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Granular cell myoblastoma: positive staining for carcinoembryonic antigen

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SUMMARY An immunoperoxidase technique for the detection of carcinoembryonic antigen was applied to 10 cases of granular cell myoblastoma. Consistent, strong, intracytoplasmic granular staining, which can be easily interpreted, was obtained in all cases. Schwannomas, neurofibromas, dermatofibromas, and leiomyomas were negative. The test is helpful in confirming doubtful cases. The results tend to support the suggestion that granular cell myoblastoma is derived from perineural rather than endoneural cells.

In a recent study of carcinoembryonic antigen (CEA) in benign and malignant breast lesions using the immunoperoxidase technique, a case of granular cell myoblastoma included in the series stained very strongly for CEA (Shousha and Lyssiotis, 1978). This prompted us to review and stain other granular cell myoblastomas from various sites in an attempt to investigate the consistency of the results and the possibility of using the test in the diagnosis, and explaining the histogenesis, of these lesions.

Cases and methods

Ten cases diagnosed as granular cell myoblastoma were found in the files of the Histopathology Departments of the Charing Cross and Royal Free Hospitals during the period 1965 to 1976. The diagnosis was confirmed by the examination of sections stained with haematoxylin and eosin, periodic acid-Schiff, reticulin and phosphotungstic acid. Sections 5 microns thick were cut from stored paraffin-embedded blocks of tissue. The sections were then deparaffinised in xylene and stained for CEA using the peroxidase-labelled antibody sandwich technique, as described in detail elsewhere (Shousha and Lyssiotis, 1978). Briefly, endogenous peroxidase was blocked with 30% hydrogen peroxide in methanol, and nonspecific background staining was reduced by 1:5 normal swine serum. The sections were then treated consecutively with 1:50 rabbit anti-CEA serum, normal swine serum, and peroxidase-labelled swine anti-rabbit IgG. Thorough washing with Tris-saline buffer, pH 7·6, was carried out between the various steps. The end product was stained with 3, 3′ diaminobenzidine tetrahydrochloride. The sections were then counterstained with celestine blue and mounted in Diatex. Some cases were pretreated with periodic acid before the specific sera were applied (Isaacson and Judd, 1977). All the sera were obtained from Dakopatts A/C (Denmark).

The controls used included: (1) sections of CEA-positive colonic carcinoma treated similarly; (2) sections from two cases each of Schwannoma, neurofibroma, dermatofibroma, and leiomyoma stained in the same way; (3) sections of colonic carcinoma and granular cell myoblastoma subjected to the same procedure except for replacement of the rabbit anti-CEA serum by normal swine serum; and (4) sections of colonic carcinoma and granular cell myoblastoma treated in the same way after absorp-

Table *Sex, age, and site of 10 granular cell myoblastomas*

Case	*Sex/Age*	*Site*	*Year of diagnosis*
1	F 66	Skin (axillary fold)	1965
2	F 53	Tongue	1966
3	F 48	Tongue	1967
4	F 52	Skin (left ring finger)	1972
5	F 29	Skin (abdomen)	1973
6	F 35	Breast	1975
7	F 53	Skin (suprasternal fossa)	1976
8	F 41	Skin (thigh)	1976
9	F 35	Tongue	1976
10	M 19	Skin (forehead)	1976

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tion of the rabbit anti-CEA serum with a 1:50 solution of CEA in 0·05 M sodium phosphate buffer, pH 7·5. CEA-2B (Code R42) was kindly supplied by Dr G. T. Rogers of the Medical Oncology Department, Charing Cross Hospital. For method of extraction and characterisation see Rogers *et al.* (1976).

Results

CONTROLS

Sections of colonic carcinoma stained strongly positive for CEA. Negative results were obtained when the anti-CEA serum was replaced by normal swine serum, and the reaction was markedly reduced to a very faint staining when the anti-CEA serum was absorbed by CEA before application to sections of colonic carcinoma and granular cell myoblastoma. No difference in the amount of stain deposit or the intensity of its colour was noticed between slides pretreated with periodic acid and those not so treated. Sections of Schwannomas, neurofibromas, dermatofibromas, and leiomyomas were all negative. Striated muscle, smooth muscle, nerve bundles, and fibroblasts were also negative.

GRANULAR CELL MYOBLASTOMA

Nine of the 10 patients with granular cell myoblastoma were women (Table). Their ages ranged from 29 to 66 years. The only male patient was 19 years old. Six of the tumours were from the skin, three from the tongue, and one from the breast (Table).

All the cells examined from the 10 tumours stained intensely for CEA. The staining was in the form of fine, dark brown, densely packed cytoplasmic granules (Figs 1 and 2). Because every tumour cell was stained, in contrast to all other types of cells present in sections, the limits of the tumour were clearly drawn, and groups of tumour cells outside the main tumour mass were easily detected (Fig. 3). Cases that were difficult to identify on haematoxylin and eosin stained sections stood out clearly after staining for CEA (Fig. 4). Whenever nerves were present, tumour cells were closely surrounding them or their sheaths (Fig. 5). Tumour cells were sometimes seen within nerve sheaths (Fig. 5).

Discussion

Our results show that the immunoperoxidase tech-

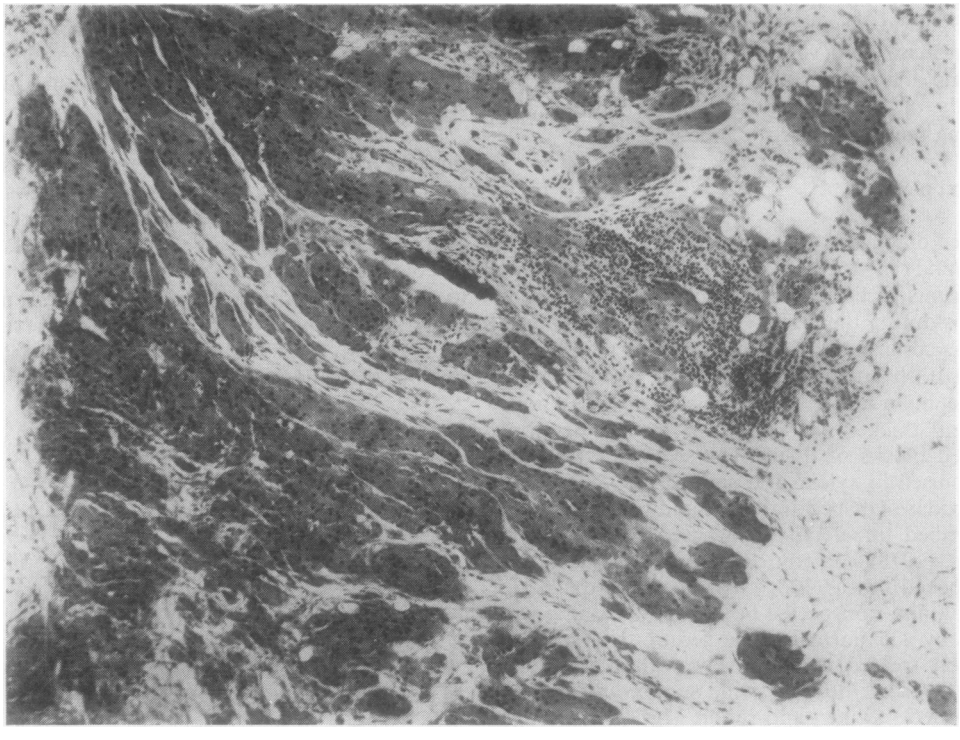

Fig. 1 *Granular cell myoblastoma, breast (case 6). CEA positively stained tumour cells extending into fat. Immunoperoxidase × 40.*

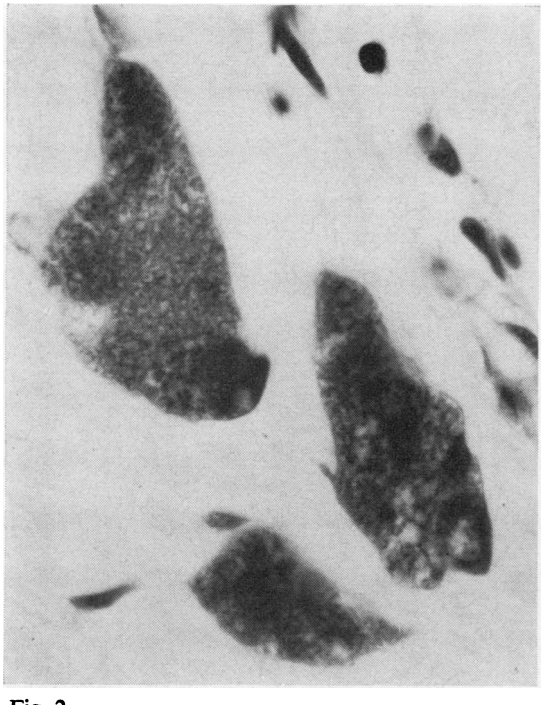

Fig. 2

Fig. 2 *Granular cell myoblastoma, axillary fold (case 1). An oil immersion, high-power view of three tumour cells packed with darkly stained, CEA-positive cytoplasmic granules. Immunoperoxidase* × *735.*

Fig. 3 *Granular cell myoblastoma, suprasternal fossa (case 7). Small groups of tumour cells seen in fibrous tissue outside main tumour mass. Immunoperoxidase* × *63.*

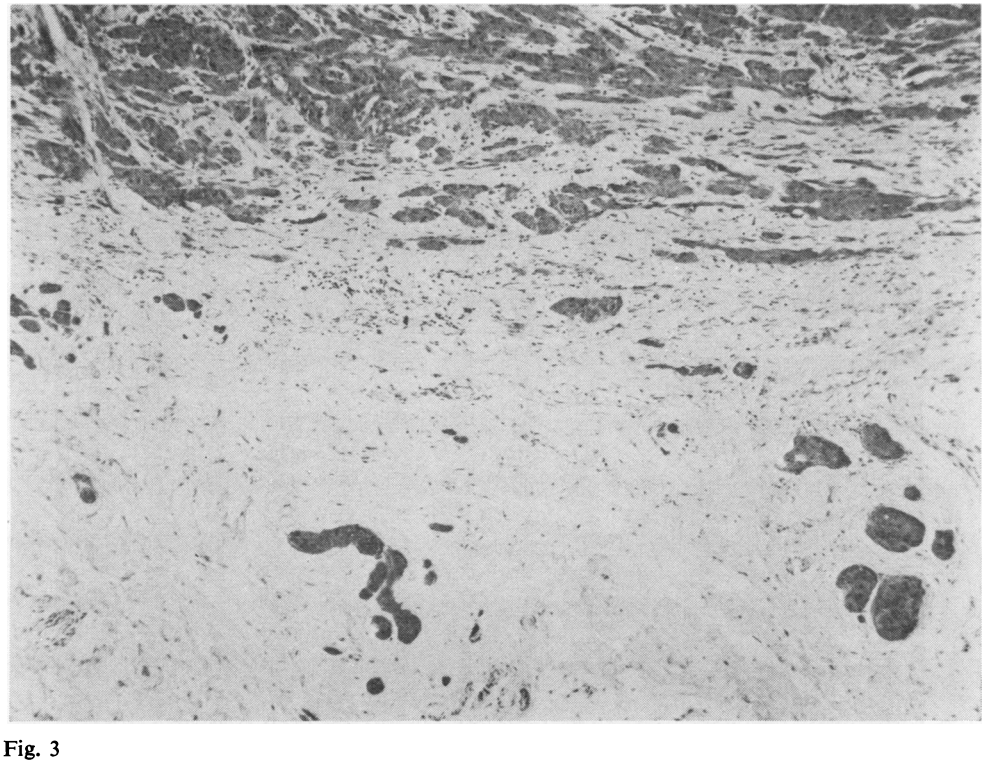

Fig. 3

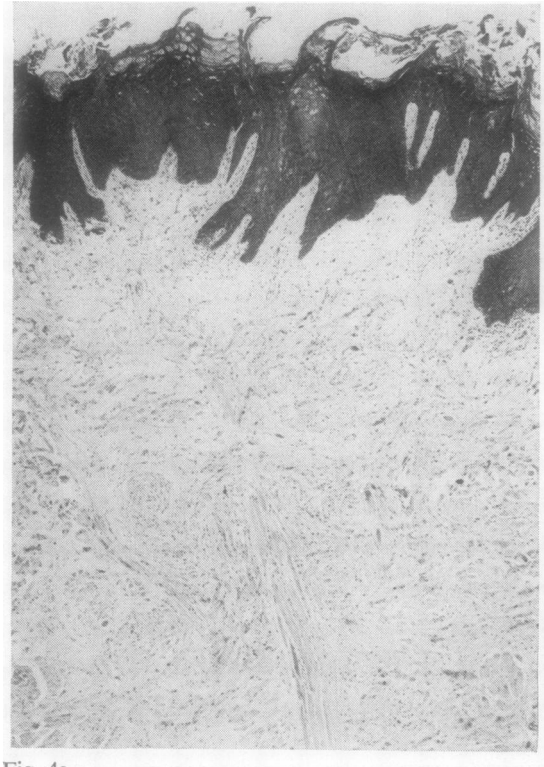

Fig. 4a

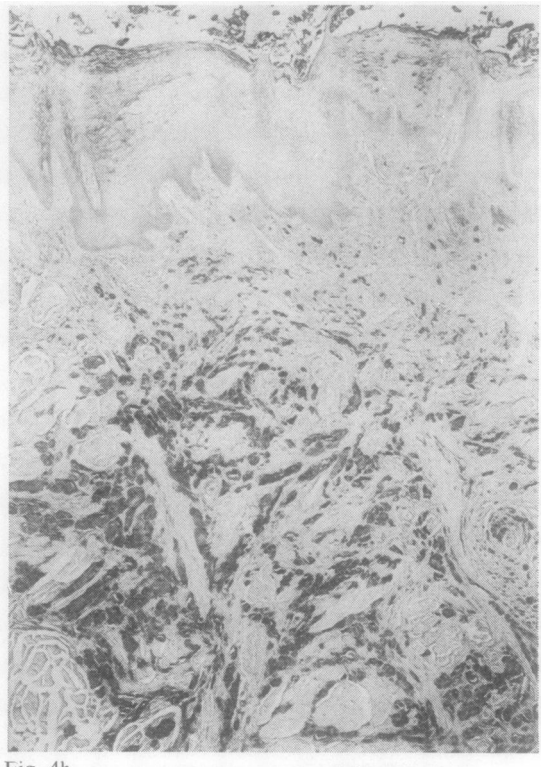

Fig. 4b

Fig. 4 *Granular cell myoblastoma, tongue (case 2). (A) Haematoxylin and eosin × 30. (B) Immunoperoxidase × 30. Note how tumour cells stand out clearly after staining for CEA.*

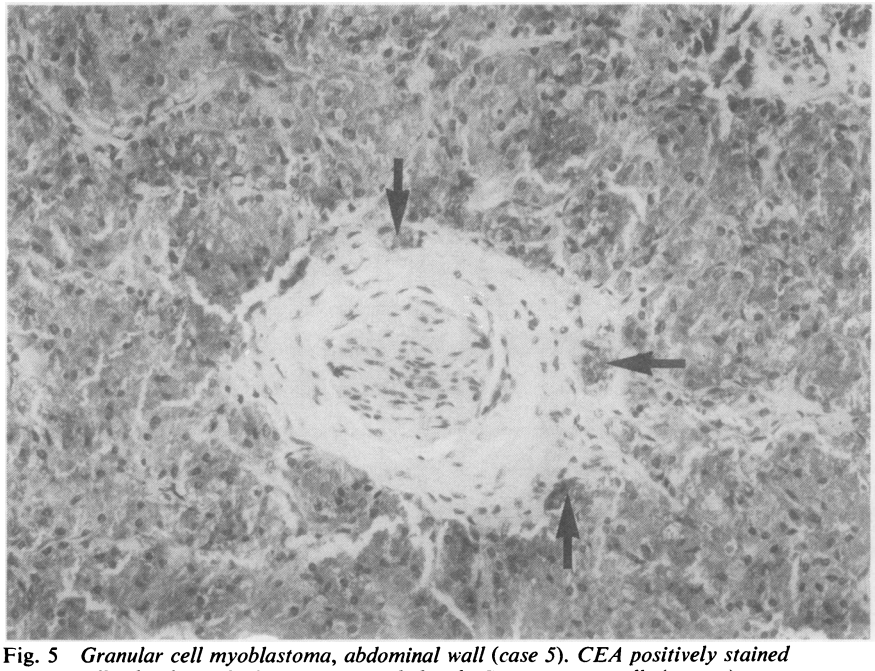

Fig. 5 *Granular cell myoblastoma, abdominal wall (case 5). CEA positively stained tumour cells closely applied to a perineural sheath. Some tumour cells (arrows) appear within the outer layer of the sheath. Immunoperoxidase × 170.*

nique for staining CEA can be used for confirming the diagnosis of granular cell myoblastoma and for differentiating it from morphologically similar tumours. It can also be used to identify doubtful cases, which may appear as degenerated muscle or frayed bundles of fibrous tissue (Fig. 4), and to define clearly the extent of excision.

The various controls used indicated the relative high specificity of the anti-CEA serum used, and it seems unlikely that the staining obtained is the result of a cross reaction between the serum and granular cell components. However, biochemical assays of tumour extracts are required to confirm these results. Serum CEA levels of patients with these tumours may not show a significant increase above the accepted normal levels as the majority of these tumours are benign, non-progressive lesions that do not invade blood vessels or lymphatics (Lo Gerfo *et al.*, 1971; Ashley, 1978).

It is difficult to explain why granular cell myoblastoma, a benign lesion, should give such a strongly positive immunohistochemical reaction for CEA. High CEA cellular content so far has been demonstrated histologically only in epithelial cells, mostly malignant (Goldenberg *et al.*, 1976). Granular cell myoblastoma is not an epithelial tumour, and the cases included in this study were not malignant. However, the production of appreciable amounts of CEA, and other tumour-associated antigens and factors, by adult, tumour cells is thought to be the result of reactivation of specific genes known to be active during embryonic and fetal life (Coggin and Anderson, 1974). Thus, the presence of CEA in granular cell myoblastoma may be related to the embryonic nature of the tumour cells (Ashley, 1978). This nature, which is implied in the suffix 'blastoma' attached to the name of the tumour, was recently supported by ultrastructural and histochemical findings, suggesting that these cells are of an undifferentiated mesenchymal type (Aparicio and Lumsden, 1969; Sobel *et al.*, 1973).

Sobel *et al.* (1973) also believe that this cell of origin is the precursor of Schwann cells. This explains the constantly observed close association of these tumours with nerves. However, there are authors who believe that granular cell myoblastoma is specifically derived from Schwann cells (Fisher and Wechsler, 1962; Garancis *et al.*, 1970), and others who suggested perineural, rather than endoneural, cells of origin. These included perineural fibroblasts (Pearse, 1950) and histiocytes (Azzopardi, 1956). Smooth muscle fibres were proposed as the origin of tumours arising in the gastrointestinal tract (Churg and Work, 1959) and urinary bladder (Christ and Ozzello, 1971), and pituicytes for tumours arising in the neurohypophysis (Burston *et al.*, 1962).

The presence, in our study, of CEA positively stained cells within the perineural sheath (Fig. 5), the absence of any similar cells among the endoneural structures, and the negativity of Schwann cells and endoneural fibres, together with the tumours thought to be derived from them, namely, Schwannoma and neurofibroma, lend some support to the suggestion that granular cell myoblastoma is derived from primitive perineural rather than endoneural cells.

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