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Differential expression and prognostic value of HMGA1 in pancreatic head and periampullary cancer

Jill A. van der Zee [a,*], *Timo L.M. ten Hagen* [a], *Wim C.J. Hop* [b], *Herman van Dekken* [c], *Bilyana M. Dicheva* [a], *Ann L.B. Seynhaeve* [a], *Gerben A. Koning* [a], *Alexander M.M. Eggermont* [a], *Casper H.J. van Eijck* [a]

[a] *Laboratory of Experimental Surgical Oncology, Section Surgical Oncology, Department of Surgery, Erasmus Medical Center, Rotterdam, The Netherlands*

[b] *Department of Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands*

[c] *Department of Pathology, Erasmus Medical Center, Rotterdam, The Netherlands*

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ABSTRACT

The high mortality rate and minimal progress made in the treatment of pancreatic cancer over the last few decades, warrant an alternative approach. Treatment protocols should be individualised to the patient guided by prognostic markers. A particularly interesting target would be the architectural transcription factor high mobility group A1 (HMGA1), that is low or undetectable in normal tissue, induced during neoplastic transformation and consequently often exceptionally high in cancer. The aim of the current study was therefore to determine the differential expression of HMGA1 in pancreatic head and periampullary cancer and investigate its relation with outcome.

HMGA1 expression was determined by immunohistochemistry on original paraffin embedded tissue from 99 pancreatic head- and 112 periampullary cancers (with R0). Expression was investigated for associations with recurrence free (RFS), cancer specific (CSS) and overall survival (OS) and conventional prognostic factors.

HMGA1 was expressed in 47% and 26% of pancreatic head- and periampullary cancer, respectively and associated with poor RFS, CSS and OS in periampullary cancer. CSS 5 years following surgery was 25% and 44% for patients with tumours which were positive or negative for HMGA1 protein, respectively. HMGA1 expression was not associated with survival in pancreatic head cancer.

In conclusion HMGA1 was identified as an independent prognostic marker predicting poor outcome in periampullary cancer. Although expressed to a higher extent as compared to periampullary cancer, HMGA1 was not associated with survival in pancreatic head cancer.

1. Introduction

With yearly mortality rates equalling incidence and a 5-year survival rate following diagnosis of 5%, pancreatic cancer remains a serious health problem.[1] Almost 80% of patients present with major vessel involvement or distant metastasis, precluding them from resection, currently still the only potential for cure. Even following the resection most patients

* *Corresponding author:* Address: Erasmus Medical Center, 's Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. Tel.: +31 10 7040704; fax: +31 10 7044746.

E-mail addresses: j.a.vanderzee@erasmusmc.nl (J.A. van der Zee), c.vaneijck@erasmusmc.nl (C.H.J. van Eijck).

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will develop recurrent disease.[2] Despite attempts to improve outcome by several adjuvant chemo- and/or radio-therapy regimens, little to no progress has been made in the last few decades.[3] Specialists therefore claim that therapy should be individualised to the patient, guided by prognostic markers. One such candidate marker could be high mobility group A 1 (HMGA1).

HMGA1 proteins, originally discovered in HeLa cells,[4] are nuclear DNA-binding proteins which by interacting with the transcription machinery and altering chromatin structure, regulate the transcription of a multitude of genes.[5,6] HMGA1 has three isoforms encoded by the same gene, however generated through an alternative splicing mechanism.[7,8] HMGA1 is increased during embryogenesis and becomes low or undetectable in normal adult tissue.[9] However, increased levels have also been observed in rat thyroid cell lines following transformation with oncogenes.[10,11] Furthermore, neoplastic transformation was associated with HMGA1 expression in human prostate-,[12,13] thyroid-,[14,15] colorectal-,[16–20] cervix-,[21] pancreas-,[22–24] gastric-,[25] ovary-,[26] breast-,[27] liver-,[28] lung-,[29] uterine-[30] and head and neck-[31] tissue and blood.[32] A relation with worse pathological factors was observed in some of these.[12,13,18,20,28,33] Interestingly, following orthotopic injection of human pancreatic cancer cells, increased HMGA1 expression was observed in metastasis as compared to the primary tumour. A relation of HMGA1 expression with disease progression[13,20,31] and poor survival[24,28,29] was observed in some clinical studies. Of interest as well is the differential expression of HMGA1 between different neuroblastic tumours and different testicular germ cell tumours.[34,35]

The expression of HMGA1 proteins in cancer cells and not in their normal counterparts makes it a particular interesting target for therapy. Adenovirus mediated suppression of HMGA1 inhibited cell growth in carcinoma cells derived from human thyroid, lung, colon and breast, however had no effect on normal thyroid cells in vitro. Furthermore, adenovirus-mediated suppression of HMGA1 in vivo reduced thyroid tumour size.[36] This growth inhibitory effect mediated by the suppression of HMGA1 was confirmed in pancreatic cancer.[37,38] Liau and co-workers showed that silencing of HMGA1-decreased anoikis resistance and cellular-invasiveness in vitro, metastatic potential in vivo and increased sensitivity to gemcitabine both in vitro and in vivo.[38–40] They also provided evidence suggesting HMGA1 to be an independent predictor of poor postoperative survival in patients with pancreatic adenocarcinoma.[24]

The evidence for an important role of HMGA1 proteins in tumour progression and the differential expression between subtypes of some tumours, prompted us to investigate the differential expression of HMGA1 in pancreatic head and the prognostically more favourable periampullary cancer and explore the relation with the outcome in both tumour types.

2. Patients and methods

2.1. Patient Population

Retrospectively, 231 patients treated for pancreatic adenocarcinoma with curative intend at Erasmus Medical Center between 1987 and 2008 who had no microscopically residual tumour (R0) were identified. Tumours were classified by location having its origin either in the pancreatic head or periampullary region, the latter group comprising tumours originating in the Ampulla of Vater or the distal common bile duct. Tumour samples originating before the new 2002 UICC TNM classification were re-evaluated according to these new criteria.

Representative tumour areas were encircled on original haematoxylin/eosin slides by a GI pathologist (HvD) with special expertise in pancreatic pathology and staining was performed on corresponding formalin fixed, paraffin embedded tissue.

During the above-mentioned period two randomized control studies were ongoing in our centre. Between September 1987 and April 1995, 17 patients were randomized to the treatment arm of the EORTC 40891 trial, receiving two courses of 5-FU as a continuous infusion (max 1500 mg/day) followed by radiotherapy (20 Gy). From June 2000 up to its closure in March 2007, 32 patients were randomized to the treatment arm of a trial combining intra-arterial chemotherapy and radiotherapy. Patients received six cycles of intra-arterial mitoxantrone (10 mg/m^2), folinic acid (170 mg/m^2/day), 5-FU (600 mg/m^2/day) and cisplatinum (60 mg/m^2), the first cycle followed by radiotherapy (54 Gy). These trials and the results have been described in detail elsewhere.[41,42]

At the time of the present report, the median follow-up duration was 19 months (range 0–192 months). Recurrence free survival (RFS) was defined as the time from resection to first proof of disease recurrence (locally, distant or both) or to death without relapse. Overall survival (OS) was computed as the number of months from resection to death of any cause as registered by the social security death index (SSDI), whereas for cancer specific survival (CSS) only the pancreatic cancer related deaths were counted. Patients who died in hospital following procedure related complications were excluded from analysis with respect to survival as we consider their death to be unrelated to the studied tumour biology and would have introduced a confounding influence on survival analysis.

2.2. HMGA1 expression by immunohistochemistry

Immunohistochemistry was performed according to the protocol used in clinical practice at our institution and was optimised for HMGA1.

Briefly, 4 μM sections were deparaffinized in xylene and rehydrated through decreasing ethanol series ending in distilled water. Antigen retrieval was performed by microwave heating (20 min preheating followed by 20 min of cooking) in Tris–EDTA buffer pH 9.0. Endogenous peroxidase activity was quenched using 0.3% hydrogen peroxide (H_2O_2) in PBS for 20 minutes. Sections were incubated overnight at 4 °C with a polyclonal mouse antibody to the full length HMGA1 protein (B01, Abnova Corporation Taipel, Taiwan) at 500× dilution in Dako REAL antibody diluent (S2022, Dako), which reduces background staining without any need for additional blocking steps. This was followed by incubation with the secondary antibody (Dako REAL Envision HRP Rabbit/Mouse) for 30 min at RT. Immunostaining was developed by immersion in

diaminobenzidine. Slides were washed extensively between each of the above steps. Nuclei were counterstained with Harris Haematoxylin, followed by dehydration, fixation and finally covered using Leica multistainer and robotic coverslipper (ST5020 and CV 5030, Leica Microsystems B.V., Rijswijk, Netherlands). Positive and negative controls were included in each run.

2.3. Tissue evaluation

Slides were examined and scored separately by three observers (J.A.v.d.Z.; B.M.D. and T.L.M.t.H.) blinded to both clinical and pathological data. HMGA1 expression was quantified using a visual grading system based on the extent of staining. Immunoreactivity in the nucleus was evaluated. HMGA1 was absent (<10% nuclei from ductal cells positive), present in low quantities (⩾10% and <50% nuclei positive) or present in high quantities (⩾50% positive nuclei) in the nuclei of tumour cells. Discrepant scores were resolved by consensus.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 15.0 for Windows.

Differences in distribution of categorical clinico-pathological parameters between groups were compared with Chi-square or Fisher's exact tests when appropriate.

The distributions of RFS, CSS and OS were estimated using Kaplan Meier curves. Univariate associations were tested using the Log-rank test. Cox- regression models were used to test if relations between HMGA1 expression and outcome were independent of other established prognostic factors (T status, nodal involvement and tumour differentiation). By the use of interaction terms it was investigated whether prognostic effects of HMGA1 differed between pancreatic head and periampullary cancers.

All p values reported are two sided and values ⩽.05 were considered statistically significant.

3. Results

3.1. Patient population

Tissue blocks were available from 222 out of 231 patients. Eleven slides could not be scored due to poor quality. As a result immunostaining was correlated with established prognostic factors for 211 cases. The age of the patients ranged from 36 to 87 years (median 65 years) and the study population included slightly more males than females (122 vs 89). Ninety-nine tumours originated in the head of the pancreas whereas 112 patients had periampullary cancer. Nine patients died during postoperative stay and were thus excluded from survival analyses.

3.2. High mobility group A1 (HMGA1) expression

HMGA1 was heterogeneously expressed between and within tumours (Fig. 1). Staining was predominantly nuclear; however some perinuclear granulation was also observed. Since relatively few patients showed high HMGA1 expression (11 and 6 patients for respectively pancreatic head and periampullary cancer), low and high expression were combined for further analysis. Nuclear immmunostaining was present in 47% (46/99) of pancreatic head tumours and 26% (29/112) of periampullary tumours ($p = .003$).

3.3. Pathologic correlations

HMGA1 expression was not associated with any of the conventional prognostic factors in the total group of pancreatic cancers ($p = .29$, .24 and .28 for respectively T-, N- and differentiation status). Analysing pancreatic head and periampullary cancer separately gave similar results.

3.4. Clinical correlations

Patients with pancreatic head cancer had considerably worse prognosis following curative resection when compared to patients treated for periampullary cancer ($p < .001$ for RFS, CSS as well as OS). Only 17% of pancreatic head cancer patients

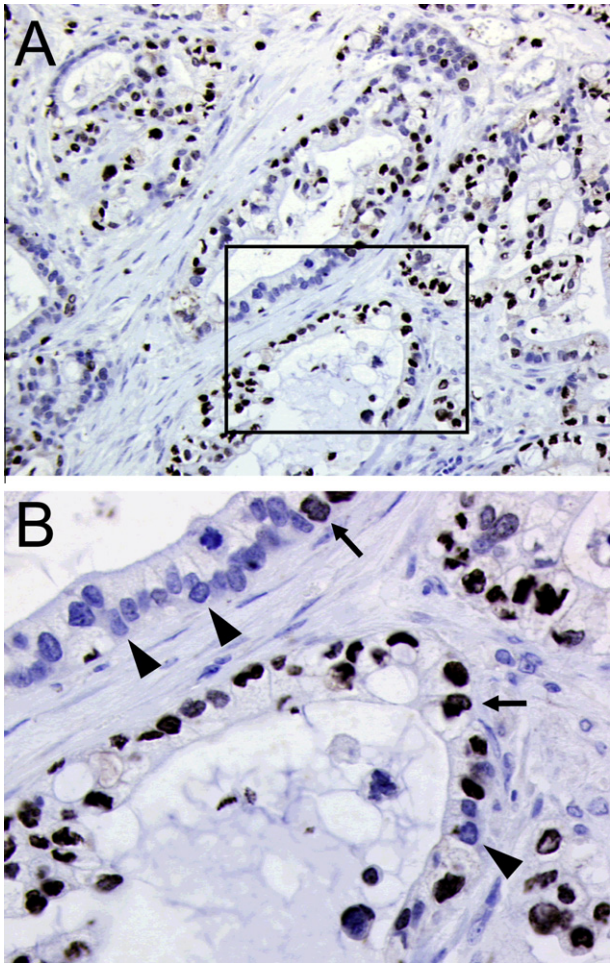

Fig. 1 – Periampullary cancer tissue immunohistochemically stained with HMGA1 antibody. (A) The tumour shows heterogeneous expression of HMGA1 (10× magnification). (B) Magnification of area depicted in (A) clearly showing both positive (arrow) and negative (arrowhead) nuclear staining (40× magnification).

were alive 5 years following curative resection as compared to 40% of patients treated for periampullary cancer. Survival analyses with respect to HMGA1 expression were therefore separated for the two tumour types.

In univariate analysis the presence of HMGA1 was significantly associated with cancer specific ($p = .019$) and overall survival ($p = .017$) and showed a trend for an association with recurrence free survival ($p = .053$) in periampullary cancer (Figs. 2–4). No significant associations were found in pancreatic head cancer ($p = .91$, .56 and .93 for respectively RFS, CSS and OS). Following correction for other conventional prognostic factors such as tumour extension, nodal involvement and degree of differentiation, HMGA1 expression proved an independent prognostic factor predicting a poor outcome following curative resection of periampullary cancer (Table 1). Multivariate analysis in pancreatic head cancer also showed that HMGA1 protein levels had no effect on outcome (Table 2).

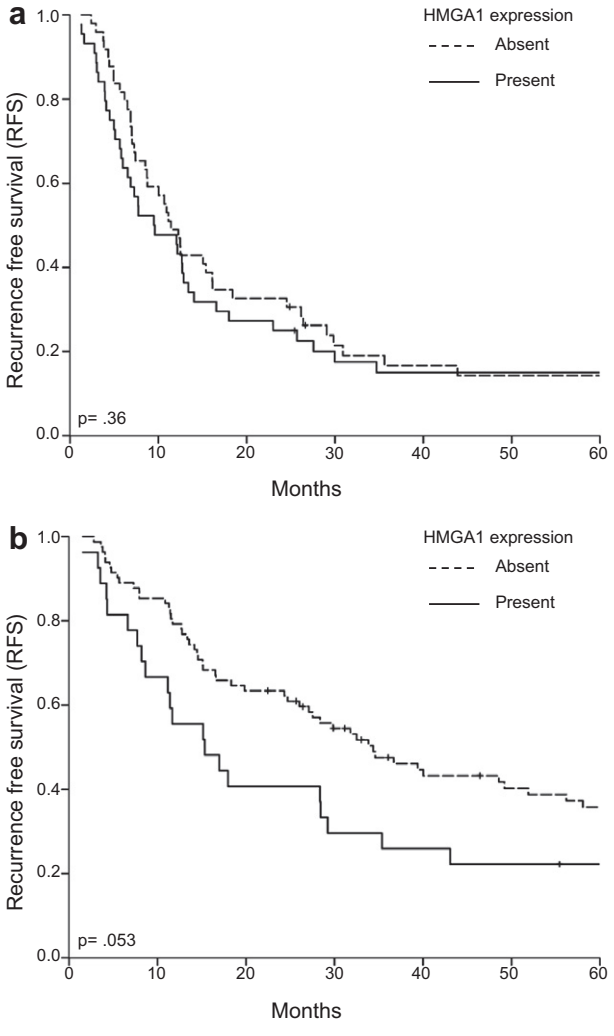

Fig. 2 – Kaplan Meier curves of recurrence free survival (RFS) of patients treated for respectively pancreatic head (a) and periampullary cancer (b) show a trend for shorter RFS in patients with periampullary tumours expressing HMGA1 compared to those with tumours that lack HMGA1 ($p = .053$). No difference in RFS was observed for patients with pancreatic head cancer ($p = .36$).

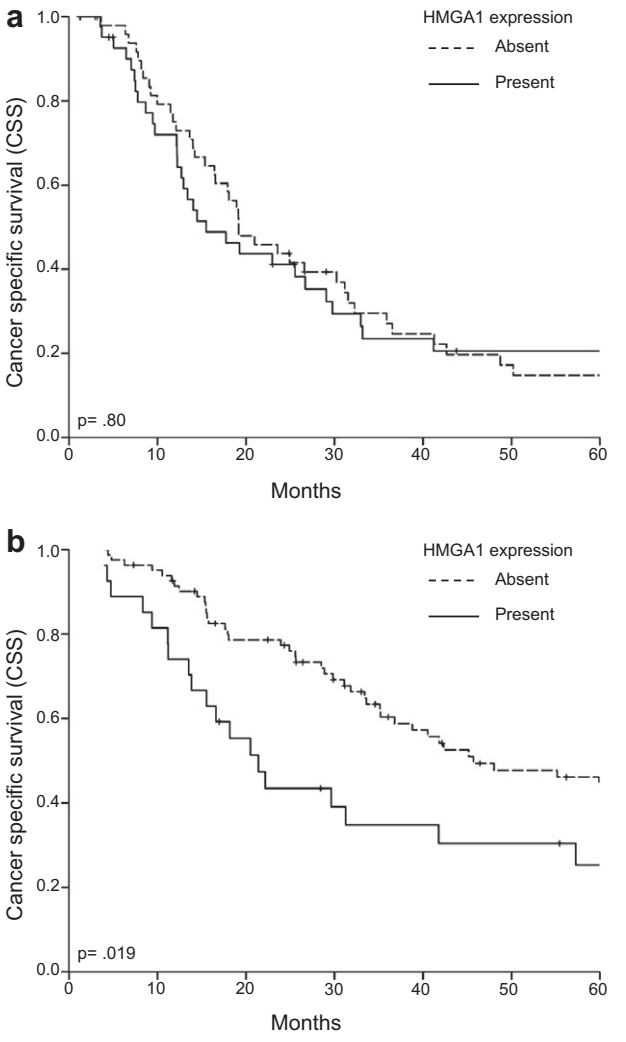

Fig. 3 – Kaplan Meier curves of cancer specific survival (CSS) of patients treated for respectively pancreatic head (a) and periampullary cancer (b) show shorter CSS in patients with periampullary cancer expressing HMGA1 compared with patients with tumours lacking expression ($p = .019$). No difference in CSS was observed for patients with pancreatic head cancer ($p = .80$).

Further analysis showed that the adjusted HR for CSS in Tables 1 and 2 differed significantly between the two tumour types (HR = 2.01 versus 0.86 for periampullary and pancreatic head cancer respectively; $p = .034$). For the other two outcome measures however, the adjusted HR did not significantly differ between the two tumour locations ($p = .14$ and .11 for, respectively RFS and OS).

4. Discussion

This is the first study to date reporting the differential expression of HMGA1 in pancreatic head and periampullary cancer.

Our results are obtained in the largest series of pancreatic cancer studies thus far and in contrast to some of the prior reports: Abe and co-workers showed that all 15 pancreatic duct cancers investigated were positive (that is at least 20% of

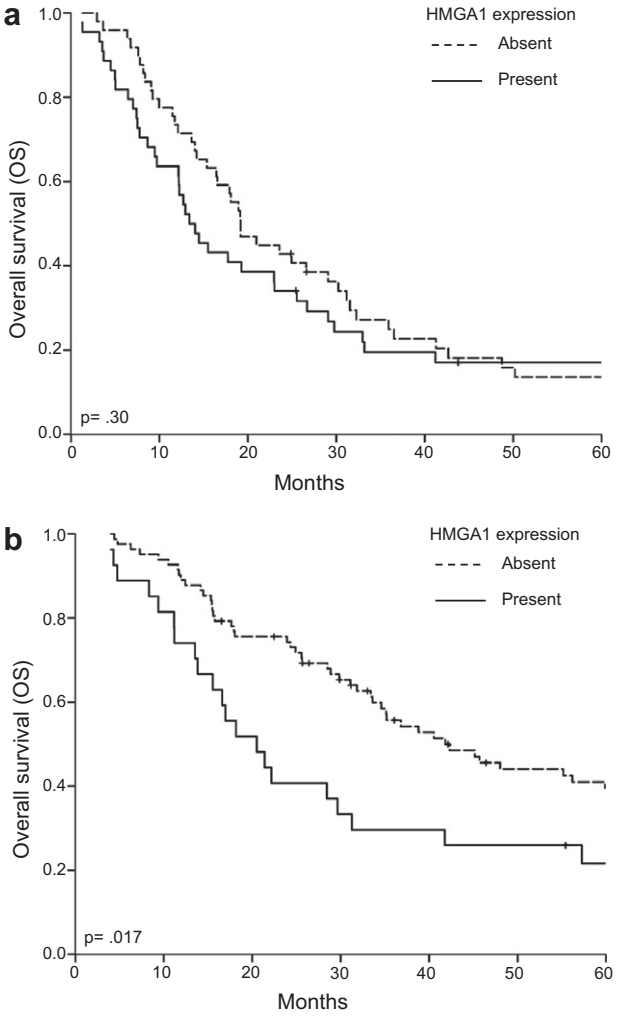

Fig. 4 – Kaplan Meier curves of overall survival (OS) of patients treated for respectively pancreatic head (a) and periampullary cancer (b) show shorter OS in patients with periampullary cancer expressing HMGA1 compared to those with tumours that lack HMGA1 (p = .017). No difference was observed for patients with pancreatic head cancer (p = .30).

nuclei positive) for HMGA1, whereas this protein was absent in nuclei of normal pancreas cells.[22] Liau and coworkers observed a 93% positive rate for HMGA1 with a cut-off level of 5%.[24] There is no consensus on the definition of positive with respect to HMGA1 immunoreactivity, multiple cut-off levels have been reported in literature. We therefore decided to take a cut-off level in between the 20% and the 5% used by Abe and Liau, respectively. With a cut-off level of 10%, forty-seven and 26% of respectively pancreatic head and periampullary cancers were identified positive for HMGA1 in our study cohort. An explanation for the different expression levels observed in our study cohort when compared to the ones reported by Abe and Liau is not obvious. With respect to differences in patient populations, in our patient cohort fewer patients presented with nodal involvement when compared to the group described by Liau and co-workers, however neither study showed a significant relation of HMGA1 with the presence or absence of positive lymph nodes (p = .20 and .08 in respectively theirs and our cohort). Furthermore, although another antibody was used to stain for HMGA1, the antibody used in the current study recognises both HMG-R and HMGIY. We initially performed immunostaining with three different antibodies, including the one used by Liau and co-workers, however satisfactory results were only achieved with the antibody currently used. In other cancers percentages ranging from 13% up to 95% were observed with the same cut-off used by us.[17,26–28,31,43] The different expression levels observed in pancreatic head and periampullary cancer are in line with previous studies in neuroblastoma and testis, showing different expression levels in the different subtypes of these cancers.[34,35]

We observed that besides different expression levels between the two types of pancreatic cancer, HMGA1 was identified as an independent predictor of outcome in only one of the two cancer types. Lack of HMGA1 was associated with increased relapse free-, cancer specific- and overall survival in periampullary cancer. Five year cancer specific survival rates were 25% and 44% for patients with tumours respectively positive and negative for HMGA1 protein. HMGA1 expression did not determine outcome in pancreatic head cancer. This is in

Table 1 – Multivariate analysis periampullary cancers.

Factor	N	RFS				CSS				OS			
		%5 year	HR	95% CI	p	%5 year	HR	95% CI	p	%5 year	HR	95% CI	p
Tumour extension					.12				.16				.09
T 1/2[a]	40	52				56				53			
T 3/4	67	20	1.50	0.90–2.50		30	1.52	0.85–2.71		25	1.56	0.93–2.62	
Nodal involvement					.001				.003				.012
No[a]	51	51				56				51			
Yes	58	16	2.28	1.41–3.69		25	2.33	1.34–4.04		21	1.87	1.15–3.04	
Differentiation					.024				.072				.13
Well[a]	18	58				71				64			
Moderately	72	30	1.11	0.56–2.18	.77	37	1.34	0.59–3.06	.49	32	1.25	0.62–2.52	.54
Poorly	19	16	2.41	1.09–5.34	.031	23	2.53	1.00–6.42	.051	21	2.14	0.94–4.88	.07
HMGA1													
Absent[a]	82	36				44				40			
Present	27	22	1.77	1.05–2.96	.031	25	2.01	1.15–3.53	.015	22	1.88	1.12–3.17	.018

[a] Reference category.

Table 2 – Multivariate analysis pancreatic head cancers.

Factor	N	RFS				CSS				OS			
		%5 years	HR	95% CI	p	%5 years	HR	95% CI	p	%5 years	HR	95% CI	p
Tumour extension					.24				.23				.34
T 1/2[a]	13	23				25				23			
T 3/4	78	13	1.50	0.77–2.92		16	1.59	0.75–3.39		14	1.39	0.71–2.72	
Nodal involvement					.016				.003				.004
No[a]	44	27				31				28			
Yes	49	3	1.78	1.11–2.86		3	2.15	1.30–3.55		2	2.01	1.25–3.23	
Differentiation					.08				.042				.08
Well[a]	15	33				38				33			
Moderately	60	10	1.96	1.02–3.75	.043	13	1.89	0.92–3.92	.09	11	1.92	1.01–3.66	.048
Poorly	17	12	2.27	1.06–4.86	.034	12	2.96	1.27–6.86	.012	12	2.25	1.05–4.82	.037
HMGA1													
Absent[a]	49	14				15				14			
Present	44	15	0.98	0.62–1.52	.91	21	0.86	0.53–1.41	.56	17	1.02	0.65–1.60	.93

[a] Reference category.

contrast to the observation made by Liau and co-workers. They observed a 12 times higher risk of death in the 93% of patients with tumours positive for HMGA1 compared to the remaining 7% lacking HMGA1 expression in their tumours when adjusted for age, gender, tumour size and differentiation, lymph node status and lymphovascular invasion.[24] The different expression levels of HMGA1, but even more so the information that the relation of HMGA1 with prognosis is restricted to periampullary cancer, suggest that these two tumour types not only differ with respect to prognosis, but also clearly seem to have different molecular behaviour. This is strengthened by similar observations for two other tumour markers, Bag-1 and TS, conversely for these the prognostic effect was restricted to pancreatic head cancer (data not shown).

In conclusion, in the current study the multifunctional architectural transcription factor HMGA1 proved to be an independent marker predicting poor survival in periampullary cancer. The lack of expression in normal tissue makes HMGA1 a particularly interesting target for therapy. The absence of an association with outcome in pancreatic head cancer makes HMGA1 a less interesting target for the treatment of this type of pancreatic cancer.

Conflict of interest statement

None declared.

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