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Saitama Medical University International Medical Center
Department of Gynecologic Oncology
Breaking Through

Claudin-6 Expression in Primary and Recurrent Epithelial Ovarian Cancer: A Potential Therapeutic Target for High-Grade Serous Ovarian Cancer

Daisuke Shintani[1], Mieko Hanaoka[1], Ryuichi Nakamura[2], Miyano Arasaki[1], Shogo Yamaguchi[1], Takahiro Komatsu[3], Mitsutake Yano[4], Akira Yabuno[1], Shigehiro Yagishita[4], Masanori Yasuda[5], Motoko Nagata[2], and Kosei Hasegawa[1]

Departments of [1]Gynecologic Oncology and [5]Diagnostic Pathology, Saitama Medical University International Medica Center, Hidaka, Saitama, Japan
[2]Translational Research Laboratories and [3]Translational Science Department I, Daiichi Sankyo Co., Ltd., Shinagawa-ku, Tokyo, Japan
[4]Division of Molecular Pharmacology, National Cancer Center Research Institute, chuo-ku, Tokyo, Japan

Introduction

Claudin (CLDN) comprises 27 family members, with the majority involved in tight junctions and cell-to-cell adhesion of epithelial cell sheets, playing a significant role in cancer initiation and progression.

Objectives

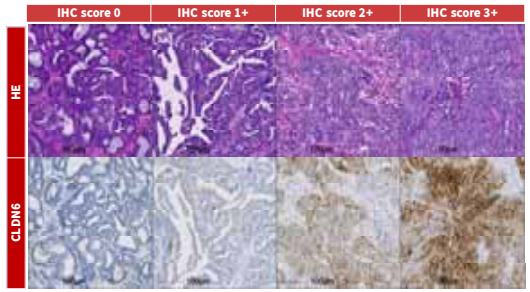
Targeting CLDNs, such as CLDN18.2 for gastric cancer and CLDN6 for solid tumors, has recently garnered attention as a potential avenue for new cancer therapies, demonstrating antitumor activity. However, limited data are available regarding the frequency of CLDN6 expression and its relationship to clinical factors in epithelial ovarian cancer (EOC). This study aims to elucidate the clinical significance of CLDN6 expression in EOC.

Methods

We performed immunohistochemical (IHC) staining for CLDN6 expression on a total of 233 EOC surgical samples (including 181 primary and 52 recurrent tumors) and 54 ascites cell blocks. CLDN6 IHC was conducted using the Leica BONDIII staining platform with the anti-CLDN6 antibody (＃18865 Immuno-Biological Laboratories Co.,Ltd.). The CLDN6 IHC scoring followed the HER2 detection guidelines for gastric cancer (0, 1+, 2+, 3+), with a score of ≥1+ intensity in ≥10% of tumor cells considered positive.

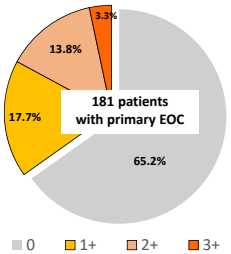
Results

Figure 1. Representative CLDN6 IHC images based on the scoring system



- A four-tiered scoring system of IHC expression.
- Membrane expression of CLDN6 stained in ≥10% of tumor cells using a microscope: 0 for negative cases; 1+ for weak intensity; 2+ for moderate intensity; and 3+ for strong intensity.

Figure 2. CLDN6 IHC scoring chart in primary EOC patients (N= 181)



- 181 patients with primary EOC for CLDN6 expression by IHC.
- 63 patients (34.8%) tested positive.
- In which, 32 (17.7%), 25 (13.8%), 6 (3.3%) cases were scored as 1+, 2+ and 3+.

Table 1. The relationship between CLDN6 positive expression and clinicopathological features (N= 181)

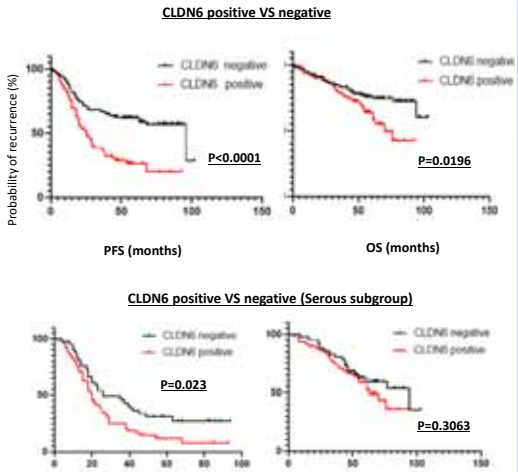
Factors	Positive CLDN6 expression/ tumor examined	Percentage(%)	P value
Age(y)			
<60	27/97	27.8	0.0582
≧60	37/84	44.0	
FIGO Stage			
1/2	16/90	17.8	<0.0001
3/4	48/91	52.7	
Histological type			
High grade serous	49/90	54.4	<0.0001
Endometrioid	10/32	31.2	
Clear cell	3/49	6.1	
Mucinous	2/10	20.0	
PDS※1 or NAC※2			
PDS	60/111	54.5	0.0002
NAC	28/70	40.0	
Residual Tumor			
Yes	43/84	51.2	<0.0001
No	21/97	21.6	
CA125(Median:437.8)			
High(>Median)	39/89	43.8	0.0189
Low(≦Median)	25/92	27.1	

Chi-square test analysis for the relationship between CLDN6 positive expression and clinicopathological features

※1 PDS: primary debulking surgery ※2 NAC: neoadjuvant chemotherapy

- The detection rate of CLDN6 expression was associated with serous type histology (p< 0.0001), NAC cases (p= 0.0002) and prognostic factors such as residual tumor (p<0.0001), advanced stage (p< 0.0001) and high CA125 value (p= 0.0189)

Figure 3. . Kaplan-Meier survival curves for progression-free survival (PFS) and overall survival (OS) according to the CLDN6 detection status in EOC patients



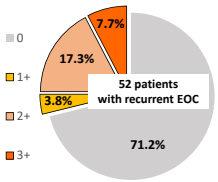
- CLDN6 detection was associated with shorter progression-free survival and overall survival in EOC patients by long-rank test (p<0.0001, p=0.0196, respectively).
- CLDN6 detection of HGSC subgroup was associated with shorter progression-free survival in EOC patients by long-rank test (p=0.023).

Table 2. Multivariate cox regression analysis for progression-free survival and overall survival in primary EOC cancer patients including CLDN6 expression (N= 63)

		PFS			OS		
		HR	95%CI	P-value	HR	95%CI	P-value
CLDN6	Positive vs Negative	1.46	0.80-2.68	0.2127	1.05	0.44-2.48	0.9
Histology	Serous vs Others	0.678	0.166-2.587	0.563	2.22	1.03-4.81	0.0451
FIGO Stage	III+IV vs Ⅰ+Ⅱ	4.23	2.01-8.91	0.0001	8.62	2.88-25.79	0.0001
Residual tumor	Yes vs No	3.06	1.47-6.34	0.0026	2.82	1.08-7.37	0.0337

- Multivariate analysis revealed that FIGO stage and Residual tumor remained independently associated with progression free survival and overall survival.

Figure 4. CLDN6 IHC scoring chart in recurrent EOC patients (N= 52)



- 52 patients with recurrent EOC for CLDN6 expression by IHC.
- 15 patients (28.8%) tested positive.
- In which, 2 (3.8%), 9 (17.3%), 4 (7.7%) cases were scored as 1+, 2+ and 3+.

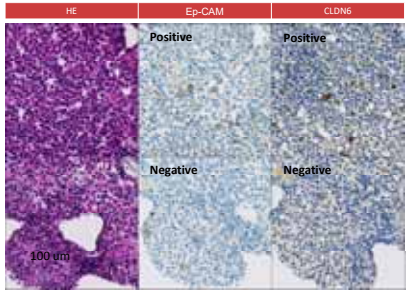
Table 5. The relationship between CLDN6 expression and clinicopathological features in recurrent EOC patients (N= 52)

Factors		positive CLDN6 expression / tumor examined	percentage(%)	P value
FIGO stage at initial diagnosis	Ⅰ+Ⅱ	8/34	23.5	0.1117
	III+IV	7/18	38.9	
Histological type	High grade serous	10/24	41.2	0.0126
	Endometrioid	1/4	25.0	
	Clear cell	2/19	10.5	
	Mucinous	0/2	0	
	Others	2/3	66.7	
Recurrent site	Intrapelvic	8/28	28.9	0.9931
	Extrapelvic	4/7	57.1	
	Lymph node	2/8	25.0	
	Distant metastasis	1/9	11.1	
Time from last chemotherapy	< 12w	5/13	38.5	0.0691
	12-36w	9/22	40.9	
	≥36w	1/17	5.9	
CA125 (MEDIAN: 47.3)	low	5/26	19.2	0.1273
	high	10/26	38.4	
Number of previous chemotherapy	0	4/16	25.0	0.0031
	1	6/29	20.7	
	≥2	5/17	29.4	

Chi-square test analysis for the relationship between CLDN6 positive expression and clinicopathological features

- Higher frequency of CLDN6 expression was associated with serous type histology (p=0.0126) and more previous chemotherapies (p=0.0031).
- Higher trends for the time from last chemo between 36w or more and less than 36w, but regardless of sites of recurrence.

Figure 5 . CLDN6 and Ep-CAM IHC staining images in ascites



- CLDN6 positive in ascites was confirmed by Ep-CAM positive as tumor cells.
- Tumor cells less than 10% seemed to be CLDN6 staining positive, thus it revealed CLDN6 negative

Table 6 . CLDN6 expression and clinicopathological features in ascites of EOC patients

Factors	Ascites in primary	Ascites in recurrent
Positive CLDN6 expression	20/33 (60.6%)	10/21 (47.6%)
FIGO Stage		
1/2	0/2 (0%)	—
3/4	20/31 (64.5%)	
Histological type		
High grade serous	15/22 (68.1%)	7/14 (50.0%)
Endometrioid	0/2 (0%)	0/1 (0%)
Clear cell	2/5 (40.0%)	2/5 (40.0%)
Mucinous	0/0 (0%)	0/0 (0%)
others	4/6 (66.7%)	1/5 (20%)

- We performed IHC for CLDN6 in 54 primary and recurrent ascites cell blocks.
- CLDN6 positivity in ascites from primary tumors seems to be higher than that in solid tumors

Conclusion

- CLDN6 expression is observed in both primary and recurrent EOC tumors of all histotypes, with a higher prevalence in high-grade serous carcinoma and stage III/IV tumors.
- CLDN6 detection was associated with shorter progression-free survival and overall survival in EOC patients
- CLDN6 positivity in ascites from naïve treatment patient shows higher trend than that in solid tumors.
- These findings highlight CLDN6 as a promising therapeutic target for high-grade serous ovarian cancer.

Contact information: Daisuke Shintani MD., Ph.D. E-mail: dshin@saitama-med.ac.jp
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