

論文要旨等報告書

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学位論文題名	Expression of human β -defensins -1, -2, and -3 in non-inflamed pseudocyst, mucoceles.(ヒト非炎症性嚢胞におけるペータディフェンシン-1、-2、-3の発現)
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学位論文内容の要旨

The human mucocele is one of the most common oral lesions generally formed by rupture of an excretory duct of the minor salivary gland in the proper layer of the oral mucosa. Once the mucocele was formed, the glandular structure of the minor salivary gland disappears gradually. The mucous storage increases, thereafter, and the blood cells become to be infiltrated in the lumen of pseudocyst. Defensins are known as one of the biophylaxis factors (Barnathan et al., 1997), that show antimicrobial activities against bacteria, fungi and enveloped virus. Defensins are small cationic peptides and are divided into α -, β - and θ -defensins. Human α -defensins have been indentified 8 subtypes, 4 of which are found in neutrophili azurophil granules, called human neutrophil defensin (Gantz et al., 1985). Human β -defensins have been divided into 4 subtypes and are, however, usually produced by epithelial cells in many organs (Harder et., 1997). The expression of human β -defensin has mostly been found in infected and inflamed diseases generally. For instance, few studies demonstrated the expression of HBDs in the minor salivary gland indicated the immunoreactivity of HBDs only in the epithelial cells of the duct (Abiko et al., 2003). However, the expression of HBDs has not been investigated in non-inflamed and non-infected pseudocysts like mucocele. Therefore, the expressions of human β -defensin-1, -2 and -3 in mucocele were investigated in this study to establish the relationship between the expression of cytokines and HBDs in mucocele and to determine the possible expression mechanism of HBDs in mucoceles.

Twenty eight specimens of mucocele from the lower lip of patients with mucosal cyst were investigated in this study. Informed consent was obtained from each patient according to the Guidelines of Okayama University Hospital prior to this study. After surgical operation, the samples were fixed by 10% formaline buffered with 0.1M phosphate for 6 hours at room temperature and dehydrated by graded concentrations of ethanol prior to embedding in Paraplast+. Serial sections (7 μ m thickness) were cut and mounted on silane coated slides glass. Antigen retrieval method was also performed by using a kitchen microwave oven with a power of 360W to activate antigens after deparaffinization. Irradiation was performed using the scheme of 3 cycles of 2min irradiation and 15 sec interval of cooling on ice. In order to reduce non specific background, the sections were blocked with normal serum. Polyclonal rabbit antibodies directed against HBD-1, -2, -3 (Peptide Institute Inc., Osaka, Japan), IL-1 β , TGF- α , INF- γ (Abcam, Japan) and TLR-2 (Biochain, CA, USA), were used with ABC Reagent Kits (Vector Laboratories, Burlingame, CA, USA). Monoclonal mouse antibody

was directed against TNF- α (Abcam, Japan) and involucrine (Via Gramsci, Roma, Italia) and keratin (Cymbus Biotechnology, Hampshire, UK) were used as negative controls. To block endogenous peroxidase activity, 1M sodium azide was added to the color reaction medium. After immunoreactions were completed, sections were stained with methylgreen. May-Grünwald's staining was also used to distinguish the neutrophils from the other cells. The floating cells were identified by their characteristic morphological appearances. To calculate the positive cell ratio of the immunoreactions, a temporary square frame (TS, 100 μm x 50 μm) was randomly placed on the microscopic field in each mucocoele. Each temporary square were divided into two equal squares (50 μm x 50 μm), the inside unit (central part of the cyst) and the outside unit (peripheral part of the cyst). The number of cells was randomly counted in 50 TSs from each mucocoele sections to compare signal intensities between both units. The signal intensity in both units was compared for statistical analysis and then Student's t-tests were performed to analyze the differences between inside and outside units.

The expression of HBDs, TNF- α , TGF- α and TLR-2 were found in mucocoeles but no signals for IL1- β and INF- γ were found. Floating cells with positive signals for HBD-2 were found in all mucocoele specimens. However, 93% and 73% specimens also exhibited positive immunoreactivities for HBD-1 and -3 in floating cells of mucocoele, respectively. Positive signals for anti HBD-1, -2 and -3 were observed in the cytoplasm of neutrophils, lymphocytes or macrophages. The expression of HBD-1 and -2 have also been found in the endothelial cells of blood vessels surrounded mucocoele and in the epithelial cells of the excretory duct of minor salivary gland. The population of anti HBD-2 positive cells per mucocoele was the highest of all, whereas the population of anti HBD-3 retained the lowest percentage. The signal intensity of positive cells located close to center of pseudocyst was significantly higher than those located in peripheral areas of pseudocysts. The population of positive cells located close to the center was higher than those located in peripheral areas. There was no significant difference in the cell density between the inside and outside units of mucocoeles. No positive neutrophils for anti involucrin or anti keratin were found. The expression of TNF- α and TGF- α has been found in the floating cells in mucocoeles, in the endothelial cells of blood vessels and in the epithelial cells of the excretory duct of minor salivary glands. However, TLR-2 was expressed in the floating cell of mucocoele and in the epithelial cell of the excretory duct of minor salivary gland.

It has been reported that TNF- α induced the expression of HBD-1 and -2 in gingival keratinocytes (Joly et al., 2005) and TGF- α induced the expression of HBD-3 in human keratinocytes (Sorensen et al., 2003). It could be speculated that the expression of HBDs in non-inflamed pseudocyst, mucocoele is a consequence of an up-regulation by cytokines, like TNF- α and TGF- α . It has also been able to consider that TLR-2 might induce the expression of HBD-2 in lung epithelial cells. Whereas, the signal intensities of them showed anti-gradient expressions. These hypothetical observations indicate that TLR-2 in mucocoele could open an opportunity to start the possible expression mechanism which might be associated with some ligands like TNF- α . Further work will be done to find some other ligands which will aid in the determination the possible expression mechanism of HBDs in non-inflamed and in non-infected pseudocyst, mucocoeles.

論文審査結果の要旨

デフェンシン類は、ヒトばかりでなく多くの生物に存在する抗菌ペプチドの代表例である。1985年に Ganz がヒト好中球中にアルファデフェンシンを見だし、その後、ヒト上皮中にベータデフェンシン(HBD)が、見いだされると皮膚科をはじめ多くの医系研究者に注目されるようになった。歯科関係においては、Mizukawa や Abiko により、唾液や口腔粘膜病変の組織にアルファデフェンシンやベータデフェンシンが見いだされた。現在まで HBD は、主に炎症性組織の上皮に発現する抗菌ペプチドであるとされるが、その発現メカニズムは、不明な点も多い。

そこで、今回の研究は非炎症性偽嚢胞である粘液嚢胞をテーマに選び、HBD-1, -2, -3 と HBD を誘導するとされる TNF- α , IL-1 β , TLR-2 の発現を免疫組織学的に検討し、炎症以外の発現メカニズムを明らかにすることを目的としている。

申請論文は、以下の内容を示すものであった。

1 粘液嚢胞において、浮遊細胞で HBD-1, -2, -3 の発現を認めた。HBD-2 の発現は、全ての症例の粘液嚢胞中の浮遊細胞に認めたが、HBD-1 と HBD-3 は、それぞれ 93%と 73%の症例で発現がみられた。また、その発現強度は、HBD-2 が最も強く、次に、HBD-1 であり、HBD-3 が最も弱かった。

2 嚢胞の内腔を周辺部と中央部に分けると、浮遊細胞における陽性細胞の分布は、周辺部よりも中央部において、より多く認められ、強い染色強度を示す例が多かった。粘液嚢胞における HBD の発現と TNF- α , IL-1 β , TLR-2 との関連は、明らかでなかった。

本研究により、HBD は、非炎症性嚢胞である粘液嚢胞でも、発現が確認された。従って HBD は、炎症以外の未知のメカニズムによっても制御される可能性を示唆した。

ゆえに、この論文は、博士(学術)の学位授与に値するものと判定した。