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# Expression of cancer stem cell-related markers in ovarian carcinoma and its clinical significance

# 난소암 조직에서 암줄기세포 관련 인자의 발현 및 임상적 의의

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김 미 경

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**February 2013**

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**Seoul National University**

**College of Medicine**

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**by**
**Mi-Kyung Kim**

**A thesis submitted to the Department of Obstetrics and Gynecology in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medicine at Seoul National University College of Medicine**

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# Expression of cancer stem cell-related markers in ovarian carcinoma and its clinical significance

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# ABSTRACT

# Expression of cancer stem cell-related markers in ovarian carcinoma and its clinical significance

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**Objectives:** To evaluate the expression of cancer stem cell (CSC)-related markers in human epithelial ovarian cancers of different subtypes and to assess their predictive and prognostic significance.

**Materials and Methods:** Immunohistochemical analyses of 18 CSC-related proteins which include CSC surface markers, stemness markers, Notch and Hedgehog signaling molecules were performed in 103 cases of invasive epithelial ovarian tumors. The differences in clinicopathologic variables according to the immunoreactivity for CSC-related proteins were evaluated using chi-square test or Student's t-test as appropriate. Survivals were also evaluated and compared using Kaplan-Meier method and log-rank test.

**Results:** CSC-related proteins were differentially expressed according to the histologic subtypes. The serous adenocarcinomas were characterized by high expression of CD24, Nanog and Snail, whereas the expression of CD44, Shh and Gli-1 was more common in mucinous adenocarcinomas. However, none of the CSC-related proteins was associated with other clinicopathologic variables, including stage and response to platinum-based chemotherapy, and survival outcomes.

**Conclusions:** Although none of the CSC-related molecules served as independent predictive and prognostic markers, the frequency of tumor cells positive for these markers varies substantially according to the histologic subtype.

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**Keywords:** ovarian carcinoma, cancer stem cell, immunohistochemistry, biomarker, prognosis

**Student number:** 2010-31136

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# INTRODUCTION

Ovarian cancer is the seventh leading cause of cancer deaths in women worldwide, and is the most lethal gynecologic malignancy (1). Despite advances in surgery and chemotherapy, overall cure rate has remained approximately 30%. The poor clinical outcome mainly comes from the high percentage of cases being diagnosed at an advanced stage and frequent emergence of chemoresistance. Recent evidences have suggested that subpopulations of tumor cells with stem-like phenotypes, cancer stem cells (CSCs), may contribute to the emergence of chemoresistance and metastasis (2, 3). In various cancers including breast, pancreatic cancers, and gliomas, the increased frequency of tumor cells showing stem cell-like phenotypes was reported to be related to the poor survival outcomes (4-6).

In ovarian cancers, the presence of CSC-like tumor cells has been also suggested through identifying tumor-initiating cells (TICs) isolated by several CSC markers, including CD133, CD44, and CD24 (7-9). However, these studies used cultured primary cells or multiply passaged xenografts, which could not properly represent the actual frequency of TICs, partly from the different tumor microenvironments which may affect the phenotypes and frequency of TICs (10).

Therefore, the present study analyzed the differential expression of CSC-related markers according to the histologic subtypes of epithelial ovarian cancer and the correlations between the CSC markers by immunohistochemistry in human epithelial ovarian cancer tissues. In addition,

the predictive and prognostic significance of CSC-related markers was evaluated.

# MATERIALS AND METHODS

## Patients

Between 2003 and 2009, a total of 110 patients were diagnosed with primary epithelial ovarian carcinomas and treated at Seoul National University Bundang Hospital. Of these patients, 4 patients who received neoadjuvant chemotherapy before surgery were excluded from the study analysis because chemotherapy might be able to affect the proportions of chemoresistant tumor cells or CSCs. Additionally, 3 patients diagnosed with rare histologic subtypes, including squamous cell and small cell carcinomas, were excluded. Consequently, 103 patients were eligible for the study analysis. Clinicopathologic data, including age, the International Federation of Gynecology and Obstetrics (FIGO) stage, surgical procedures, the extent of residual disease, histologic subtype, grade, adjuvant chemotherapy, and survival outcomes, were evaluated by reviewing medical charts and pathologic records.

## Tissue samples

Tissue microarrays (TMAs) were constructed from core biopsies (diameter 2 mm) of formalin-fixed paraffin-embedded primary ovarian cancer specimens using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). Three core biopsies were taken from each individual specimen.

## Immunohistochemistry

The expression of CSC-related proteins, including CSC surface markers, stemness proteins, Notch and hedgehog signaling molecules, and EMT-related proteins, was evaluated through immunohistochemistry. CSC markers consisted of CD133, CD44, CD24, and CD117. Notch signaling molecules included Notch1, Notch3, and Jagged-1, and Hedgehog signaling molecules included PTCH, Shh, Smo, and Gli-1. Snail and slug were also evaluated as EMT proteins.

Immunohistochemistry was carried out on TMA blocks described above. Sections (4 μm) from array blocks were dewaxed in xylene and rehydrated through serial dilutions of ethanol and distilled water. Antigen retrieval was performed in a standard pressure cooker method. After blocking endogenous peroxidase activity and applying non-specific background blocking serum, sections were incubated with the appropriate antibody to detect the specific immunoreactivity for each CSC-related protein. Antibodies used for the present study and their corresponding detection kits are summarized in Table 1.

## Staining evaluation

For each immunostaining, both the percentage of positive cells and staining intensity from 1 to 3 (1 weak, 2 moderate, and 3 strong) were examined. Since three cores were taken from each tumor, the average value was used for the study analysis. For analysis of clinicopathologic variables and survival

outcomes according to the CSC-related proteins, expression of each molecule was categorized into two groups: low vs. high expression. In principle, the cutoff for high expression of each marker was determined based on the criteria used in previous studies. The cutoff value for each molecule is listed in Table 1.

## Statistical analysis

The differences in clinicopathologic variables according to the immunoreactivity for CSC-related proteins were evaluated using chi-square test or Student's t-test as appropriate. Survivals were also evaluated and compared using Kaplan-Meier method and log-rank test or Breslow test as appropriate. Progression-free survival (PFS) was defined as the time interval from surgery to the first evidence of recurrence or death from any cause, whichever occurred first. Overall survival (OS) was defined as the time from surgery to death from any cause. To identify independent prognostic factors, Cox regression analysis was performed. A p-value of less than 0.05 was considered to indicate statistical significance, and all tests were two-sided. The statistical analysis was performed using SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL).

Table 1. Primary antibodies and cutoffs for high immunoreactivity

| Antibody (Clone) | Clonality | Dilution | Source (cat.No.) | Expression in cancer cells | Cutoffs (%) | Refs |
|---|---|---|---|---|---|---|
| CD133 | Rabbit monoclonal | 1:50 | Cell signaling (#3663) | cytoplasmic | >0 | (6) |
| CD24 (24C02) | Mouse monoclonal | 1:200 | Neomarker (MS-1279) | cytoplasmic | ≥10 | (19) |
| CD44 (DF1485) | Mouse monoclonal | 1:200 | Novocastra (NCL-CD44-2) | membranous | ≥10 | (12) |
| CD117 (c-kit) | Rabbit polyclonal | 1:200 | DAKO (A4502) | rare, cytoplasmic +/- membranous | >0 | |
| Nestin | Mouse monoclonal | 1:100 | Millipore (MAB5326) | cytoplasmic +/- membranous | ≥10 | (20) |
| Nanog | Rabbit monoclonal | 1:100 | Cell signaling (#4093) | cytoplasmic | ≥5 | (21) |
| Oct4 | Rabbit monoclonal | 1:40 | Cell signaling (#2890) | rare, nuclear | >0 | |
| Sox-2 | Rabbit monoclonal | 1:50 | Cell signaling (#3579) | nuclear | >0 | (22) |
| Bmi-1 | Rabbit polyclonal | 1:600 | Epitomics (S2983) | nuclear | >0 | |
| Notch1 | Rabbit monoclonal | 1:300 | Cell signaling (#3608) | cytoplasmic + membranous | ≥10 | (23) |
| Notch3 | Rabbit polyclonal | 1:100 | Santa-cruz (sc-5593) | nuclear | >0 | (17, 18) |
| Jagged-1 | Rabbit polyclonal | 1:50 | Santa-cruz (sc-8303) | cytoplasmic | ≥10 | |
| PTCH | Goat polyclonal | 1:50 | Santa-cruz (sc-6149) | cytoplasmic | >50 | (24) |
| SHH | Rabbit monoclonal | 1:500 | Epitomics (1843-1) | cytoplasmic | >50 | (24) |
| Smo | Rabbit polyclonal | 1:100 | Novus (NLS2666) | cytoplasmic +/- membranous | >50 | (24) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Gli1 | Rabbit polyclonal | 1:100 | Thermo (PAI-22557) | nuclear | >0 | (25) |
| Snail | Rabbit polyclonal | 1:800 | Abcam (ab17732) | nuclear | >50 | (26) |
| Slug | Rabbit polyclonal | 1:100 | Abcam (ab27568) | cytoplasmic | >50 | (26) |

# RESULTS

## Patient characteristics

The characteristics of 103 patients with epithelial ovarian cancer who received upfront staging operation are summarized in Table 2. The serous type was the most frequently diagnosed histologic subtype (59.2%), followed by mucinous (16.5%), clear cell (12.6%) and endometrioid type (9.7%). Most of the patients were diagnosed with stage I (35.9%) and stage III (45.6%) diseases. The majority of patients (85.5%) received paclitaxel/platinum chemotherapy after the debulking surgery.

During the median follow-up period of 40.7 months (range, 1-97 months), there occurred 48 recurrences and 29 deaths. The 2-year progression-free survival and 5-year overall survival rates were 62% and 67.7%, respectively.

## CD133, CD44, CD24, and CD117 expression in primary human ovarian carcinoma specimens

The immunoreactivity of CD133 and CD24 were detected in the apical membrane and the cytoplasm of tumor cells, whereas CD44 immunostaining was mainly membranous (Figure 1). For the study analysis, only cytoplasmic staining was considered positive for CD133 and CD24. CD117 was rarely expressed in ovarian cancer samples (n=11; 10.7%).

CD133 and CD44 were more frequently expressed in non-serous types, whereas CD24 staining was more consistently observed in serous adenocarcinomas (72.1%; p=0.017; Table 3). CD133 was more frequently

expressed in endometrioid (50%) and clear cell adenocarcinomas (69.2%), and CD44 was commonly expressed in the mucinous subtype (82.4%). The histologic subtype was the only independent determinant of the expression of CSC markers (CD133, CD44, and CD24) on multivariate analysis, which included histologic subtype, stage, peritoneal seeding, and residual disease status, as covariates. Between the CSC markers, there were no statistically significant correlations.

When the analysis of the expression profiles of CSC markers was limited to the serous ovarian carcinomas, their immunopositivity were not associated with any clinicopathologic variables in serous adenocarcinomas, except the more frequent CD44 expression in high-grade tumors (Table 4). In addition, response to chemotherapy and platinum sensitivity were not different according to the expression of CSC markers. Moreover, CSC markers were not associated with survival outcomes (Figure 2).

When analyzing the associations between stem cell markers (CD133, CD44, and CD24) and stemness-related proteins in serous adenocarcinomas (Table 5), CD44 positivity was inversely correlated with the expression of stemness proteins (CD44 vs. Sox-2, p=0.046; CD44 vs. Nestin, p=0.020). In addition, negative relationship between CD133 and snail immunoreactivity was observed.

Table 2. Patient characteristics (N=103)

| Variables | N (%) |
|---|---|
| Age, median +/- SD (range) | 52.0 +/- 13.8 (23-91) |
| Stage | |
| I | 37 (35.9) |
| II | 13 (12.6) |
| III | 47 (45.6) |
| IV | 6 (5.8) |
| Histology | |
| Serous | 61 (59.2) |
| Mucinous | 17 (16.5) |
| Endometrioid | 10 (9.7) |
| Clear cell | 13 (12.6) |
| Others* | 2 (1.9) |
| Grade | |
| 1 | 20 (19.4) |
| 2 | 37 (35.9) |
| 3 | 45 (43.7) |
| Unknown | 1 (1.0) |
| Residual disease | |
| none | 49 (47.6) |
| <1cm | 13 (12.6) |
| 1-2cm | 9 (8.7) |
| >2cm | 26 (25.2) |
| unknown | 6 (5.8) |
| Adjuvant chemotherapy | |
| None | 14 (13.6) |
| Paclitaxel/Carboplatin | 85 (82.6) |
| Paclitaxel/Cisplatin | 3 (2.9) |
| Others | 1 (1.0) |
| Chemo cycles, mean+/-SD | 6.05 +/- 2.66 |

| | |
|---|---|
| 2-year PFS | 62.0% |
| 5-year OS | 67.7% |

PFS, progression-free survival; OS, overall survival

*Other histologic types include undifferentiated (n=1) and transitional cell carcinoma (n=1).

Table 3. Expression of cancer stem cell markers (CD133, CD44, and CD24) according to the clinicopathologic variables (N=103)

| Variables | CD133 | | p-value | CD44 | | p-value | CD24 | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | |
| Histology | | | 0.007 | | | 0.007 | | | 0.017 |
| Serous | 43 (72.9) | 16 (27.1) | | 39 (63.9) | 22 (36.1) | | 17 (27.9) | 44 (72.1) | |
| Mucinous | 14 (87.5) | 2 (12.5) | | 3 (17.6) | 14 (82.4) | | 11 (64.7) | 6 (35.3) | |
| Endometrioid | 5 (50) | 5 (50) | | 3 (30) | 7 (70) | | 7 (70) | 3 (30) | |
| Clear cell | 4 (30.8) | 9 (69.2) | | 5 (38.5) | 8 (61.5) | | 6 (46.2) | 7 (53.8) | |
| Others | 2 (100) | 0 (0) | | 1 (50) | 1 (50) | | 1 (50) | 1 (50) | |
| Stage | | | 0.538 | | | 0.172 | | | 0.047 |
| I | 22 (59.5) | 15 (40.5) | | 13 (35.1) | 24 (64.9) | | 22 (59.5) | 15 (40.5) | |
| II | 9 (69.2) | 4 (30.8) | | 8 (61.5) | 5 (38.5) | | 1 (7.7) | 12 (92.3) | |
| III | 33 (73.3) | 12 (26.7) | | 27 (57.4) | 20 (42.6) | | 17 (36.2) | 30 (63.8) | |
| IV | 4 (80) | 1 (20) | | 3 (50) | 3 (50) | | 2 (33.3) | 4 (66.7) | |
| Grade | | | 0.701 | | | 0.060 | | | 0.050 |
| 1 | 15 (75) | 5 (25) | | 7 (35) | 13 (65) | | 13 (65) | 7 (35) | |
| 2 | 25 (67.6) | 12 (32.4) | | 24 (64.9) | 13 (35.1) | | 14 (37.8) | 23 (62.2) | |
| 3 | 27 (64.3) | 15 (35.7) | | 20 (44.4) | 25 (55.6) | | 15 (33.3) | 30 (66.7) | |
| Residual disease | | | 0.596 | | | 0.683 | | | 0.247 |
| None | 30 (61.2) | 19 (38.8) | | 20 (40.8) | 29 (59.2) | | 25 (51) | 24 (49) | |
| <1cm | 8 (66.7) | 4 (33.3) | | 6 (46.2) | 7 (53.8) | | 5 (38.5) | 8 (61.5) | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1-2cm | 6 (75) | 2 (25) | | 5 (55.6) | 4 (44.4) | | 4 (44.4) | 5 (55.6) | |
| >2cm | 19 (76) | 6 (24) | | 14 (53.8) | 12 (46.2) | | 7 (26.9) | 19 (73.1) | |
| LN metastasis | | | 0.215 | | | 0.428 | | | 0.087 |
| No | 49 (64.5) | 27 (35.5) | | 39 (51.3) | 37 (48.7) | | 35 (46.1) | 41 (53.9) | |
| Yes | 18 (78.3) | 5 (21.7) | | 11 (42.3) | 15 (57.7) | | 7 (26.9) | 19 (73.1) | |
| Peritoneal seeding | | | 0.392 | | | 0.630 | | | 0.208 |
| No | 38 (63.3) | 22 (36.7) | | 28 (45.9) | 33 (54.1) | | 28 (45.9) | 33 (54.1) | |
| <2cm | 6 (66.7) | 3 (33.3) | | 6 (60) | 4 (40) | | 5 (50) | 5 (50) | |
| >2cm | 24 (77.4) | 7 (22.6) | | 17 (53.1) | 15 (46.9) | | 9 (28.1) | 23 (71.9) | |
| Preoperative CA-125, mean+/-SD | 940.7 +/- 1645.1 | 589.5 +/- 978.4 | 0.268 | 1094.5 +/- 1869.7 | 544.7 +/- 820.0 | 0.058 | 484.2 +/- 965.6 | 1053.0 +/- 1690.2 | 0.053 |

Table 4. Expression of cancer stem cell markers (CD133, CD44, and CD24) according to the clinicopathologic variables in serous adenocarcinomas (n=61)

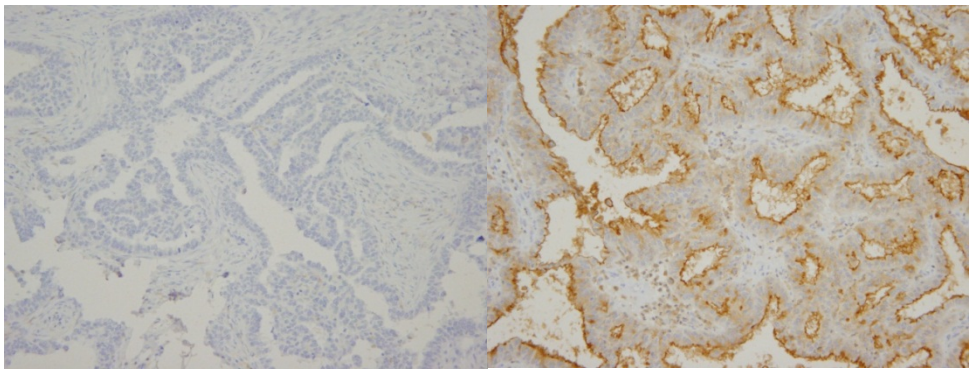
| Variables | CD133 | | p-value | CD44 | | p-value | CD24 | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | |
| Stage | | | 0.647 | | | 0.698 | | | 0.406 |
| I/II | 16 (69.6) | 7 (30.4) | | 14 (60.9) | 9 (39.1) | | 5 (21.7) | 18 (78.3) | |
| III/IV | 27 (75) | 9 (25) | | 25 (65.8) | 13 (34.2) | | 12 (31.6) | 26 (68.4) | |
| Grade | | | 0.807 | | | 0.003 | | | 0.237 |
| 1 | 3 (75) | 1 (25) | | 4 (100) | 0 (0) | | 2 (50) | 2 (50) | |
| 2 | 17 (68) | 8 (32) | | 21 (84) | 4 (16) | | 9 (36) | 16 (64) | |
| 3 | 22 (75.9) | 7 (24.1) | | 14 (45.2) | 17 (54.8) | | 6 (19.4) | 25 (80.6) | |
| LN metastasis | | | 0.445 | | | 0.294 | | | 0.604 |
| No | 30 (69.8) | 13 (30.2) | | 29 (67.4) | 14 (32.6) | | 13 (30.2) | 30 (69.8) | |
| Yes | 12 (80) | 3 (20) | | 9 (52.9) | 8 (47.1) | | 4 (23.5) | 13 (76.5) | |
| Responses | | | 0.265 | | | 0.264 | | | 0.256 |
| CR/PR | 33 (70.2) | 14 (29.8) | | 32 (65.3) | 17 (34.7) | | 15 (30.6) | 34 (69.4) | |
| SD/PD | 3 (100) | 0 (0) | | 1 (33.3) | 2 (66.7) | | 0 (0) | 3 (100) | |
| Platinum sensitivity | | | 0.692 | | | 0.062 | | | 0.526 |
| Sensitive | 21 (67.7) | 10 (32.3) | | 19 (59.4) | 13 (40.6) | | 11 (34.4) | 21 (65.6) | |
| Intermediate | 11 (78.6) | 3 (21.4) | | 12 (85.7) | 2 (14.3) | | 3 (21.4) | 11 (78.6) | |
| Resistant | 4 (80) | 1 (20) | | 2 (33.3) | 4 (66.7) | | 1 (16.7) | 5 (83.3) | |

Table 5. Correlations between cancer stem cell markers and stemness and epithelial-mesenchymal transition proteins in serous adenocarcinomas (n=61)

| Variables | CD133 | | p-value | CD44 | | p-value | CD24 | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | |
| Bmi-1 | | | 0.647 | | | 0.366 | | | 0.856 |
| - | 27 (62.8) | 9 (56.3) | | 22 (56.4) | 15 (68.2) | | 10 (58.8) | 27 (61.4) | |
| + | 16 (37.2) | 7 (43.8) | | 17 (43.6) | 7 (31.8) | | 7 (41.2) | 17 (38.6) | |
| Sox-2 | | | 0.770 | | | 0.046 | | | 0.856 |
| - | 26 (60.5) | 9 (56.3) | | 20 (51.3) | 17 (77.3) | | 10 (58.8) | 27 (61.4) | |
| + | 17 (39.5) | 7 (43.8) | | 19 (48.7) | 5 (22.7) | | 7 (41.2) | 17 (38.6) | |
| Nanog | | | 0.080 | | | 0.501 | | | 0.867 |
| Low | 9 (20.9) | 7 (43.8) | | 12 (30.8) | 5 (22.7) | | 5 (29.4) | 12 (27.3) | |
| High | 34 (79.1) | 9 (56.3) | | 27 (69.2) | 17 (77.3) | | 12 (70.6) | 32 (72.7) | |
| Nestin | | | 0.477 | | | 0.020 | | | 0.195 |
| Low | 12 (27.9) | 6 (37.5) | | 8 (21.1) | 11 (50.0) | | 3 (18.8) | 16 (36.4) | |
| High | 31 (72.1) | 10 (62.5) | | 30 (78.9) | 11 (50.0) | | 13 (81.3) | 28 (63.6) | |
| Snail | | | 0.005 | | | 0.435 | | | 0.528 |
| Low | 1 (2.3) | 4 (25) | | 4 (10.3) | 1 (4.5) | | 2 (11.8) | 3 (6.8) | |
| High | 42 (97.7) | 12 (75) | | 35 (89.7) | 21 (95.5) | | 15 (88.2) | 41 (93.2) | |
| Slug | | | 0.091 | | | 0.501 | | | 0.867 |
| Low | 28 (65.1) | 14 (87.5) | | 12 (30.8) | 5 (22.7) | | 5 (29.4) | 12 (27.3) | |
| High | 15 (34.9) | 2 (12.5) | | 27 (69.2) | 17 (77.3) | | 12 (70.6) | 32 (72.7) | |

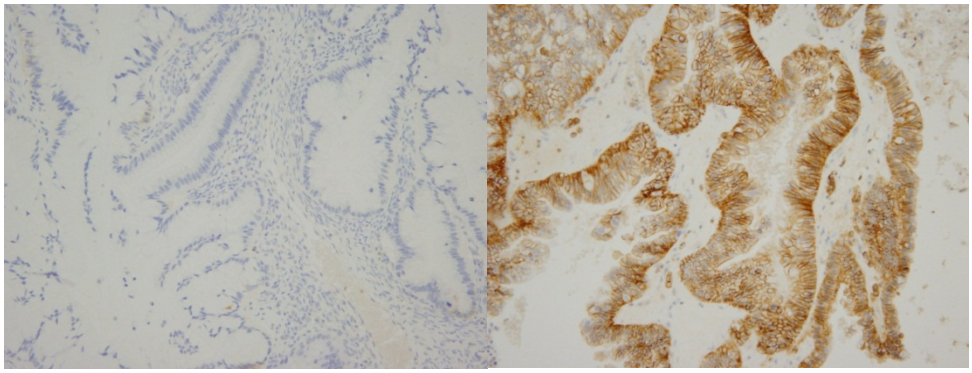
(A)

CD133- CD133+



(B)

CD44- CD44+



(C)

CD24- CD24+

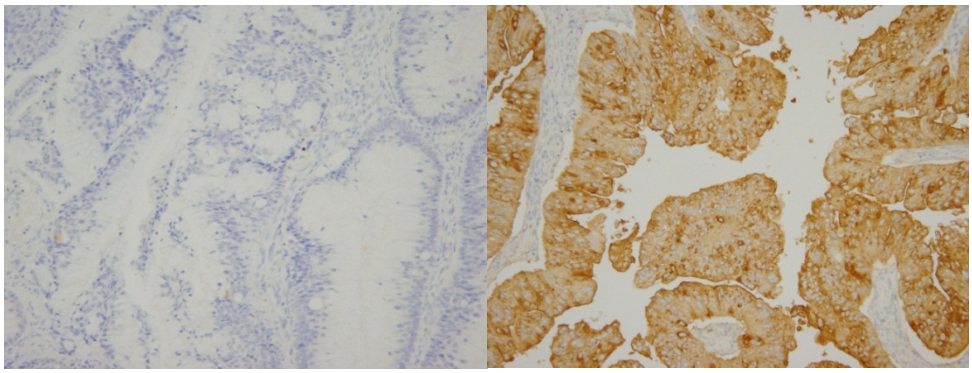


**Figure 1. Immunohistochemical staining patterns for cancer stem cell markers in ovarian cancer tissues.**

(**A**) CD133, (**B**) CD44, and (**C**) CD24. All figures are 200x magnification.

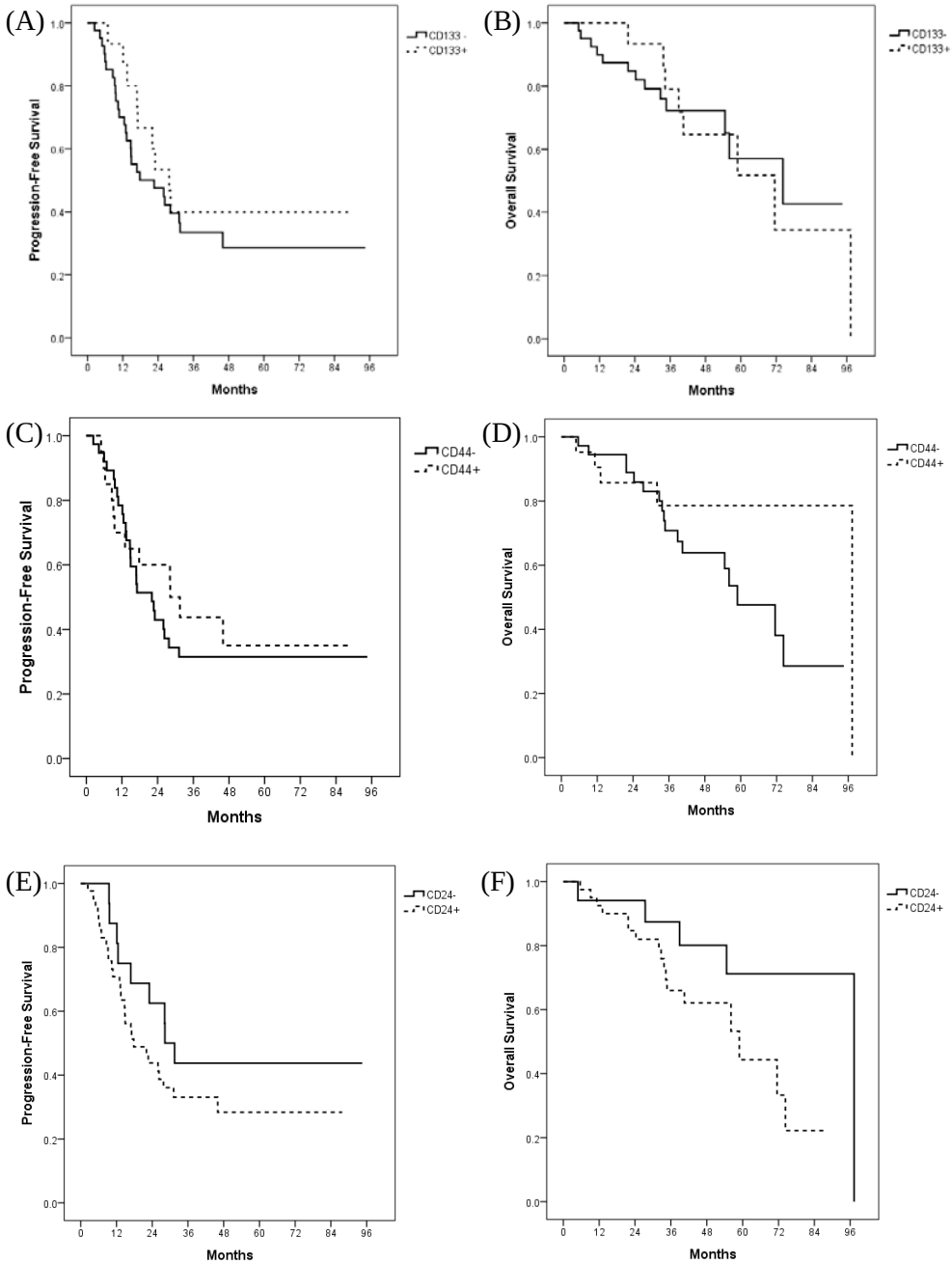



**Figure 2. Survivals of patients with serous adenocarcinomas according to the expression of cancer stem cell markers.**

(A) Progression-free survivals (PFS) and (B) Overall survivals (OS) according to CD133 expression (p=0.363 and p=0.930). (C, D) PFS and OS according to CD44 expression (p=0.545 and p=0.257). (E, F) PFS and OS according to CD24 expression (p=0.211 and p=0.091).

## Expression profiles of Notch signaling pathway proteins in ovarian carcinomas

Notch1 was mainly localized to the cytoplasm and cell membrane of tumor cells, whereas Notch3 was frequently observed in the cytoplasm and focally in the nucleus. Jagged-1 was stained diffusely in the cytoplasm (Figure 3). Notch1 was highly expressed in serous, endometrioid, and clear cell adenocarcinomas ($p<0.001$; Table 6). However, Notch1 expression was not associated with other clinicopathologic variables, including stage, grade, lymph node metastasis, and peritoneal seeding in serous adenocarcinomas. In mucinous adenocarcinomas, by contrast, Notch1 was more frequently expressed in the advanced stage and high-grade tumors ($p=0.051$ and $p=0.032$, respectively). Notch3 and Jagged-1 were not differentially expressed according to the histologic subtypes ($p=0.265$ and $p=0.531$, respectively; Table 6). Moreover, their immunopositivity was not associated with any other clinicopathologic variables (Table 6). In addition, Notch signaling proteins were not correlated with the CSC markers, including CD133, CD44 and CD24 (Table 7).

In serous adenocarcinomas, Notch signaling molecules were not associated with survival outcomes (PFS, $p=0.383$; OS, $p=0.622$). However, in mucinous adenocarcinomas, patients with Jagged-1+ mucinous ovarian cancer showed a trend of better PFS and OS ($p=0.025$ and $p=0.031$, respectively), although its expression did not serve as an independent prognostic factor when corrected by stage ($p=0.973$ and $p=0.996$, respectively).

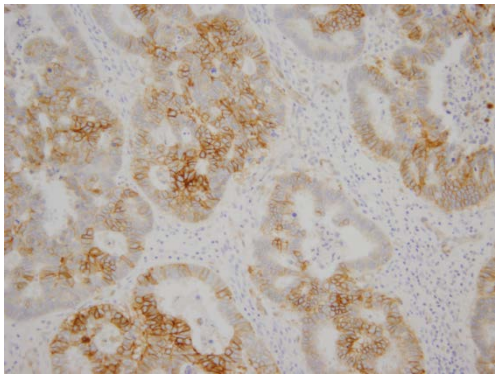
Table 6. Expression of Notch signaling molecules (Notch1, Notch3, Jagged-1) according to the clinicopathologic variables (N=103)

| Variables | Notch1 | | p-value | Notch3 | | p-value | Jagged-1 | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | |
| Histology | | | <0.001 | | | 0.265 | | | 0.531 |
| Serous | 18 (29.5) | 43 (70.5) | | 52 (85.2) | 9 (14.8) | | 24 (39.3) | 37 (60.7) | |
| Mucinous | 14 (82.4) | 3 (17.6) | | 11 (64.7) | 6 (35.3) | | 3 (17.6) | 14 (82.4) | |
| Endometrioid | 3 (30) | 7 (70) | | 7 (70) | 3 (30) | | 3 (30) | 7 (70) | |
| Clear cell | 2 (15.4) | 11 (84.6) | | 9 (69.2) | 4 (30.8) | | 4 (30.8) | 9 (69.2) | |
| Others | 0 (0) | 2 (100) | | 2 (100) | 0 (0) | | 1 (50) | 1 (50) | |
| Stage | | | 0.771 | | | 0.057 | | | 0.631 |
| I | 14 (37.8) | 23 (62.2) | | 26 (70.3) | 11 (29.7) | | 10 (27) | 27 (73) | |
| II | 3 (23.1) | 10 (76.9) | | 8 (61.5) | 5 (38.5) | | 4 (30.8) | 9 (69.2) | |
| III | 18 (38.3) | 29 (61.7) | | 41 (87.2) | 6 (12.8) | | 19 (40.4) | 28 (59.6) | |
| IV | 2 (33.3) | 4 (66.7) | | 6 (100) | 0 (0) | | 2 (33.3) | 4 (66.7) | |
| Grade | | | 0.022 | | | 0.589 | | | 0.319 |
| 1 | 12 (60) | 8 (40) | | 14 (70) | 6 (30) | | 5 (25) | 15 (75) | |
| 2 | 14 (37.8) | 23 (62.2) | | 30 (81.1) | 7 (18.9) | | 16 (43.2) | 21 (56.8) | |
| 3 | 11 (24.4) | 34 (75.6) | | 36 (80) | 9 (20) | | 14 (31.1) | 31 (68.9) | |
| LN metastasis | | | 0.838 | | | 0.046 | | | 0.606 |
| No | 28 (36.8) | 48 (63.2) | | 56 (73.7) | 20 (26.3) | | 25 (32.9) | 51 (67.1) | |
| Yes | 9 (34.6) | 17 (65.4) | | 24 (92.3) | 2 (7.7) | | 10 (38.5) | 16 (61.5) | |
| Peritoneal seeding | | | 0.206 | | | 0.149 | | | 0.506 |
| No | 19 (31.1) | 42 (68.9) | | 44 (72.1) | 17 (27.9) | | 19 (31.1) | 42 (68.9) | |
| <2cm | 6 (60) | 4 (40) | | 9 (90) | 1 (10) | | 5 (50) | 5 (50) | |
| >2cm | 12 (37.5) | 20 (62.5) | | 28 (87.5) | 4 (12.5) | | 11 (34.4) | 21 (65.6) | |

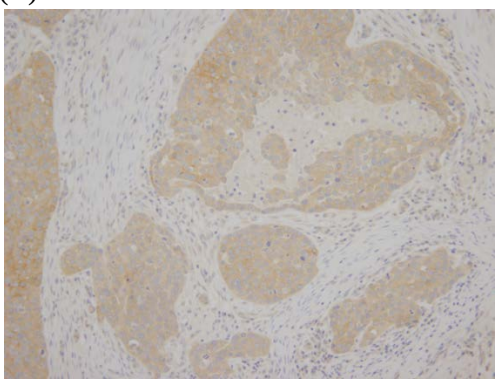
Table 7. Correlations between cancer stem cell markers and Notch and Hedgehog signaling molecules in serous adenocarcinomas (n=61)

| Variables | CD133 | | p-value | CD44 | | p-value | CD24 | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | | Low, n(%) | High, n(%) | |
| Notch1 | | | 0.122 | | | 0.383 | | | 0.214 |
| Low | 10 (23.3) | 7 (43.8) | | 13 (33.3) | 5 (22.7) | | 7 (41.2) | 11 (25) | |
| High | 33 (76.7) | 9 (56.3) | | 26 (66.7) | 17 (77.3) | | 10 (58.8) | 33 (75) | |
| Notch3 | | | 0.317 | | | 0.349 | | | 0.682 |
| - | 36 (83.7) | 15 (93.8) | | 32 (82.1) | 20 (90.9) | | 15 (88.2) | 37 (84.1) | |
| + | 7 (16.3) | 1 (6.3) | | 7 (17.9) | 2 (9.1) | | 2 (11.8) | 7 (15.9) | |
| Jagged-1 | | | 0.137 | | | 0.720 | | | 0.856 |
| Low | 15 (34.9) | 9 (56.3) | | 16 (41) | 8 (36.4) | | 7 (41.2) | 17 (38.6) | |
| High | 28 (65.1) | 7 (43.8) | | 23 (59) | 14 (63.6) | | 10 (58.8) | 27 (61.4) | |
| PTCH | | | 0.004 | | | 0.383 | | | 0.718 |
| Low | 3 (7) | 6 (37.5) | | 7 (17.9) | 2 (9.5) | | 3 (17.6) | 6 (14) | |
| High | 49 (93) | 10 (62.5) | | 32 (82.1) | 19 (90.5) | | 14 (82.4) | 37 (86) | |
| Shh | | | 0.052 | | | 0.348 | | | 0.809 |
| Low | 20 (46.5) | 3 (18.8) | | 13 (33.3) | 10 (45.5) | | 6 (35.3) | 17 (38.6) | |
| High | 23 (53.5) | 13 (81.3) | | 26 (66.7) | 12 (54.5) | | 11 (64.7) | 27 (61.4) | |
| Smo | | | 0.473 | | | 0.605 | | | 0.007 |
| Low | 31 (72.1) | 13 (81.3) | | 29 (74.4) | 15 (68.2) | | 8 (47.1) | 36 (81.8) | |
| High | 12 (27.9) | 3 (18.8) | | 10 (25.6) | 7 (31.8) | | 9 (52.9) | 8 (18.2) | |
| Gli-1 | | | 0.498 | | | 0.053 | | | 0.753 |
| - | 40 (93) | 14 (87.5) | | 33 (84.6) | 22 (100) | | 15 (88.2) | 40 (90.9) | |
| + | 3 (7) | 2 (12.5) | | 6 (15.4) | 0 (0) | | 2 (11.8) | 4 (9.1) | |

(A) Notch1



(B) Notch3



(C) Jagged-1

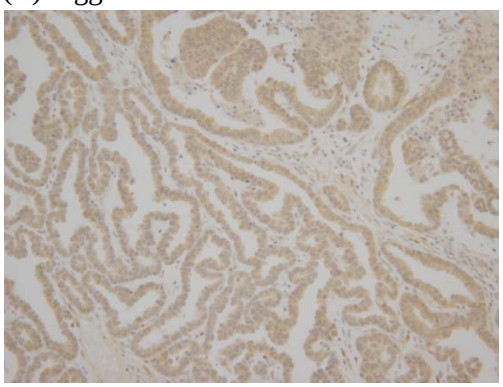


**Figure 3**. **Representative immunohistochemical stainings for Notch proteins.** All figures are 200x magnification.

## Expression profiles of Hedgehog signaling pathway proteins in ovarian carcinomas

Most of the hedgehog signaling molecules, including PTCH, Shh, and Smo, demonstrated cytoplasmic staining (Figure 4). Gli-1 showed cytoplasmic immunoreactivity with occasional nuclear staining.

Between the hedgehog molecules, there were statistically significant correlations among Shh, Smo, and Gli-1 expression (Shh and Gli-1, $p=0.003$; Smo and Gli-1, $p<0.001$). However, PTCH was not correlated with any of the three other hedgehog molecules.

Among hedgehog proteins, Shh and Gli-1 were highly expressed in mucinous adenocarcinomas (100% and 47.1%, respectively; Table 8). PTCH and Smo, however, were not differentially expressed according to the histologic subtypes ($p=0.283$ and 0.162, respectively).

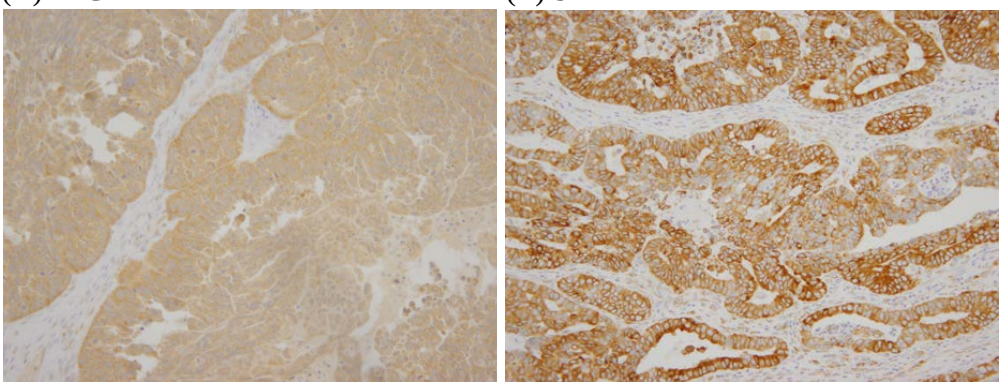
When evaluating the relationships between CSC markers and hedgehog molecules in serous adenocarcinomas, CD133 expression was inversely correlated with PTCH immunopositivity ($p=0.004$; Table 7). CD24 also showed inverse correlation with Smo ($p=0.007$)

In serous adenocarcinomas, the expression profiles of hedgehog molecules were not associated with any clinicopathologic variables, except the PTCH expression which showed a significant correlation with platinum sensitivity ($p=0.013$). Survival outcomes also failed to show statistically significant difference according to the expression of PTCH, Shh, Smo, and Gli-1 ($ps>0.05$).

Table 8. Expression of Hedgehog signaling molecules (PTCH, Shh, Smo, Gli-1) according to the clinicopathologic variables (N=103)

| Variables | PTCH | | p-value | Shh | | p-value | Smo | | p-value | Gli-1 | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Low,n(%) | High,n(%) | | Low,n(%) | High,n(%) | | Low,n(%) | High,n(%) | | Low, n(%) | High,n(%) | |
| Histology | | | 0.283 | | | 0.023 | | | 0.162 | | | 0.001 |
| Serous | 9 (15) | 51 (85) | | 23 (37.7) | 38 (62.3) | | 44 (72.1) | 17 (27.9) | | 55 (90.2) | 6 (9.8) | |
| Mucinous | 6 (35.3) | 11 (64.7) | | 0 (0) | 17 (100) | | 7 (41.2) | 10 (58.8) | | 9 (52.9) | 8 (47.1) | |
| Endometrioid | 3 (30) | 7 (70) | | 5 (50) | 5 (50) | | 5 (50) | 5 (50) | | 10 (100) | 0 (0) | |
| Clear cell | 4 (30.8) | 9 (69.2) | | 6 (46.2) | 7 (53.8) | | 8 (61.5) | 5 (38.5) | | 12 (92.3) | 1 (7.7) | |
| Others | 0 (0) | 2 (100) | | 1 (50) | 1 (50) | | 1 (50) | 1 (50) | | 2 (100) | 0 (0) | |
| Stage | | | 0.544 | | | 0.071 | | | 0.008 | | | 0.753 |
| I | 9 (24.3) | 28 (75.7) | | 9 (24.3) | 28 (75.7) | | 16 (43.2) | 21 (56.8) | | 30 (81.1) | 7 (18.9) | |
| II | 1 (7.7) | 12 (92.3) | | 6 (46.2) | 7 (53.8) | | 9 (69.2) | 4 (30.8) | | 12 (92.3) | 1 (7.7) | |
| III | 10 (21.7) | 36 (78.3) | | 20 (42.6) | 27 (57.4) | | 37 (78.7) | 10 (21.3) | | 41 (87.2) | 6 (12.8) | |
| IV | 2 (33.3) | 4 (66.7) | | 0 (0) | 6 (100) | | 3 (50) | 3 (50) | | 5 (83.3) | 1 (16.7) | |
| Grade | | | 0.003 | | | 0.046 | | | 0.203 | | | 0.002 |
| 1 | 5 (25) | 15 (75) | | 2 (10) | 18 (90) | | 10 (50) | 10 (50) | | 12 (60) | 8 (40) | |
| 2 | 14 (37.8) | 23 (62.2) | | 15 (40.5) | 22 (59.5) | | 27 (73) | 10 (27) | | 34 (91.9) | 3 (8.1) | |
| 3 | 3 (6.8) | 41 (93.2) | | 17 (37.8) | 28 (62.2) | | 27 (60) | 18 (40) | | 41 (91.1) | 4 (8.9) | |
| LN metastasis | | | 0.757 | | | 0.108 | | | 0.207 | | | 0.450 |
| No | 16 (21.1) | 60 (78.9) | | 22 (28.9) | 54 (71.1) | | 45 (59.2) | 31 (40.8) | | 66 (86.8) | 10 (13.2) | |
| Yes | 6 (24) | 19 (76) | | 12 (46.2) | 14 (53.8) | | 19 (73.1) | 7 (26.9) | | 21 (80.8) | 5 (19.2) | |
| Peritoneal seeding | | | 0.774 | | | 0.962 | | | 0.168 | | | 0.840 |
| No | 13 (21.3) | 48 (78.7) | | 21 (34.4) | 40 (65.6) | | 34 (55.7) | 27 (44.3) | | 52 (85.2) | 9 (14.8) | |
| <2cm | 3 (30) | 7 (70) | | 3 (30) | 7 (70) | | 7 (70) | 3 (30) | | 8 (80) | 2 (20) | |
| >2cm | 6 (19.4) | 25 (80.6) | | 11 (34.4) | 21 (65.6) | | 24 (75) | 8 (25) | | 28 (87.5) | 4 (12.5) | |

(A) PTCH (B) Shh



(C) Smo (D) Gli-1

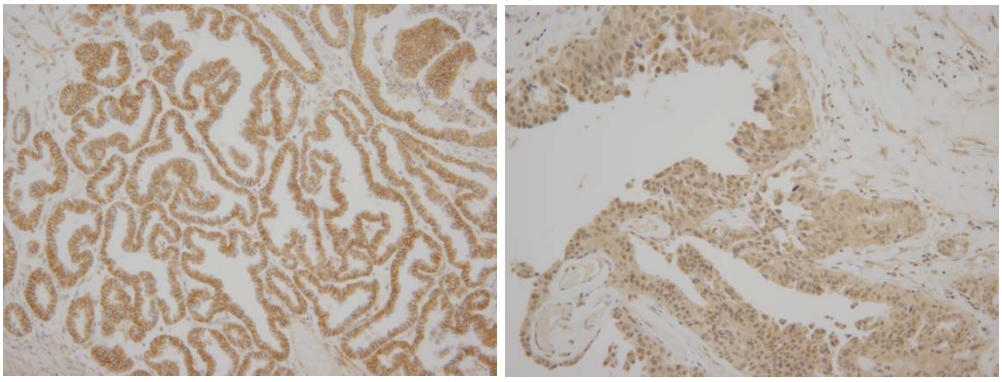


**Figure 4**. **Representative immunohistochemical stainings for Hedgehog proteins.** All figures are 200x magnification.

# DISCUSSION

In the present study, the expression profiles of CSC-related proteins were evaluated comprehensively in ovarian cancer tissues, and it was found that CSC markers were differentially expressed according to the histologic subtypes. The serous adenocarcinomas were characterized by high expression of CD24, Nanog and Snail. In contrast, the expression of CD44, Shh and Gli-1 was more common in mucinous adenocarcinomas. Although the expression profiles of CSC-related molecules were found to be subtype-specific, none of these molecules served as independent predictive or prognostic markers. These findings suggest that CSC-related molecules or pathways might have different roles in tumor progression depending on the subtypes of ovarian cancer.

Most of the previous studies on the expression profiles of CSC markers reported that the increased frequency of tumor cells with stem cell-like phenotypes was related to the poor prognosis in various cancers, including breast, pancreatic cancers, and gliomas (4-6). In ovarian cancers, the presence of CSCs has been also suggested by several researchers through identification of subpopulations of tumor-initiating cancer cells using different CSC markers, such as CD133, CD44, and CD24 (7-9). However, the prognostic significance of these CSC markers in ovarian cancer has been debated. CD24 expression was reported to be related to shortened survival, whereas CD133 and CD44 expression was not shown to be associated with survival outcomes (11-13). Most of these studies had limitations of not evaluating the differential

expression of CSC markers according to the different histologic subtypes. In a study by Kobel et al., ovarian cancer subtypes demonstrated substantially different biomarker expression profiles and their expression did not differ across stage within each subtype, supporting the hypothesis that ovarian cancer subtypes are different disease entities (14). The present study included various histologic subtypes of ovarian cancer, which enabled the comparison of the distribution of histologic subtypes and survival outcomes according to the CSC markers more relevantly. Consequently, it was demonstrated that the expression of CSC-related proteins was substantially different between subtypes, but was not independently related to survival outcomes as well as response to chemotherapy.

The activation of developmental signaling pathways, including Notch and Hedgehog pathways, has been implicated in the development of human malignancies (15, 16). In addition, the roles of these pathways have been recently suggested in the maintenance of CSC phenotypes (3). In ovarian cancer, these signaling pathways have been frequently observed to be overexpressed. Notch3 and Jagged-1 have been reported to be associated with intraperitoneal dissemination and chemoresistance (17, 18). In addition, Hedgehog signaling was reported to be activated in ovarian cancers and Gli-1 expression was shown to be an independent prognostic marker (16). However, there have been few studies evaluating the relationships between CSC markers and developmental signaling pathways. In the current study, associations between CSC markers and Notch or Hedgehog signaling proteins were evaluated. As a result, CD133 and CD24 were shown to be inversely

correlated with PTCH and Smo, respectively, whereas other signaling molecules were not associated with CSC markers.

In the present study, the evaluation of the underlying mechanisms was limited due to the immunohistochemical analysis. In addition, the retrospective study design might cause selection biases. However, the current finding of the differential distribution of tumor cells expressing CSC-related markers may provide useful information regarding the patient selection for targeted therapy against stem-like tumor cells. Moreover, some clues on connections between CSC markers and specific developmental signaling pathways were suggested in this study.

In summary, CSC markers were differentially expressed according to the histologic subtypes, with CD24 highly expressed in serous type and CD44 more frequently expressed in non-serous type. Although the expression profiles of CSC-related molecules were found to be subtype-specific, none of these molecules served as independent predictive or prognostic markers. Alterations in the frequency and distribution of cells with stem cell-like features according to the histologic subtypes have clinical implications for designing clinical trials of selective targeting of these distinct tumor cell populations.

# REFERENCES


1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90.
2. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105-11.
3. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008;8(10):755-68.
4. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 2008;100(9):672-9.
5. Maeda S, Shinchi H, Kurahara H, Mataki Y, Maemura K, Sato M, et al. CD133 expression is correlated with lymph node metastasis and vascular endothelial growth factor-C expression in pancreatic cancer. Br J Cancer 2008;98(8):1389-97.
6. Zeppernick F, Ahmadi R, Campos B, Dictus C, Helmke BM, Becker N, et al. Stem cell marker CD133 affects clinical outcome in glioma patients. Clin Cancer Res 2008;14(1):123-9.
7. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 2009;8(1):158-66.
8. Curley MD, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, et al. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells 2009;27(12):2875-83.
9. Gao MQ, Choi YP, Kang S, Youn JH, Cho NH. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. Oncogene 2010;29(18):2672-80.

10. Stewart JM, Shaw PA, Gedye C, Bernardini MQ, Neel BG, Ailles LE. Phenotypic heterogeneity and instability of human ovarian tumor-initiating cells. Proc Natl Acad Sci U S A 2011;108(16):6468-73.
11. Kristiansen G, Denkert C, Schluns K, Dahl E, Pilarsky C, Hauptmann S. CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. Am J Pathol 2002;161(4):1215-21.
12. Sillanpaa S, Anttila MA, Voutilainen K, Tammi RH, Tammi MI, Saarikoski SV, et al. CD44 expression indicates favorable prognosis in epithelial ovarian cancer. Clin Cancer Res 2003;9(14):5318-24.
13. Ferrandina G, Martinelli E, Petrillo M, Prisco MG, Zannoni G, Sioletic S, et al. CD133 antigen expression in ovarian cancer. BMC cancer 2009;9:221.
14. Kobel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med 2008;5(12):e232.
15. Ranganathan P, Weaver KL, Capobianco AJ. Notch signalling in solid tumours: a little bit of everything but not all the time. Nat Rev Cancer 2011;11(5):338-51.
16. Liao X, Siu MK, Au CW, Wong ES, Chan HY, Ip PP, et al. Aberrant activation of hedgehog signaling pathway in ovarian cancers: effect on prognosis, cell invasion and differentiation. Carcinogenesis 2009;30(1):131-40.
17. Park JT, Chen X, Trope CG, Davidson B, Shih Ie M, Wang TL. Notch3 overexpression is related to the recurrence of ovarian cancer and confers resistance to carboplatin. Am J Pathol 2010;177(3):1087-94.
18. Choi JH, Park JT, Davidson B, Morin PJ, Shih Ie M, Wang TL. Jagged-1 and Notch3 juxtacrine loop regulates ovarian tumor growth and adhesion. Cancer Res 2008;68(14):5716-23.
19. Sano A, Kato H, Sakurai S, Sakai M, Tanaka N, Inose T, et al. CD24 expression is a novel prognostic factor in esophageal squamous cell carcinoma. Ann Surg Oncol 2009;16(2):506-14.

20. Kawamoto M, Ishiwata T, Cho K, Uchida E, Korc M, Naito Z, et al. Nestin expression correlates with nerve and retroperitoneal tissue invasion in pancreatic cancer. Hum Pathol 2009;40(2):189-98.
21. Pan Y, Jiao J, Zhou C, Cheng Q, Hu Y, Chen H. Nanog is highly expressed in ovarian serous cystadenocarcinoma and correlated with clinical stage and pathological grade. Pathobiology 2010;77(6):283-8.
22. Leis O, Eguiara A, Lopez-Arribillaga E, Alberdi MJ, Hernandez-Garcia S, Elorriaga K, et al. Sox2 expression in breast tumours and activation in breast cancer stem cells. Oncogene 2012;31(11):1354-65.
23. Wang M, Wang J, Wang L, Wu L, Xin X. Notch1 expression correlates with tumor differentiation status in ovarian carcinoma. Med Oncol 2010;27(4):1329-35.
24. Chen X, Horiuchi A, Kikuchi N, Osada R, Yoshida J, Shiozawa T, et al. Hedgehog signal pathway is activated in ovarian carcinomas, correlating with cell proliferation: it's inhibition leads to growth suppression and apoptosis. Cancer Sci 2007;98(1):68-76.
25. Li Y, Yang W, Yang Q, Zhou S. Nuclear localization of GLI1 and elevated expression of FOXC2 in breast cancer is associated with the basal-like phenotype. Histol Histopathol 2012;27(4):475-84.
26. Hotz B, Arndt M, Dullat S, Bhargava S, Buhr HJ, Hotz HG. Epithelial to mesenchymal transition: expression of the regulators snail, slug, and twist in pancreatic cancer. Clin Cancer Res 2007;13(16):4769-76.

## 국문 초록

# 난소암 조직에서 암줄기세포 관련 인자의 발현 및 임상적 의의

서울대학교 대학원
의학과 산부인과학 전공
김미경

**목적:** 난소암의 서로 다른 조직학적 유형에 따른 암줄기세포 관련 인자의 발현 양상을 분석하고 암줄기세포 관련 인자의 발현과 항암 화학요법에 대한 반응 및 예후와의 연관성을 살펴보고자 하였다.

**방법:** 2003 년부터 2009 년까지 분당서울대학교병원에서 상피성난소암으로 일차적 병기설정술을 시행받은 103 명의 환자를 대상으로 연구를 진행하였으며, 대상 환자들의 난소암 파라핀 블록을 확보하여 면역조직화학 분석을 시행하였다. 암줄기세포 관련 인자 중 암줄기세포 표면항원 (CD133, CD44, CD24, CD117), 줄기세포 특성과 관련된 인자 (Bmi-1, Nestin, Nanog, Oct4, Sox-2), Notch 신호전달체계 관련 인자 (Notch1, Notch3, Jagged-1), Hedgehog 신호전달체계 관련 인자 (PTCH, Shh, Smo, Gli-1), 및 상피-배엽간 이행 (epithelial-mesenchymal transition) 관련 인자 (Snail, Slug) 등 총 18 가지의 항원에 대한 면역

조직화학 분석을 시행하였다. 각 암줄기세포 관련 인자의 발현과 임상병리학적 특성 간의 연관성을 chi-square test 및 Student's t-test 를 통해 평가하였다. 각 인자의 발현에 따른 무진행생존율 및 전체생존율의 차이는 Kaplan-Meier method 및 log-rank test 을 통해 분석하였다.

**결과:** 암줄기세포 관련 인자들은 각 난소암의 조직학적 유형에 따라 차별적으로 발현함이 관찰되었다. 장액성 난소암의 경우 CD24, Nanog 및 Snail 의 발현이 다른 유형에 비해 높게 관찰된 반면, 점액성 난소암에서는 CD44, Shh 및 Gli-1 이 더 빈번히 발현되었다. 연구에 포함된 모든 암줄기세포 관련 인자들은 조직학적 유형을 제외한 기타 임상병리학적 변수, 즉 병기, 백금기반 항암화학요법에 대한 반응성, 생존율과는 통계학적으로 유의한 연관성이 없었다.

**결론:** 상피성 난소암에서 암줄기세포 관련 인자들은 예후예측을 위한 생물표지자로서의 의의는 낮으나, 조직학적 유형에 따라 차별화된 발현 양상을 보여 향후 암줄기세포 표적 치료법 개발 시 적절한 환자군 선택에 기초 자료를 제공할 수 있을 것으로 사료된다.

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**주요어 :** 난소암, 암줄기세포, 면역조직화학, 생체표지자, 예후

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