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# Granular cell tumors contain myelin-associated glycoprotein

## An immunohistochemical study using Leu 7 monoclonal antibody

**J. Smolle [1], K. Konrad [2] and H. Kerl [1]**

[1] Department of Dermatology, University of Graz, Auenbruggerplatz 8, A-8036 Austria
[2] Department of Dermatology (I), University of Vienna, Austria

**Summary.** An immunohistochemical staining procedure using Leu 7 (HNK-1) monoclonal antibody was used to study the distribution of myelin-associated glycoprotein in granular cell tumours. Positive reactions were noted in 10 of the 13 tumours investigated. This observation supports the concept that granular cell tumours are of Schwann cell origin.

**Key words:** Granular cell tumor – Myelin-associated glycoprotein – Leu-7 antibody

## Introduction

Since the original description of Abrikossoff in 1926 (Abrikossoff 1926), the histogenesis of 'granular cell myoblastoma' has remained questionable. Myoblastic, fibroblastic, histiocytic and neural crest origins have been discussed (Feyrter 1935; Fisher and Wechsler 1962; Armin et al. 1983; Bedetti et al. 1983; Enzinger and Weiss 1983; Penneys et al. 1983).

Evidence for Schwann cell derivation has been based on histological (Bedetti et al. 1983), histochemical (Feyrter 1935), electron microscopic (Fisher and Wechsler 1962; Bedetti et al. 1983) and immunohistological (Armin et al. 1983; Penneys et al. 1983) observations. Two antigenic determinants shared by Schwann cells and granular tumor cells have been previously descirbed: S-100 protein, primarily detected in nervous tissue (Armin et al. 1983), and myelin basic protein, a quantitative major component of myelin sheaths (Penneys et al. 1983). We investigated the distribution of another neural antigen, myelin associated glycoprotein, in granular cell tumors. Leu 7 (HNK-1) antibody served as primary reagent in the immunoperoxidase procedure. It was initially raised against human natural killer cells

*Offprint requests to:* J. Smolle at the above address

(Abo and Balch 1981), but subsequent studies demonstrated specific binding to neural structures (Schuller-Petrovic et al. 1983) and preparations of myelin-associated glycoprotein (McGarry et al. 1983).

## Material and methods

*Specimens.* 13 cases of granular cell tumour originating from the skin (10 tumours) and mucous membranes (3 tumours) were evaluated. Paraffin sections were stained with H&E and with immunoperoxidase methods demonstrating S-100 protein and myelin-associated glycoprotein.

*Monoclonal antibody.* Leu 7 monoclonal antibody was purchased from Becton-Dickinson. In brief, BALB/c mice were immunized by HSB-2 lymphoblastoid cells (Abo and Balch 1981) and antibody-secreting hybridomas were produced according to the method of Köhler and Milstein (1975). The antibody has been shown to react with isolated myelin-associated glycoprotein (McGarry et al. 1983).

*Immunoperoxidase staining procedure.* We applied a two step immunoperoxidase sandwich method (Huber et al. 1983). Sections were deparaffinized and rehydrated, incubated with Leu 7 monoclonal antibody diluted 1:100 for 30 min, rinsed in phosphate buffered saline, covered by peroxidase-conjugated rabbit-antimouse immunoglobulin (DAKO laboratories) and washed again. Colour reaction was achieved by amino-ethyl-carbazole and hydrogen peroxide. Slides were counterstained in Mayer's haematoxylin and mounted in glycerine gelatine. In order to increase the staining reaction, a triple layer technique using a peroxidase-conjugated swine-anti-rabbit immunoglobulin (DAKO laboratories) as the third step, and delipidinization in acetone for 20 min (Schuller-Petrovic et al. 1983), were additionally applied in 5 cases. S-100 protein was demonstrated by a rabbit anti S-100 serum (DAKO) followed by a swine anti rabbit horseradish peroxidase conjugate (DAKO).

*Controls.* Normal peripheral nerves served as positive controls. Negative controls were obtained by omisson of the primary antibody and by application of an irrelevant antibody (OKT 3, Ortho Pharmaceutical Corporation) or normal mouse serum (Behring).

## Results

In H&E stained sections the tumours revealed typical diagnostic features (large polygonal cells, granular eosinophilic cytoplasm, oval nuclei and occasionally pseudoepitheliomatous hyperplasia of the epidermis).

Immunohistological staining of myelin-associated glycoprotein demonstrated various amounts of positive cells in 10 of 13 tumours ranging from less than 5% up to 30% (Fig. 1 and Fig. 2). The three negative granular cell tumours originated from the tongue (1 case) and trunk (2 cases), respectively. The cells containing myelin-associated glycoprotein were either grouped or randomly distributed. Single positive cells infiltrating surrounding tissue were also observed. On higher manification, a distinct granular cytoplasmic staining pattern was evident. Narrow cytoplasmic extensions with intensely stained granules were seen in most tumors. In 7 of 13 tumours small bundles of nerve fibers appeared in the periphery of the neoplastic tissue. Occasionally single nerve fibers were distributed between granular tumour cells. Application of the triple layer technique as well as delipidinization did not influence the staining pattern.

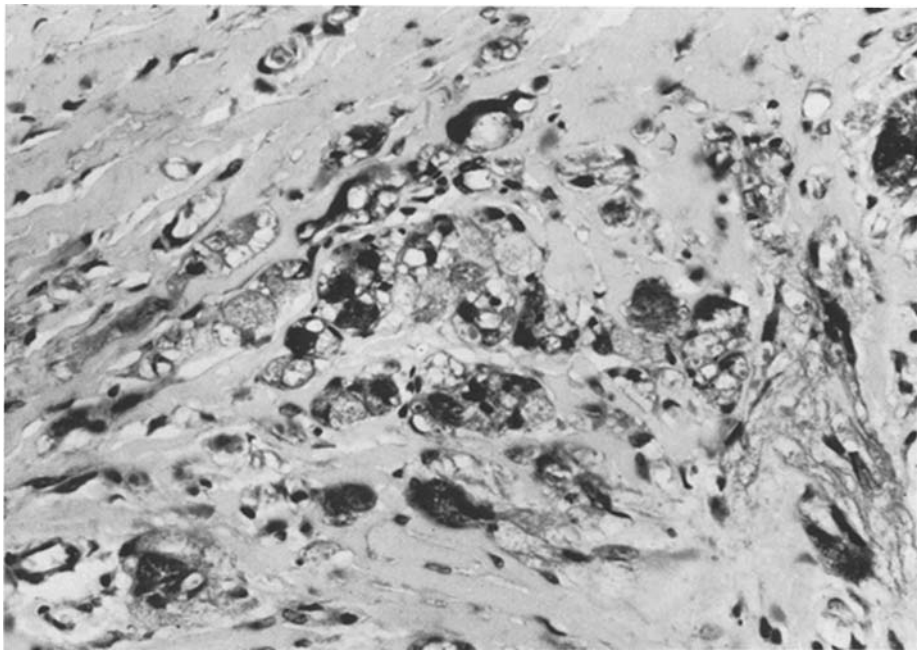

**Fig. 1.** Granular cell tumor. Immunohistological demosntration of myelin-associated glycoprotein. 250 ×

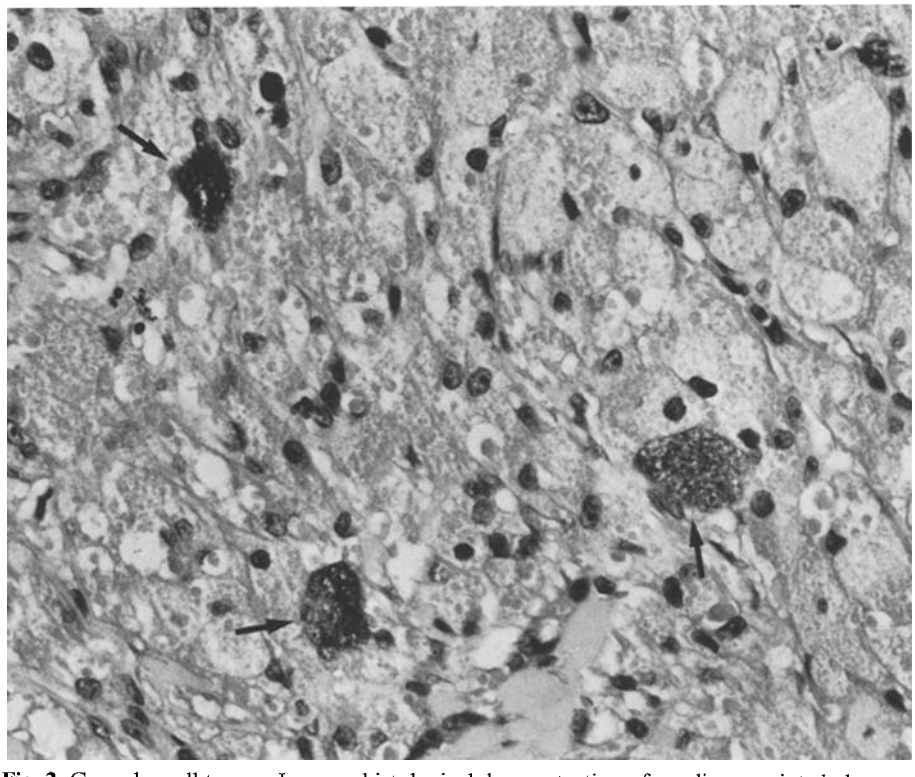

**Fig. 2.** Granular cell tumor. Immunohistological demonstration of myelin-associated glycoprotein: Positive cells (arrows) reveal a typical granular staining pattern. 250 ×

Immunohistological demonstration of S-100 protein labelled 100% of tumour cells in all cases, including those which were negative for myelin-associated glycoprotein.

## Discussion

Feyrter suggested a neural crest origin for 'granular cell myoblastoma' in 1935 – because of certain staining properties of the tumor cells. Subsequent electron microscopic studies by various authors revealed ultrastructural features in favour of Schwann cell derivation: basement membranes around single cells, cytoplasmic extensions, and whorls of membranes (Fisher and Wechsler 1962; Bedetti et al. 1983). The granules resemble autophagic vacuoles or residual bodies.

The immunohistological detection of S-100 protein (Armin et al. 1983) and myelin basic protein (Penneys et al. 1983) demonstrated similarity between Schwann cells and granular tumor cells on a molecular base. Our own observations confirm the results obtained with S-100 protein antisera.

Using Leu 7 monoclonal antibody we were able to demonstrate another independent neural antigen, namely myelin-associated glycoprotein. This substance is usually present in myelinated fibers and in a variety of neurogenic tumours (Lipinski et al. 1983). The characteristic granules obviously contain myelin-related antigens. We conclude that granular cell tumours are closely related to Schwann cells, which presumably retain their synthetic capacity. The Leu 7 positive tumor cells probably represent a higher level of Schwannian differentiation when compared with negative cells. However, we cannot rule out the possibility that the appearently negative granular cells contain myelin-associated glycoprotein in small amounts which are beyond the sensitivity of our method.

S-100 protein, which is in common use in surgical pathology, is obviously not restricted to neural tissue. It has been demonstrated in T-zone histiocytes, lipocytes, salivary glands and sweat glands (Kahn et al. 1984). We therefore consider the demonstration of myelin-associated glycoprotein by Leu 7 monoclonal antibody as a valuable additional measure to assess the neurogenic origin of neoplastic tissues.

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