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Expression of ABCA2 protein in human vestibular schwannoma and peripheral nerve☆

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Abstract

ABCA2, which belongs to the A subclass of the ATP-binding cassette (ABC) transporter superfamily, is predominantly expressed in the cytoplasm of oligodendrocytes and Schwann cells, the myelin-forming cells in brain and peripheral nerve, respectively. Here, we demonstrate by immunoblot and immunohistochemistry that ABCA2 is expressed in benign vestibular schwannomas, which contain neither axons nor compact myelin. The expression patterns of ABCA2 in combination with other markers showed phenotypic heterogeneity in schwannomas. The majority of cells in fascicular Antoni type A areas coexpressed ABCA2, Ca^{2+}-binding protein S100β, and p75 nerve growth factor receptor. In contrast, considerably varied expression levels of ABCA2 and p75 were more prominent in hypocellular type B areas than in type A areas. These data suggest that ABCA2 may be useful as a marker for cellular characterization of schwannomas.

Keywords: ABC transporters; Schwann cells; Myelin sheath; Neurilemmoma; Nerve sheath tumor; S100β

1. Introduction

ABCA2, which we recently cloned and characterized in rat [1], is a member of the A subclass of the ATP-binding cassette (ABC) transporter superfamily, which is proposed to be involved in ATP-dependent transport of lipids such as cholesterol [2,3]. We have shown that ABCA2 is expressed at high levels in brain white matter [1]. Immunoblot and immunohistochemical studies in situ and in cultured cells demonstrated that ABCA2 is not expressed in glial fibrillary acidic protein (GFAP)-positive astrocytes, in CD11b-positive microglia, or in NG2 chondroitin sulfate proteoglycan-positive progenitor cells, but is predominantly expressed in cytosolic components (late endosomes/lysosomes) of oligodendrocytes [4–6]. In addition, ABCA2 begins to be expressed in oligodendrocytes as they elaborate myelin segments just before compact myelin formation [6], suggesting possible involvement of ABCA2 in a function related closely to production of a myelin component. We also have found that ABCA2 is expressed in 2′:3′-cyclic nucleotide-3′-phosphodiesterase (CNP)-positive Schwann cells in adult rat peripheral nerve [5]. In the present study, the expression of ABCA2 in human Schwann cells was investigated using benign vestibular schwannomas as well as normal peripheral nerve.

Vestibular schwannoma is one of the most common, benign intracranial tumors, originating from the vestibular branch of the acoustic nerve [7]. Benign vestibular schwannoma consists entirely of Schwann cells lacking compact myelin [7–10]. Histologically, schwannoma tissue typically features two areas, a fascicular (Antoni type A) area comprised of compact tissue with elongated spindle

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Table 1
Diagnosis of schwannoma examined

Patient no.	Diagnosis	Age	Sex	Size of tumor (cm)
1	Right VS	34	Male	2
2	Right VS	41	Male	2
3	Left VS	44	Female	3
4	Bilateral VS	48	Female	3.5
5	Left VS	53	Female	2
6	Right VS	40	Male	4
7	Left VS	56	Male	3.8

VS—benign vestibular schwannoma.

cells in interlacing or whorled bundles, and a hypocellular (type B) area with looser texture and some cyst formation [7]. In the present study, we show that ABCA2 is expressed in human schwannomas as well as in Schwann cells. The expression patterns of the novel marker ABCA2 in combination with conventional markers were examined to characterize the schwannoma cells in Antoni type A and type B areas.

2. Materials and methods

2.1. Patients

Schwannoma samples were taken from 7 patients with vestibular schwannoma (Table 1). Normal peripheral nerves were obtained from 3 adult patients: facial nerve from a patient with trauma and greater auricular nerve transected for prophylactic upper neck dissection from patients with cancer in the maxillary sinus, in Akita University School of Medicine with approval of the Akita University Institutional Committee. Neuropathological assessment of schwannoma was established by the institutional pathologists. Three to six pieces of schwannoma tissue were examined from each patient for histochemical or immunoblot analyses.

2.2. Immunohistochemistry

Vestibular schwannoma and normal nerve tissues were fixed with 4% paraformaldehyde, and cut in frozen sections (actual thickness estimated by confocal microscopy was 7–8 μm) using a cryostat at −20 °C. A total 285 slices obtained from 10 patients were analyzed for immunohistological studies. Double immunofluorescence labeling was performed as described previously [6]. In brief, the sections were reacted with primary antibodies at 4 °C for 3 days: a polyclonal rabbit antibody against ABCA2 (1:2500) [1], monoclonal mouse antibodies to S100β (S2532, Sigma, St Louis, MO; 1:1000), neurofilament 200 (N0142, Sigma; 1:5000), myelin basic protein (MBP) (MAB382, Chemicon International, Temecula, CA; 1:50), and polyclonal goat antibodies against nerve growth factor receptor p75 (sc-6188, Santa Cruz Biotechnology, Santa Cruz, CA; 1:500). The secondary antibodies used were indocarbocyanine (Cy3)-conjugated goat anti-rabbit immunoglobulin (Ig) G antibody (Amersham Pharmacia Biotech, Piscataway, NJ, 1:2000) for anti-ABCA2 antibody, fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG antibody (F2883, Sigma; 1:100) for anti-S100β, anti-neurofilament

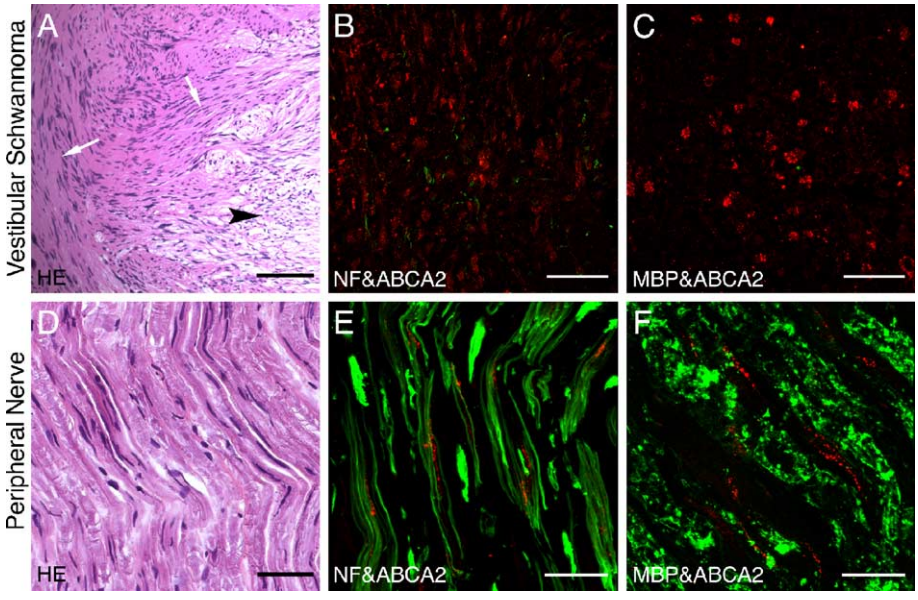

Fig. 1. Expression of ABCA2 in human vestibular schwannoma and peripheral nerve. (A) H&E staining of vestibular schwannoma tissue containing typical Antoni type A (white arrows) and type B (black arrowhead) areas. (B) Double immunofluorescence labeling of schwannoma tissues with anti-neurofilament 200 (NF; FITC, green) and anti-ABCA2 (Cy3, red), showing no expression of neurofilament. (C) Similar to panel (B), but with anti-myelin basic protein (MBP; FITC, green) and anti-ABCA2 (Cy3, red). (D–F) Similar to panels (A–C), but for human greater auricular nerve tissues. ABCA2-expressing cells are seen in peripheral nerve where neurofilament and MBP are abundantly expressed. Scale bars = 200 μm in panel (A), 50 μm in panels (B–D), 20 μm in panels (E–F).