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ORIGINAL ARTICLE

Immunohistochemical study of GLUT-1 in oral peripheral nerve sheath tumors

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AIM: To investigate the immunoexpression and diagnostic applicability of human erythrocyte-type glucose transporter protein (GLUT-1) in oral peripheral nerve sheath tumors.

MATERIAL AND METHODS: Specimens diagnosed as oral peripheral nerve sheath tumors archived in the Oral Pathology Service of Universidade Federal de Minas Gerais from 1966 to 2006 were evaluated. Thirty-four lesions were included: 15 traumatic neuromas, 11 neurofibromas, four neurilemmomas, and four malignant peripheral nerve sheath tumors (MPNST). One case of neurofibroma was associated with neurofibromatosis type I. Immunohistochemistry for S-100 and GLUT-1 was performed. S-100 was immunopositive in all lesions.

RESULTS: Benign lesions were immunopositive for GLUT-1 except in two (18.2%) cases of neurofibromas. In the traumatic neuroma, the perineuriums were immunopositive for GLUT-1. In the neurofibroma, the immunoreactivity was heterogeneous. Immunopositivity was observed at levels of 54.5% in the periphery of the lesion, 9.1% in the center, and 18.2% in both. The neurilemmoma demonstrated immunopositivity in the capsule. One case (25%) of MPNST presented GLUT-1 positive stain in occasional cells distributed homogeneously in all the tumor area.

CONCLUSION: GLUT-1 is a useful marker for perineurial cells and should be included in the oral peripheral nerve sheath tumors immunophenotyping thus aiding in the correct diagnosis of these lesions.

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Keywords: neuroma; neurofibroma; neurilemmoma; nerve sheath neoplasm; glucose transporter type 1; immunohistochemistry

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Clinical relevance

This is the first large study of GLUT-1 immunoexpression in oral peripheral nerve sheath tumors, including benign and malignant specimens. GLUT-1 is a useful marker for perineurial cells and may be helpful in immunophenotyping of oral peripheral nerve sheath tumors, thus aiding in the correct diagnosis of these lesions.

Introduction

Traumatic neuroma, neurofibroma, neurilemmoma, and malignant peripheral nerve sheath tumor (MPNST) share a common neural origin, but otherwise present microscopic and clinical diversity. The traumatic neuroma consists of a proliferation of Schwann cells and axons inside nerve fascicles. The neurofibroma is characterized by a mixture of perineurial cells, Schwann cells, and endoneurial fibroblasts. The neurilemmoma is composed only of Schwann cells. The MPNST is composed of spindle cells that resemble Schwann cells (Enzinger and Weiss, 1995; Chrysomali *et al*, 1997), but the cellular constituents of MPNST are not completely known (Hirose *et al*, 2003).

The classification of the peripheral nerve sheath tumors is important because they may be associated with syndromes, such as neurofibromatosis type I and multiple endocrine neoplasia type III (Wright and Jackson, 1980; Chrysomali *et al*, 1997; Pilavaki *et al*, 2004). Immunohistochemistry is an auxiliary method that has contributed to the differential diagnosis of many oral lesions, for example, salivary gland tumors (Araújo *et al*, 2000) or oral benign vascular lesions (Johann *et al*, 2007). The immunohistochemical markers used in peripheral nerve sheath tumors described in the medical literature include: S-100, CD34, and epithelial membrane antigen (EMA). S-100 has been useful in identifying Schwann cells (Weiss *et al*, 1983; Johnson *et al*, 1988; Chrysomali *et al*, 1997; Hirose *et al*, 2003). CD34 identifies endoneurial fibroblasts in peripheral

nerve sheath tumors (Chrysomali *et al*, 1997; Hirose *et al*, 2003). EMA has been found in the perineurial cells of peripheral nerve sheath tumors (Hirose *et al*, 2003).

The human erythrocyte-type glucose transporter (or glucose transporter type 1 – GLUT-1) is a transmembrane protein that facilitates the glucose transporter. It is normally expressed in erythrocytes, blood–brain barriers, the liver, the placenta, as well as in reactive germinal centers of lymphoid tissues and renal tubules (Younes *et al*, 1996). Hirose *et al* (2003) concluded in their immunohistochemical study with 43 peripheral nerve sheath tumors that GLUT-1 is a useful marker of perineurial cells. However, these authors do not report the localization of the lesions. Also, GLUT-1 was used as a marker for perineurial cells in sclerosing perineurioma of the hand (Yamaguchi *et al*, 2003). The goal of this study was to investigate the immunoexpression and the applicability of GLUT-1 in the diagnosis of oral peripheral nerve sheath tumors.

Materials and methods

Institutional Ethical Board

The protocol of this study was approved by the Committee of Bioethics in Research at the Universidade Federal de Minas Gerais – UFMG (016/03).

Specimens

All specimens diagnosed as oral peripheral nerve sheath tumors archived in the Oral Pathology Service at UFMG from 1966 to 2006 were analyzed. The diagnosis of the tumors were based on Enzinger and Weiss (1995) and immunohistochemical criteria. The traumatic neuroma consisted of well-delimitated lesions composed of a disorganized proliferation of nerve fibers in a fibrous connective tissue (Figure 1a). The neurofibroma represented a circumscribed neoplasm composed of a bundle of fusiform cells with wavy and hyperchromatic nuclei (Figure 1b). The neurilemmoma presented two patterns, surrounded by a capsule: Antoni A (Figure 1c), composed of Schwann cells arranged in palisade surrounding the Verocay's body, and Antoni B, less organized and less cellular. The MPNST consisted of lesions composed of a proliferation of pleomorphic and hyperchromatic cells arranged in hypocellular areas which alternated with other hypercellular areas. Perineurium-like structures were observed (Figure 1d) (Hirose *et al*, 2003). The oral peripheral nerve sheath tumors studied were: traumatic neuroma (15 cases), neurofibroma (11 cases), neurilemmoma (four cases), and MPNST (four cases). One case of neurofibroma was associated with neurofibromatosis type I. One case of the MPNST has been previously reported (Fernandes *et al*, 2006).

Immunohistochemistry

The immunohistochemistry for GLUT-1 was made using the streptavidin–biotin standard protocol. The paraffin-embedded blocks were cut in sections of 4 μm, deparaffinized, and dehydrated. For antigen retrieval, the specimens were immersed in a 10 mM citrate buffer (pH 6.0, 30 min at 98°C). Avidin and biotin were blocked (Miller *et al*, 1999). To block the endogenous

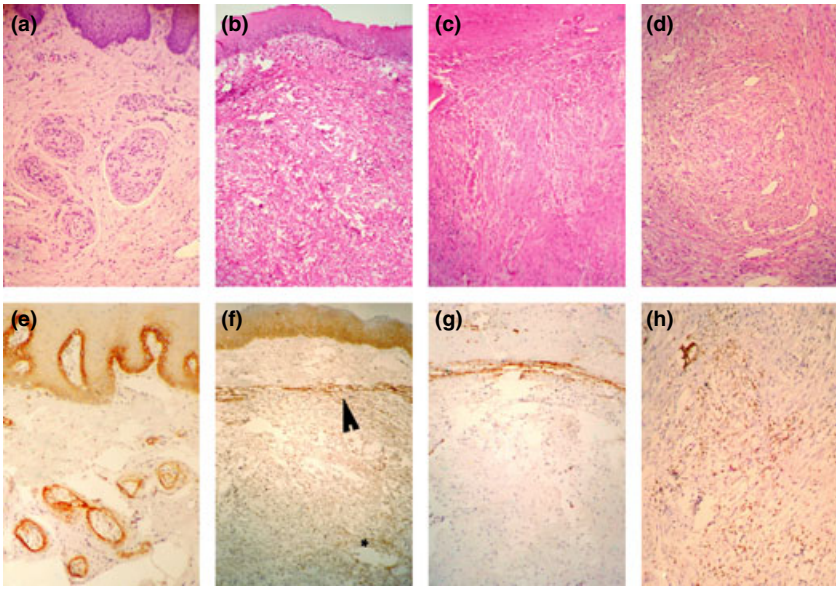

Figure 1 (**a**) The oral traumatic neuroma was characterized by a matrix of connective tissue, presenting a disorderly proliferation of nerve fibers (Hematoxylin–eosin, 200× original magnification). (**b**) The oral neurofibroma was characterized by a circumscribed lesion composed of spindle cells with hyperchromatic and wavy nuclei arranged in interlacing bundles (Hematoxylin–eosin, 200× original magnification). (**c**) The oral neurilemmoma was characterized by the Antoni A pattern, composed of palisading Schwann cells surrounding the Verocay's body (Hematoxylin–eosin, 200× original magnification). (**d**) Perineurium-like structure of the MPNST (Hematoxilin-eosin, 200× original magnification). (**e**) In the oral traumatic neuroma, GLUT-1 immunopositive perineurial cells surrounded the proliferating nerve fascicles (streptavidin–biotin, 200× original magnification). (**f**) In oral neurofibroma was observed GLUT-1 immunopositive cells in the periphery (arrow) and in the center (asterisk) of the lesion (streptavidin–biotin, 200× original magnification). (**g**) GLUT-1 was positive in the capsules of the oral neurilemmoma (streptavidin–biotin, 200× original magnification). (**h**) GLUT-1 positive stain in occasional cells distributed homogeneously in perineurium-like structure (streptavidin–biotin, 200× original magnification)

peroxidase activity, 0.3% hydrogen peroxidase was used. Specimens were incubated with the GLUT-1 primary antibody at a 1:200 dilution (Dako Corporation®, Carpinteria, CA, USA) for 18 h at 4°C. The primary antibody was detected using an LSAB+ system, HRP Peroxidase Kit (Dako Corporation®) and 3,3′-diaminobenzidine tetrahydrochloride chromogen (Sigma Chemical, St Louis, MO, USA). As erythrocytes always stain positive for GLUT-1, they were the internal positive control. External positive controls included: the placenta (GLUT-1 positive in erythrocytes, trophoblast and microvascular endothelial cells) and the normal mucosa (GLUT-1 positive in perineurial cells of nerve fibers).

The immunohistochemistry for the S-100 protein was performed in all cases. Streptavidin–biotin standard protocol was also used, but without antigen retrieval. The dilution used to incubate the primary antibody S-100 was 1:700 (Dako Corporation®, Carpinteria, CA, USA) for 18 h at 4°C. All tumors were immunopositive for S-100.

In cases of MPNST, immunohistochemistry was performed for AE1/AE3 (M3515, 1:100 dilution), HHF35 (M0635, 1:100 dilution), and α-smooth muscle actin (M0851, 1:50 dilution). These antibodies were obtained from Dako Corporation® Carpinteria, CA, USA. In the immunohistochemistry for AE1/AE3 and α-smooth muscle actin, antigen retrieval was performed using a 10 mM citrate buffer, pH = 6.0, for 30 min at 98°C. The primary antibody incubation was for 18 h at 4°C. MPNST presented negativity for AE1/AE3, HHF35, and α-smooth muscle actin.

Results

The GLUT-1 positive stain was identified in cellular membranes, presenting a brownish color. All cases of traumatic neuromas presented positive stains for GLUT-1 in perineurial cells that involved proliferating nerve fascicles (Figure 1e). Nine of eleven (81.8%) cases of neurofibroma were immunopositive for GLUT-1. Six (54.5%) cases showed positive cells only in the periphery of the lesion, two (18.2%) cases also showed positive cells in the center (Figure 1f), and one (9.1%) case demonstrated positive cells only in the center of the lesion. There was no difference in GLUT-1 expression between neurofibroma associated or no with neurofibromatosis type I. The neurilemmomas showed two cases (50%) with the Antoni A pattern and two other cases (50%) with both Antoni A and B patterns. All cases were encapsulated and, in their capsules, fusiform cells were immunoreactive for GLUT-1 (Figure 1g). One case (25%) of MPNST presented GLUT-1 positive stain in occasional cells distributed homogeneously in all the tumor area, including in the perineurium-like structures (Figure 1h).

Discussion

The diagnosis of the oral peripheral nerve sheath tumors evaluated was in accordance with the histological analysis and immunoexpression for the S-100 protein. The marker S-100 was chosen in this study because it has been widely used in the identification of Schwann cells and is considered to be an important auxiliary in the diagnosis of peripheral nerve sheath tumors (Weiss *et al*, 1983; Johnson *et al*, 1988; Chrysomali *et al*, 1997; Hirose *et al*, 2003).

Other immunohistochemical markers may help in the diagnosis of peripheral nerve sheath tumors, such as CD34 and EMA. Previous studies considered GLUT-1 to be an effective marker for perineurial cells (Yamaguchi *et al*, 2003). Hirose *et al* (2003) performed an immunohistochemical study on GLUT-1 in peripheral nerve sheath tumors without reporting the localization of the lesions. The current paper approaches for first time a broad study that investigates the GLUT-1 expression in oral peripheral nerve sheath tumors, including benign and malignant specimens.

In the traumatic neuroma, the immunopositive reaction to GLUT-1 could be observed in the area surrounding the proliferating nerve fascicles. This stain pattern is expected since the perineurial cells of the traumatic neuroma were localized around the nerve fascicles, which supports findings from Hirose *et al* (2003). This GLUT-1 immunoexpression confirms the reactive nature of this lesion.

The GLUT-1 immunoexpression was diverse in the neurofibroma. Two lesions were not immunopositive for GLUT-1. Positive cases demonstrated a heterogeneity varying the number of positive cells and alternating the immunoreactivity in the peripheral area, in the central area or in both. These results reassert the cellular composition of the neurofibroma. It is believed that the neurofibroma presents a mixture of cells (Wright and Jackson, 1980; Enzinger and Weiss, 1995). Chrysomali *et al* (1997) performed an immunohistochemical study on benign peripheral nerve sheath lesions and found that some markers were immunoreactive in neurofibromas: S-100 identified Schwann cells, CD34 identified endoneurial fibroblasts and EMA, and GLUT-1 identified perineurial cells. Hirose *et al* (2003) mentioned, in their study, a positive stain for GLUT-1 in a small quantity of cells which were localized in perineurium-like structures. In the present study, the presence of perineurial cells was identified in 81.8% of the cases shown to be immunopositive for GLUT-1. In all lesion, the presence of the Schwann cell (S-100 positive) add to the GLUT-1 stain pattern, which in turn aids in shedding light on the cellular composition of the neurofibroma. Moreover, this shows that both cell types (perineurial cells and Schwann cells) proliferate in this lesion.

Neurilemmoma was immunopositive for GLUT-1 in the fusiform cells of the lesion capsules, in accordance with Hirose *et al* (2003). This stain pattern is also observed in immunoreactions to EMA (Chrysomali *et al*, 1997; Hirose *et al*, 2003). The immunopositivity for GLUT-1/EMA observed in the capsule suggests that it originates from a perineurium (Hirose *et al*, 2003). This fact could indicate that the lesion in fact grows inside a nerve fascicle.

In one case (25%) of MPNST was observed positive stain in occasional lesional cells distributed homogeneously in all the tumor area. Hirose *et al* (2003) found only two GLUT-1 immunopositive lesions (33%) of conventional MPNST in the seven unspecified localization tumors reviewed. Schwann cells seems to be the constituent cells of the majority of MPNST, but other cellular types as perineurial cells and fibroblasts may compose the MPNST (Hirose *et al*, 1998, 2003). Furthermore, MPNST may in fact exhibit a proliferation of variable cellular components, which, in our case, include the proliferation of perineurial cells. Also, as the immunostain pattern of GLUT-1 was homogeneous distributed in all the tumor area of MPNST and in benign lesions this stain was focally distributed or absent, this finding may be helpful to distinguish benign and malignant specimens of peripheral nerve sheath tumors.

In conclusion, GLUT-1 is a marker for perineurial cells and may be helpful in oral peripheral nerve sheath tumor immunophenotyping, thus aiding in the correct diagnosis of these lesions.

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