구강평편태선의 면역조직화학 어레이에서 검색된 E-cadherin/β-catenin의 신호전달경로 이상

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〈Abstract〉

Deranged Expression of E-cadherin/β-catenin Signaling Pathway Detected by Immunohistochemical Array in Oral Lichen Planus

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Oral lichen planus (OLP) is an atypically keratinized and ulcerative lesion, producing severe pain and discomforts in the involved patients. Nevertheless, the etiological factor or the pathogenetic mechanism has not been clearly elucidated. In the present study, the different gene expressions were screened in 21 cases of OLP by immunohistochemical (IHC) array method using 80 antibodies, and found that the pathway of E-cadherin/β-catenin was abnormally expressed compared to the other essential genetic pathways. Particularly, the expressions of eIF5A, DHS, and DOHH, which are the biomarkers of protein translation, were remarkably reduced, nevertheless the expression of β-catenin was strongly positive in the 7 cases among 21 cases of OLP. The other expressions of p53, BCL-2, MDM-2, PAKT, BAX, BAK, BAD, NFkB, HO-1, etc, were usually weak or sparse, while the expressions of PCNA, CDK4, and HSP-70 were markedly increased. Taken together, it is presumed that the overexpression of β-catenin indicates the derangement of E-cad/β-catenin/NFkB pathway, causing the transcription of cellular proliferating genes in downstream events, i.e., PCNA and CDK4, and that it may be eventually relevant to the malignant potential of OLP epithelial cells. It is also suggested that the activation of β-catenin/TCF/LEF1 pathway be closely relevant to the immunological reaction of OLP with the accumulation of T-cells underneath the mucosal epithelium.

Key words : E-cadherin/β-catenin overexpression, Malignant potential, T-cell accumulation

Ⅰ. Introduction

OLP is a chronic, immunological mucocutaneous disease with a wide range of clinical manifestations. Since OLP is considered a pre-malignant condition[1], many authors investigated its pathogenesis using different methods. Histopathological diagnosis of OLP is not easy, since some cases of epithelial dysplasia may present traits which are very similar to those from OLP[2].

OLP can be a source of severe morbidity and has a small potential to be malignant. The diagnosis of OLP can be made from the clinical features if they are sufficiently characteristic, particularly if typical skin or other lesions are present, but biopsy is recommended to confirm the diagnosis and to exclude dysplasia and malignancy[3]. The prevalence of OLP, as an oral pre-malignant lesion, is 1-2% population. Lateral border of tongue, dorsal tongue, gingiva, hard palate and vermilion

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border are common sites and the lesions appear as reticular, plaque-like, and papular intraoral types. Atrophic and erosive types are known as intraoral pre-malignant types of OLPs[4].

As the OLP is also considered as a common chronic inflammatory disorder with a low risk of malignancy, some author suggested that a genetic predisposition linked to Th1 cytokine polymorphisms may promote the T cell-mediated immunological response to an induced antigenic change that is supposed to lead to OLP lesions. Other putative etiologic factors widely included the amalgam restorations, hepatitis C virus, poor oral hygiene, poor dental condition, and smoking, etc[5]. Thus the diagnosis of OLP can be made from the clinical features if they are sufficiently characteristic, but biopsy is recommended to confirm the diagnosis, to exclude dysplasia and malignancy, and if active treatment is required[6].

Although the potential for malignant transformation of OLP is still controversial, many authors reported the expression of proteins related to cell proliferation and apoptosis in OLP to evaluate the true potential for malignant transformation of OLP[7]. The Ki-67 labeling index (LI) was significantly higher in OLP compared with normal controls. A particularly large number of OLP lesions (64%) were p53 positive. No association was, however, found with p53 expression and the Ki67 LI. Atrophic and flat epithelia had a quantitatively higher LI, which did not significantly differ from acanthotic lesions[8]. Mitotic count, Ki-67 and cyclin D1 labeling indices in the basal and suprabasal layers of OLP mucosa were elevated together with the increase of p53, p21, Cip1 and TUNEL indices[9].

As the oral leukoplakia is a premalignant lesion exhibiting severe keratinization, proliferation, and acanthosis of epithelium, it should be differentially diagnosed from OLP. In this study a representative case of oral leukoplakia was used as a positive control for the OLPs examined by IHC array method in order to know the malignant potential of OLP in comparison with oral leukoplakia.

Particularly, the OLP lesions are transient from the early stage to the late stage depending on the severity of inflammatory cell infiltration and epithelial degeneration, disclosing variable features of reticular, erosive, and atrophic type, respectively. The changes of protein expressions in the epithelia of OLPs were observed transiently from the early stage to late stage of OLPs. Therefore, the present study also demonstrated the transient change of protein expression in the different types of OLP in comparison with the protein expressions in a leukoplakia used as a control.

II. Materials and methods

Total 21 cases of OLP, filed in the Department of Oral Pathology, Gangneung-Wonju National University Dental Hospital (GWNUDH) were examined to know the pathogenesis of OLP (Table 1). The OLPs were divided into three types, i.e., reticular type, erosive type, atrophic type, according to the pathological diagnosis. The representative three cases from each type of OLP were performed for IHC array study, which were preserved well to show the typical features of OLP and were less degenerative in histological observation. The biopsy specimens were fixed in 10% neutral formalin, embedded in paraffin, and sectioned in 4 μm thickness. The serial microsections were routinely stained with hematoxylin & eosin, and followed by immunohistochemical (IHC) array. The immunostaining was performed with indirect triple sandwich method using 80 antisera, i.e., proliferation-related proteins [cyclin dependent kinase 4 (CDK4), c-Myc, Max, mitotic protein monoclonal 2 (MPM-2), p16, p21, p53, Rb-1, proliferating cell nuclear antigen

(PCNA), nuclear factor kappa B (NFkB)], apoptosis-related proteins [B-cell leukemia/lymphoma-2 (BCL-2), BCL-2 associated X-protein (BAX), BCL-2 antagonist of cell death (BAD), BID (BCL-2 interacting protein), FAS-associated via death domain (FADD), FAS (CD95/Apo1), FAS ligand (FASL), FLIP, poly-ADP ribose polymerase (PARP), caspase-3, caspase-8, caspase-9], growth factor-related oncoproteins [hepatocyte growth factor-a (HGFa), epithelial growth factor receptor(EGFR), transforming growth factor b1 (TGF-b1), pAKT, c-erbB2, Janus Kinase-1 (JAK2), N-RAS, son of sevenless-1 (SOS-1)], Wnt signaling oncoproteins [adenomatous polyposis Coli (APC), β-catenin, E-cadherin, Wnt-1], tumoroncoproteins [carcinoembryonic antigen (CEA), deleted in malignant brain tumors 1 (DMBT1), maspin, pivotal integration site 1 (PIM1), survivin, signal transducer and activator of transcription-3 (STAT3), neurofibromin-1 (NF-1), SOX9, E2F-1], immunology related proteins [CD3, CD20, CD68, tumor necrosis factor-a (TNFa), CD68, immunoglobulin kappa(IgK), interleukin 6(IL-6), IL-8, LL-37, lysozyme, natural killer cell-1R (NK-1R)], angiogenesis- related proteins [angiogenin, CD31, vascular endothelial growth factor (VEGF), hypoxia inducible factor (HIF), von Willebrand Factor (vWF)], extracellular matrix proteins [laminina5, matrix metallo- protease-1 (MMP-1), MMP-2, MMP-9], protective proteins [hemeoxygenase 1 (HO-1), heat shock protein 70 (HSP-70), HSP-90, transglutaminase 2 (TGase 2), TGase 4], protein translation genes [deoxyhypusine dehy- drogenase (DOHH) and eukaryotic translation initiation factor 5A (eIF5A)], cytoskeletal proteins[α-actin, α-smooth muscle actin (a-SMA), cytokeratin 14 (CK14), S-100, α-tubulin, vimentin, pancytokeratin (pan-K), mucin 1], as listed in Table 1. The immunostaining of α-actin or α-tubulin (1:100

Table 1. Antibodies used in this study

Groups	Number	Antibodies
Proliferation-related proteins	12	CDK4*, c-Myc*, MAX#, MPM-2*, p16*, p21*, PCNA$
		p53*, p63*, Rb-1*, MPM2*, NFkB*
Apoptosis-related proteins	12	BAX*, BAD*, BCL-2*, BID, FADD*, FAS*, FASL*, FLIP
		PARP*, caspase-3@, caspase-8@, caspase-9@
Growth factor-related proteins	8	HGFa*, EGFR#, TGF-b1@, pAKT$, c-erbB2#, JAK2$
		N-RAS$, SOS#
Wnt signaling proteins	4	APC*, β-catenin#, E-cadherin#, Wnt-1#
Tumor oncoproteins	8	CEA$, DMBT1$, maspin*, PIM1@, survivin@, STAT3*,
		NF-1*, SOX9#. E2F-1*
Immunology related proteins	10	CD3#, CD20#, CD68*, TNFa*, IgK*, IL-6*, IL-8*, LL-37*,
		Lysozyme$, NK-1R*
Angiogenesis-related proteins	5	angiogenin*, CD31*, VEGF@, HIF@, VWF*
ECM proteins	5	laminina5*, MMP-1$, MMP-2$, MMP-9*, integrin a4
Protective proteins	5	HO-1*, HSP-70*, HSP-90$, TGase 2$, TGase 4$
Protein translation proteins	2	DOHH$, eIF5A$
Cytoskeletal proteins	8	α-actin*, α-SMA#, CK14*, S-100#, α-tubulin*, vimentin*
		pan-K*, mucin 1*
Total	80	

*Santa Cruz Biotechnology, USA; #DAKO, Denmark: $Neomarkers, CA,USA; @ ZYMED,CA, USA

dilution, DAKO, Denmark) was used as a control to normalize the expression levels of different proteins. Background cross reaction was minimized by the negative control staining using no primary antibody in the same immunohistochemical procedures. For the analysis of IHC array the histological images of similar representative tumor sections (x100) were captured under same exposure condition by digital camera (DP-70, Olympus, Japan), and compared the distribution and intensity of the immunohistochemical reaction among the OLPs and a leukoplakia using Image Quant version 5.2 program (Molecular Dynamic, USA). The usage of the biopsy specimens filed in the Department of Oral Pathology, GWNUDH was approved by Life Ethic Committee of GWNU (S2008-033).

Ⅲ. Results

The OLPs used in this study includes 21 cases occurred in buccal cheek (n=14), gingiva (n=8), buccal vestibule (n=6), tongue (n=2), and palate (n=1), were predominant in female (14 cases out of 21 cases), and showed the mean age of occurrence, about 57 years old. In the histological observation 21 cases of OLPs were divided into three groups, i.e., 8 cases of reticular type, 9 cases of erosive type, and 4 cases of atrophic type (Table 2 and 3).

In the histological observation of 21 OLPs the different pathological features, i.e, hydropic degeneration of the basal layer, necrotic keratinocytes in the epithelium, epithelial atypia of hyperplasia or hyperkeratosis, acanthosis, serrated ridge, fibrin deposition in the epithelium, epithelial erosion, neutrophil infiltration into epithelium, subepithelial lymphocytic infiltrate, plasma cells in the connective tissue were estimated depending on the severity of each finding, and turned out that the late stage OLPs, usually found in erosive and atrophic type OLPs, became aggravated into dysplastic and degenerative changes (Table 4). Therefore, 8 cases of reticular type OLP were classified into the early stage OLP, 9 cases of erosive type OLP were classified into the intermediate stage OLP, 4 cases of atrophic type OLP were classified into the late stage OLP. A representative leukoplakia was also used as a control, The IHC array was performed to know the protein expressions in 21 OLPs in comparison with the same IHC array of a representative leukoplakia, which showed only mild inflammatory cell infiltration and minimum basal cell hyperplasia. The IHC arrays of OLPs showed the depressed expression of protein translation factors, i.e., eIF5A, DHS, DOHH compared to the IHC array of leukoplakia. Consequently almost of proteins related to cytodifferentiation and protective proteins were generally weaker in the epithelia of OLPs than the epithelium of leukoplakia. Particularly, the expression of β-catenin was occasionally strong in the spinous layer of OLP epithelium in 7 cases out of 21 cases, and the expressions of PCNA and CDK4 were consistently increased in the late stage of OLPs (Fig. 1).

The IHC expressions of different proteins, including PCNA, eIF5A, PARP, E-cadherin, and β-catenin increased in the early, intermediate, and late stage of OLPs. The early stage OLPs showed the zonal infiltration of small round cells underneath the thin and hyperkeratotic epithelium, and the late stage OLPs showed the severe accumulation of small round cells underneath the thin epithelium of OLP. The basal cells and spinous cells of the early stage OLPs were frequently positive for PCNA, which was similar to the PCNA expression in the leukoplakia examined in this study as a control. Whereas the expression of PCNA was gradually increased in the intermediate and late stage of OLPs as the severity of OLP increased. Particularly, the positive reaction of PCNA was found in the suprabasal to spinous layer of OLP

Table 2. Pathological diagnosis of oral lichen planus (OLP)

	Sample No.*	Sex	Age	Lesion site	Type
1	S2011-065	F	68	Both buccal cheek	Atrophic LP
2	S2011-101	F	69	Rt. Mx. posterior gingiva	Atrophic LP
3	S2010-018	F	74	Border of tongue	Reticular LP
4	S2009-123	M	61	Cheek and palate	Reticular LP
5	S2009-339	M	55	Both Mx. and Mn. vestibular area	Erosive LP
6	S2008-229	F	52	Both buccal cheek and tongue	Erosive LP
7	S2008-281	F	62	Lt. Mx. vestibular area	Erosive LP
8	S2008-300	F	55	Lt. buccal cheek	Reticular LP
9	S2007-313	F	49	#47 gingival vestibule	Reticular LP
10	S2007-316	F	39	Both buccal cheek	Atrophic LP
11	S2007-377	F	59	Both buccal cheek	Atrophic LP
12	S2006-084	F	56	#46,47 buccal vestibule and cheek	Reticular LP
13	S2006-109	M	51	#45-48 gingival vestibule	Reticular LP
14	S2006-362	F	70	Rt. buccal cheek	Reticular LP
15	S2005-045	F	78	Lt. buccal cheek	Erosive LP
16	S2005-112	M	42	Lt. buccal cheek/Rt. retromolar area	Reticular LP
17	S2005-161	F	59	Both buccal cheek	Erosive LP
18	S2004-024	M	60	Lt. buccal cheek	Erosive LP
19	S2004-032	M	65	Lt. buccal cheek	Erosive LP
20	S2003-100	F	39	#24-26 gingival vestibule	Erosive LP
21	S2003-307	M	44	Both buccal cheek	Erosive LP

*The file numbers from Department of Oral Pathology, GWNUDH

Table 3. Different types of OLPs used in this study

Type		Reticular LP	Erosive LP	Atrophic LP	Total
Sex	F/M	5/3	5/4	4/0	14/7
Age	F/M	61/51	58/56	59/-	59/54
	Total	57	57	59	57
Location	Buccal cheek	5	7	3	15
	Tongue	1	0	0	1
	Gingiva	2	0	1	3
	Vestibule	0	2	0	2
	Total	8	9	4	21

*The types of OLPs were defined by histopathological observation in H&E stain.

epithelium (Fig. 2A).

EIF5A, a biomarker of protein translation, was strongly positive in the early stage of OLP, gradually decreased in the intermediate stage, and rarely observed in the late stage. In the early stage of OLP the expression of eIF5A was diffusely positive in the cytoplasms from basal cells to superficial keratinocytes, while in the intermediate stage of OLP, as the severity of OLP increased, the expression of eIF5A was disappeared in the epithelium of OLP, and became sparse in the late stage of OLP (Fig. 2C).

PARP, a biomarker of apoptosis, was weak in the early stage, gradually increased in the intermediate stage, and became strong in the late stage. In the early stage of OLP the PARP was rarely expressed in the epithelium, and became condensed in the suprabasal cells and spinous cells of the late stage OLPs (Fig. 2D).

E-cadherin, a biomarker of epithelial attachment, was strong in the early stage, gradually decreased in the intermediate stage, and rarely observed in the late stage. In the early stage of OLP the E-cadherin was diffusely positive in the epithelium, and usually condensed in the cell membrane and cytoplasms of the spinous cells of OLP epithelium. The positive reaction of E-cadherin was gradually decreased in the intermediate and late stage of OLPs, it became sparse in the basal cells and weak in the cell membranes of spinous cells (Fig. 2E).

β-catenin, a transcription factor, was weak in the early stage, gradually increased in the intermediate stage, and became condensed in the basal and spinous cells of the late stage OLPs. In the early stage OLPs the β-catenin was diffusely and weakly positive in the epithelium, and became gradually condensed in the spinous cells of the intermediate stage OLPs. The positive reaction was consistently positive in the suprabasal and spinous cells of the late stage OLPs, which was usually strong in their cytoplasms (Fig. 2F). Occasionally the expression of β-catenin was strongly condensed in the cytoplasms of

Table 4. Histological changes of mucosal epithelium in different types of OLPs

**Histological features	Reticular	Erosive	Atrophic	Total
Hydropic degeneration of the basal layer	*1.90.7	0.50.4	1.80.5	1.40.8
Necrotic Keratinocytes in the epithelium	1.20.6	1.70.5	1.10.6	1.40.6
Epithelial atypia	0.90.2	1.70.5	1.10.6	1.20.6
Epithelial hyperplasia	2.40.5	0.70.4	1.10.6	1.51.0
Epithelial hyperkeratosis	1.50.9	0.40.4	2.30.5	1.51.1
Acanthosis	1.80.8	0.70.4	1.80.5	1.51.2
Serrated ridges	1.90.7	0.30.4	0.60.5	1.51.3
Fibrin deposit in the epithelium	0.90.2	0.20.3	1.30.5	1.51.4
Epithelial erosion	0.30.4	1.80.8	0.90.9	1.51.5
Neutrophils in the epithelium	1.60.9	1.80.8	0.90.3	1.51.6
Subepithelial lymphocytic infiltrate	1.90.9	2.70.5	1.10.6	1.51.7
Plasma cells in the connective tissue	1.10.4	1.30.8	0.80.3	1.51.8

* -: none (0), : rare (0.5), +: mild (1), ++: moderate (2), +++: severe (3) (calculated mathematically)

** The representative histological features of OLPs.

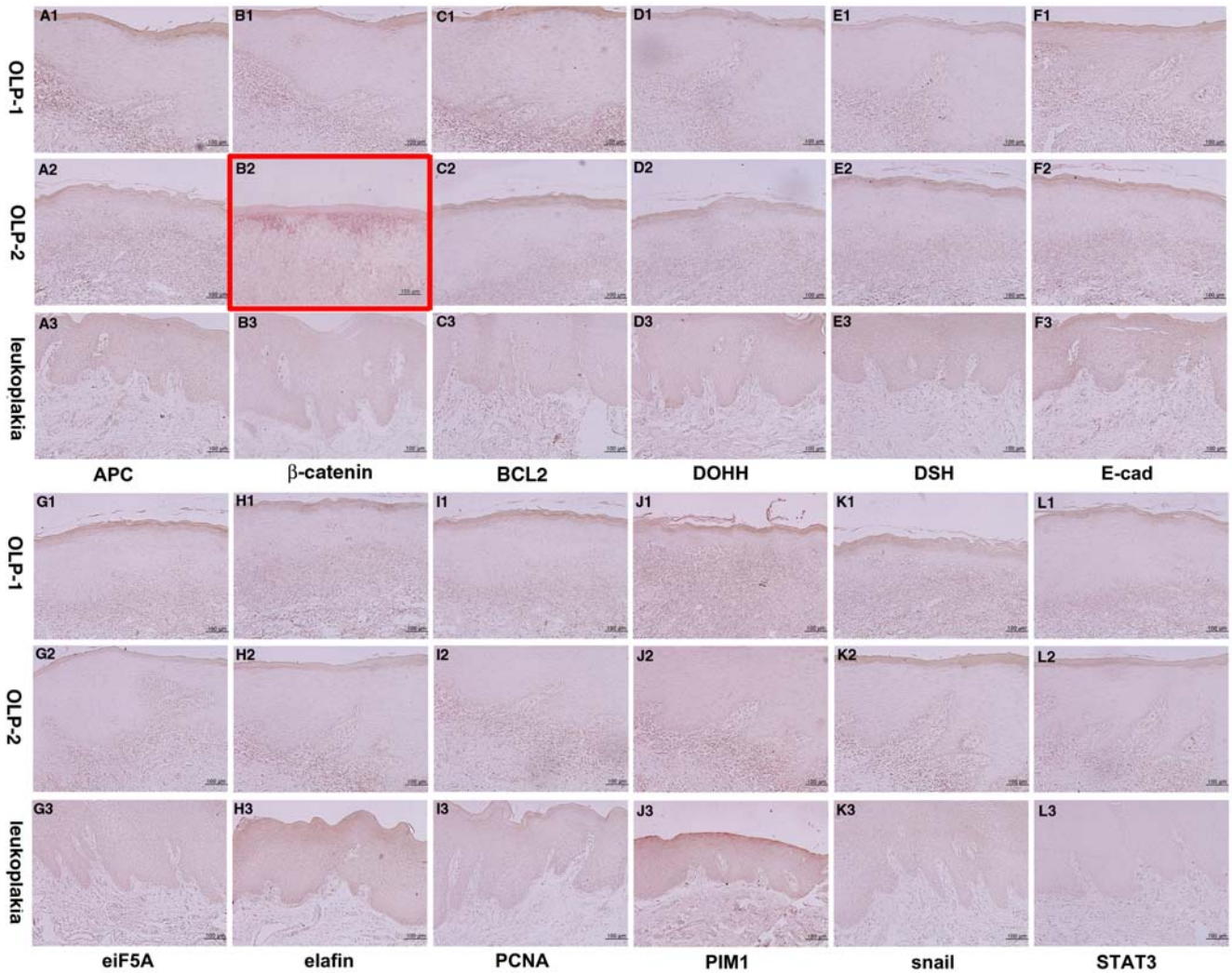

Fig. 1. IHC array of OLP compared to that of leukoplakia. OLP-1: Case 1 of OLP in the early stage, found in reticular type OLP. OLP-2: Case 2 of OLP in the early stage, found in erosive type OLP. Leukoplakia: Representative one with mild inflammatory cell infiltration and minimum basal cell hyperplasia, used as a control. Immunohistochemistry performed simultaneously. A. APC, B. β-catenin, C. BCL2, D. DOHH, E. DSH, F. E-cad, G. eiF5A, H. elafin, I. PCNA, J. PIM1, K. snail, L. STAT3

acanthotic keratinocytes, and subsequently became slightly positive in the nuclei of some keratinocytes (Fig. 2G).

In the microscopic observation in higher magnification the expression of E-cadherin became weak in the basal cells and basement membrane in the intermediate stage of OLP, which were transient from the early stage to the late stage of OLPs. β-catenin expression in the basal cells also appeared, which was usually condensed in some basal and suprabasal cells. On the other hand, the expression of PARP was usually localized in the basal cells, and the positive reaction became strong in some basal and suprabasal cells in the late stage of OLP (Fig. 3).

In the IHC array the epithelia of OLPs showed dramatic decrease of proteins related to the cyto-differentiation and cellular protection but gradual increase of proteins related to the cellular proliferation and apoptosis. On the other hand, the leukoplakia used as a control lesion showed the frequent positive reaction of PCNA and strong positive reaction of eIF5A, DHS, DOHH, elafin, TGase-2, E-cadherin, etc., in the proliferating

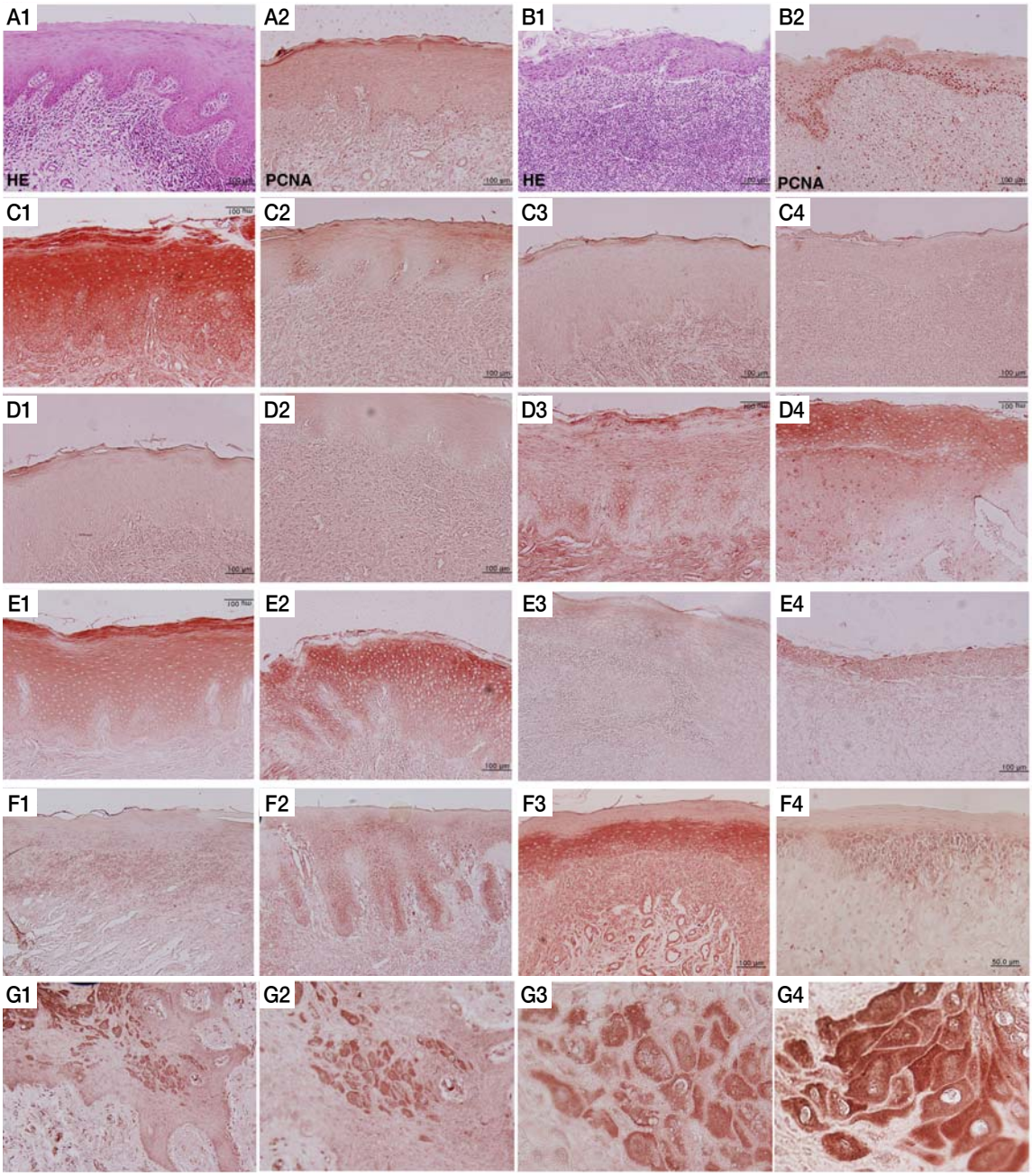

Fig. 2. IHC expressions of different proteins, including PCNA, eiF5A, PARP, E-cadherin, and β-catenin in the early, intermediate, and late stage of OLP. A. The early stage OLPs found in reticular type. A1. The zonal infiltration of small round cells underneath the hyperkeratotic epithelium (hematoxylin and eosin stain). A2. PCNA, a few basal cells were positive. B. The late stage OLPs found in erosive type. B1. The accumulation of small round cell infiltration underneath the thin epithelium (hematoxylin and eosin stain). B2. PCNA, many basal cells and spinous cells were positive. C. eiF5A, strong in the early stage (C1), gradually decreased in the intermediate stage (C2 and C3), and rarely observed in the late stage (C4). D. PARP, weak in the early stage (D1), gradually increased in the intermediate stage (D2 and D3), and became strong in the late stage (D4). E. E-cadherin, strong in the early stage (E1), gradually decreased in the intermediate stage (E2 and E3), and rarely observed in the late stage (E4). F. β-catenin, weak in the early stage (F1), gradually increased in the intermediate stage (F2 and F3), and became condensed in the basal and spinous cells in the late stage (F4). G. β-catenin, overexpression in some acanthotic epithelial cells, low magnification (G1 and G2) and high magnification (G3 and G4) showed mainly condensed in their cytoplasms and also weakly positive in their nuclei.

epithelium.

In the IHC array using 80 antibodies for 21 cases of OLP most of protein expressions were gradually decreased as the histological features of OLP were aggravated, while the proteins related to cellular proliferation, i.e., PCNA, CDK4, remained strongly positive in basal and suprabasal cells of OLPs. Particularly the expression of β-catenin was irregularly positive in some spinous cells of OLP epithelium in 7 cases out of 21 cases.

Ⅳ. Discussion

The epithelium of OLP becomes gradually ulcerated and repeatedly regenerated, and resulted in atrophied mucosal epithelium which is weak in a simple trauma[10]. In the signaling pathway coordinates the β-catenin expression is enhanced by E-cadherin, α-actin, and α-tubulin, and inhibited by p53/p21/APC in the upstream, and it enhances the expressions of COX, MMP1,2,7, 9,11,12,14, c-Myc, CcnD1, TCF/LEF1, and inhibits the expression of Wnt1 in the downstream (Fig. 4)[11-16]. However, the present study showed that the expression of eiF5A, DHS, and DOHH, which are biomarkers of protein translation, were reduced in the epithelia of OLPs. Especially, they become sparse in the severely ulcerated epithelia found in the late stage of OLP. The reduction of protein translation in OLPs may imply that there happens generalized decrease of gene expressions necessary to the cellular protection and cyto-differentiation. Nevertheless, the overexpression of β-catenin found in some epithelia of present OLPs can propagate the activation of NFkB, TCF, LEF1, and followed by the induction of cellular proliferation and immunological signaling[7,17,18].

Although the pathogenesis of OLP is still not elucidated clearly, the characteristic gene expressions of E-cadherin/β-catenin/NFkB and E-cadherin/β-catenin/TCF/LEF1 pathways are meaningful to explain the pathological findings of OLPs. For example, the hypertrophic reticular keratinization of OLP epithelium, known as Wickham striae, may be relevant to the irregular and focal proliferation of OLP epithelium via E-cadherin/β-catenin/NFkB pathway, and the zonal infiltration of lymphocytes into the submucosal area in the absence of specific antigen sources and complex immunological reaction may be relevant to the expression of E-cadherin/β-catenin/TCF/LEF1 pathway (Fig. 5).

Clinically, it is characterized by the presence of lineal reticular papules histologically by liquefaction degeneration of the basal layer of the epithelium associated with an inflammatory infiltrate with a "band-like" disposition on the lamina propria, composed primarily of T lymphocyte cells. Its pathogenicity is associated with deregulation of the cellular immune system, where the activated cytotoxic CD8+ and CD4+ T helper lymphocytes induce apoptosis of the epithelial cells. Classically it has been considered a precancerous condition, although the malignant transformation does not exceed 1% of cases. In recent years the differential diagnosis between OLP and oral lichenoid lesions (OLL) has become important, since the latter might have a greater malignant potential[19]. It was also reported that T cells bearing these TCRs are involved in the pathogenesis of OLP, and that IL-5 and TNF- may participate in its inflammatory processes[20].

OLP is characterized among other features by apoptosis of basal keratinocytes. T cells may orchestrate inflammatory cell responses in OLP via CD40-CD40L interactions. As basal keratinocytes downregulate CD40, they may escape CD40-CD40L-induced apoptosis in OLP. On the other hand, loss of E-cadherin in OLP may contribute to the destruction of basal cells and also to the T-cell migration into the epithelial compartment[21].

A pivotal role in the pathogenesis of long-lasting

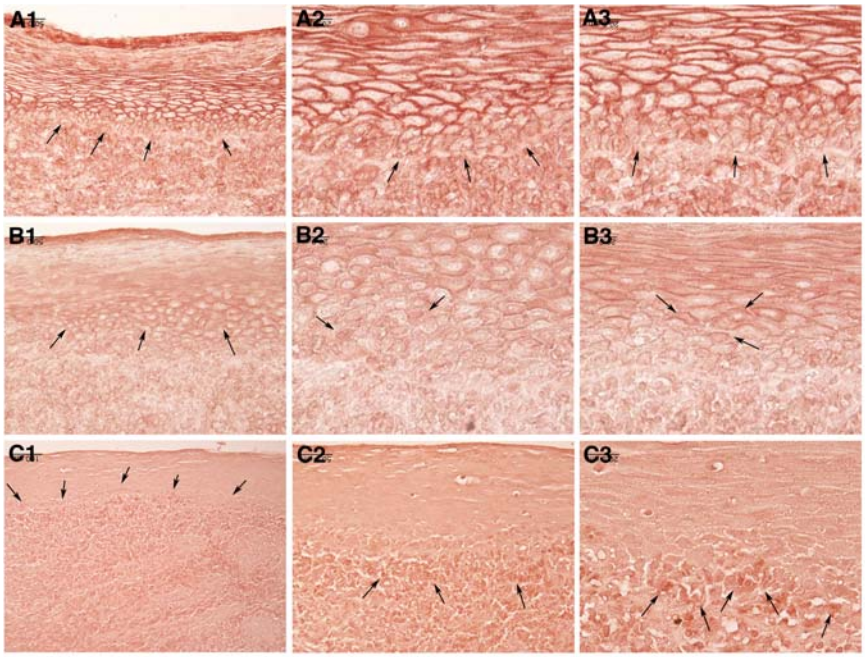

Fig. 3. IHC expressions of E-cadherin, β-catenin, and PARP in OLPs. A. E-cadherin. A1. The expression of E-cadherin in the basal layer (arrows) was gradually disappeared. A2 and A3. High magnification of A1. The positive reaction became weak in the basal cells and basement membrane (arrows). B. β-catenin. B1. The expression of β-catenin in the basal cells (arrows) was clearly positive. B2 and B3. High magnification of B1. The positive reaction was condensed in some basal and suprabasal cells (arrows). C. PARP. C1. The expression of PARP was localized in the basal layer (arrows). C2 and C3. High magnification of C1. The positive reaction became strong in some basal and suprabasal cells (arrows).

(-,+)	Gene Coordinate	(+,+)
E-cadherin α-actin α-tubulin	**enhance**	COX→antiapoptosis survivin→cell survival MMP1,2,7,9,11,12,14→proliferation c-Myc→proliferation CcnD1→proliferation TCF/LEF1→T-cell infiltration
upstream	**β-catenin**	**downstream**
p53→p21→APC	**inhibit**	
(-,-)		(+,-)

Fig. 4. Signaling pathway coordinate of β-catenin. The enhanced or inhibited expressions of upstream or downstream genes centered to β-catenin can be summarized into a scheme of signaling pathway coordinate of β-catenin. The β-catenin expression is enhanced by E-cadherin, α-actin, and α-tubulin, and inhibited by p53/p21/APC in the upstream, and it enhances the expressions of COX, MMP1,2,7,9,11,12,14, c-Myc, CcnD1, TCF/LEF1, and inhibits the expression of Wnt1 in the downstream.

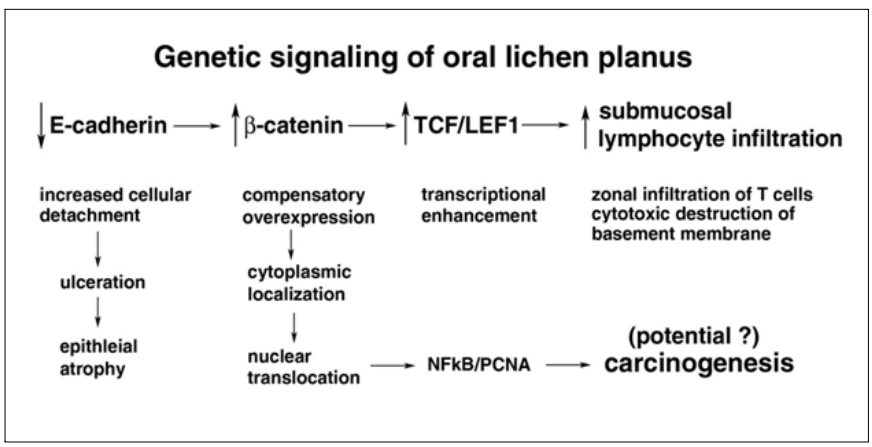

Fig. 5. Genetic signaling for OLP. The schematic flow chart of genetic signaling pathway showed the close relationship between signaling molecules and histopathological changes. In the OLPs the submucosal infiltration of T-cells and severe proliferation of basal and suprabasal layer keratinocytes are caused by the overexpression of TCF/LEF1 and NFkB/PCNA, respectively, both of which are similarly signaled by the overexpression of β-catenin.

inflammatory processes is played by the activation of nuclear factor kappa B (NFkB), a primary transcription factor which binds to promoter regions of different genes encoding immune and pro-inflammatory mediators. As proposed for other chronic inflammatory disorders associated with increased NFkB activity, the involvement of NFkB in the pathogenesis of OLP could be considered for selective therapeutic inhibitory targeting[22].

Identification of a disease-specific and possibly pathogenic T-cell receptor (TCR) in OLP is one of the most important steps to reveal the pathogenic antigen recognized by the T cells and thereby elucidate the pathogenesis and etiology of OLP. In the polymerase chain reaction-based and single-strand conformation polymorphism analyses the unique T-cell populations bearing TCRV beta 2, V beta 6, or V beta 19 gene products tend to expand in OLP lesions as a consequence of in situ stimulation with a restricted epitope of either a nominal antigen on the MHC molecule for the majority of the V beta families, even if only in minor populations, or of a common super antigen for the minority of the V beta families[23].

Both antigen-specific and non-specific mechanisms may be involved in the pathogenesis of OLP. Antigen-specific mechanisms in OLP include antigen presentation by basal keratinocytes and antigen-specific keratinocyte killing by CD8+ cytotoxic T-cells. Non-specific mechanisms include mast cell degranulation and matrix metalloproteinase (MMP) activation in OLP lesions. These mechanisms may combine to cause T-cell accumulation in the superficial lamina propria, basement membrane disruption, intra-epithelial T-cell migration, and keratinocyte apoptosis in OLP. OLP chronicity may be due, in part, to deficient antigen-specific TGF-beta1-mediated immunosuppression. The normal oral mucosa may be an immune privileged site (similar to the eye, testis, and placenta), and breakdown of immune privilege could result in OLP and possibly other autoimmune oral mucosal diseases. Recent findings in mucocutaneous graft-versus-host disease, a clinical and histological correlation OLP suggests the involvement of α, CD40, FAS, MMPs, and mast cell degranulation in the disease pathogenesis. Potential roles for oral Langerhans cells and the regional lymphatics in OLP lesion formation and chronicity are rebated by many authors[24-30]. However, the carcinogenesis in OLP may be regulated by the integrated signal from various tumor inhibitors (TGF-β1, α, γ, IL-12) and promoters (MIF, MMP-9)[28].

Current evidence suggests that immunological mechanisms are involved in OLP pathogenesis. The immunological events of OLP implicated the activated epithelia that comprise antigen-presenting Langerhans cells, also associated with the increased number of CD4+, CD8+, CD1a+ cells in OLP. The expressions of ICAM-1 and VCAM-1 were significantly higher in OLP. Those cells through ICAM-1 and LFA-1 promote epithelial destruction. Afterwards, cytokine production, ICAM-1 and VCAM-1 expression can activate CD8+ lymphocytes leading to the chronic form of OLPs[31,32].

The OLP usually concerns a benign skin disorder without involvement of other organ systems. Its course is generally limited to less than a year. Classic OLP is characterized by pruritic, violaceous, plane papules which occur most commonly on the inside of the wrists, the lower back, the lower legs and the perimalleolar region of adults aged between 30-60 years. Frequently, oral and genital mucous membrane lesions are involved. Erosive mucosal lesions are particularly painful and long-lasting. Many clinical variants have been described ranging from lichenoid drug eruptions to associations with graft-versus-host disease. The cause of OLP is still unknown, although an immunopathological pathogenesis with T-lymphocytes directed against basal keratinocytes or the basal membrane zone is assumed. However, multiple

therapeutic options exist: local and systemic corticosteroids, psoralens with ultraviolet A light, retinoids, cyclosporin[33].

The p63 gene encodes six different proteins which are required for development of ectodermally derived tissues such as oral mucosa, salivary glands, teeth and skin. p63 is highly expressed in squamous cell carcinoma of the head and neck, whereas decreased expression is seen in OLP. β-catenin, E-cadherin and epidermal growth factor receptor (EGFR) are p63-related proteins, and abnormalities in their expressions suggested they are involved in development of squamous cell carcinoma of the head and neck[11]. However, in this study we found only weak expression of p63 in OLP similar to p53 expression.

On the other hand, there was a prominent expression of FASR/FASL in OLP with a rather uniform distribution throughout the inflammatory cell infiltration. In the epithelium, the FASR/FASL expression was more abundant in the basal cell area compared to the suprabasal cell layer[34]. Desmocollin-1 and E-cadherin expressions were also detected in 24.4% (20/82) of the each sample. Of the positive samples, only eight specimens expressed both desmocollin-1 and E-cadherin. Strong desmocollin-1 and E-cadherin expression was found in 8.5% and 3.7% of OLP biopsies, respectively. Desmocollin-1 expression increased the risk of dysplasia 31.8-fold (95% confidence intervals, 3.6-280.9; p=0.0001), while E-cadherin was significantly related to cancer (odds ratio=5.13; 95% CI 3.3-8.1; p=0.001). In univariate survival analysis, desmocollin-1 was a significant predictor of both cancer (log-rank test; p=0.033) and dysplasia (p=0.0001), while E-cadherin predicted the development of cancer (p=0.0001). Neither CDK-1 nor RAD-51 had any predictive value. Importantly, desmocollin-1 retained its value as the only independent predictor of dysplasia in the multivariate model (adjusted Hazard Ratio=44.13; 95% CI 3.7-525)[6]. In atrophic OLP, desmocollin-1 is a powerful predictor of an important intermediate endpoint marker (dysplasia) in the causal pathway toward oral cancer[13].

Eventually, it is known that the focal loss of E-cadherin expression was observed in basal keratinocytes in 88% of the OLP specimens investigated, in all epithelial dysplasias and oral squamous cell carcinoma. Therefore, it is agreeable that neither aneuploidy, COX-2 expression, nor loss of E-cadherin expression, were significant reliable markers, but the present study demonstrated the transient loss of E-cadherin expression in the different types of OLPs, accompanying the transient overexpression of β-catenin and PCNA in the late stage of OLP.

In this study we found the expression of E-cadherin was significantly decreased in OLPs.

Particularly, we identified that the expression of β-catenin expression was abnormally increased in 7 cases out of 21 cases of OLPs by IHC array method. This overexpression of β-catenin was coincident with the increased expression of PCNA and CDK4 in the late stage of OLP. Therefore, with the genetic analysis of signaling pathway coordinates the implications of β-catenin overexpression is disclosed with the relevant gene expressions of NFkB, TCF/LEF1, APC, and Wnt1, and also provides the potential target genes for gene therapy of OLP (Fig. 6).

In conclusion, to know the potential evidences of the immunological reaction and the premalignant proliferation of OLP lesion the IHC array was performed using 21 cases of OLPs, and resulted in the generalized depression of gene expression, which was represented by the reduction of translation factors of eIF5A, DHS, and DOHH. Nevertheless, the expression of β-catenin was clearly increased in 7 cases out of 21 cases, implicating an important pathogenetic role of β-catenin in OLP. Therefore, it is presumed that the disharmony of E-cadherin/β-catenin pathway may indicate the pathogenetic mechanism of OLP, resulted in the nuclear translocation

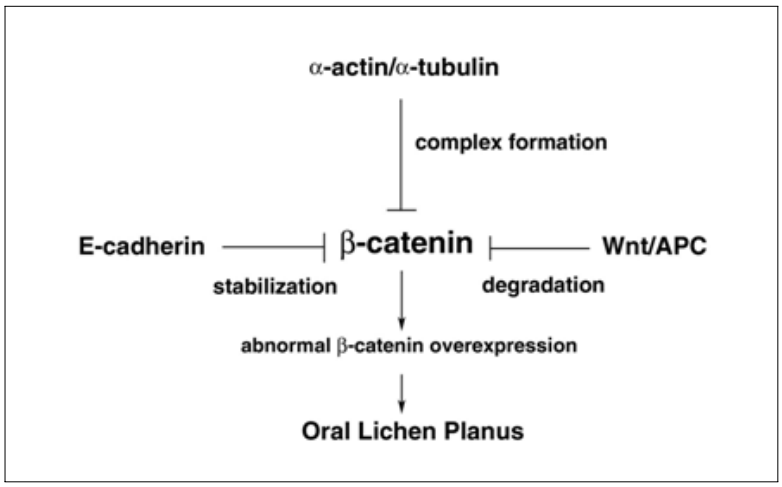

Fig. 6. The key molecule, β-catenin, is tightly regulated by E-cadherin, α-actin/α-tubulin, Wnt/APC, and etc. Therefore, the abnormal expression of β-catenin is able to be controlled by multiple gene therapies targeting E-cadherin, α-actin/α-tubulin, Wnt/APC.

of β-catenin and followed by the transcription of TCF, LEF1, NFkB, PCNA, etc. And more, the zonal infiltration of T-cells into submucosal area seems to be relevant to the activation of E-cadherin/β-catenin/TCF/LEF1 pathway. The premalignant potential of OLP seems to be relevant to E-cadherin/β-catenin/cyclin 1/NFkB pathway.

We also suggest that the β-catenin is an important candidate gene for gene therapy of OLP, which is controllable by the signalings of E-cadherin, α-actin/α-tubulin, Wnt/APC expressions, etc. However, because the above results were simply obtained from the IHC array performed in this study, the different pathogenetic mechanisms of OLP relevant to the overexpression of β-catenin should be investigated more by different molecular biological methods to elucidate the real signaling pathways of β-catenin in OLPs.

V. References

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