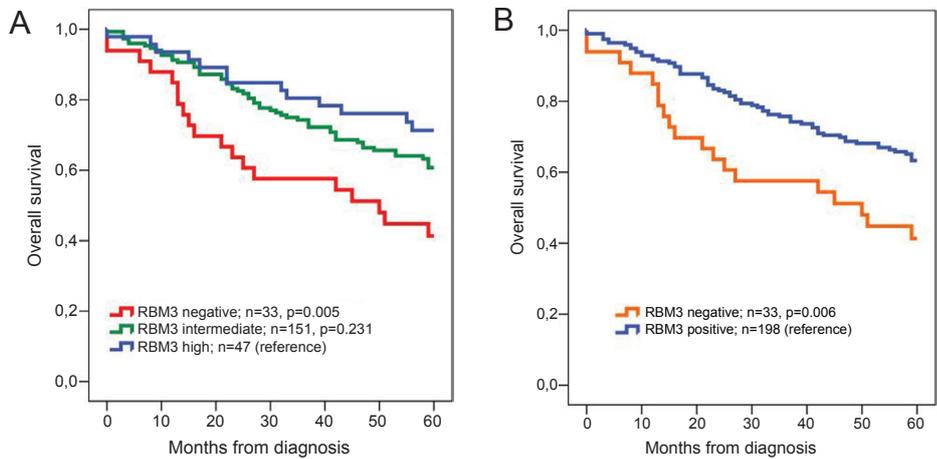


Figure 3

Figure 3 shows Kaplan-Meier analysis of 5-year overall survival of the 231 patients in the cohort diagnosed with stage Ta or T1 disease. Patients were stratified according to RBM3 expression, either according to negative, intermediate, and high expression (A), or according to negative vs. positive expression (B).



Colorectal cancer

In colorectal cancer, a major clinical problem is to identify patients in need of adjuvant therapy. The only curative treatment today is surgery, but adjuvant treatment may prolong patient survival, and is recommended for patients with stage III and high-risk stage II disease. However, not all high-risk stage II patients benefit from adjuvant treatment, thus to categorize these patients is of utmost importance. A biomarker that provides additional information on risk assessment would be a great step to improve patient care.

RBM3 has been shown to be a prognostic marker in colorectal cancer in two independent patient cohorts, with a significantly improved survival for patients with high levels of RBM3 expression in their tumors⁸ (see Figure 4). This means that analysis of RBM3 expression could be a prognostic factor for better stratification of patients, leading to more appropriate treatment for patients with colorectal cancer. When analyzing patients with stage II disease only, similar results can be seen as for the full cohort (Figure 5), indicating that analysis of RBM3 expression may be a valuable tool in deciding whether or not stage II colorectal cancer patients should receive adjuvant treatment for their disease.

It could be concluded that RBM3 is a prognostic marker that may aid in determining if patients should receive adjuvant treatment, and also the intensity of the treatment.

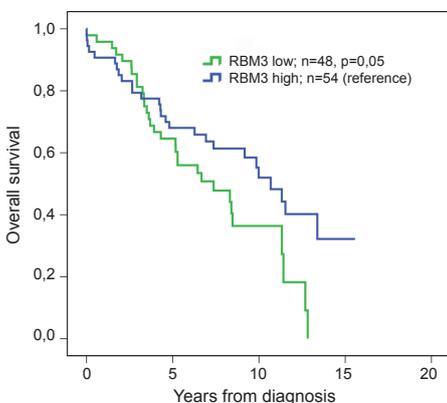


Figure 5 Figure 5 shows Kaplan-Meier analysis of overall survival of the 102 patients in the cohort diagnosed with stage II disease, stratified according to RBM3 expression.

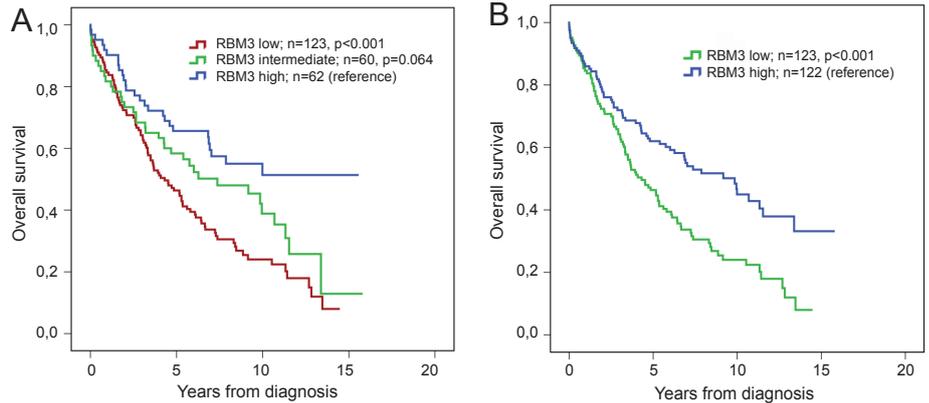


Figure 4 Figure 4 shows Kaplan-Meier analysis of overall survival of 305 colorectal cancer patients. Patients were stratified according to RBM3 expression, either according to low, intermediate or high expression (A), or according to low versus high expression (B).

RBM3 as a treatment predictive biomarker

During analysis of RBM3 as a prognostic biomarker in ovarian cancer, it was speculated about the potential connection between sensitivity to platinum-based therapies and RBM3 expression⁹. Cisplatin treatment is a cornerstone in ovarian cancer treatment and most patients in the cohorts analyzed were thus likely treated with cisplatin. The ovarian cancer cell lines A2780 and A2780-Cp70 (a cisplatin resistant cell line derived from A2780) can be used as a model system for studies related to cisplatin treatment and response. Studies of these two cell lines revealed that RBM3 expression (assessed by Western Blot as well as IHC analysis) was significantly higher in the cisplatin sensitive A2780 cell line than in the cisplatin resistant cells (Figure 6). This finding was also confirmed by mRNA analysis of RBM3 mRNA levels in the two cell lines. When the RBM3 gene was silenced by siRNA-mediated knock-down

in the cisplatin sensitive A2780 cells, they became significantly less sensitive to cisplatin treatment as can be seen in Figure 7, where the viability of RBM3-high cells is lower than that of RBM3-low cells. In a clinical setting, this finding would correspond to improved survival for cisplatin-treated patients with tumors expressing high levels of RBM3 as compared to cisplatin-treated patients with low levels of RBM3 expression in their tumors.

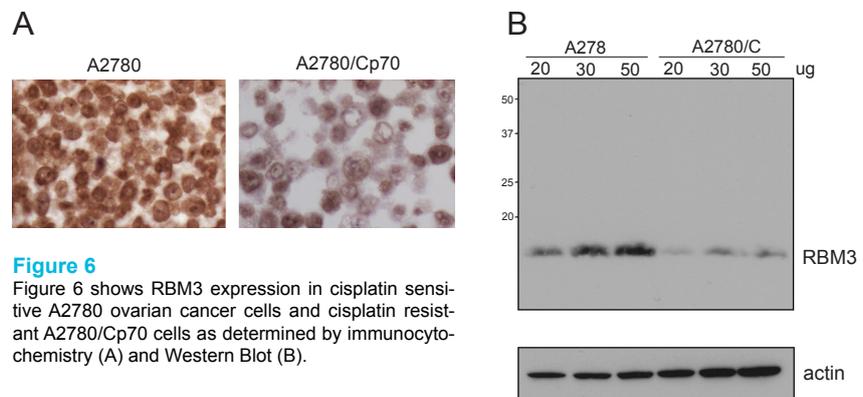


Figure 6 Figure 6 shows RBM3 expression in cisplatin sensitive A2780 ovarian cancer cells and cisplatin resistant A2780/Cp70 cells as determined by immunocytochemistry (A) and Western Blot (B).



The results from the in vitro studies above were further verified in a colorectal cancer cohort, where patient survival according to RBM3 expression was compared between patients receiving no adjuvant treatment, non platinum based adjuvant treatment, and platinum based adjuvant treatment. Comparing the patients with tumors expressing high levels of RBM3 in the three different groups, it can be seen that patients receiving platinum based adjuvant treatment had a markedly increased survival compared to patients receiving no or non platinum based adjuvant treatment (Figure 8).

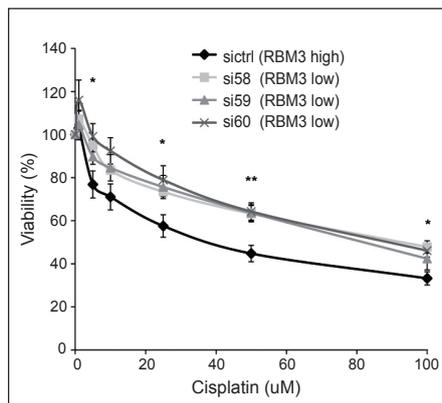


Figure 7

Figure 7 shows viability of the ovarian cancer cell line A2780 when exposed to increasing concentrations of cisplatin. The control cells (labeled sictrl) show a significantly reduced viability compared to cells whose RBM3 gene was silenced by siRNA transfection using three different constructs (labeled si58, si59, and si60 respectively).

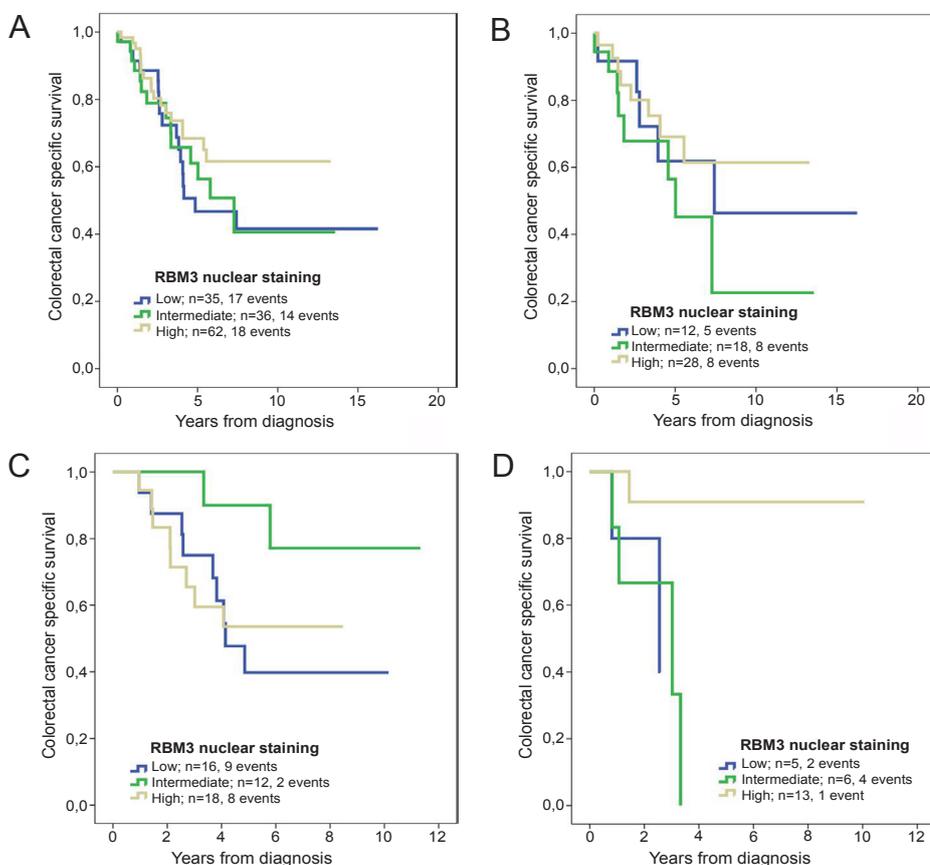


Figure 8

Figure 8 shows Kaplan-Meier analysis of colorectal cancer specific survival of 113 curatively treated patients with stage III-IV disease stratified according to RBM3 expression (low, intermediate, or high expression). In Figure 8A, all patients are represented. For further analysis, patients were divided into three groups by adjuvant treatment modality; no adjuvant treatment (B), non platinum based adjuvant treatment (C), and platinum based adjuvant treatment (D).

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Summary

- RBM3 is a prognostic marker in several cancers, with a high RBM3 expression indicating improved patient survival.
- RBM3 is a treatment predictive marker, with a high RBM3 expression indicating response to platinum-based therapies and a better overall survival.
- Determining the RBM3 protein expression may improve patient care by aiding in deciding on the proper treatment for cancer patients.