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Review

Bioactive peptides hidden in human salivary proteins

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ABSTRACT

Background: Extensive peptidomic studies of human saliva have resulted in considerable advances in the field of proteomics. As the next generation in salivary research, a comprehensive understanding of the biological functions of *in vivo* peptides generated by proteolysis in the oral cavity has been long awaited. A cyclopedic functional analysis of salivary peptides may bring promising therapeutic agents and novel clinical applications.
Highlight: (1) This review article refers to bioactive peptides hidden in salivary parent proteins. (2) Functions of the peptides as anti-microbial, anti-viral, wound-closing, and anti-pain are described. (3) Biological significances of the repeated structures in salivary proline-rich proteins are emphasized.
Conclusion: Human salivary proteins have the ability to generate bioactive peptides upon proteolytic cleavage.

Contents

1. Introduction

To develop diagnostic systems based on human salivary protein components, extensive proteomic and peptidomic studies have been performed involving whole saliva (WS), parotid saliva (PS), submandibular/sublingual saliva (SM/SL-S), and exosomes in WS over the past decade. Previous studies of the human salivary proteome identified 914 proteins in the PS proteome [1], 917 in the SM/SL-S

Abbreviations: N-, amino-; C-, carboxyl-; PRP, proline rich protein; a, acidic; b, basic; g, glycosylated

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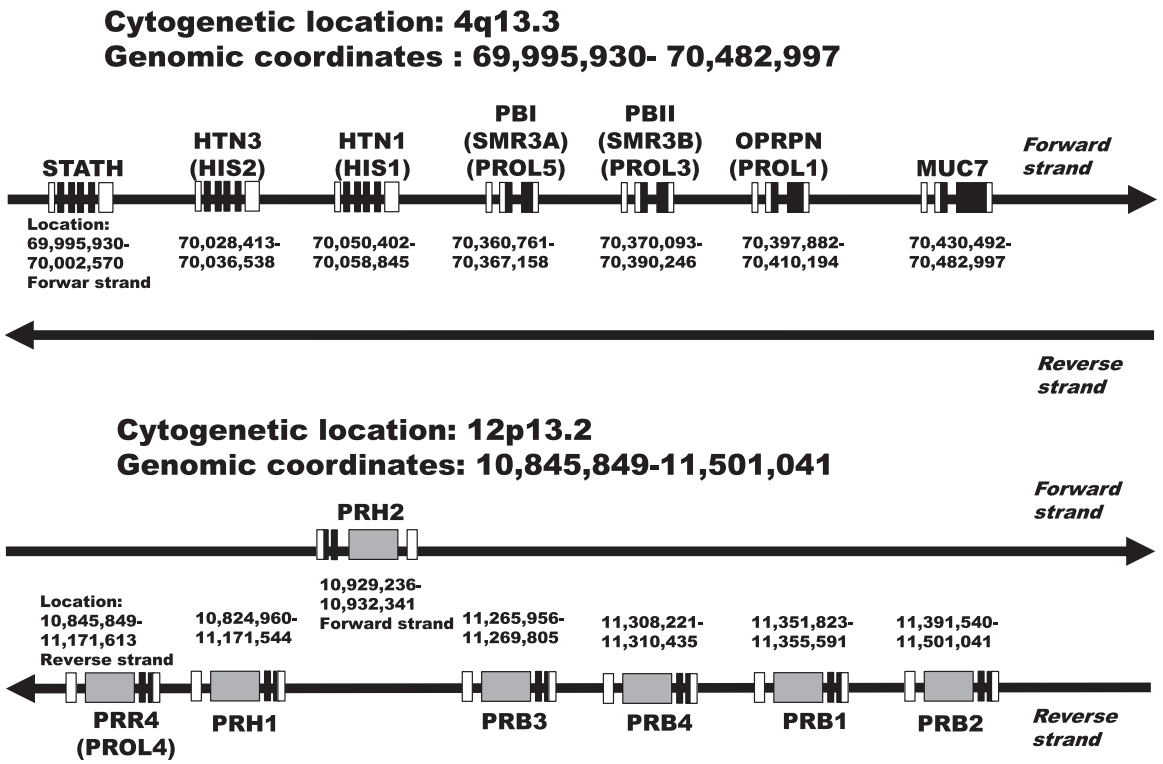

Fig. 1. Chromosomal localization of the genes encoding salivary (or lacrimal) proteins. Open squares indicate noncoding exon regions. Closed squares denote coding exon regions. Gray squares indicate exons encoding the PRPs-specific repeating unit $G(P)_nG(K/R)PQ$ and its related sequences.

proteome [1], 491 in the parotid exosome [2], 187 in two types of exosomes in WS [3], and 56 in minor salivary gland secretions [4]. WS proteins in the oral cavity predominantly originate from three major salivary glands: the parotid, submandibular, and sublingual glands. Many proteins have been identified in WS, including acidic (a) proline-rich proteins (PRPs), basic (b) PRPs, glycosylated (g) PRPs, P-B, cystatins, histatins, statherin, mucous glycoproteins, mucin 5 (MG1), mucin 7 (MG2), immunoglobulins, amylase, and agglutinin [5]. Multiple variations based on genetic polymorphisms have been observed for each protein [6].

According to the NCBI gene database (http://www.ncbi.nlm.nih.gov/gene/), salivary proteins, including statherin, histatin 3, histatin 1, P-B1, P-B, BPLP, and mucin 7, are encoded by a set of clustered genes localized on chromosome 4 [Cytogenetic location: 4q13.3, Genomic coordinates: 4: 69,995,930-70,482,997; - *STATH* (encoding statherin) - *HTN3* (alias *HIS2*; encoding histatin 3) - *HTN1* (*HIS1*; histatin 1) - *PBI* (*SMR3A* or PROL5; *P-B1* or SMR3A) - *PBII* (SMR3B or PROL3; *P-B* or SMR3B) - *OPRPN* (*PROL1*; basic proline-rich lacrimal protein BPLP) - *MUC7* (mucin 7) -]. Moreover, lacrimal proline-rich protein (LPRP), two aPRPs, one bPRP, and three gPRPs are encoded by another gene cluster [Cytogenetic location: 12p13.2, Genomic coordinates: 12: 10,845,849-11,501,041; - *PRR4* (alias *PROL4*; encoding LPRP) - *PRH1* (encoding aPRP) - *PRH2* (aPRP) - *PRB3* (gPRP) - *PRB4* (gPRP) - *PRB1* (bPRP) - *PRB2* (gPRP) -]. The *PRH2* gene is located in the forward strand of chromosome 4 but the other six genes are in the reverse strand.

Most of the established biological functions of saliva such as antimicrobial properties, wound repairing, pain control, buffering, dilution and cleaning, digestion, lubrication, and protection of tooth enamel are granted by parent proteins and occasionally their processed forms [7]. These biological functions are related not only to oral health, but also to systemic health. The environment of the oral cavity, the "port of entry" of the gastrointestinal tract, is likened to a high-performance and elaborate incubator because the pH and temperature in the cavity and gastrointestinal tract are precisely controlled. The oral cavity contains culture media rich in nutrients supplied by dietary foods, in which notable proteolytic events take place on salivary and dietary proteins. The predominate peptide fragments present in WS have been reported to be derived from aRRPs, bPRPs, gPRP, P-B, statherin, and histatins [8]. Oppenheim et al. [8] have discovered unique proteases in WS that cleave preferentially after a glutamine residue for the tripeptide sequence -KPQ- in aPRPs, bPRPs, and gPRP. The PRP-specific repeating unit, $G(P)_nG(K/R)PQ$, and its related sequences are excised by proteases. These excised peptides display completely different functions than the parent proteins [9,10] and were of unknown relevance until now. It has been gradually recognized that the peptide fragments created by proteases from oral epithelial cells, bacteria, and a serum-like gingival crevicular transudate, play important roles in both the oral cavity and further downstream in the alimentary canal. Despite the identification of more than 4000 different salivary peptides and protein species [11], physiological functions of salivary peptides are beginning to be understood and only recently have applications of these peptides been examined.

In this review article, we summarize the bioactive peptides identified so far that are hidden in major human salivary proteins.

2. A gift from the human genome project to salivary research

2.1. Multiple proteins are produced by one gene

Brief maps of two gene clusters encoding major salivary proteins on chromosome 4q13.3 and chromosome 12p13.2 are illustrated in Fig. 1 and are based on data resulting from the human genome project (http://www.ncbi.nlm.nih.gov/gene/). These achievements revealed that alternative splicing causes multiple exon combinations, including coding- and non-coding DNA sequences, thereby many transcripts can be generated from a single gene. In some cases, an unexpected exon comprised of coding- and/or non-coding sequences located far from the exon in an established gene can be combined.

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