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Cancer–testis antigen expression in primary cutaneous melanoma has independent prognostic value comparable to that of Breslow thickness, ulceration and mitotic rate ☆

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ABSTRACT

To determine the effect of Cancer–Testis Antigen (CTAg) expression on the natural history of primary cutaneous melanoma we compared its impact on prognosis with that of known prognostic factors and its relationship with other clinicopathologic characteristics.

The immunohistochemical expression of three CTAgs (MAGE-A1, MAGE-A4 and NY-ESO-1) in 348 cases of stage I and stage II primary cutaneous melanoma was analysed and correlated with clinicopathologic characteristics, relapse free survival (RFS) and overall survival (OS). A Cox proportional hazards regression model was used to analyse factors which independently predicted RFS.

All three CTAgs were significantly co-expressed with each other ($P < 0.001$). The median RFS for patients with CTAg-negative tumours and CTAg-positive tumours was 72 months and 45 months, respectively, ($P = 0.008$). Univariate analysis demonstrated that the impact of CTAg expression on RFS was comparable in magnitude to that of Breslow thickness, ulceration and tumour mitotic rate. Multivariate Cox regression analysis indicated that CTAg expression was a powerful independent predictor of RFS (risk ratio (RR) = 1.715, 95% confidence interval (CI) = 0.430–0.902, $P = 0.010$). In contrast, CTAg expression was demonstrated to have no prognostic impact on overall survival.

This study demonstrates CTAg expression in primary cutaneous melanoma is a strong independent predictor of RFS and it is comparable to other known important prognostic factors. CTAg expression has no relationship with overall survival, suggesting anti-melanoma immunity directed towards CTAg expression may contribute to the natural history of the disease. In view of these results, further investigation of the function of CTAgs and their potential use in therapeutic targeting is warranted.

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1. Introduction

Cutaneous melanoma is a major and increasing public health issue worldwide and its incidence continues to rise in individuals of European origin.[1] In the United States it is predicted to be the fifth and sixth most common cancer in men and women, respectively, in 2009.[2] Wide local excision of the primary tumour is currently the standard treatment for cutaneous melanoma[3] and is successful particularly for patients with thin tumours.[4] In many melanoma treatment centres, sentinel lymph node biopsy is offered to patients with melanomas >1 mm thick or to those with melanomas <1 mm thick if other adverse prognostic factors are present. Surgery is also considered the mainstay for treating melanoma that has metastasised to regional lymph nodes.[5] For patients with widespread metastatic disease, the outcome is poor,[4] single agent or combination chemotherapy has little efficacy[6] and at present treatment is directed at palliation of symptoms rather than curing the tumour.[7]

With such limited therapeutic options for advanced metastatic disease, interest in vaccine development is high. Antigens that are relatively specific for cancer, known as cancer–testis antigens (CTAgs), have been demonstrated to serve as targets through their presentation on HLA molecules for immune recognition by cytotoxic T lymphocytes (CTLs).[8] CTAgs are expressed by many different tumours as well as by spermatogonia and trophoblast but not by other normal somatic cells.[9] Previous work involving CTAgs has focused on assessment of the expression patterns of these antigens in cancers[10–12] as well as the development, use and immune response monitoring of cancer vaccines in the clinical setting.[13] However, very little is currently known about the correlation of CTAg expression with clinical data such as known prognostic factors and survival in melanoma patients. Most previous studies are disadvantaged by the small number of tumours studied and/or the limited numbers of antigens tested.[12,14]

This study assessed CTAg expression in a large series of primary cutaneous melanomas and correlated the expression of these antigens with other clinico-pathologic features, relapse free survival (RFS) and overall survival (OS).

2. Materials and methods

2.1. Patients and clinical specimens

Archival formalin-fixed, paraffin-embedded tissue blocks of stage I or stage II primary cutaneous melanomas from patients treated at the Austin Health Melanoma Clinic (Melbourne, Australia) or the Melanoma Institute Australia (MIA) (Sydney, Australia) were retrieved. Sections were cut and stained with hematoxylin–eosin to confirm the diagnosis of primary melanoma and then immunohistochemistry was performed as described below. All protocols were approved by the Austin Health Human Research Ethics Committee.

2.2. Antibodies

Monoclonal antibodies to MAGE-A1[15] and NY-ESO-1[16] were produced by the Biological Production Facility at the Ludwig Institute for Cancer Research (Melbourne, Australia). MA454 (MAGE-A1) was diluted 1:50 for staining. E978 (NY-ESO-1)[17] was used at 3 μg/ml. The monoclonal antibody supernatant 57B (Dr. G. Spagnoli, Surgical Research Centre, Basel, Switzerland) was diluted 1:100 for staining. Monoclonal antibody 57B was raised against MAGE-A3. 57B was originally reported to detect only the MAGE-A3 protein[15] however it was later observed to recognise other CTAgs.[17] In paraffin-embedded tissue sections, 57B has been shown to recognise MAGE-A4 predominantly regardless of the expression of other MAGE genes[18] and therefore for this study it was reported as such.

2.3. Immunohistochemistry

Four-micron thick sections of formalin-fixed paraffin-embedded tissue were cut and then dried at 37 °C overnight. Slides were dewaxed in xylene and rehydrated through alcohols. Water bath antigen retrieval was performed for 30 min using EDTA buffer pH 8.0 (NeoMarkers, Fremont, CA) for MA454 and E978 or citrate buffer pH 6.0 (NeoMarkers, Fremont, CA) for 57B staining. Antibody binding and counterstaining was performed as previously described.[16] The chromogen used was 3-amino-9-ethyl-carbazole (Sigma–Aldrich, St. Louise, MO) and the application of Crystal Mount (Biomeda, Foster City, CA) preceded dehydration and mounting in DePeX (BDH 36125). Known antigen-positive tumours were used as positive controls. Negative substitution controls included replacing the primary antibody with the antibody diluent solution and an IgG matched control antibody.

2.4. Interpretation

Tumour cell CTAg expression was estimated microscopically by two pathologists (D.M. and S.D.) and Ludwig Institute for Cancer Research clinical investigators (I.D.D. and J.S.C.). Slides were scored by eye as a percentage of tumour cells staining for each antigen and assigned to one of two groups: positive (any cells staining) and negative (no staining).

2.5. Human sera

Human sera were obtained from 313 patients treated at the Austin Health Melanoma Clinic (Melbourne, Australia) under informed consent and approved by the Austin Health Human Research Ethics Committee. Sera was assigned to one of four groups, AJCC classified stage I, II, III or IV. All sera were collected prior to surgery for the relevant stage of disease.

2.6. ELISA to detect serum NY-ESO-1 autoantibodies

Maxisorp 96-well plates (Nunc, Roskilde, Denmark) were coated with 0.05 μg/well recombinant NY-ESO-1 (Dr. Roger Murphy, Ludwig Institute for Cancer Research, Melbourne, Australia), in phosphate buffered saline (PBS) and incubated

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