Check for updates

Pre-Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(11) 4722–4730
© The Author(s) 2018
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/0300060518798261
journals.sagepub.com/home/imr

Ectopic transglutaminase 1 and 3 expression accelerating keratinization in oral lichen planus

Katsumitsu Shimada[1], Takanaga Ochiai[1] and Hiromasa Hasegawa[2]

Abstract

Objective: Oral lichen planus (OLP) characterized by interface mucositis frequently shows hyper-keratinization. To clarify mechanisms of excess keratinization, we investigated key molecules for cornified cell envelope (CE).

Methods: Involucrin (IVL), loricrin (LOR), transglutaminase 1 (TGase 1) and transglutaminase 3 (TGase 3) were immunohistochemically examined in 20 specimens of OLP; five specimens of buccal mucosa served as controls. Subsequently, the data were statistically analyzed.

Results: IVL in OLP was localized in the cell membrane, in contrast to its localization in the cytoplasm in controls. No positive reaction indicative of LOR was noted in any specimens. Although the TGase 1 localization in controls was restricted to the upper three-quarters of the membrane, the localization in OLP was in both membrane and in the cytoplasm of full thickness mucosal layers. The TGase 3 localization pattern was dramatically altered from cytoplasmic to membranous in OLP.

Conclusion: Our data suggest that aberrant TGase 1 and TGase 3 localization and distribution are closely related to hyper-keratinization in OLP. This is the first report of ectopic transglutaminase localization in OLP.

Keywords

Transglutaminase, ectopic localization, hyperkeratosis, oral lichen planus, loricrin, involucrin, mucositis

Date received: 6 June 2018; accepted: 9 August 2018

[1]Department of Oral Pathology, Matsumoto Dental University, Nagano, Japan
[2]Hard Tissue Pathology Unit, Graduate School of Oral Medicine, Matsumoto Dental University, Nagano, Japan

Corresponding author:
Hiromasa Hasegawa, Hard Tissue Pathology Unit, Graduate School of Oral Medicine, Matsumoto Dental University, 1780 Gobara Hiroka, Shiojiri, Nagano 399-0781, Japan.
Email: hirohasegawa@384.jp

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Introduction

Oral lichen planus (OLP) is a chronic inflammatory disease caused by T-lymphocytes, which occurs in the epithelial-connective tissue interface. Although its etiology has not yet been clarified, various conditions such as genetic disorders, stress, drug use, exposure to dental materials, or hepatitis C virus infection are involved in this disease.[1] This disease is characterized by the combination of its clinical and histopathological features.[2,3]

Histologically, OLP shows hyperkeratosis, a thickened ortho/para-keratinized layer constituting the clinical features of reticular-type OLP accompanied by band-like lymphocytic infiltration and liquefaction degeneration. In OLP, keratin 13 (K13) disappears, while keratin 10 (K10), which is localized in the keratinized squamous epithelium, is abnormally expressed.[4] Bloor et al. reported that mRNA expression levels of both K1 and K10 were up-regulated with increasing lymphocytic infiltration.[5] Thus, excessive keratinization is a characteristic phenomenon that represents abnormal differentiation of the squamous epithelium in OLP. Both the aggregation of keratin fibers, such as K1 and K10, and the formation of cornified cell envelope (CE) result in keratinization. In normal epidermis, CE is an insoluble 15-nm-thick structure that forms beneath the plasma membrane in differentiating keratinocytes. It includes two functional parts: one comprises covalently cross-linked protein (10-nm-thick) that comprises the backbone of the envelope; the other comprises covalently linked ceramide lipids (5-nm-thick). In the early phase of CE formation, transglutaminase 1 (TGase 1) and involucrin (IVL) are expressed along with K1 and K10 in lower keratinocytes. Subsequently, TGase 1 cross-links IVL and intercellular ceramide lipids on the cell membrane. Finally, TGase 1 binds together cross-linked IVL and loricrin (LOR)–small proline-rich protein (SPR) complexes that were previously combined by transglutaminase 3 (TGase 3).[6]

As described above, TGase 1 and TGase 3 are key molecules associated with CE formation. Immunohistologically, non-keratinizing oral epithelium shows cytoplasmic expression of IVL, while the epithelium demonstrates ectopic membranous localization in OLP.[7] To our knowledge, the mechanism of excessive keratinization has not been determined. To resolve this issue, we examined the localization of CE-related proteins, such as IVL, LOR, TGase 1, and TGase 3, in OLP.

Materials and methods

Case selection

We selected specimens from 130 cases from the archives of our surgical pathology laboratory that were diagnosed with OLP during the period from 2000 to 2013. All cases had been diagnosed in accordance with a set of modified World Health Organization diagnostic criteria,[2,3] which comprised clinically bilateral and lace-like network features and histological features of band-like lymphocytic infiltration and liquefaction degeneration in the basal cell layer without epithelial dysplasia. Finally, 20 specimens, which were bilateral and reticular, and which originated from patients without histories of drug- or metal-induced allergy and hepatitis C virus infection, were chosen. All OLP specimens showed typical histopathological features with band-like lymphocytic infiltration and varying degrees of basal cell injury and keratinization. Five specimens of buccal mucosa that served as controls were obtained from the mucosa of patients with vascular malformation (four cases) or from the non-cancerous portion of squamous cell carcinoma (one case), without either of the

following: 1) inflammatory or atypical changes, and 2) medical histories of allergy and hepatic infection. These control specimens showed no or slight keratinization. The average age of control and OLP patients was 49.8 and 60.7 years, respectively.

This study was approved by the Ethics Committee of Matsumoto Dental University (approval number 209) and conducted according to the principles of the Declaration of Helsinki (version 2002). Informed consent was obtained in the form of opt-out because all samples constituted archived paraffin-embedded tissues.

Immunohistochemistry

Deparaffinized and hydrated 3-μm-thick sections were pretreated in accordance with the manufacturer's recommendations. A high-temperature unmasking technique was performed by autoclaving at 121°C for 15 minutes in 0.01 M sodium citrate buffer solution (pH 6.0); some sections were digested with proteinase K (DAKO, Glostrup, Denmark). As shown in Table 1, IVL (Leica Novocastra, Newcastle, UK), LOR (GeneTex, Irvine, CA, USA), TGase 1 (Abnova, Taipei, Taiwan), and TGase 3 (Sigma-Aldrich, St. Louis, MO, USA) were used as primary antibodies. IVL, LOR, and TGase 1 were incubated at 4°C for 24 hours, while TGase 3 was incubated for 60 minutes at room temperature. As secondary antibody, Nichirei MAX-PO Multi (Nichirei, Tokyo, Japan) was incubated for 30 minutes at room temperature with HISTOSTAINER® (Nichirei). After visualization with 3-3′-diaminobenzidine tetrahydrochloride (DAKO), sections were counterstained with hematoxylin. Negative control slides were processed with phosphate-buffered saline, rather than primary antibodies. Subsequently, indirect immunofluorescence was performed with the above-mentioned primary antibodies and Alexa Fluor® 488-conjugated F (ab') 2-goat anti-mouse IgG (H+L) secondary antibody (Thermo Fisher Scientific, Waltham, MA, USA). To confirm co-expression of IVL and TGase 3, Alexa Fluor® 488-conjugated F (ab') 2-goat anti-mouse IgG (H+L) secondary antibody and Alexa Fluor® 647-conjugated F (ab') 2-goat anti-rabbit IgG (H+L) secondary antibody (Thermo Fisher Scientific) were used for indirect double immunofluorescence. Immunofluorescence images were taken with a fluorescence microscope BZ-X710 (KEYENCE Corporation, Osaka, Japan).

Table 1. Panel of antibodies used.

Antibody	Clone	Company	Dilution	Pretreatment
IVL	SY5	Novocastra	1:100	ProK#
LOR	–	GeneTex	1:500	ProK#
TGase1	–	Abnova	1:160	ProK#
TGase3	–	SIGMA	1:200	HRM*

IVL: Involucrin, LOR: loricrin, TGase1: transglutaminase 1, TGase3: transglutaminase 3.
*Heat antigen-retrieval method, #Proteinase K digestion.

Immunohistochemical analyses of CE-related protein reactions

After evaluation of immunoreactions, positive reactions of all antibodies were analyzed by using two approaches, as described below.

Intracellular localization pattern of positive reaction

Immunostaining indicated that CE-related proteins were either cytoplasmic or membranous. Therefore, we examined the frequencies of positive cases of CE-related proteins, divided into cytoplasmic and membranous patterns. Sporadic/faint positive reactions and positive reactions in more than half of the cells in any layers were

designated as “negative” and “positive,” respectively. Positive cases per all cases examined were calculated as values (%).

Intraepithelial distribution patterns of positive reactions

We also examined the extent of CE-related proteins in the epithelium of control and OLP specimens. The epithelium was equally divided into quarters from upper to lower (superior, upper middle, lower middle, and inferior parts). On the basis of immunostaining results, the distribution patterns of immunoreactions were classified into four types: upper quarter (1/4), upper half (1/2), upper three-quarters (3/4), and full thickness (4/4) patterns. Values (%) were numbers of each pattern per numbers of cases.

Statistical analysis

To compare values between control and OLP specimens, values of localization and distribution patterns were analyzed by using Fisher’s exact test and corrected by Holm’s method. All were analyzed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (www.r-project.org).[8]

Results

Immunohistochemical reactions of CE-related proteins

In controls, positive reactions indicative of IVL and TGase 3 localization were mostly found in the cytoplasm surrounding perinuclear areas, while TGase 1 was limited to the membrane. In OLP specimens, all antibodies (with the exception of LOR) showed varying degrees and frequencies of both membranous and cytoplasmic-positive reactions (Figure 1). The nuclear positive reaction of TGase 3 was consistently noted in the middle portions of both control and OLP specimens (Figure 1c). LOR reactions were negative in all layers of all specimens examined (data not shown). There were no verifiable reactions in negative controls among all examined antibodies.

Double immunofluorescence images demonstrated cytoplasmic expression of both IVL and TGase 3 in controls. In contrast, OLP specimens showed co-expression of IVL and TGase 3 on the membrane (Figure 2).

Intracellular localization patterns of positive reaction

IVL was localized in the cytoplasm in all controls, but in only 20.0% of OLP specimens. Conversely, membranous IVL-positive reactions were noted in 80.0% of OLP specimens, but in no control specimens. There were no cytoplasmic TGase 1 reactions in controls, whereas 70.0% of OLP specimens were membranous-positive. Membranous reactivity of TGase 1 was consistently observed in all control and OLP specimens. In contrast, cytoplasmic TGase 3 immunoreactivity was demonstrated in all control specimens, whereas only 15% of OLP specimens exhibited cytoplasmic TGase 3; 85% of OLP specimens showed membranous TGase 3 localization, but this was absent among controls. With the exception of LOR and membranous TGase 1, all differences were statistically significant ($p < 0.01$ or less) between control and OLP specimens (Table 2).

Intraepithelial distribution patterns of positive reactions

Although most cases showed upper three-quarter or full thickness patterns among both control and OLP specimens, there were some exceptional patterns of IVL and TGase 3 distribution that showed a tendency toward restriction in the upper parts

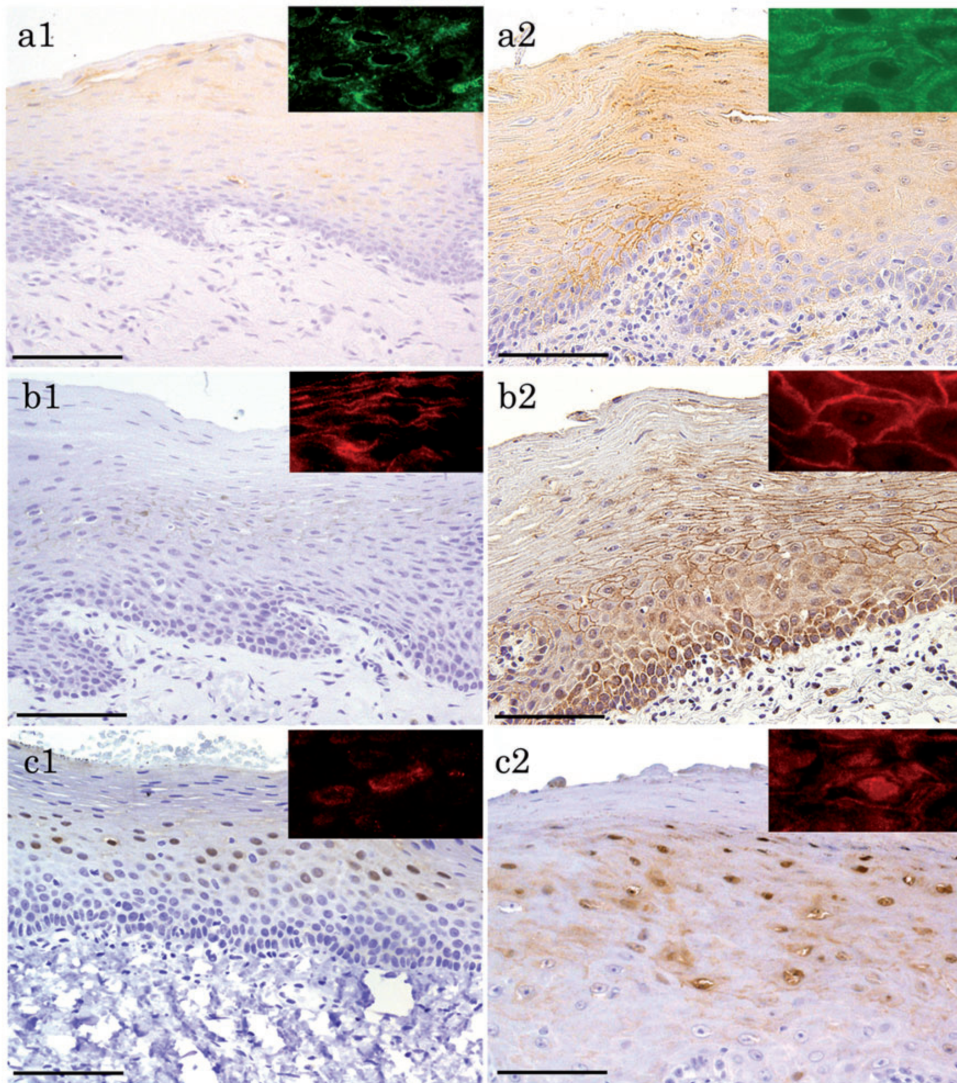

Figure 1. Immunohistochemistry of CE-related proteins: IVL is cytoplasmic-positive in the upper portion of the spinous layer of control specimens (a1), and membranous-positive in the full thickness layers of OLP specimens (a2). TGase 1 in control specimens is membranous-positive from the middle spinous to the superficial layers (b1). TGase 1 in OLP specimens is cytoplasmic-positive in the lower portion, in addition to a membranous-positive reaction from basal to spinous layers (b2). TGase 3 in control specimens is nuclear- and cytoplasmic-positive from lower spinous to superficial layers (c1). In OLP specimens, TGase 3 is nuclear-, cytoplasmic-, and membranous-positive from middle spinous to superficial layers (c2). All bars are 100 μm and all inserted figures are high-magnified views of immunofluorescence.
CE: cornified cell envelope, IVL: Involucrin, OLP: oral lichen planus, TGase1: transglutaminase 1, TGase3: transglutaminase 3.

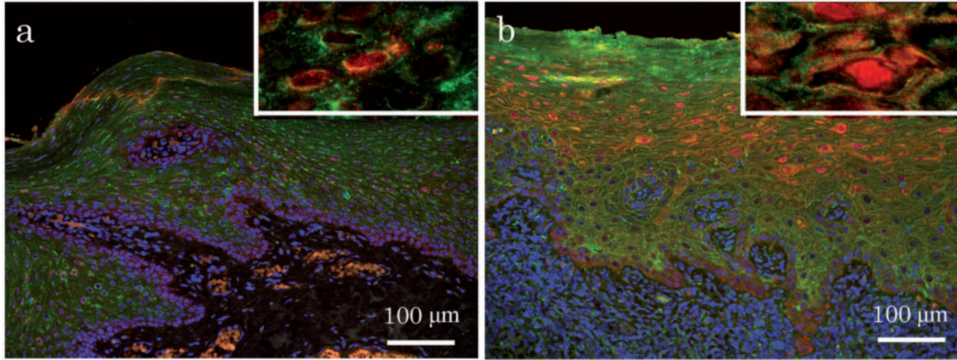

Figure 2. Co-expression of IVL and TGase 3 in OLP: Double immunofluorescence for IVL and TGase 3 reveals no yellowish membranous reactions in control specimens (a). In OLP specimens, an overlay image of IVL and TGase 3 shows yellowish membranous reactions distributed through the upper-half of the spinous layer (b). All bars are 100 μm and all inserted figures are high-magnified views.
IVL: Involucrin, OLP: oral lichen planus, TGase1: transglutaminase 1, TGase3: transglutaminase 3.

Table 2. Immunoreactive patterns.

		Intracellular localization		Intraepithelial distribution			
Antibody	Specimen	Cytoplasmic	Membranous	Upper 1/4	Upper 1/2	Upper 3/4	Full 4/4
IVL	Cont	100.0	0.0	0.0	0.0	40.0	60.0
	OLP	20.0***	80.0**	2.5	5.0	5.0	87.5
LOR	Cont	0.0	0.0	0.0	0.0	0.0	0.0
	OLP	0.0	0.0	0.0	0.0	0.0	0.0
TGase1	Cont	0.0	100.0	0.0	0.0	100.0	0.0
	OLP	70.0**	100.0	0.0	0.0	17.5***	82.5***
TGase3	Cont	100.0	0.0	0.0	0.0	40.0	60.0
	OLP	15.0 ***	85.0**	5.0	12.5	32.5	50.0

IVL: Involucrin, LOR: loricrin, OLP: oral lichen planus, TGase1: transglutaminase 1, TGase3: transglutaminase 3
Upper 1/4: upper quarter pattern, Upper 1/2: upper half pattern, Upper 3/4: upper three-quarters pattern, Full 4/4: full thickness pattern.
Values represent numbers of each pattern per numbers of cases (%). **$p<0.01$, ***$p<0.001$.

(Table 2). The distributions of TGase 1 significantly differed ($p<0.001$) between control and OLP specimens, but those of IVL and TGase 3 did not.

Discussion

In this study, we showed altered localization patterns of IVL, TGase 1, and TGase 3 between specimens from OLP cases and controls. The reticulo-white type of OLP, according to Andreasen's classification, shows various histopathological changes, including saw-tooth appearance, acanthosis, and acceleration of keratinization, in addition to band-like T cell infiltration.[1] Excessive keratinization is a characteristic phenomenon representing abnormal differentiation of the squamous epithelium in OLP. Therefore, understanding the

mechanism of this status is important for clarifying this complex disease, and it is critical to determine the key to abnormal keratinization.

IVL was reported to localize aberrantly on the cell membrane in OLP.[7] This ectopic localization is consistent with the cross-linkage of IVL after transfer to the cell membrane in the early phase of CE formation of epidermal keratinocytes.[9] Specifically, unless IVL transfers to the membrane, CE cannot be formed in keratinocytes. The buccal mucosa used as control in our study showed only cytoplasmic IVL expression, which appears to represent typical non-keratinizing squamous epithelium. Importantly, there was a statistically significant increase in the frequency of membranous localization in OLP. Transfer of IVL to the membrane in 80% of OLP specimens, which mimics IVL localization in the epidermis, may be a cause of excess keratinization in the inflamed buccal mucosa. Therefore, proteins that contribute to membranous localization of IVL may be important in supporting excess keratinization in OLP.

Although it was not statistically significant, the pattern of IVL localization in OLP specimens was 87.5% full thickness, compared with 60% full thickness in control specimens. Similar to our results, McCullough and Radden reported that the IVL-positive area maximally increased when the grades of lymphocytic infiltration and epithelial injury were highest.[7] The phenomenon of IVL expression in the lower epithelium is thought to derive from the scraping of lower epithelial cells, such as basal and parabasal cells. EGFR induces IVL production,[10] such that IVL might be widely expressed as a result of chronic inflammatory reaction. However, IVL cannot accelerate keratinization alone.

After IVL cross-linkage to the cell membrane, the complex of LOR and SPRs, bound by TGase 3, transfers to the cell membrane, reinforcing the CE.[11] In this study, LOR was absent in both buccal mucosa and OLP. Epidermal keratinocytes exhibit greater expression of LOR than SPRs,[9] while LOR is a minor component in oral keratinocytes.[12] Therefore, the negative staining results could be related to an extremely small amount of LOR. Excess keratinization of OLP might be influenced by other proteins, rather than by LOR.

TGase 1 and TGase 3 are important enzymes for cross-linking CE-related proteins, such as IVL and LOR.[11] In cutaneous keratinocytes, soluble-type TGase 1 localized in the cytoplasm is the precursor of a TGase 1 with weak enzymatic activity.[13–15] After partial degradation and anchoring to the cell membrane, TGase 1 activity increases.[16–18] In *in vitro* studies, human epidermal keratinocytes exhibited TGase 1 and TGase 3 that were restrictively degraded by calcium-dependent cytoplasmic proteases, such as μ/m-calpains.[14] According to quantitative analysis in the foreskin, the undegraded or full-length form of TGase 1 is the dominant form of TGase 1 in basal cells, while the degraded complex-form of TGase 1 is dominant in upper keratinocytes.[13,15] This evidence suggests that a small amount of active TGase 1 is also present and active in basal and parabasal cells. Therefore, the expression of TGase 1 and TGase 3 in the lower portion of the epithelium could be meaningful for both cross-linking and transferring of IVL.

The localization patterns of TGase 1 also differed between control and OLP specimens. In contrast to the controls that showed only a membranous pattern, TGase 1 in 70% of OLP specimens was localized on the membrane and in the cytoplasm of basal to spinous layers. These results showed that the cytoplasmic or full-length form of TGase 1 is expressed and contributes to cross-linking and transferring of IVL in basal and parabasal cells, as mentioned above. Considering the

TGase 1 expression pattern in skin (i.e., in the cytoplasm of basal to spinous layers, and in the membrane of the upper epidermis),[13] it seems that the TGase 1 expression in OLP closely resembles that of the epidermis. Thus, cytoplasmic TGase 1 in basal layers accelerates IVL membrane transfer; consequently, IVL in OLP indicates the epidermal type localization.

Although the distribution patterns of TGase 3 did not significantly differ between control and OLP specimens, the TGase 3 localization pattern was dramatically altered, from cytoplasmic to membranous, in 85% of OLP specimens, which is substantially different from that of epidermal TGase 3. Interestingly, this abnormal change has not been previously reported. With respect to epidermal CE formation, TGase 3 that is unanchored to the cell membrane promotes the formation of intra-chain cross-links in LOR and cross-links between LOR and SPRs; however, it only participates in the early phase of CE assembly in the cytoplasm.[9,12,19] The biological function of membranous TGase 3 is an important question, but has not been resolved in oral epithelium. Immunofluorescence microscopy clearly revealed co-expression of TGase 3 and IVL, which suggests that TGase 3 anchoring occurs in the cell membrane in OLP. The precise role of TGase 3 is unknown, but we suspect that membranous localization of TGase 3 contributes to hyperkeratinization in OLP.

Although numerous reports have been published regarding TGase or CE formation, only limited data have been provided to resolve the mechanisms of excess keratinization in oral diseases. Currò et al.[20] reported that mRNA expression levels of *TGASE1* and *TGASE3* were significantly reduced in the gingival epithelium of chronic periodontitis patients, compared with healthy controls. Reduced mRNA expression is generally consistent with pathological conditions, in that the inflamed epithelium shows degenerative and necrotic changes. Notably, similar alteration of mRNA expression might be observed in erosive-type OLP. As shown in an *in vitro* experiment, a lower growth rate was associated with reduced expression of differentiation markers, such as involucrin and filaggrin, whereas overexpression of wild-type *TGASE1* led to accelerated growth.[21] Interestingly, phenotypic analysis with *TGase1* and *TGase2* double-knockout mice showed that absence of the *TGase1* gene resulted in defective CE assembly and keratinization.[22] Considering these reports, TGase 1 and TGase 3 appear to be crucial for keratinization and differentiation of keratinocytes. To resolve the precise roles of TGase 1 and TGase 3 in reticulo-white type OLP, mRNA expression and regulatory genes, including *AP1*, *AP2*, *Sp1*, *Ets*, and *POU*,[23] should be examined.

In conclusion, our study clearly showed altered localization patterns, from membranous to both membranous and cytoplasmic in widely distributed TGase 1, and from cytoplasmic to membranous in TGase 3. Thus, the distribution of abnormal transglutaminases in buccal-mucosal epithelium promotes membranous IVL translocation, resulting in hyperkeratosis. This is the first report of the ectopic localization of TGase 1 and TGase 3 in OLP.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Ismail SB, Kumar SK and Zain RB. Oral lichen planus and lichenoid reactions:

etiopathogenesis, diagnosis, management and malignant transformation. *J Oral Sci* 2007; 49: 89–106.
2. van der Meij EH, Schepman KP and van der Waal I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; 96: 164–171.
3. Rad M, Hashemipoor MA, Mojtahedi A, et al. Correlation between clinical and histopathologic diagnoses of oral lichen planus based on modified WHO diagnostic criteria. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 107: 796–800.
4. Jacques CM, Pereira AL, Maia V, et al. Expression of cytokeratins 10, 13, 14 and 19 in oral lichen planus. *J Oral Sci* 2009; 51: 355–365.
5. Bloor BK, Seddon SV and Morgan PR. Gene expression of differentiation-specific keratins (K4, K13, K1 and K10) in oral non-dysplastic keratoses and lichen planus. *J Oral Pathol Med* 2000; 29: 376–384.
6. Eckert RL, Sturniolo MT, Broome AM, et al. Transglutaminase function in epidermis. *J Invest Dermatol* 2005; 124: 481–492.
7. McCullough MJ and Radden BG. Involucrin expression in some oral lichenoid lesions. *J Oral Pathol Med* 1992; 21: 367–369.
8. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; 48: 452–458.
9. Steinert PM and Marekov LN. Initiation of assembly of the cell envelope barrier structure of stratified squamous epithelia. *Mol Biol Cell* 1999; 10: 4247–4261.
10. Efimova T and Eckert RL. Regulation of human involucrin promoter activity by novel protein kinase C isoforms. *J Biol Chem* 2000; 21: 1601–1607.
11. Candi E, Tarcsa E, Idler WW, et al. Transglutaminase cross-linking properties of the small proline-rich 1 family of cornified cell envelope proteins. Integration with loricrin. *J Biol Chem* 1999; 12: 7226–7237.
12. Lee CH, Marekov LN, Kim S, et al. Small proline-rich protein 1 is the major component of the cell envelope of normal human oral keratinocytes. *FEBS Lett* 2000; 21: 268–272.
13. Kim SY, Chung SI, Yoneda K, et al. Expression of transglutaminase 1 in human epidermis. *Invest Dermatol* 1995; 104: 211–217.
14. Hitomi K, Yamagiwa Y, Ikura K, et al. Characterization of human recombinant transglutaminase 1 purified from baculovirus-infected insect cells. *Biosci Biotechnol Biochem* 2000; 64: 2128–2137.
15. Kim SY, Chung SI and Steinert PM. Highly active soluble processed forms of the transglutaminase 1 enzyme in epidermal keratinocytes. *J Biol Chem* 1995; 28: 18026–18035.
16. Phillips MA, Qin Q, Mehrpouyan M, et al. Keratinocyte transglutaminase membrane anchorage: analysis of site-directed mutants. *Biochemistry* 1993; 19: 11057–11063.
17. Rice RH, Rong XH and Chakravarty R. Proteolytic release of keratinocyte transglutaminase. *Biochem J* 1990; 15: 351–357.
18. Steinert PM, Kim SY and Chung SI. The transglutaminase 1 enzyme is variably acylated by myristate and palmitate during differentiation in epidermal keratinocytes. *J Biol Chem* 1996; 18: 26242–26250.
19. Hitomi K, Presland RB, Nakayama T, et al. Analysis of epidermal-type transglutaminase (transglutaminase 3) in human stratified epithelia and cultured keratinocytes using monoclonal antibodies. *J Dermatol Sci* 2003; 32: 95–103.
20. Currò M, Matarese G, Isola G, et al. Differential expression of transglutaminase genes in patients with chronic periodontitis. *Oral Dis* 2014; 20: 616–623.
21. Zhang SQ, Li CX, Gao XQ, et al. Identification and functional characterization of a novel transglutaminase 1 gene mutation associated with autosomal recessive congenital ichthyosis. *Int J Dermatol* 2016; 55: 201–207.
22. Pitolli C, Pietroni V, Marekov L, et al. Characterization of TG2 and TG1-TG2 double knock-out mouse epidermis. *Amino Acids* 2017; 49: 635–642.
23. Kypriotou M, Huber M and Hohl D. The human epidermal differentiation complex: cornified envelope precursors, S100 proteins and the 'fused genes' family. *Exp Dermatol* 2012; 21: 643–649.