Nerve growth factor receptor immunoreactivity in human benign peripheral nerve sheath tumor*

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Summary. In situ expression of nerve growth factor (NGF) receptors in human dermal and plexiform neurofibroma, schwannoma and traumatic neuroma was examined by an immunohistochemical method using a monoclonal anti-human NGF receptor antibody. Immunoreactivity for the NGF receptor was observed on the principal cells of both neurofibroma and schwannoma. Immunostaining by the anti-S-100β protein antibody in adjacent sections suggested that the vast majority of NGF receptor-positive cells were also positive for S-100β protein. In traumatic neuroma, staining for the NGF receptor was more intense in the perineurium than in the endoneurial cells.

Key words: Nerve growth factor receptor — S-100β protein — Neurofibroma — Schwannoma — Von Recklinghausen disease

Nerve growth factor (NGF) is a polypeptide that is essential for the differentiation and survival of sympathetic and neural crest-derived sensory neurons [14, 15, 42]. Monoclonal antibodies, which specifically recognize the rat or human NGF-binding proteins, are currently used for immunohistochemical detection of the NGF receptor in tissue section and cultured cells [19, 26, 27, 29, 39, 40]. In addition to sympathetic and sensory neurons [38], NGF receptors are present in non-neuronal cells such as normal Schwann cells maintained in culture or Schwann cells in vivo at embryonal and neonatal stage [3, 25, 40, 47, 49]. Recently, the presence of NGF receptors in vivo and in vitro have been demonstrated in neurofibromas of patients with von Recklinghausen’s disease and traumatic neuromas [19, 27–29, 36, 46]. However, a vast majority of the cells in these tumors and their characteristics are often indistinguishable from normal Schwann cells [19, 33, 36]. In this study, we extended the research in this line by examining various human nerve sheath tumors including dermal and plexiform neurofibroma, schwannoma and traumatic neuroma by using monoclonal antibody recognizing human NGF receptors.

Materials and methods

Tissue specimens were obtained from 39 patients with benign peripheral nerve sheath tumors and 4 patients with traumatic neuroma. However, these tissue specimens were obtained during operations performed for clinical reasons unrelated to this study. The patients consisted of 8 patients with dermal neurofibroma, 5 patients with plexiform neurofibroma, 12 patients with acoustic neurinoma, 1 patient with vagus nerve schwannoma, 6 patients with spinal root schwannoma and 7 patients with cutaneous schwannoma. The specimens were immediately fixed in a 10% buffered-formalin solution at pH 7.4 and thereafter embedded in paraffin. These paraffin sections were then stained with hematoxylin and eosin. For NGF receptor immunohistochemistry, tissue sections were deparaffinized with xylene and washed first with 100% alcohol and then with a 50% alcohol dilution. The sections were washed several times in 100 mM phosphate-buffered saline (PBS) and thereafter incubated overnight at 4°C with the NGF receptor monoclonal antibody ME20-4 (diluted 1:20 with 1% bovine serum albumin). Monoclonal antibody ME20-4 was generously provided by Dr. Alzano Ross, Wistar Institute, Philadelphia. Following the first incubation period, the sections were washed again with the PBS and incubated for 3 h at 25°C with biotinylated horse anti-mouse IgG immunoglobulin. The sections were then washed and incubated for 2.5 h at 25°C with a complex of avidin and biotinylated horseradish peroxidase. After several washes, the sections were incubated for 5 min in 0.05% 3,3′-diaminobenzidine/0.01% H₂O₂. Using this system of fixation and immunostaining, ME20-4 did not stain organs which were obtained as biopsies or resected surgical specimens, such as...
skeletal muscles, small intestine, stomach, lung, uterus and kidneys. To ascertain the specificity of immunoreactivity of ME20-4, an indifferent monoclonal antibody (monoclonal antibody against thyroglobuline) [17] was also applied to the section adjacent to the ME20-4-treated section, and no positive staining was noted (Fig. 1 B). Immunohistochemistry for the S-100β protein was in some cases performed as described for the NGF receptor. The monovalent polyclonal rabbit IgG fraction for S-100β protein was generously provided by Dr. K. Kato, Department of Biochemistry, Aichi Prefectural Colony Institute, Japan [9].

Results

All eight dermal neurofibromas, five of which were associated with von Recklinghausen's disease, were composed of principally fusiform or often twisted cells with wiry cell processes. They were also composed of minor components of lymphocytes, mast cells and cells with large oval or round nuclei which probably correspond to fibrocytes (Fig. 1 A). The five plexiform neurofibromas had a less cellular population consisting of cells which were similar to those of dermal neurofibromas (Fig. 2 A). The cells of a neurofibroma were variably stained with antibodies to the NGF receptor (Figs. 1 C, 2 B) and the S-100β protein (Figs. 1 D, 2 C). Most of the spindle-shaped cells were strongly positive for NGF receptors and the S-100β protein (Table 1).

However, NGF receptor-negative or S-100β protein-negative cells with slender elongated nuclei were also noted. Cells with large oval nuclei, most of which were considered to be fibrocytes and cells with round

Fig. 1 A–D. Immunohistochemical findings on the sections of a dermal neurofibroma. A The spindle-shaped cells were separated by collagen fibrils and a mucinous matrix. B Indifferent monoclonal antibody (monoclonal antibody against thyroglobuline) was applied to the section from the adjacent tissue shown in A. No positive staining was obtained. C Immunohistochemical localization of nerve growth factor (NGF) receptor in the section adjacent to B. NGF receptor staining was localized on the spindle-shaped cell. Some of the cells exhibiting elongated slender nuclei stained only a little. D Immunohistochemical localization of the S-100β protein in the section adjacent to C. The S-100β protein was heavily stained in spindle-shaped cell, but some of the cells with elongated slender nuclei showed little immunoreactivity. A H&E, B–D with methyl green counterstain; A–D × 272