รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัย

การแสดงออกของ tight junction proteins ใน oral squamous cell carcinoma, odontogenic cyst และ tumor

Expression of tight junction proteins in oral squamous cell carcinoma, odontogenic cyst and tumor

โดย

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ชื่อเรื่อง : การแสดงออกของ tight junction proteins ใน oral squamous cell carcinoma, odontogenic cyst และ tumor

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Claudin และ occludin เป็นโปรตีนสำคัญในกลุ่มของ tight junctional complex มีหลายรายงานที่พบการเปลี่ยนแปลงการแสดงออกของ claudin และ occludin ในมะเร็งชนิดต่างๆ จุดประสงค์ของการศึกษาฉบับนี้ก็เพื่อตรวจสอบการแสดงออกของ claudin และ occludin ในมะเร็งชนิดสความัสเซลล์ในช่องปาก ถุงน้ำที่มีจุดกำเนิดมาจากฟัน และเนื้องอกที่มีจุดกำเนิดมาจากฟันและความสัมพันธ์ของการแสดงออกของโปรตีนเหล่านี้กับข้อมูลทางคลินิกและพยาธิวิทยา

ในการศึกษานี้เราใช้ตัวอย่างมะเร็งชนิดสความัสเซลล์ในช่องปากจำนวน 45 ชิ้นถุ งน้ำที่มีจุดกำเนิดมาจากฟัน และเนื้องอกที่มีจุดกำเนิดมาจากฟันจำนวน 15 ชิ้น ข้อมูลทางคลินิกของผู้ป่วยรายละเอียดทางจุลพยาธิวิทยาและข้อมูลการติดตามผลการรักษาถูกนำมาทบทวน และวิเคราะห์ เทคนิคอิมมูโนฮิสโตเคมีถูกนำมาใช้ในการหาการแสดงออกของโปรตีนที่ว่านี้

การศึกษาพบการแสดงออกของ claudin-1 และ claudin-4 ในมะเร็งชนิดสความัสเซลล์ในช่องปากเป็นจำนวน 86.7 และ 80% ตามลำดับ ส่วนใหญ่พบว่ามีการแสดงออกของโปรตีนนี้น้อยกว่า 25% นอกจากนี้ยังพบว่า การแสดงออกของ claudin-1 มีความเกี่ยวข้องกับ grading ของเนื้องอก การพบการลุกลามเข้าไปที่เส้นประสาทและหลอดเลือด การแพร่การจายไปยังต่อมน้ำเหลืองและ staging ของเนื้องอกด้วย ในขณะที่ไม่พบความสัมพันธ์ในทางคลินิกและพยาธิวิทยาของเนื้องอกกับการแสดงออกของ claudin-4 ดังนั้น claudin-1 จึงน่าจะมีบทบาทสำคัญในการดำเนินโรคของมะเร็งชนิดสความัสเซลล์ในช่องปาก และสามารถใช้เป็นตัวพยากรณ์โรคได้อีกด้วย

Title : Expression of tight junction proteins in oral squamous cell carcinoma, odontogenic cyst and tumor

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Claudin and occluding constitute a group of principal proteins forming the tight junctional complex. The altered expression of selected claudins and occluding has been reported in several human cancers. The purpose of this study was to investigate the expression of claudin and occludin in oral squamous cell carcinoma (OSCC), odontogenic cyst and tumot and examine their relationship with patient clinical-pathologic features. Fortyfive OSCC and 15 odontogenic cyst and tumor cases were enrolled. Patient clinical, pathologic and follow-up data were reviewed and the claudin and occluding expression were analyzed immunohistochemically. Only positive claudin-1 and claudin-4 immunoreactivities were noted in 86.7 and 80 % of OSCC cases, respectively. The majority of cases showed the staining in less than 25 % of cancer cells. The increased claudin-1 expression was significantly associated with the high pathologic grade, the presence of microscopic perineural invasion, vascular invasion, nodal metastasis, and advanced clinical stage. No relationship between various clinico-pathologic parameters and differential claudin-4 expression was observed. Claudin-1 may play a role in OSCC progression and could serve as a prognostic marker of advanced disease.

กิตติกรรมประกาศ

ผู้วิจัยขอขอบพระคุณมหาวิทยาลัยรังสิตที่มอบทุนวิจัยให้

ขอขอบพระคุณ คณบดี คณะทันตแพทยศาสตร์ มหาวิทยาลัยรังสิต ศ คลินิก พลเรือตรีหญิง สุชาดา วุฒเกนก ที่สนับสนุนให้ข้าพเจ้าได้ทำวิจัยต่อเนื่องหลังจากจบการศึกษา และให้กำลังใจมาตลอดเวลาที่ทำงานที่คณะทันตแพทยศาสตร์ มหาวิทยาลัยรังสิต

ขอขอบพระคุณ ศ. ทญ. ดร. สมพร สวัสดิสรรพ์ ที่สนับสนุนในเรื่องการทำวิจัย และเป็นแรงบันดาลใจมาตลอด

ขอขอบพระคุณ ผศ.ทพ.ดร. เอกรัฐ ภัทรธราธิป ที่ช่วยร่วมกันทำวิจัยจนสำเร็จลุล่วง

ขอขอบพระคุณ คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และบุคลากรในภาควิชาทันตพยาธิวิทยา และสถาบันพยาธิวิทยา กรมการแพทย์ ที่เอื้อเฟื้อสถานที่ในการทำวิจัยร่วมกันตั้งแต่เริ่มต้น

สุดท้ายทุกความดีที่ได้รับ ข้าพเจ้าขอมอบให้ แม่ พ่อ คู่ชีวิต และครอบครัว อันเป็นที่รักที่สุดในชีวิต

สารบัญ

สารบัญตาราง รูปภาพ

บทที่ 1
บทนำ

ความเป็นมาและความสำคัญของปัญหา

Intercellular junctions are crucial structures for physiologic functions in eukaryotes. Tight junctions (TJs) (1), one of the intercellular junction, are the most apical components of the junctional complexes. They play a main role for controlling cellular polarity and acting as a cellular barrier for ions, water and proteins transportation (2). TJs are also believed to be involved in signaling cascades (3) that control cell growth and differentiation . Several studies indicate that TJs are thought to play critical roles in the neoplastic process owing to their activity as couplers of the extracellular milieu to intracellular signaling pathways and the cytoskeleton (2). Abnormalities of tight junction permeability allow increased diffusion of nutrients and other factors that promote tumor growth and/or survival. Moreover, changes in TJs have been noted as an early event in tumor metastasis (4).

No study, to the best of our knowledge, has been done on the expression of claudins 1,4,5,7 and occludin in odontogenic cyst and tumor together with oral squamous cell carcinoma.

วัตถุประสงค์ของการวิจัย

Therfore, our aim was to analyze the distribution and staining patterns of claudins 1, 4, 5, and 7 and occludin in oral squamous cell carcinoma (OSCC), odontogenic cyst and tumor. Analysis of the expression of these proteins with the clinical status will also be analyzed statistically.

ขอบเขตของการวิจัย

ด้านเนื้อหา

Only expression of claudins 1,4,5,7 and occludin in odontogenic cyst and tumor together with oral squamous cell carcinoma were analyzed

ประชากรและกลุ่มตัวอย่าง

45 of OSCC and 15 of odontogenic cyst and tumor specimens from biopsy or surgical specimen at the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University since 2006-2012 will be enrolled for the study.

ระยะเวลาที่ดำเนินการวิจัย

Two and a half year.

ประโยชน์ที่คาดว่าจะได้รับ

1. The distribution and staining patterns of claudins 1, 4, 5, and 7 and occludin in oral squamous cell carcinoma (OSCC), odontogenic cyst and tumor
2. Analysis of the expression of these proteins with the clinical status were analyzed statistically

บทที่ 2
เอกสารและงานวิจัยที่เกี่ยวข้อง

Tight junctions comprise three major transmembrane proteins, namely claudins, occludin, and junctional adhesion molecules. Currently, at least 24 different members of the claudin family are known in humans, and claudins are essential for the “fence” and “gate” functions in epithelium and endothelium (5). Occludin, the first isolated and ubiquitously expressed transmembrane TJ protein, is not essential for TJ formation and function but may play a role in cellular signaling. Claudins are essential for the barrier function of epithelia and endothelia as they are thought to be responsible for the paracellular ionic selectivity seen in epithelium (2, 4, 5).

Claudin, discovered in 1998, are the main sealing proteins of TJs. They are connected with the actin cytoskeleton and participate in intracellular signaling. Expression of claudins may vary in different cells and tissues of the body. For example, claudin 2 is found in murine liver and kidney (6) but not in lung tissue while claudin 4 is found in murine lung and kidney but not in the liver (7). Variable expression of claudins has also been reported in rat liver and pancreatic cells (8). Recent studies have shown that considerable changes in TJ proteins expressions are associated with various carcinomas. Neoplastic cells frequently exhibit both structural and functional disorganization in their tight junctions. Downregulation or upregulation of claudins might have a role in cancer development. Claudin expression has been described in benign and malignant tumors, particularly in epithelial and endothelial cancers. The altered expression of some claudns has also been found in many human carcinomas such as those of the breast (9-12), ovary (11, 12), prostate (13, 14), liver (15, 16) and stomach (17, 18). Many of these studies have used either immunoreactivity intensity or quantity as the criterion for assessing the expression of the claudins in the various tumors studied. Claudin expression has been also shown to have prognostic value in these tumors (9-18).

In epithelial tumors, loss or gain in claudin expression has been associated with biologic behavior in some tumor types (19-24). However, the mechanism by which overexpression of claudins may contribute to tumor progression and aggressiveness is less clear. It has been suggested that a possible mechanism for this is that up-regulation and/or aberrant tissue expression of claudins may directly interfere with TJ formation and function and thereby contribute to neoplasia (2). Neverthelss, relatively few studies have described the expression of claudins or their relationship with tumor activity or behavior in oral cancer and odontogenic cyst or tumor.

Occludin expression decreased progressively in parallel with the increase in carcinoma grade, and the decreased occludin expression correlated with myometrial invasion and lymph node metastasis in endometrial carcvinoma (25). About squamous cell carcinoma, there are only few studies showing expression or clinical correlation of occludin staining (24, 26, 27).

บทที่ 3
วิธีดำเนินการวิจัย

Materials and methods

45 of OSCC and 15 of odontogenic cyst and tumor specimens from biopsy or surgical specimen at the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University since 2006-2012 were enrolled for the study. The specimen included OSCC, odontogenic cyst and tumor from various sites such as tongue, buccal mucosa, gingiva, palate, retromolar, lip and others.

Histopathologic slides were prepared from formalin-fixed, paraffin-embedded archival specimens. The tissue sections were cut at 4-µm thick, initially stained with hematoxylin and eosin (H&E) and examined under light microscope by two oral pathologists, both to confirm the diagnosis and grading of tumor. The tissue sections and patients' history were reviewed. Other clinical data such as patients age, gender, lymph node status and recurrent were collected. Cases were excluded if the specimen included any other associated pathology (e.g. chronic fungal, bacterial infection or other tumors).

Paraffin-embedded blocks from the tumor with adjacent areas and control tissue specimens were cut at 4-µm thick and place on lysine coated slides and then processed using standard immunohistochemical technique.

For immunohistochemical study, the tissue sections slides weretreated with a boiling solution of freshly prepared Tris EDTA buffer, pH 9.0 in microwave oven for 10 min. After cooling down to room temperature, the tissue sections were blocked the nonspecific reaction with normal goat serum at the dilution of 1:100 for 10 min. The sections were incubated in a moist chamber at 4ºC overnight with the primary antibodies.

Each primary antibody specific to claudin 1,4,5,7 and occludin were used at the dilution of 1:100. Then, slides will berinsed in Tris-buffered saline twice before being treated with goat anti-rabbit horseradish peroxidase (HRP) conjugated (secondary antibody) at dilution of 1:100 for 60 min at room temperature. Individual squamous cell carcinoma, odontogenic cyst and tumor specimen were treated in the same manner but with the omission of the primary antibody served as internal experimental controls. As positive controls, nonneoplastic kidney, breast, skin, and liver samples were used.

The immunohistochemical reaction were visualized by developing the slides in 3, 3′ diaminobenzidine tetrahydrochloride (Vector Laboratories, USA) and counter-stained with Mayer's hematoxylin. The tissue sections were then dehydrated, cleare and mount. The experiment were performed in triplicate.

The sections were evaluated under a Nikon Eclipse 800 microscope (Nikon Corporation, Japan) with a magnification of ×200. Staining will bescored by two oral pathologists, by evaluating both the percentage of stained cells within representative regions of each specimen and the intensity of the stain. Slides were randomly reviewed so as to minimize possible bias. For claudin 1,4,5,7 and occludin expression, only plasma membrane of malignant epithelial cells or tumor cells or cystic epithelium were regarded as claudin 1,4,5,7 and occludin positive staining.

The intensity of the stain was on the following scale:

0, no staining seen;

1, mild staining;

2, moderate staining;

3, intense staining.

The area of staining was evaluated as follows:

0, no stained cells in any microscopic field;

1, less than 25% of lesional cells stained positively;

2, between 25 and 50% of lesional cells stained positively;

3, between 50 and 75% of lesional cells stained positively;

4, greater than 75% of lesional cells stained positively.

The sum between area and intensity of staining were used for statistical analysis as described by Brennan *et al*. For example, if the intensity of stain was graded as 3, the area of staining is graded as 4, the sum is 3 plus 4 which is 7. In this analysis, the minimum score were zero and the maximum were seven.

For the analyses of claudin and occludin expressions, cases were divided into 2 groups, the low expression group (cases with less than 50% of positive cancer cells) and the high expression group (cases with more than 50% of positive cancer cells).

Expression of claudin 1,4,5,7 and occludin in OSCC, odontogenic cyst and tumor and the association with clinical and histopathological data were reported and analyzed by Non-parametric Mann-Whitney test for two group differences, Kruskal-Wallis test for three group differences and Spearman Rank correlation for correlation analysis. All statistical analyses will performed with SPSS statistical software package version (latest edition) (SPSS, Chicago).

บทที่ 4
ผลการวิเคราะห์ข้อมูล

Results

Patient characteristics

Only claudin-1 and claudin-4 were observed in the oral squamous cell carcinoma specimen. Occludin staining were most negative in OSCC while both claudin and occludin were totally absent in odontogenic cyst and tumors.

The clinical and pathological characteristics of 45 OSCC patients are present in Table 1. Briefly, there were 22 male and 23 female patients with a mean age of 65.82+12.10 years (range= 44-86 years). The majority of lesions were located on gingiva (37.8%), followed by floor of mouth (17.8%), tongue (15.6%) and buccal mucosa (13.3%). Ten patients reported local recurrence (22.2%). Twenty-four patients (53.3%) had regional lymph node involvement, and 2 patients had distant metastasis (4.4%). The majority of patients were classified as TNM stage I (40.0%), followed by stage IV (31.1%). Microscopically, 53.3% of cases was graded as well-differentiated, followed by moderately differentiated (33.3%) and poorly differentiated (13.3%).

Table 1 : Clinical and pathological detail of the patients

Clinical and pathologic variables		Number of patient(N)
Sex	Male	22(48.9%)
	Female	23(51.1%)
Age (years)	Mean +- SD (65.82 +-12.10)	
	Range (44-86)	
Site	Gingiva	17(37.8%)
	Floor of mouth	8(17.8%)
	Tongue	7(15.6%)
	Buccal mucosa	6(13.3%)
	Alveolar mucosa	4(8.9%)
	Hard palate	3(6.7%)
T stage	T1	24(53.3%)
	T2	11(24.4%)
	T3	5(11.1%)
	T4	5(11.1%)
N stage	N0	21(46.7%)
	N1	13(28.9%)
	N2	7(15.6%)
	N3	4(8.9%)
Distant metastasis	Absence	35(77.8%)
	Presence	10(22.2%)
Recurrence	Absence	35(77.8%)
	Presence	10(22.2%)
Pathologic grade	Well differentiated	24(53.3%)
	Moderately differentiated	15(33.3%)
	Poorly differentiated	6(13.3%)
Perineural invasion	Absence	28(62.2%)
	Presence	17(37.8%)

Vascular invasion	Absence	26(57.8%)
	Presence	19(42.2%)
TNM staging	Stage I	18(40.0%)
	Stage II	6(13.3%)
	Stage III	7(15.6%)
	Stage IV	14(31.1%)

Expression of claudin and occluding in OSCC and odontogenic cyst and tumor

A membranous staining pattern of cancer cells was noted in all positive cases for both proteins. The staining was more intense in the central squamous cells than the peripheral basal cells of tumor nests. Only claudin-1 and claudin-4 were observed in the oral squamous cell carcinoma specimen. The claudin-1 immunoreactivity was observed in 86.7% of cases. The majority of them showed the positive staining in less than 25% of cancer cells (level 1+; 33.3%), followed by the staining in 26-50% of cells (level 2+; 26.6%). The positive immunoreactivity of claudin-4 appeared less frequent (80.0%) than that of claudin-1. More than 60% of cases showed claudin-4 positivity in less than 25% of cancer cells, and no case stained more than 75% of cancer cells. (Figure 1)

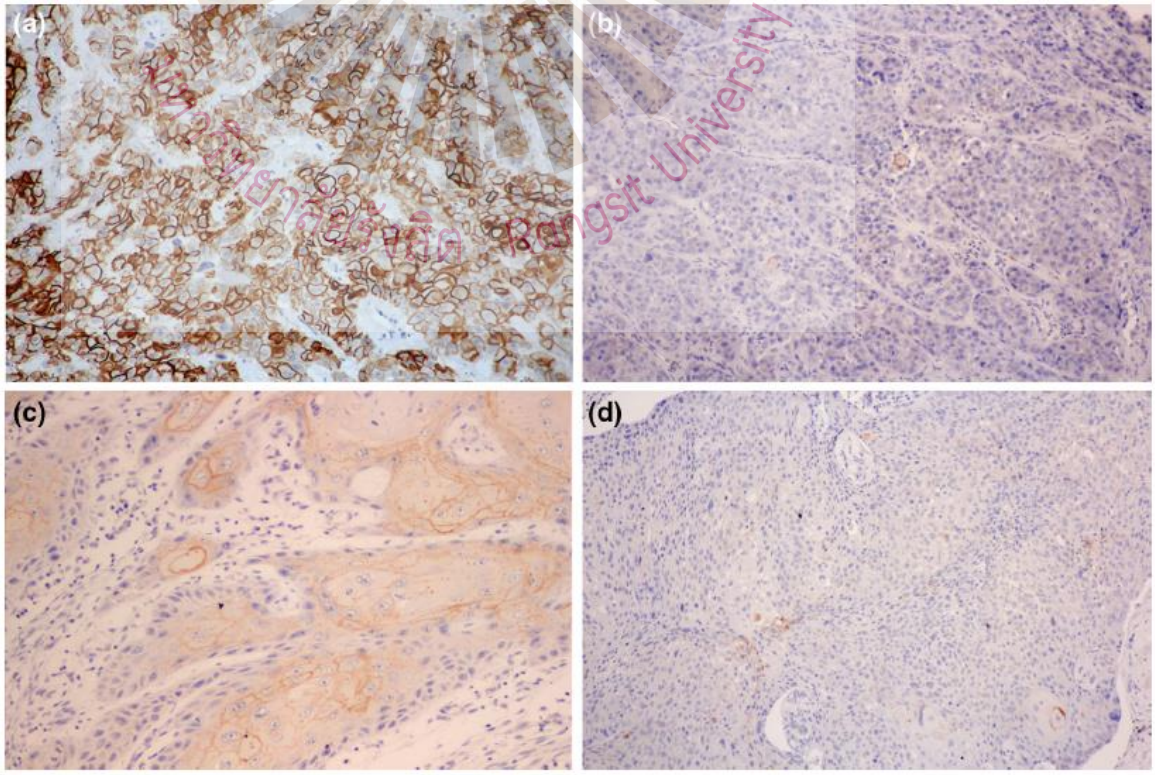

Figure 1 : Representative photomicrographs of claudin-1 and claudin-4 immunoreactivity in OSCC (Magnification x100). A) High claudin-1 expression; B) Low claudin-1 expression; C) High claudin-4 expression; D) Low claudin-4 expression**.**

In addition, the correlation between the expression levels of claudin-1 and claudin-4 was examined. The statistically significant positive relationship was noted between the expression patterns of both proteins (P=0.03) (Table 2).

Table 2 : Immunohistochemical staining of OSCC cells

Staining / Marker	IHC staining				
	0	**1+**	**2+**	**3+**	**4+**
Claudin 1	6(13.3%)	15(33.3%)	12(26.6%)	3(6.7%)	9(20%)
Claudin 4	9(20.0%)	30(66.7%)	2(4.4%)	4(8.9%)	0(0%)
Occludin	41(91.1%)	4(8.9%)	0(0%)	0(0%)	0(0%)

Relationships between the claudin-1 and claudin-4 expressions and the clinico-pathologic features

For the analyses of claudin expressions, cases were divided into 2 groups, the low expression group (cases with less than 50% of positive cancer cells) and the high expression group (cases with more than 50% of positive cancer cells). No sex or age difference was observed between the two groups.

Results are shown on Table 3. Significantly, the increased claudin-1 expression was associated with the high pathologic grade (P=0.02), high T stage (P=0.01), the presence of microscopic perineural (P=0.03) and vascular (P=0.04) invasions, regional lymph node involvement (P=0.02) and the advanced TNM stage (P=0.00). On the contrary, no statistically significant relationship was noted between the claudin-4 expression and all clinico-pathologic features examined

Table 3 : Relationship between claudin-5 and claudin-7 expression and clinico-pathologic features of OSCC patients

Clinico pathologic parameter		Claudin 1 expression			Claudin 4 expression		
		Low	High	P value	Low	High	P value
Sex	Male	18	4	0.21	20	2	1
	Female	15	8		21	2	
Age (years)	<65	17	4	0.28	19	2	0.98
	>65	16	8		22	2	
TNM staging	Stage1-2	23	1	0	23	1	0.33
	Stage3-4	0	11		18	3	
Tumor size	T1-T3	32	8	0.01	37	3	0.39
	T4	1	4		4	1	
Lymph node involvement	Absence	19	2	0.02	20	1	0.61
	Presence	14	10		21	3	
Distant metastasis	Absence	32	11	0.47	40	3	0.132
	Presence	1	1		1	1	
Recurrence	Absence	25	10	0.71	32	3	1
	Presence	8	2		9	1	
Pathologic grade	Well differentiated	21	3	0.02	23	1	0.33
	Moderately/poorly differentiated	12	9		18	3	
Perineural invasion	Absence	24	4	0.03	27	1	0.14
	Presence	9	8		14	3	
Vascular invasion	Absence	22	4	0.04	23	3	0.63
	Presence	11	8		18	1	

Survival analysis

The follow up period ranged from 8 to 119 months (median=38 months). At the end of the follow-up period, seventeen patients died of OSCC, 2 patients died of other causes and the remaining 26 patients were alive with no disease. The advanced clinical staging was strongly correlated with the poor overall patient survival (P=0.01). Regarding claudin expressions, the univariate survival analysis showed a tendency towards the association of the higher claudin-1 expression and a shorter survival

time (Figure 2), however, this did not reach statistically significant level (P=0.07). Claudin-4 expression showed no statistically significant association with cancer-specific survival of patients (P=0.85).

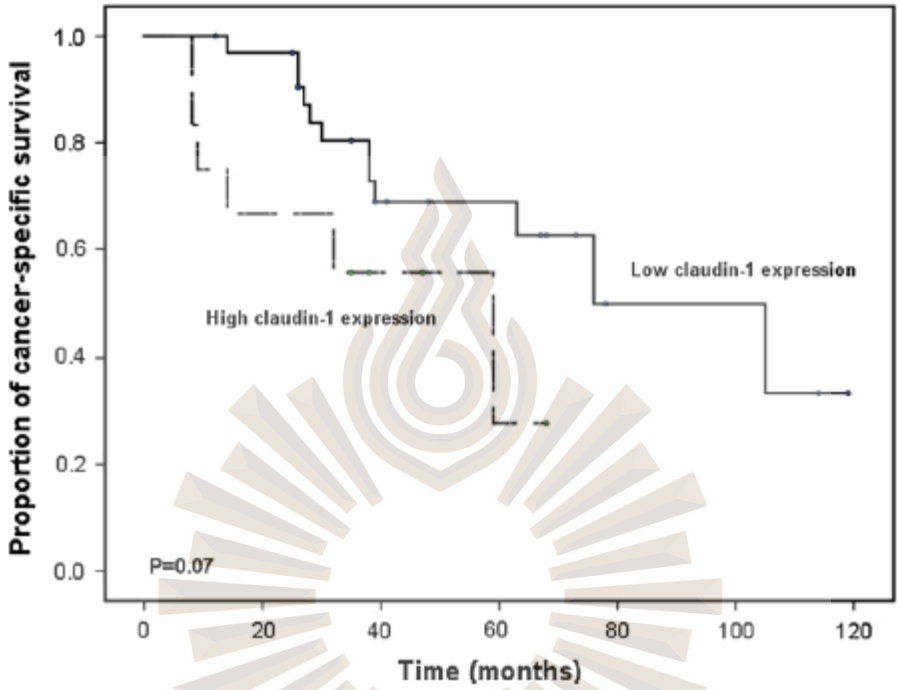

Figure 2 : Kaplan-Meier curve of OSCC patients with low (less than 50% positivity) versus high (more than 50% positivity) expression of claudin-1. (P=0.07)

บทที่ 5
สรุปและอภิปรายผล

Discussion

An increasing number of studies has demonstrated the changes in the expression levels of different claudins in a variety of human cancers. Claudin-1 is perhaps the most studied protein among claudin family members. The reduced expression of claudin-1 was observed in breast and prostate cancers[10-12], however, a greater number of other cancers including gastric, thyroid, pancreatic, urothelial and cervical cancers instead showed the increased claudin-1 expression[13-16]. This suggests that this protein may have tissue-specific functions and its roles in various cancers may be different depending on the type of cancer cells and/or the nearby cancer environment.

In this study, we reported the potential role of claudin-1 in OSCC. The overexpression of claudin-1 was observed in the more advanced diseases and associated with the invasive histopathologic features. This indicates that claudin-1 may be either directly or indirectly involved in the progression of this cancer.

The underlying mechanisms of claudin-1 in the progression of several cancers are not completely understood and become a basis of recent molecular studies. To date, no claudin gene mutation has been reported. Instead, recent evidence suggested that claudins may be involved in cancer progression through the complex interaction with several extracellular matrix elements. In the expression cloning study, claudin-1 was shown to increase matrix metalloproteinase-2 (MMP-2) activity via its interaction with membrane-typed matrix metalloproteinase-1 (MT1-MMPs) and enrich the localization of MMP-2 on the cell surface[35]. This could enhance the invasive potential of cancer cells through the degradation of extracellular matrix components, including the basement membrane. In colon carcinomas, the claudin-1 upregulation was associated with the increased cancer cell migration and MMP-2 and MMP-9 activities. In contrast, inhibition of claudin-1 in colonic cancer cells decreased their invasive/metastatic potential, promoted apoptosis and reduced cell survival[32]. The development of intraoral SCC is different from that of other cancers. Among the significant predisposing factors are tobacco use, alcohol consumption, betel nut chewing and human papilloma virus (HPV) infection. In an individual, oral mucosal tissues of different sites are to some extent exposed to these similar types of carcinogenic agents. As a result, we did not find statistically

significant differences of either claudin-1 or claudin-4 expression in different intraoral tissues in this study.

A handful of studies reported the altered claudin-1 expression in OSCC[25, 33, 31]. Compared to the normal oral mucosa, the claudin-1 expression was altered in different grades of oral epithelial dysplasias and squamous cell carcinoma[26, 31]. In contrast to the tissue microarray study by Lourenco et al.[31] which reported the low-to-absent claudin-1 expression in moderately/poorly differentiated OSCCs, we found that the increased claudin-1 expression is significantly associated with higher pathologic grade. This discrepancy of results may partly be related to tissue sampling error from the tissue microarray technique. In the present study, the entire tissue specimen from each case was analyzed and some variations of claudin staining in different areas of the section were noted. Therefore, sampling a selected portion of the specimens may not be entirely representative of the lesions. In conjunction with our finding, Dos Reis et al. found that the increased claudin-1 gene expression was associated with the increased angiolymphatic and perineural invasions[33], the prognostically relevant histopathologic features of OSCC[2].

A study of SCC of the lower lip revealed that the claudin-1 expression was higher in metastatic and advance-staged cases[27]. This findings is consistent with our results, even though in our study all lesions originated from the intraoral sites. The pathogenesis of OSCC of the lower lip is considered different from that of intraoral sites, due to some different predisposing factors involved, in particular the sunlight exposure. This suggests that claudin-1 may be involved in one of the common pathways in the development of the head and neck SCC.

The molecular insight of the contribution of claudin-1 in OSCC progression also pointed towards its role in cell-extracellular matrix interaction. Oku et al. found that the inhibition of claudin-1 expression in OSCC cell lines diminished the cancer cell invasion and the degradation of laminin-5, an important component of the basement membrane, through MMP-2 and MT1-MMP inactivation[34]. Overall, it appears that claudin-1 could be a potential marker of OSCC invasiveness. The high expression of this protein is related to the more progressive lesions and consequently poor clinical outcome of patients.

A number of studies reported the overexpression of claudin-4 in a variety of cancers[19, 17, 14, 18, 20, 12, 21-23]. The claudin-4 up-regulation was shown to stimulate MMP-2 activity in ovarian carcinoma cells and promote cancer invasion[36]. A strong correlation of claudin-4 expression and poor patient survival was also reported in a few cancers, such as gastric adenocarcinoma and

endometrial carcinoma[37, 15]. In addition, based on a recent gene expression profiling study, claudin-4 was found to be a predictive marker for the poor response to radiation therapy of patients with head and neck SCC[38]. However, we did not find any significant relationship with the claudin-4 expression and patient clinical-pathologic features and survival data. Therefore, our data do not support the prognostic role of altered claudin-4 in patients with OSCC.

Occludin is believed to be not essential for TJ formation and function but may play a role in cellular signaling. The expression of occludin in our study is mostly negative which corresponds with study in tongue squamous cell carcinoma (24). While study in hepatocellular carcinoma(28), urothelial carcinoma show occludin expression without clinic-pathological impact(29).

In conclusion, the present study demonstrates the claudin-1 expression in OSCC and its clinical implications. The high claudin-1 expression in cancer cells is significantly associated with the high pathologic grade, increased perineural/vascular invasion, increased propensity of lymph node metastasis and advanced clinical stage of tumor. These results suggest that claudin-1 may play a role in the progression of OSCC. Notably, the claudin-1 expression assessed immunohistochemically may be a potential indicator of advanced diseases in these patients.

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