High-mobility group protein B1 is an independent predictor of poor survival in ovarian cancer

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Introduction

HMGB1 has recently been implicated in a number of human cancers including colon, gastric, lung, and liver. The role of HMGB1 (a chromatin binding protein) in processes relevant to cancer cell survival include autophagy (Tang et al, 2010; Sun & Tang, 2014), genome stability (Liu et al), angiogenesis (Yang et al, 2014), invasion and metastasis (Yan et al, 2012). HMGB1 has a complex range of functions depending in part on its subcellular and extracellular localisation, redox state, and interaction with other cell surface receptors. Extracellular secretion of HMGB1 can maintain tumour cell autophagy (by binding to beclin-1)(Kang et al, 2010), as well as recruit and activate immune cells. HMGB1 expression can also regulate the mitochondrial bioenergetics of cancer cells by enhancing complex I activity, ATP production and subsequent proliferation and migration (Kang et al, 2014). The redox status of HMGB1 is important in modulating its function (Rubartelli & Lotze, 2007). HMGB1 contains three cysteines at positions C23, C45 and C106 that can be modified. The all reduced form of HMGB1 is a chemoattractant that mediates leukocyte recruitment. The disulphide form has cytokine (but not chemokine) activity and can bind TLR4. The fully oxidised form of HMGB1 induced by reactive oxygen species (ROS) has neither cytokine nor chemoattractant activity.

However the prognostic value of HMGB1 in ovarian cancer remains unclear. There is an important need to understand the context-dependent role of HMGB1 as either an anti- or pro-tumourogenic protein in ovarian cancer. To achieve this, the expression and prognostic value of HMGB1 was examined using two independent tissue microarrays. A large cohort of test (n=360) and validation (n=194) tumour samples were analysed to determine the effect on survival and the utility of HMGB1 as an independent prognostic marker.

Methods

Patient samples

This is a retrospective study with patients comprehensively staged according to the International Federation of Obstetricians and Gynecologists (FIGO) staging system for ovarian cancer. Clinical details of test (n=360) (Popple et al, 2012) and validation (n=194) (Abdel-Fatah et al, 2013) cohorts have been previously described. This work was approved by the Derby Royal Hospital Ethics Committee and Nottingham Research Ethics Committee.

Tissue Microarray and immunohistochemistry

Tissue microarrays were constructed as described previously (Woolston et al, 2010; Popple et al, 2012; Abdel-Fatah et al, 2013). Immunohistochemical staining was performed using a routine streptavidin–biotin peroxidase method. Sections were incubated with a Rabbit anti HMGB1 mAb (clone D3E5) (New England Biolabs, Hitchin, UK). Pearson's χ2-tests were used to determine the significance of associations between categorical variables. Survival rates were calculated using the Kaplan–Meier method; differences between survival curves were tested using the log-rank test. The Cox proportional-hazards model was used for multivariate analysis in order to calculate the Hazard ratios and independent significance of individual factors. In all cases two-sided P-values of <0.05 were considered as statistically significant.

Statistical analysis

Statistical analysis was performed using SPSS20 statistical software (SPSS Inc., Chicago, IL, USA). Pearson's χ2-tests were used to determine the significance of associations between categorical variables. Survival rates were calculated using the Kaplan–Meier method; differences between survival curves were tested using the log-rank test. The Cox proportional-hazards model was used for multivariate analysis in order to calculate the Hazard ratios and independent significance of individual factors. In all cases two-sided P-values of <0.05 were considered as statistically significant.

Results

In the test cohort 360 ovarian tumours were stained for HMGB1 (Table 1). 10% could not be evaluated due to the absence of enough tissue core or no evaluable tumour cells (i.e. all stroma) in the core. Of the 316 evaluable ovarian tumours stained with a HMGB1 specific antibody, only 23/31660 (7%) tumours failed to stain. A further 42/316 (13%) stained weakly, 251/316 (79%) stained strongly (Figure 1). Kaplan-Meier analysis showed there was a correlation of HMGB1 expression and overall survival with low expression of HMGB1 being protective (p=0.002). This was replicated in the second cohort (overall survival p=0.077, progression free survival p=0.023) (Figure 2). The data indicates that there was a correlation of HMGB1 expression and survival where low expression of HMGB1 gave an almost 2 fold increase in survival time from 55.7 months to 104.2 months (Table 2) After multivariate analysis HMGB1 remained an independent prognostic factor (p=0.006) (Table 3). In a multivariate model FIGO stage (p<0.0001), response to chemotherapy (p<0.0001), and HMGB1 expression (p=0.006) were independent predictors of patient survival.

Table 1. Clinicopathological variables for the test patient cohort (n=360) and cores stained for HMGB1.

Variable	Categories	Frequency of total cohort (%), n=360	Frequency of the HMGB1-stained cohort (%), *n*=316
SEER age characteristics	<30 years at diagnosis	2 (<1)	1 (<1)
	30–60 years at diagnosis	143 (40)	127 (40)
	>60 years at diagnosis	212 (59)	190 (59)
	Unknown	3 (<1)	3 (1)
Macroscopic residual disease	Absent	143 (40)	126 (39)
	Present	201 (56)	180 (56)
	Unknown	16 (4)	15 (5)
FIGO stage	I	95 (26)	88 (27)
	II	38 (11)	34 (11)
	III	175 (49)	155 (48)
	IV	40 (11)	33 (10)
	Unknown	12 (3)	11 (3)
Histological type	Serous carcinoma	178 (49)	159 (50)
	Mucinous cystoadenocarcinoma	35 (10)	31 (10)
	Endometrioid	42 (12)	39 (12)
	Clear cell	25 (7)	25 (8)
	Undifferentiated	54 (15)	47 (15)
	Others	26 (7)	20 (6)
Serous tumour grade	High	160 (44)	142 (44)
	Low	18 (5)	17 (5)
Tumour grade of all other tumours	Well differentiated (3)	100 (28)	91 (28)
	Moderately differentiated (2)	39 (11)	35 (11)
	Poorly differentiated (1)	20 (6)	19 (6)
	Unknown	23 (6)	17 (5)
Adjuvant therapy	No	101 (28)	92 (29)
	Yes	249 (69)	220 (69)

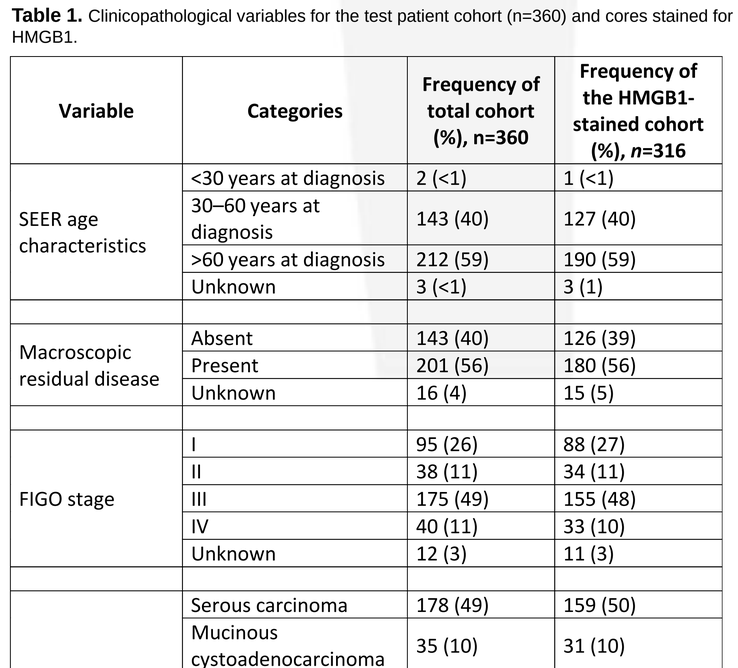

Figure 1. Representative photomicrographs of ovarian TMA cores immunohistochemically strained for HMGB1. The level of expression ranged from (A) Negative, (B) Weak, (C) Intermediate and (D) Strong expression.

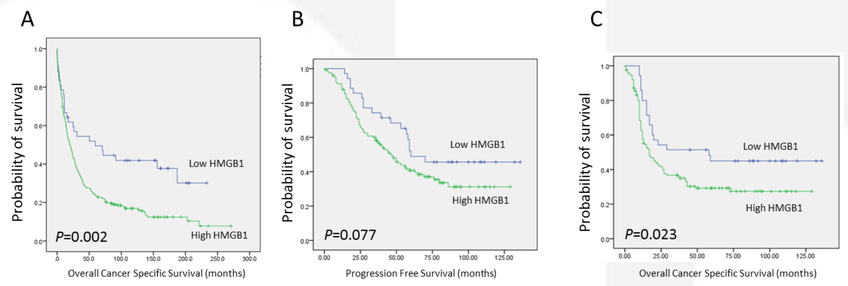

Figure 2. Kaplan Meier curves showing overall ovarian cancer specific survival in (A) the test cohort (high expression with cut point value >4) and (B) overall ovarian cancer specific survival and (C) progression free survival in ovarian cancer patients in the validation cohort (high expression with cut point value >42)

Table 2. Mean survival time in relation to HMGB1 expression.

Expression	Mean survival time (months) in relation to HMGB-1 expression			
	Estimate (months)	95% confidence interval		
		Lower bound	Upper bound	*P*-value
Low	104.2	73.2	135.1	0.002
High	55.7	45.4	65.9	
Overall	63.7	53.2	74.1	

Table 3. Multivariate analysis for overall cancer specific survival in 316 consecutive patients with epithelial ovarian cancer.

	Exp(*B*)	95% CI for Exp(*B*)		*P*-value
		Lower	Upper	
FIGO stage				
Stage 1	1			<.001
Stage 2	3.350	1.918	5.852	
Stage 3	7.886	4.896	12.704	
Stage 4	10.021	5.810	17.284	
Histological type				
				0.467
Borderline	1			
Clear cell OVCA	1.130	0.479	2.667	
Mucinous OVCA	1.695	0.767	3.744	
Endometrioid OVCA	1.339	0.606	2.962	
Serous OVCA	1.594	0.796	3.195	
Undifferentiated OVCA	1.598	0.761	3.354	
Other OVCA	3.180	0.960	10.533	
Adjuvant therapy				
No	1			<.001
Yes	0.361	0.240	0.541	
HMGB1				
Low	1			0.006
High	1.921	1.205	3.064	

Abbreviations: CI=confidence interval; FIGO=International Federation of Gynecology and Obstetrics.

The analysis is based on Cox multivariate regression model.

P-values <0.05 are accepted to be significant

Discussion

Our results indicate that high expression of HMGB1 is deleterious in ovarian cancer. The role of DAMPs in cancer is complex and is likely to be tumour specific as well as contingent on the redox state of HMGB1 , its subcellular localisation and the expression of corresponding ligands. Gene expression data from previous work suggest HMGB1 may correlate with poor survival in ovarian cancer (Chen *et al*, 2012). However, to our knowledge this is the first large scale analysis of HMGB1 protein expression in ovarian cancer with validation in an independent second cohort.

Our work using large test and validation cohorts from ovarian cancer patients demonstrates that HMGB1 represents an independent prognostic marker of poor prognosis and that better understanding of HMGB1 in ovarian carcinoma pathogenesis may allow the rationale design of agents that target the HMGB1 pathway in ovarian cancer.

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