Antibodies to skeletal muscle actin and their reactivity with malignant melanoma of the choroid

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SUMMARY Imprints of choroidal malignant melanoma cells were treated with serum containing antibodies to a purified preparation of actin derived from skeletal muscle. Evidence of a positive reaction, as shown by indirect immunofluorescence, substantiated an impression based on morphological criteria that choroidal melanoma cells contain actin protein. The significance of this protein in tumour biology is discussed, and a possible interference by antiactin antibodies in the immunodiagnosis of choroidal melanoma is highlighted.

Actin is a globular contractile protein with a molecular weight of about 48 000. Together with myosin it plays a fundamental role in muscular contraction.

Skeletal muscle fibres are composed of a macromolecular complex of polymerised actin and myosin organised into interdigitating filaments which are able to slide over one another. The interdigitations are responsible for the characteristic banded pattern of skeletal muscle fibres. Smooth muscle fibres similarly contain actin and myosin, which may, however, be present either in filamentous (F actin) or soluble globular forms (G actin). Furthermore the filaments do not have the banded spatial arrangement of striated muscle but can lie either parallel or at right angles to each other.

Recent studies1-4 show that actin, although principally found in muscle fibres, is present in almost all eukaryotic cells, where it can be demonstrated in at least 2 forms: as bundles of filaments or as a randomly orientated meshwork of filaments.5 Apart from providing structural support (cytoskeleton), the contractile proteins take part in other mechanochemical activities of the cell such as cell movement and mitotic division.

The presence of actin in cells which normally display only limited mobility suggests that it performs functions additional to those required in cell movement, such as endocytosis, exocytosis, cell division, and in-vitro attachment to glass surfaces in tissue culture.46-8

The content and molecular organisation of actin varies in different types of cells as well as being modified by the physiological state of the cell.9-11

It has been shown that actin is present in large amounts in malignant tumours arising from some epithelial tissues,9 thus implying a possible role in the invasive properties of a malignant tumour. Whether this is true of cancers in general and of malignant melanoma of the choroid in particular is not known. A striking feature of actin in non-muscle cells is that it aggregates into filaments which can change their pattern and appearance in a matter of hours.11 Although some of these changes have been noted in pathological tissues,9 they have not been studied in detail, and their significance is largely obscure.

Antibodies reacting with smooth muscle antigens were first described in patients with chronic active hepatitis.12 The smooth muscle antibodies (SMA) in such sera were subsequently shown to react with nonmuscle tissues, including the endothelial cells of renal glomeruli,13 hepatocytes,12 14 15 thyroid cells,16 17 fibroblasts,18 19 and platelets.20 21 All these cell types are now known to contain microfilaments which morphologically resemble the actin filaments seen in muscle tissue. Similar antibodies are now known to occur in the sera of patients with other inflammatory conditions such as cytomegalovirus infection,22 mycoplasma pneumonial infection,23 infectious mononucleosis,24 acute and chronic hepatitis,25 26 and uveitis.27 They are also a feature of a variety of neoplastic states which include basal and squamous cell carcinomas,9 Burkitt's lymphoma, nasopharyngeal carcinoma,28 29 carcinoma of the breast,30 and malignant melanoma of the skin.9 31

In a previous communication, using an indirect
immunofluorescence test, we showed that sera from patients with a variety of inflammatory eye diseases may similarly contain smooth muscle antibodies.37 These sera produced equally strong cytoplasmic fluorescence when tested against choroidal melanoma cell impressions,32 which led us to examine the tumour cells for the presence of contractile proteins.

In an ultrastructural study we showed the presence of varying numbers of microfilaments in the cytoplasm of cells from a single malignant choroidal melanoma, but more recently we have demonstrated them in 21 of 22 similar tumours. These microfilaments, ranging in diameter between 5 and 7 nm, were arranged in loose bundles and aligned essentially parallel to the surfaces of the cells, and were consistent morphologically with actin.38

In the present communication we describe the reactivity of melanoma cells with antibodies specifically raised against pure actin. This study proves for the first time that choroidal melanoma cells contain actin protein comparable to that found in contractile organs.

Methods and materials

Actin was extracted from the skeletal muscle of an albino rabbit. Initially a crude extract was obtained by a method essentially similar to that described by Iyengar and Weber.33 Finely minced muscle tissue was soaked in 50% glycerol containing 0-1 M KC1 and 1 mM MgCl₂ at pH 7 for 3 days. The suspension was filtered and squeezed to remove all the soluble components. The residue was then soaked in a solution containing 0-6 M KC1 and 1 mM adenosine triphosphate in distilled water at pH 6-4 for 24 hours. The insoluble residue obtained after filtration was then treated for 2 successive periods of 30 minutes each in 0-3 M KC1 at pH 7 and 0-1 M KC1 at pH 9 respectively. Finally the residue was treated 4 times with distilled water at pH 9. The final residue was dehydrated in acetone and dried overnight. All procedures were carried out in the cold room at a temperature of 4°C.

Purification of actin

The dried powder thus obtained contained actin which was further purified using the aggregation-disaggregation principle.34

Actin was extracted from the acetone-dried powder by suspending it in ice-cooled Tris buffer (2 mM Tris HC1 (hydroxymethyl methylamine hydrochloride) 0-2 mM ATP, 0-5 mM mercaptoethanol and 0-2 mM CaCl₂ at pH 8) for 30 minutes. The solution was passed through a coarse sintered glass funnel, and the filtrate was further cleared by centrifugation at 10 000 g for 1 hour. To this supernate KC1 and MgCl₂ were added to give a final concentration of 50 mM KC1 and 2 mM MgCl₂. The solution was kept on ice for 2 hours in order to polymerise the actin (F actin). More KC1 was then added to the solution to bring its concentration to 0-6 M and left for 90 minutes. The solution was centrifuged for 3 hours at 80 000 g, and the resulting actin protein pellet was resuspended in buffer and dialysed with vigorous stirring for 3 days at 4°C, changing to fresh buffer every 24 hours.

The protein concentration in the final solution was measured using a modification of the Lowry technique.35

Identification of actin and the assessment of its purity was carried out by sodium dodecyl sulphate polyacrylamide gel electrophoresis.38 37 Pure actin protein (obtained by the courtesy of Dr M. Owen,
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National Institute for Medical Research, Mill Hill, London) and other standard proteins of known molecular weight were used as markers. The protein bands in the gel were fixed overnight in 50% trichloroacetic acid and then stained with 0.1% Coomassie brilliant blue solution.

Actin solution was allowed to ‘age’ by storing for one week at 4°C, to increase its antigenicity, and then lyophilised for storage at −20°C.

Preparation of Antiserum
Albino rabbits were used to raise antiaction antibodies. Before immunisation the serum of each animal was tested for the presence of antibodies to smooth muscle and other autoantibodies by the conventional immunofluorescence test, and only the animals whose sera gave negative results were used.

Each animal received 1 mg of ‘aged’ actin redissolved in 0.25 ml of distilled water and emulsified with an equal volume of Freund’s complete adjuvant (Difco Laboratories). The dose was divided between intramuscular and intraperitoneal injections. The animals were bled after 3 weeks and a booster injection was given. Three weeks later the animals were bled again.

Standardisation Procedure
The antisera thus raised were tested for the presence of antiactin antibodies by a standard indirect immunofluorescence test using frozen sections of a composite block consisting of portions of liver, kidney, skeletal muscle, stomach and salivary gland from a rat. Fluorescein labelled antirabbit immunoglobulin was obtained from Nordic Diagnostic and the sections were examined under a Zeiss epifluorescence microscope equipped with an FITC interference filter.

Control studies were carried out by using sera obtained from animals prior to immunisation as well as antiactin sera absorbed with purified actin.

Reactivity with Choroidal Malignant Melanoma Cells
Eyes with choroidal malignant melanomas were opened as soon as possible after enucleation (up to a maximum of 5 hours), and the cut surface of the tumour was pressed against multispot slides to give a monolayer of tumour cell imprints. The slides were snap frozen and stored in liquid nitrogen for further use. Cell impressions from 8 different choroidal melanomas were used in this study to test for the presence of actin in tumour cells.

Results
The purity of the actin protein preparation was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (Fig. 1).

The presence of antiactin antibody in the rabbit sera was confirmed by the immunofluorescent staining patterns obtained when the sera were tested against the smooth muscle cells of the blood vessel wall, the muscularis mucosae and smooth muscle fibres between the glands of the stomach,

Fig. 2 Indirect immunofluorescence staining of choroidal malignant melanoma cell imprints treated with antiactin antibodies. The bright cytoplasmic fluorescence is indicative of the presence of actin protein (× 135; inset × 450).
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the myoepithelial cells of the parotid gland, liver cells in the region of the cell membranes (polygonal staining pattern), and the actin-containing I bands of skeletal muscle. In each case the staining pattern corresponded to that reported by Trenchev et al. using antiactin antibodies.

The antiactin sera gave strong cytoplasmic fluorescence with all 8 choroidal malignant melanoma cell impressions. As well as diffuse fluorescence of the cytoplasm, filamentous fluorescence was also observed in the region of the cell processes (Figs. 2 and 3).

The patterns of staining described above were diminished when the sera were absorbed against actin prior to testing, the reduction being most marked when the sera were tested in high dilutions.

Discussion

In an earlier study we reported the presence of intracytoplasmic microfilaments of 4–7 nm diameter in choroidal malignant melanoma cells and suggested that these filaments were probably actin. It was also speculated then that the ‘false’ positive immunofluorescence obtained by testing the sera of patients with nonmalignant eye diseases against the cells of a malignant melanoma might be due to the smooth muscle antibody demonstrated in the serum of these patients.

In the present study by subjecting the melanoma cells to an antiserum raised against a known purified preparation of actin we have been able to demonstrate with some certainty that choroidal malignant melanoma cells contain actin protein. Moreover, since the pattern of immunofluorescence assumed filamentous and diffuse forms, it is reasonable to suppose that the actin was present in either a filamentous or a depolymerised globular state.

The present study reinforces our claim that the results of immunological tests for the diagnosis of choroidal melanoma based on reactions between patient’s serum and preparations of uveal melanoma cells need to be interpreted with circumspection. This is not to say that antibodies to tumour-specific antigens are not a feature of patients with uveal malignancy but rather to emphasise that before such a claim can be made it is essential to exclude the presence of antibodies to actin and, possibly, other cell components.

The significance of the contractile protein filaments in the tumour cells is unknown, though it is reasonable to speculate that they are related to the motility of the cells and hence to the capacity for infiltration and invasion characteristic of malignant tumours.

References

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