

Urological Oncology

Correlation between Claudins Expression and Prognostic Factors in Prostate Cancer

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Purpose: The purpose of this study was to evaluate the correlation between the expression of claudins and prognostic factors in patients with prostate cancer.

Materials and Methods: The subjects of this study were 48 patients who had undergone surgery for prostate cancer. The Gleason score (6 or lower, 7 or higher), prostate-specific antigen (PSA) level, T stage, biochemical recurrence, local recurrence, and distant metastasis were compared according to the expression of claudin-1 and claudin-5 in prostate cancer.

Results: In the group with a low expression of claudin-1, the Gleason score was 7 points or higher in 18 cases (82%) and 6 points or lower in 4 cases (18%). In the group with a high expression of claudin-1, the Gleason score was 7 points or higher in 13 cases (50%) and 6 points or lower in 13 cases (50%). Thus, the low-expression group had more cases with a Gleason score of 7 or higher ($p=0.022$). The group with a low expression of claudin-5 also had more cases with a Gleason score of 7 or higher ($p=0.011$). The mean PSA values in the groups with a low and high expression of claudin-1 were 9.6 ng/ml and 5.6 ng/ml, respectively ($p=0.007$). A low expression of claudin-5 was also associated with a high PSA value ($p=0.002$). There was no statistical difference in the expression of claudin-1 and claudin-5 by T stage, biochemical recurrence, local recurrence, or distant metastasis.

Conclusions: The low expression of claudin-1, claudin-5 was associated with a Gleason score of 7 or higher and a high PSA value in prostate cancer.

Key Words: Claudin 1; CLDN5 protein; Prostate-specific antigen; Prostatic neoplasms

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INTRODUCTION

The incidence of prostate cancer has increased greatly in recently years with the popularization of the prostate-specific antigen (PSA) test and the increase in the growth of the elderly population. According to research, prostate cancer is the fifth most frequent malignant tumor in Korean men [1]. Most of these tumors are adenocarcinoma, and their histological aggressiveness is evaluated by using the Gleason grading system, whereby grades are evaluated according to changes in glandular architecture. Glandular architecture is partly influenced by cellular polarity and cell-to-cell contacts, and it could thus be hypothesized that changes and dysregulations of proteins mediating cellular contacts might influence the histology and Gleason grade [1,2].

The tight junctional proteins located in the apical and basolateral sections of cells act as a gateway and a barrier for epithelial and endothelial cells. They separate the lumen or glandular architecture in the body from the outside and maintain homeostasis and cellular polarity by controlling paracellular permeability and osmotic pressure [3-5]. Furthermore, they are also known to participate in the control of the proliferation, differentiation, and functions of cells as well as in intercellular communication.

The tight junctional proteins consist of three types of integral membrane proteins: occludins, claudins, and junctional adhesion molecules [6]. Claudins are the tight junctional proteins expressed in epithelial and endothelial cells [3,4]. The structural or functional deficiency or loss of these proteins is known to be an important process in the development and metastasis of cancer [7]. Currently, more than

40 tight junctional proteins have been discovered, and 24 claudins expressed specifically for each tissue are known [8]. Because of the abnormal control of claudin proteins, different expression levels of specific claudins have been reported in various tumors, including colorectal cancer [9], pancreatic cancer [10], breast cancer [11,12], ovarian cancer [13], and prostate cancer [14].

We investigated the correlation between the expression of claudins and prognostic factors in patients who underwent surgery for prostate cancer.

MATERIALS AND METHODS

1. Subjects

The subjects of this study were 48 patients with a diagnosis of prostate cancer who underwent retropubic radical prostatectomy between January 2004 and December 2008. The age of the patients, Gleason score, PSA level, T stage, biochemical recurrence, local recurrence, and distant metastasis were examined, and the average follow-up period was 36 months (range, 12-112 months). One pathologist evaluated the stage of the primary tumor and the Gleason score of the tumors by observing samples stained with H&E under an optical microscope. Biochemical recurrence was defined as an increase in PSA above 0.2 ng/ml, twice or more in succession, during the follow-up period.

2. Immunohistochemical staining

The pathologist redyed the H&E samples of the subjects to mark the representative tumor sites and collected two cores with a 3 mm diameter from the paraffin block for each subject to produce a tissue microarray. The tissue microarray paraffin block was cut into 4 µm thick slices, which were attached to slides. They were then soaked in xylene for 5 minutes three times for paraffin removal, passed through a moisturizing process, and washed with distilled water. For preprocessing for recovery of antigenicity, the citrate buffer solution (pH 6.0) was used for the slides for claudin-1 immunostaining, and the TE buffer solution (pH 9.0) was used for the claudin-5 immunostaining. For staining, an autostainer (Lab Vision, autostainer360) was used with the LP kit (LabVision). For the primary antibody, claudin-1 (Abcam, #15098) and claudin-5 (Invitrogen, #18-7394) were diluted to 1:500 and 1:200, respectively, and allowed to react for 40 minutes at room temperature. Meyer's hematoxylin was used for contrast staining, and an optical microscope was used for observation.

3. Evaluation of immunohistochemical staining

A predominantly membranous pattern of expression was observed in the epithelial elements for each of the claudin-1 and claudin-5 proteins. Immunoreactivity was interpreted without prior knowledge of any of the clinicopathologic parameters. The intensity and distribution were considered in the semiquantitative assessment of the membranous staining pattern. The intensity of staining was subjectively graded as negative or positive (+1, weak; +2,

moderate; +3, intense; +4, strong). Furthermore, the distribution of staining in the tumor cells was graded as +1 if the distribution of staining was 10% or less in the tumor cells, +2 if it was 10-50%, +3 if it was 50-75%, and +4 if it was more than 75%. The same weights were given to the intensity and distribution of staining, and they were divided into low-expression and high-expression groups on the basis of the median value of the sum of these two values.

4. Statistical analysis

The Gleason score (6 or lower, 7 or higher), PSA level, T stage, biochemical recurrence, local recurrence, and distant metastasis were compared according to the expression of claudin-1 and claudin-5 in prostate cancer. The chi-square test and SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) were used for analysis. Statistical significance was based on a p-value less than 0.05.

RESULTS

1. Clinicopathologic characteristics

The primary tumors of the patients were staged as follows: T2 for 40 cases (83%) and T3 for 8 cases (17%). Gleason scores were 6 or lower in 17 cases (35%) and 7 or higher in 31 cases (65%). The mean PSA was 7.4 ng/ml (range, 3.2-18.4 ng/ml). Biochemical recurrence was observed in 6 cases (12%), local recurrence in 4 cases (8%), and distant metastasis in 4 cases (8%) (Table 1). The correlation between claudin expression and PSA and Gleason score are summarized in Table 2.

2. Expression profiles of claudin-1

The group with a low expression of claudin-1 consisted of

TABLE 1. Clinicopathological characteristics of the patients

Variables	No. of patients (%)
Mean age (years)	63 (51-76)
Mean PSA (ng/ml)	7.4 (3.2-18.4)
Gleason score	
≤6	17 (35)
≥7	31 (65)
T stage	
T2	40 (83)
T3	8 (17)
T4	0 (0)
Biochemical recurrence	
No	42 (88)
Yes	6 (12)
Local recurrence	
No	44 (92)
Yes	4 (8)
Distant metastasis	
No	44 (92)
Yes	4 (8)

PSA: prostate-specific antigen

22 cases (46%), and the group with a high expression of claudin-1 consisted of 26 cases (54%). In the group with a low expression of claudin-1, 18 cases (82%) had a Gleason score of 7 points or higher, and 4 cases (18%) had a score of 6 points or lower. In the group with a high expression of claudin-1, 13 cases (50%) had a Gleason score of 7 points or higher, and 13 cases (50%) had a score of 6 points or lower. Thus, the low-expression group had more cases with a Gleason score of 7 or higher ($p=0.022$) (Fig. 1). The mean PSA values were 9.6 ng/ml and 5.6 ng/ml for the groups with a low and high expression of claudin-1, respectively. The mean PSA value of the low-expression group was statistically higher ($p=0.007$). T stage ($p=0.442$), biochemical recurrence ($p=0.274$), local recurrence ($p=0.861$), and distant metastasis ($p=0.382$) were not significantly different by expression of claudin-1 (Table 3).

3. Expression profiles of claudin-5

The group with a low expression of claudin-5 consisted of 17 cases (35%), and the group with a high expression of claudin-5 consisted of 31 cases (65%). In the group with a low expression of claudin-5, 15 cases (88%) had a Gleason score of 7 points or higher, and 2 cases (12%) had a score of 6 points or lower. In the group with a high expression of claudin-5, 16 cases (52%) had a Gleason score of 7 points or higher, and 15 cases (48%) had a score of 6 points or lower. Thus, the low-expression group had more cases with a Gleason score of 7 or higher ($p=0.011$) (Fig. 2). The mean PSA values were 9.8 ng/ml and 6.1 ng/ml for the groups with a low and high expression of claudin-5, respectively. The mean PSA value of the low-expression group was statistically higher ($p=0.002$). T stage ($p=0.112$), biochemical recurrence ($p=0.425$), local recurrence ($p=0.524$), and distant metastasis ($p=0.649$) were not significantly different by expression of claudin-5 (Table 3).

TABLE 2. Claudin expression in prostate adenocarcinoma and in relation to Gleason score and PSA value

Variables	Claudin-1 expression		Claudin-5 expression	
	Low (n=22)	High (n=26)	Low (n=17)	High (n=31)
PSA value (ng/ml)				
< 4	0	4	0	4
4-10	15	22	11	26
> 10	7	0	6	1
Gleason score				
5 <	1	2	1	2
6	3	11	1	13
7	13	4	8	9
8	2	2	2	2
9	2	5	4	3
10	1	2	1	2

PSA: prostate-specific antigen

DISCUSSION

It is known that many factors are involved in the development, advancement, and metastasis of malignant tumors. Among them, disintegration of the cytoskeleton and changes in intercellular adhesion materials are attracting interest as factors related to the pathogenesis of tumor cells and as prognostic factors of cancer. The loss of intercellular adhesion in tumor cells is known as an important process for the intrusion and metastasis of tumor cells to the peripheral matrix [7].

Cell binding is involved in the interconnection of cells and the maintenance of stable interrelations between cells. Components include zonula adherens, desmosomes, zonula occludens, and tight junction [15]. Tight junctional proteins are located in the apical and basolateral sections of cells, which play an important part in the maintenance of polarity of cells, control of paracellular transportation, and barrier functions. The molecular biological structure of the tight junction is divided into two groups: integral membrane proteins, which include occludins, claudins, and junctional adhesion molecules, and peripheral membrane proteins, which include zonulae occludens, cingulin, symplekin, pilt, and MAGI-1 [16]. Claudin was first discovered in 1998, and at least 24 subtypes have been identified. It has been reported that the increased or decreased expression of specific claudins influences the development of various cancers [8].

Normal cells distinctively express various claudin proteins, but some subtypes of claudin have tissue-specific distributions. Claudin-4 is expressed in lungs and kidney, but not in liver. In contrast, claudin-2 is normally ex-

TABLE 3. Comparison of clinicopathological characteristics according to claudin-1 and claudin-5 expression

Variables	Claudin-1 expression		p-value	Claudin-5 expression		p-value
	Low (n=22)	High (n=26)		Low (n=17)	High (n=31)	
Mean PSA (ng/ml)	9.6	5.6	0.007	9.8	6.1	0.002
Gleason score						
≤ 6	4	13	0.022	2	15	0.011
≥ 7	18	13		15	16	
T stage						
T2	17	23	0.442	12	28	0.112
T3	5	3		5	3	
Biochemical recurrence						
No	18	24	0.274	14	28	0.425
Yes	4	2		3	3	
Local recurrence						
No	20	24	0.861	15	29	0.524
Yes	2	2		2	2	
Distant metastasis						
No	21	23	0.382	16	28	0.649
Yes	1	3		1	3	

PSA: prostate-specific antigen

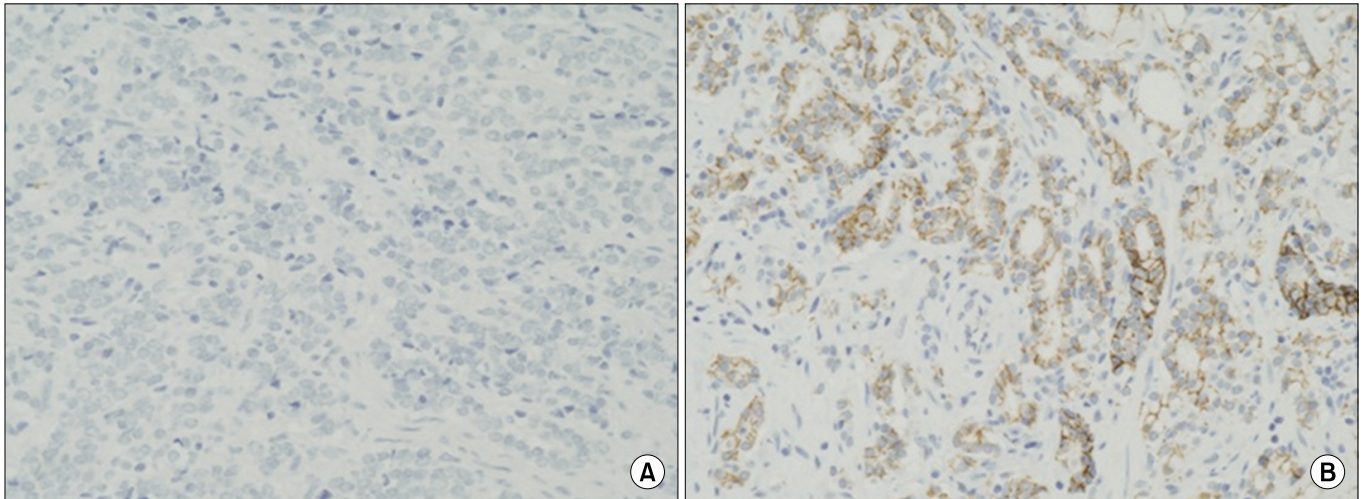


FIG. 1. Claudin-1 expression in prostate malignancy tissues (anti-claudin-1 antibody, x400). (A) Loss of membranous claudin-1 expression in a case of high-grade PCa. (B) High expression of membranous claudin-1 protein in a case of low-grade PCa. PCa: prostate cancer.

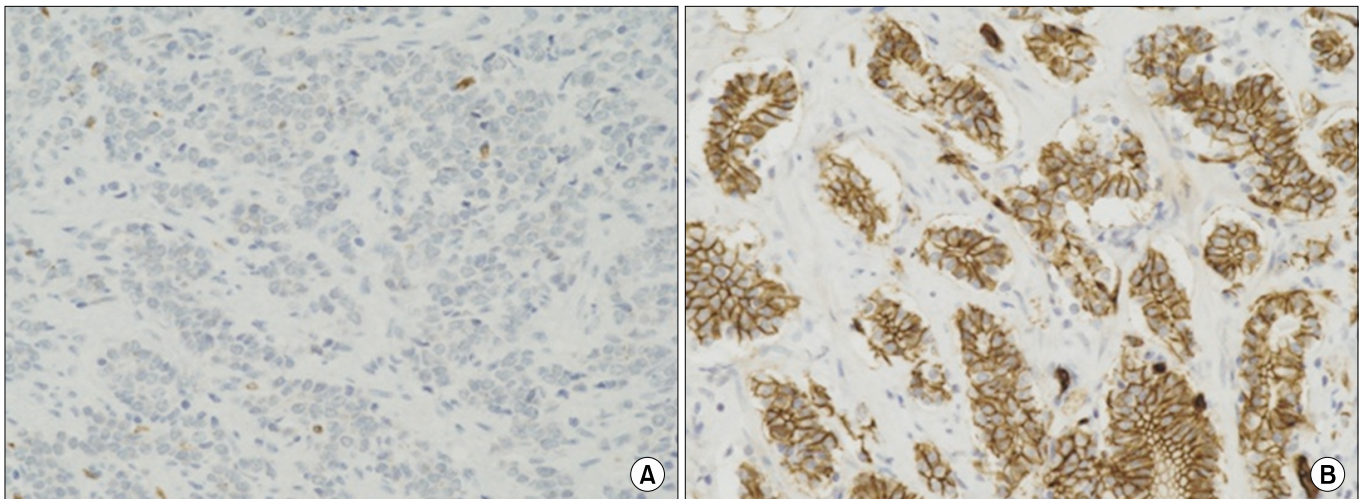


FIG. 2. Claudin-5 expression in prostate malignancy tissues (anti-claudin-5 antibody, x400). (A) Loss of membranous claudin-5 expression in a case of high-grade PCa. (B) High expression of membranous claudin-5 protein in a case of low-grade PCa. PCa: prostate cancer.

pressed in liver, but not in lungs and kidney. Furthermore, claudins exhibit highly tissue-specific distributions by carcinoma. Hornsby et al studied the expression of claudin-7 in renal cell carcinoma (RCC) and reported a higher expression of claudin-7 in chromophobe RCC than in clear-cell RCC and papillary RCC [17]. In a study about the correlation between the expression of claudin-7 and the grade of breast cancer, Kominsky et al reported that the low expression of claudin-7 is associated with the high grade of breast cancer [11]. Tokes et al also reported the loss of claudin-1 proteins in breast cancer cells and argued that claudin-1 is involved in the infiltration and metastasis of breast cancer [12]. In a study of the correlation between the expression of claudin-4 and pancreatic cancer, Nichols et al reported a higher expression of claudin-4 in pancreatic can-

cer and metastatic pancreatic cancer tissues than in normal pancreatic tissues [18]. Lee et al reported that the decreased expression of claudin-4 and E-cadherin in gastric cancer is associated with oncological low differentiation and metastasis [19]. In the case of colorectal cancer, the decreased expression of claudin-1 is known to be associated with the malignancy of tumors [20]. In addition, many studies of the correlation of claudin with ovarian cancer, prostate cancer, and other cancers have been conducted [13,14]. The correlations between tumors and the expression of claudin are not uniform, and the expression profiles are diverse.

Malignant cells are generally known to have structural or functional anomalies in tight junctions [21]. This causes the loss of cell cohesion and infiltration and a decrease in

differentiation, which influence the infiltration and metastasis of tumor cells to the peripheral matrix. This is supported by the findings of many studies, i.e., a loss of expression of tight junctional proteins is evident in carcinoma. However, the prediction that the progression of an infiltrative carcinoma decreases the expression of claudin has not been supported in many studies. Choi et al reported two reasons the expression of claudin increases in carcinoma: first, claudin proteins may increase because of a secondary reaction to the loss of cell adhesion in the process of malignant degeneration; second, claudin proteins may increase because of amplification by claudin gene mutation [22]. According to various reports, the loss of tight junctional proteins weakens the normal intercellular adhesion that separates cancer cells and initiates metastasis. However, there is doubt about whether the loss of some tight junctional proteins affects the change in intercellular adhesion and the differentiation of cancer cells.

This study found that the low expression of claudin-1 was associated with a high Gleason score. The low expression of claudin-5 was also found to be associated with a high Gleason score. As mentioned above, the Gleason score was related to the degree of tumor differentiation and to the formation of glandular architecture in tumors. The disintegration of glandular architecture and the formation of malignant tumor tissues occur simultaneously, which decreases the formation and frequency of tight junctional proteins in malignant tumor cells. As a result, the expression of claudin seems to decrease in tissues with a high Gleason score. In both the claudin-1 and claudin-5 groups, no correlations were found with prognostic factors such as T stage, biochemical recurrence, local recurrence, and distant metastasis. However, many previous studies reported a correlation between prognostic factors of prostate cancer and the abnormal expression of claudin. Regarding the correlation between prostate cancer and abnormal expression of claudin-1, -2, -3, -4, -5, and -7, Väre et al reported that the low expression of claudin-1 and claudin-5 was strongly correlated with Gleason scores of 7 or higher, as was observed in the present study [2]. Furthermore, Väre et al reported that the low expression of both claudin-1 and claudin-5 was associated with a high TNM stage or local advanced cancer. In a study of the correlation between the abnormal expression of claudin-1, -3, -4, and -7 and the prognostic factors of prostate cancer, Sheehan et al reported that the decreased expression of claudin-1 and -7 was associated with tumors with histological low differentiation, and a decreased expression of claudin-1 was associated with biochemical recurrence [23]. On the other hand, they reported that the increased expression of claudin-3 and claudin-4 was associated with high-stage or advanced tumors and found through multivariate analysis that the decreased expression of claudin-1 was a predictive factor that was independent of the recurrence of tumor. According to Landers et al, compared with prostatic hyperplasia tissues, the expression of claudin-4 was high in prostate cancer and metastatic prostate carcinoma [24]. Furthermore, they reported

that the increased expression of claudin-4 is a useful indicator of cancer diagnosis and is a therapeutic target for metastatic prostate carcinoma [24].

This study found that the PSA value in the groups with a low expression of claudin-1 and claudin-5 was statistically significantly higher than that in the high-expression group. Few studies of the correlation between the PSA value and the expression of claudin have been conducted. Zheng et al reported that claudin-7 controls the expression of PSA [25]. In a study of the correlation between prostate cancer and the abnormal expression of claudin-1, -2, -3, -4, -5, and -7, Väre et al reported that in all claudins, the PSA value of the low-expression group (i.e., the group with a low immunohistochemical score) was higher than that of the high-expression group (83.3 ± 87.3 ng/ml vs. 32.9 ± 41.1 ng/ml, $p=0.01$) [2]. They stated that the low concentration of claudin in prostate cancer tissues caused the outflow of more tumor cells, which increased the PSA value.

As mentioned above, many studies have reported on the specific expression of claudins in prostate cancer, and the results of our study also support the findings of these previous studies. However, unlike previous studies, our study found no correlation of claudin expression with prognostic factors of prostate cancer, such as T stage, biochemical recurrence, local recurrence, and distant metastasis. The reason for this seems to be the small number of cases and the difficulty of investigating the patients' prognosis because of the short follow-up period of this study. Therefore, a long-term study with more patients is required.

CONCLUSIONS

The low expression of claudin-1 and claudin-5 was associated with a Gleason score of 7 or higher and with a high PSA value in prostate cancer. Therefore, it is believed that a loss of these proteins would affect the development, progression, and prognosis of prostate cancer. More long-term studies are required to reveal the correlation between claudins and the prognostic factors of prostate cancer.

Conflicts of Interest

The authors have nothing to disclose.

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