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Novel Classification of Ovarian Cancer Based on Transcription Factors That Are Closely Associated with Ovarian Development, Differentiation, and Growth

Mitsumasa Osakabe[1]; Noriyuki Yamada[1]; Hiroaki Itamochi[3]; Tsukasa Baba[2]; Noaki Yanagawa[1]; Tamotsu Sugai[1]*

[1]Department of Molecular Diagnostic Pathology, School of Medicine, Iwate Medical University, 2-1-1, Shiwagun'yahabachou, 028-3695, Japan.

[2]Department of Obstetrics and Gynecology, School of Medicine, Iwate Medical University, 2-1-1, Shiwagun'yahabachou, 028-3695, Japan.

[3]Department of Clinical Oncology, School of Medicine, Iwate Medical University, 2-1-1, Shiwagun'yahabachou, 028-3695, Japan.

***Corresponding Author(s): Tamotsu Sugai**

Department of Molecular Diagnostic Pathology, School of Medicine, Iwate Medical University, 2-1-1, Shiwagun'yahabachou, 028-3695, Japan.

Tel: +81-19-613-7111 & +81-19-611-8071;

Email: tsugai@iwate-med.ac.jp & tsugai@cocoa.ocn.ne.jp

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Abstract

Background and objectives: The traditional classification of Ovarian Cancer (OC), which includes High-Grade Serous Carcinoma (HGSC), Endometrioid Carcinoma (EC), Clear Cell Carcinoma (CCC), and Mucinous Carcinoma (MC), has been used worldwide. However, a new classification should be possible, based on immunohistochemical expression of Transcription Factors (TFs) that promote growth and differentiation of tumor cells.

Materials and methods: We examined the immunohistochemical expression patterns of 11 selected TFs (p53, WT1, Estrogen Receptor (ER), Progesterone Receptor (PgR), pRb, β-catenin, p63, GATA3, androgen receptor, SALL4, and cdx2) in HGSC, EC, CCC, and MC. In addition, we stratified the expression patterns of TFs using hierarchical cluster analysis.

Results: Consequently, the expression patterns were classified into three subgroups based on the expression level of each TF as measured using an auto-image analyzer. A significantly high frequency of HGSC was found in subgroup 1, whereas CCC and MC were most common in subgroup 3. However, there was no significant difference in the frequency of EC between subgroups. In addition, TFs that could contribute to the segregation of the subgroups were WT1, p53, ER, and PgR. Finally, we found through multivariate analysis that subgroup 3, which was characterized by low expression of all of the selected markers, showed a worse prognosis than subgroups 1 and 2.

Conclusion: We suggest that TFs may allow for stratification of patients into risk categories for overall survival and that histological type may largely depend on the TF expression pattern subgroup.

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Introduction

Ovarian Cancer (OC) is the leading cause of death from gynecological cancer because most are at an advanced stage at the time of diagnosis [1]. Despite their initial chemosensitivity, relapses are frequent, and the median overall survival is less than 5 years [2]. One probable explanation for the disappointing results with OC is that its histological and molecular natures are heterogeneous [3]. Previous studies have shown that there are two types of OC based on clinicopathological and molecular findings [3-5]. Whereas type I OC is typically low grade, better differentiated, genomically stable, and characterized by the absence of *TP53* mutation, type II OC is a high-grade serous carcinoma that is closely associated with *TP53* mutation and high genomic instability [6]. In addition, OC has been histologically subclassified into High-Grade Serous Carcinoma (HGSC), Endometrioid Carcinoma (EC), Clear Cell Carcinoma (CCC), and Mucinous Carcinoma (MC) [7-9]. This classification scheme suggests a heterogeneous nature among ovarian cancer cells. Although these classifications have been widely used worldwide [8], a new classification may be required, one that is based on functional markers dependent on growth and differentiation. The advantage of a classification that is based on the functional activity is to provide objectivity in addition to histological classification. In addition, immunohistochemical markers are more likely to be useful in evaluating function in diagnostic pathology. Although there are many factors contributing to the heterogeneity of cell growth and differentiation, transcriptional factors that regulate cell growth and differentiation may be particularly useful.

Transcription Factors (TFs) are commonly deregulated in the pathogenesis of human cancer and are a major factor in cancer cell dependencies that are compelling therapeutic targets [10-12]. TFs have been identified as drivers of cancer, including fusion proteins that arise in various subtypes of leukemia (e.g., PML-RARα) [13]. In addition, the activation of TFs can promote stem cells to form terminally differentiated cells [14]. In cancer, a terminally differentiated cell may undergo dedifferentiation to a stem cell, following which it becomes a new and altered differentiated cell [14]. Accordingly, those TFs that are associated with ovarian tumor cell development, differentiation, and growth may play an essential role in ovarian carcinogenesis.

As far as we are aware, our research is the first to study the clinicopathological significance of TFs in OC using immunohistochemical staining. We propose a new classification of OC that is defined by expression levels of TFs that we selected based on their roles in ovarian development and the differentiation and growth of tumor cells.

Materials and methods

Patients

A total of 183 cases of primary epithelial ovarian neoplasms, including HGSC, CCC, EC, and MC, were examined from pathology files of Iwate Medical University. Tissue specimens were obtained from patients undergoing surgery for ovarian carcinomas between 2008 and 2017. Slides stained with hematoxylin and eosin were reviewed in each tumor case, and all tumors were classified into the four above-mentioned histological subtypes according to the World Health Organization criteria [9]. Clinicopathological findings including age, FIGO stage, mortality, and recurrence, are shown in Table 1 [15,16]. Although the proportion of non-HGSC tumors was higher than expected (CCC, 27.9%; EC, 24.6%), there was no pre-selection in the present study.

Patients with OC who were enrolled in this study underwent postoperative chemotherapy according to National Comprehensive Cancer Network (mainly Taxol and carboplatin) [17]. The median durations of follow-up for overall survival and metachronous metastasis (disease-free survival) were 1319 days (range, 95-3542 months) and 819 days (27-3542), respectively.

Tissue microarray construction

The tissue microarrays were assembled using a manual tissue array (Azumaya Co., Tokyo, Japan). Five mm tissue cores were taken from each targeted lesion and placed into a recipient block containing 12 cores: 10 cancer tissues, and 2 controls (normal uterine and ovary tissue). After construction, sections were cut and stained with hematoxylin and eosin on the initial slides to verify the histologic diagnosis. Additional serial sections were cut from the tissue microarray blocks for immunohistochemical staining.

Selection of antibodies

We selected the TFs to be studied on the basis of their involvement in ovarian development and the differentiation and growth of tumor cells. We first chose SALL4, which is expressed in blastocysts. Müllerian ducts differentiate into four components, including fallopian tube (expressing WT1), uterine corpus (expressing Estrogen Receptor (ER) and Progesterone Receptor (PgR)), uterine cervix (expressing p63 for squamous cell differentiation), and upper vagina (also expressing p63 for squamous cell differentiation). Furthermore, β-catenin was added for gonadal differentiation. Next, Androgen Receptor (AR) was examined to assess defeminization. In addition, GATA3 and CDX2 regulate early embryonic development of the ovary. In particular, GATA 3 is thought to be associated with trophoblastic differentiation. Finally, overexpression of p53 and high expression of pRb were selected for assessment of tumor proliferation, though these two markers may not be related to normal ovarian development. However, overexpression of TFs p53 and pRb is important to identify neoplastic progression. Antibodies against TFs that are readily available, reliable, and reproducible will be required in routine pathology. The antibodies we chose for this study met those criteria.

Immunohistochemistry and scoring

Immunohistochemistry was performed on 4-µm sections from tissue microarrays on a DAKO Envision platform. The antibodies used for immunohistochemical staining are provided in Supplementary Table 1.

Quantitative analysis of p53, WT1, ER, PgR, pRb, β-catenin, p63, GATA3, AR, SALL4, and cdx2 expression was performed using digital pathology with Aperio software (Leica Biosystems) as previously described [18,19]. Tissue sections were scanned on an Aperio AT2 scanner with an average scan time of 120 s (compression quality: 70). Images were analyzed using color deconvolution and colocalization. The Aperio Pixel Count v9 Algorithm in Aperio Image Analysis software (for cytoplasmic analysis) was used, and the Nuclear v9 algorithm was applied to detect the nuclear staining of individual tumor cells in the selected regions for nuclear analysis. The intensity of staining was measured on a continuous scale from 0 (black) to 255 (bright white) and was automatically calculated by the software as the ratio of positively stained nuclei to all nuclei (negative, well, moderate, strong, and very strong). Greater than "moderate intensity" (moderate, strong, and very strong) was considered to be positive. Stained areas were color-separated from hematoxylin-

counterstained sections and measured by the software. Then, the score for the area of the positively stained cells (percentage of positive cells) was based on the average score observed in 10 hot spots at 400×. After immunohistochemical examination, we characterized p53 staining into three known patterns: null, diffuse strong pattern, and cytoplasmic [20]. Although the cytoplasmic staining pattern is occasionally encountered in routine practice, it was not found in the present study. The null pattern is divided into two categories that result in negative staining, one with a stop codon and one with no mutation [20,21]. However, because we did not perform genetic sequencing for p53 gene mutation, we could not distinguish the stop codon pattern from the no mutation pattern. Finally, another pathologist performed a double check for image reading.

Hierarchical analysis of the expression of transcription factor

We used open-access Cluster 3.0 software (bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) to cluster the samples according to the TF expression levels, thereby achieving maximal homogeneity for each group and the greatest difference between the groups. The clustering algorithm was set to centroid linkage clustering, which is the standard hierarchical clustering method used in biological studies.

Statistical analysis

Data obtained for histological subtype, FIGO stage, mortality, and recurrence based on each subgroup were analyzed using Fisher exact tests with the aid of JMP Pro 13.0 software (SAS Institute Inc., Cary, NC, USA). If statistical differences between the 3 groups were found, statistical analysis between two groups was further performed using Fisher exact tests (JMP Pro 13.0 software) with Bonferroni correction. For statistical analysis of the expression of p53, WT1, ER, PgR, pRb, β-catenin, p63, GATA3, AR, SALL4, and cdx2 in each subgroup, we used Mann-Whitney U-tests. For survival analysis, the Kaplan-Meier method and the log-rank test were used. Univariate and multivariate analyses were conducted with the Cox proportional hazard model to identify statistical differences for the prediction of overall survival and disease-free survival. A value of $p < 0.05$ was taken to indicate a statistically significant difference, and the Confidence Interval (CI) was determined at the 95% level. Statistical analyses were performed with the JMP Pro 13.0 software package (SAS Institute, Inc., Cary, NC, USA) for Mac.

Results

We screened whether heterogeneous TF expression was observed within each tumor type (HGSC, CCC, EC, and MC). Although heterogeneous expression of TFs was variably found in some tumor tissue, we selected representative tissue without necrosis or hemorrhage for tissue microarray study.

Hierarchical clustering based on the expression level of each TF

We performed hierarchical clustering based on the expression level of each TF to evaluate differences in the expression patterns in patients with OC (Figure 1). Three distinct expression patterns were stratified, and the horizontal lines denote "relatedness" between samples. We found that the frequency of HGSC was significantly higher in subgroup 1 than in subgroups 2 and 3. In addition, a significant difference in the frequency of HGSC between subgroups 2 and 3 was found. However, there was no significant difference in the frequency of EC between each subgroup. Next, significantly higher frequencies of CCC and MC were found in subgroup 3 compared with the frequencies in subgroups 1 and 2. Finally, although the frequency of stage I was significantly higher in subgroup 2 than in either subgroup 1 or subgroup 3, there was a significant difference in the frequency of stage III between subgroup 1 and 2 or 3 (subgroup 1 > 2 and 3). Finally, there was a significant difference in the frequency of stage IV between subgroups 2 and 3 (subgroup 3 > subgroup 2). The detailed data are summarized in Table 2.

Association of individual TFs with each subgroup

First, WT1 and p53 were significantly overexpressed in subgroup 1, compared with subgroups 2 and 3. Second, the ER expression level was significantly higher in subgroup 1 than in subgroups 2 and 3 (subgroup 1 > subgroup 2 and 3). Third, the PgR expression level was significantly higher in subgroup 2 than in subgroup 1 and 3, and there was a significant difference in the expression level between subgroup 1 and 3. The expression of cdx2 was significantly higher in subgroup 2 than in subgroups 1 and 3. Furthermore, there was a significant difference in the expression level of cdx2 between subgroups 1 and 3, 1 and 2, and 2 and 3 (subgroup 2 > 3 > 1). Additionally, β-catenin expression was significantly higher in subgroup 2 than in subgroups 1 and 3. Finally, p63 expression was significantly different in subgroup 2 than in subgroup 3. The detailed data are shown in Figure 2.

Survival analyses with each subgroup

Kaplan-Meier analyses were performed to determine and compare the overall 5-year survival rates and disease-free survival. Overall survival and disease-free survival were correlated with subgroup 1 and 3 compared with subgroup 2 (supplementary Figure 1a and 1b). There were also significant differences in overall survival and disease-free survival between subgroups 1 and 2 (supplementary Figure 1a and 1b).

Association of clinicopathological variables and TF expression patterns with survival using univariate and multivariate analyses

To determine whether the clinicopathological variables (age, histological subtype, and FIGO stage) and expression patterns of examined markers were independent predictors of overall survival and disease-free survival among patients with OC, we used univariate analysis for preliminary screening of the variables, followed by a Cox proportional hazard model of the risk of mortality with the significant univariate predictors.

In overall survival, the univariate analysis (Table 3) identified 4 factors - stage III vs stage I, stage IV vs stage I, subgroup 2 vs 1, and subgroup 2 vs 3 - as being associated with increased overall survival in patients with OC. Table 3 shows the three factors (stage III, stage IV, subgroup 3) that were retained in the multivariate Cox proportional hazard model. We found that subgroup 3 versus 2 (HR, 7.75; 95% CI, 1.64-138.67; $P = 0.0051$) remained significant predictors of overall survival, even after controlling for the other variables. These results are summarized in Table 3.

In disease-free survival, the univariate analysis (Table 4) identified 6 factors - HGSC versus EC, HGSC vs MC, stage III vs stage I, stage IV vs stage I, subgroup 2 vs 1, and subgroup 2 vs 3 - as being associated with increased disease-free survival in patients with OC. Table 4 shows the two factors (stage III, IV) that were retained in the multivariate Cox proportional hazard model. Unfortunately, we found that subgroup 3 was not an independent significant predictor of disease-free survival. These results are depicted in Table 4.

Table 1: Clinicopathological findings for ovarian carcinomas in this study.

Histological type of tumor											
	Total (%)		HGSC (%)		CCC (%)		EC (%)		MC (%)		*p*-value
Total samples	183		70	(38.2)	51	(27.9)	45	(24.6)	17	(9.3)	
Age (years) (mean ± SD)	56.8±12.0		58.4±12.0		52.7±10.8		57.8±11.4		58.4±12.0		
FIGO stage											<0.0001
Stage I	78	(42.6)	9	(12.9)*†‡	29	(56.9)*	26	(57.8)†	14	(82.4)‡	
Stage II	20	(10.9)	10	(14.3)	5	(9.8)	5	(11.1)	0	(0.0)	
Stage III	66	(36.1)	43	(61.4)	12	(23.5)	8	(17.8)	3	(17.6)	
Stage IV	19	(10.4)	8	(11.4)	5	(9.8)	6	(13.3)	0	(0.0)	
Mortality	47	(25.7)	19	(27.1)	19	(37.3)	6	(13.3)	3	(17.6)	
Recurrence	81	(44.3)	40	(57.1)§	27	(52.9)¶	10	(22.2)§¶	4	(23.5)	0.0003

Abbreviations: HGSC: High Grade Serous Carcinoma; CCC: Clear Cell Carcinoma; EC: Endometrioid Carcinoma; MC: Mucinous Carcinoma; SD: Standard Deviation; FIGO: International Federation of Gynecology and Obstetrics. *†‡: *P*-Value <0.0001. §: *P*-Value 0.0002. ¶: *P*-Value 0.0031.

Table 2: Clinicopathological findings according to each subgroup.

Cluster Subgroup							
	Subgroup 1	(%)	Subgroup 2	(%)	Subgroup 3	(%)	*p*-value
Total samples	53	(30.0)	30	(16.4)	100	(54.6)	
Age (years) (mean ± SD)	57.7±11.7		55.1±12.2		56.9±12.2		
Histological subtype							<0.0001
High grade serous carcinoma	44	(83.0)*, †	3	(10.0)*, ‡	23	(23)†, ‡	
Clear cell carcinoma	0	(0.0)*	2	(6.7)‡	49	(49.0)*, ‡	
Endometrioid carcinoma	9	(17.0)	23	(76.6)	13	(13.0)	
Mucinous carcinoma	0	(0.0)*	2	(6.7) ‡	15	(15.0)*, ‡	
FIGO stage							<0.0001
Stage I	6	(11.3)*, ‡	20	(66.7)*, §	52	(52.0) ‡, §	
Stage II	5	(9.4)	6	(20.0)	9	(9.0)	
Stage III	36	(68.0)*	3	(10.0)*, ‡	27	(27.0)‡	
Stage IV	6	(11.3)	1	(3.3)*	12	(12.0)*	
Mortality	15	(28.3)*	1	(3.3)*, ‡	31	(31.0)‡	0.0035
Recurrence	31	(58.5)*	3	(10.0)*, ‡	47	(47.0)‡	<0.0001

Abbreviations: SD: standard deviation; FIGO: International Federation of Gynecology and Obstetrics; *, $p<0.01$; †, $p<0.05$, ‡, $p<0.01$; §, $p<0.01$.

Table 3: Univariate and Multivariate Cox proportional hazards model analysis of overall survival in ovarian cancer patients.

	Univariate		Multivariate	
	HR (95% CI)	*P Value*	HR (95% CI)	*P Value*
Age	1.10 (0.32-3.80)	0.88		
Histological subtypes				
High grade serous carcinoma	1 [Reference]			
Endometrioid carcinoma	0.48 (0.18-1.14)	0.10		
Clear cell carcinoma	1.58 (0.83-3.00)	0.16		
Mucinous carcinoma	0.70 (0.16-2.06)	0.55		
FIGO stage				
Stage I	1 [Reference]		1 [Reference]	
Stage II	1.11 (0.25-3.57)	0.87		
Stage III	2.99 (1.50-6.36)	0.0017	2.80 (1.40-5.75)	0.0035
Stage IV	4.51 (1.82-10.91)	0.0017	4.23 (1.74-9.81)	0.0022
Subgroups				
Subgroup 2	1 [Reference]		1 [Reference]	
Subgroup 1	9.27 (1.88-167.65)	0.0029	4.71 (0.90-86.81)	0.0709
Subgroup 3	10.33 (2.22-183.89)	0.0006	7.75 (1.64-138.67)	0.0051

FIGO: International Federation of Gynecology and Obstetrics; CI: Confidence Interval.

Table 4: Univariate and Multivariate Cox proportional hazards model analysis of disease free survival in ovarian cancer patients.

	Univariate		Multivariate	
	HR (95% CI)	***P Value***	**HR (95% CI)**	***P Value***
Age	0.64 (0.26-1.63)	0.35		
Histological subtypes				
Serous carcinoma	1 [Reference]		1 [Reference]	
Endometrioid carcinoma	0.33 (0.15-0.63)	0.0005	0.56 (0.26-1.07)	0.08
Clear cell carcinoma	1.05 (0.64-1.70)	0.84		
Mucinous carcinoma	0.38 (0.11-0.94)	0.0352	0.72 (0.21-1.84)	0.52
FIGO stage				
Stage I	1 [Reference]		1 [Reference]	
Stage II	1.21 (0.40-3.10)	0.71		
Stage III	4.97 (2.86-9.08)	<0.0001	3.96 (2.28-7.09)	<0.0001
Stage IV	5.89 (2.83-12.12)	<0.0001	4.91 (2.36-9.96)	<0.0001
Subgroups				
Subgroup 2	1 [Reference]		1 [Reference]	
Subgroup 1	7.39 (2.64-30.81)	<0.0001	2.00 (0.64-8.90)	0.26
Subgroup 3	5.76 (2.11-23.72)	0.0001	2.53 (0.85-10.93)	0.10

FIGO: International Federation of Gynecology and Obstetrics; CI: confidence interval.

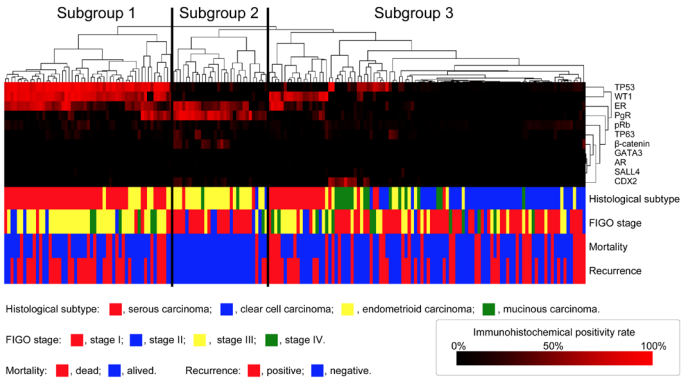

Figure 1: Hierarchical cluster analysis by expression patterns of selected TFs in ovarian cancers.

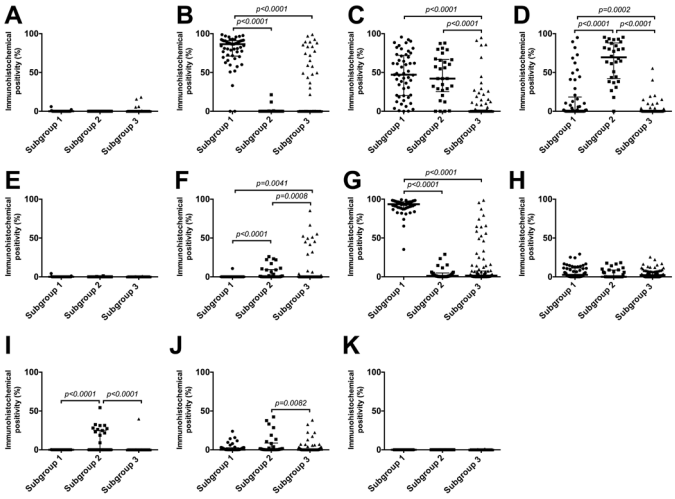

Figure 2: Immunohistochemical expression level of each marker based on subgroups stratified using a cluster analysis. A. SALL4; B, WT1; C. ER; D. PgR; E. AR; F. cdx2; G. p53; H. pRb; I. catenin; J. p63; K. GATA3.

Discussion

In the present study, we examined whether OC could be classified according to the specific expression pattern of the TFs we selected. We found that TF expression patterns could be classified into three subgroups in OCs we examined. Histologically, subgroup 1 was associated with HGSC, whereas subgroup 3 was closely associated with CCC and MC. Meanwhile, subgroup 2 was not associated with any specific histological type. This finding is interesting in that the histological type of OC generally depends on the expression pattern of the selected TFs. Moreover, this finding may suggest that MC and CCC have common characteristics associated with the TF expression patterns. That said, subgroup 3 included four histological subtypes (HGSC, MC, CCC, and MC), so we also suggest that subgroup 3 shares four histological types of OC.

The Wilms' tumor gene WT1 plays complex roles in the development of the organs of the genitourinary tract and mesothelium, as well as in Wilms' tumors [22,23]. Although its biological role remains unclear, most serous carcinomas of the ovary have been shown to express WT1 [22]. It is difficult to differentiate HGSC from EC, especially with poorly differentiated tumors, but WT1 may assist in this distinction given that the WT1 staining pattern differs between HGSC and EC in OC [24]. Accordingly, WT1 may also be helpful in differentiating subgroup 1 (in which HGSC is the main cancer) from subgroup 2 (that characterizes EC). In addition, mutation of *TP53*, which is detected by p53 overexpression in histopathology [25], may also be useful to distinguish subgroup 1 from subgroup 2. However, although there was a statistical difference in expression level between subgroups 1 and 3, both WT1 and p53 showed a heterogeneous expression ranging from low to high in the present study. Therefore, the diagnostic ability of WT1 and p53 to differentiate subgroup 1 from subgroup 3 may be limited. In addition, the negative expression of p53 found in subgroup 3 may result from the null type having a premature stop codon, given that HGSC is reported to have a high frequency of *TP53* mutations containing stop codon mutation (>90%) [20,21].

Expression of ER and PgR is useful to examine hormone sensitivity occurring in tumor cells [26]. Although high expression of ER was commonly observed in both subgroups 1 and 2, PgR characterized subgroup 2 compared to subgroups 1 and 3 in the present study. In addition, subgroup 2 showed a good prognosis compared to subgroups 1 and 3. According to the current results, EC with a pattern of ER (-)/PgR (+) may suggest an excellent prognosis for subgroup 2. However, it is unclear why high expression of PgR in EC reflects a good prognosis.

Subgroup 3 was correlated with overall survival in multivariate analysis. However, no correlation of any subgroup with disease-free survival was found in the present study. Although it is known that patients with HGSC show frequent recurrence and worse survival [4,5], subgroup 1 in which most tumors were HGSC was not retained in multivariate analysis. On the other hand, subgroup 3, which includes HGSC, EC, CCC, and MC, was retained as a prognostic factor in multivariate analysis. Subgroup 3 was characterized by low expression levels of all TFs we examined. This finding suggests that inactivation of these TFs results in decreasing survival. Conversely, subgroup 2, which consisted primarily of MC, demonstrated a good prognosis in the present study. According to this result, a new classification using the selected TFs may be able to distinguish a subgroup with a poor prognosis from one with a good prognosis. Thus, the current findings are interesting in that a functional classification could define different subgroups irrespective of differences in histological subtype.

There are some limitations to this study. First, this study lacks a second cohort to validate the current results. Although a second cohort for validation will be required, the collection of cases without pre-operative chemotherapy is limited.[2] Second, we could not register patients with OC who did not receive treatment with single chemotherapy. In chemotherapeutic treatment of OC, most patients undergo a combination of multiple chemotherapies, so it is very difficult to find OC patients who did not receive multiple chemotherapy. This finding may be undeniable that the current result is not affected by additional treatment. Third, we used TFs that we selected for the present study. Although there are various other TFs to evaluate ovarian carcinogenesis, we selected TFs that are closely associated with ovarian development, differentiation, and growth of tumor cells. We believe that these TFs may be helpful to classify OC in terms of their functional aspect.

In conclusion, we examined the expression pattern of TFs that we selected based on their involvement in ovarian development, differentiation, and growth of tumor cells. Three distinct patterns (subgroups 1-3) were discerned in the present study. Subgroup 1 was characterized by HGSC, whereas CCC and MC were associated with subgroup 3; MC did not belong to any subgroup. In addition, subgroup 3, which is characterized by low expression of all TFs we examined, was correlated to worse prognosis, whereas subgroup 2 was closely associated with good prognosis. A new classification based on the expression of TFs may be helpful for pathological classification of OC. From a practical perspective, the four TFs are recommended for classifying HGSC, CCC, EC, and MC ovarian cancers. However, further study will be required in the near future.

Conflict of interest statement: We declare that we have no conflicts of interest.

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Availability of data and materials: The data that support the findings of this study are available on request from the corresponding author.

Author contributions and disclosure statement: M. Osakabe (first author) constructed the figures and tables and performed the statistical analyses. N. Yamada performed immunohistochemical examination. H. Itamochi and T. Baba assisted with the clinical data. N. Yanagawa supported the in-terpretation of the histological findings. T. Sugai (corresponding author) contributed to the preparation of the manuscript, including all aspects of the data collection and analysis.

Ethics approval and consent to participate: This use of the clinical materials for research purposes was approved by the Institutional Ethics Committee of Iwate Medical University (MH2018-577).

Patient consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Trial Registration: Not applicable, because this article does not contain any clinical trials.

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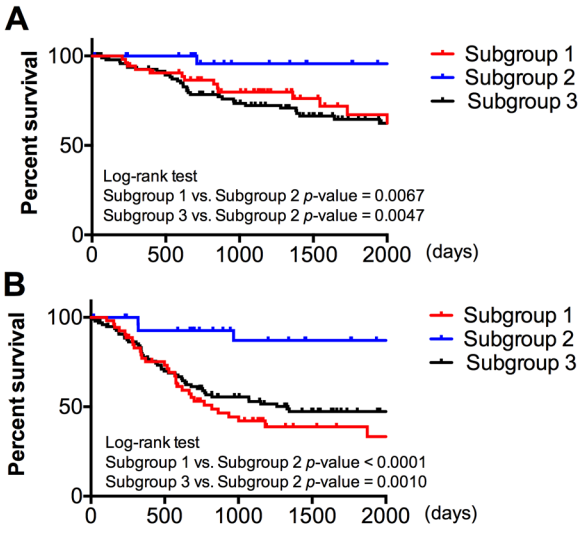
Supplementary Figure & Table



Supp Figure 1: Kaplan-Meier survival analyses of each subgroup in the cohort of patients with CRC (log-rank test). **a**. Overall survival; **b**. Disease-free survival.

Supp Table 1: List of primary antibodies.

Antibody	Clone	Supplier	Dilution	Antigen retrieval
p53	DO-7	Dako	RTU	HR
WT1	6F-H2	Dako	RTU	HR
ER	EP1	Dako	RTU	HR
PgR	PgR636	Dako	RTU	HR
pRb	Ser807/811	Cell Signaling	1:300	HR
β-catenin	β-catenin1	Dako	RTU	HR
p63	DAK-p63	Dako	RTU	HR
GATA3	L50-823	Biocare Medical	1:400	Microwave
AR	AR27	Thermo fisher	RTU	HR
SALL4	6E3	Sigma Aldrich	1:2500	HR
cdx2	DAK-CDX2	Dako	RTU	HR