Age-related changes in microvillar cells of rat olfactory epithelium

Bum Sun Kwon a,b, Min Kyu Kim c, Woo Ho Kim c, Jung-Soo Pyo d, Young Hee Cheon d, Choong Ik Cha a, Seon Young Nam e, Tai-Kyoung Baik d,∗, Byung Lan Lee a,∗∗

a Department of Anatomy, Seoul National University College of Medicine, 28 Yongon-dong, Jung-gu, Seoul 110-799, South Korea
b Department of Rehabilitation Medicine, Dankook University College of Medicine, Cheonan 330-714, South Korea
c Department of Pathology, Seoul National University College of Medicine, Seoul 110-799, South Korea
d Department of Anatomy and Neurosciences, Eulji University School of Medicine, Daejon 301-832, South Korea
e Laboratory of Radiation Effect, Radiation Health Research Institute, Korea Hydro and Nuclear Power Co., Seoul 132-703, South Korea

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Abstract

The nature and function of microvillar cells (MVCs) of the mammalian olfactory epithelium (OE) are little understood. Previous studies have examined MVC morphology in the developing and mature OE, but not in the aged OE. The present study investigated the effect of aging on MVCs of the OE in male Sprague–Dawley rats using histological and immunohistochemical methods. OE of aged rats contained MVCs with marked hypertrophy and swollen end-feet, which reached the basement membrane. Such MVC features were not observed in the young OE. These MVC changes were more conspicuous in proximity to severely degenerated olfactory receptor neurons (ORNs) and supporting cells. The ratio of the number of MVCs to that of supporting cells increased with aging; however, MVCs in the aged OE were not proliferating cell nuclear antigen-immunoreactive. In addition, the total cell population was decreased in the aged OE. Thus, our results suggest that MVCs are non-neuronal and that they are more resistant to aging compared to ORNs and supporting cells.

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Keywords: Olfactory epithelium; Microvillar cell; Hypertrophy; Aged rat
The effect of aging on MVCs has not been previously reported. The purpose of the present study was to seek new insights into MVC function by examining age-related changes in MVCs using histological and immunohistochemical methods.

Specimens were obtained from young (3 months old, n=5) and aged (25 months old, n=5) healthy male Sprague-Dawley rats. All animals were treated according to the NIH guide for the care and use of Laboratory Animals (NIH Publication No. 86-23, revised in 1996). Animals were perfused transcardially under ethyl ether anesthesia with a flush solution of 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS, pH 7.4. Nasal cavities were removed and immersed in the same fixative at 4°C overnight, and then decalcified in an RDO decalcifier solution (Apex Engineering Products Corp., Plainfield, IL, USA) for 10 h. Specimens were then sectioned in the coronal plane at a thickness of 5 mm at the level of the second upper molar teeth, embedded in 5% gelatin and sectioned at a thickness of 200 μm using a vibrating microtome.

For immunohistochemistry for proliferating cell nuclear antigen (PCNA) and glial fibrillary acidic protein (GFAP), 200 μm sections were embedded in paraffin, resectioned at a thickness of 6 μm, deparaffinized and then treated with 0.01 M citrate buffer (pH 6.0) for 15 min in a microwave oven for antigen retrieval and with 3% hydrogen peroxide for endogenous peroxidase inhibition. To determine whether MVCs proliferate in the aged OE, sections were incubated with 1:100 monoclonal mouse anti-PCNA (Dako, Glostrup, Denmark). In addition, to observe the astroglial nature of MVCs, sections were incubated with 1:200 goat anti-GFAP (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). For OX-42 immunohistochemistry to detect the microglial nature, specimens were infiltrated with 30% sucrose overnight, cut into 10 μm sections with a cryostat, thawed and mounted on gelatin-coated slides. The remaining immunostaining procedures were the same as that for GFAP except for the primary antibody, which was mouse anti-OX-42 solution (1:200) (Serotec Inc., Raleigh, NC, USA). Subsequently, tissue sections were incubated with corresponding biotinylated secondary antibodies and with the avidin–biotin–peroxidase complex of an ABC kit (Vector Labs, Burlingame, CA, USA). Visualization was performed with 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO, USA). To rule out non-specific background staining, primary antibodies were replaced with the non-immune normal host animal serum of the corresponding secondary antibody.

For histological observation, 200 μm sections were fixed with 2.5% glutaraldehyde, post-fixed in 1% osmium tetraoxide, and embedded in Epon 812 (Ted Pella, Redding, CA, USA). Semithin sections were then cut and stained with toluidine blue. Morphological analysis was performed on sections where the complete crests and turbinate were clearly present. For morphological analysis, the OE of the crest was divided into four regions: (1) the OE of the concave surface (cC); (2) the proximal convex surface (cvP); (3) the middle convex surface (cvM) and (4) the distal convex surface (cvD) (Fig. 1A and B). Morphological features including a superficial location, a tapered apex with microvilli, pale nucleus and cytoplasm were used as the criteria for the determination of MVC. The ratio of MVCs to supporting cells was quantified and the thickness of the OE was analyzed by measuring the distance from basement membrane to apical surface at the midpoints of each of the above regions of OE. Relative densities of cell types (i.e. ORNs, supporting cells,
basal cells and MVCs) were quantified by cell counts within a high-power field (1000×) of a light microscope (Olympus, Tokyo, Japan). Difference of means between young and aged OE was analyzed using a Student’s t-test with p < 0.05 used as the threshold for statistical significance.

For transmission electron microscopy (TEM), thin sections were prepared from the same blocks as those used for light microscopy, stained with uranyl acetate and lead citrate and examined on 3 mm grids with 200 square meshes using a JEM-1200EX II transmission electron microscope (JEOL, Tokyo, Japan).

MVCs in semithin sections were easily identified with toluidine blue staining (Fig. 1C–J), which was not the case with hematoxylin–eosin stained materials (data not shown). Region-specific histological differences were seen along the OE. MVCs in the young OE were located slightly superficial to supporting cell nuclei and typically showed a tapered apex and lighter nucleus and cytoplasm than surrounding supporting cells (arrows in Fig. 1C, E, G and I). In young rats, the OE was thicker and contained more cells in the convex region (cv) than in the concave region (cC) (Fig. 1A, C, E, G and I, Table 1). In the aged OE (Fig. 1B, D, F, H and J), MVCs showed marked hypertrophy and were frequently observed, especially in cvP and cvD regions where supporting cells and ORNs were severely degenerated (Fig. 1D and H). Conversely, enlarged MVCs were less frequently observed in cvM and cC of the aged OE where supporting cells and ORNs showed less degeneration (Fig. 1F and J). In addition, the single cytoplasmic process of enlarged MVCs descended to the basement membrane, which was not evident in young rats (double arrow in Fig. 1H).

Transmission electron micrographs of the young OE (Fig. 2A and B) demonstrated ultrastructural characteristics of the MVC such as an apical surface covered by microvilli, the cell apex with a narrow neck, and electron-lucent cytoplasm which are consistent with those described by Moran et al. [8]. Also observed were the cytoplasmic process originating from the basal pole of the MVC and passing toward the lamina propria as well as the indented nucleus of the MVC previously reported by Andres [1]. Fig. 2C shows an aged OE with a hypertrophied MVC surrounded by degenerating OE cells. Also a dilated apical surface of a MVC covered by microvilli was seen.

Table 1 shows OE thicknesses and cell population data. The convex surfaces of aged OEs were thinner and showed lower cell densities than those of young OEs (p < 0.05). Although thinning and scant cell populations were previously reported to be characteristic findings in the aged OE [10,11],

PCNA immunohistochemistry (Fig. 3) showed that, in the young OE, PCNA immunoreactivity was frequently observed in the nuclei of horizontal basal cells adjacent to the basal lamina, but less frequently and more weakly observed in the aged OE. However, in the nuclei of MVCs, PCNA immunoreactivity was not observed in either the young or the aged OE.

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Thus, Loo et al. [6] reported regional differences severe with age and overwhelm the regenerative capacity of that environmental insults could accumulate or become more anterior part than in the posterior part of OE. They suggested F1 hybrid rats, more severe changes in the proliferation of showed that, in barrier-raised Fischer 344X Brown Norwayences in aged rat OE were described by Loo et al. [6] . They suggested that the cytoplasmic process of hypertrophied MVCs was demonstrated in the aged OE, which pertrophied MVCs was shown more frequently near the metaplastic areas, especially in the cvP and cvD of the crest (data not shown) where the ORNs and supporting cells showed severe degenerative changes. Since supporting cells of the OE seemed to act as glia [16] and MVCs have morphologic characteristics similar to supporting cells such as apical microvilli and a cytoplasmic process reaching the basement membrane, we wondered whether MVCs also act as glia and influence the survival of ORNs. However, MVCs were not immunoreactive for GFAP or OX-42 (data not shown).

In the present study, the finding of an increased ratio of MVCs to supporting cells in the aged OE could be attributable to cell division of MVCs; however, the lack of PCNA immunoreactivity in the nuclei of MVCs of either young or aged OE suggests otherwise. The increased ratio of MVCs to supporting cells may have been due entirely to degeneration of surrounding supporting cells without significant proliferation of MVCs, which is supported by a decrease in the total cell population in the aged OE.

In conclusion, in the present study, the morphology of hypertrophied MVCs was demonstrated in the aged OE, which suggests that MVCs are non-neuronal. In addition, the ratio of MVCs to supporting cells increased, and total cell population decreased in the aged OE. Thus, MVCs seem to be more resistant to aging compared to ORNs and supporting cells. Since there are several other cell types that also have microvilli, further investigation of MVCs in aged OE is needed to understand the mechanism of age-related olfactory dysfunction.

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References


Table 2

Comparison of the MVC to supporting cell ratios of young and aged OEs in four separate regions of the crests (n = 10).

<table>
<thead>
<tr>
<th>Context surface</th>
<th>Concave surface</th>
</tr>
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<tbody>
<tr>
<td>Young OE</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td>Aged OE</td>
<td>0.56 ± 0.28††</td>
</tr>
<tr>
<td></td>
<td>0.52 ± 0.26††</td>
</tr>
</tbody>
</table>

Data represent mean ± S.D.
† p < 0.05 by Student’s t-test compared with the young OE.
†† p < 0.05 by Student’s t-test compared with the concave surfaces.