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Original article

ADAM 10 expression in primary uveal melanoma as prognostic factor for risk of metastasis

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ABSTRACT

Uveal melanoma is the most frequent primary intraocular neoplasm in adults. Although malignant melanoma may be located at any point in the uveal tract, the choroid and ciliary body are more frequent locations than the iris. In the present study, we examined ADAM10 expression levels in primary uveal melanoma both with and without metastasis, and we evaluated their association with other high risk characteristics for metastasis in order to assess if ADAM10 can be used to predict metastasis. This study included a total of 52 patients, 23 men and 29 women, with uveal melanoma. A significantly high expression of ADAM-10 was seen in patients with metastasis (11/13, 84.6%), but not in patients without metastasis (15/39, 38.5%). In conclusion we found that ADAM10 expression was associated with a more rapid metastatic progression confirming its role in uveal melanoma metastasis.

1. Introduction

Uveal melanoma is the most frequent primary intraocular neoplasm in adults. Rare cases are reported in adolescents, children, and even infants [1]. It has been suggested that most of them arise from preexisting benign nevi [2]. Other risk factors for uveal melanoma (UM) include a fair complexion, light irides, oculodermal and ocular melanocytosis, and type 1 neurofibromatosis [3]. Although malignant melanoma may be located at any point in the uveal tract, the choroid and ciliary body are more frequent locations than the iris. Visual disturbance caused by retinal detachment is usually the more frequent presenting complaint. Nevertheless, frequently the patient remains asymptomatic until the tumour has grown enough to become necrotic and produce complications such as endophtalmitis, massive intraocular hemorrhage and secondary glaucoma. Microscopically, UMs have been divided into three types: 1) spindle cell; 2) epithelioid cell; and 3) mixed cell type [4]. Cytogenetically, common alterations include loss of 1p, monosomy 3, and loss of 6q, and gain of 6p and 8q [5]. UM is biologically distinct from cutaneous melanoma by a very strong propensity to metastasize the liver. Local treatment of uveal melanoma has improved, with increased use of conservative methods and preservation of the eye, but survival rates have remained unchanged. The median time from diagnosis of UM until death is 47 months and only 5 months in patients with metastatic disease [6].

A disintegrin and metalloproteases (ADAMs) are transmembrane proteins with broad tissue distribution. ADAMs are multidomain proteins that comprise a prodomain, a zinc metalloprotease domain, a disintegrin domain, a cysteine-rich region, an epidermal growth factor-like region, a transmembrane domain and a cytoplasmic domain. ADAMs play critical roles in physiological, inflammatory and pathological conditions. ADAMs are implicated in the control of membrane fusion and in cell–cell and cell-matrix interactions by the binding capacity of the disintegrin domain to specific integrins [7]. ADAMs are also able to induce the release of proprotein ectodomains like membrane-anchored cytokines and growth factors [7]. An increasing body of evidence indicates that disintegrin-metalloprotease proteins are involved in the formation

Abbreviations: UM, uveal melanoma.

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and progression of a variety of cancers [8] through the regulation of cell adhesion, migration and invasion. A disintegrin and metalloproteinases ADAM10 and ADAM17 are known to be upregulated in several types of malignancies and participate in progression. In particular, ADAM10 is highly expressed in cutaneous melanoma [9–11], where it plays a role in invasion and is considered to be a potential target for therapy. Immunohistochemical analysis on tissue microarrays indicated that ADAM10 expression was significantly elevated in melanoma metastasis compared with primary melanomas.

In the present study, we examined ADAM10 expression levels in primary uveal melanoma both with and without metastasis, and we evaluated their association with other high risk characteristics for metastasis in order to assess if ADAM10 can be used to predict metastasis.

2. Materials and methods

The authors performed a retrospective analysis of clinical records and formalin-fixed, paraffin-embedded (FFPE) tissue specimens of all cases of primary choroidal and/or ciliary body melanoma treated by primary enucleation at the Eye Clinic, University of Catania, Catania, Italy during the eight years up to May 2014. Enucleations were performed in the case of tumours not suitable for radiotherapy procedures, such as plaque brachytherapy or proton beam radiotherapy. Cases were excluded if the paraffin blocks containing the tumour could not be located for the preparation of additional slides for the immunohistochemical staining study, representative tumour tissue was not present in the paraffin blocks, if the tumour was completely necrotic, or the tumour had been treated previously by a method such as plaque radiotherapy or proton beam radiotherapy, which might have altered the histopathologic features and immunoreactivity of the tumours. Formalin-fixed and paraffin-embedded tissue specimens were obtained from the surgical pathology files at the Anatomic Pathology, Department G.F. Ingrassia, University of Catania, Catania, Italy. From formalin-fixed and paraffin-embedded tissue specimens multiple sections (at least 5) were obtained. Due to the retrospective nature of the study, no written informed consent from patients was obtained. The research protocols were approved by the Local Medical Ethical Committee (University of Catania) and conformed to the ethical guidelines of the Declaration of Helsinki. In order to use uniform criteria, all histological slides were evaluated by two pathologists (RC and LP). The study consisted of 39 uveal melanomas without metastasis and 13 uveal melanomas with metastasis. From clinical charts the following data were collected: size and location of the tumour evaluated through ophthalmoscopy and A and B scan ultrasonography The presence of metastasis was assessed using standard modalities, including physical examination, liver ultrasound and total body computed tomography. The mitotic rate was measured by counting the number of mitoses in 40 high-power fields. Tumor infiltrating lymphocytes were reported as absent, focal (single cluster of lymphocytes), multifocal (multiple clusters of lymphocytes), or diffuse (lymphocytes seen throughout tumour).

2.1. Immunohistochemistry

Sections were processed as previously described [12]. Briefly, the slides were dewaxed in xylene, hydrated using graded ethanols and were incubated for 30 min in 0.3% H_2O_2/methanol to quench endogenous peroxidase activity then rinsed for 20 min with phosphate-buffered saline (PBS; Bio-Optica, Milan, Italy). The sections were heated (5 min × 3) in capped polypropylene slide-holders with citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0; Bio-Optica, Milan, Italy), using a microwave oven (750 W) to unmask antigenic sites. The blocking step was performed before application of the primary antibody with 5% bovine serum albumin (BSA; Sigma, Milan,Italy) in PBS for 1 h in a humid chamber. BSA was used as a blocking agent to prevent non-specific binding of the antibody. Then, the sections were incubated overnight at 4 °C with rabbit polyclonal anti-ADAM10 antibody (ab1997; Abcam, Cambridge, United Kingdom), diluted 1:200 in PBS (Sigma, Milan, Italy) and MIB-1, a monoclonal antibody directed against the Ki-67 antigen (M7240; Dako Corporation, Glostrup, Denmark), diluted 1:75 in PBS (Sigma, Milan, Italy). The secondary antibody, biotinylated anti-rabbit antibody was applied for 30 min at room temperature, followed by the avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for a further 30 min at room temperature. The immunoreaction was visualized by incubating the sections for 4 min in a 0.1% 3,3′-diaminobenzidine (DAB) and 0.02% hydrogen peroxide solution (DAB substrate kit, Vector Laboratories, CA, USA). The sections were lightly counterstained with Mayer's hematoxylin (Histolab Products AB, Göteborg, Sweden) mounted in GVA mountant (Zymed Laboratories, San Francisco, CA, USA) and observed with a Zeiss Axioplan light microscope (Carl Zeiss, Oberkochen, Germany).

2.2. Evaluation of immunohistochemistry

Immunostained slides were separately evaluated by two pathologists (RC and LP), who were blinded to patient identity, clinical status and group identification, using a light microscope.

The ADAM10-staining status was identified as either negative or positive. Immunohistochemistry positive staining was defined as the presence of brown chromogen detection within the cytoplasm or in the membrane. Spleen tissue was identified as positive control to test the specific reaction of primary antibodies used in this study at a protein level. Negative control sections were processed like the experimental slides, except that they were incubated with PBS instead of the primary antibody.

Stain intensity and the proportion of immunopositive cells were assessed by light microscopy. Intensity of staining (IS) was graded on a scale of 0–3, according to the following assessment: no detectable staining = 0, weak staining = 1, moderate staining = 2, strong staining = 3, as described previously [12]. The percentage of ADAM10 immunopositive cells (Extent Score (ES)) was independently evaluated by two investigators and scored as a percentage of the final number of 100 cells in five categories: <5% (0); 5–30% (+); 31–50% (++); 51–75% (+++), and >75% (++++). Counting was performed at 200× magnification. The staining intensity was multiplied by the percentage of positive cells to obtain the intensity reactivity score (IRS).

MIB-1 labeling index was evaluated in the highest immunoreactivity fields. It was expressed as percentage and was determined by dividing the number of positive staining nuclei by 1000 tumour cells. Cell nuclei were considered to be positive if there was any nuclear staining present, regardless of the intensity and distribution within the nucleus.

2.3. FISH

FISH was performed with DNA probes specific for the centromere of chromosome 3 (probe: α-sat3; Cytocell, Cambridge, UK) and for region 3p24.3-p25 (probe: RP11-322M13). It was classified as successful and reliable if a minimum of 200 cells in interphase could be assessed.