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S-100 antigen labels neoplastic cells in liposarcoma and cartilaginous tumours

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Summary. S-100 antigen, originally believed to be unique to the nervous system, has recently been found in cell types of non-neuroectodermal origin such as chondrocytes and adipocytes. These findings suggested the possibility of detecting the antigen in tumours derived from such cells. Using the PAP method and an anti-ox brain S-100, the antigen was found in the cells of human chondrosarcomas, chondroblastomas and liposarcomas. In contrast, fibrous histiocytomas and fibrosarcomas, tested to verify the cellular specificity of the S-100 immunoreaction, did not exhibit S-100-containing cell types. The present data indicate the usefulness of the S-100 antigen as a diagnostic and investigative tool in defined neoplasms of non-neuroectodermal origin, such as chondroid tumours and liposarcoma.

Key words: S-100 antigen – PAP method – Liposarcoma – Cartilaginous tumours

The S-100 antigen, originally isolated from the brain (Moore 1965), is currently regarded as a heterogeneous group of proteins with similar antigenic sites, conserving a close immunological relationship between different species (for review, see Zomzely-Neurath and Walker 1980). The biological role of S-100 is still unknown, although its common evolution with the family of Ca^{2+}-binding proteins (Isobe et al. 1982), such as calmodulin, troponin C and intestinal calcium binding protein, suggests that it might be involved in Ca^{2+}-mediated processes. In the nervous system it is found primarily in the cytoplasm and nucleus of glial cells (for review, see Zomzely-Neurath and Walker 1980) and in this respect it has been used as a diagnostic tool in neurological diseases (Michetti et al. 1979; Jacque et al. 1979; Michetti et al. 1980). The detection of S-100 in melanocytes (Cocchia et al. 1981) opened the possibility of labelling melanotic and amelanotic malignant

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melanoma tissue (Cocchia et al. 1981; Cochran et al. 1982; Nakajima et al. 1982; Springall et al. 1983). Recently, S-100 has been found in normal cell types of non-neuroectodermal origin including chondrocytes and adipocytes (Stefansson et al. 1982; Michetti et al. 1983). This finding offers the possibility of detecting the antigen in tumours derived from such cells. The present study provides data indicating the presence of S-100 in neoplastic cells of such non-neural tumours.

Materials and methods

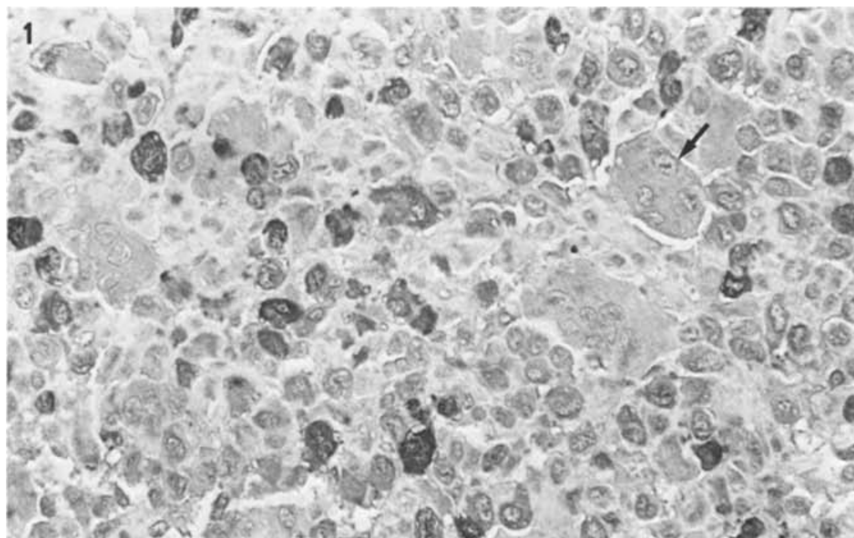
Preparation of S-100 and antisera. The S-100 protein was prepared from ox brain according to the procedure of Moore (1965). The purity was checked by immunodiffusion and disc electrophoresis by the methods of Ornstein (1964) and Davis (1964). In addition, the fast moving band of an unstained gel was recognized as S-100 as described elsewhere (Donato and Michetti 1974). A specific antiserum to S-100 protein from ox brain was obtained from rabbit and characterized according to Zuckerman et al. (1970). The serological specificity of the antiserum was shown by double diffusion and by quantitative microcomplement fixation assay employing soluble protein extracts of a variety of tissues as antigens. Sheep antiserum to rabbit IgG was produced by intramuscular injection of a total of 600 mg IgG, as described by Sternberger et al. (1970). Chromatographically pure immunoglobulins from rabbit serum and from sheep antiserum against rabbit IgG were prepared as described by Harboe and Ingild (1973) and their dilution was referred to the starting volume of the respective serum. For control experiments, preimmune rabbit serum or anti-S-100 antiserum absorbed as described below with purified ox S-100 was employed. Anti-S-100 antiserum was incubated for 1 h at 37° C with S-100 antigen (10 µg/ml) and the antigen-antibody reaction was continued overnight at 4° C. The procedure was repeated once with an additional treatment of 10 µg/ml of antigen. The completeness of the reaction between antigen and antibody was evaluated by quantitative precipitin determination and by immunodiffusion (Harboe and Ingild 1973).

Immunohistochemical procedure. Tissues from human chondroblastoma (3 cases), chondrosarcoma (4 cases), liposarcoma (2 well differentiated, 2 myxoid, 1 fibroblastic, 1 pleomorphic), fibrous histiocytoma (2 cases) and fibrosarcoma (2 cases), obtained at biopsy, were fixed in 10% formalin buffered with 0.1 M phosphate pH 7.4 for 24 h, washed in phosphate buffer and embedded in paraffin. Three µm sections were deparaffinized in xylol, treated with 0.3% hydrogen peroxide in methanol for inhibition of endogenous peroxidase activity and finally processed for the immunoperoxidase reaction using unlabelled antibody PAP method. Dilutions of the reagents in 0.1 M phosphate buffered saline (PBS), pH 7.4, and duration of reactions were as follows: anti-ox brain S-100 (1:1,000) or preimmune rabbit serum (1:1,000) or anti-ox brain S-100 absorbed with S-100 antigen (1:1,000), overnight; sheep anti-rabbit IgG (1:50) for 1 h; PAP complex (Dako-immunoglobulins a/s Copenhagen, Denmark) (1:100), for 1 h. Peroxidase reaction was developed with freshly made 0.03% 3-3′diaminobenzidine 4 HCl (Sigma Chemical Co., St. Louis, MO, USA) solution in 0.05 M Tris-HCl buffer, pH 7.4,

Fig. 1. Cellular area of human chondroblastoma treated with anti anti-ox brain S-100. HaE counterstaining. Some neoplastic cells appear to be stained. Multinucleated giant cells (*arrows*) are devoid of reaction product. ×250

Fig. 2. Chondroid area of human chondroblastoma treated with anti-ox brain S-100 antiserum. HaE counterstaining. Immunostaining is confined to neoplastic cells scattered in the homogeneous intercellular matrix. ×250

Fig. 3. Human chondrosarcoma treated with anti-ox brain S-100. HaE counterstaining. All neoplastic cells appear to be intensely stained. ×250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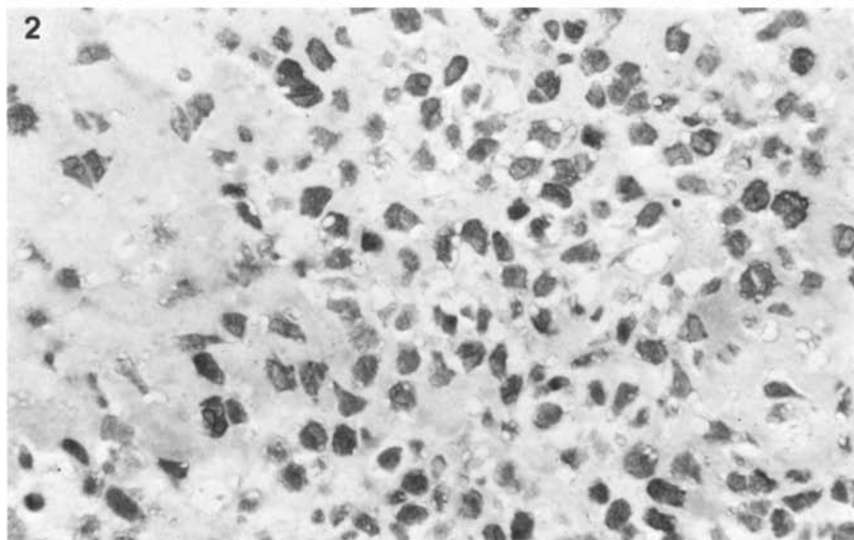
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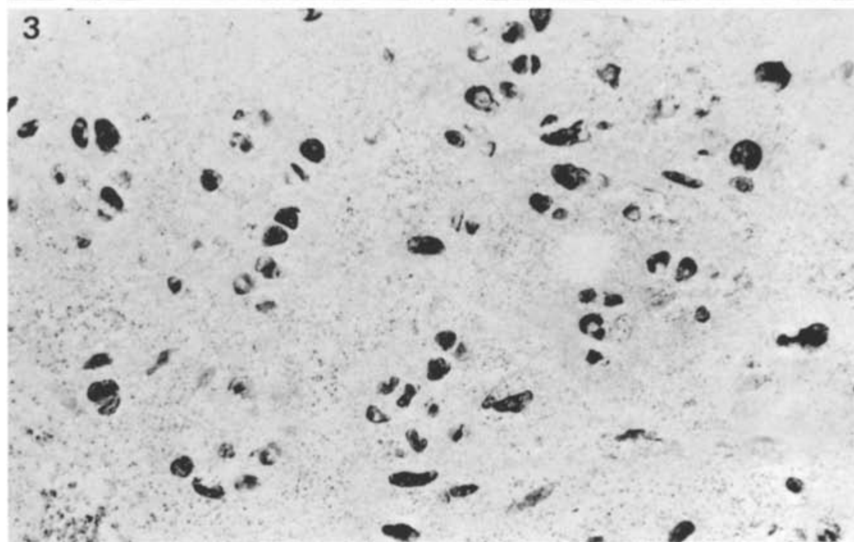
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containing 0.05% hydrogen peroxide. At the end of each step the sections were washed 3 times in PBS over a period of 30 min. Some sections were lightly counterstained with haematoxylin for tissue orientation.

Results

In cellular areas of all chondroblastomas treated with anti-ox brain S-100 antiserum various degrees of immunostaining were observed in the cytoplasm of cells with polygonal or rounded outlines, considered to be chondroblasts (Levine and Bensch 1972), while no reaction product was present in multinucleated cells (Fig. 1). In cartilaginous foci, on the other hand, a uniformly darker reactivity appeared in the cytoplasm of all cells (Fig. 2). Likewise, in all cases of chondrosarcoma examined, all chondrocytes were heavily stained in the cytoplasm, irrespective of their degree of atypia (Fig. 3). By scoring sections, also not counterstained with haematoxylin, a conclusive assessment on the presence of S-100 in nuclei of neoplastic cells of chondrosarcoma and chondroblastoma could not be obtained. Control sections of chondroblastoma and chondrosarcoma, treated with preimmune normal rabbit serum or with anti-S-100 antiserum absorbed with the antigen, exhibited no reaction (Fig. 4).

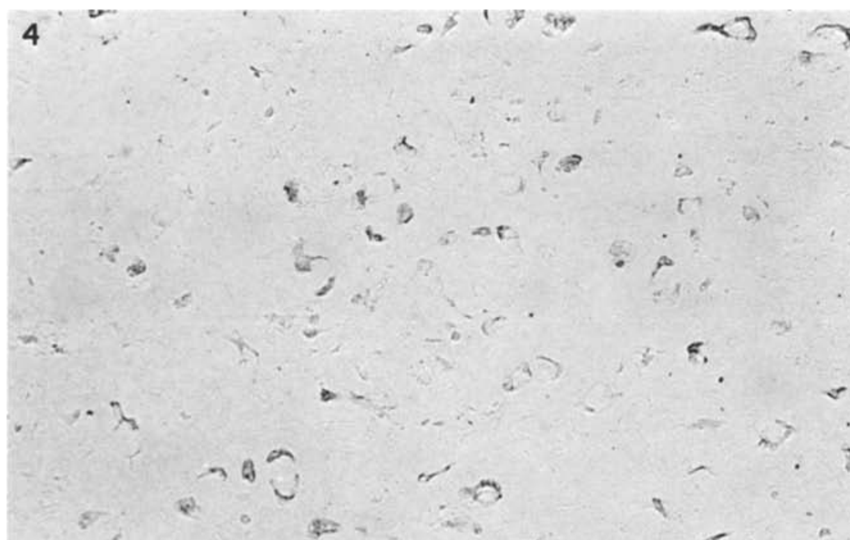
When different liposarcomas were treated with the anti-S-100 antiserum, immunoreactivity was detected in all cases and all neoplastic cell types appeared to be stained, irrespective of their degree of differentiation (Fig. 5). In well differentiated liposarcomas reaction product was confined to the thin cytoplasmic rim of fat cells and to nuclei, which were sometimes clearly atypical. Nuclear and cytoplasmic staining was observed in the multivacuolated lipoblastic cells of myxoid liposarcomas as well as in spindle-shaped cells of the one case with a prominent fibroblastic pattern. In the pleomorphic liposarcoma case, both the bizzarre giant cells as well as lipoblastic cells showed cytoplasmic and nuclear reaction product. Control sections of liposarcoma appeared to be unstained (Fig. 6).

In order to verify the cellular specificity of the S-100 immunoreaction, fibrous histiocytomas and fibrosarcomas, cells also derived from mesenchyme, but known to be devoid of S-100 (Cocchia et al. 1981) were treated

Fig. 4. Control section of human chondrosarcoma treated with preimmune rabbit serum. HaE counterstaining. No reaction is detectable. ×250

Fig. 5. Human liposarcoma treated with anti-ox brain S-100 antiserum. HaE counterstaining. Both vacuolated (*arrow*) and non-vacuolated (*double arrow*) neoplastic cells appear to contain S-100. ×250

Fig. 6. Control section of human liposarcoma treated with preimmune rabbit serum. HaE counterstaining. No reaction is detectable. ×250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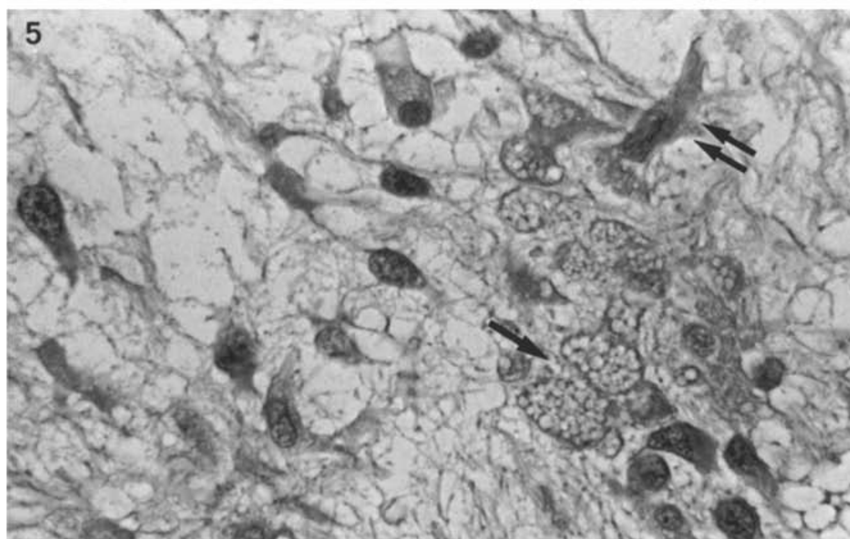
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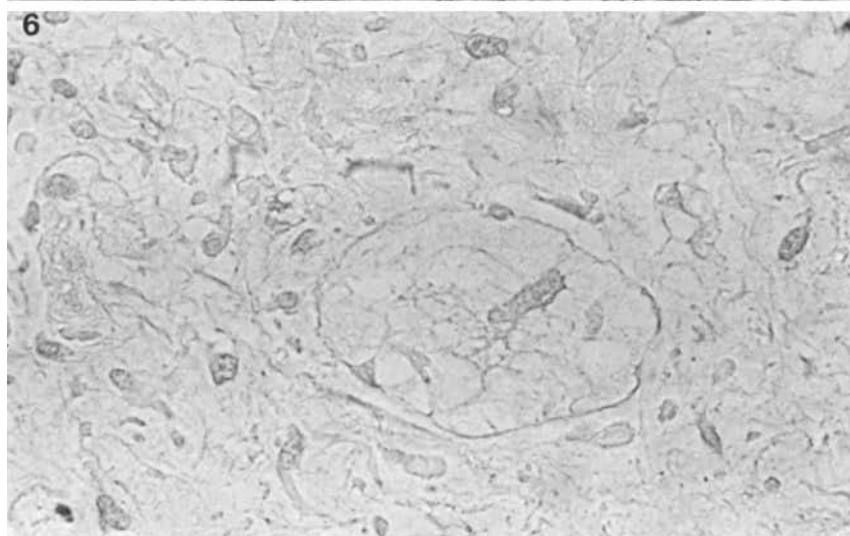
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with the same anti-S-100 antiserum. No reaction was present, as expected (not shown).

Discussion

The present study extends the observations of S-100 antigen-containing tumours beyond neuroectodermal tissues. The usefulness of S-100 protein as a marker in the diagnosis of tumours by immunohistochemistry is based on the presence of this antigen in a restricted number of definite cell types in different tumours (Nakazato et al. 1982; Stefansson et al. 1982; Kahn et al. 1983; Kindblom et al. 1983).

As is well established in neuroectodermal-derived tumours (Jacque et al. 1979; Springall et al. 1983), S-100 appears to be expressed by neoplastic cell types corresponding to normal S-100-containing cells (Stefansson et al. 1982; Michetti et al. 1983) also in liposarcomas, chondroblastomas and chondrosarcomas. In this respect, the present data confirm the well known tissue origin of the neoplasms under examination.

In tumours of cartilaginous origin the immunostaining appeared to be heavier in chrondrocytes than in chondroblasts, although more detailed studies will be needed in order to define any possible relationship between the expression of the antigen and the degree of cell maturity. Furthermore, our present data do not allow any suggestion concerning relationships of S-100 expression with the degree of cell atypia. On the same way our observations on liposarcomas do not suggest any correlation between the amount of S-100 and the degree of malignancy. Nevertheless, the absence of S-100 from other tumours of mesenchymal origin, such as fibrous histiocytomas or fibrosarcomas, indicates that S-100 is an investigative and diagnostic tool both for liposarcoma and cartilaginous tumours. In particular, a differential diagnosis between pleomorphic liposarcoma and malignant fibrous histiocytoma, which sometimes can be confused, could be reached by S-100 immunostaining.

The S-100 antigen detected in normal rat adipose tissue has been shown to be immunologically identical to rat brain S-100 (Michetti et al. 1983), while information is lacking concerning the degree of immunochemical relationship between S-100 in cartilage and nervous tissue. Keeping in mind that S-100 probably constists of a family of acidic proteins with similar antigenic sites but with structural differences (for review, see Zomzely-Neurath and Walker 1980), it would be interesting to investigate whether the molecule(s) responsible for the S-100 immunostaining for each neural and extra-neural neoplasm corresponds to one or more of the S-100 molecules present in the normal tissues, or is a different molecule bearing the S-100 antigenic sites.

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