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# Difference in P57 Expression Among Four Histological Types of Epithelial Ovarian Carcinoma.

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P57 is known to have anti-tumor activity through inhibition of cyclin-dependent kinases. We performed immunohistochemical analysis on expression of P57 in 92 cases of epithelial ovarian carcinoma（EOC）, of which, 31, 14, 18 and 29 were diagnosed as serous, mucinous, endometrioid（EC）and clear cell carcinoma（CCC）, respectively. Of 92 cases of EOC, 6 and 28 were positive for P57 in the nucleus and the cytoplasm, respectively. Five cases of 29 CCC（17.2%）were positive in the nucleus, which was significantly greater than in non-CCC（p = 0.03）. In contrast, positive cases in the cytoplasm were significantly greater in EC（55.6 and 23.0% for EC and non-EC, respectively, p = 0.01）. Expression patterns of nuclear and cytoplasmic p57 showed no correlations with clinical stage of EOC. Clinicopathological significance of P57 expression in EOC would need further investigation.

Keywords: epithelial ovarian carcinoma, p57, immunohistochemistry, clear cell carcinoma, endometrioid carcinoma

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## INTRODUCTION

The cyclin-dependent kinase inhibitor, P57, negatively regulates cell division cycle and keeps cells at the G0 period［1］. Although P57 is expressed in various types of cells in a fetus, the expression is lost in most cells after birth［1］.

On the other hand, P57 was reported to be expressed at a low level in some malignant tumors, such as esophageal squamous cell carcinoma, colorectal carcinoma, hepatocellular carcinoma, bile duct carcinoma, pancreas carcinoma and breast carcinoma［2-11］. P57 was suggested to play an important role as a tumor suppressor in those carcinomas since expression of P57 inversely correlated with the histological grade and/or the clinical stage of carcinomas［1］.

Several reports showed that P57 was expressed in epithelial ovarian carcinoma（EOC）as well. Most of those reports, however, focused on the correlation of P57 expression with the clinical stage and the prognosis of EOCs［12-16］. Accordingly, the correlation of the EOC histology with P57 expression was not yet elucidated despite that the histology was an important prognostic factor for EOC［17］.

EOC has 4 major histological subtypes; endometrioid carcinoma（EC）, serous carcinoma（SC）, mucinous carcinoma（MC）and clear cell carcinoma（CCC）. Differential diagnosis was not easy in some cases, and immunohistochemistry using particular antibodies have been applied to histological diagnosis of EOC［17］. In the routine practice of pathological diagnosis, we found that a case of CCC was positively stained with P57, which prompted us to

study expression of P57 in different histological types of EOC. In this study, we performed an immunohistochemical analysis of P57 in 92 EOC cases to examine the potential usefulness of P57 in the differential diagnosis of EOC.

## MATERIALS AND METHODS

### *Cases*

We collected histological samples of 92 EOC cases surgically resected either at Shimane University Hospital, Hamada Medical Center or Matsue Red Cross Hospital between January 2011 and July 2016. In 92 cases, there were 31 high-grade SCs (HGSCs), 29 CCCs, 18 ECs and 14 MCs. SC is categorized into the low and the high grade, which are different from each other in terms of etiology and prognosis [18]. As we had only two cases of the low grade SC, we used only HGSCs in the present study. Patients' age, clinical stage and histological grade were obtained from medical records. Histological grade of CCC was not determined according to the standardized diagnostic protocol [19]. As this was a retrospective study, we had a difficulty to obtain informed consent from individual patients. We therefore applied an opt-out policy under the permission of the ethical committee of Shimane University. All the personal information that could identify the patients was not collected nor used in the study. The study protocol was approved by the ethical committee of Shimane University Faculty of Medicine (#20161130-1), of Hamada Medical Center (#2817), and of Matsue Red Cross Hospital (#320).

### *Immunohistochemical studies*

Paraffin sections (4 μm in thickness) were deparaffined and endogenous peroxidase was inhibited by incubation with 3% $H_2O_2$ for 5 min. Histological slides were then autoclaved at 120℃ for 10 min and then incubated at 90℃ for 10 sec in citrate buffer (10 mM, pH 6) to retrieve antigens. The slides were washed with phosphate-buffered saline and blocked with 5% bovine serum albumin at a room temperature for 20 min.

The slides were incubated overnight with a mouse monoclonal anti-P57 (1:200, sNCL-p57, clone 25B2, Novocastra/Leica, Nassloch, Germany) at 4℃, followed by 1- to 3-hour incubation with a secondary antibody (EnVision, DAKO/Agilent, Santa Clara, CA). Staining was done with diaminobenzene by avidin-biotin peroxidase complex method. Nuclei were counterstained with hematoxylin.

Cases were interpreted as 'nuclear and cytoplasmic P57-positive' when more than 5% of tumor cells showed clear brown-yellow granules in the nucleus and the cytoplasm, respectively. In some cases, approximately 1800 cells were examined for P57 expression and counted the positive cells for reference.

Expression of Napsin A (1:200, mouse monoclonal antibody, clone; IP64, Novacastra/Leica), WT1 (1:25, mouse monoclonal antibody, clone;6P-H2, DAKO/Agilent), and AMACR (1:100, rabbit monoclonal antibody, clone;13H4, DAKO/Agilent) were examined by immunostaining.

### *Statistics*

The associations between P57 expression and all variables were assessed using the contingency table analysis, Fisher's exact test and logistic regression analysis. $p < 0.05$ was considered statistically significant. JMP (ver. 12, SAS Institute, Cary, NC) was used in statistical analyses.

## RESULTS

Fig. 1 indicates typical CCC cases positive for P57. We found that positive cases expressed P57 either in the nucleus or in the cytoplasm (Figs. 1c and d). It was of note that only one case was positive for P57 both in the nucleus and the cytoplasm. In all the positive cases, the prevalence of positive cells were less than 30% of tumor cells. Clinico-pathological characteristics of the cases according to their histology are summarized in Table 1. Clinical stage was significantly greater in HGSC than in other histological types, suggesting rapid progression of this type of carcinoma [17]. This was further supported by the fact of greater histological grade of SC. Immunohistochemical feature of each type of EOC stained with Napsin A, WT1 and AMACR were consistent with that in previous reports [17, 20-22].

Table 2a compares clinico-pathological characteristics between cases with and without nuclear P57

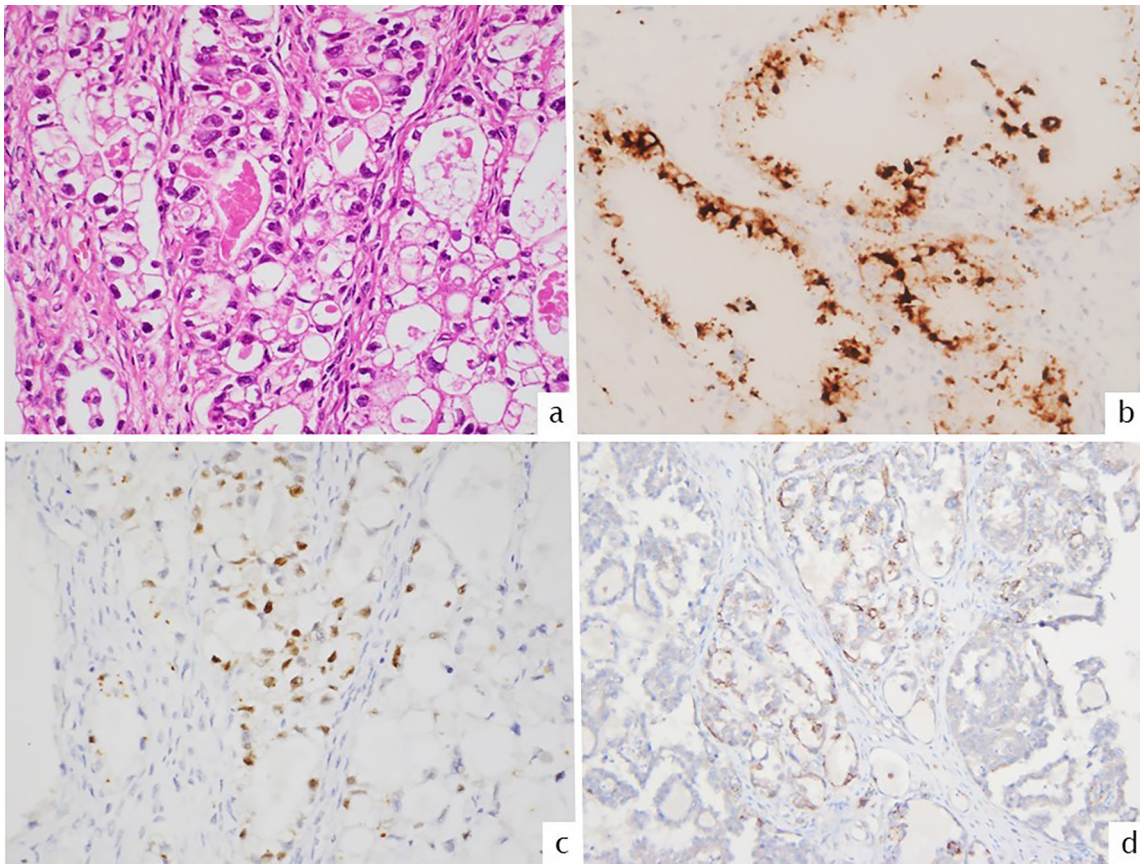


Fig 1. P57 expression in CCC
a) Histological feature of CCC is shown in HE staining. Tubular pattern is seen in this region. b) Expression of Napsin A in the same case. c) Nuclear expression of P57 in the same case. d) Cytoplasmic expression of P57 in another case of CCC. Papillary pattern is seen in this region.

Table 1. Clinico-pathological data of studied cases

| | CCC | HGSC | EC | MC | P |
|---|---|---|---|---|---|
| N | 29 (31.5%) | 31 (33.7%) | 18 (19.6%) | 14 (15.2%) | |
| Age, years | 63.2 (42-92) | 65.6 (40-81) | 61.3 (42-80) | 59.3 (30-81) | 0.37 |
| Clinical stage[a] | | | | | <0.0001* |
| I | 21 (72.4%) | 4 (12.9%) | 15 (88.2%) | 13 (92.9%) | |
| II | 2 (6.9%) | 0 (0%) | 1 (5.9%) | 0 (0%) | |
| III | 5 (17.2%) | 22 (71.0%) | 1 (5.9%) | 1 (7.1%) | |
| IV | 0 (0%) | 4 (12.9%) | 0 (0%) | 0 (0%) | |
| Histological grade[a] | | | | | <0.0001* |
| G1 | - | 0 (0%) | 3 (17.6%) | 9 (64.3%) | |
| G2 | - | 10 (34.4%) | 12 (70.6%) | 3 (21.4%) | |
| G3 | - | 19 (65.6%) | 2 (11.8%) | 2 (14.3%) | |
| Immunohistochemistry | | | | | |
| Napsin A, positive % | 93.1 | 9.7 | 11.1 | 21.4 | <0.0001* |
| WT1, positive % | 3.5 | 93.6 | 38.9 | 0 | <0.0001* |
| AMACR, positive % | 93.1 | 38.7 | 61.1 | 76.9 | <0.0001* |

[a]: Percentages in blankets were calculated in each of the four histological types.
Abbreviations: CCC, clear cell carcinoma; HGSC, high grade serous carcinoma; EC, endometrioid carcinoma; MC, mucinous carcinoma.
* :statistically significant

Table 2. clinico-pathological feature of P57-positive and -negative cases

a) Nuclear expression

| | positive | negative | P |
|---|---|---|---|
| N | 6 (6.5%) | 86 (93.5%) | |
| Age, years | 56.8 (43-71) | 63.4 (30-92) | 0.19 |
| Clinical stage | | | 0.20 |
| I | 6 (11.3%) | 47 (88.7%) | |
| II | 0 (0%) | 3 (100%) | |
| III | 0 (0%) | 29 (100%) | |
| IV | 0 (0%) | 4 (100%) | |
| Histology | | | 0.03* |
| CCC | 5 (17.2%) | 24 (82.8%) | |
| HGSC | 0 (0%) | 31 (100%) | |
| EC | 0 (0%) | 18 (100%) | |
| MC | 1 (7.1%) | 13 (92.9%) | |

b) Cytoplasmic expression

| | positive | negative | P |
|---|---|---|---|
| N | 27 (29.3%) | 65 (70.7%) | |
| Age, years | 61.3 (42-81) | 63.7 (30-92) | 0.37 |
| Clinical stage | | | 0.99 |
| I | 16 (30.2%) | 37 (69.8%) | |
| II | 1 (33.3%) | 2 (66.7%) | |
| III | 8 (27.6%) | 21 (72.4%) | |
| IV | 1 (25.0%) | 3 (75.0%) | |
| Histology | | | 0.04* |
| CCC | 5 (17.2%) | 24 (82.8%) | |
| HGSC | 8 (25.8%) | 23 (74.2%) | |
| EC | 10 (55.6%) | 8 (44.4%) | |
| MC | 4 (28.6%) | 10 (71.4%) | |

Abbreviations: CCC, clear cell carcinoma; HGSC, high grade serous carcinoma; EC, endometrioid carcinoma; MC, mucinous carcinoma.
*: statistically significant

expression. We identified 5 positive cases out of 29 cases of CCC, which was significantly greater than in non-CCC (17.2 and 1.6% for CCC and non-CCC, respectively, p = 0.01 by Fisher's exact test). In contrast, for cytoplasmic P57, a greater prevalence of positive cases was obtained in EC than in non-EC (55.6 and 23.0% for EC and non-EC, respectively, p = 0.01 by Fisher's exact test, see Table 2b). Further, the logistic regression analysis confirmed the effects of the histology on nuclear and cytoplasmic P57 expression under adjustment with patients' age and the clinical stage (Table 3). Despite that P57 expression was not associated with the clinical stage in the univariate analysis (Table 2), clinical stage was included in the logistic analysis for cytoplasmic P57 expression because previous studies indicated a positive association between P57 expression and clinical stage [2, 3, 5, 6, 8, 10, 13, 14, 16]. Clinical stage was not included in the analysis for nuclear P57 expression because of several empty categories (Table 2a). Even if clinical stage was included, the histology (CCC vs. others) showed a significant effect on the probability of the nuclear P57 expression (B = 1.46 ± 0.67, p = 0.03). Histological grade was not included in the models because the information on CCC was not available (see Materials and Methods).

## DISCUSSION

In this study using 92 cases of EOC, we found that cases with nuclear P57 expression were more prevalent in CCC while cases with cytoplasmic P57 expression were more prevalent in EC. This result was interesting if biological characteristics of EOC as well as regulatory roles of P57 in cell proliferation were considered. In addition, only one case examined here expressed P57 both in the nucleus and the cytoplasm, which was an interesting observation in terms of regulatory mechanisms of intracellular P57 distribution in cancer cells. In spite of such interesting features of P57 expression, P57 did not seem a reliable marker in the histological diagnosis of EOC because less than 30% of tumor cells were positive even at a maximum.

P57 is, like P21 and P27, a cyclin-dependent kinase inhibitor, which becomes active after translocation into the nucleus [1, 23, 24]. It was therefore generally accepted that a lower level of P57 expression in the nucleus was an indicator of a poor prognosis in various cancers [1-11, 23, 24]. We found, however, no correlations of nuclear P57 expression with the clinical stage in EOC (Table 2). This result might be inconsistent with previous observations. However, as we did not have information about the prognosis of the examined cases, it

Table 3. Logistic regression analysis on parameters influencing P57 expression in EOC

a) Nuclear expression

| Parameters | B | SE | OR | P |
|---|---|---|---|---|
| Age | 0.07 | 0.05 | 1.07 | 0.15 |
| Histology, CCC vs. others | 1.37 | 0.58 | 15.6 | 0.02* |

b) Cytoplasmic expression

| Parameters | B | SE | OR | P |
|---|---|---|---|---|
| Age | 0.02 | 0.02 | 1.02 | 0.39 |
| Clinical stage, I vs. others | -0.12 | 0.26 | 0.8 | 0.65 |
| Histology, EC vs. others | 0.68 | 0.3 | 3.8 | 0.03* |

Abbreviations: B, partial regression coefficient; SE, standerd error; OR, odds ratio; EOC, epithelial ovarian carcinoma; CCC, clear cell carcinoma; EC, endometrioid carcinoma
*: statistically significant

was not feasible to refer to effects of P57 expression on the prognosis in this study. It is necessary to perform a longitudinal study to clarify this point.

In contrast to nuclear P57 expression, cases positive for cytoplasmic P57 expression were significantly greater in EC than in other histological types (Tables 2 and 3). These observations suggested that the difference in intracellular P57 expression might reflect biological or pathogenetic feature of EOC. Cytoplasmic expression of P57 was implicated as an indicator of a good prognosis in spite of some inconsistent observations [24-28]. Cytoplasmic P57 was reported to affect various functions in cancer cells such as motility of cells, stabilization of the actin cytoskeleton, inhibition of apoptosis, and suppression of invasion and metastasis [1, 23, 28]. Although the present study failed to support the relationship between cytoplasmic P57 expression and clinical stage of EOC, further cell biological studies are warranted to clarify roles of cytoplasmic P57 in biological behavior of EOC.

We found five reports on expression of P57 in EOC [12-16]. Among them, three used polyclonal antibodies [12-14], and only two used a monoclonal antibody [15, 16]. Of the two, one studied cases of clinical stage III [15]. The other mainly studied SC though cases of different stages were included [16]. In contrast to those studies, we collected nearly 100 cases of EOC, including four histological types at different clinical stages. In addition, we examined both cytoplasmic and nuclear P57 expression. Accordingly, this is so far the most comprehensive study on P57 expression in EOC. Despite of the advantage above, we have some limitations in this study; the sample size is not yet large enough to cover all the clinical stages, and the study design, i.e., a cross-sectional study, was not suitable to refer to the causal relationship between P57 expression and the prognosis of patients. It is necessary to prepare a large-scale longitudinal study for further analyses of the role of P57 in EOC, in particular, in CCC and EC.

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***Declaration of Conflicting Interests***

The authors declare no conflict of interests.

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