Human Pathology (2015) 46, 991–999

Human
PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Protease-activated receptor 2 modulates proliferation and invasion of oral squamous cell carcinoma cells☆,☆☆

Kamal Al-Eryani DDS, PhD[a,b], Jun Cheng MD, PhD[a], Tatsuya Abé DDS[a,c], Satoshi Maruyama DDS, PhD[c], Manabu Yamazaki DDS, PhD[a], Hamzah Babkair DDS[a], Ahmed Essa DDS[a], Takashi Saku DDS, PhD[a,c,*]

[a]*Division of Oral Pathology, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8514, Japan*
[b]*Department of Oral Medicine and Pathology, Faculty of Dentistry, Sanaa University, Sanaa, Yemen*
[c]*Oral Pathology Section, Department of Surgical Pathology, Niigata University Hospital, Niigata 951-8520, Japan*

Received 13 December 2014; revised 8 March 2015; accepted 11 March 2015

Keywords:
PAR-2;
Oral mucosa;
Squamous cell carcinoma;
Carcinoma in situ;
Cell proliferation;
Cell invasion

Summary Based on our previous finding that protease-activated receptor 2 (PAR-2) regulates hemophagocytosis of oral squamous cell carcinoma (SCC) cells, which induces their heme oxygenase 1–dependent keratinization, we have formulated a hypothesis that PAR-2 functions in wider activities of SCC cells. To confirm this hypothesis, we investigated immunohistochemical profiles of PAR-2 in oral SCC tissues and its functional roles in cell proliferation and invasion in SCC cells in culture. The PAR-2 expression modes were determined in 48 surgical tissue specimens of oral SCC. Using oral SCC–derived cell systems, we determined both gene and protein expression levels of PAR-2. SCC cell proliferation and invasive properties were also examined in conditions in which PAR-2 was activated by the synthetic peptide SLIGRL. PAR-2 was immunolocalized in oral SCC and carcinoma in situ cells, especially in those on the periphery of carcinoma cell foci (100% of cases), but not in normal oral epithelia. Its expression at both gene and protein levels was confirmed in 3 oral SCC cell lines including ZK-1. Activation of PAR-2 induced ZK-1 cell proliferation in a dose-dependent manner. PAR-2–activated ZK-1 cells invaded faster than nonactivated ones. The expression of PAR-2 is specific to oral malignancies, and PAR-2 regulates the growth and invasion of oral SCC cells.
© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Early detection of early stage oral cancer, especially squamous cell carcinoma (SCC), often translates to a satisfactory clinical outcome [1]. To this end, we have developed several aids for the evidence-based histopathologic diagnosis of oral carcinoma in situ (CIS), which is the most critical disease entity among oral borderline malignancies. The precise diagnosis of CIS at surgical margins

☆ Competing interest: None.
☆☆ This work was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS KAKENHI grant nos. 25305035 and 25462849 to J. C. and 26305032 and 23406038 to T. S.) and by a Follow-up Research Fellowship from the Japan Student Services Organization (K. A.).
* Corresponding author. Division of Oral Pathology, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-dori, Chuo-ku, Niigata 951-8514, Japan.
E-mail address: tsaku@dent.niigata-u.ac.jp (T. Saku).

http://dx.doi.org/10.1016/j.humpath.2015.03.003
0046-8177/© 2015 Elsevier Inc. All rights reserved.

might determine prognoses of individual patients because according to our recent studies, CISs would recur as CISs or SCCs in 39.2 months [2] or in 12.3 months [3], when CISs are left behind at surgery.

To avoid subjectivities in the differential diagnosis of oral CIS, we have introduced combined immunohistochemistry for keratin (K) 19, K13, and Ki-67 for diagnostic criteria of CIS with the conceptual background that the second basal cell layer is the proliferating center of the oral mucosal epithelia and that the basal cell differentiation is determined with K19 expression and the prickle cell differentiation is recognizable with K13 expression [4]. We have also demonstrated the genetic mechanism for the loss of K13 as well as the reciprocal loss of K13 and emergence of K16/K17 in oral CIS [5,6]. To consolidate our diagnostic criteria of oral CIS, we have introduced some other immunohistochemistry for podoplanin [7,8], perlecan [9-12], matrix metalloproteinase (MMP) 7 [10], tenascin [12], and β-catenin/E-cadherin [11] as diagnostic aids. More recently, we have also emphasized the formation of intraepithelial blood vessels as one of the histopathologic characteristics of oral CIS and as a driving force for dyskeratosis among cancer cells [13].

Seeking the molecular mechanism of abnormal keratinization in oral CIS and SCC, we have come to find that the expression levels of K17 and K10 were elevated in round-shaped dyskeratosis in CIS as well as in keratin pearls in SCC by hemoglobin derived from extravasated erythrocytes via intraepithelial blood vessels [13,14]. Interestingly, this hemophagocytosis activity is mediated by protease-activated receptor 2 (PAR-2) through heme oxygenase 1 activation pathways [14], whereas PAR-2 had previously been known to up-regulate keratinocyte phagocytosis [15]. PAR-2 is one of the four PAR family members. The PAR family members are G-protein–coupled receptors that regulate cellular motility, growth and differentiation, and gene transcription [16]. PARs are uniquely activated by cleavage of their NH_2-terminal domains by serine proteinases for exposing new NH_2-termini, which further function as tethered ligand domains for their activation sites [17].

Although PAR-2 is the least understood among the four family members, it has been reported to be expressed in various cell types, including keratinocytes or epithelial cells of the kidney and intestines, vascular endothelial cells, and neural and muscle cells [18]. Trypsin has been considered as its main activator, whereas in vitro, the synthetic peptide SLIGKV, which mimics the NH_2-terminal portion of the human receptor, and SLIGRL, a murine homolog, are known to activate PAR-2 [19]. Functionally, PAR-2 activation is implicated in a broad spectrum of pathophysiologic processes, including regulation of vascular [20] and inflammatory [21] responses through cell membrane trafficking. In addition, recent reports have emphasized that PAR-2 is involved in cancer cell behaviors in the breast [22], stomach [23], prostate [24], pancreas [25], colon [26], and cervix uteri [27]. However, in terms of its detailed roles, more extensive studies are needed to elucidate whether PAR-2 could be a neoplastic phenotype.

As mentioned above, it remains totally unknown how PAR-2 functions in oral SCC or CIS in terms of their growth or invasion, other than its hemophagocytosis-related expression, which we have determined in oral SCC cells [14]. The aims of this study are to characterize PAR-2 expression profiles in the oral mucosal epithelia from normal, dysplastic, CIS, and SCC stages and to determine the effect of PAR-2 activation by agonist peptides on oral SCC cells in their proliferation and invasion potentials.

2. Materials and methods

2.1. Tissue samples

A total of 48 surgical specimens of oral SCC, in which areas of normal epithelia and foci of epithelial dysplasia and CIS were simultaneously contained, were randomly selected from surgical pathology files of the Division of Oral Pathology, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences, during the 15-year period from 1997 to 2010. The surgical specimens were fixed in 10% formalin and routinely embedded in paraffin. One set of 5-μm serial sections was stained with hematoxylin and eosin and used to reevaluate the pathologic diagnosis. The other sets were used for immunohistochemical investigations with the antibodies described below. The experimental protocol for analyzing surgical material was reviewed and approved by the Ethical Board of the Niigata University Graduate School of Medical and Dental Sciences (Oral Life Science).

2.2. Antibodies

A monoclonal mouse antibody against human PAR-2 (clone SAM11, IgG2a) was purchased from Zymed Laboratories (South San Francisco, CA). Rabbit polyclonal antibodies against mouse immunoglobulin G (IgG) was obtained from Dako (Glostrup, Denmark). Antibodies against K10, K13, K17, K19, Ki-67, and perlecan were used for objective categorization of dysplasia, CIS, and SCC, as described elsewhere [5-8].

2.3. Enzyme immunohistochemistry

Deparaffinized sections were autoclaved in citrate buffer (pH 6.0) at 121°C for 10 minutes to restore antigenicity. After antigen retrieval treatments, the sections were rinsed in 0.01 M phosphate-buffered saline (PBS) and treated in 0.3% H_2O_2 in methanol at room temperature for 30 minutes for blocking endogenous peroxidase activities. The sections were then incubated in 5% skimmed milk in PBS containing 0.05% Triton X-100 (PBST) at 37°C for 30 minutes to block

Download English Version:

https://daneshyari.com/en/article/4132836

Download Persian Version:

https://daneshyari.com/article/4132836

Daneshyari.com