Abstract

Involvement of RNase 7 in Oral inflammatory diseases and

Oral squamous cell carcinoma

2019

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1. Introduction

RNase 7, an antimicrobial peptide with ribonuclease activity, belongs to the RNase A superfamily and was first identified in healthy skin. It is most highly expressed RNase A superfamily in keratinocytes derived from skin. It is inducible upon stimulation of microbes and pro-inflammatory cytokines. The expression of RNase 7 is well documented in human skin epithelial health and diseases, however there is no information about expression profile of RNase 7 in oral epithelial health and disease. The localization pattern of RNase 7 in the oral epithelium may be different from that in skin. Information about role of RNase 7 in oral cancer has not been shown, despite other epithelial antimicrobial peptides (AMPs) have been often related to malignant potential of cancers. Therefore, the present study investigated the localization of RNase 7 in normal, malignant and oral inflammatory diseases, and involvement of RNase 7 in the malignant potential of oral squamous cell carcinoma (OSCC).

2. Materials and methods

Tissue sections of normal oral mucosa surrounding the fibrous polyps, oral lichen planus, radicular cyst and OSCC were included in this study. The localizations of RNase 7 were determined by immunohistochemistry (IHC) using anti-RNase 7antibody (anti Ribonuclease 7 antibody [CL0224]; Abcam). Four different types of OSCC cell lines (BSC-OF, SAS, OSC-19 and HSC-2) were used in this study. Immortalized normal human keratinocytes cell line (HaCaT) was used as a control. mRNA and protein expressions of RNase 7 were determined by qRT-PCR and immunocytochemistry respectively, in OSCC cells. The quantitative detection of RNase 7 protein was evaluated by ELISA assay (Hycult biotech). In order to examine whether decreased expression of RNase 7 in the OSCC cells make them alter their malignant characteristics, RNase 7 was knockdowned by transfecting with siRNA (Invitrogen) in OSCC cells (siRNA-OSCC). Cell proliferation was compared between siRNA-OSCC and the control using cell proliferation assay kit (Invitrogen). siRNA-OSCC were allowed to invade matrigel invasion chamber (Corning, life sciences) and cell numbers were compared with the control. MMP 9 expression was evaluated by qRT-PCR to examine whether the expression of MMP 9 was involved in promotion of invasion of siRNA-OSCC. The expression of involucrin and K14 was determined as a marker for differentiation and undifferentiation respectively, by qRT-PCR. Statistical evaluations were done by Mann Whitney U test and student-*t* test.

3. Results

The superficial layers of the keratinized layer of the hard palate, dorsum of tongue, and attached gingiva showed staining for RNase 7. Strong expression of RNase 7 was observed in the surface layers of orthokeratinized /parakeratinized epithelia in the inflamed oral epithelia of oral lichen planus. The inflamed oral epithelia obtained from radicular cyst showed weak staining for RNase 7 in the lining epithelium. Intense immunoreaction for RNase 7 was observed in the keratinized layers of epithelial pearls of OSCC. Expression of RNase 7 was weak to moderate in non-keratinized tumor cells of OSCC. By qRT-PCR, the mRNA expression level of RNase 7 was significantly higher in OSCC cell lines (BSC-OF, SAS, OSC-19 and HSC-2) than in the control ($p < 0.05$). RNase 7 protein was found in the OSCC cell by the immunocytochemical staining. RNase 7 protein was detected in both conditioned medium and cell extract derived from each cell line. The concentration of RNase 7 protein was significantly higher in the OSCC cells than in the control ($p < 0.05$). The cell number was significantly higher in siRNA-BSC-OF, -SAS, -HSC-2 and -OSC-19 than those in the negative control at 24h ($p < 0.05$). The number of invading cells was significantly higher in siRNA-BSC-OF, -SAS and -HSC-2 than those in the control ($p < 0.05$). We observed whether MMP 9 were involved in those promotion of invasiveness. The mRNA expression level of MMP 9 was significantly higher in siRNA-BSC-OF, -SAS and -HSC-2 than those in the control ($p < 0.05$). The mRNA expression level of involucrin was significantly higher in HSC-2 and SAS than in siRNA-HSC-2 and -SAS ($p < 0.05$). All siRNA-OSCC showed significantly higher expression levels of keratin 14 than the control did ($p<0.05$).

4. Conclusion

This is the first study to demonstrate that RNase 7 is expressed in oral epithelial health and diseases. The localization patterns of RNase 7 were similar to that of other epithelial AMPs including beta-defensins. The epithelial AMPs may be involved in oral epithelia diseases in a coordinated manner. Decreased expression of RNase 7 in OSCC promoted their growth and invasion. RNase 7 may contribute to suppression of malignant potential of OSCC.