Acta histochemica

Zeitschrift für
histologische Topochemie
Supplementband XXXVIII

Herausgegeben von
Joachim-Hermann Scharf, Halle,
Gerhard E. Voigt, Lund,
Werner Linß, Jena

Verhandlungen der Gesellschaft für Histochemie
auf dem XXX. Symposion in Gargellen, Montafon (Österreich)
vom 21. bis 24. September 1988

Cytochemical Markers of Neural and Endocrine Cells

Herausgegeben von Jan Drukker, Maastricht

Mit 129 Abbildungen und 34 Tabellen

Gustav Fischer Verlag Jena · 1990

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NCAM expression in endocrine cells

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With 4 figures

Introduction

Cell adhesion is one of the primary processes of morphogenesis involved in several stages of development to determine embryonic form. A number of cell adhesion molecules (CAMs) have been described and are named according their presence in different tissues. The best-known is NCAM, the neutral cell adhesion molecule (Thiery et al., 1977). This membrane associated glycoprotein has been shown to exist in brain as three closely related polypeptides (NCAM-180, NCAM-140 and NCAM-120) i.e. of Mrs 180, 140 and 120 kD. They are characterized by an identical or similar extracellular domain which contains the amino-terminal end, the homophilic binding site and the region with large amounts of sialic acid. NCAM-120 is anchored in the membrane via a phospholipid. NCAM-140 and NCAM-180, which are characterized by a transmembraneous segment, differ in the length of their cytoplasmic domains. While NCAM is widely distributed in the embryo (Crossin et al., 1985; Daniloff et al., 1986), in the adult it has been reported to be essentially restricted to cell of the central and peripheral nervous system, with the exception of skeletal muscle (Covault and Sanes, 1986) and chick lung epithelium (Crossin et al., 1985). The present study demonstrates that NCAM Is much more widely distributed in the adult than had been originally supposed. We have extended our investigations on endocrine cells of neural and non-neural origin and on endocrine cell lines. The cellular distribution of NCAM was studied immunocytochemically, and the various NCAM determinants expressed were characterized by immunoblots.

Materials and methods

Cell culture: The rat insulinoma (RIN A 2) and the pheochromocytoma cell lines (PC 12) were cultured as previously described (Lind et al., 1987).

Immunocytochemistry: Adult rats were perfused with 4% formaldehyde in phosphate buffer (0,12 M), and the excised tissues postfixed in the same solution. 50 µm thick vibratome sections and cells were incubated in rabbit polyclonal anti-NCAM-antiserum (diluted 1:1,000; kindly supplied by C. Goridis) and then in peroxidase-conjugated sheep anti-rabbit IgG (dilution 1:100). Bound immunoglobulin was detected with diaminobenzidine and hydrogen peroxide. Cells and slices destined for electron microscopy were subsequently osmicated and embedded in Spurr resin.

Immunochemistry: Adult rat tissues and cells were homogenized in sample buffer (62,5 mM) Tris/2% SDS) and the proteins separated electrophoretically under reducing conditions (Laemmli, 1979). After transfer to nitrocellulose (Towbin et al., 1979) the strips were incubated

with polyclonal rabbit anti-NCAM antiserum (dilution 1:1,000), and the bound antibody detected with iodinated protein A. For the quantification of the NCAM polypeptides the optical densities of the immunostained bands were determined by computer-assisted analysis (I. P. S., Kontron, FRG).

Results and discussion

The cellular distribution of NCAM was studied in seven different rat tissues and cell lines at both the light microscopical and ultrastructural levels. The analysis included: posterior lobe, intermediate lobe and anterior lobe of the hypophysis, adrenal medulla, and pancreas, and phenochromocytoma (PC 12) and insulinoma (RIN) cell lines.
Light microscopy of vibratome sections of the examined tissues and cells immunostained with anti-NCAM all showed prominent cell surface labelling (Langley et al., 1987, 1988). With the exception of the intermediate lobe, the staining was always very intense: neurohypophysis was the most intensely stained tissue studied. In the adenohypophysis all different types of secretory cells were intensely stained; immunocytochemistry of the adrenal medulla showed that both the adrenergic and noradrenergic cells were positive for NCAM as reported earlier (Langley and Aunis, 1984). PC 12 cells, a tumour cell line derived from a rat pheochromocytoma, were strongly surface labelled, in accordance with data provided by other groups (Jørgensen and Richter-Landsberg, 1983; Prentice et al., 1987).
The most remarkable result was obtained by staining pancreatic tissue (Fig. 1), an endocrine organ considered not to be derived from the neural crest (La Douarin, 1988).

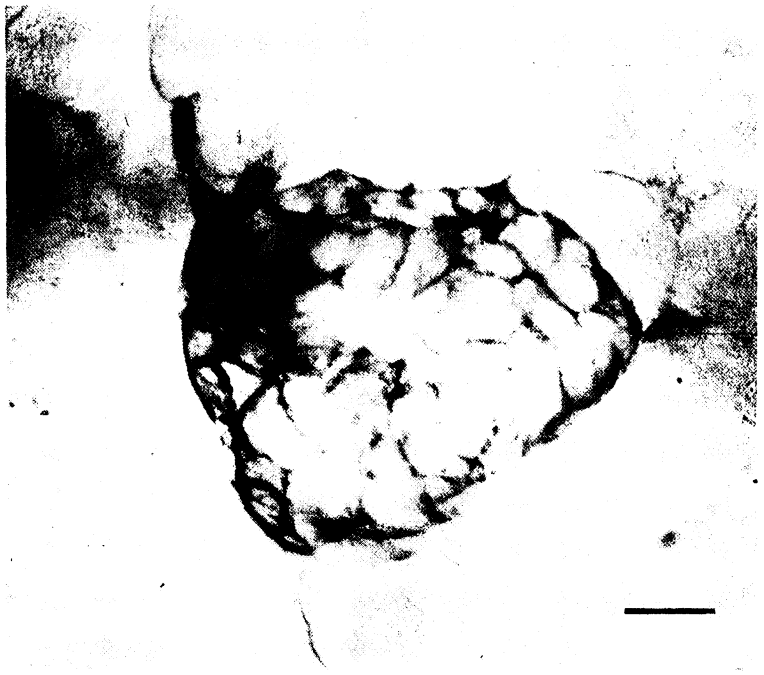

Fig. 1. Adult rat pancreas immunoperoxidase labelled for NCAM. It is evident that the reaction product is limited to the islets of Langerhans. Scale bar, 20 μm.

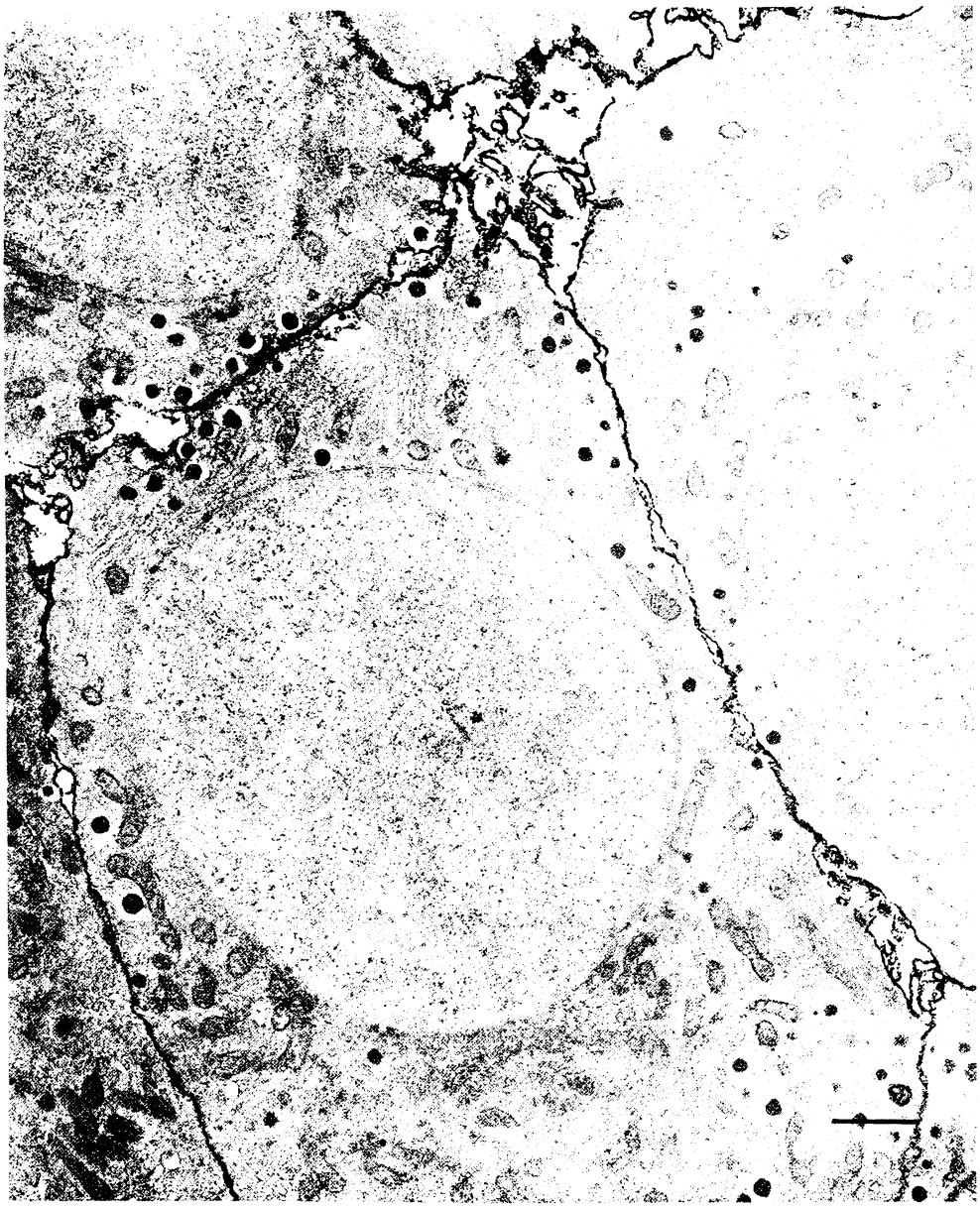

Fig. 2. Electron micrograph of a pancreatic islet labelled with anti-NCAM-antibody. Only the surface membrane is immunoreactive. No intracellular staining is detectable (section not counterstained). Scale bar, 1 μm.

The immunoreactivity was restricted to the islets of Langerhans, the acinar cells of the exocrine pancreas remaining unstained. It can thus be concluded that NCAM is expressed by endocrine cells of both neural and non-neural origins. Immunocytochemical studies of the closely related RIN cells, a tumour cell line derived from a rat insulinoma, demonstrated again the presence of NCAM antigenic determinants at the cell membrane.

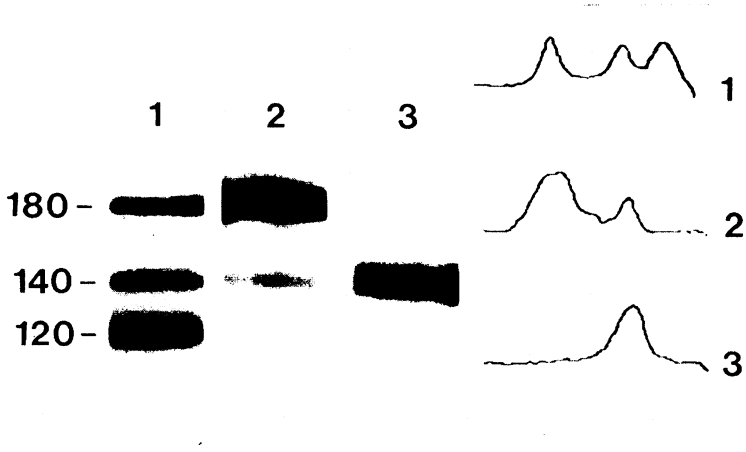

Fig. 3. Immunological identification of NCAM in cerebellum (lane 1), neuro- (lane 2) and adenohypophysis (lane 3). The optical densitis of the immunostained bands were quanticated. Control immunoblots with normal rabbit serum revealed none of the specific immunoreactive bands at 180, 140 and 120 kD. Molecular masses (kD) of NCAM are indicated.

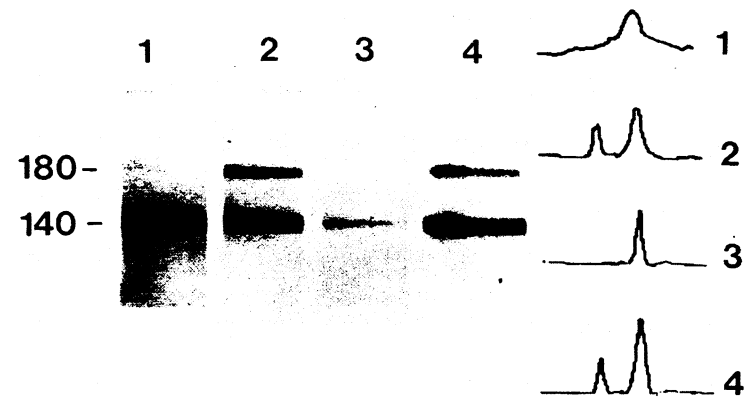

Fig. 4. Immunological identification of NCAM in adrenal medulla (lane 1), PC 12 cells (lane 2), pancreatic islets (lane 3) and RIN cells (lane 4). The optical densities of the immunostained bands were quantificated. Molecular masses (kD) of NCAM are indicated.

Electron microscopy of the immunostained sections confirmed the results obtained by light microscopy: N-CAM was found to be expressed by all the endocrine cells studied, distributed over the entire surface. Significant intracellular labelling was not noted. Controls employing normal rabbit serum produced none of these specific staining features. Fig. 2 demonstrates the staining of the pancreatic islet cells by antigenic structures.

Comparison of the immunoblots of the endocrine cells and tissues showed marked differences in the pattern of the three different NCAM glycoproteins. Cerebellum (Fig. 3, lane 1) was used as a reference. The presence of the three antigenic determinants NCAM-180, NCAM-140 and NCAM-120 is well documented (Langley et al., 1982;

Edelman, 1984). The pattern of the neurohypophysis (Fig. 3, lane 2) when compared to cerebellum, clearly differed in the amount of the 3 glycoproteins. This endocrine tissue expressed NCAM-140, high contents of NCAM-180 and only trace levels of NCAM-120 (Langley et al., 1988). In extracts of the adenohypophysis (Fig. 3, lane 3) and the adrenal medulla (Fig. 4, lane 1) only the 140 kD variant was present. By contrast, immunoblots of the pheochromocytoma cell line (PC 12, Fig. 4, lane 2) were characterized by an additional immunoreactive band of molecular weight 180 kD, which is in agreement with data reported by Friedlander et al. (1986). The relative amounts of the two NCAM determinants varied in the different cell extracts. Isolated pancreatic islets (Fig. 4, lane 3) contained only the 140 kD NCAM-immunoreactive band whereas the pattern of the insulinoma cell line (Fig. 4, lane 4) ressembled that observed in PC 12 cells: The RIN cell extracts showed mainly a band of 140 kD with a less prominent band of 180 kD. Compared to the corresponding tissues it is evident that both tumour cell lines express an additional NCAM-180 polypeptide, suggesting that the malignant transformation is associated with a change in the regulation of NCAM mRNA processing.

As shown immunocytochemically, all endocrine cells examined express NCAM at their surface. However the pattern of the three NCAM polypeptides observed was different. It may be concluded that the expression of NCAM in the adult is a common feature of endocrine cells, irrespective of their embryonic origin, though the pattern of antigenic determinants is very different from that of central nervous system. In addition, with the exception of neurohypophysis, the NCAM endocrine phenotype is characterised by a predominance of NCAM-140.

Acknowledgements. We thank Dr. G. Ahnert-Hilger for generously providing PC 12 and RIN cells, Dr. K. Schilling, H. Frey and W. Podschuweit for their help with densitometric scans and Mrs. M. Rudolf for expert technical assistance.

This work was supported by Deutsche Forschungsgemeinschaft (Gr 681), by Forschungsschwerpunkt no. 24 of the State of Baden-Württemberg, and by a twinning exchange PROCOP fellowship.

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