자궁 경부 편평세포암에서 Tight Junction 단백인 Claudin-1과 Claudin-7의 발현에 관한 연구

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Expressions of Tight Junction Protein Claudin-1 and Claudin-7 in Squamous Cell Carcinoma of the Uterine Cervix

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Objective : Claudins have been shown to be up-regulated in various cancers and have been suggested as possible biomarkers and targets for cancer therapy. The aims of this study were to evaluate the expression patterns of claudin-1 and claudin-7 in squamous cell carcinoma of the uterine cervix and investigate the different expressions according to presence of lymph node metastasis in theses carcinoma.

Methods : Tumor specimens were obtained from each 20 patients with invasive squamous cell carcinoma (ISCC) with and without lymph node (LN) metastasis. Normal cervical tissues as control were obtained from 10 patients with benign uterine disease. Immunohistochemical analysis was performed with antibodies to claudin-1 and claudin-7.

Results : The expressions of claudin-1 and claudin-7 were undetectable in normal exocervical mucosa, but had variable staining in endocervical mucosa. The expressions of both claudin-1 and claudin-7, exhibiting mainly membranous staining, were significantly up-regulated in all of the ISCC in comparison to the normal control in similar manner ($P<0.001$ and $P<0.001$, respectively). The expressions of claudin-1 and claudin-7 were not significantly different between ISCC with and ISCC without LN metastasis ($P=0.54$ and $P=0.53$, respectively). However there was increasing tendency of expressions in ISCC with LN metastasis in comparison to ISCC without LN metastasis.

Conclusion : These results suggest that claudin-1 and claudin-7 may play a significant role in carcinogenesis of squamous cell carcinoma of the uterine cervix and may represent novel markers for this disease.

Key Words : Cervical neoplasm, Tight junctions, Claudin-1, Claudin-7, Metastasis

INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide, with approximately 400,000 new cases being diagnoses each year despite existence of screening methods.[1] Metastasis is the primary cause of fatality in cervical cancer patients. Although it is believed there are a number of events contributing to the process of metastasis, it is widely accepted that the loss of cell-to-cell adhesion in neoplastic epithelium is necessary for invasion of surrounding stromal elements and subsequent metastatic events.[2]

Cell-to-cell adhesion in epithelial cell sheets is maintained mainly through two types of junctions: adherens junctions and tight junctions (TJs). TJs are intercellular junctions adjacent to the apical end of the lateral membrane surface.[3] They have two functions, the barrier function and the fence function. The barrier function of TJs regulates the passage of ions, water, and various macromolecules, even cancer cells, though paracelluar spaces.[4-6] The barrier function is thus relevant to edema, jaundice, diarrhea, and blood-borne metastasis. On the other hand, the fence function maintains cell polarity. In other words, TJs work as a fence to prevent intermixing of molecules in the apical membranes with those in the lateral membrane. This function is deeply involved in cancer cell biology, in terms of loss of cell polarity.[6-8]

From extensive investigations[3,9] into the molecular components of the TJ structure, it has become apparent that the junctions are composed of three regions: (i) the integral membrane proteins occludin, claudins, junctional adhesion molecules; (ii) the peripheral proteins zonula occluden-1, -2, -3; and (iii) proteins associated with TJs. Claudins contain four transmembrane domains and two extracellular loops through which they bind to claudins on adjacent cells.[10] Normal cells typically express multiple claudin proteins, but some family members exhibit a tissue specific distribution. For example, claudin-5 is the main form in endothelial cells,[9,11] suggesting that they may contribute to functional differences, whereas the type of claudin may be a determining factor in the tightness of the TJs.[9,12] Currently, at least there are 20 known members of the claudin family.[6]

As TJs exist between the stromal, the endothelium and the tumor cells, TJs are the first structure blocking the way for cancer cells to metastasis. For the cancer cell to spread, the TJ structure must be disturbed and broken to allow penetration of the cancer cells.[8] Although changes in the permeability of TJs have been observed in several types of cancer, little is known about the role of claudins in cervical cancer.

In this study we used an immunohistochemical assay to evaluate the expression patterns of claudin-1 and claudin-7 in cervical squamous cell carcinoma and to investigate whether differences in expression of these molecules exist during lymph node (LN) metastasis.

METHODS

1. Tissue specimens

This study was carried out using stored paraffin-embedded tissue blocks from patients who were treated by radical hysterectomy and pelvicparaaortic lymphadenectomy in the Department of Obstetrics and Gynecology, Samsung Medical Center, Sungkyunkwan University School of Medicine between 1995 and 2002. One designated gynecological pathologist reviewed all cases in regard to histological type, histological grading, and LN status. Paraffin-embedded tissues were divided into two groups: primary cervical cancer tissues with or without LN metastasis. Tissue blocks from 20 patients in each group were available for immunohistochemistry of claudin-1 and claudin-7. As normal control, we obtained 10 normal cervical tissues from paraffin-embedded tissue blocks from patients who had simple hysterectomy for myoma uteri.

2. Immunohistochemistry

Immunostaining was performed under standard avidin-biotin complex peroxidase method by use of DAKO Tech Mate100. Used antibodies are claudin-1 (dilution: 1:50, Zymed Laboratories Inc., San Francisco, CA) and claudin-7 (dilution: 1:50, Zymed Laboratories Inc., San Francisco, CA) and heat-induced antigen retrieval was applied for a pretreatment.

3. Interpretation of stains

A specialized pathologist who was blind to clinical features of the patients, reviewed slides and evaluated immunohistochemical data. The intensity of staining was scored on a scale from 0 to 3+: 0, no staining; 1+, less than 50%; 2+, 50-90%; 3+, more than 90%. The distribution of cell staining was also assessed. The percentage of cells expressing claudin-1 and claudin-7 was estimated by dividing the number of positively

stained tumor cells by the total number of tumor cells per high-power field.

4. Statistical analysis

Statistical calculations were carried out using SPSS for Windows version 11.0. Student t-test and Fisher's exact test were used for correlation analysis and the least significant difference test using ranks was applied for multiple comparison among 3 groups. A P value <0.05 was considered statistically significant.

RESULTS

Clinicopathological features of the patients are described in Table 1. The expressions of claudin-1 and claudin-7 were undetectable in normal exocervical mucosa (Fig. 1A and 1D, respectively), but had variable staining in endocervical mucosa (Fig. 1B and 1E, respectively). Both claudin-1 and claudin-7 were strongly expressed in all cases of invasive squamous cell carcinomas (ISCC) and most of tumor cells showed intense membranous staining pattern (Fig. 1C and 1F). The expressions of claudin-1 and claudin-7 were significantly different between normal control and all ISCC groups ($P<0.001$ and $P<0.001$, respectively). However the expressions of claudin-1 and claudin-7 were not significantly different between two tumor groups according to LN metastasis ($P=0.54$ and $P=0.53$, respectively). However there was an increasing tendency of claudin-1 and claudin-7 expressions for ISCC with LN metastasis compared to ISCC without LN metastasis (Table 2 and Table 3, respectively).

Table 1. Clinicopathological features of cervical squamous cell carcinoma with or without lymph node (LN) metastasis

	LN negative (N=20)	LN positive (N=20)	P
Age (years, mean SD)	50.1±11.68	46.9±9.94	0.36
Tumor size (cm, mean±SD)	3.69±1.36	4.23±1.75	0.28
Parametrial invasion (%)	0	10	0.13
Full thickness invasion (%)	25	45	0.02
Serum TA-4 (ng/ml, mean±SD)	2.05±0.58	5.31±5.77	0.03
FIGO stage			0.58
IB1	6	7	
IB2	5	6	
IIA	9	7	

Table 2. The expression of claudin-1 in normal cervical squamous epithelium, invasive squamous cell carcinoma (ISCC) without lymph node (LN) metastasis, and with LN metastasis

Histologic grade	0	1+	2+	3+
Normal	10/10 (100%)			
ISCC, LN(-)[a]	-	3/20 (15%)	6/20 (30%)	11/20 (55%)
ISCC, LN(+)[b]	-	2/20 (10%)	6/20 (30%)	12/20 (60%)

[a-b] *(P=0.54) by multiple comparison*

Table 3. The expression of claudin-7 in normal cervical squamous epithelium, invasive squamous cell carcinoma (ISCC) without lymph node (LN) metastasis, and with LN metastasis

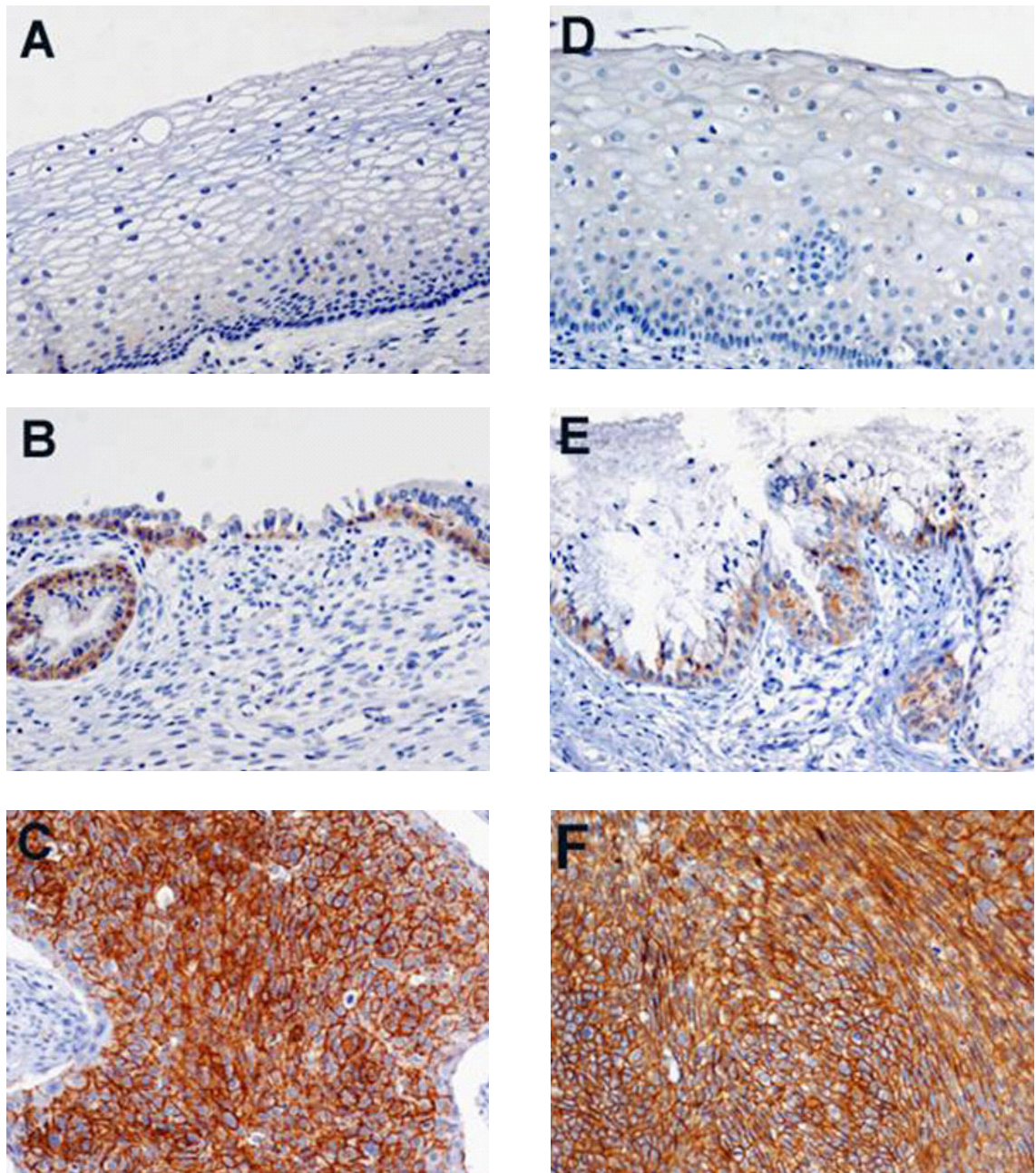| Histologic grade	0	1+	2+	3+
Normal	10/10 (100%)			
ISCC, LN(-)[a]	-	2/20 (10%)	5/20 (25%)	13/20 (65%)
ISCC, LN(+)[b]	-	1/20 (5%)	4/20 (20%)	15/20 (75%)

[a-b] *(P=0.53) by multiple comparison*

Fig. 1. Immunohistochemical staining of claudin-1 and claudin-7 in cervical tissues. (A–C) claudin-1. (A) Normal cervical squamous epithelium showing no immunostaining. (B) Normal endocervical mucosa showing positive staining. (C) Invasive squamous cell carcinoma (ISCC) showing intense immunostaining. (D–F) claudin-7. (D) Normal cervical squamous epithelium showing no immunostaining. (E) Normal endocervical mucosa showing positive staining. (F) ISCC showing intense immunostaining. Immunohistochemical locations of claudin-1 and claudin-7 were mainly membranous and patterns of immunostaining were almost identical in both (Original magnification ×400).

DISCUSSION

The aim of present study was to evaluate the expression patterns of claudin-1 and claudin-7 in cervical squamous cell carcinoma and to investigate whether differences in their expression of these exist during LN metastasis. The expressions of claudin-1 and claudin-7 were undetectable in normal squamous cell epithelium of the exocervix. Both claudin-1 and claudin-7 were strongly expressed in all cases of squamous cell carcinomas and also there was a significant difference between normal control and all ISCC groups ($P<0.001$ and $P<0.001$, respectively). However the expressions of claudin-1 and claudin-7 were not significantly different between two tumor groups according to LN metastasis ($P=0.54$ and $P=0.53$, respectively), although there was an increasing tendency of expressions in ISCC with LN metastasis compared to ISCC without LN metastasis (Table 2 and Table 3, respectively).

Results of the present study regarding claudin-1 correspond with the recent study of cDNA and tissue microarrays in cervical cancer which revealed that the expression of claudin-1 was low in normal basal epithelial cells and elevated mildly in LSIL and increased significantly in HSIL and squamous cell carcinoma by in situ hybridization.[13] Claudin-1 is expressed in human mammary epithelial cells, but is only expressed in low or undetectable levels in several established breast cancer cell lines.[14-16] The expression profile of claudin-1 in non-malignant versus tumor-derived cells has made this gene interesting for its involvement in tumorigenesis, namely as a suppressor for mammary epithelial proliferation. The loss of their expression in breast cancer cell lines is believed to be not due to genetic mutations in the human claudin-1 gene, both in sporadic and familial breast cancer compared to normal controls.[16] It appears that other regulatory or epigenetic factors may be involved in the down-regulation of the claudin-1 gene during breast cancer development.[16]

Kominsky et al. reported that the expression of claudin-7 was lost in both preneoplastic and invasive ductal carcinoma of the breast, occurring predominantly in high-grade lesions.[17] Claudin-7 expression was also frequently lost in lobular carcinoma in situ (LCIS), correlating with the increased cellular discohesion observed in LCIS. Additionally, the majority of invasive ductal carcinoma cases displaying a low claudin-7 expression had a positive lymph node metastasis. Taken together, these findings suggest that the loss of claudin-7 may aids in tumor cell dissemination and augments metastatic potential.[17] Also in cDNA microarray study for head and neck cancer, claudin-7 was down-regulated in head and neck squamous cell carcinoma compared to normal cells.[18] However in our study, claudin-7 was highly expressed in squamous cell carcinoma of the cervix in comparison to the normal control.

Why the expressions of claudin-1 and caudin-7 are increased in cervical caner and what is the cause of these different results in comparison to breast, head and neck cancer? It is not entirely clear why claudin-1 and claudin-7 are up-regulated in cervical cancer but the answer to the above question may lie in the tissue-specific action of claudins in different tissues.

Evaluating the roles of claudin-1 and claudin-7 in cervical and other cancers may provide new opportunity for cancer therapy. In fact, several reports suggest that the targeting prostate and pancreatic cancer cells expressing claudin-3 and claudin-4 with *Clostridium perfringens* enterotoxin, a bacterial toxin that specifically binds these transmembrane proteins,[19,20] may provide novel strategies against tumors resistant to conventional therapy.[21,22]

In conclusion, this study demonstrates that the expressions of the TJ protein claudin-1 and claudin-7 were increased in ISCC in comparison to the normal control with statistical significance and showed increasing tendency in ISCC with LN metastasis than ISCC without LN metastasis. Especially, the evaluation for claudin-7 in cervical cancer has been a novel study until now. We suggest that claudin-1 and claudin-7 may be involved in carcinogenesis of uterine cervical cancer and they may be used as biomarkers for detection of cervical carcinoma.

REFERENCES

1. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. CA Cancer J Clin 1999; 49: 33-64.
2. Jiang WG, Puntis MC, Hallett MB. Molecular and cellular basis of cancer invasion and metastasis: implications for treatment. Br J Surg 1994; 81: 1576-90.
3. Jiang WG, Martin T.A, Llaffafian I, Mansel R.E. Tight junctions, a critical structure in the control of cancer invasion and metastasis. 1st ed. Netherlands: Kluwer Academic Publishers; 2000.
4. Anderson JM, Van Itallie CM. Tight junctions: closing in on the seal. Curr Biol 1999; 9: R922-4.
5. Tsukita S, Furuse M. Pores in the wall: claudins constitute tight junction strands containing aqueous pores. J Cell Biol 2000; 149: 13-6.
6. Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. Prog Biophys Mol Biol 2003; 81: 1-44.
7. Sawada N, Murata M, Kikuchi K, Osanai M, Tobioka H, Kojima T, Chiba H. Tight junctions and human diseases. Med Electron Microsc 2003; 36: 147-56.
8. Martin TA, Jiang WG. Tight junctions and their role in cancer metastasis. Histol Histopathol 2001; 16: 1183-95.
9. Fanning AS, Mitic LL, Anderson JM. Transmembrane proteins in the tight junction barrier. J Am Soc Nephrol 1999; 10: 1337-45.
10. Morita K, Sasaki H, Furuse M, Tsukita S. Endothelial claudin: claudin-5/TMVCF constitutes tight junction strands in endothelial cells. J Cell Biol 1999; 147: 185-94.
11. Liebner S, Fischmann A, Rascher G, Duffner F, Grote EH, Kalbacher H, et al. Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. Acta Neuropathol (Berl) 2000; 100: 323-31.
12. Tsukita S, Furuse M. Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell Biol 1999; 9: 268-73.
13. Chen Y, Miller C, Mosher R, Zhao X, Deeds J, Morrissey M, et al. Identification of cervical cancer markers by cDNA and tissue microarrays. Cancer Res 2003; 63: 1927-35.
14. Swisshelm K, Machl A, Planitzer S, Robertson R, Kubbies M, Hosier S. SEMP1, a senescence-associated cDNA isolated from human mammary epithelial cells, is a member of an epithelial membrane protein superfamily. Gene 1999; 226: 285-95.
15. Hoevel T, Macek R, Mundigl O, Swisshelm K, Kubbies M. Expression and targeting of the tight junction protein CLDN1 in CLDN1-negative human breast tumor cells. J Cell Physiol 2002; 191: 60-8.
16. Hoevel T, Macek R, Mundigl O, Swisshelm K, Kubbies M. Genomic organization of claudin-1 and its assessment in hereditary and sporadic breast cancer. Hum Genet 2000; 107: 249-56.
17. Kominsky SL, Argani P, Korz D, Evron E, Raman V, Garrett E, et al. Loss of the tight junction protein claudin-7 correlates with histological grade in both ductal carcinoma in situ and invasive ductal carcinoma of the breast. Oncogene 2003; 22: 2021-33.
18. Al Moustafa AE, Alaoui-Jamali MA, Batist G, Hernandez-Perez M, Serruya C, Alpert L, et al. Identification of genes associated with head and neck carcinogenesis by cDNA microarray comparison between matched primary normal epithelial and squamous carcinoma cells. Oncogene 2002; 21: 2634-40.
19. Fujita K, Katahira J, Horiguchi Y, Sonoda N, Furuse M, Tsukita S. Clostridium perfringens enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein. FEBS Lett 2000; 476: 258-61.
20. McClane BA. Clostridium perfringens enterotoxin and intestinal tight junctions. Trends Microbiol 2000; 8: 145-6.
21. Long H, Crean CD, Lee WH, Cummings OW, Gabig TG. Expression of Clostridium perfringens enterotoxin receptors claudin-3 and claudin-4 in prostate cancer epithelium. Cancer Res 2001; 61: 7878-81.
22. Michl P, Buchholz M, Rolke M, Kunsch S, Lohr M, McClane B, et al. Claudin-4: a new target for pancreatic cancer treatment using Clostridium perfringens enterotoxin. Gastroenterology 2001; 121: 678-84.

국문초록

목적 : Claudins은 다양한 암종에서 상향 조절된다고 알려져 있으며 이들 암에서 생물학적 지표 (biomarker)와 치료의 표적이 될 수 있을 것으로 생각되고 있다. 본 연구의 목적은 자궁경부 편평세포암에서 claudin-1과 claudin-7의 발현 양상을 규명하고 또한 이들 단백이 임파전이에 관여하는지를 규명하고자 한다.

연구 방법 : 종양조직은 임파전이의 유무에 따라 자궁경부 편평세포암 조직에서 20명씩의 환자로부터 파라핀 고정조직을 얻었고, 정상 자궁경부 조직은 양성 자궁 질환을 가진 10명의 환자로부터 채취되었다. Claudin-1과 claudin-7에 대한 항체를 이용하여 이들 조직에서 면역조직화학적 분석(immunohistochemical analysis)을 시행하였다.

결과 : 정상 외자궁경부(exocervix) 점막에서는 claudin-1과 claudin-7의 발현을 관찰할 수 없었으나, 내자궁경부(endocervix) 점막에서는 다양한 염색 양상을 보였다. 그러나 모든 자궁경부암 조직에서 claudin-1과 claudin-7의 발현은 주로 세포막에서 보였으며, 정상 자궁경부 조직에 비해 유의하게 증가된 양상을 보였다($P<0.001$ and $P<0.001$, respectively). 자궁경부암 조직에서 림프절 전이 여부에 따른 claudin-1과 claudin-7의 발현은 통계적으로 유의한 차이를 보이지 않았으나 임파전이가 있는 군에서 발현이 증가되는 양상을 보였다($P=0.54$ and $P=0.53$, respectively).

결론 : 본 연구의 결과에서 claudin-1과 claudin-7은 자궁경부 편평세포암의 발암과정에 관여할 것으로 생각되고 또한 이 질환에 특징적인 표식자가 될 수 있음을 보여 주었다. 향후 tight junction 및 claudin 유전자에 대한 연구는 자궁경부암의 조기진단 및 치료에 많은 기여를 할 것으로 생각된다.

중심단어 : 자궁경부암, Tight junction, Claudin-1, Claudin-7, 전이