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EXPRESSions

Expression of Caveolin-1 in the Rat Temporomandibular Joint

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ABSTRACT: This immunocytochemical study revealed the expression of caveolin-1, a major protein of caveolae, in the rat temporomandibular joint. In the synovial lining layer, immunoreactive products for caveolin-1 were detected on the cell membrane of the fibroblast-like type B cells, as confirmed by immunocytochemistry for heat shock protein 25. The cells in the articular disk, the articular layer and zone of proliferation of the mandibular condyle also showed intense immunoreactions for caveolin-1.

Key words: temporomandibular joint, rat, synovial lining cell, caveolae, caveolin-1

INTRODUCTION

Caveolae are flask-shaped invaginations of the plasma membrane that play important roles in cholesterol transport (Smart et al., 1996), transcytosis (for review, see Stan 2002), signal transduction (for review, Quest et al, 2004), and tumor suppression (Lee et al, 1998). Caveolin is a major component protein of the caveolae (Rothberg et al., 1992), which has three main isoforms--caveolin-1, 2, 3 (for review, Cohen et al, 2004). The localization of caveolin-1 has been reported to show a distribution pattern similar to caveolin-2 in the tissues, while caveolin-3 is restricted to muscle tissue (Tang et al., 1996). In particular, caveolin-1 has been regarded as a biochemical marker for the caveolae because mice with a null mutation of caveolin-1 completely lack any caveolae formation (Drab et al, 2001).

The synovial membrane in the temporomandibular joint (TMJ) consists of a cellular lining and a connective tissue sublining layer. Its synovial lining layer contains two types of synovial lining cells--macrophage-like type A and fibroblast-like type B cells (for review, Nozawa-Inoue et al., 2003)--as reported in other systemic joints (for review, Iwanaga et al., 2000). The type A cell is characterized by numerous vacuoles, lysosomes, nuclei with rich heterochromatin, and cell surface filopodia while the type B cell possesses a well-developed rough endoplasmic reticulum (rER), a nucleus with less chromatin, and cytoplasmic long projections. In addition, some ultrastructural investigations have pointed out that the fibroblast-like type B cells had invaginations reminiscent of caveolae in their cell membrane (Graabæk, 1984; Iwanaga et al., 2000; Nozawa-Inoue et al, 2003). However, both the nature and the functional significance of this structure remain unclear.

Previous studies have confirmed the expression of caveolin-1 in various kinds of cells including adipocytes, endothelial cells, and fibroblasts in most tissues (for review, Quest et al, 2004). An analysis of the human fibroblast-like synoviocytes of patients suffering from

arthritis has reported caveolin-1-immunoreactivity in cultured cells (Riemann et al., 2001). To date, however, no information is available regarding the expression of caveolin-1 in the normal TMJ in vivo. In the present study, therefore, we aimed to demonstrate the localization of caveolin-1 in the rat TMJ by immunocytochemical techniques. Furthermore, a double immuno-staining using antibodies to caveolin-1 and heat shock protein 25 (Hsp25) was employed to identify the relationship between caveolin-1 reactive cells and fibroblast-like type B cells because Hsp25 has been reported as a specific cellular marker for the type B cells (Nozawa-Inoue et al., 1999; Andoh et al., 2001; Ikeda et al., 2004).

MATERIALS AND METHODS

All experiments were performed under the guidelines of the Niigata University Intramural Animal Use and Care Committee (approval number 150).

Five Wistar rats (4 weeks old, weighing approximately 100 g) were anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg) and perfused with a fixative containing 4% paraformaldehyde and 0.025% glutaraldehyde in a 0.067M phosphate buffer (pH 7.4). After decalcification, the removed tissue blocks were equilibrated in a 30% sucrose solution for cryoprotection and embedded in OCT compound (Leica, Nussloch, Germany). Serial sagittal sections including the TMJ were cut at a thickness of 7 μm in a cryostat (HM-500; Carl Zeiss, Jena, Germany) and mounted onto silane-coated glass slides.

The cryostat sections were processed for immunocytochemistry for caveolin-1 using a commercially available avidin-biotin-complex (ABC) kit (Vector Lab. Inc., Burlingame, CA, USA). The sections were reacted overnight at 4˚C with a monoclonal antiserum against caveolin-1 (1: 600; BD Transduction Laboratory, San Diego, CA) which recognizes caveolin-1 from the human, mouse, rat, dog, and chick (manufacture's instruction). The bound primary antibody was then localized using a biotinylated anti-mouse IgG (1: 100; Vector Lab. Inc.) and subsequently with ABC conjugated with peroxidase (Vector Lab. Inc.) for 90 min each at room temperature. Final visualization used 0.04% 3, 3'-diaminobenzidine tetrahydrochloride and 0.002% H_2O_2 in a 0.05 M tris-HCl buffer (pH 7.6). Immunoreacted sections were counter-stained with methylene blue.

Some immunostained sections without counter-staining were post-fixed in 1% OsO_4 reduced with 1.5% potassium ferrocyanide for 1 h at 4˚C, dehydrated in an ascending series of ethanol, and finally embedded in epoxy resin (Epon 812; Taab, Berkshire, UK). Plastic sections, 1 μm thick, were stained with 0.03% methylene blue. Ultrathin sections were briefly

double-stained with uranyl acetate and lead citrate, and examined in a transmission electron microscope (H-7000; Hitachi Co. Ltd, Tokyo, Japan).

In fluorescent immunohistochemistry, sections were incubated with the same primary and secondary antibodies, and then reacted with avidin D-fluorescein (Vector Lab. Inc.). For demonstration of Hsp25 localization in the synovial membrane, they were further incubated with a rabbit polyclonal antiserum against Hsp25 (1:5000; Stressgen Biotechnologies, Victoria, Canada), followed by biotinylated goat anti-rabbit IgG (Vector Lab. Inc.) and avidin D-rhodamine (Vector Lab. Inc.). After rinsing, the single- or double-labeled sections were cover-slipped with a Vectashield mounting medium with 4', 6-diamidino-2-phenylindole (DAPI) (Vector Lab. Inc.), and finally examined in a confocal laser scanning microscope (LSM 510; Carl Zeiss).

Immunocytochemical controls were performed by: 1) replacing the primary antisera with non-immune sera or phosphate-buffered saline; and 2) omitting the anti-mouse/rabbit IgG. These control sections did not reveal any immunoreaction. The characterization of Hsp25-antiserum has been previously reported (Nozawa-Inoue, 1999).

RESULTS AND DISCUSSION

Immunocytochemistry for caveolin-1 demonstrated an intense immunoreaction in various cellular elements in the rat TMJ, including the synovial lining cells, vascular endothelial cells, fibroblasts, stromal cells in the articular disk, and a part of the cells in the mandibular condyle (Figs. 1, 2). The inner surface of the articular capsule, except for the temporal bone, the mandibular condyle and the articular disk, was covered with a synovial membrane. The synovial membrane protruded in the postero-superior potion to form synovial folds extending into the articular cavity (Fig. 1a). Throughout the synovial membrane, the synovial lining layer consisted of caveolin-1-immunopositive and negative cells (Fig. 1b). Several fibroblasts in the sublining tissue and vascular endothelial cells also had a positive immunoreaction for caveolin-1 in their cell membranes (Fig. 1b). Ultrastructurally, immunoreactive products for caveolin-1 were observed as electron dense products. The immunoreaction was localized on the cell membrane of the synovial lining cells which had well-developed rER, a large nucleus with less heterochromatin, and numerous flask-shaped invaginations in their cell membrane (Fig. 1c). These flask-shaped structures were intensely immunoreacted with the caveolin-1 antibody (Fig. 1d), suggesting that they were caveolae. Furthermore, a double-labeling immunohistochemistry using an additional antibody to Hsp25 demonstrated that the caveolin-1-positive lining cells expressed Hsp25-immunoreactivity (Figs. 2a-c). The caveolin-1- and Hsp25-immunoreactions appeared dot-like on the cell membrane and diffusely in the cytoplasm, respectively. On the other hand, the lining cells without the caveolin-1-immunoreaction had lysosomes and surface folds like filopodia (Fig. 1c), indicating that they were macrophage-like type A cells. This evidence indicates the synovial lining cells reactive to caveolin-1 were fibroblast-like type B cells.

Most of the articular disk and a part of the mandibular condyle also contained

caveolin-1 positive cells (Figs. 2d, e). Since the terminology for the cartilaginous layer of the rat condyle has not been well established yet, we divided the mandibular condyle into four zones based on the classification by Carlson (1994): the articular layer, zones of proliferation, maturation, and hypertrophy. Immunostaining revealed the localization of caveolin-1-immunoreactivity in many cells in the articular layer and zone of proliferation but only in a few cells in the zones of maturation and hypertrophy (Fig. 2e). To our knowledge, this is the first report to reveal a presence of caveolin-1 in the fibroblast-like type B cells, stromal cells in the articular disk and mandibular condyle in normal rat TMJ.

The functional significance of caveolin-1 in the TMJ remains unclear. It may regulate calcium transduction and/or transport cholesterol in their cells the same as in other mammalian cells under normal conditions. Interestingly, caveolin-1 is known to inhibit directly inducible nitric oxide synthases (iNOS) activity in vivo (Garcia-Cardena et al., 1997). The activation of the iNOS is involved in the pathogenesis of inflammatory arthritis, including rheumatoid arthritis (Grabowski et al., 1997; Pelletier et al., 1999). In the temporomandibular joint, recent studies have described the existence of iNOS in the human inflamed synovial membrane (Homma et al., 2001; Takahashi et al., 2003) and the interleukin-1β stimulated rabbit fibrocartilage cells (Agarwal et al., 2001). Supposing that caveolin-1 inhibits the iNOS activity in the normal TMJ as well as long bone joint, it can be assumed that the interaction between caveolin-1 and iNOS may affect the pathophysiology of arthritis in TMJ. Further investigation is needed for clarifying the functional significance of the caveolin-1 under normal conditions.

Notably, the mandibular condyle showed caveolin-1-immunoreactivity in the cells in the articular layer and zone of proliferation (Fig. 2e), consistent with previous reports on its expression in the flattened proliferating cells, not maturative chondrocytes in the rat tibial

(Schwab et al., 1999) and the avian tibiotarsal cartilage (Hollins et al., 2002). These findings imply the possible involvement of caveolin-1 in proliferation and differentiation during chondrogenesis. Its detailed functions during chondrogenesis constitute one subject for a future study.

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Figure legends

Figure 1.

a. Expression of caveolin-1 immunoreactions in the rat TMJ. Frozen sagittal section, counter-stained with methylene blue. An arrow indicates the anterior direction. A few synovial folds protrude into the articular cavity, in particular in the posterior potion. Intense immunoreactivity for caveolin-1 is observed in the synovial membrane (arrowheads). A part of the cells in the surface of mandibular condyle (C) and temporal bone (T), and the stromal cells in the articular disk (D) also show caveoloin-1-immunoreaction. **b.** Caveolin-1 immunoreactivity in the synovial membrane. Plastic section, counter-stained with methylene blue. The synovial lining layer consists of a one- to three-layered arrangement of immuno-positive and -negative lining cells. Immunoreactions are localized in the cell membrane of the lining cells with round and clear nuclei (open arrowheads). Several fibroblasts in the sublining tissue (arrow) and endothelial cells (arrowheads) are also positive in caveolin-1-immunoreaction. **c.** Immunoelectron micrograph of synovial membrane. The fibroblast-like type B cells (B) with caveolin-1 immunoreactivity contain much rough endoplasmic reticulum and a large nucleus with less heterochromatin. Immunonegative macrophage-like type A cells (A) are characterized by heterochromatin-rich nuclei, lysosomes, and surface filopodia (arrows). **d.** Higher magnification of the boxed area in **c**. Immunoreactive materials assemble in the flask-shaped invaginations of the cell membrane. Asterisk: articular cavity. Scale bars = a: 600 μm, b: 10 μm, c: 5 μm, d: 0.2 μm.

Figure 2.

a-c. Confocal microscopic images of a double stained section by antisera against caveolin-1 (top

panel), Hsp25 (middle panel), and a merged image (bottom panel) in the synovial membrane. The synovial lining cells with cytoplasmic Hsp25-immunoreaction (red) show caveolin-1-immunoreactivity (green) around the cell membrane. **d.** Confocal microscopic images of the cells in the articular disk. Caveolin-1 positive cells (green) appear stellate in their profile. **e.** Distribution of caveolin-1 immunoreactions (green) in the mandibular condyle. The rat mandibular condyle is divided into four layers from the surface: the articular layer (A), zones of proliferation (P), maturation (M), and hypertrophy (H). The cells in the articular layer and the zone of proliferation show intense immunoreactions. Asterisk: articular cavity. Scale bars = a-d: 25 μm, e: 100 μm.