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Association of galectin-3 expression with melanoma progression and prognosis

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ABSTRACT

Aims: Galectin-3 plays an important role in adhesion, proliferation, differentiation, angiogenesis and metastasis in multiple tumours. To investigate the role of galectin-3 in melanoma pathogenesis we examined the expression of galectin-3 in melanocytic lesions and analysed the correlation between galectin-3 expression and clinicopathologic factors including patient survival and BRAF mutation status.

Methods: We evaluated the expression of galectin-3 in 53 cases of benign naevi, 31 cases of dysplastic naevi, 59 in-situ melanomas, 314 cases of primary melanoma and 69 metastatic melanomas using tissue microarray and immunohistochemistry.

Results: Marked differences in expression of galectin-3 were seen between different categories of melanocytic lesions (ANOVA $p < 0.0001$). An increase in expression of galectin-3 between benign naevi and thin primary melanomas and a progressive decrease in expression between thin primary melanomas and thicker melanomas or metastatic melanomas was seen. Strong galectin-3 expression was associated with improved overall survival ($p = 0.002$ and $p = 0.0002$ for cytoplasmic and nuclear expression, respectively) and melanoma-specific survival ($p = 0.017$ and $p = 0.003$ for cytoplasmic and nuclear expression, respectively). A multifactorial Cox regression analysis suggested that galectin-3 expression was an independent prognostic marker for overall survival in melanoma (risk ratio 0.73, 95% CI 0.547–0.970, $p = 0.031$ for cytoplasmic expression and risk ratio 0.76, 95% CI 0.587–0.985, $p = 0.036$ for nuclear expression). No association between galectin-3 expression and BRAF mutation status was observed.

Conclusion: This study suggests that galectin-3 is a marker of progression in melanocytic lesions and a novel prognostic marker in primary melanoma.

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1. Introduction

Melanoma is the most lethal form of skin cancer and an increasingly common disease worldwide.[1] Although early melanomas are often cured by surgery up to 20% of patients develop metastatic disease.[2] The outlook for patients with metastatic melanoma remains dismal with response rates to chemotherapy of less than 20% and a median survival of less than 12 months.[1] There are now some encouraging data however on the efficacy of BRAF inhibitors in patients with tumours that carry the BRAF V600E mutation, where over 80% of patients were seen to achieve a response in a phase 1 trial.[3]

Several lines of evidence suggest that the pathogenesis of melanoma is a multistep process that may include the phases' benign naevus, dysplastic naevus, in-situ melanoma, radial and vertical growth phase melanoma and metastatic melanoma.[4] Although the mechanisms that mediate the transition between each step of the pathway remain largely unknown a number of key proteins involved in proliferation, control of apoptosis and invasion have been implicated.[5] These include tumour suppressor genes (p16 and PTEN), oncogenes (BRAF and N-Ras), cell adhesion molecules (E-cadherin), and metalloproteinases (mmp-2).

A number of clinical and histologic factors have been found to be associated with melanoma prognosis and these form the basis for the AJCC TNM staging of melanoma.[2] These factors include Breslow (lesion) thickness, mitotic rate and presence of ulceration. The best current prognostic marker, lesion thickness, is not an accurate indicator of biological behaviour as a significant minority of patients with very thin (<1 mm) melanomas go on to develop metastatic disease.[2] This has led to an extensive search for novel prognostic markers in melanoma.

Galectin-3 is a member of the family of lectins which selectively binds β-galactosidase residues. It is a chimeric molecule consisting of both carbohydrate recognition and collagen-like domains. It is predominantly localised in the cytoplasm although it may translocate to the nucleus or be secreted from the cell by ectocytosis. Galectin-3 plays an important role in adhesion, proliferation, differentiation, angiogenesis and metastasis in multiple tumours.[6] Both pro- and anti- apoptotic activities of galectin-3 have been found depending on the type of tumour studied. A recent study has suggested that galectin-3 expression may be associated with melanoma progression and may have some potential as a prognostic marker.[7] It is being investigated as a potential therapeutic target in multiple tumour types.[8]

The aim of this study was to investigate the expression of galectin-3 in a large series of melanocytic lesions and to correlate the expression with clinical and histologic features.

2. Methods

2.1. Cases

Four hundred and eighty-one patients with cutaneous melanoma from South-East Scotland diagnosed between 1993 and 1997 were selected from the Scottish Melanoma Group database. Patients were excluded if tissue blocks were unavailable or there was insufficient tissue for coring as judged by the pathologist. A total of 524 cases of melanocytic lesions including 55 benign naevi, 31 dysplastic naevi, 59 melanoma in situ, 350 primary melanoma and 71 metastatic melanoma were included. The local ethics committee granted ethical approval for this study (REC reference number: 06/S1103/9).

2.2. Construction of TMAs

H&E-stained sections were reviewed by a pathologist in order to select representative areas of tissue for coring. 0.6 mm^2 tissue cores were sampled and mounted into recipient paraffin blocks using a manual tissue arrayer (Beecher Instruments). In samples where sufficient material was present in duplicate, and in the case of thicker tumours, triplicate or quadruplicate, cores were taken. A total of 20 TMA blocks were constructed and serial 2 μm thick sections were cut from each block.

2.3. Immunohistochemistry

A standard 3,3-diaminobenzidine tetrahydrochloride (DAB)-based immunohistochemical labelling protocol was used to detect galectin-3 using the BondMax™ (Vision BioSystems, Newcastle, United Kingdom (UK)) automated immunohistochemistry system as per the manufacturer's instructions. The galectin-3 antibody used was a mouse monoclonal antibody (Novocastra™, product code NCL-Gal3). For each staining run a negative, no-antibody control was included.

Galectin-3 expression was assessed by staining intensity, frequency and location. Stained sections were examined without knowledge of the outcome of individual cases. The histoscore method was used where a weighted score was calculated by multiplying the percentage of cells stained (0–100%) by the staining intensity (0, 1, 2, 3) to give a maximum histoscore of 300.[9] Slides were examined by two independent pathologists and the average of the scores given by the two pathologists was calculated to give an overall score for each core. A separate score was given for the cytoplasmic and nuclear compartments. Where replicate scores from 1 case were available the average score from the cores was calculated.

2.4. Solar elastosis scoring

Solar elastosis scoring was performed on H&E-stained sections using an adaptation of a previously used method.[10] The breakdown of the scores are as follows: 1: no elastotic fibres, 2: rare elastotic fibres, 3: scarcely scattered elastotic fibres between collagen bundles, 4: scattered elastotic fibres between collagen bundles, 5: densely scattered elastotic fibres between collagen bundles, 6: densely scattered elastotic fibres between collagen bundles with occasional bushels, 7: densely scattered elastotic fibres between collagen bundles with some bushels, 8: densely scattered elastotic fibres between collagen bundles mostly as bushels, 9: focal formation of amorphous deposits of blue-grey material with lost

Table 1 – Clinicopathologic characteristics of primary melanoma cases. Included patients (n = 314) are those with galectin-3 scores and excluded (n = 167) are those without scores due to lack of or unsuitable tissue for scoring. SSM = superficial spreading melanoma, LMM = lentigo-maligna melanoma. p-Values are shown for the following analyses: Welch two sample t-test for age; Pearson's chi-squared test for sex, site, histology, ulceration, status; Wilcoxon rank sum test for Breslow.

Variable	Included Patients No. (%)	Excluded Patients No. (%)	
Sex			
M	121 (39)	63 (38)	$p = 0.96$
F	193 (61)	104 (62)	
Breslow			
⩽1.0 mm	180 (57)	103 (62)	
1.01–2.0 mm	63 (20)	20 (12)	
2.01–4.0 mm	40 (13)	17 (10)	
>4.0 mm	31 (10)	27 (16)	$p = 0.11$
Site			
Sun exposed	42 (13)	40 (24)	
Sun protected	264 (84)	124 (74)	
Acral	8 (3)	3 (2)	$p = 0.02$
Ulceration			
Present	67 (21)	31 (19)	
Absent	195 (62)	117 (70)	
Incipient	42 (13)	15 (9)	
Unknown	10 (3)	4 (2)	$p = 0.29$
Type			
SSM	240 (76)	104 (62)	
Nodular	39 (12)	26 (16)	
LMM	25 (8)	20 (12)	
Acral	8 (3)	12 (7)	
Other	2 (1)	5 (3)	$p = 0.002$
Status			
Alive	249 (79)	123 (74)	
Dead (melanoma)	44 (14)	23 (14)	
Dead (other)	21 (7)	21 (13)	$p = 0.45$

fibre architecture, 10: moderate formation of amorphous deposits of blue-grey material with lost fibre architecture, 11: large formation of amorphous deposits of blue-grey material with lost fibre architecture.

2.5. BRAF V600E mutation detection by restriction fragment length polymorphism (RFLP)

DNA was isolated from 0.6 mm^2 cores of formalin-fixed paraffin embedded melanoma tissue using a QIA quick micro column kit (Qiagen, UK) and then amplified using nested polymerase chain reaction (PCR) with the following primer pairs. Pair 1; forward, 5′CTACTGTTTTCCTTTACTTACTACACCTCAGA and reverse, 5′CTGATTTTTGTGAATACTGGGAA. Pair 2; forward, 5′TAAAAATAGGTGATTTTGGTCTAGCTCTAG and reverse, 5′GGCCAAAAATTTAATCAGTGGA. Presence of the BRAF V600E mutation was detected by RFLP using an adaptation of a previously published method.[11] Fifteen microlitres of PCR product was digested with XbaI for 2 h at 37 °C and fragments were separated by agarose gel electrophoresis. The liberation of a 100 bp fragment from the 125 bp PCR product indicated the presence of a BRAF V600E mutation. Sensitivity of the BRAF assay was assessed by mixing BRAF mutant and wild-type DNA isolated from cell lines of known BRAF V600E genotype at varying concentrations.

2.6. Statistics

Differences in expression of galectin-3 between different types of melanocytic lesions were assessed by analysis of variance (ANOVA) with the Tukey method used to establish confidence intervals for pair-wise groups. The Kaplan–Meier method and the log-rank test were used to evaluate correlations between protein staining and patient survival. Cox proportional hazards models were used to develop a multifactorial survival model for primary melanoma in terms of both conventional risk factors and protein expression. The relationship between galectin-3 expression and other variables was assessed by the Welsh T-test where two groups were present and by ANOVA when more than two groups were present.

3. Results

3.1. Clinicopathologic features of TMAs

Fifty-three cases of normal naevi, 31 cases of dysplastic naevi, 59 in-situ melanomas, 314 cases of primary melanoma and 69 metastatic melanomas could be evaluated for galectin-3 expression. A number of cores (two naevi, 36 primary melano-

mas and two metastatic melanomas) were unevaluable due to insufficient tissue or excessive pigment being present in the cores which made scoring impossible. Clinical and pathologic characteristics of the primary melanoma samples that were 'included' in the TMA and those that were 'excluded' are shown in Table 1. The median duration of follow up was 5.4 years.

The 'included' and 'excluded' patient groups were similar in respect of sex, Breslow, ulceration, and status. The mean age of the 'included' patients was 53.3 and for the 'excluded' was 54.4 (Welch two sample *t*-test, $p = 0.29$). A lower proportion of 'included' samples were from sun-exposed sites ($p = 0.02$) and histologically there were fewer acral melanomas included ($p = 0.002$).

3.2. *Expression of galectin-3 during melanoma progression*

Marked differences in expression of both cytoplasmic/membranous and nuclear galectin-3 were seen between various melanocytic lesions (ANOVA $p < 0.0001$) (Fig. 1). An increased expression of galectin-3 between benign naevi and thin melanomas and a fall in expression between thin melanoma, thicker melanomas and metastases was seen (Fig. 2). The increase in expression of galectin-3 between benign naevi and thin primary melanomas was observed in both cytoplasmic and nuclear compartments. The progressive decrease in expression between thin primary melanomas and thicker

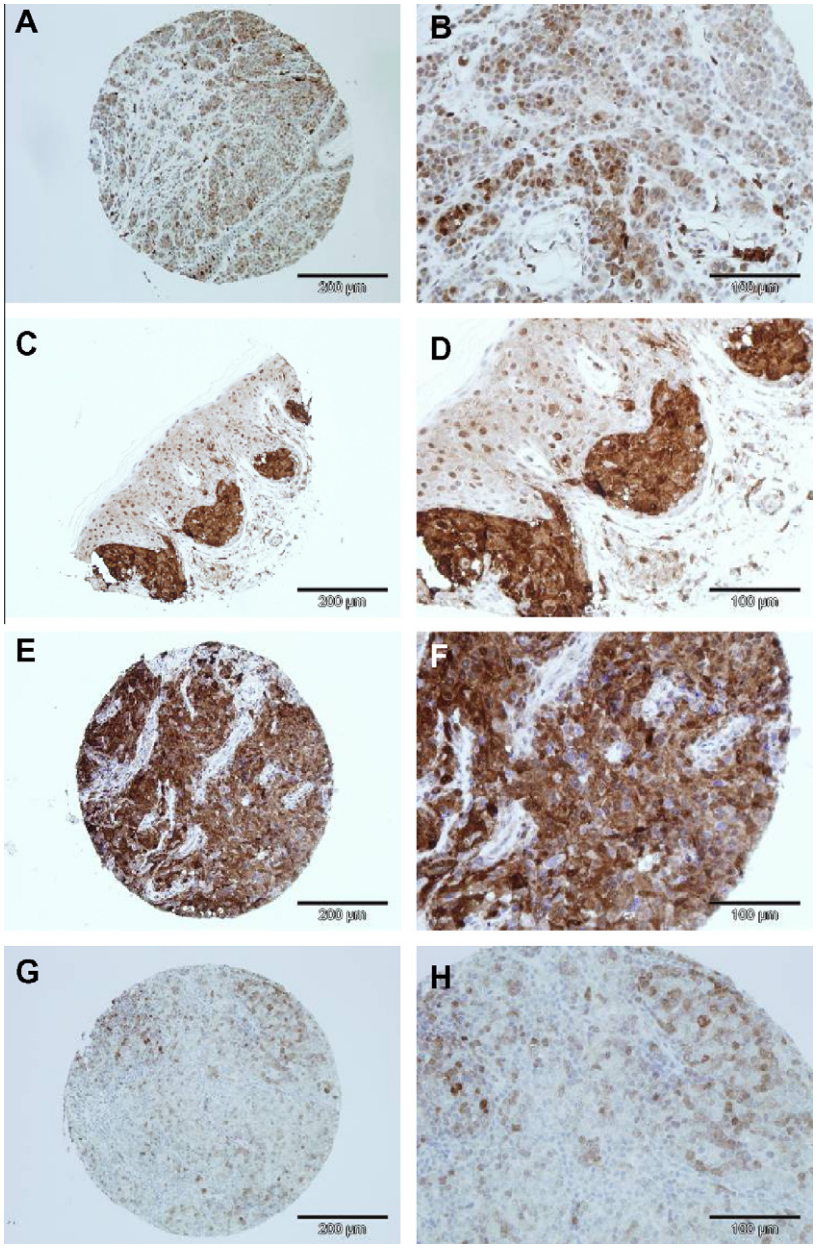

Fig. 1 – Galectin-3 immunohistochemistry. (A and B) Naevus, (C and D) dysplastic naevus, (E and F) primary melanoma, (G and H) metastatic melanoma. The galectin-3 histoscores for cytoplasmic/membranous staining for (A and B), (C and D), (E and F) and (G and H) were 80, 220, 200 and 40 and for nuclear staining were 20, 220, 80 and 40, respectively. Magnification is 200× for (A), (C), (E) and (G) and 400× for (B), (D), (F) and (H).

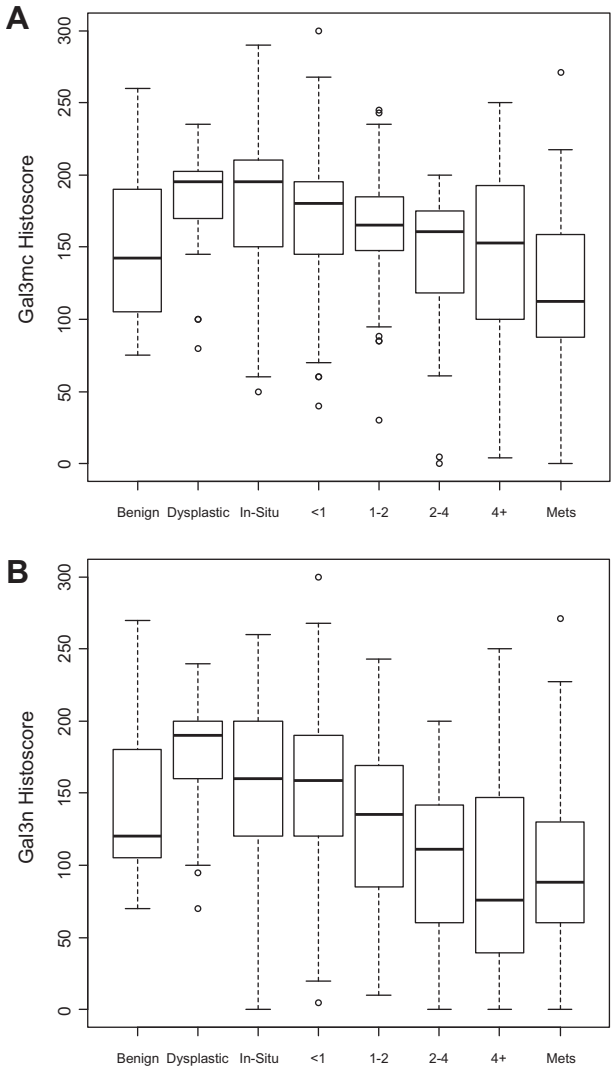

Fig. 2 – Expression of galectin-3 during melanoma progression. (A) Cytoplasmic and membranous galectin-3, (B) nuclear galectin-3. Each box represents the interquartile range (1st quartile to 3rd quartile). The median is marked with a bold line. The dotted lines represent the extent of 1.5× the interquartile range. <1, 1–2, 2–4 and >4 represent the Breslow thickness (mm). Open circles represent outliers.

primary melanomas or metastatic melanomas was more evident for nuclear staining. On formal statistical testing a significant difference in cytoplasmic galectin-3 expression was seen between thin melanomas (<1 mm) and metastatic melanomas ($p < 0.0005$), whereas for nuclear galectin-3 significant differences in expression were seen both between thin melanomas (<1 mm) and thicker melanomas (1–2 mm or >4 mm) or metastatic melanomas ($p < 0.0005$), respectively.

3.3. *Survival analysis*

To evaluate whether expression of galectin-3 correlated with prognosis in patients with primary melanoma, Kaplan–Meier survival curves were constructed using overall and melanoma-specific survival (Fig. 3). The data revealed that strong galectin-3 expression was correlated with both increased overall and disease-specific survival. This correlation was found with both cytoplasmic galectin-3 expression (log rank $p = 0.002$ for overall survival and $p = 0.017$ for melanoma-specific survival) and for nuclear galectin-3 expression (log rank $p = 0.0002$ for overall survival and $p = 0.003$ for melanoma-specific survival). The most favourable survival was seen in those patients with the highest nuclear galectin-3 expression and the least favourable survival was seen in those with the lowest nuclear galectin-3 expression. The clear separation of both overall and melanoma-specific survival curves for each quartile for nuclear galectin-3 staining was particularly striking (Fig. 3).

A multifactorial Cox regression analysis was performed in order to assess whether cytoplasmic or nuclear galectin-3 expression were independent prognostic markers for melanoma (Table 2). The multifactorial analysis included age, sex, Breslow, ulceration and presence of elastosis. Both cytoplasmic and nuclear galectin-3 expression predicted overall survival independently of other clinicopathologic parameters ($p = 0.031$ and $p = 0.036$, respectively). Significance for melanoma-specific survival was not reached ($p = 0.079$ for cytoplasmic galectin-3 and $p = 0.055$ for nuclear galectin-3). Independent prognostic markers for worse melanoma-specific survival were presence of ulceration ($p = 0.008$ for cytoplasmic galectin-3 and $p = 0.009$ for nuclear galectin-3), increased Breslow ($p = 0.014$ for cytoplasmic galectin-3 and $p = 0.025$ for nuclear galectin-3) and absence of marked elastosis ($p = 0.026$ for cytoplasmic galectin-3 and $p = 0.013$ for nuclear galectin-3).

A number of additional exploratory analyses were performed in order to assess the relationship between galectin-3 expression and other known prognostic factors. It was confirmed that decreased nuclear galectin-3 expression was associated with increased patient age ($p = <0.001$), higher Breslow thickness ($p < 0.001$) and presence of ulceration ($p < 0.001$). Decreased cytoplasmic galectin-3 expression was associated with increased age ($p < 0.006$) and increased Breslow ($p < 0.001$).

3.4. *BRAF mutation analysis*

The relationship between galectin-3 expression and presence of the BRAF V600E mutation was investigated in 115 primary melanoma patients and 29 metastatic melanoma patients. The remainder of the samples were inadequate for isolation of tumour DNA. The sensitivity of the assay was such that it could detect the mutant BRAF gene if it was present in at least 10% of cells isolated (Fig. 4). In primary patients, 23 melanomas (20%) were positive for the BRAF V600E mutation, 21 were heterozygous and two were homozygous whilst in metastatic melanomas, 20 (69%) were BRAF V600E mutants, with three being homozygous. The presence of the BRAF V600E mutation in primary samples was associated with the presence of pigmentation; 39% of BRAF V600E melanomas were heavily pigmented compared to 11% of melanomas where the mutation was not detected (Fisher's exact test $p = 0.003$). While 65% and 80%, respectively, of BRAF V600E positive primary melanomas had cytoplasmic and nuclear galectin-3 histoscores in the lowest two quartiles, compared to 43% and 57% in primaries where the mutation was not detected, the

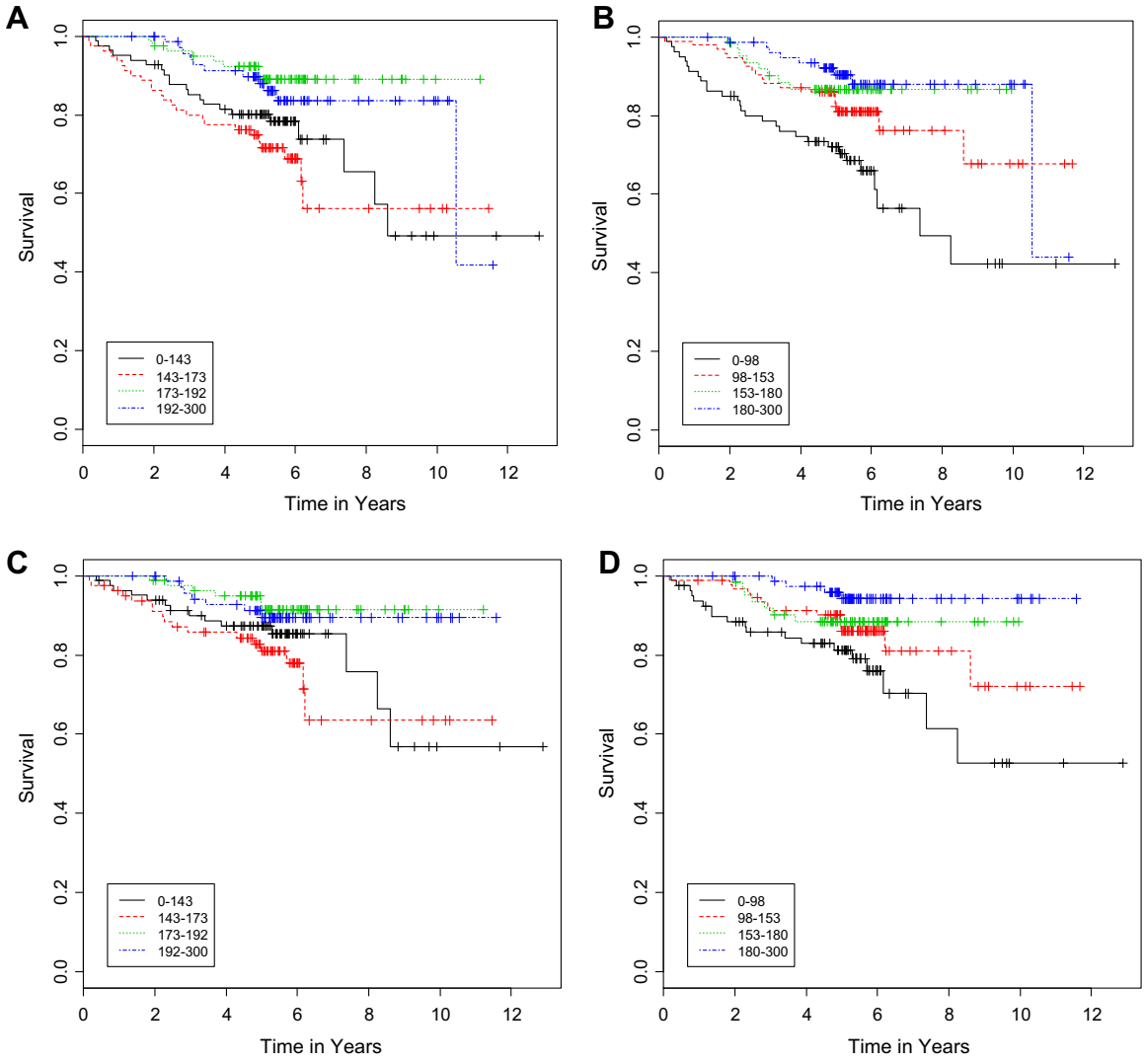

Fig. 3 – Kaplan–Meier survival analysis for galectin-3. Overall survival for cytoplasmic (A) and nuclear galectin-3 (B); melanoma-specific survival for cytoplasmic (C) and nuclear galectin-3 (D). The histoscore range for each galectin-3 quartile is in the bottom left hand corner of each graph.

differences were not statistically significant. The BRAF V600E mutation was also not associated with age, tumour thickness (Breslow), presence of ulceration or degree of solar elastosis. Although patients with the BRAF V600E mutation appeared to have lower overall and melanoma-specific survival (Fig. 5A and B), the differences were not significant (log rank $p = 0.20$ and 0.25, respectively). However, the BRAF V600E mutation was significantly associated with a shorter time to relapse (Fig. 5C, log rank $p = 0.02$, hazard ratio 2.62, 95% CI 1.25–9.80).

4. Discussion

This study used tissue microarrays to investigate galectin-3 as a potential marker of melanoma progression and prognosis. Although a number of previous melanoma TMA studies have identified novel prognostic markers in melanoma including p-Akt,[12] PUMA,[13] osteopontin,[14] nuclear receptor coactivator-3[15] and nm23[16] the majority of these studies included mostly thick melanomas.[8,12,15,16] In our study all stages of melanoma progression were represented including a large number of thin melanomas. The comparison of clinicopathologic features of the cases included in this study versus those that were excluded confirmed that there were few differences between the groups in sex, age, Breslow, presence of ulceration and histological type. The only statistically significant differences between the 'included' and 'excluded' groups were in the site and type of melanoma. This was in part due to the presence of fewer acral melanomas as a result of tissue being used in a previous immunohistochemistry study. The TMA constructed in this study is therefore representative of the wider melanoma population.

Galectin-3 has been reported to be expressed by monocytes, macrophages, and several epithelial tissues including mammary, colonic and kidney tissue and has been found to be overexpressed in several pathological conditions including

Table 2 – Cox regression analysis of the impact of various factors on overall and melanoma-specific survival. (A) Cytoplasmic and membranous galectin-3. (B) Nuclear galectin-3. Coding of variables; sex was coded as 1, female and 2, male. Ulceration was coded as 1, absent and 2, present. Breslow was coded as 1, ⩽1.00 mm versus 2, 1.01–2.00 mm, 2.01–4.00 mm and >4.00 mm. Risk ratios represent the comparison of variable 2 versus 1. Galectin-3, age and elastosis were included as continuous variables; risk ratio is per 50 unit increase in galectin-3 histoscore or 1 unit increase in elastosis score or 1 year increase in age.

Variable	Overall survival			Melanoma-specific survival		
	Risk ratio	95% CI	*p*	Risk ratio	95% CI	*p*
A						
Galectin-3 (mc)	0.729	0.547–0.970	0.031	0.729	0.512–1.051	0.079
Age	1.022	1.000–1.045	0.050	1.01	0.988–1.039	0.19
Sex	0.945	0.510–1.752	0.858	0.496	0.234–1.054	0.068
Ulceration	4.699	1.649–13.389	0.004	5.62	1.578–19.993	0.008
Breslow 1–2 mm	1.843	0.676–5.026	0.232	3.795	0.945–15.235	0.060
Breslow 2–4 mm	2.824	0.892–8.939	0.077	6.529	1.470–29.005	0.014
Breslow >4 mm	1.627	0.409–6.483	0.490	2.686	0.418–17.274	0.298
Elastosis	0.887	0.757–1.039	0.137	0.772	0.615–0.969	0.026
B						
Galectin-3 (n)	0.759	0.587–0.985	0.036	0.744	0.552–1.000	0.055
Age	1.023	1.001–1.046	0.040	1.01	0.988–1.038	0.313
Sex	0.976	0.526–1.809	0.938	0.505	0.239–1.068	0.074
Ulceration	4.421	1.567–12.473	0.005	5.35	1.532–18.688	0.009
Breslow 1–2 mm	1.694	0.611–4.701	0.311	3.418	0.835–13.000	0.087
Breslow 2–4 mm	2.508	0.772–8.142	0.126	5.658	1.247–25.673	0.025
Breslow >4 mm	1.625	0.406–6.492	0.492	2.528	0.395–16.194	0.328
Elastosis	0.870	0.746–1.014	0.075	0.757	0.608–0.942	0.013

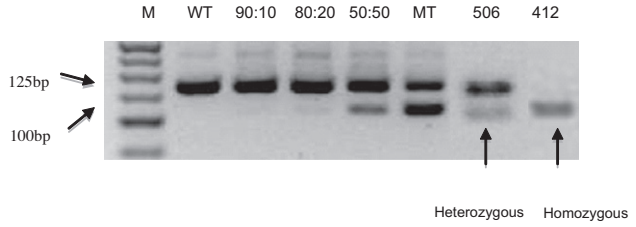

Fig. 4 – BRAF V600E RFLP assay. Lanes 2–6 are samples with various concentrations of wild-type (WT):mutant (MT) DNA; lanes 7 and 8 are melanoma samples with heterozygous and homozygous BRAF V600E mutations, respectively.

human atherosclerosis and cancer. Data on the expression of galectin-3 in cancer are conflicting. Decreased expression of galectin-3 has been detected in breast cancer, colonic cancer, prostate cancer and head and neck cancer compared to that of corresponding normal tissues.[17–20] In contrast, galectin-3 expression increased in pancreatic, vulvar and colonic carcinomas compared to normal tissues.[21]

The relationship between galectin-3 expression and survival in cancer is also somewhat conflicting. Elevated galectin-3 is associated with worse prognosis in hepatocellular carcinoma, colorectal cancer and tongue cancer.[22–24] In contrast reduced galectin-3 is associated with unfavourable prognosis in gastric cancer, clear cell renal carcinoma, cholangiocarcinoma and pancreatic cancer.[25–28]

Our study found higher expression of galectin-3 in primary melanomas compared to naevi and a progressive fall in expression of galectin-3 between thin melanomas, thicker melanomas and metastases. The depletion in galectin-3 expression was particularly evident in the nuclear compartment. Our survival analysis revealed that elevated galectin-3 expression was associated with an improved overall and melanoma-specific survival in patients with primary melanoma. This finding was confirmed for both cytoplasmic and nuclear expression of galectin-3. On multivariate analysis it was found that galectin-3 was an independent prognostic marker for overall survival although statistical significance was not reached for melanoma-specific survival. Other independent prognostic markers for worse melanoma-specific survival were presence of ulceration, increased Breslow and absence of marked elastosis. It should be noted that some of the prognostic effects of galectin-3 may be due to the association of reduced galectin-3 expression with increased age, higher Breslow and presence of ulceration.

This study adds to a growing literature that has investigated the role of galectin-3 in melanoma progression and prognosis. Abdou et al. published a small study of 20 benign naevi and 21 primary melanomas.[29] Whole tissue sections were stained using the same antibody as used here and scoring was performed for both cytoplasmic and nuclear compartments using the histoscore method. They found an increased expression between naevi and primary melanomas which is consistent with our findings. No association was found between galectin-3 expression and Breslow thickness or overall survival. Vereecken et al. published a study of 15 benign naevi, 89 primary melanoma and 11 melanoma metastases.[30] They stained whole sections with the same antibody as used here. They found increased expression of galectin-3 between benign naevi and primary melanoma and lower expression in thick melanoma and metastases compared to thin melanomas, which is again consistent with our findings, although no survival data were presented. Prieto et al. published a study of 17 benign naevi, 18 dysplastic naevi, 23 primary melanomas

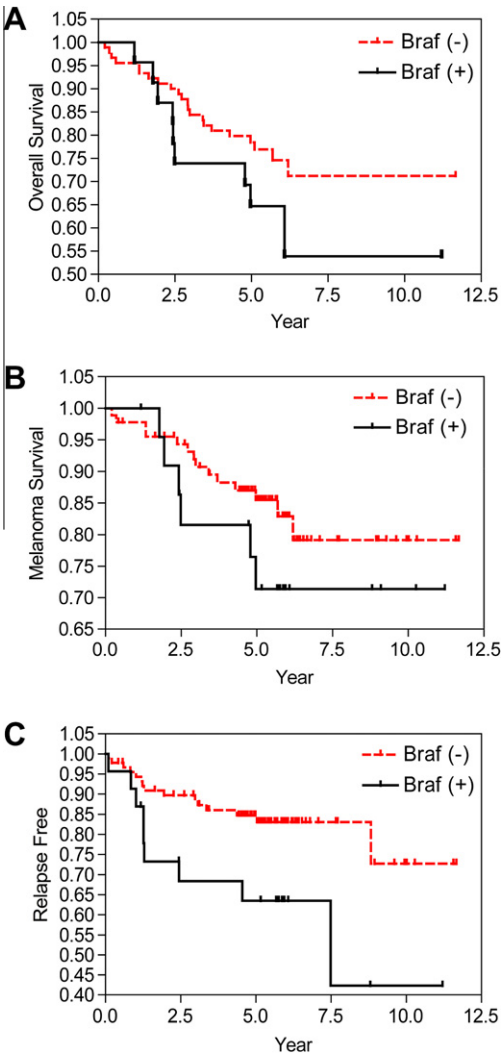

Fig. 5 – Kaplan–Meier survival analysis for BRAF V600E. Overall survival (A), melanoma-specific survival (B) and time to melanoma relapse (C) are shown for patients with (+) and without (–) the BRAF V600E mutation.

and 31 metastatic melanomas.[7] A tissue microarray was used and the antibody (TIB 166) was different from the one used in our study. Separate scores were given for frequency and intensity of nuclear and cytoplasmic staining, respectively. They found an increased expression of galectin-3 between naevi and primary melanomas. Expression appeared highest in metastatic melanomas, unlike our finding of lower expression in metastatic melanomas. No relationship was found between galectin-3 expression and Breslow thickness and no statistically significant association between galectin-3 and disease-free survival was found. Buljan et al. published a study of 104 primary melanomas. They used whole tissue sections, rather than tissue cores, and used the same antibody as used here.[31] The scoring system employed was different to ours as, firstly, an immunohistochemical staining index (ISI) was used based on a combination of frequency and intensity of staining and, secondly, no attempt was made to score the nuclear and cytoplasmic compartments separately as had been done in our and other studies. Despite the differences in methodology it remains surprising that Buljan et al.'s study found an association between increased expression of galectin-3 and increased Breslow thickness. The paper did not however give a breakdown of the differences in expression between different Breslow groups so it is impossible to comment further as to whether there was one outlying group that was driving the difference, or whether there was a stepwise change in galectin-3 expression between different Breslow groups as had been seen in our study. There were also no naevi or metastases included to allow a comparison with these groups. Although no results were presented for overall survival an association between increased galectin-3 and reduced recurrence-free survival was seen which was again, unlike our study. Galectin-3 was however not associated with recurrence-free survival on multi-variate testing. The reasons for this difference is unclear, although it does seem remarkable that in the group of patients with low galectin-3 expression, none of the patients developed melanoma metastases, or died from melanoma over a follow up period of up to 8 years. This is a finding that has not been seen in any of the previous melanoma studies, nor indeed in studies of other cancers and, although intriguing, should be interpreted with caution. Our finding of increased galectin-3 expression being associated with improved survival is consistent with the findings of several studies of other cancer types.[25–28] The different conclusions between the Buljan study and ours emphasise the need to perform well-designed prospective trials which incorporate novel biomarkers in order to confirm which markers are the most reliable indicators of melanoma progression or prognosis.

Given the current interest in BRAF inhibition as a novel therapeutic strategy in patients with metastatic melanoma the relationship between galectin-3 expression and the presence of the BRAF V600E mutation was also investigated. In the sub-set of samples for which BRAF V600E mutation status could be assessed, no association between galectin-3 expression and presence of the BRAF V600E mutation was found. It should be noted that the prevalence of BRAF V600E mutations in primary melanomas that we reported was 20%, which is at the lower end of the range reported in previous studies, whereas the higher frequency (69%) found in metastatic melanomas was more similar to other reports.[32–34] The low mutation frequency seen in our primary melanomas may relate to the sensitivity of the method being insufficient to detect a low frequency of BRAF V600E cells in an excess of normal tissue. A further interesting finding was that two of our primary tumours only contained a BRAF V600E allele. Although it is common for melanoma cell lines to lack a normal BRAF allele, we are not aware that this has been reported previously in primary tumours. The BRAF V600E mutation was found to be associated with increased pigmentation in our primary melanomas, which is consistent with results of previous studies.[34,35] Previous studies have also reported correlations with age and degree of solar elastosis, but no such associations were seen in our study, possibly because the sample size was too small.[35,36] This was one of the largest melanoma TMA studies to be carried out so far and was unique in containing a high proportion of thin melanomas so that it more closely resembled the distribution of primary tumour thicknesses observed at diagnosis. However, the consequence was that

many of the tumours contained insufficient material for BRAF V600E mutation detection in addition to providing cores for the TMA. The BRAF V600E mutation was associated with a significantly shorter relapse free period, but the failure to see an association with survival means that this result should be interpreted with caution.

There is growing evidence that galectin-3 is involved in a number of key aspects of regulation of cellular homeostasis and it could be hypothesised that different functions of galectin-3 including control of apoptosis and adhesion may be responsible for the findings in this study. In thin melanomas high levels of galectin-3 may contribute to resistance to apoptosis which is key hallmark of melanoma cells.[37] During progression to thicker melanomas metalloproteinases are released by the cell and may use galectin-3 as a substrate.[38] Melanoma progression may therefore be associated with a depletion of intracellular stores and as a consequence a decrease in galectin-3 expression may be associated with an increase in the risk of metastatic spread and a worse overall prognosis in primary melanoma.

In conclusion, our results suggest that galectin-3 may have an important role in melanoma pathogenesis and is a promising marker of prognosis in primary melanoma. These results require validation in a separate cohort of patients.

Conflict of interest statement

None declared.

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