Expression of Yes-associated protein 1 and its clinical significance in ovarian serous cystadenocarcinoma

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Abstract. Yes-associated protein 1 (YAP1) is a key transcriptional regulator in the Hippo signaling pathway that plays a critical role in the development and progression of several types of malignancies, including ovarian cancer. Herein, we investigated the expression of YAP1 and its clinical significance in a large population of patients with ovarian serous cystadenocarcinoma (OSC), which is the most common form of epithelial ovarian neoplasm, using the TCGA database. Surprisingly, cross-cancer mRNA expression and alterations in YAP1 were higher in OSC than in those of other types of cancers in the TCGA database. YAP1 mRNA expression was significantly higher in OSC compared with normal ovarian samples, and was higher in stages III and IV, than stages I and II. The level of YAP1 protein, which is mainly localized to the nucleus, was also higher in stage IV as compared with stages I, II and III. However, the protein level of pYAP1, which is inactive and is localized to the cytoplasm, was not significantly different between stages. The ratio of pYAP/YAP, which shows higher activity at a low ratio, was lower in stage III than in stages I and II. High YAP and low pYAP levels were significantly correlated with a poor prognosis in patients with OSC. The mRNA and protein expression of YAP1 were significantly increased in the proliferative subtype as compared to the differentiated, immunoreactive and mesenchymal subtypes. According to bioinformatics analysis, YAP1 is most highly correlated with the cell cycle. TGF-β signaling and WNT signaling were significantly increased in the high YAP1 group according to gene set enrichment analysis. Taken together, our results suggest that not only high YAP1 expression but also its subcellular distribution may be associated with poor overall survival in patients with OSC.

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Introduction

Yes-associated protein (YAP), along with the transcriptional co-activator TAZ, is a main downstream effector of the Hippo pathway, which regulates tissue homeostasis, organ size, regeneration and tumorigenesis (1). In mammalian systems, the Hippo pathway is composed of the core kinase complexes mammalian Ste2-like kinases 1/2 and large tumor suppressor kinases 1/2 (2). The main function of the Hippo pathway is to negatively regulate the activity of YAP and TAZ, to promote cellular proliferation, and to induce anti-apoptotic genes via interactions with various transcription factors (2-4). When the Hippo pathway is active, the inhibitory mammalian Ste2-like kinases/large tumor suppressor kinases phosphorylate YAP and TAZ. Phosphorylation leads to nuclear exclusion of YAP and TAZ. Then, YAP and TAZ are sequestered and subjected to proteasomal degradation in the cytoplasm; also, gene expression of YAP- and TAZ-driven molecules is suppressed (4,5). Overexpression of YAP1 has been found in various types of cancers (6-9), and may lead to oncogenic transformation of immortalized epithelial cells (10). The expression and role of YAP1 in cancer is cell type-dependent (11,12). Overexpression of YAP was observed in 62% of hepatocellular carcinomas and 72.6% of colorectal cancers, and was found to be an independent predictor associated with poor disease-free survival and overall survival (13). In 66.3% of non-small cell lung cancers, YAP was found to be overexpressed, and was

associated with reduced overall survival (14). Several studies reported that YAP1 is overexpressed in ovarian cancer (6) and acts as an oncogene (15). Zhang *et al* reported that high levels of nuclear YAP1 correlate with poor prognosis in ovarian cancer patients with clear cell carcinoma (15). Another study showed that YAP1 is highly expressed in serous/endometrioid cystadenocarcinomas, and is positively associated with patient prognosis (16). However, the role of YAP1 as an oncogene has not yet been fully investigated in a large group of ovarian serous cystadenocarcinoma (OSC) patients, who account for the largest proportion of malignant ovarian cancer cases (17,18). Therefore, in the present study, we investigated the expression of YAP1 and determined its clinical significance in OSC.

Materials and methods

Gene expression profiles. Level 3 mRNA expression data from 8 normal and 590 OSC samples were obtained from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/).

Analysis of mRNA microarray data. The raw data was initially analyzed using R software (v.3.2.5;http://www.r-project.org/). The chip data was normalized using the RankNormalize module in GenePattern (http://www.broadinstitute.org/cancer/software/genepattern). GeneNeighbors and ClassNeighbors, modules programmed in GenePattern (http://www.broadinstitute.org/cancer/software/genepattern), were used to select genes closely related to YAP1 (19). cBioportal (http://www.cbioportal.org/) was also used to analyze cross-cancer alterations in YAP1.

Functional enrichment analysis. The DEGs were imported into the Database for Annotation, Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov/) (20) in order to perform Gene Ontology (GO) functional enrichment analysis. Gene set enrichment analysis (GSEA) was used to enrich the mRNAs predicted to have a correlation with pathway in C2, curated gene set enrichment analysis (21,22). GO analysis encompasses 3 domains: biological processes, cellular components and molecular functions. $P<0.05$ was considered to indicate statistical significance.

Statistical analysis. The distributions of characteristics between the 2 groups were compared using the t-test for continuous variables (or the Kolmogorov-Smirnov test when the expected frequency within any cell was <5), and the χ^2 test (or Fisher's exact test when the expected frequency within any cell was <5) for categorical variables. The distributions of characteristics between 3 or more groups were compared using ANOVA. Cumulative event (death) rate was calculated by the Kaplan-Meier method, using the time to the first event as the outcome variable. Probability of and calculated risk for recurrence were determined by actuarial analysis. The criteria for statistical analysis were date of operation and date of death. Survival curves were compared by the log-rank test for various recurrence factors and Cox's model for multivariate analysis. A P-value of<0.05 was considered statistically significant. Statistical analyses were performed using the Prism 5.0 software (GraphPad Prism Software, La Jolla, CA, USA), and the Statistical Package for Social Sciences for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Cross-cancer mRNA expression and alterations in the YAP1 gene. YAP1 mRNA expression in cases of OSC was higher than in 21 other cancer types recorded in the TCGA database. mRNA expression of YAP1 was lowest in acute myeloid leukemia (Fig. 1). Cross-cancer alteration was investigated in 21 types of cancer, and YAP1 expression in OSC was the greatest among the 21 types of cancers recorded in the TCGA.

YAP1 mRNA expression in OSC. The present study examined YAP1 mRNA expression in OSC compared with 8 normal control samples (Fig. 2). Clinicopathological information of the patients is shown in Table I. YAP1 mRNA expression was significantly higher in cases of OSC compared to normal controls (Fig. 2A). YAP1 mRNA expression was higher in stages III and IV compared to earlier stages (Fig. 2B). When comparing YAP1 mRNA expression in 4 subtypes of ovarian cancer, differentiated, immunoreactive, mesenchymal and proliferative, and in 2 subtypes of ovarian cancer, integrated mesenchymal and epithelial subtypes (23,24), YAP1 mRNA expression in the proliferative subtype was significantly higher than that in the differentiated, immunoreactive and mesenchymal subtypes (Fig. 2C). However, there was no significant difference in expression between the integrated mesenchymal subtype vs. the integrated epithelial subtype (Fig. 2D).

YAP1 protein expression in OSC. When a comparison was conducted between stages of ovarian cancer, YAP1 protein expression was only significantly higher in stage IV compared to stages I, II and III (Fig. 3A). The proliferative and differentiated subtypes showed significantly higher protein expression than did the immunoreactive subtype (Fig. 3B). However, there was no significant difference in YAP1 protein level between the integrated epithelial and mesenchymal subtypes (Fig. 3C). The phosphorylated form of YAP1, at serine 127 (pYAP), which is inactivate and is localized to the cytoplasm, did not show any significant differences in protein expression (Fig. 3D). pYAP in the immunoreactive subtype was significantly lower than that in other subtypes; however, the pYAP/YAP ratio, which indicates higher YAP1 activity when it is lower, was lower in stage III than in stage I (Fig. 3E and G). There was no significant difference in the pYAP/YAP ratio between the subtypes of ovarian cancer (Fig. 3H and I).

GeneNeighbors of YAP1. The range of YAP1 mRNA expression in the 590 OSC samples was 2.12 ($\log_2$) to 9.78 ($\log_2$), with a fold-change of 4.61. The 100 genes that were most highly correlated with YAP1 were selected using GeneNeighbors (Fig. 4A), and classified using DAVID. The genes were classified into 3 groups based on biological processes, cellular components and molecular functions. GO terms with significant differences ($P<0.05$) were: i) biological process, ii) cellular components, and iii) molecular functions. Genes highly expressed in OSC were mainly associated with the cell cycle (cell cycle process, cell cycle and cell cycle phase) and protein complexes (protein localization, protein complex

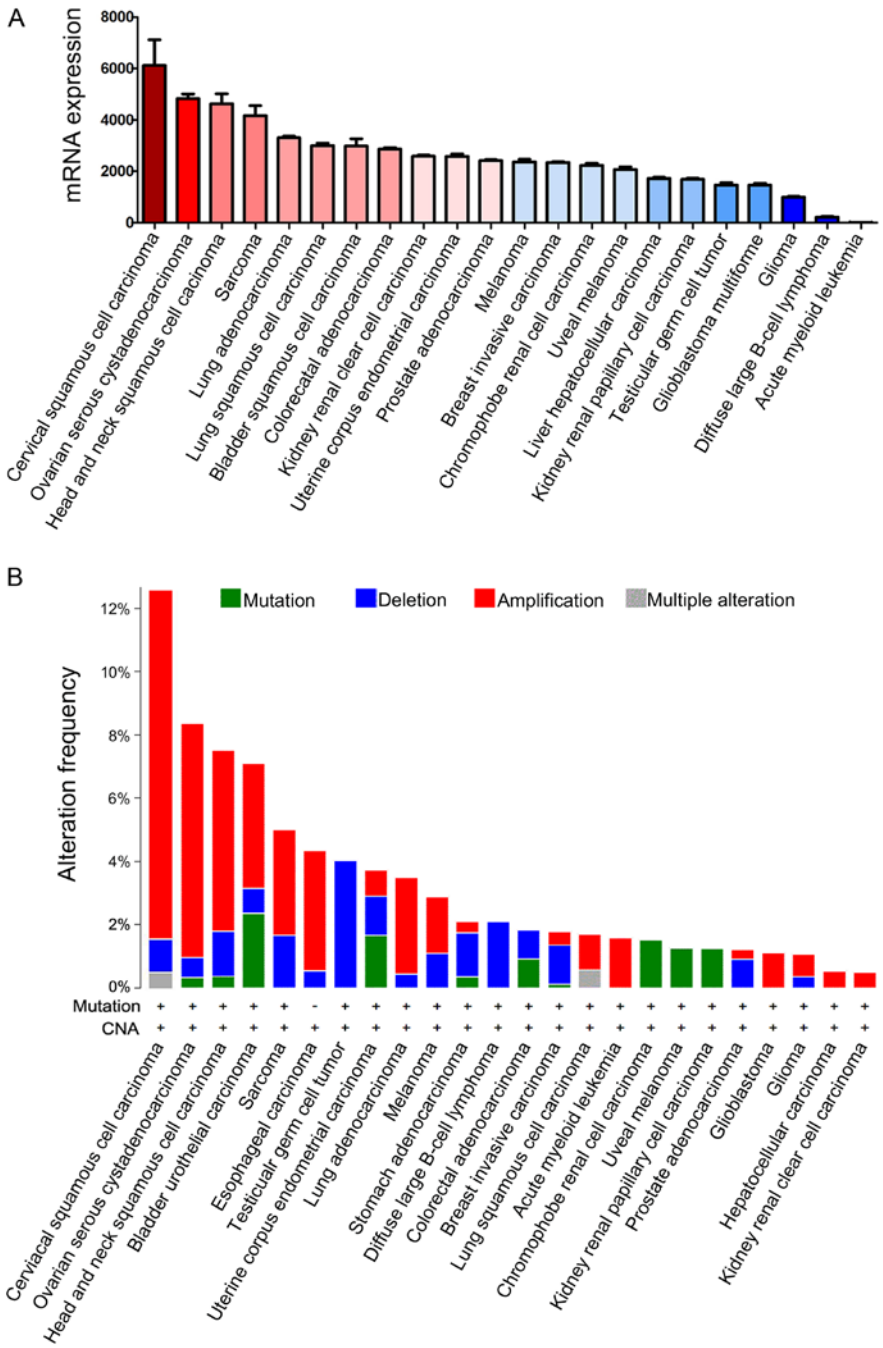


Figure 1. Cross-cancer mRNA expression of YAP1. (A) The data depict the mRNA expression of YAP1 in different cancer types based on the TCGA (https://tcga-data.nci.nih.gov/tcga/) data portal. (B) The data depict the frequency of alterations in YAP1 across different cancer types based on the TCGA. Potential alterations include mutations, deletions, amplification or multiple alterations. Data were obtained from the cBio database for cancer genomics (http://cbioportal.org/public-portal/).

biogenesis and protein complex assembly) when analyzed by biological process (Fig. 4B). Genes highly expressed in OSC were mainly associated with the cytosol and ubiquitin ligase complexes when analyzed by cellular components. Genes highly expressed in OSC were mainly associated with ATP-dependent peptidase activity when analyzed by molecular function. In addition, when genes were analyzed according to cell signaling pathway [Kyoto Encyclopedia of Genes and Genomes (KEGG)], 5 signaling pathways had significant P-values. The analysis illustrated the importance of the ATM signaling pathway, the role of BRCA1, BRCA2 and ATR in cancer susceptibility, the Cdc25 and Chk1 regulatory pathways that respond to DNA damage, regulation of cell cycle progression by Plk3, and RB tumor-suppressor/checkpoint signaling in response to DNA damage.

ClassNeighbors of YAP1 upregulated and downregulated in OSC. Analysis using ClassNeighbors yielded 2 classes of OSC: Class A contained the top 59 (10%) YAP1-upregulated OSC samples and Class B contained the 59 (10%) most YAP1-downregulated OSC samples (Fig. 5A). Of the 17,814 probe sets, the 200 genes that were most strongly

Table I. Clinicopathological information of the ovarian serous cystadenocarcinoma patients of The Cancer Genome Atlas (TCGA).

	mRNA YAP expression			YAP protein expression			Phosphorylated YAP protein expression		
Feature	Total	2X Down	2X Up	Low	Intermediate	High	Low	Intermediate	High
No. of patients	563	205	83	137	138	137	137	138	137
Mean age (years)	59.7	60.2	58.8	61.1	59.7	61.3	61.7	58.5	58.9
Stage									
I	16	9	0	5	3	3	3	9	2
II	27	11	4	6	7	8	10	4	7
III	440	152	66	108	105	110	110	109	103
IV	85	30	13	16	22	16	14	14	23
Tumor grade									
G1	6	4	0	1	0	2	2	2	1
G2	65	29	7	15	20	16	15	17	22
G3	478	166	75	112	117	118	117	116	113
Surgical outcome									
Optimal	369	125	55	86	87	91	85	86	88
Suboptimal	142	56	17	30	38	36	39	36	37
Vital status									
Living	269	100	37	60	65	61	62	66	67
Deceased	291	103	45	76	73	75	75	71	68

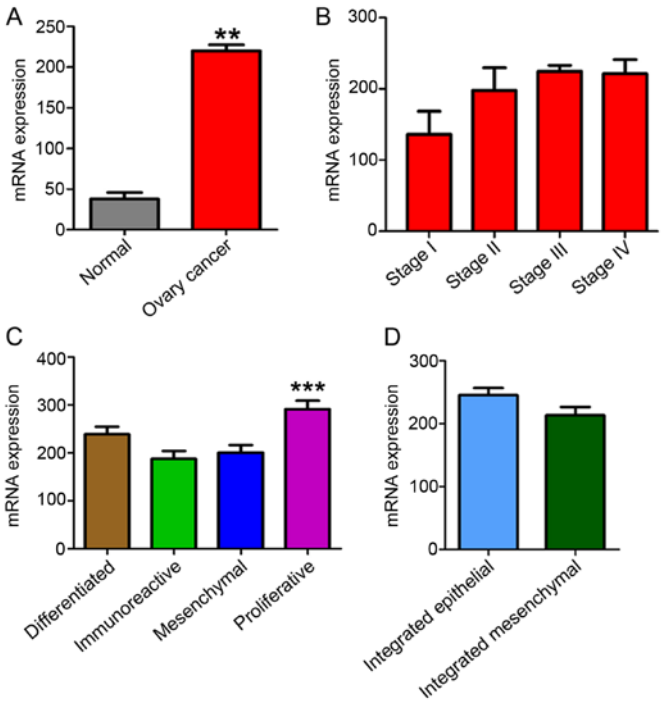


Figure 2. (A-D) YAP1 mRNA expression in ovarian serous adenocarcinonoma. mRNA microarray data of YAP1 in normal controls and ovarian serous cystadenocarcinoma patients, obtained from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). mRNA microarray data of YAP1 in various cell types of epithelial ovarian carcinoma, obtained from the CCLE data portal (http://www.broadinstitute.org/ccle/); **P<0.01 and ***P<0.001. One way ANOVA was performed for comparisons between more than 2 groups, and t-tests were performed for comparisons between 2 groups.

correlated and most highly expressed in Classes A and B were selected. DAVID analysis classified these genes into groups based on GO terms: i) biological processes, ii) cellular components, iii) molecular functions, and iv) the KEGG

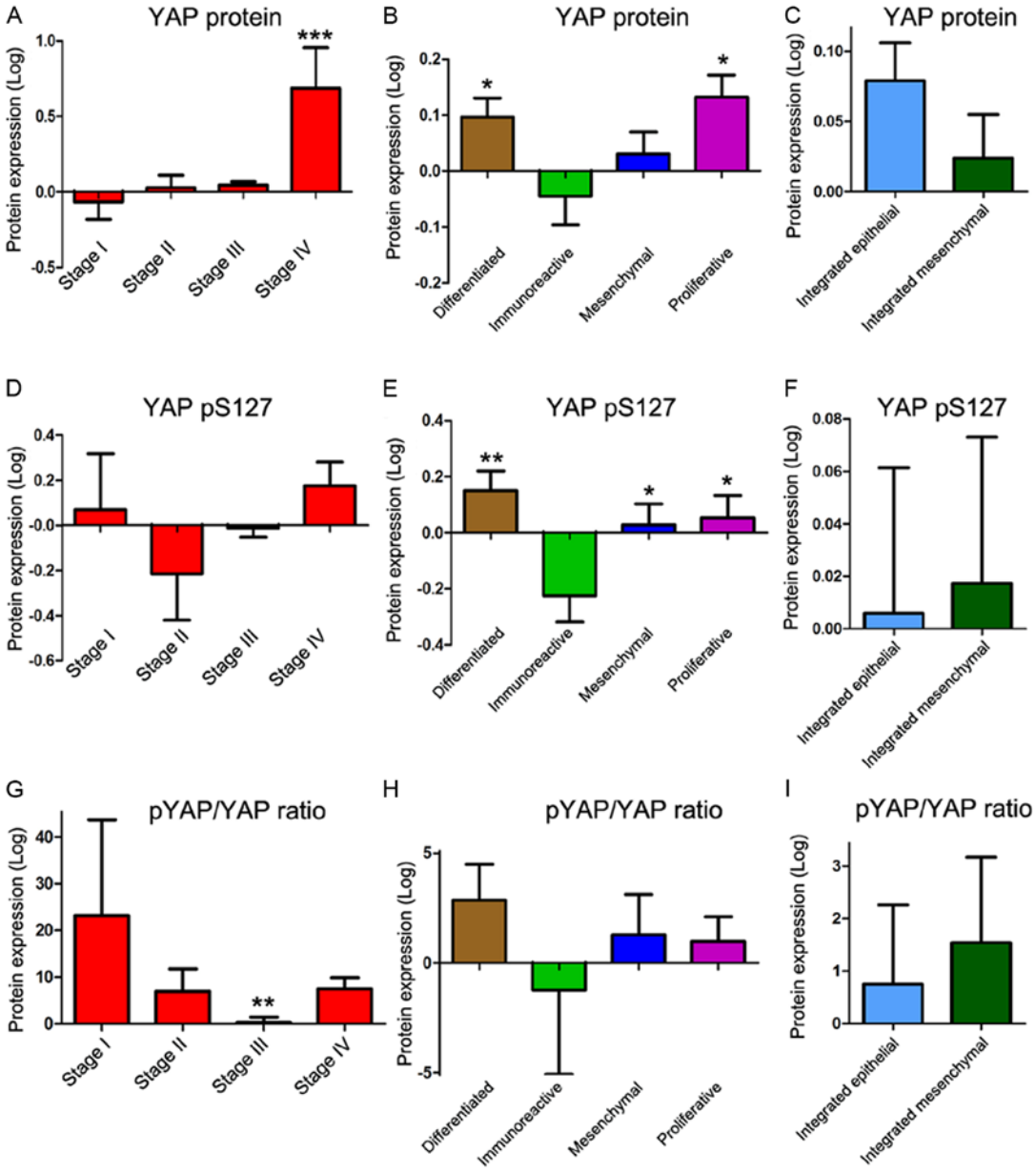


Figure 3. (A-I) YAP1 protein expression in ovarian serous adenocarcinonoma. Protein expression data of YAP1 in ovarian serous cystadenocarcinoma, obtained from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/); *P<0.05, **P<0.01 and ***P<0.001. One way ANOVA was performed for comparisons between more than 2 groups, and t-tests were performed for comparisons between 2 groups.

pathway (Fig. 5B and C and Table II). Genes highly expressed in Class A were mostly associated with DNA recombination and the cell cycle (biological processes), intracellular organelle lumen (cellular components), and RNA and nucleotide binding (molecular functions) (Fig. 5B). Genes highly expressed in Class B were mostly associated with nucleosome and chromatin assembly (biological processes), nucleosomes and the respiratory chain (cellular components), and NADH dehydrogenase (molecular functions) (Fig. 5C).

In addition, GSEA was performed in order to investigate the significantly enriched pathways that differed between Classes A and B. In Class A, pathways involving tight junctions, endometrial cancer, WNT signaling, TGF-β signaling, adherent junctions, basal cell carcinoma and prostate cancer were significantly enriched when compared with Class B. In Class B, pathways involved with primary immunodeficiency, systematic lupus erythematosus, the intestinal immune network for IgA production, regulation of autophagy, autoimmune thyroid disease and natural killer cell-mediated cytotoxicity were enriched (Table III). In Class A, WNT (25) and TGF-β signaling (26) were related to cancer progression (Fig. 6A). Immune-related signaling pathways were related to Class B (Fig. 6B).

Survival analysis. In order to determine the prognostic significance of YAP1 expression in patients with OSC, we assessed the correlation between YAP mRNA and protein expression profiles and clinically significant characteristics: survival, tumor stage, grade and residual disease status. Initially, Kaplan-Meier curves were used to plot overall

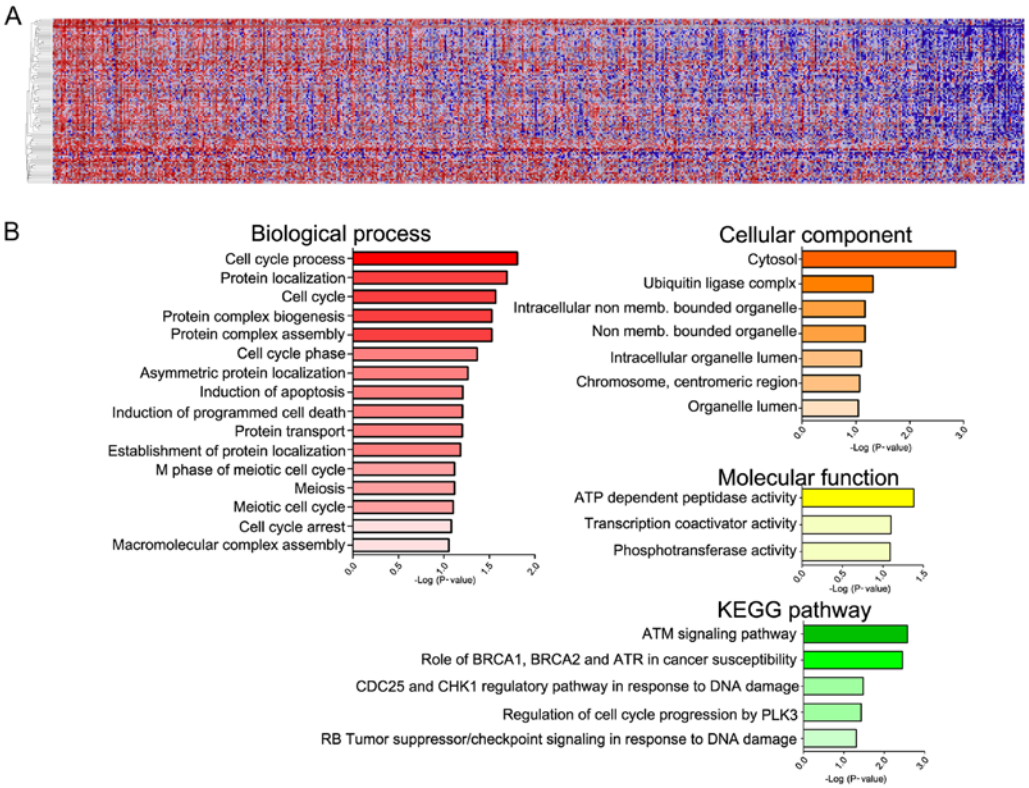


Figure 4. GeneNeighbors of YAP1 in 590 ovarian serous cystadenocarcinoma samples. Hierarchical clustering of YAP1 GeneNeighbors in ovarian serous cystadenocarcinoma. Ovarian serous cystadenocarcinoma samples are arranged in decreasing order of YAP mRNA expression. Colors in the heat map represent expression relative to the mean expression value, with red indicating higher expression and blue indicating lower expression. (A) GeneNeighbors of YAP1 are shown in the column. (B) GeneNeighbors were characterized as biological processes, cellular components, molecular function and KEGG pathway-related.

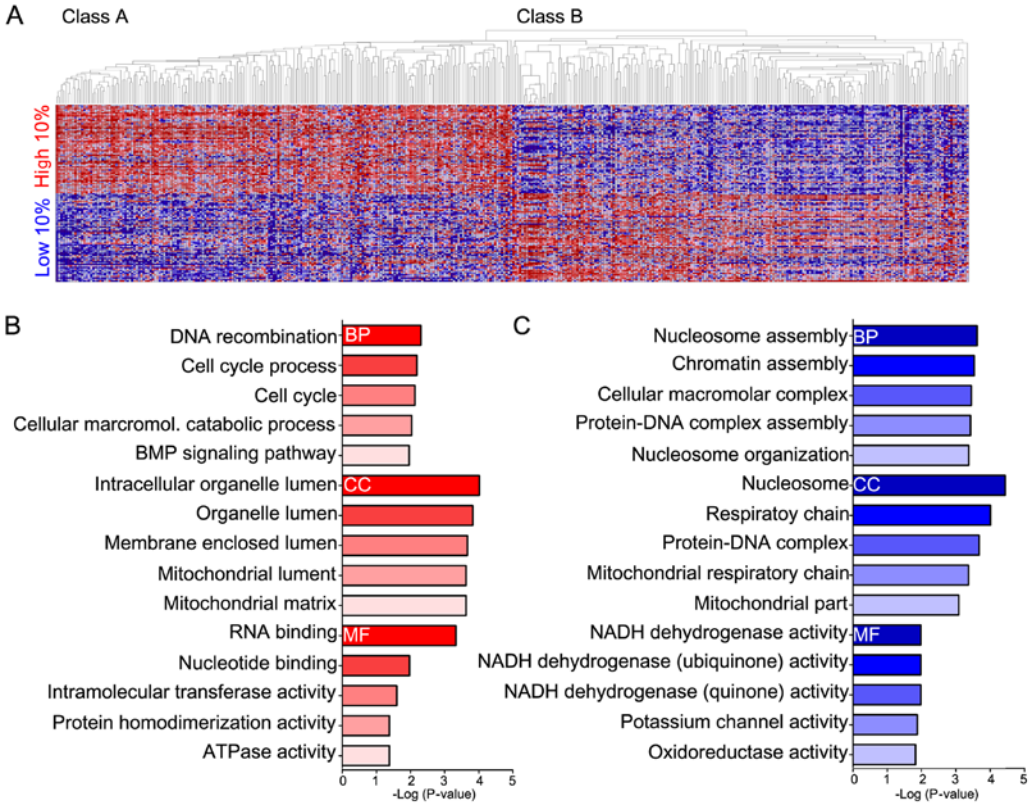


Figure 5. ClassNeighbors of YAP1-related genes in 2 classes of ovarian serous cystadenocarcinoma samples. Hierarchical clustering of differentially expressed genes (top 10%) upregulated and downregulated in OSC cases according to Pearson distance. (A) Colors in the heat map represent expression relative to the mean expression value, with red indicating higher expression and blue indicating lower expression. (B and C) Genes in classes A and B were divided into biological processes, cellular components and molecular functions.

Table II. DAVID analysis of ClassNeighbors.

A, Class A

Term	Count	%	P-value
Biological process (BP)			
GO:0006310~DNA recombination	6	3.24	0.005
GO:0022402~cell cycle process	14	7.57	0.006
GO:0007049~cell cycle	17	9.19	0.007
GO:0044265~cellular macromolecule catabolic process	16	8.65	0.009
GO:0030509~BMP signaling pathway	4	2.16	0.011
GO:0008104~protein localization	18	9.73	0.011
GO:0022403~cell cycle phase	11	5.95	0.012
GO:0000077~DNA damage checkpoint	4	2.16	0.014
GO:0009451~RNA modification	4	2.16	0.014
GO:0000075~cell cycle checkpoint	5	2.70	0.015
GO:0009057~macromolecule catabolic process	16	8.65	0.017
GO:0031570~DNA integrity checkpoint	4	2.16	0.017
GO:0007126~meiosis	5	2.70	0.020
GO:0051327~M phase of meiotic cell cycle	5	2.70	0.020
GO:0010719~negative regulation of epithelial to mesenchymal transition	2	1.08	0.021
GO:0051321~meiotic cell cycle	5	2.70	0.021
GO:0065003~macromolecular complex assembly	14	7.57	0.023
GO:0007178~transmembrane receptor protein serine/threonine kinase signaling pathway	5	2.70	0.023
GO:0007131~reciprocal meiotic recombination	3	1.62	0.026
GO:0045596~negative regulation of cell differentiation	7	3.78	0.026
GO:0015031~protein transport	15	8.11	0.029
GO:0010771~negative regulation of cell morphogenesis involved in differentiation	2	1.08	0.031
GO:0045184~establishment of protein localization	15	8.11	0.031
GO:0051276~chromosome organization	11	5.95	0.032
GO:0051222~positive regulation of protein transport	4	2.16	0.033
GO:0050821~protein stabilization	3	1.62	0.035
GO:0043933~macromolecular complex subunit organization	14	7.57	0.036
GO:0016567~protein ubiquitination	5	2.70	0.037
GO:0002377~immunoglobulin production	3	1.62	0.039
GO:0016071~mRNA metabolic process	9	4.86	0.041
GO:0002440~production of molecular mediator of immune response	3	1.62	0.042
GO:0006974~response to DNA damage stimulus	9	4.86	0.043
GO:0032446~protein modification by small protein conjugation	5	2.70	0.050
Cellular component (CC)			
GO:0070013~intracellular organelle lumen	33	17.84	0.000
GO:0043233~organelle lumen	33	17.84	0.000
GO:0031974~membrane-enclosed lumen	33	17.84	0.000
GO:0031980~mitochondrial lumen	10	5.41	0.000
GO:0005759~mitochondrial matrix	10	5.41	0.000
GO:0000794~condensed nuclear chromosome	5	2.70	0.001
GO:0000793~condensed chromosome	6	3.24	0.007
GO:0005829~cytosol	22	11.89	0.009
GO:0031981~nuclear lumen	23	12.43	0.012
GO:0030135~coated vesicle	6	3.24	0.015
GO:0000228~nuclear chromosome	6	3.24	0.017
GO:0044429~mitochondrial part	12	6.49	0.020
GO:0005694~chromosome	10	5.41	0.025

Table II. Continued.

A, Class A

Term	Count	%	P-value
GO:0005654~nucleoplasm	15	8.11	0.030
GO:0042645~mitochondrial nucleoid	3	1.62	0.033
GO:0009295~nucleoid	3	1.62	0.033
GO:0031090~organelle membrane	17	9.19	0.041
GO:0042175~nuclear envelope-endoplasmic reticulum network	7	3.78	0.046
Molecular function (MF)			
GO:0003723~RNA binding	18	9.73	0.000
GO:0000166~nucleotide binding	33	17.84	0.011
GO:0016866~intramolecular transferase activity	3	1.62	0.025
GO:0042803~protein homodimerization activity	8	4.32	0.041
GO:0016887~ATPase activity	8	4.32	0.041
GO:0019237~centromeric DNA binding	2	1.08	0.047

B, Class B

Term	Count	%	P-value
Biological process (BP)			
GO:0006334~nucleosome assembly	7	3.91	0.000
GO:0031497~chromatin assembly	7	3.91	0.000
GO:0034621~cellular macromolecular complex subunit organization	13	7.26	0.000
GO:0065004~protein-DNA complex assembly	7	3.91	0.000
GO:0034728~nucleosome organization	7	3.91	0.000
GO:0006091~generation of precursor metabolites and energy	12	6.70	0.000
GO:0022900~electron transport chain	7	3.91	0.001
GO:0006323~DNA packaging	7	3.91	0.001
GO:0034622~cellular macromolecular complex assembly	11	6.15	0.002
GO:0006812~cation transport	15	8.38	0.002
GO:0006333~chromatin assembly or disassembly	7	3.91	0.002
GO:0006119~oxidative phosphorylation	6	3.35	0.004
GO:0045454~cell redox homeostasis	5	2.79	0.004
GO:0006811~ion transport	17	9.50	0.006
GO:0043281~regulation of caspase activity	5	2.79	0.009
GO:0006120~mitochondrial electron transport, NADH to ubiquinone	4	2.23	0.009
GO:0052548~regulation of endopeptidase activity	5	2.79	0.011
GO:0052547~regulation of peptidase activity	5	2.79	0.012
GO:0015672~monovalent inorganic cation transport	9	5.03	0.018
GO:0006917~induction of apoptosis	9	5.03	0.019
GO:0012502~induction of programmed cell death	9	5.03	0.019
GO:0042981~regulation of apoptosis	16	8.94	0.020
GO:0042775~mitochondrial ATP synthesis coupled electron transport	4	2.23	0.021
GO:0042773~ATP synthesis coupled electron transport	4	2.23	0.021
GO:0043067~regulation of programmed cell death	16	8.94	0.022
GO:0010941~regulation of cell death	16	8.94	0.023
GO:0030001~metal ion transport	11	6.15	0.024
GO:0051336~regulation of hydrolase activity	9	5.03	0.025
GO:0006813~potassium ion transport	6	3.35	0.026
GO:0022904~respiratory electron transport chain	4	2.23	0.029
GO:0043933~macromolecular complex subunit organization	14	7.82	0.034
GO:0042127~regulation of cell proliferation	15	8.38	0.035

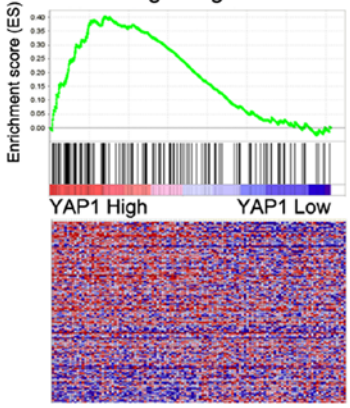
Table II. Continued.

B, Class B			
Term	Count	%	P-value
GO:0008285~negative regulation of cell proliferation	9	5.03	0.035
GO:0043065~positive regulation of apoptosis	10	5.59	0.036
GO:0007268~synaptic transmission	8	4.47	0.037
GO:0043068~positive regulation of programmed cell death	10	5.59	0.037
GO:0010942~positive regulation of cell death	10	5.59	0.038
GO:0050728~negative regulation of inflammatory response	3	1.68	0.041
GO:0044093~positive regulation of molecular function	12	6.70	0.043
GO:0006325~chromatin organization	9	5.03	0.044
GO:0050727~regulation of inflammatory response	4	2.23	0.045
Cellular component (CC)			
GO:0000786~nucleosome	7	3.91	0.000
GO:0070469~respiratory chain	7	3.91	0.000
GO:0032993~protein-DNA complex	7	3.91	0.000
GO:0005746~mitochondrial respiratory chain	6	3.35	0.000
GO:0044429~mitochondrial part	16	8.94	0.001
GO:0044455~mitochondrial membrane part	7	3.91	0.002
GO:0019866~organelle inner membrane	11	6.15	0.002
GO:0005739~mitochondrion	22	12.29	0.002
GO:0005740~mitochondrial envelope	12	6.70	0.003
GO:0005743~mitochondrial inner membrane	10	5.59	0.003
GO:0000785~chromatin	8	4.47	0.004
GO:0031966~mitochondrial membrane	11	6.15	0.006
GO:0009897~external side of plasma membrane	7	3.91	0.007
GO:0045271~respiratory chain complex I	4	2.23	0.008
GO:0005747~mitochondrial respiratory chain complex I	4	2.23	0.008
GO:0030964~NADH dehydrogenase complex	4	2.23	0.008
GO:0031967~organelle envelope	14	7.82	0.009
GO:0031975~envelope	14	7.82	0.009
GO:0009986~cell surface	9	5.03	0.023
GO:0031090~organelle membrane	19	10.61	0.023
GO:0044427~chromosomal part	9	5.03	0.039
Molecular function (MF)			
GO:0003954~NADH dehydrogenase activity	4	2.23	0.010
GO:0008137~NADH dehydrogenase (ubiquinone) activity	4	2.23	0.010
GO:0050136~NADH dehydrogenase (quinone) activity	4	2.23	0.010
GO:0005267~potassium channel activity	6	3.35	0.013
GO:0016655~oxidoreductase activity, acting on NADH or NADPH, quinone or similar compound as acceptor	4	2.23	0.015
GO:0047485~protein N-terminus binding	4	2.23	0.043
GO:0030955~potassium ion binding	5	2.79	0.047

survival in samples with mRNA expression that was either 2-fold upregulated or downregulated (Fig. 7). YAP1 mRNA expression was not significantly associated with patient prognosis in OSC (Fig. 7A). To determine whether YAP and pYAP distribution are associated with overall patient survival in OSC, YAP and pYAP expression levels were categorized as high, intermediate and low, since neither YAP nor pYAP alone were associated with OSC prognosis. Among 9 categories studied, the category of high YAP and low pYAP showed the poorest prognosis (Fig. 7B). The category of high YAP and low pYAP showed significantly poorer prognosis than did the category of high YAP and high pYAP and the category of intermediate YAP and intermediate pYAP (Fig. 7C and D).

A

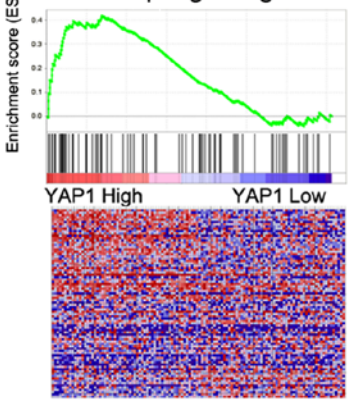
Wnt signaling



Normal p-value	0.019
FDR q-value	0.34
FWER p-value	0.67

Gene symbol	Rank in gene list	Rank metric score	Running ES
DKK4	3540	0.146	0.403
PPP2R1A	3453	0.150	0.402
RHOA	3427	0.151	0.397
NKD1	3387	0.153	0.392
WNT71	2821	0.178	0.391
FRAT2	2720	0.182	0.389
TP53	2607	0.187	0.388
TBL1XR1	3362	0.154	0.387
DVL1	3331	0.155	0.382
WNT10A	2595	0.188	0.380

TGF-β signaling

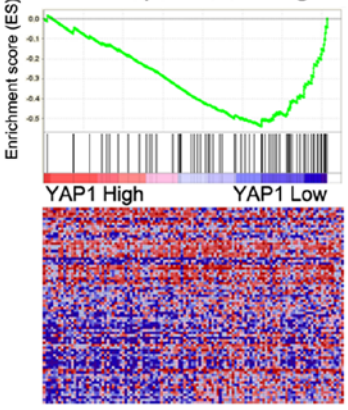


Normal p-value	0.028
FDR q-value	0.29
FWER p-value	0.80

Gene symbol	Rank in gene list	Rank metric score	Running ES
PPP2R1A	3453	0.150	0.421
RHOA	3427	0.151	0.412
BMP4	3383	0.153	0.405
ID4	3255	0.158	0.402
PPP2R1B	1650	0.241	0.398
SMAD2	3240	0.158	0.392
SMAD3	2025	0.218	0.391
ROCK1	2500	0.192	0.390
TGFBR2	3176	0.162	0.385
SMAD4	1609	0.244	0.385

B

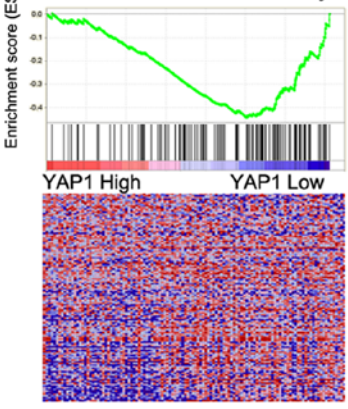
Hematopoietic cell linage



Normal p-value	0.028
FDR q-value	0.38
FWER p-value	0.50

Gene symbol	Rank in gene list	Rank metric score	Running ES
GP1BA	13684	-0.146	-0.532
TPO	13699	-0.146	-0.522
ITGA3	13700	-0.146	-0.512
IL1A	14188	-0.163	-0.506
GP5	13728	-0.147	-0.503
IL11	13916	-0.154	-0.502
IL4	14303	-0.168	-0.501
CD1D	14428	-0.171	-0.495
ITGA5	14449	-0.173	-0.484
CD1C	14556	-0.176	-0.478

Natural killer cell mediated cytotoxicity



Normal p-value	0.042
FDR q-value	0.46
FWER p-value	0.80

Gene symbol	Rank in gene list	Rank metric score	Running ES
SHC1	12573	-0.112	-0.437
IFNA5	12630	-0.113	-0.434
HLA-E	12945	-0.122	-0.433
ICAM1	12700	-0.115	-0.432
BID	13168	-0.130	-0.432
ULBP1	12783	-0.118	-0.430
PIK3R5	13224	-0.131	-0.428
FAS	12956	-0.123	-0.427
PRF1	13309	-0.133	-0.425
RAET1G	13412	-0.137	-0.424

Figure 6. (A) GSEA analysis of Class A and B. WNT and TGF-β signaling were significantly enriched in Class A. (B) Hematopoietic cell lineage pathway and natural killer mediated cytotoxicity pathway were significantly enriched in Class B.

Table III. Gene set enrichment analysis (GSEA) of Class A and Class B.

Name	Size	ES	NES	NOM p-val
A, Class A				
KEGG_TIGHT_JUNCTION	125	0.38	1.63	0.004
KEGG_ENDOMETRIAL_CANCER	52	0.49	1.67	0.014
KEGG_WNT_SIGNALING_PATHWAY	147	0.40	1.63	0.019
KEGG_SELENOAMINO_ACID_METABOLISM	23	0.55	1.62	0.025
KEGG_LYSINE_DEGRADATION	43	0.49	1.64	0.025
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	41	0.54	1.60	0.026
KEGG_TGF_BETA_SIGNALING_PATHWAY	82	0.42	1.57	0.028
KEGG_ADHERENS_JUNCTION	73	0.46	1.62	0.032
KEGG_BASAL_CELL_CARCINOMA	55	0.51	1.69	0.036
KEGG_PROSTATE_CANCER	87	0.37	1.48	0.049
B, Class B				
KEGG_ARACHIDONIC_ACID_METABOLISM	51	-0.43	-1.58	0.010
KEGG_PRIMARY_IMMUNODEFICIENCY	34	-0.61	-1.73	0.026
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	114	-0.61	-1.86	0.027
KEGG_HEMATOPOIETIC_CELL_LINEAGE	79	-0.54	-1.71	0.029
KEGG_ALPHA_LINOLENIC_ACID_METABOLISM	17	-0.54	-1.53	0.034
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	43	-0.51	-1.60	0.038
KEGG_REGULATION_OF_AUTOPHAGY	32	-0.44	-1.51	0.039
KEGG_AUTOIMMUNE_THYROID_DISEASE	47	-0.54	-1.62	0.042
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	128	-0.44	-1.57	0.043

ES, enrichment score; NES, normalized enrichment score; NOM p-val, norminal p-value.

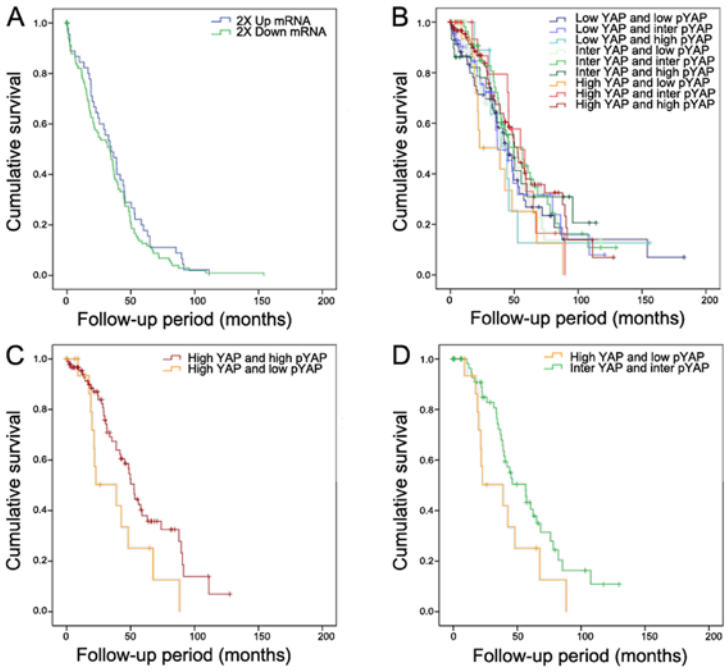


Figure 7. Survival analysis. High YAP and low pYAP protein expression were correlated with poor prognosis. Kaplan-Meier analysis of the association between YAP mRNA and protein expression, and overall survival. (A) Kaplan-Meier curves were used to plot overall survival with mRNA expression. YAP and pYAP expression levels were categorized as high, intermediate and low. (B) Among 9 categories, the category of high YAP and low pYAP showed the poorest prognosis. (C) P=0.042. (D) P=0.065. P-value was determined by log-rank tests.

Discussion

In the present study, alterations in the YAP1 gene in cases of OSC were found to be higher than that in various other cancer types. YAP1 mRNA expression was significantly higher in OSC compared with normal ovarian samples, and was higher in stages III and IV than in stages I and II. YAP1 protein, which mainly localized to the nucleus, was also expressed more highly in stage IV than in stages I ,II and III. However, the protein level of pYAP1, which is localized to the cytoplasm, was not significantly different between stages. The ratio of pYAP/YAP, which indicates higher activity at a low ratio, was lower in stage III than in stages I and II. When considering OSC subtypes, YAP1 mRNA and protein expression in the proliferative subtype was significantly higher than that in the differentiated, immunoreactive and mesenchymal subtypes. However, there was no significant difference in YAP1 mRNA or protein expression between the integrated mesenchymal and the integrated epithelial subtypes. In bioinformatic analysis, YAP1 was mainly correlated with the cell cycle. TGF-β and WNT signaling were significantly increased in the high-YAP1 class as assessed by gene set enrichment analysis. Finally, high-YAP and low-pYAP were associated with poor overall survival in cases of OSC.

Elevated YAP1 expression and nuclear localization have been observed in multiple types of human cancers, including liver, colon, lung and prostate cancer (6-8,27). In hepatocellular carcinoma, YAP1 was found to be an independent prognostic marker for overall and disease-free survival (13). In epithelial ovarian cancer, subcellular levels of YAP1 showed an exceptionally strong association with poor prognosis; high levels of nuclear YAP or low levels of cytoplasmic phosphorylated YAP1 were associated with poor prognosis (28). Patients with both high levels of nuclear YAP and low levels of phosphorylated YAP had an ~50% lower 5-year survival rate, and this combination served as an independent prognostic marker for survival (28). In accordance with previous findings, we showed that high YAP and low pYAP were associated with a poor prognosis. High YAP1 expression and its subcellular distribution may be related to poor overall survival in OSC. This finding should be confirmed in further studies.

The Cancer Genome Atlas Research Network separates OSC into 4 subtypes (immunoreactive, differentiated, proliferative and mesenchymal) based on mRNA analysis (24). Yang *et al* found that the integrated epithelial and mesenchymal subtypes were associated with poor overall survival based on miRNA analysis of OSC patients (23). In the present study, we revealed that YAP1 mRNA and protein expression in the proliferative subtype was significantly higher than that in the differentiated, immunoreactive and mesenchymal subtypes. However, there was no significant difference in YAP1 mRNA and protein expression between the integrated mesenchymal subtype and the integrated epithelial subtype. Molecular subgroups of ovarian cancer have been poorly examined and need to be further elucidated.

To verify the involvement of YAP1 in OSC, we performed bioinformatic analysis. This analysis revealed that cell cycle- and protein localization-related genes were highly correlated with YAP1 in 563 OSC patient samples (Fig. 4A). In addition, ClassNeighbors analysis classified YAP1-expressing OSC into Class A, which expresses genes associated with DNA recombination, cell cycle and RNA binding (Fig. 5B) and Class B, which expresses genes associated with nucleosome assembly, the respiratory chain, and NADH dehydrogenase activity (Fig. 5C). Class A genes enhance cell cycle-related functions, while Class B genes enhance nucleosome and oxidative phosphorylation pathways. GSEA was performed to investigate significantly enriched pathways that differed between Classes A and B. In Class A, pathways involving tight junctions, WNT and TGF-β signaling, and adherens junctions were more active than they were in Class B. In Class B, pathways involving primary immunodeficiency, systematic lupus erythematosus, intestinal immune network for IgA production, regulation of autophagy, and natural killer cell-mediated cytotoxicity were enriched (Table III). In Class A, WNT signaling (25) and TGF-β signaling (26) were related to cancer progression.

In conclusion, we investigated alterations in YAP1 gene expression in OSC, which was higher than that in 20 other types of cancers. mRNA expression and protein levels of YAP1 were significantly higher in advanced-stage OSC. High YAP and low pYAP were significantly correlated with poor prognosis in OSC. High YAP expression level and also its subcellular distribution may be associated with overall patient survival in OSC.

Acknowledgements

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