



A New Cancer Biomarker Discovered

Need for Biomarkers in Cancer Prognosis

Cancer is a common disease and a major cause of death in the Western world. In general, incidence rates increase with age for most cancer forms. As human populations continue to live longer due to an improvement of the general health status, cancer will affect an increasing number of individuals. A major problem today is that, due to the lack of accurate prognostic and treatment predictive biomarkers, cancer patients are often either overtreated or given inadequate treatment, causing unnecessary discomfort. Thus, there is an urgent need for new molecular markers that allow for better stratification of cancer patients into different risk categories.

RBM3 and the Human Protein Atlas

The RNA binding protein, RBM3, was initially identified as a potential biomarker within the Human Protein Atlas (HPA) project (proteatlas.org).^{1,2} Immunohistochemical analysis of tumor samples from 12 breast cancer patients revealed that RBM3 was moderately expressed in two of the tumor samples, weakly expressed in six samples, and four samples showed no staining at all. Researchers further investigated the expression in larger breast cancer cohorts and the results were recently published.³ The expression of RBM3 was shown to be associated with a prolonged survival. New data from extended Tissue Microarrays (TMAs) show that RBM3 is a biomarker of good prognosis in several other cancer forms as well, here demonstrated in malignant melanoma, epithelial ovarian cancer and colon cancer.

Prestige Antibodies®, powered by Atlas Antibodies, are validated every day and are used to study the expression of the corresponding proteins in samples representing 46 different normal human tissues, 20 most common cancer types, and 59 different cells and cell lines. Subcellular localization studies are performed in three cell lines for each antibody, using confocal microscopy and immunofluorescence staining. All resulting tissue and cell images are publicly available on the Human Protein Atlas web portal (proteatlas.org). In total, more than 700 immunohistochemistry (IHC), immunocytochemistry (ICC), immunofluorescence (IF) and Western blot (WB) images are presented for each Prestige Antibody. Each year protein expression and localization data of ~2,500 new proteins are added to the portal.

Breast Cancer

Breast cancer is by far the most frequent cancer in women. Surgery is the primary curative treatment for breast cancer patients, often in combination with adjuvant therapy. However, adjuvant therapy is associated with substantial costs and sometimes severe side effects, and physicians have identified reduction of overtreatment as the major clinical need in breast cancer treatment today. Thus, the stratification of patients into different prognostic categories is of great importance as it may aid physicians in selecting the most appropriate treatment for a given patient.

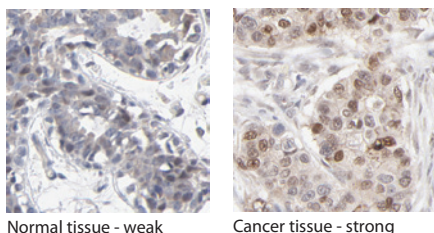


Figure A: Immunohistochemical analysis using the Anti-RBM3 antibody (HPA003624) shows weak expression in normal breast tissue and differential expression, varying from weak to strong, in tumor breast samples.

RBM3 was identified on the Human Protein Atlas (proteatlas.org) where it shows a weak expression pattern in normal breast tissue, but a stratified pattern in tumor samples (see **Figure A**). In a recent publication,³ RBM3 protein expression was evaluated in 241 tumor samples from a cohort of 500 premenopausal women with stage II invasive breast cancer (see **Figure B**). RBM3 expression was associated with small, low-grade, estrogen receptor (ER)-positive tumors, and it is an independent predictor of recurrence free survival (RFS) in ER-positive patients. It could be concluded that RBM3 is a good prognosis marker in breast cancer.

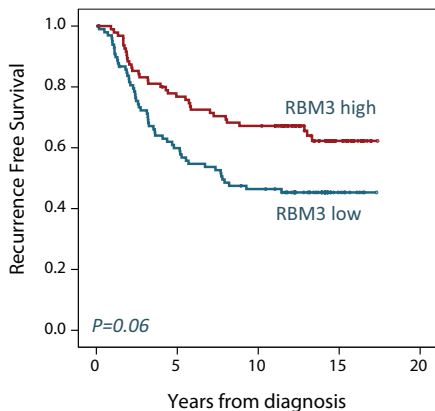


Figure B: The figure shows Kaplan-Meier (survival) analysis of RFS according to RBM3 expression for ER-positive breast cancer patients. Patients were split into two groups based on high and low RBM3 expression. The analysis showed that high levels of RBM3 in ER positive tumors, using a cut-off of 75% positive nuclei, was associated with an improved RFS ($P = 0.06$). Cox multivariate regression analysis (adjusted for NHG (I-II vs III), nodal status 0 vs 1 and tumor size: $</>20$ mm) revealed that high RBM3 nuclear expression (fraction of cells stained $> 75\%$) was associated with an improvement in RFS (RR = 0.56, 95% CI: 0.36-0.90, $P = 0.02$).

Malignant Melanoma

Malignant melanoma is a common skin tumor with a rapidly increasing incidence rate. Survival rates are high, but melanomas tend to metastasize relatively early, and for patients with metastatic melanoma prognosis is poor, with a five-year survival rate of less than 10%. Today, there are no validated biomarkers for use in the clinical setting that are able to give prognostic information and estimate the risk for metastatic disease.

RBM3 protein expression was evaluated in tumor samples from a cohort of 157 patients surgically treated for primary cutaneous malignant melanoma (manuscript in preparation). RBM3 was identified to show a differential expression pattern in different skin tumor samples (see **Figure C**). Also, a correlation between RBM3 expression and number of metastases was observed (see **Figure D**).

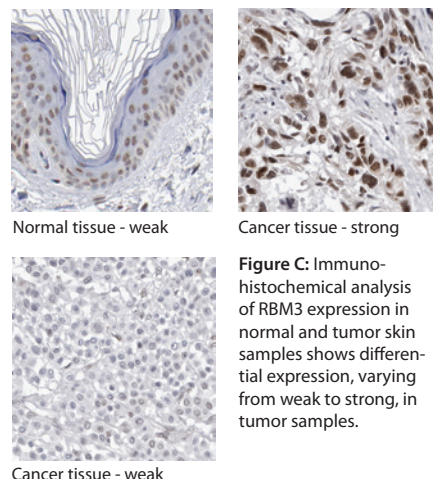


Figure C: Immunohistochemical analysis of RBM3 expression in normal and tumor skin samples shows differential expression, varying from weak to strong, in tumor samples.

It could be concluded that analysis of RBM3 expression may aid physicians in identifying patients with a high risk of metastatic disease that should be more closely monitored. This might mean a less intense treatment for high-RBM3 patients, and more aggressive treatment for low-RBM3 patients.

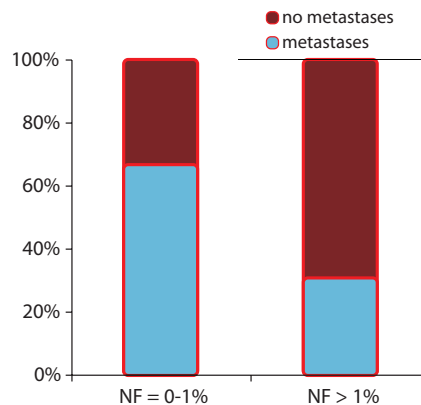
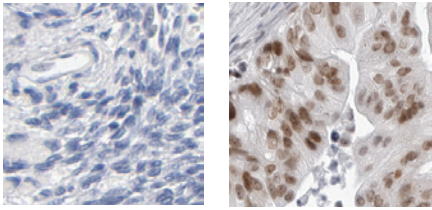


Figure D: The figure shows the proportion of subjects having metastases in the group of RBM3-low primary tumors (nuclear fraction (NF) 0–1%) and for the group of RBM3-high primary tumors (NF > 1%). Low RBM3 expression in the primary tumor corresponds to a probability of having metastasis of approximately 50%, whereas the probability was approximately 30% in case of RBM3-high primary tumors.

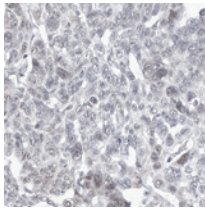
Epithelial Ovarian Cancer

Epithelial ovarian cancer (EOC) is one of the most common forms of cancer worldwide, and a leading cause of death from gynecological malignancy. Treatment with curative intent involves surgery and postoperative platinum-based chemotherapy in combination with paclitaxel. However, relapse is common, and side effects of platinum-based chemotherapy are severe.



Normal tissue - weak

Cancer tissue - strong



Cancer tissue - weak

Figure E: Immunohistochemical analysis of RBM3 expression in normal and tumor ovarian samples shows differential expression, varying from weak to strong, in tumor samples.

RBM3 protein expression was evaluated in tumor samples from a cohort of 154 patients surgically treated for primary invasive EOC.⁴ It was shown that RBM3 expression is weak in normal ovary tissue, but shows a stratified pattern in tumor samples (see **Figure E**).

It could be concluded that RBM3 is a prognostic marker that may aid physicians in determining which type of adjuvant treatment is appropriate. This might mean a less intensive treatment for high-RBM3 patients with early-stage cancer, and more intensive treatment for low-RBM3 patients (see **Figure F**).

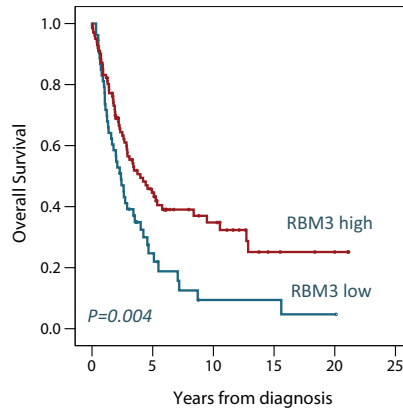


Figure F: The figure shows Kaplan-Meier (survival) analysis of overall survival (OS) according to RBM3 expression for EOC cancer patients. Patients were split into two groups based on high and low RBM3 expression. The analysis showed that high levels of RBM3, using a cut-off of 75% positive nuclei, was associated with an improved OS ($P = 0.004$). Cox multivariate regression analysis (adjusted for age: continuous, stage: I-II vs III-IV and grade: I-II vs III) revealed that high RBM3 nuclear expression (fraction of cells stained $> 75\%$) was associated with an improvement in OS (RR = 0.61, 95% CI: 0.40-0.92, $P = 0.017$).

Colon Cancer

Colorectal cancer is one of the most common types of cancer, and it accounts for about 10% of cancer deaths in Europe and the USA. Surgery is the only curative treatment today, but metastatic disease is common and this hampers successful treatment. Currently, patients with advanced disease are routinely given chemotherapy as adjuvant treatment.

RBM3 protein expression was evaluated in 274 tumor samples from a cohort of 331 retrospectively identified cases from patients who underwent curative resection for sigmoid colon cancer (see **Figure G**) (manuscript in preparation). Immunohistochemical analysis of RBM3 expression showed a differential expression in colon tumor samples (see **Figure H**).

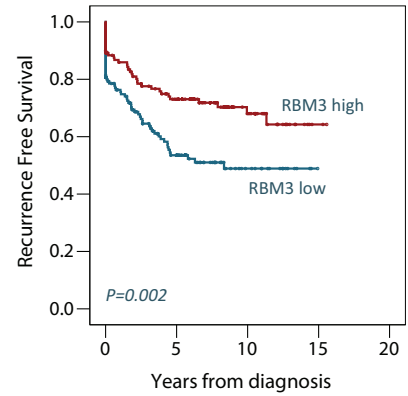
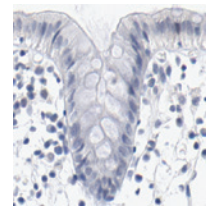
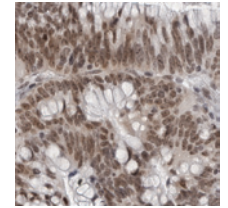


Figure G: The figure shows Kaplan-Meier (survival) analysis of recurrence free survival (RFS) according to RBM3 expression for patients diagnosed with cancer of the sigmoid colon. Patients were split into two groups based on high and low RBM3 expression. The analysis showed that expression of RBM3 in the tumor, using a cut-off of 1% positive nuclei, was associated with an improved RFS ($P = 0.002$). Cox multivariate regression analysis (adjusted for age ($>/\leq 75$ years), gender, stage (I-II vs III-IV) and differentiation: high- intermediate vs low) revealed that nuclear expression of RBM3 (fraction of cells stained $> 1\%$) was associated with an improvement in RFS (RR = 0.49, 95% CI: 0.36-0.68, $P < 0.001$).

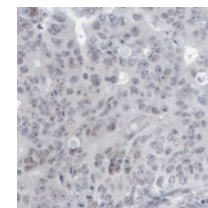
It could be concluded that RBM3 is a prognostic marker that may aid in determining if patients should receive adjuvant treatment, and also the treatment intensity level.



Normal tissue - weak



Cancer tissue - strong



Cancer tissue - weak

Figure H: Immunohistochemical analysis of RBM3 expression in normal and tumor colon samples shows differential expression, varying from weak to strong, in tumor samples.

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Summary

- The Human Protein Atlas is an excellent tool for identification of new biomarkers
- By the use of Prestige Antibodies, RBM3 was recently identified on the Human Protein Atlas as a prognostic biomarker in breast cancer and later shown to be a promising biomarker in several other cancer forms as well
- RBM3 is a biomarker for stratification of cancer patients into different risk categories in several major cancer forms including breast, ovarian, and colon carcinoma, as well as malignant melanoma
- Increased, mainly nuclear, expression of RBM3 protein is associated with an improved overall and recurrence-free survival in cancer patients
- One hypothesis is that high RBM3 expression confers a defense against continued uncontrolled growth and thus explains the good prognostic impact; whereas, a low expression probably indicates that this defense mechanism has been lost, which is associated with a poor prognosis

References

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- (4) Ehlén, Å., *et al.*, Expression of the RNA-binding protein RBM3 is associated with a favourable prognosis and cisplatin sensitivity in epithelial ovarian cancer. *J. Transl Med*, **8**:78 (2010).



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