Increased Expression of Actin-like Protein in Human and Ethylnitrosourea-induced Tumors of the Nervous System

B. H. Toh, R. Qvist, V. B. Randell, and W. L. Elrick

Department of Pathology and Immunology, Monash University Medical School [B. H. T., R. Q., V. B. R.], and Department of Neurosurgery, Alfred Hospital [W. L. E.], Melbourne 3181, Australia

SUMMARY

Twenty-one human intracranial tumors comprising 15 astrocytomas and 6 meningiomas and 26 ethylnitrosourea-induced rat neural tumors comprising 7 astrocytomas and 9 schwannomas were examined by indirect immunofluorescence for reactivity with a human anti-actin antibody. In cryostat sections both human and rat astrocytomas showed an increased reaction with the anti-actin antibody compared to normal astrocytes, and the reaction with astrocytomas was greater than that with meningiomas. Malignant rat schwannomas also showed prominent anti-actin staining contrasting with the negative reaction in normal Schwann cells. These in vivo observations were paralleled by concurrent studies with impression films and in vitro monolayer cultures of tumor tissue. The results, reviewed in the light of previous studies of anti-actin antibody reactivity with other nonneural tumors, suggest that an enhanced actin expression in vivo may be a general feature of the neoplastic state and that this increased expression may be more pronounced in malignant than in benign tumors.

INTRODUCTION

Using indirect immunofluorescence and a specific human anti-actin antibody (18, 19), we have demonstrated an increased expression of cytoplasmic actin in chemically induced tumors originating from skin (21), glia (22), kidney (7, 20), and liver (17). Independent confirmation of this phenomenon has been provided by others for spontaneous human skin and breast tumors (5, 6). Furthermore, ultrastructural studies have shown that this increased actin expression corresponds to prominent bands of microfilaments in the same (5, 6, 20) or similar (11) tumor type. We (17, 20–22) and others (5, 6, 11) have suggested that the increased actin expression in the form of contractile microfilaments may provide a mechanism for increased tumor cell motility and that the latter may contribute toward tumor invasiveness.

In the present study we have examined a larger series of spontaneous human and chemically induced rat astrocytomas with a human anti-actin antibody (18, 19). The study was carried out to test our previous observation of enhanced actin expression in astrocytomas (22) and to compare malignant schwannomas with normal Schwann cells as well as malignant astrocytomas with benign meningiomas with regard to the expression of actin.

MATERIALS AND METHODS

Neural Tissues. Twenty-one human intracranial tumors were obtained from patients at craniotomy. These comprised 15 astrocytomas and 6 meningiomas. Histologically, the astrocytomas were classified (22) as: Grade I, 1; Grade II to III, 4; and Grade IV, 10. Meningiomas were classified (15) as: meningotheliomatous, 1; fibroblastic, 5.

Sixteen rat neural tumors were obtained from the offspring of pregnant rats treated with a single i.v. injection of ethylnitrosourea, 10 mg/kg (16). The tumors, excised 121 to 342 (mean, 202) days after birth, comprised 7 astrocytomas and 9 schwannomas.

For comparison with anti-actin antibody staining of neural tumors, normal rat brain with attached meninges and normal rat sciatic nerve were obtained from adult rats (200 to 250 g).

Fresh specimens of the above tissue were snap-frozen in isopentane-liquid nitrogen at −160°C and examined for reactivity with anti-actin antibody.

Tissue culture monolayers and impression films of neural tumors were also prepared by previously described methods (22) and examined for reactivity with anti-actin antibody.

Human Anti-actin Antibody. The anti-actin antibody obtained from a patient with active chronic hepatitis was characterized by reactivity with smooth muscle (8); skeletal muscle (10, 18); liver parenchymal cells (4); thymic medulla (3); bursa medulla (1); renal glomeruli, brush border, and peritubular fibrils of renal tubules (20, 23); gastric parietal cells (2); and central nervous system synaptic endings (19). The strongest reactivity was obtained with smooth muscle, in which a titer of 256 was obtained.

Immunohistology. Cryostat sections (6 μm), impression films, and tissue culture monolayers were examined by sandwich immunofluorescence tests (13) with anti-actin antibody at a dilution of 1:8. The conjugate for immunofluorescent tracing of bound human immunoglobulin was a fluorescein isothiocyanate-labeled goat anti-human-γ-globulin with a fluorescein:protein molar ratio of 4.0 and a protein content of 0.8 g/100 ml. Before use, the conjugate was appropriately absorbed (22) so that by itself it gave no...
staining reaction on test sections, impression films, or monolayer cultures. After immunofluorescent staining, the microscopic preparations were examined by dark-ground UV fluorescent microscopy with a condenser fitted with a toric lens and a colorless barrier filter.

Specificity of the tests was established by failure to obtain staining with normal control serum or anti-actin antibody serum neutralized by smooth (17, 20) or skeletal tumor cells showed prominent diffuse staining of the cytoplasmic staining of the cytoplasmic staining mainly in the cell periphery and nucleoli (Fig. 1). Necrotic tissue did not stain. The enhanced reaction of tumor tissue stood in marked contrast to the weaker reaction of the adjacent normal brain tissue (Fig. 2). Astrocytomas belonging to differing grades of malignancy gave similar staining reactions. Impression films showed that the staining reaction of tumor cells was clearly located in the cytoplasm and processes of tumor astrocytes (Fig. 3). Likewise, monolayer cultures of tumor cells showed prominent diffuse staining of the cytoplasm and processes of multipolar cells (Fig. 4).

**Astrocytomas.** Human and ethylnitrosourea-induced rat astrocytomas gave similar staining reactions with anti-actin antibody. Cryostat sections of these tumors showed cytoplasmic staining mainly in the cell periphery and nucleoli (Fig. 1). Necrotic tissue did not stain. The enhanced reaction of tumor tissue stood in marked contrast to the weaker reaction of the adjacent normal brain tissue (Fig. 2). Astrocytomas belonging to differing grades of malignancy gave similar staining reactions. Impression films showed that the staining reaction of tumor cells was clearly located in the cytoplasm and processes of tumor astrocytes (Fig. 3). Likewise, monolayer cultures of tumor cells showed prominent diffuse staining of the cytoplasm and processes of multipolar cells (Fig. 4).

**Human Meningiomas.** Four meningiomas gave negative reactions of tumor cells with anti-actin antibody; prominent staining of tumor blood vessels stood in striking contrast to the negatively stained tumor tissue (Fig. 5). Two fibroblastic meningiomas gave staining of tumor cell processes (Fig. 6). Impression films and tissue culture monolayers (Fig. 7) gave mainly weak to negative reactions; where staining was present it was restricted to the perinuclear area and to the processes of occasional tumor cells. Normal meninges did not stain.

**Ethylnitrosourea-Induced Rat Schwannomas.** Cryostat sections of rat schwannomas reacted with anti-actin antibody, giving a staining pattern similar to that obtained with the astrocytomas, i.e., cytoplasmatic fluorescence, particularly in the region of the cell periphery (Fig. 8). Monolayer cultures and impression films (Fig. 7) showed that the antibody reacted with the cell body and processes of tumor cells. Normal Schwann cells in sections of sciatic nerve (Fig. 10) did not stain; staining in this nerve was restricted to the perineurium and the vasa nervorum (Fig. 10).

**Serum Titrations.** Titers of the anti-actin antibody against tumor tissue gave titers ranging between 64 and 256 for astrocytomas and schwannomas. In contrast, titers for the 2 positively staining fibroblastic meningiomas were 8 and 16, emphasizing the stronger reactivity of astrocytomas and schwannomas over meningiomas.

**DISCUSSION**

In a previous study, we have demonstrated that the cytoplasm of cells in human and ethylnitrosourea-induced rat astrocytomas showed increased binding to a human anti-actin antibody compared to the corresponding normal tissue (22). The present report confirms this observation and extends the results to show that enhanced anti-actin antibody reactivity of tumor over normal cells is also seen with ethylnitrosourea-induced rat schwannomas. However, in contrast to astrocytomas, human meningiomas gave mainly a negative or weak reaction in frozen sections of tissues in vivo as well as in monolayer cultures of tumor cells in vitro. This lesser reactivity of benign compared to malignant tumors has also been observed previously in skin tumors (21).

The results of the present study and those of previous studies on the reactivity of anti-actin antibody with chemically induced and/or spontaneous skin (5, 6, 21), kidney (7, 20), liver (17), and breast (5, 6) tumors suggest that an increased expression of cytoplasmic actin may be a general feature of all tumors. Further, these studies suggest that the enhanced actin expression may be associated with the growth potential of the tumor because it is more pronounced in malignant than in benign tumors and in poorly differentiated rather than well-differentiated tumor cells (17, 21). Ultrastructural studies in kidney (20) and skin (5, 6, 11) tumors have shown that the increased actin expression correlates well with the presence of prominent sheath microfilaments. Since the total content of actin in tumor cells seems to be no different from that in normal cells (24), the observations suggest that neoplastic transformation may be associated with the enhanced formation of actin-like microfilaments from a nonfilamentous precursor in the cytoplasm.

We (20) and McNutt (11) have pointed out that the results of the preceding studies are in direct conflict with those of others on the expression of actin in in vitro cultures of viral-transformed cells (11, 14, 24). It is of particular interest that McNutt, who initially documented a decrease in microfilaments in in vitro cultures of SV40-transformed BALB/c 3T3 cells (11), has recently reported an increase in microfilaments in in vivo studies of human basal cell carcinomas (12). These divergent reports stress the need to carry out concurrent in vivo as well as in vitro studies of actin in tumor cells, especially since all the previous reports of decreased actin in viral-transformed cells (11, 14, 24) have been made in in vitro studies of monolayer cultures of these cells.

**ACKNOWLEDGMENTS**

We thank Dr. C. R. Lucas of the Fairfield Infectious Diseases Hospital for the human anti-actin antibody serum.

**REFERENCES**


Fig. 1. Cryostat section of human astrocytoma reacted with anti-actin antibody, showing cytoplasmic staining of tumor cells mainly localized to the cell periphery. The nucleoli of some tumor cells are also stained. × 200.

Fig. 2. Cryostat section of ethylnitrosourea-induced rat astrocytoma reacted with anti-actin antibody, showing peripheral cytoplasmic staining. The adjacent normal brain tissue shows much weaker staining. × 320.

Fig. 3. Impression film of human astrocytoma reacted with anti-actin antibody, showing staining of the tumor cell cytoplasm and process. × 320.

Fig. 4. Monolayer culture of human astrocytoma reacted with anti-actin antibody, showing staining of the cytoplasm and processes of a multipolar tumor cell. × 320.

Fig. 5. Cryostat section of human meningotheliomatous meningioma reacted with anti-actin antibody, showing mainly negative staining of tumor cells. Blood vessels in the body of the tumor are intensely fluorescent. × 200.

Fig. 6. Cryostat section of human fibroblastic meningioma reacted with anti-actin antibody, showing staining of tumor cell processes. × 200.

Fig. 7. Monolayer culture of human fibroblastic meningioma reacted with anti-actin antibody, showing staining of tumor cell cytoplasm and processes. × 200.

Fig. 8. Cryostat section of ethylnitrosourea-induced rat schwannoma reacted with human anti-actin antibody, showing cytoplasmic staining of tumor cells. × 320.

Fig. 9. Impression film of ethylnitrosourea-induced rat schwannoma reacted with human anti-actin antibody, showing staining of the cytoplasm and long processes of tumor cells. × 320.

Fig. 10. Cryostat section of normal rat sciatic nerve reacted with human anti-actin antibody, showing staining of the vasa nervorum. Normal Schwann cells do not stain. × 200.
Increased Expression of Actin-like Protein in Human and Ethynitrosourea-induced Tumors of the Nervous System


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/12/4280

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.