EUROPEAN JOURNAL OF CANCER 48 (2012) 865–874

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.ejconline.com

Association of galectin-3 expression with melanoma progression and prognosis

Ewan R. Brown [a,*], *Tamasin Doig* [b], *Niall Anderson* [c], *Thomas Brenn* [b], *Val Doherty* [d], *Yan Xu* [e], *John M.S. Bartlett* [f], *John F. Smyth* [a], *David W. Melton* [e]

[a] *Edinburgh Cancer Centre, NHS Lothian, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*
[b] *Department of Pathology, NHS Lothian, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*
[c] *Public Health Sciences Section, Division of Community Health Sciences, The University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG, UK*
[d] *Department of Dermatology, NHS Lothian, Level 1, Lauriston Building, Lauriston Place, Edinburgh EH3 9HA, UK*
[e] *Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*
[f] *Endocrine Cancer Group, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*

ARTICLE INFO

Article history:
Available online 7 November 2011

Keywords:
Melanoma
Galectin-3
Prognosis
BRAF

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.

* *Corresponding author.*
E-mail address: Ewan.Brown@luht.scot.nhs.uk (E.R. Brown).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.09.003

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