Title

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Age-related Differences in Localization of Beta-defensin-2 in Human Gingival Epithelia

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Abstract
Defensins are known to play an important role in defense against bacteria. It is also known that immunity against infection is compromised with age. The purpose of this study was to evaluate the localization of human beta-defensin (HBD)-2 in human gingival epithelia according to age. Gingival epithelia in maxillary premolar buccal normal regions was immunohistochemically stained for HBD-2. Specimens were divided into two groups: 6 cases in a young group (<20 years old) and 7 cases in an elderly group (>50 years old). Expression of HBD-2 in gingival epithelium in young subjects was mostly detected in the superficial layer of the parakeratinized layer, while some areas of the spinous cell layer in elderly subjects were positive for HBD-2, as was the superficial layer. Two cases in the young group and 5 cases in the elderly group immunoreacted with HBD-2 in the spinous cell layer. Furthermore, immunoreaction was stronger in the elderly group. The results revealed HBD-2 positive cells in spinous cells in the elderly group and in the parakeratinized layer in the young group.

Key words: Beta-defensin-2—Gingival epithelium—Aging

Introduction
Defensins are single-chain, strongly cationic peptides with a molecular weight of 34 kDa. They can be classified into alpha- and beta-defensins based on the sequence relationship of their three intermolecular disulfide bonds. Alpha-defensins are expressed in neutrophils and intestinal Paneth cells. Human beta-defensins (HBDs) are mainly produced by the epithelial cells of many organs, including skin, lung, kidney, pancreas, uterus, eye, and nasal and oral mucosa. Expression of the first three HBDs (HBD1–3) is well documented in oral tissues. HBD-1 is constitutively expressed, and may be modulated by inflammation. HBD-2 and HBD-3 are inducible in cells with stimulation by pro-inflammatory cytokines such as IL-1beta, TNF-alpha, IFN-gamma and microorganisms. Therefore, HBD-1 may influence normal epithelial interactions with the commensal flora, and HBD-2 and HBD-3 may participate in the host defense response to enteric microbes. HBD-2 was originally detected in
the skin of patients with psoriasis\textsuperscript{8,14}. Anti-
microbial activity of natural beta-defensins has
been observed against \textit{Pseudomonas aeruginosa},
\textit{Candida albicans} and so on. 

Localized expression of HBD-2 was shown in
both normal and inflamed oral epithelia\textsuperscript{12}. 
Although HBD-2 is expressed only in the pres-
ence of infection or inflammation in most
tissues, including skin, trachea and gut epi-
thelium, it is expressed in normal uninfamed
oral epithelia\textsuperscript{8,11,14}. Therefore, oral epithelia
may be the most appropriate site for obser-
vation of individual differences in HBD-2
expression. The production of defensins is an
ancient defense mechanism. It is elicited by
contact between microorganisms and epithe-
リアル cells, and is completely independent of
leukocyte-dependent immune defense mecha-
nisms\textsuperscript{13}. HBD-2 plays an important role the
defense against bacteria. However, immunity
against infection is compromised with age, and
there have been few reports about changes in
the localization of HBD-2 associated with aging.
Therefore, the purpose of this study was to
evaluate the localization of HBD-2 expression
in human gingival epithelia according to age.

Materials and Methods

Thirteen healthy specimens of gingival
mucosa obtained from biopsies performed
following diagnosis of epulis in the maxillary
premolar buccal region were used in this study.
The specimens were divided into two groups:
one from subjects under 20 years of age as a
young group (11-year-old female, 14-year-old
female, 17-year-old female, two 17-year-old
males, 19-year-old male) and the other from
subjects over 50 years of age as an elderly group
(two 59-year-old females, 72-year-old female,
two 72-year-old males, 74-year-old female, 78-
year-old male). Specimens were fixed in buff-
ered 10\% formalin, and then processed and
embedded in paraffin. The embedded tissues
were oriented such that approximately 5\mu m
sections could be cut at 90 degrees to the
epithelial surface. Specimens were chosen after
confirmation that they were without candidi-
asis or other microinfections at the surface
of the regions to be observed using PAS stain-
ing (data not shown). Immunohistochemical
staining was then performed using a primary
antibody to HBD-2 (1:100, Rabbit anti-beta
defensin-2 (human), Peptide, Japan), and the
specimens were observed by light microscopy
at a site distant from the lesions. Informed
consent was obtained for use of all specimens.

Results

HBD-2 expression was found in the cyto-
plasm of epithelial cells located throughout
the upper spinous cell layer to the parakera-
tinized layer of the gingiva. Expression of
HBD-2 in the gingival epithelium from young
subjects was mostly in the superficial layer
of the parakeratinized layer (Fig. 1a), while
some areas of the spinous cell layer from
elderly subjects were positive for HBD-2, as
was the superficial layer (Fig. 1b). Two cases in
the young group (Table 1) and 5 cases in the
elderly group (Table 2) immunoreacted with
HBD-2 in the spinous cell layers. Furthermore,
HBD-2 immunoreaction was stronger in the
elderly group.

Discussion

HBD-2 positive expression in the spinous
cell layer suggests keratinization in elderly
people. Sawaki \textit{et al.}\textsuperscript{12} found HBD-2 expression
in the cytoplasm of epithelial cells throughout
the upper spinous cell layer to the parakerat-
inized layer of the epithelia, both in normal
skin and in buccal skin with candidiasis. Dale
\textit{et al.} reported that in adult human gingiva,
HBD-2 expression occurred within well dif-
ferentiated cells in the upper spinous and
granular cell layers\textsuperscript{15}. In this study, HBD-2
expression in the young group was mainly
detected in the superficial layer of the gingival
epithelium. Although HBD-2 is reportedly
active against Gram-negative bacteria, it is also
well known to exert an anti-microbial effect
against \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}
169 and Candida albicans. On the other hand, it is known that the prevalence of Candida albicans is higher in the elderly. Feng et al. obtained results supporting the hypothesis that HBD controls fungal colonization through hyphal induction, direct fungicidal activity and inhibition of candidal adherence. In other words, candida and other microorganisms upregulate HBD-2 activity, widening localization of HBD-2. The higher frequency of bacteria carriers in elderly people is high, may be the reason for observation of HBD-2-positive cells in the spinous cell layer in elderly people. Although specimens without apparent candidiasis were used in this study, these results suggest that the difference in expression of HBD-2 in elderly subjects and young subjects is a response to microorganisms. The turnover of oral epithelium in elderly people is slower, and single cell keratinization, metabolic abnormality in protein differentiation, can often be observed in the spinous cell layer in elderly people. This suggests that keratinocytes age before keratinization and single cell keratinization can be seen in the spinous cell layer. We believe this to be the reason that HBD-2 positive cells were observed in spinous cells in the elderly group, and in the parakeratinized layer in the young group.

Table 1 HBD-2-positive area in young group

<table>
<thead>
<tr>
<th>age sex</th>
<th>11</th>
<th>14</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>19</th>
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<tbody>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>spinous layer</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2 HBD-2-positive area in elderly group

<table>
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<tr>
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<th>59</th>
<th>72</th>
<th>72</th>
<th>72</th>
<th>74</th>
<th>78</th>
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</thead>
<tbody>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>spinous layer</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1 Immunohistochemical staining of HBD-2 (Original magnification ×40) and H-E staining in inset a: 14-year-old female; note that HBD-2 immunoreacted in superficial layer of parakeratinized layer. b: 74-year-old female; note that HBD-2 reacted not just in superficial layer, but in spinous cell layer also.
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References


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