〔Mini Review〕

Epithelium−derived antimicrobial peptide RNase 7 in oral health and disease : A mini−review

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Abstract

The oral epithelium is normally protected from various stimuli within the diverse environment of the oral cavity. This review focuses on RNase 7, a potent epithelium−derived antimicrobial peptide, and discusses its importance in oral health and disease. RNase 7 exhibits high antimicrobial activity against a broad spectrum of microorganisms. The expression of RNase 7 in the oral epithelium is induced by stimulation with oral bacterial biofilms and several cytokines. Studies have identified numerous beneficial effects of this peptide, such as the prevention of bacterial infections and its use as a potential therapeutic agent. However, there is little information on the alterative expression and roles of RNase 7 in oral inflammatory disease and cancer, which merits further investigations.

Introduction

Antimicrobial peptides (AMPs) are small, positively charged, amphipathic structures possessing both hydrophobic and hydrophilic regions of variable amino acid composition (length < 100 amino acids) (Brogden, 2005). They are also known as host defense peptides because of their essential role in the innate immunity system. AMPs exhibit broad−spectrum antimicrobial activities against various microorganisms, including Gram−positive and Gram−negative bacteria, fungi and viruses (Hancock & Diamond, 2000). Several AMPs are known to be effective against multi−drug resistant bacteria, and possesses a low propensity for resistance development (van t Hof W et al., 2001). Among the 112 host defense peptides identified in humans, the cathelicidins, defensins and ribonuclease families are the most prominent (Wang et al., 2009). RNase A superfamily is one of the best−characterized ribonucleases and consists of 13 subtypes (Becknell et al., 2015). Among them, RNase 7 is most abundantly found in the skin and expressed in various epithelial tissues such as the genitourinary, respiratory, and gastrointestinal tissues (Amatngalim et al., 2015 ; Eberhard J et al., 2008 ; Harder & Schroder, 2002 ; Spencer et al., 2011). RNase 7 expression has been reported in the oral epithelium, and it is suggested to be involved in host defense in the oral cavity (Eberhard et al., 2008). Although certain types of host defense peptides including cathelicidins, defensins and ribonucleases may contribute to causing several diseases, there is little information about the involvement of RNase 7 in oral epithelial diseases. Therefore, this mini−review mainly focuses on the roles of RNase 7 in epithelial health and disease with emphasis on its involvement in oral epithelial diseases.

Overview of RNase 7

All 13 RNase subtypes are encoded in a single gene cluster in chromosome 14. RNases 1–8 are called as canonical RNases (true RNases), while the remaining five (RNase 9–13) are known as non−canonical RNases (Boix & Nogues, 2007 ; Cho et al., 2005 ; Sorrentino, 2010), which lack the catalytic triad and may therefore be incapable of catalyzing RNA degradation. Besides their ribonucleolytic activity, canonical RNases have been shown to play roles in angiogenesis, neurotoxicity, immunomodulation, and host defense

(Boix & Nogues, 2007 ; Goo & Cho, 2013 ; Simanski et al., 2012 ; Rosenberg, 2008). RNase 7 is a canonical RNases found most abundantly in the skin (Harder & Schroder, 2002), where the keratinocytes are the major source of RNase 7 and the RNase inhibitor (RI). Structurally, RNase 7 is composed of three α−helices and two triple−stranded antiparallel β−sheets (Harder & Schroder, 2002 ; Huang et al., 2007). The lysine residue from flexible N and C terminal cationic cluster has a key role in antimicrobial activities ; RNase 7 has the special capacity to permeate and disrupt the bacterial membrane independent of its RNase activity, thus differentiating it from the other RNases (Huang et al., 2007). Instead of interacting with internal microbial targets, RNase 7 forms a complex with the surface proteins on some bacteria (Torrent et al., 2010).

Role of RNase 7 in host defense of oral cavity

The oral epithelium is constantly exposed to various microorganisms, including commensals and pathogens present in the diverse environment of the oral cavity (Presland & Dale, 2000). A healthy oral status is maintained by the innate immune system, which augments physical and chemical barriers in the oral epithelium (Dale, 2002 ; Ganz, 2004 ; Eberhard et al., 2008 ; Rakoff−Nahoum et al., 2004). The rigid intercellular connections between the epithelial cells act as a physical barrier, while AMPs produced by the epithelial cells function as a chemical barrier against pathogenic organisms (Abiko Y et al., 2007 ; Chung et al., 2007). AMPs often play a significant role in adaptive immunity by recruiting adaptive immune cells (Abiko Y et al., 2007). As shown in Figure 1, the RNase 7 peptide is mainly localized in the keratinized layers of normal skin and oral epithelium (Scola et al., 2012 ; Rademacher et al., 2016). The expression of RNase 7 is inhibited when it forms a complex with RI in the basal and lower spinous layers. Furthermore, RNase 7 activity is unveiled following the degradation of RI by serine proteases in the stratum corneum, thereby exerting its antimicrobial effects during the process of keratinocyte differentiation (Fig.2) (Abtin et al., 2009). The expression of RNase 7 can be further enhanced by incubation with TNFα, IL−1β or interferon γ (IFNγ), or by subjection to microbial challenge with *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Trichophyton rubrum* (Harder & Schroder, 2002). RNase 7 expression has also been induced by bacterial biofilms and protozoa in gingival and corneal epithelial cells, respectively (Eberhard et al., 2008 ; Otri et al., 2010). RNase 7 activity is more pronounced in early formed biofilms than in matured biofilm (Eberhard et al., 2008), and the induction of this peptide depends on the types of bacterial species present in the biofilm (Eberhard et al., 2009).

RNase 7 in oral inflammatory diseases

There is very little information about the involvement of RNase 7 in oral inflammatory diseases, thus far. Several types of microbes are known to induce the expression of this peptide (Harder & Schroder, 2002 ; Rademacher et al., 2016) ; alternatively, upregulated RNase 7 expression has been observed in other inflammatory diseases without microbial infection. In psoriasis (chronic inflammatory skin disease), RNase 7 was found to be expressed in the psoriatic scales, thus contributing to the low rates of infection. (Harder & Schroder, 2005 ; Rademacher et al., 2016). The increased expression of RNase 7 is induced by its immuneregulatory effects on activated T cells and on cytokines such as TNF−α, and IFN−γ (Kopfnagel et al., 2017). Atopic dermatitis also presented with increased levels of RNase 7 expression, which may have been induced by the disturbed skin barrier (Harder et al., 2010).

Expression of T cell cytokines including TNF−α and IFN −γ plays a role in the pathogenesis of oral lichen planus ;

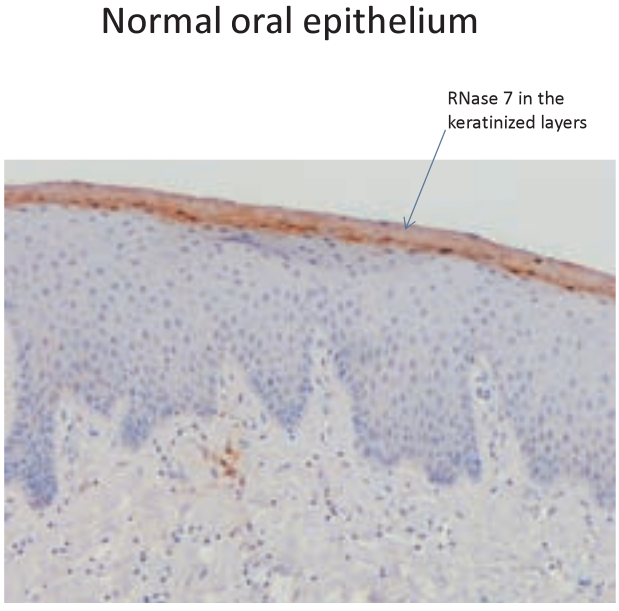

Figure 1. Normal oral epithelium
Immunohistochemical staining showing positive staining of RNase 7 in the keratinized layers of the normal oral epithelium.

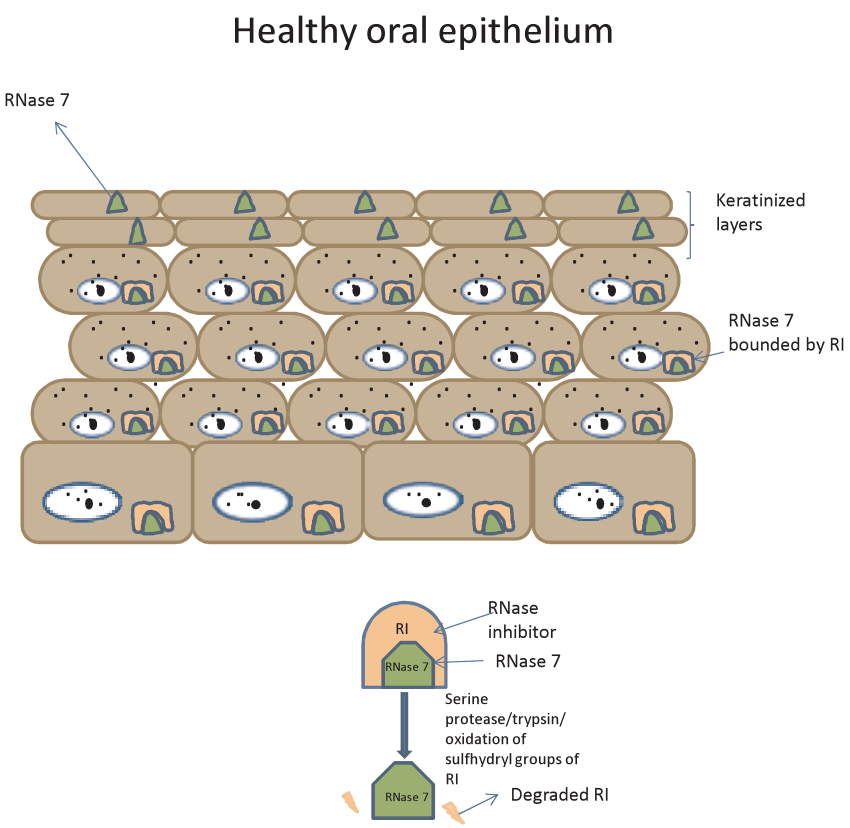

Figure 2. Healthy oral epithelium
RNase 7 is constitutively expressed in the keratinized layer but inhibited by the horseshoe −shaped RNase inhibitor (RI) in other keratinocytes in healthy oral epithelium. RNase 7 is released after degradation of the RNase inhibitor (RI). RI is degraded by protease and by the oxidation of its sulfhydryl groups, thus enabling RNase 7 activity.

moreover, disturbed skin barriers due to T cell infiltration are often observed in this condition (Nogueira et al., 2015). Further studies investigating the significance of increased RNase 7 expression levels in the pathogenesis of oral lichen planus are warranted.

Role of RNase 7 in tumors

Certain types of AMPs demonstrate anticancer activities, whereas others may exhibit oncogenic activities (Mader & Hoskin, 2006 ; Gasper et al., 2013) such as the disruption of the cell membrane. The cancer cell membrane typically carries a net negative charge, which has an affinity towards cationic AMP (Cruciani et al., 1991). AMPs kill cancer cells due to differences in cell membrane composition, cell surface area, and fluidity when compared with the normal cells (Leuschner & Hansel, 2004 ; Hoskin & Ramamoorthy, 2008). Thus, they may be clinically applied as potent anticancer agents that kill the cancer cells and prevent metastasis without causing damage to normal cells and tissues (Papo & Shai, 2005). The expression level of RNase 7 in cutaneous squamous cell carcinoma (SCC) has been found to decrease with the malignant transformation indicating its role as a tumor suppressor gene (Scola et al., 2012). On the other hand, some AMPs have been found in progressing tumors, and may be suitable for use as a tumor marker. In head and neck cancer, the gene expression level of RNase 7 was higher in SCC than in normal tissues (Muehleisen et al., 2012). Furthermore, the expression level of peptide was higher in SCC than in basal cell carcinoma, which presented with lower malignant potential in the skin (Muehleisen et al., 2012). These reports imply that RNase 7 may function as an oncogene. The phenotype of the tumor cells varies among the different types and sites of tumors. The expression levels and functions of RNase 7 may depend on the location and types of the tumor. Therefore, further studies are needed to better understand the mechanisms underlying the variations in RNase 7 expression in different types of carcinoma. Studies in the oral cavity, which is largely polluted by several microbes, can provide more about inflammatory diseases and their association with carcinoma. Knowledge

about the details of the expression patterns and functions of RNase 7 in oral carcinoma may prove useful for the development of cancer−targeted immune therapy.

Future aspects of RNase 7

RNase 7 is produced by the oral epithelium, and is known to possess broad−spectrum antimicrobial activities against oral pathogenic microbes. Studies undertaken in the area of oral health have identified numerous beneficial effects of this peptide, such as prevention of bacterial infections and development of potential therapeutic agents ; however, there is not enough evidence about its effect in oral diseases including inflammatory diseases and tumors. Other types of epithelial antimicrobial peptides such as beta−defensins are directly involved in cancer prevention and in causing allergy as well as autoimmune diseases (Prado−Montes de Oca E, 2010). Nevertheless, owing to limited information regarding the involvement of RNase 7 in oral diseases, further investigations clarifying its role are needed (Fig 3).

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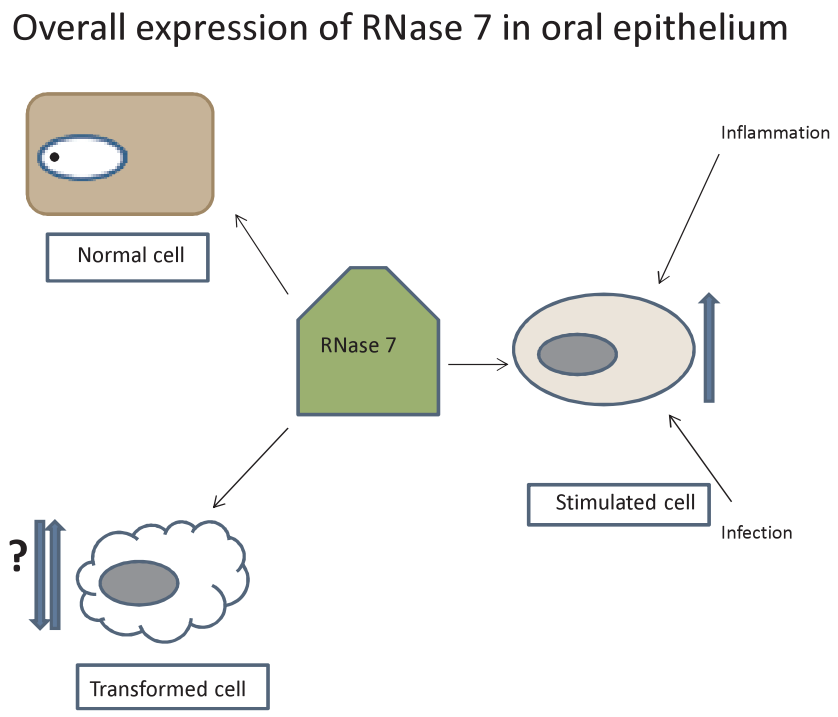

Figure 3. Overall expression of RNase 7 in the oral epithelium
Expression of RNase 7 is constitutive in normal epithelial cells, upregulated during inflammation and infection, and either upregulated or downregulated in tumors.

antimicrobial peptides and toll-like receptors is increased in tinea and pityriasis versicolor. Mycoses 57 : 147−152, 2014.

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