Journal of Cancer Research and Clinical Oncology (2019) 145:873–879
https://doi.org/10.1007/s00432-019-02850-1

ORIGINAL ARTICLE – CANCER RESEARCH

Prevalence and clinical significance of RBM3 immunostaining in non-small cell lung cancers

Nathaniel Melling[1] · Kai Bachmann[1] · Bianca Hofmann[1] · Alexander Tarek El Gammal[1] · Matthias Reeh[1] · Oliver Mann[1] · Christoph Moebius[2] · Marco Blessmann[2] · Jakob Robert Izbicki[1] · Katharina Grupp[1,2]

Received: 25 September 2018 / Accepted: 23 January 2019 / Published online: 13 February 2019

Abstract

Introduction Aberrant expression of RNA-binding motif protein 3 (RBM3) has been suggested as a prognostic biomarker in several malignancies.

Materials and methods This study was performed to analyse the prevalence and clinical significance of RBM3 immunostaining in non-small cell lung cancers (NSCLCs). Therefore, we took advantage of our tissue microarray (TMA) containing more than 600 NSCLC specimens.

Results While nuclear RBM3 staining was always high in normal lung tissue, high RBM3 staining was only seen in 77.1% of 467 interpretable non-metastatic NSCLCs. Reduced RBM3 staining was significantly associated with advanced pathological tumor stage (pT) in NSCLCs ($p = 0.0031$). Subset analysis revealed that the association between reduced RBM3 staining and advanced pT stage was largely driven by the histological subgroup of lung adenocarcinoma (LUACs) ($p = 0.0036$). In addition, reduced RBM3 expression predicted shortened survival in LUAC patients ($p = 0.0225$).

Conclusions In summary, our study shows that loss of RBM3 expression predicts worse clinical outcome in LUAC patients.

Keywords RBM3 · Lung adenocarcinoma · Squamous cell lung carcinoma · Large cell lung carcinoma · Non-small-cell lung carcinoma · Immunohistochemistry

Introduction

Non-small cell lung cancer (NSCLC) is a heterogeneity disease, and to date, specific clinical factors and tumor stage are established as prognostic markers. Nevertheless, prognosis within stage may vary significantly. Therefore, the identification of prognostic markers to predict patient prognosis in NSCLC is desperately needed.

The growing interest in stress-response pathways and its dysfunction reflects its potential role for cancer. The cold-inducible RNA-binding protein 3 (RBM3) is a member of the cold-shock protein family induced by lowering the temperatures (Al-Fageeh et al. 2009) and hypoxia (Wellmann et al. 2004). RBM3 binds to both DNA and RNA (Wright et al. 2001) and is involved in maintenance of DNA integrity, including DNA-dependent replication, DNA replication, chromatin remodeling, DNA integrity checkpoints (Ehlén et al. 2011), and regulation of RNA (Derry et al. 1995). In cancers, the role of RBM3 is obviously variable, since both pro-oncogene as well as anti-oncogene roles have been described (Zhou et al. 2017).Thus, RBM3 has been associated with survival (Kita 2002; Wellmann et al. 2010) and proliferation (Lleonart 2010; Sureban et al. 2008; Zeng et al. 2009), as well as with cell apoptosis (Martínez-Arribas et al. 2006).

Immunohistochemical studies suggested RBM3 as a prognostic biomarker in several malignancies (Grupp et al. 2014; Olofsson et al. 2015; Jögi et al. 2009; Jonsson et al. 2011a, b, 2014; Hjelm et al. 2011; Boman et al. 2013; Ehlén et al. 2010; Melling et al. 2016; Nodin et al. 2012). However, its prognostic role in NSCLC remains elusive. To further

✉ Katharina Grupp
k.grupp@uke.de

[1] General-, Visceral and Thoracic Surgery Department and Clinic, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[2] Department of Plastic-, Reconstructive and Aesthetic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

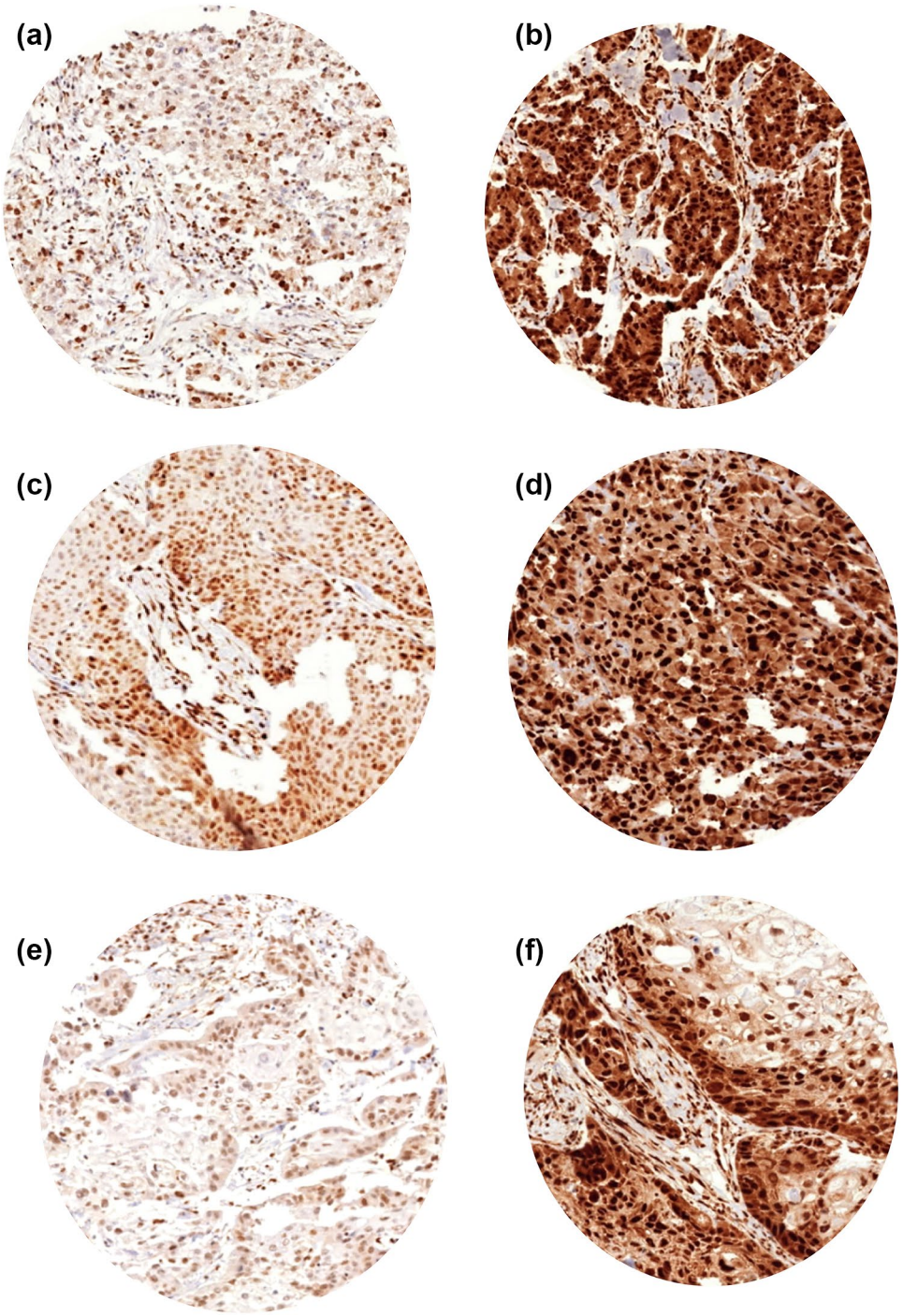

Fig. 1 Immunohistochemical staining with RBM3 protein in LUACs, LCLCs, and SQCCs of the lung. Low and high RBM3 expression in LUACs (**a, b**), LCLCs (**c, d**), and SQCCs (**e, f**) of the lung

assess the prevalence and clinical significance of RBM3 expression in NSCLCs and its histological subgroups, we took advantage of our TMA containing more than 600 NSCLC specimens.

Materials and methods

Patients and TMA construction

Lung cancer TMAs with a total of 619 non-small cell lung cancer specimens were included in this study. Patients were treated at the Department of General-, Visceral and Thoracic

Table 1 Association between RBM3 immunohistochemistry and clinico-pathological variables in NSCLCs

	On TMA (*n*)	RBM3 expression level			*p*
		Analyzable (*n*)	Low (%)	High (%)	
All tumors	577	467	22.91	77.09	
Histology					
Adenocarcinoma	202	164	21.34	78.66	0.4282
Large cell carcinoma	129	101	19.8	80.2	
Squamous cell carcinoma	246	202	25.74	74.26	
Tumor stage					
pT1	161	119	11.76	88.24	0.0031
pT2	209	264	24.91	75.09	
pT3	52	41	34.15	65.85	
pT4	46	26	29.73	70.27	
Nodal stage					
pN0	254	210	19.05	80.95	0.1933
pN1	161	135	28.89	71.11	
pN2	106	82	21.95	78.05	
pN3	16	14	28.57	71.43	

Surgery at the University Medical Center Hamburg-Eppendorf due to lung cancer. The TMAs contained 619 NSCLC specimens, including 227 LUACs, 134 LCLCs, and 258 lung SQCCs. Regarding the clinico-pathological data, 174 of the NSCLCs were staged pT1, 323 specimens were staged pT2, 53 tumors pT3, and 50 tumors were staged pT4. Furthermore, 264 of the NSCLCs were lymph nodal negative, while 293 specimens were lymph node positive. The median age of the patients was 63 years (range 29–91 years). 444 of the included patients were male and 177 of the patients were female. For statistical analysis, patients with metastasis disease were excluded. TMA construction was as described (Mirlacher et al. 2010). In brief, hematoxylin and eosin-stained sections were made from each block to define representative tumor regions. One tissue cylinder with a diameter of 0.6 mm was then punched from the tumor on the "donor" tissue block using a home-made semi-automated precision instrument and brought into empty recipient paraffin blocks. Four µm sections of the resulting TMA blocks were transferred to an adhesive coated slide system (Instrumedics Inc., Hackensack, New Jersey). The utilization of tissues and clinical data was according to the Hamburger Krankenhaus Gesetz (§ 12 HmbKHG) and approved by our local Ethical Committee.

Immunohistochemistry

Freshly cut TMA sections were analyzed on 1 day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121 C in pH 7.8 Tris-EDTA-Citrate buffer. Primary antibody specific for RBM3 (polyclonal rabbit, HPA003624; Sigma-Aldrich; at 1/150 dilution) was applied at 37 °C for 60 min. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer´s directions. RBM3 staining was analyzed by one person (KG) experienced in immunohistochemisty. Since nuclear staining was typically paralleled by similar or slightly lower cytoplasmic staining, only nuclear staining was considered. For statistical analyses, the staining results were categorized into two groups: low and high immunostaining.

Statistical analysis

Statistical calculations were performed with JMP® 10.0.2 software (2012 SAS Institute Inc., NC, USA). Contingency tables and the Chi-square test were performed to search for associations between molecular parameters and tumor phenotype. Survival curves were calculated according to Kaplan–Meier. The Log-rank test was applied to detect significant survival differences between groups. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular, and clinical variables.

Results

Technical issues

Immunohistochemical analysis of RBM3 protein was successful in 80.3% (497/619) of NSCLCs. A total of 19.7% (122/619) tissue samples were excluded from analysis,

Table 2 Association between RBM3 immunohistochemistry and clinico-pathological variables in LUACs (a), LCLCs (b), and SQCCs (c) of the lung

	RBM3 expression level			*p*
	Analyzable (*n*)	Low (%)	High (%)	
(a)				
All cancers	164	21.34	78.66	
Tumor stage				
pT1	51	5.88	94.12	
pT2	87	25.29	74.71	
pT3	11	36.36	63.64	
pT4	13	38.46	61.54	0.0036
Nodal stage				
pN0	85	20	80	
pN1	33	30.3	69.7	
pN2	30	16.67	83.33	
pN3	4	50	50	0.3169
(b)				
All cancers	101	19.8	80.2	
Tumor stage				
pT1	28	17.86	82.14	
pT2	55	20	80	
pT3	9	44.44	55.56	
pT4	9	0	100	0.0772
Nodal stage				
pN0	47	12.77	87.23	
pN1	21	28.57	71.43	
pN2	19	26.32	73.68	
pN3	7	14.29	85.71	0.3698
(c)				
All cancers	202	25.74	74.26	
Tumor stage				
pT1	40	15	85	
pT2	123	26.83	73.17	
pT3	21	28.57	71.43	
pT4	15	40	60	0.2291
Nodal stage				
pN0	78	21.79	78.21	
pN1	81	28.4	71.6	
pN2	33	24.24	75.76	
pN3	3	33.33	66.67	0.7922

because the tissue spots were missing on the tissue microarray slide or did not contain unequivocal tumor cells.

Clinico-pathological data of NSCLCs

A total of 619 NSCLCs, including 227 LUACs, 134 LCLCs, and 258 lung SQCCs, were included in this study. 174 of the NSCLC specimens were staged pT1, 323 specimens were staged pT2, 53 tumors pT3, and 50 tumors pT4. 264 of the NSCLCs were lymph nodal negative and 293 were lymph node positive. For statistical analysis, only patients without distant metastasis were included ($n=577$).

RBM3 immunostaining was decreased in NSCLCs

RBM3 immunostaining was predominantly localized in the nucleus of the cells, and was typically paralleled by lower cytoplasmatic staining-level RBM3. Nuclear RBM3 staining was always high in benign lung epithelium. In NSCLCs, high RBM3 staining was only seen in 77.1% of tumors and was reduced in 22.9% of analyzable tumor samples. Representative images of RBM3 immunostaining are given in Fig. 1.

Decreased RBM3 expression is linked to advanced pathological tumor stage in NSCLCs

RBM3 expression results were compared to clinico-pathological variables of NSCLCs. In the analysis of all NSCLCs, decreased RBM3 staining was significantly correlated with advanced pathological tumor stage ($p=0.0031$; Table 1). Histological subtype analysis of NSCLCs revealed that the association of RBM3 with advanced tumor stage was largely driven by LUACs ($p=0.0036$; Table 2).

RBM3 expression was not associated with clinical outcome in patients with NSCLCs

RBM3 expression was unrelated to prognosis in the analysis of all NSCLCs ($p=0.4363$; Fig. 2a). However, histological subgroup analysis revealed that RBM3 expression was linked to clinical outcome in the subset of LUACs ($p=0.0225$; Fig. 2b), but was unrelated to clinical outcome in patients with LCLCs ($p=0.8585$; Fig. 2c) and SQCCs ($p=0.6503$; Fig. 2d).

Discussion

The results of our study show that loss of RBM3 expression predicts aggressive LUAC tumor phenotype and shortened survival of LUAC patients.

We became interested in the RBM3 protein, because its expression is dysregulated and linked to clinical outcome in several cancer types. The role of RBM3 is obviously variable between tissues and cancer types. In some tumor types, such as prostate cancer (Grupp et al. 2014) and astrocytoma (Zhang et al. 2013), RBM3 is overexpressed in cancerous in comparison with benign tissue and RBM3 overexpression

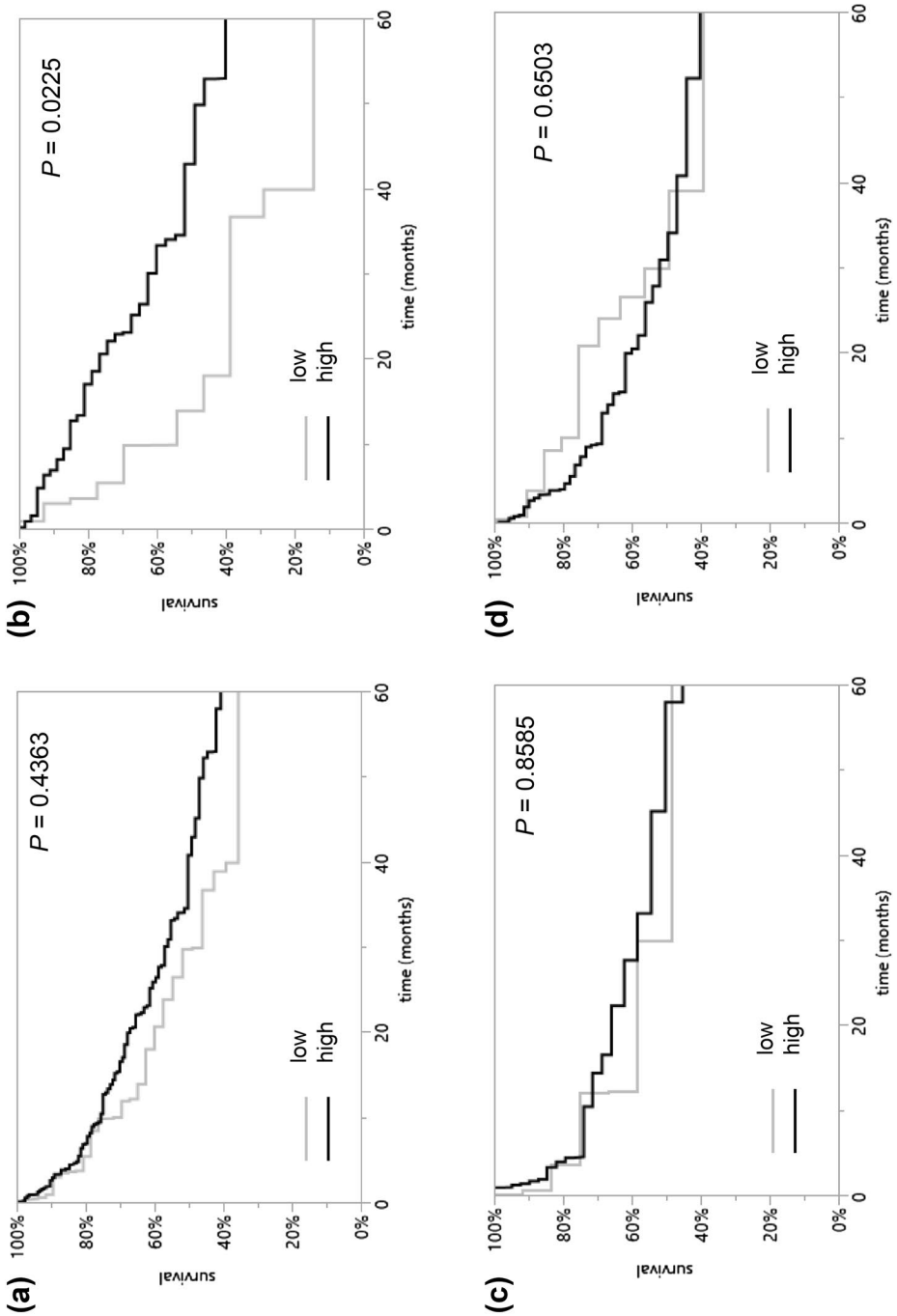

Fig. 2 Kaplan–Meier curves for overall survival of NSCLC patients. Associations of RBM3 immunostaining with overall survival of all NSCLC patients (**a**) and the subsets of LUAC (**b**), LCLC (**c**), and SQCC (**d**) patients

has been linked to adverse tumor phenotype (Grupp et al. 2014; Zhang et al. 2013) and poor clinical outcome (Grupp et al. 2014). In other tumor types, such as colorectal cancers (Melling et al. 2016), RBM3 expression is reduced in cancer as compared to normal tissue and loss of RBM3 expression has been linked to poor clinical outcome (Melling et al. 2016). So far, RBM3 expression has not been systematically analysed in NSCLCs.

Our data demonstrate that RBM3 staining is reduced in a subset of NSCLC. The combination of high RBM3 staining in benign with reduced expression in a small subset of NSCLC with unfavourable tumor phenotype and clinical outcome in LUACs argues for a role of RBM3 during lung carcinogenesis. RNA-binding proteins have been suggested as mediators of cancer traits and it is becoming increasingly clear that changes in gene expression, which are mastered by RNA-binding proteins, contribute to tumor development and confer competitive advantage to cancerous cells (Wurth and Gebauer 2014). To date, only few studies have focused on cancerogeneity of RNA-binding proteins in detail, and hence, our understanding of the role of RNA-binding proteins in cancer progression is still rudimentary. Recently, in vitro and in vitro studies of the RBM3 proteins in tumorigenesis have suggested pro-oncogenic as well as anti-oncogenic functions (Zhou et al. 2017). While some authors suggested that RBM3 might induce cell proliferation (Lleonart 2010; Sureban et al. 2008; Zeng et al. 2009) and survival (Kita 2002; Wellmann et al. 2010), others suggested that RBM3 might be linked to the proapoptotic BAX gene (Martínez-Arribas et al. 2006). Further studies will be needed to fully elucidate the role of RBM proteins in cancer and to clarify the reasons for their variable roles in different entities.

Recently, RBM3 expression had been suggested as a prognostic biomarker in several malignancies, including melanoma (Jonsson et al. 2011; Nodin et al. 2012), prostate (Grupp et al. 2014; Jonsson et al. 2011a, b), testicular non-seminomatous germ cell (Olofsson et al. 2015), breast (Jögi et al. 2009), esophageal (Jonsson et al. 2014), gastric (Jonsson et al. 2014), colorectal (Hjelm et al. 2011; Melling et al. 2016), bladder (Boman et al. 2013), and ovarian (Ehlén et al. 2010) cancers. Here, we demonstrate that RBM3 expression is also linked to clinical outcome in LUACs. Thus, there might be useful role of RBM3 measurement in the assessment of lung adenocarcinoma patients.

In summary, the present study shows that reduced RBM3 expression is linked to aggressive tumor features and poor clinical outcome in patients with LUACs.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Al-Fageeh MB et al (2009) Cold-inducible RNA binding protein (CIRP) expression is modulated by alternative mRNAs. RNA (New York, N.Y.) 15:1164–1176

Boman K et al (2013) Decreased expression of RNA-binding motif protein 3 correlates with tumour progression and poor prognosis in urothelial bladder cancer. BMC Urol 13:17

Derry JMJ et al (1995) RBM3 a novel human gene in Xp11.23 with a putative RNA-binding domain. Hum Mol Genet 4:2307–2311

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

Ehlén Õ et al (2011) RBM3-regulated genes promote DNA integrity and affect clinical outcome in epithelial ovarian cancer. Transl Oncol 4:212

Grupp K et al (2014) High RNA-binding motif protein 3 expression is an independent prognostic marker in operated prostate cancer and tightly linked to ERG activation and PTEN deletions. Eur J Cancer (Oxford, England: 1990) 50:852–861

Hjelm B et al (2011) High nuclear RBM3 expression is associated with an improved prognosis in colorectal cancer. Proteom Clin Appl 5:624–635

Jögi A et al (2009) Nuclear expression of the RNA-binding protein RBM3 is associated with an improved clinical outcome in breast cancer. Modern Pathol 22:1564–1574

Jonsson L et al (2011a) High RBM3 expression in prostate cancer independently predicts a reduced risk of biochemical recurrence and disease progression. Diagn Pathol 6:91

Jonsson L et al (2011b) Low RBM3 protein expression correlates with tumour progression and poor prognosis in malignant melanoma: an analysis of 215 cases from the Malmö Diet and Cancer Study. J Trans Med 9:114

Jonsson L et al (2014) High expression of RNA-binding motif protein 3 in esophageal and gastric adenocarcinoma correlates with intestinal metaplasia-associated tumours and independently predicts a reduced risk of recurrence and death. Biomarker Res 2:11

Kita H (2002) Modulation of polyglutamine-induced cell death by genes identified by expression profiling. Hum Mol Genet 11:2279–2287

Lleonart ME (2010) A new generation of proto-oncogenes: cold-inducible RNA binding proteins. Biochimica et biophysica acta 1805:43–52

Martínez-Arribas F et al (2006) Positive correlation between the expression of X-chromosome RBM genes (RBMX, RBM3, RBM10) and the proapoptotic Bax gene in human breast cancer. J Cell Biochem 97:1275–1282

Melling N et al (2016) Loss of RNA-binding motif protein 3 expression is associated with right-sided localization and poor prognosis in colorectal cancer. Histopathology 68:191–198

Mirlacher M et al (2010) Recipient block TMA technique. Methods Mol Biol (Clifton NJ) 664:37–44

Nodin B et al (2012) High MCM3 expression is an independent biomarker of poor prognosis and correlates with reduced RBM3 expression in a prospective cohort of malignant melanoma. Diagn Pathol 7:82

Olofsson S-E et al (2015) Low RBM3 protein expression correlates with clinical stage, prognostic classification and increased risk of treatment failure in testicular non-seminomatous germ cell cancer. PloS one 10:e0121300

Sureban SM et al (2008) Translation regulatory factor RBM3 is a proto-oncogene that prevents mitotic catastrophe. Oncogene 27:4544–4556

Wellmann S et al (2004) Oxygen-regulated expression of the RNA-binding proteins RBM3 and CIRP by a HIF-1-independent mechanism. J Cell Sci 117:1785–1794

Wellmann S et al (2010) The RNA-binding protein RBM3 is required for cell proliferation and protects against serum deprivation-induced cell death. Pediatr Res 67:35–41

Wright CF et al (2001) Vaccinia virus late transcription is activated in vitro by cellular heterogeneous nuclear ribonucleoproteins. J Biol Chem 276:40680–40686

Wurth L, Gebauer F (2014) RNA-binding proteins, multifaceted translational regulators in cancer. Biochim Biophys Acta 1849(7):881–886. https://doi.org/10.1016/j.bbagrm.2014.10.001

Zeng Y et al (2009) Down-regulating cold shock protein genes impairs cancer cell survival and enhances chemosensitivity. J Cell Biochem 107:179–188

Zhang H-T et al (2013) Differential expression of the RNA-binding motif protein 3 in human astrocytoma. Chin Med J 126:1948–1952

Zhou R-B et al (2017) RNA binding motif protein 3: A potential biomarker in cancer and therapeutic target in neuroprotection. Oncotarget 8:22235–22250