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Carcinoembryonic antigen like antigen in granular cell myoblastomas

An immunohistochemical study

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Summary. A series of granular cell myoblastomas (GCM) and other benign and malignant tumours of soft tissue were examined for cytoplasmic content of carcinoembryonic antigen (CEA) by the two-layer conjugated immunoperoxidase technique. Using a commercial rabbit anti-CEA serum only granular cell myoblastomas showed positive cytoplasmic reaction. Pretreatment with periodic acid made this reaction less intense, but when the commercial rabbit anti-CEA serum was absorbed with tissue powder from normal human spleen the positive reaction was totally abolished. It is concluded that the positivity of GCM for CEA using commercial rabbit anti-CEA serum is due to the content of non-specific cross-reacting antigen (NCA) and maybe other cross-reacting glycoproteins in this tumour, and not to CEA as claimed in a previous study.

Key words: Carcinoembryonic antigen (CEA) – Granular cell myoblastoma – Immunohistochemistry

Introduction

In a previous study of carcinoembryonic antigen (CEA) in granular cell myoblastomas (GCM) and other benign soft tissue tumours using immunoperoxidase technique only GCM showed positive reaction (Shousha and Lyssiotis 1979). The use of commercial antibodies in immunohistochemical methods, however, may require absorption procedures to avoid false positive results due to cross-reacting substances. As a cytoplasmic content of CEA is an unexpected finding in GCM in regard to the postulated origin of this tumour, we have investigated a series of GCM and other benign and malignant soft part tumours for cytoplasmic CEA.

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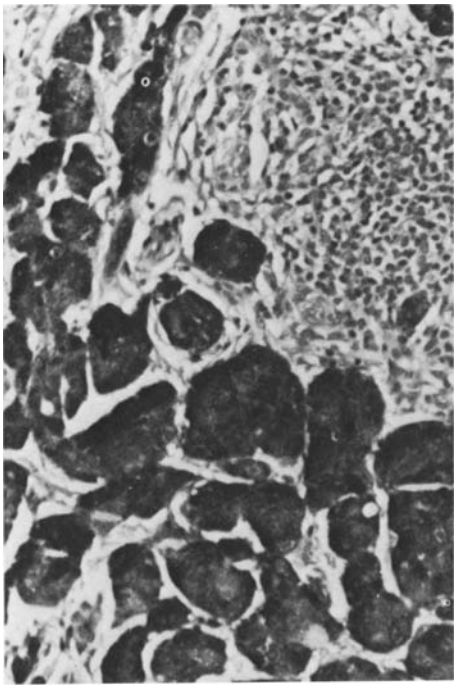

Fig. 1. Granular cell myoblastoma. Strong cytoplasmic positivity of tumour cells surrounding a lymphatic nodule. (Immunoperoxidase using commercial rabbit anti-CEA serum. Haematoxylin counterstaining × 400)

Materials and methods

The material consists of 13 consecutive cases of granular cell myoblastomas from the files of the department of pathology, Aalborg and Holstebro Hospitals. From all cases paraffin blocks were available.

In addition, six cases of dermatofibrosarcoma protuberans, six cases of dermatofibroma, seven cases of neurofibroma and six cases of malignant fibrous histiocytoma from the files of department of pathology, Aalborg Hospital were also examined.

From each case deparaffinised sections were investigated by the two-layer conjugated immunoperoxidase method. The method consists of the following steps.

1) Endogenous peroxidase is blocked with three per cent H_2O_2 in methanol.

2) Sections were incubated for 30 min with 1:50 rabbit anti-CEA serum containing 20% normal swine serum to reduce background staining. The working dilution of 1:50 for the rabbit anti-CEA serum was chosen after titration.

3) Incubation with peroxidase labelled swine anti-rabbit IgG.

4) Peroxidase activity was developed with 3,3′diaminobenzidine tetrahydrochloride.

Thorough washing in phosphate buffered saline pH 7.4 (PBS) was carried out between each step. The sections were counterstained with haematoxylin. All sera were obtained from DAKO, Copenhagen, Denmark.

As the rabbit anti-CEA serum from DAKO cross-reacts with non-specific cross-reacting antigen (NCA) sections from all GCM were also examined by replacement of rabbit anti-CEA serum with rabbit anti-CEA serum absorbed with tissue powder from normal human spleen. The tissue powder was prepared by the method described by Nairn (1969).

All GCM were also examined by the substitution of one per cent periodic acid for hydrogen peroxide in the staining procedure. Controls included sections treated with normal rabbit serum instead of rabbit anti-CEA serum in the first step of the staining procedure and sections of CEA-positive colonic adenocarcinoma.

The absorbed rabbit anti-CEA serum was tested by its ability to abolish staining of granulocytes and monocytes both containing NCA and therefore stained by the commercial rabbit anti-CEA serum. Sections from normal human spleen pretreated with periodic acid served as control of the negative staining of granulocytes and monocytes after incubation with unabsorbed anti-CEA serum. A section from each GCM was treated by step one and four to control blocking of endogenous peroxidase.

Results

Sections from colonic adenocarcinomas stained intensely for CEA whether absorbed or unabsorbed rabbit anti-CEA sera were used in the immunoperoxidase method. GCM also stained strongly for CEA when unabsorbed rabbit anti-CEA serum was employed (Fig. 1), but sections from all GCM showed consistently negative reaction when the rabbit anti-CEA serum had been absorbed with tissue powder from normal human spleen. Pretreatment of GCM with periodic acid did not abolish positive staining by the unabsorbed rabbit anti-CEA serum but in periodic acid treated sections the intensity of staining was less than in sections treated with hydrogen peroxide.

Sections from all other tumours examined showed no staining for CEA using unabsorbed rabbit anti-CEA serum.

Discussion

Our results concur with the findings of Shousha and Lyssiotis (1979) concerning the positive staining of GCM for CEA using unabsorbed rabbit anti-CEA serum in the immunoperoxidase method. Other soft tissue tumours were negative. In our study, however, it is shown that this positive staining of GCM can be abolished when the anti-serum is absorbed with tissue powder from normal human spleen. In both studies rabbit anti-CEA serum from DAKO, Denmark was used. This antibody cross-reacts with NCA. The cross-reaction is illustrated by the staining of granulocytes and macrophages containing NCA (Burtin et al. 1975).

The fact that positive staining of GCM using unabsorbed rabbit anti-CEA serum can be blocked when the serum is absorbed with tissue powder from normal spleen, rich in NCA, indicates that this reaction is due to NCA contents of GCM. The discrepancy concerning CEA content also goes for malignant granula cell myoblastomas. Robertson et al. (1981) using commercial unabsorbed anti-CEA serum found positive staining in their case while Steffelaar et al. (1982) after absorbtion procedures concluded that the positive staining of their case of malignant GCM was due to NCA content.

Glycoproteins other than NCA have, however, been found to cross-react with anti-CEA serum, but this cross-reaction could be blocked by pretreatment with periodic acid (Isaacson and Judd 1977). In our study periodic acid did not abolish positive staining of GCM with unabsorbed rabbit anti-CEA serum, but the positive reaction became less intense, while periodic acid totally abolished staining of granulocytes and monocytes. This finding indicates that GCM may also contain cross-reacting glycoproteins other

than NCA. The fact that absorption with tissue powder from human spleen abolishes the staining capacity of anti-CEA serum does not rule out this possibility since the tissue powder may contain other cross-reacting glycoproteins than NCA.

Though the positive staining reacting for CEA and GCM using commercial anti-CEA serum in our study is shown not to be due to CEA per se, this tumour differs from other soft part tumours by the contents of substances, NCA, and perhaps other glycoproteins cross-reacting with anti-CEA serum.

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