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.

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 χ² 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 inactive 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 (log2) to 9.78 (log2), 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
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.

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/).

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
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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

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

| Feature               | mRNA YAP expression | YAP protein expression | Phosphorylated YAP protein expression |
|-----------------------|---------------------|------------------------|--------------------------------------|
|                       | 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 adenocarcinoma. 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.
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, systemic lupus erythematosus, the intestinal immune network for IgA production, regulation of autophagy, auto-immune 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:00007049—cell cycle                                               | 14    | 7.57 | 0.006   |
| GO:00007049—cell cycle                                               | 17    | 9.19 | 0.007   |
| GO:0004265—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     | 5     | 2.70 | 0.023   |
| signaling pathway                                                    |       |      |         |
| 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         | 2     | 1.08 | 0.031   |
| in differentiation                                                    |       |      |         |
| 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:000228—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)

| Term                                                      | Count | %    | P-value |
|-----------------------------------------------------------|-------|------|---------|
| 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

| Biological process (BP)                                   | Count | %    | P-value |
|-----------------------------------------------------------|-------|------|---------|
| 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   |
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).

Table II. Continued.

| B, Class B | 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:0007855-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 |
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.

A, Class A

| Name                                      | Size | ES   | NES   | NOM p-val |
|-------------------------------------------|------|------|-------|-----------|
| 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

| Name                                      | Size | ES   | NES   | NOM p-val |
|-------------------------------------------|------|------|-------|-----------|
| 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, normal 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 YAPI mRNA or protein expression between the integrated mesenchymal and the integrated epithelial subtypes. In bioinformatic analysis, YAPI was mainly correlated with the cell cycle. TGF-β and WNT signaling were significantly increased in the high-YAPI 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 YAPI 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, YAPI was found to be an independent prognostic marker for overall and disease-free survival (13). In epithelial ovarian cancer, subcellular levels of YAPI showed an exceptionally strong association with poor prognosis; high levels of nuclear YAP or low levels of cytoplasmic phosphorylated YAPI 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 YAPI expression and its subcellular distribution may be related to poor overall survival in OSC. This finding should be confirmed in further studies.

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.

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