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YAP modifies cancer cell sensitivity to EGFR and survivin inhibitors and is negatively regulated by the non-receptor type protein tyrosine phosphatase 14

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SUMMARY

The Yes-associated protein (YAP) is a transcriptional factor involved in tissue development and tumorigenesis. Although YAP has been recognized as a key element of the Hippo signaling pathway, the mechanisms that regulate YAP activities remain to be fully characterized. In this study, we demonstrate that the non-receptor type protein tyrosine phosphatase 14 (PTPN14) functions as a negative regulator of YAP. We show that YAP forms a protein complex with PTPN14 through the WW domains of YAP and the PPXY motifs of PTPN14. In addition, PTPN14 inhibits YAP-mediated transcriptional activities. Knockdown of YAP sensitizes cancer cells to various anti-cancer agents, such as cisplatin, the EGFR tyrosine kinase inhibitor erlotinib, and the small-molecule antagonist of survivin, S12. YAP-targeted modalities may be used in combination with other cancer drugs to achieve maximal therapeutic effects.

Keywords

ovarian cancer; YAP; WW domain; PTPN14; EGFR; survivin; targeted therapy; erlotinib

Introduction

Our efforts have been in defining features of the malignant phenotype of erbB transformed cells [1–2]. Contact inhibition, a feature of normal cell growth, can be influenced by heteromeric associations of EGFR ectodomain forms, but the precise mechanisms involved in this phenotypic modulation were unclear. The erbB family of receptors, in particular, can

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be influenced by amphiregulin expression induced by YAP mediated transcriptional signals [3]. YAP expression can be enhanced by EGFR-mediated signals [4].

The Hippo-YAP pathway was initially characterized in *Drosophila* melanogaster as a mechanism that controls tissue growth and organ size, and its core signaling components are evolutionally conserved in mammals [5]. Several recent studies have revealed a role for this pathway in regulating cell contact inhibition, organ size control, and cancer development in mammals [6–8]. YAP, also known as Yes-associated protein 1, is a component of nuclear transcriptional complexes [9]. As a transcription factor, YAP mediates the expression of many growth-promoting or anti-apoptotic genes, including connective tissue growth factor (CTGF), cysteine-rich angiogenic inducer 61 (CYR61), cyclin E, E2F1, myc and survivin [7, 10–13].

An accumulating body of evidence indicates that YAP promotes malignant transformation in mammalian cells. For example, overexpression of YAP or its paralog, TAZ, causes epithelial-mesenchymal transition (EMT), growth factor-independent proliferation, and anchorage-independent growth [14–15]. Overexpression of YAP/TAZ also causes loss of contact inhibition [6, 15]. Gene amplification at the YAP locus is associated with breast and liver cancers [14, 16]. Indeed, overexpression of YAP strongly correlates with the neoplastic phenotype of a variety of human solid tumors and, in particular, contributes to the development of ovarian cancer and liver cancer [17–20]. Activation of YAP has been observed in greater than 60 percent of non-small cell lung cancer cases [21]. In addition, TAZ is overexpressed in NSCLC cell lines and is required for cancer cell proliferation [22]. Finally, YAP mediates hedgehog-driven neural precursor proliferation and promotes radioresistance and genomic instability in medulloblastoma[23–24].

The transcriptional activity of YAP is subject to negative regulation by cytoplasmic sequestration or ubiquitin-mediated degradation. When YAP is phosphorylated at S127 - a process that is affected by cell density – it forms a more stable complex with the 14-3-3 proteins and becomes retained in the cytoplasm [6, 25–26]. Phosphorylation of YAP at S381 by Lats1/2 primes the protein for subsequent phosphorylation at multiple sites, which then leads to polyubiquitination and degradation [27]. In contrast, sumoylation of YAP can stabilize the protein [28]. YAP activity can be also inhibited through the interactions with angiomotin (AMOT) family proteins, which lead to localization and sequestration of the YAP protein to tight junction [29–31].

The non-receptor protein tyrosine phosphatase type 14 (PTPN14) is located at the adheren junctions (AJ) in both endothelial and epithelial cells and plays a role in regulation of cell adhesion and cell growth [32–35]. PTPN14 can also be localized in the nucleus [35], suggesting that it may have nuclear targets and functions. PTPN14 can mediate the process of EMT by promoting TGF-β signaling [36]. Down regulation of PTPN14 is associated with an increase of metastatic potential in liver cancer [37]. Moreover, loss-of-function mutations of PTPN14 were discovered in clinical samples of colorectal cancers [38–39]. Although PTPN14 has been implicated as a downstream effector of Akt [40], the signaling pathways regulated by this tyrosine phosphatase have not been well characterized. In this study we show that PTPN14 binds to YAP and act as a negative regulator of YAP-mediated transcriptional activity. The

structural features involved in PTPN14-YAP interaction have been biochemically defined by mutagenesis. We also examined the role of YAP and PTPN14 in modifying cancer cell sensitivity to a variety of therapeutic agents.

Results

Identification of PTPN14 as a YAP-interacting protein

In an effort to elucidate the mechanism involved in the regulation of YAP, we performed immunoprecipitation (IP) and mass spectrometry analysis to identify the proteins that form a complex with YAP. Both NIH3T3 and MCF10A cell lines expressing HA-tagged YAP were established and used for IP. Our study isolated a number of previously reported YAP-binding partners - including the TEAD family proteins, 14-3-3 proteins, LATS1, the angiomotin proteins AMOT/AMOTL2, PATJ, LIN7C and PALS1- and several novel or not-well-studied YAP-associated proteins, including PTPN14 and MUPP1 (Table 1 and Table S1). In this report, we focus on PTPN14, a member of the non-receptor protein tyrosine phosphatase family characterized with an N-terminal FERM (4.1 protein-Ezrin-Radixin-Moesin) domain and a c-terminal phosphatase domain [41–42].

To confirm the interaction of YAP and PTPN14, HA-YAP and FLAG-PTPN14 were ectopically expressed in 293T cells and the cell lysate was subject to IP using anti-FLAG antibody (Figure 1). HA-YAP was found co-immunoprepiciptated with FLAG-PTPN14 (Figure 1A). The reciprocal co-IP study also confirmed that PTPN14 is associated with YAP (Figure 1B). Similarly, we showed that the YAP homologous protein TAZ can also interact with PTPN14 (Figure 1C).

We examined the expression patterns of YAP/TAZ and PTPN14 in ovarian cancer cell lines by Western blot analysis. YAP is expressed in all of the ovarian cancer cell lines we have tested, whereas PTPN14 can be detected in all but OV2008 cells (Figure 1D). TAZ is expressed in all the ovarian cancer cells we tested except in ES2 and OV2008 cells.

To detect the interactions between the endogenous YAP and PTPN14 proteins, we carried out IP using the ovarian cancer cell lines that express both YAP and PTPN14. Our results indicate that the endogenous PTPN14 protein can be co-immunoprecipitated with the antibody specific for the YAP protein (Fig 1E).

The WW domains of YAP interact with the PPXY motifs of PTPN14

We next sought to identify the structural features important for YAP-PTPN14 interaction. A variety of YAP and PTPN14 mutant forms were generated and used for the co-IP study (Figure 2B and 2D). Our results show that deletion of the WW domain of YAP abolishes the interaction with PTPN14, whereas other alterations of the YAP protein have no effect (Fig 2A). The WW domain is a motif of approximately 40 amino acid residues characterized by conserved tryptophan and proline residues (Rotin, 1998). The WW domains of YAP belong to a subfamily of these protein structures that recognize the proline-rich PPXY motifs found in various proteins [9, 43–46]. Our studies indicate that the region encompassing amino acid residues 456-878 of PTPN14 is required for binding to YAP and this region contains, of note, two PPXY motifs (Figure 2C).

To determine whether the PPXY motifs is required for YAP-PTPN14 interaction, we generated mutants of PTPN14, in which the first (PTPN14 A), the second (PTPN14 B) or both (PTPN14 AB) PPXY motifs were changed to “PPXA”. Our co-IP studies show that YAP-PTPN14 interaction was weakened by a single mutation and became abolished when both PPXY motifs were mutated (Figure 2E, F). In addition, each of the two WW domains of YAP can independently bind to the PPXY sequences with similar affinity (Fig 2G). Taken together, these studies demonstrate that YAP and PTPN14 interact through the WW domains of YAP and the PPXY motifs of PTPN14.

Inhibition of YAP-mediated transcription by PTPN14

To investigate whether PTPN14 affects YAP’s function as a transcription factor, we employed a luciferase reporter assay where YAP, in the presence of its co-transcription factor TEAD4, activates the transcription of the reporter gene (Figure 3A). Co-expression of PTPN14 reduced YAP and TAZ-mediated transcriptional activities (Figure 3A and 3D). The inhibitory effect of PTPN14 is dependent on the region that contains the PPXY motifs but not on the N terminal FERM domain or the C-terminal phosphatase domain (Figure 3B and 3D). In addition, PTPN14 fragment with PPXY domain is sufficient to inhibit YAP activities. Moreover, mutations of the two PPXY diminished the ability of PTPN14 to inhibit YAP-mediated transcription (Figure 3C). Our results support the notion that PTPN14 inhibits YAP/TAZ transcriptional activity through interactions mediated by the PPXY motifs.

Down regulation of YAP sensitizes ovarian cancer cell to various cancer therapeutic agents

We next explored the therapeutic potential in targeting YAP for the treatment of ovarian cancer. Stable knockdown of YAP were established in various ovarian cancer cell lines (Figure 4A). We found that ablation of YAP in ES-2 cells, which do not express TAZ (Figure 1), significantly reduced the capacity of this ovarian cancer cell line to form colonies in soft agar (Figure 4). We also investigated whether knockdown of YAP affects cancer cell invasion using the transwell assay, which measures the ability of cells to migrate and penetrate matrigel. Our results show that certain invasive properties of ES2 cells were lessened by down regulation of YAP expression (Figure 4).

We determined whether YAP modifies sensitivity to cancer therapeutic agents in ovarian cancer cells. Depletion of YAP in ES-2 cells significantly increased the cytotoxicity of cisplatin (Figure 5). We recently found that YAP is up regulated in EGFR-positive non-small cell lung cancer cells that acquired resistance to cetuximab, where knockdown of YAP re-sensitize the cells to inhibit proliferation by the anti-EGFR antibody (data not shown). In this regard, activation of YAP function may contribute to the resistant phenotype. We therefore examined whether knockdown of YAP can increase the activity of EGFR TKI inhibitor erlotinib in ovarian cancer cells. Our results indicate that a number of EGFR-positive ovarian cancer cell lines can be inhibited by erlotinib, which is further enhanced by knockdown of YAP (Figure 5).

We have developed the first small-molecule survivin inhibitor called S12 [47]. Consistent with the essential role of survivin in mitosis, S12 inhibited proliferation of all the ovarian cancer cell lines that we tested (Figure 6). Additionally, YAP depletion also increased sensitivity of ovarian cancer cell lines to S12 (Figure 6).

Because the PTPN14 fragment that contains the PPXY motifs is sufficient to bind to YAP and reduces its transcriptional activity, we tested whether ectopic expression of this region of PTPN14 can modify the efficacy of chemo therapeutic agents. Indeed, following over expression of the PPXY-containing PTPN14 fragment, the cells became more sensitive to erlotinib or S12 (Figure 7).

Discussion

We have demonstrated that PTPN14 serves as a novel YAP-binding protein. The YAP-PTPN14 interaction involves the WW domains of YAP and the PPXY motifs of PTPN14. Each of the two PPXY motifs can independently bind to the WW domains, of the long form of YAP, with similar affinity. In addition TAZ, which also has a WW domain, can also associate with PTPN14 through the PPXY motifs. These findings are consistent with a recent report by Webb et al., which indicates that the two WW domains of YAP and TAZ show similar structural features [48]. It should be noted that several proteins, including the RUNX proteins, p73, PML, SMAD1, AMOT and the c-terminal fragment of erbB4, interact with YAP through the WW domain [28–31, 49–53]. Our analysis of these YAP-binding regions showed little sequence similarities beyond the PPXY signatures.

Our results indicate that PTPN14 negatively regulates the transcriptional events mediated by YAP and TEAD4. This process is dependent on the two PPXY motifs, whereas the phosphatase domain appears to be dispensable. It has been established that phosphorylation of YAP at S127 is involved in inhibition of YAP by retaining it in the cytoplasm [6, 25–26]. We found no evidence that overexpression of PTPN14 affect the subcellular distribution of YAP immunofluorescence studies (data not shown). In addition, phosphorylation of S127 was not affected by overexpression or knockdown of PTPN14 (data not shown). Thus, it is unlikely that PTPN14 inhibits YAP function by affecting its localization within the cell. Indeed, YAP and PTPN14 can be localized to both the nucleus and the cytoplasm.

The YAP-TEAD interaction is mediated by the N-terminal region of YAP [10, 54] and this protein complex is critical for YAP-mediated cell proliferation [10, 55–56]. Importantly, the WW domains of YAP are required for both the transcriptional and oncogenic activities of the YAP-TEAD protein complex [55]. It has been proposed that these YAP activities require yet-to-be-identified proteins that bind to the WW domains of YAP. It is conceivable that PTPN14 may modulate YAP function by competing for the binding sites in the WW domains. Alternatively, it is possible that the presence of PTPN14 in the YAP containing protein complex may exert a negative effect on the transcriptional activities of the protein ensemble.

Poernbacher et al. recently reported that the drosophila PTPN14 protein can inhibit Yorkie through binding to Kibra [57]. Kibra can interact with Merlin and Expanded and act as a

component of the Hippo pathway upstream of Yorkie [58–60]. The interaction of PTPN14 with Kibra also involves the WW domain of Kibra and the PPXY sequences of PTPN14 [57]. Combined with these observations, our results support the notion that PTPN14 regulates YAP function both directly and indirectly.

We noted that PTPN21 is highly homologous to PTPN14 and also contains the PPXY motifs. It is likely that PTPN21 can inhibit YAP/TAZ functions in a similar fashion. PTPN21 is implicated as a component of the endocytic machinery that modulates cell migration, EGFR stability, and growth and motility of cancer cells [61–62] and is perhaps involved in defining sensitivity to cisplatin [63].

The finding of overexpression of YAP in a variety of cancers, together with the emerging role of YAP in malignant transformation, implicate YAP itself as a potentially attractive therapeutic target. Previous studies using a number of ovarian cancer cell lines revealed that knockdown of YAP only caused a very modest effect on cancer cell growth or drug sensitivity [19–20]. We noted that the cells used in these studies have expression of both YAP and TAZ. We also observed limited benefit of YAP knockdown in ovarian cancer cells that have co-expression of YAP and TAZ, which indicate that TAZ may compensate for the loss of YAP functions. Indeed, TAZ is overexpressed in several NSCLC cell lines and knockdown of TAZ expression suppresses cancer cell proliferation [22]. TAZ levels are also increased in human breast cancer cells and contribute to resistance to taxol [11]. We found that knockdown of YAP led to a more pronounced inhibitory effect on anchorage-independent growth in ovarian cancer cell lines that express little or no TAZ. Thus, up regulation of either YAP or TAZ may be sufficient to promote or sustain the transformed phenotypes in cancer cells.

We found that ablation of YAP expression also significantly increases cancer cell sensitivity to EGFR TKI erlotinib. Our interest in the Hippo-YAP pathway emerged from our studies of contact inhibition mediated by forms of EGFR ectodomain that affect the malignant phenotype [2]. More recent studies have shown that YAP may modulate erbB signaling. For example, the EGFR ligand amphiregulin was identified as a transcription target for YAP [3]. In addition, YAP forms a complex with the cytoplasmic fragment of erbB4 and may affect gene transcription [50, 52]. These observations may partially explain the benefit of YAP knockdown on EGFR-targeted therapy.

We have recently developed the first small-molecule survivin inhibitor and showed that it inactivates survivin function by directly binding to a pseudo-allosteric interface [47]. While the survivin antagonist showed anti-proliferative and pro-apoptotic activities to a broad spectrum of cancer cells, some cells appear to be more resistant than others. Up regulation of YAP/TAZ and survivin is commonly seen in human cancers, especially ovarian and lung cancers [17, 19, 22]. That knockdown of YAP can enhance the efficacy of the survivin inhibitor suggests that targeting simultaneous targeting both of these two molecules may prevent resistance and achieve maximal therapeutic effect.

We show that a fragment of PTPN14 containing the PPXY motifs can bind to of both YAP and TAZ and act in a dominant-negative manner to inhibit their transcriptional activities.

Forced expression of this mutant form also sensitizes cancer cells to erlotinib or S12 treatment. It would be of interest to identify the minimal PPXY signature that is sufficient to bind the WW domains of YAP or TAZ with specificity. We propose that therapeutic agents may be developed based on the structural information of the PPXY-WW domain complex. These molecular entities are expected to have the advantage of simultaneously targeting YAP and TAZ, which appear to exhibit certain levels of functional redundancy.

In summary, our studies demonstrate that PTPN14 can directly act upon YAP/TAZ and negatively regulate its transcriptional functions. Novel therapeutic strategies targeting YAP in cancer may emerge by altering the protein interactions between them and suppressing the activities of YAP or its paralog TAZ.

Materials and Methods

Cell lines

NIH-3T3, MCF10A, U2OS, and 293T cells were purchased from ATCC. NIH-3T3, U2OS and 293T were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS). MCF10A, a non-transformed mammary epithelial cell line, was cultured in DMEM/F12 medium with 100 ng/ml cholera toxin (Sigma, St. Louis, MO), 20 ng/ml epidermal growth factor (Life Technologies-Invitrogen, Grand Island, NY), 1X ITS premix (BD Biosciences; insulin, transferrin, selenous acid), 500 ng/ml hydrocortisone (Sigma), and 5% horse serum. OVCAR3 and OV2008 cells were cultured in RPMI-160 medium with 20% FBS; the other ovarian cancer cell lines (ES-2, OVCAR5, SKOV3, TOV21G, 3A, CAOV3) were cultured in DMEM with 10% FBS. OVCAR3 and OV2008 cells stably expressing YAP specific shRNA were selected and cultured in RPMI-1640 medium supplemented with 1.5 μg/mL puromycin. YAP shRNA expressing 3A and ES2 cells were cultured in DMEM with 10 μg/mL puromycin.

Antibodies

The antibodies used in this study include: anti-HA (F7), anti-myc (9E10), anti-YAP (Santa Cruz Biotechnology, Santa Cruz, CA); anti-TAZ (Cell Signaling Technology, Danvers, MA); anti-FLAG, mouse monoclonal Anti-HA-agarose, and anti-FLAG (M2)-agarose (Sigma); anti-PTPN14 (R&D); anti-MUPP1 (BD Biosciences, San Jose, CA); and anti-LATS1 (Bethyl Laboratories, Montgomery, TX). HRP-conjugated secondary antibodies against mouse or rabbit were from GE Amersham (Piscataway, NJ). Alexa Fluor 488 conjugated anti-mouse antibody was purchased from Molecular Probes.

Plasmids

pBABE YAP1 was obtained from Addgene (Addgene plasmid 15682, deposited by Dr. Brugge; [14]. This plasmid encodes the YAP protein that has two WW domains with a total of 504 amino acids, previously referred to as YAP2. YAP was subcloned into pIRESpuro-2xHA and pIRESpuro-2xMyc expression vectors, and the pIRESpuro-RFP vectors, which were created by modifying pIRESpuro (Clontech). A series of HA-YAP mutant constructs with various mutations were created by PCR-based methods. The mutants include: delta N-term (deletion of amino acids 1-57), delta WW (del. a.a. 173-262), delta

PDZ-binding (carboxyl-terminal LTWL was altered to LAWA), delta C-half (del. a.a. 291-504), delta Coiled-coil (del. a.a. 304-353), delta TEAD-binding (del. a.a. 61-90), delta SH3-binding (del. a.a. 278-290), and S127A substitution [64]. They are schematically summarized in Figure 2(A). The human PTPN14 cDNA was obtained from Openbiosystems (Thermo Scientific, Lafayette, CO) and subcloned into pCDNA3.1 (Invitrogen), pEGFPorpLVX-puro (BD Biosciences). Gal4-TEAD4 was obtained from Addgene (Kunliang Guan). pG5*luc*, pTK-R*luc*, and pBIND plasmids were purchased from Promega (Madison, WI). The pG5*luc* contains five GAL4 binding sites upstream of the firefly luciferase gene. The *Renilla* luciferase reporter (pTK-R-Luc) was used as control.

Immunoprecipitation and Western blot

293T cells were transfected with wild-type or mutant YAP and PTPN14 expressing plasmid. Cells were lysed in RIPA buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Nonidet P-40, 1% Na deoxycholate, 50 mM NaF, 1 mM Na_3VO_4, and Protease Inhibitor Cocktail) 24 hrs after transfection, followed by immunoprecipitation with anti-HA or anti-FLAG antibody. The proteins associated with the antibodies were subjected to SDS-PAGE and immunoblotted with anti-HA or FLAG antibodies.

For Western blot, total protein lysate was prepared using RIPA buffer containing protease inhibitors and phosphatase inhibitors. Protein concentrations of the lysates were measured by DC protein assay reagents (Bio-Rad Laboratories), and the samples were normalized for protein concentration and mixed with the SDS-PAGE sample buffer (final concentration 62.5 mM Tris-HCl, pH 6.8, 5% 2-Mercaptoethanol, 2% SDS, 5% Sucrose, and 0.002% Bromophenol blue). After resolved by 4–20% Tris-HCl SDS-PAGE, the proteins were transferred onto nitrocellulose membrane (Millipore). Then membrane was blocked for 1h with 1% nonfat dried milk in phosphate buffered saline containing 0.05% Tween-20 (PBST).

Mass spectrometry

Cells were lysed in NP40 buffer with 20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1% Nonidet P-40, 10% Glycerol, 50 mM NaF, 1 mM Na_3VO_4, and Protease Inhibitor Cocktail (Roche). Lysates were pre-cleared by anti-FLAG M2 affinity agarose gel (Sigma) or protein G agarose (Invitrogen), and then subjected to IP using monoclonal Anti-HA-agarose antibody (clone HA-7). Precipitated samples were washed in PBS twice and the proteins were eluted using SDS-PAGE sample buffer without 2-mercaptoethanol (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 5% sucrose, and 0.002% bromophenol blue). The samples were boiled, beads were removed by centrifugation, and then 2-Mercaptoethanol was added to a final concentration of 5%. Electrophoresis in 12% SDS-PAGE gel was done for about 15 minutes until all proteins had migrated into the gel by about 1.5 cm. Then the gels were stained with SimplyBlueSafeStain reagents (Invitrogen), and stained areas were cut out and separated into small pieces.

The mass spectrometry analysis was provided by the Proteomics Core at the University of Pennsylvania, Proteins samples were in-gel digested with trypsin. Peptides were separated by on-line chromatography, followed by mass spectrometric analyses for protein

identification by nanoLC-MS/MS method. Specifically, LTQ mass spectrometer operated by Xcalibur was employed for peptide sequencing. The database search and protein identification were performed with Mascot Search using NCBI database. In order to compare two sets of samples obtained from transfectant cells and parental cells, Sequest and Scaffold search were also employed.

Cell proliferation and viability assay

Cells seeded in white opaque 96-well plates were treated with S12 orcisplatin in 10% FBS DMEM or with erlotinib in serum-free DMEM. After 48 hrs of incubation, CellTiter-Glo assay was performed according to manufacturer's instruction (BD BioScience, San Jose, CA). The viability value was determined by Veritas microplate luminometer (Promega, Madison, WI). Student's *t* test was used for statistical analysis.

Lentivirus production

The pLenti plasmid with genes of interest, pCMV delta R8.2, and pVSVG were co-transfection to 293T cells in 10 cm dish using Lipofectamine 2000 (Invitrogen). Twelve hours after transfection, the medium was changed to 2% FBS-DMEM. Two days after transfection, the conditioned medium was collected, filtered through 0.4 μ filter, and used for infection.

Soft agar assay

Cells (5,000) were plated in complete medium with 0.5% agar in 60 mm plates in triplicate. The medium was replaced every 3 days. After 21 days, the cells were stained with 0.5 mL of 1 mg/mL P-iodonitrotetrazolium violet (2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride for 2 hours. Colonies larger than 0.5 mm were counted. Student's *t* test was used for statistical analysis.

Cell transfection

Lipofectamine 2000 (Invitrogen) was used for transfection of 293T, NIH3T3 or MCF10A cells. For establishment of cells stably expressing HA-YAP, the transfected cells were selection with puromycin (Sigma) at 1 μg/ml for NIH3T3 and 0.5 μg/ml for MCF10A. Stable expression of HA-YAP was confirmed by Western blot using anti-HA antibody.

Luciferase reporter assay

To assess functional regulation of YAP as a transcription co-activator, we performed dual luciferase assay according to the manufacturer's protocol (Promega). Sub-confluent U2OS cells on 6-well plates were transfected with a combination of plasmids as indicated in each experiment. These include the luciferase reporter plasmid pG5*luc*, pTK-R*luc* (internal control), GAL4-TEAD4, YAP, or PTPN14 constructs. Twenty-four to forty-eight hours after transfection, the cell lysates were prepared with lysis buffer, and analyzed for luciferase activities using luminometer. The activity of firefly luciferase (pG5*luc*) was normalized to that of the internal control, pTK-renilla luciferase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Drebin JA, Stern DF, Link VC, Weinberg RA, Greene MI. Monoclonal antibodies identify a cell-surface antigen associated with an activated cellular oncogene. Nature. 1984 Dec 6–12; 312(5994): 545–8. [PubMed: 6504162]
2. Yoneda T, Kumagai T, Nagatomo I, Furukawa M, Yamane H, Hoshino S, et al. The extracellular domain of p185(c-neu) induces density-dependent inhibition of cell growth in malignant mesothelioma cells and reduces growth of mesothelioma in vivo. DNA Cell Biol. 2006 Sep; 25(9): 530–40. [PubMed: 16989576]
3. Zhang J, Ji JY, Yu M, Overholtzer M, Smolen GA, Wang R, et al. YAP-dependent induction of amphiregulin identifies a non-cell-autonomous component of the Hippo pathway. Nat Cell Biol. 2009 Dec; 11(12):1444–50. [PubMed: 19935651]
4. Urtasun R, Latasa MU, Demartis MI, Balzani S, Goni S, Garcia-Irigoyen O, et al. Connective tissue growth factor autocriny in human hepatocellular carcinoma: oncogenic role and regulation by epidermal growth factor receptor/yes-associated protein-mediated activation. Hepatology. 2011 Dec; 54(6):2149–58. [PubMed: 21800344]
5. Zhao B, Li L, Lei Q, Guan KL. The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. Genes Dev. 2010 May; 24(9):862–74. [PubMed: 20439427]
6. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev. 2007 Nov 1; 21(21):2747–61. [PubMed: 17974916]
7. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, et al. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell. 2007 Sep 21; 130(6):1120–33. [PubMed: 17889654]
8. Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, et al. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol. 2007 Dec 4; 17(23):2054–60. [PubMed: 17980593]
9. Sudol M, Bork P, Einbond A, Kastury K, Druck T, Negrini M, et al. Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain. J Biol Chem. 1995 Jun 16; 270(24):14733–41. [PubMed: 7782338]
10. Zhao B, Ye X, Yu J, Li L, Li W, Li S, et al. TEAD mediates YAP-dependent gene induction and growth control. Genes Dev. 2008 Jul 15; 22(14):1962–71. [PubMed: 18579750]
11. Lai D, Ho KC, Hao Y, Yang X. Taxol resistance in breast cancer cells is mediated by the hippo pathway component TAZ and its downstream transcriptional targets Cyr61 and CTGF. Cancer Res. 2011 Apr 1; 71(7):2728–38. [PubMed: 21349946]
12. Tapon N, Harvey KF, Bell DW, Wahrer DC, Schiripo TA, Haber DA, et al. salvador Promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines. Cell. 2002 Aug 23; 110(4):467–78. [PubMed: 12202036]

13. Goulev Y, Fauny JD, Gonzalez-Marti B, Flagiello D, Silber J, Zider A. SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in Drosophila. Curr Biol. 2008 Mar 25; 18(6):435–41. [PubMed: 18313299]
14. Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, et al. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A. 2006 Aug 15; 103(33):12405–10. [PubMed: 16894141]
15. Lei QY, Zhang H, Zhao B, Zha ZY, Bai F, Pei XH, et al. TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. Mol Cell Biol. 2008 Apr; 28(7):2426–36. [PubMed: 18227151]
16. Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, et al. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell. 2006 Jun 30; 125(7):1253–67. [PubMed: 16814713]
17. Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, et al. Expression of Yes-associated protein in common solid tumors. Hum Pathol. 2008 Nov; 39(11):1582–9. [PubMed: 18703216]
18. Lee KP, Lee JH, Kim TS, Kim TH, Park HD, Byun JS, et al. The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci U S A. 2010 May 4; 107(18):8248–53. [PubMed: 20404163]
19. Zhang X, George J, Deb S, Degoutin JL, Takano EA, Fox SB, et al. The Hippo pathway transcriptional co-activator, YAP, is an ovarian cancer oncogene. Oncogene. 2011 Jun 23; 30(25): 2810–22. [PubMed: 21317925]
20. Hall CA, Wang R, Miao J, Oliva E, Shen X, Wheeler T, et al. Hippo pathway effector Yap is an ovarian cancer oncogene. Cancer Res. 2010 Nov 1; 70(21):8517–25. [PubMed: 20947521]
21. Wang Y, Dong Q, Zhang Q, Li Z, Wang E, Qiu X. Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer. Cancer Sci. 2010 May; 101(5):1279–85. [PubMed: 20219076]
22. Zhou Z, Hao Y, Liu N, Raptis L, Tsao MS, Yang X. TAZ is a novel oncogene in non-small cell lung cancer. Oncogene. 2011 May 5; 30(18):2181–6. [PubMed: 21258416]
23. Fernandez LA, Squatrito M, Northcott P, Awan A, Holland EC, Taylor MD, et al. Oncogenic YAP promotes radioresistance and genomic instability in medulloblastoma through IGF2-mediated Akt activation. Oncogene. 2011 Aug 29.
24. Fernandez LA, Northcott PA, Dalton J, Fraga C, Ellison D, Angers S, et al. YAP1 is amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic hedgehog-driven neural precursor proliferation. Genes Dev. 2009 Dec 1; 23(23):2729–41. [PubMed: 19952108]
25. Hao Y, Chun A, Cheung K, Rashidi B, Yang X. Tumor suppressor LATS1 is a negative regulator of oncogene YAP. J Biol Chem. 2008 Feb 29; 283(9):5496–509. [PubMed: 18158288]
26. Basu S, Totty NF, Irwin MS, Sudol M, Downward J. Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. Mol Cell. 2003 Jan; 11(1):11–23. [PubMed: 12535517]
27. Zhao B, Li L, Tumaneng K, Wang CY, Guan KL. A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF (beta-TRCP). Genes Dev. 2010 Jan 1; 24(1):72–85. [PubMed: 20048001]
28. Lapi E, Di Agostino S, Donzelli S, Gal H, Domany E, Rechavi G, et al. PML, YAP, and p73 are components of a proapoptotic autoregulatory feedback loop. Mol Cell. 2008 Dec 26; 32(6):803–14. [PubMed: 19111660]
29. Wang W, Huang J, Chen J. Angiomotin-like proteins associate with and negatively regulate YAP1. J Biol Chem. 2011 Feb 11; 286(6):4364–70. [PubMed: 21187284]
30. Zhao B, Li L, Lu Q, Wang LH, Liu CY, Lei Q, et al. Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein. Genes Dev. 2011 Jan 1; 25(1):51–63. [PubMed: 21205866]
31. Chan SW, Lim CJ, Chong YF, Pobbati AV, Huang C, Hong W. Hippo pathway-independent restriction of TAZ and YAP by angiomotin. J Biol Chem. 2011 Mar 4; 286(9):7018–26. [PubMed: 21224387]

32. Ogata M, Takada T, Mori Y, Uchida Y, Miki T, Okuyama A, et al. Regulation of phosphorylation level and distribution of PTP36, a putative protein tyrosine phosphatase, by cell-substrate adhesion. J Biol Chem. 1999 Jul 16; 274(29):20717–24. [PubMed: 10400706]
33. Ogata M, Takada T, Mori Y, Oh-hora M, Uchida Y, Kosugi A, et al. Effects of overexpression of PTP36, a putative protein tyrosine phosphatase, on cell adhesion, cell growth, and cytoskeletons in HeLa cells. J Biol Chem. 1999 Apr 30; 274(18):12905–9. [PubMed: 10212280]
34. Wadham C, Gamble JR, Vadas MA, Khew-Goodall Y. The protein tyrosine phosphatase Pez is a major phosphatase of adherens junctions and dephosphorylates beta-catenin. Mol Biol Cell. 2003 Jun; 14(6):2520–9. [PubMed: 12808048]
35. Wadham C, Gamble JR, Vadas MA, Khew-Goodall Y. Translocation of protein tyrosine phosphatase Pez/PTPD2/PTP36 to the nucleus is associated with induction of cell proliferation. J Cell Sci. 2000 Sep; 113(Pt 17):3117–23. [PubMed: 10934049]
36. Wyatt L, Wadham C, Crocker LA, Lardelli M, Khew-Goodall Y. The protein tyrosine phosphatase Pez regulates TGFbeta, epithelial-mesenchymal transition, and organ development. J Cell Biol. 2007 Sep 24; 178(7):1223–35. [PubMed: 17893246]
37. Niedergethmann M, Alves F, Neff JK, Heidrich B, Aramin N, Li L, et al. Gene expression profiling of liver metastases and tumour invasion in pancreatic cancer using an orthotopic SCID mouse model. Br J Cancer. 2007 Nov 19; 97(10):1432–40. [PubMed: 17940512]
38. Laczmanska I, Sasiadek MM. Tyrosine phosphatases as a superfamily of tumor suppressors in colorectal cancer. Acta Biochim Pol. 2011; 58(4):467–70. [PubMed: 22146137]
39. Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, et al. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science. 2004 May 21; 304(5674):1164–6. [PubMed: 15155950]
40. Andersen JN, Sathyanarayanan S, Di Bacco A, Chi A, Zhang T, Chen AH, et al. Pathway-based identification of biomarkers for targeted therapeutics: personalized oncology with PI3K pathway inhibitors. Sci Transl Med. 2010 Aug 4; 2(43):43–55.
41. Barr AJ, Debreczeni JE, Eswaran J, Knapp S. Crystal structure of human protein tyrosine phosphatase 14 (PTPN14) at 1.65-A resolution. Proteins. 2006 Jun 1; 63(4):1132–6. [PubMed: 16534812]
42. Smith AL, Mitchell PJ, Shipley J, Gusterson BA, Rogers MV, Crompton MR. Pez: a novel human cDNA encoding protein tyrosine phosphatase- and ezrin-like domains. Biochem Biophys Res Commun. 1995 Apr 26; 209(3):959–65. [PubMed: 7733990]
43. Chen HI, Einbond A, Kwak SJ, Linn H, Koepf E, Peterson S, et al. Characterization of the WW domain of human yes-associated protein and its polyproline-containing ligands. J Biol Chem. 1997 Jul 4; 272(27):17070–7. [PubMed: 9202023]
44. Sudol M, Chen HI, Bougeret C, Einbond A, Bork P. Characterization of a novel protein-binding module--the WW domain. FEBS Lett. 1995 Aug 1; 369(1):67–71. [PubMed: 7641887]
45. Chen HI, Sudol M. The WW domain of Yes-associated protein binds a proline-rich ligand that differs from the consensus established for Src homology 3-binding modules. Proc Natl Acad Sci U S A. 1995 Aug 15; 92(17):7819–23. [PubMed: 7644498]
46. Sudol M, Sliwa K, Russo T. Functions of WW domains in the nucleus. FEBS Lett. 2001 Feb 16; 490(3):190–5. [PubMed: 11223034]
47. Berezov A, Cai Z, Freudenberg JA, Zhang H, Cheng X, Thompson T, et al. Disabling the mitotic spindle and tumor growth by targeting a cavity-induced allosteric site of survivin. Oncogene. 2012 Apr 12; 31(15):1938–48. [PubMed: 21892210]
48. Webb C, Upadhyay A, Giuntini F, Eggleston I, Furutani-Seiki M, Ishima R, et al. Structural features and ligand binding properties of tandem WW domains from YAP and TAZ, nuclear effectors of the Hippo pathway. Biochemistry. 2011 Apr 26; 50(16):3300–9. [PubMed: 21417403]
49. Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, et al. Physical interaction with Yes-associated protein enhances p73 transcriptional activity. J Biol Chem. 2001 May 4; 276(18): 15164–73. [PubMed: 11278685]
50. Komuro A, Nagai M, Navin NE, Sudol M. WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. J Biol Chem. 2003 Aug 29; 278(35):33334–41. [PubMed: 12807903]

51. Yagi R, Chen LF, Shigesada K, Murakami Y, Ito Y. A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator. EMBO J. 1999 May 4; 18(9):2551–62. [PubMed: 10228168]
52. Omerovic J, Puggioni EM, Napoletano S, Visco V, Fraioli R, Frati L, et al. Ligand-regulated association of ErbB-4 to the transcriptional co-activator YAP65 controls transcription at the nuclear level. Exp Cell Res. 2004 Apr 1; 294(2):469–79. [PubMed: 15023535]
53. Alarcon C, Zaromytidou AI, Xi Q, Gao S, Yu J, Fujisawa S, et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. Cell. 2009 Nov 13; 139(4):757–69. [PubMed: 19914168]
54. Vassilev A, Kaneko KJ, Shu H, Zhao Y, DePamphilis ML. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes Dev. 2001 May 15; 15(10):1229–41. [PubMed: 11358867]
55. Zhao B, Kim J, Ye X, Lai ZC, Guan KL. Both TEAD-binding and WW domains are required for the growth stimulation and oncogenic transformation activity of yes-associated protein. Cancer Res. 2009 Feb 1; 69(3):1089–98. [PubMed: 19141641]
56. Chan SW, Lim CJ, Loo LS, Chong YF, Huang C, Hong W. TEADs mediate nuclear retention of TAZ to promote oncogenic transformation. J Biol Chem. 2009 May 22; 284(21):14347–58. [PubMed: 19324876]
57. Poernbacher I, Baumgartner R, Marada SK, Edwards K, Stocker H. Drosophila Pez Acts in Hippo Signaling to Restrict Intestinal Stem Cell Proliferation. Curr Biol. 2012 Feb 1.
58. Baumgartner R, Poernbacher I, Buser N, Hafen E, Stocker H. The WW domain protein Kibra acts upstream of Hippo in Drosophila. Dev Cell. 2010 Feb 16; 18(2):309–16. [PubMed: 20159600]
59. Genevet A, Wehr MC, Brain R, Thompson BJ, Tapon N. Kibra is a regulator of the Salvador/Warts/Hippo signaling network. Dev Cell. 2010 Feb 16; 18(2):300–8. [PubMed: 20159599]
60. Yu J, Zheng Y, Dong J, Klusza S, Deng WM, Pan D. Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. Dev Cell. 2010 Feb 16; 18(2):288–99. [PubMed: 20159598]
61. Carlucci A, Porpora M, Garbi C, Galgani M, Santoriello M, Mascolo M, et al. PTPD1 supports receptor stability and mitogenic signaling in bladder cancer cells. J Biol Chem. 2010 Dec 10; 285(50):39260–70. [PubMed: 20923765]
62. Carlucci A, Gedressi C, Lignitto L, Nezi L, Villa-Moruzzi E, Avvedimento EV, et al. Protein-tyrosine phosphatase PTPD1 regulates focal adhesion kinase autophosphorylation and cell migration. J Biol Chem. 2008 Apr 18; 283(16):10919–29. [PubMed: 18223254]
63. Wu ZZ, Lu HP, Chao CC. Identification and functional analysis of genes which confer resistance to cisplatin in tumor cells. Biochem Pharmacol. 2010 Jul 15; 80(2):262–76. [PubMed: 20361941]
64. Cao X, Pfaff SL, Gage FH. YAP regulates neural progenitor cell number via the TEA domain transcription factor. Genes Dev. 2008 Dec 1; 22(23):3320–34. [PubMed: 19015275]

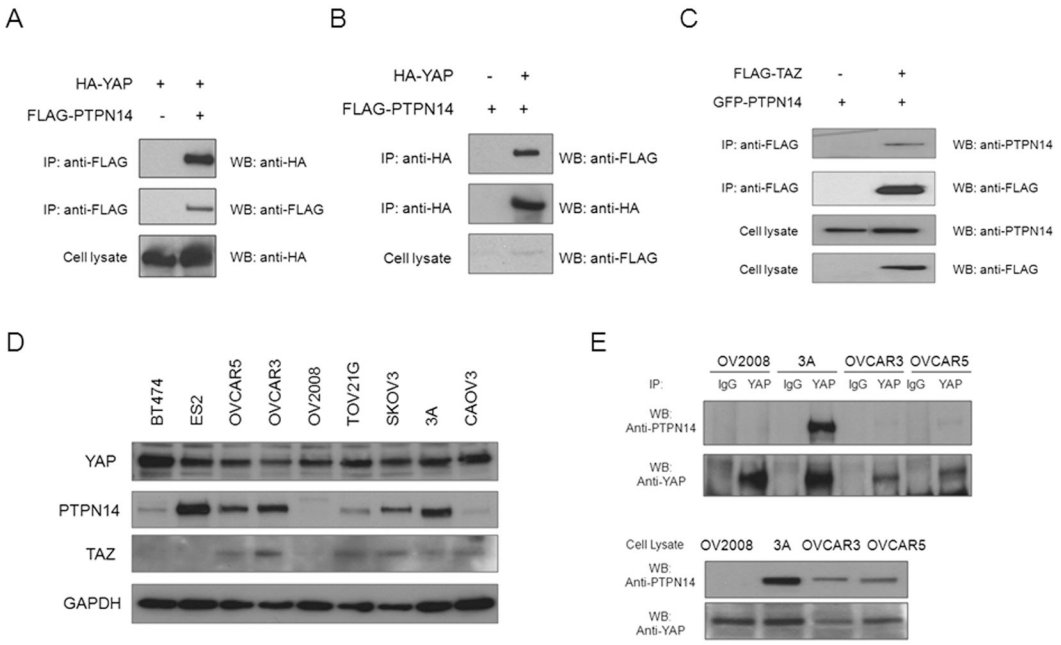

Figure 1. Identification of PTPN14 as a YAP-interacting protein

(A) 293T cells were transfected with HA-YAP in combination with FLAG-tagged PTPN14 or empty vector. The cell lysates were immunoprecipitated with the anti-FLAG antibody M2. The proteins associated with the antibody were analyzed by Western blotting with either the anti-HA (top panel) or the anti-FLAG antibody (middle panel). The levels of HA-YAP were examined with Western blot using the anti-HA antibody (bottom panel).

(B) Reciprocal co-IP analysis of YAP-PTPN14 interaction. IP was performed using anti-HA antibody followed by Western blot using anti-FLAG antibody.

(C) Co-IP analysis of TAZ-PTPN14 interaction.

(D) Western blot analysis of YAP, TAZ and PTPN14 levels in breast and ovarian cancer cells.

(E) Co-IP analysis of endogenous YAP-PTPN14 interaction. IP was performed using anti-PTPN14 antibody followed by Western blot using anti-YAP antibody (upper two panels). The levels of endogenous YAP and PTPN14 were examined by Western blot (lower two panels).

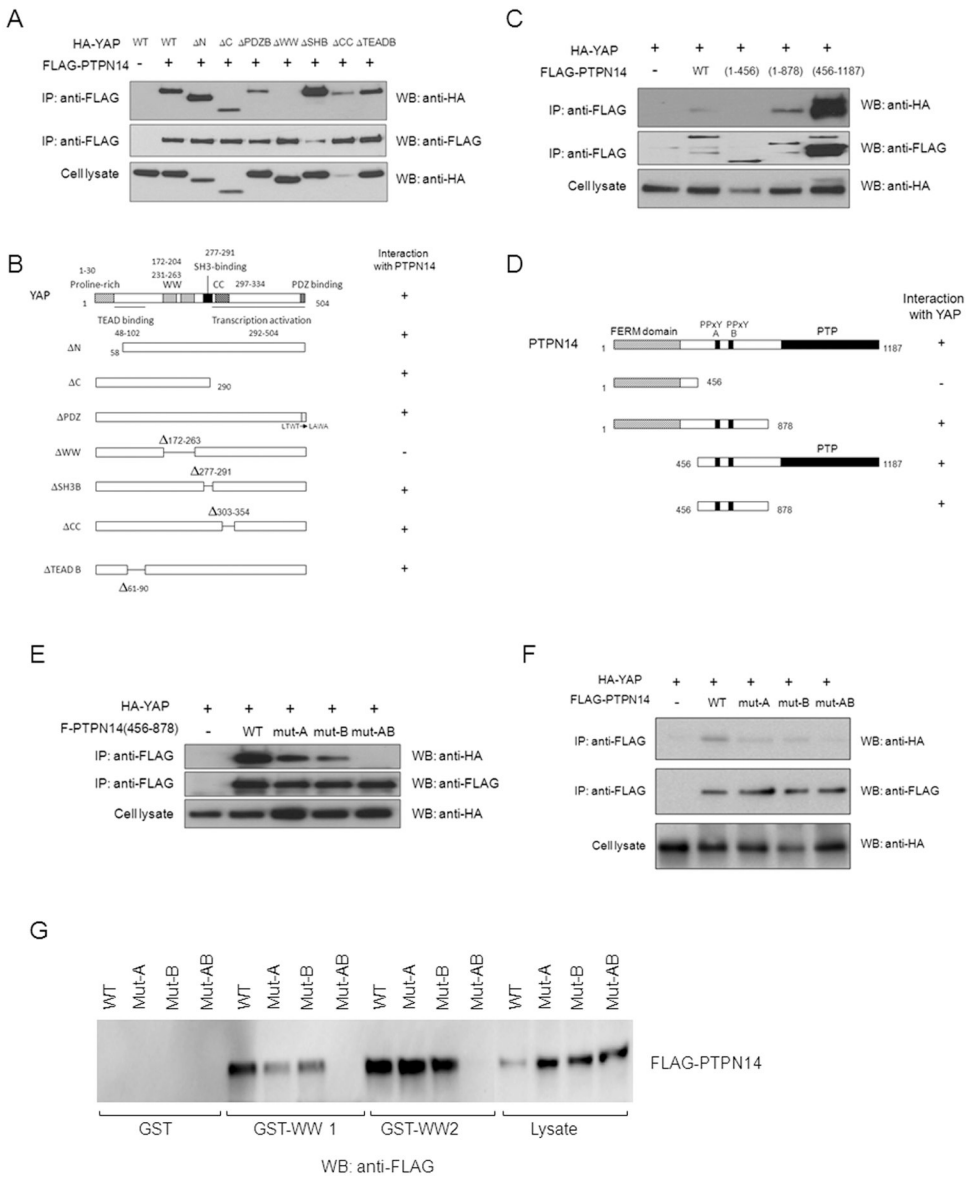

Figure 2. Mapping the structure features involved in YAP-PTPN14 interaction

(A) 293T cells were transfected with FLAG-PTPN14 in combination with HA-YAP (full length or deletion mutants). IP was performed using anti-FLAG antibody, followed by Western blot using either the anti-HA (top panel) or the anti-FLAG antibody (middle panel). The levels of HA-YAP proteins were examined with Western blot using the anti-HA antibody (bottom panel).

(B) Schematic representation of the YAP deletion mutants used and summary of the co-IP study results.

(C) 293T cells were transfected with HA-YAP in combination with FLAG-tagged PTPN14 (full length or deletion mutants). IP was performed using anti-FLAG antibody, followed by Western blot using anti-HA antibody (top panel) or re-probed with the anti-FLAG antibody (middle panel). The levels of HA-YAP proteins were examined by Western blot using the anti-HA antibody (bottom panel).

(D) Schematic representation of the PTPN14 deletion mutants used and summary of the co-IP study results.

(E) 293T cells were transfected with HA-YAP in combination with FLAG-PTPN14 fragment a.a. 456-878 (wild-type or point mutations of either one or both of the PPXY motifs). IP was performed using anti-FLAG antibody, followed by Western blot using either the anti-HA (top panel) or the anti-FLAG antibody (middle panel). The levels of HA-YAP proteins were examined by Western blot using the anti-HA antibody (bottom panel). Mut-A: mutation of the N terminal PPXY motif; Mut-B: mutation of the C terminal PPXY motif; Mut-AB: mutations of the both PPXY motifs.

(F) 293T cells were transfected with HA-YAP in combination with FLAG-PTPN14 (wild-type or point mutations of either one or both of the PPXY motifs). IP was performed using anti-FLAG antibody, followed by Western blot using either the anti-HA (top panel) or the anti-FLAG antibody (middle panel). The levels of HA-YAP proteins were examined by Western blot using the anti-HA antibody (bottom panel).

(G) 293T cells were transfected with FLAG tagged PTPN14 fragment (456-878): wild-type (WT) or point mutations). The cell lysates were incubated with purified GST fusion proteins that contain the one of WW domains of YAP (GST-WW1 and GST-WW2). The proteins that were pulled down by the glutathione beads were separated by SDS-PAGE, followed by Western blot using with anti-FLAG antibody.

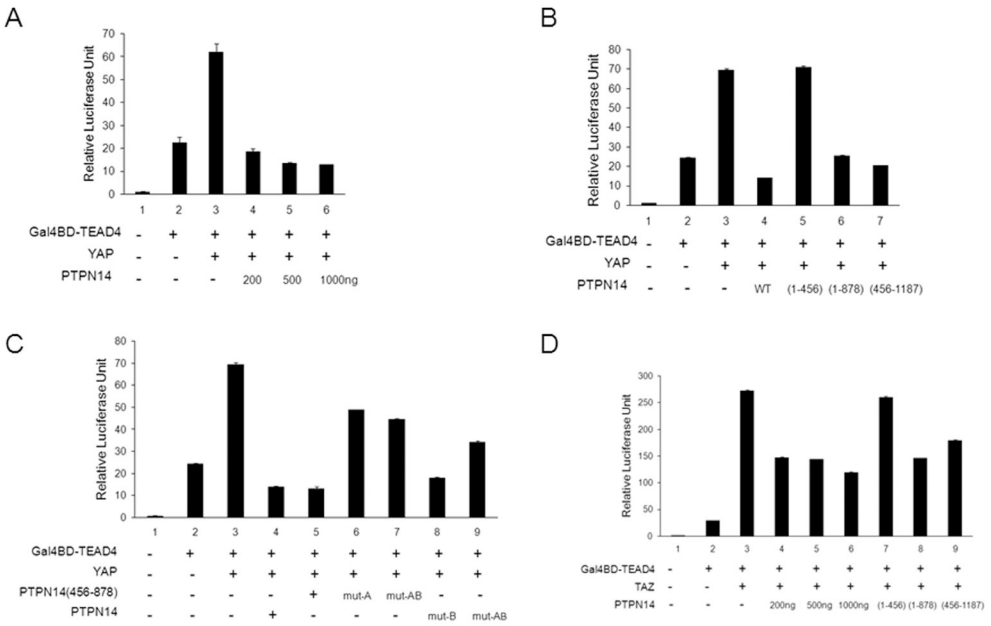

Figure 3. Inhibition of YAP/TAZ-mediated transcription by PTPN14

(A-D) U2OS cells were transfected with the reporter G5-luciferase which contains five Gal4 binding sites, the control reporter pTK-*renilla* luciferase, and the plasmids as indicated. Dual luciferase assay was performed 24 hour after transfection. Mut-A: mutation of the N terminal PPXY motif; Mut-B: mutation of the C terminal PPXY motif; Mut-AB: mutations of the both PPXY motifs.

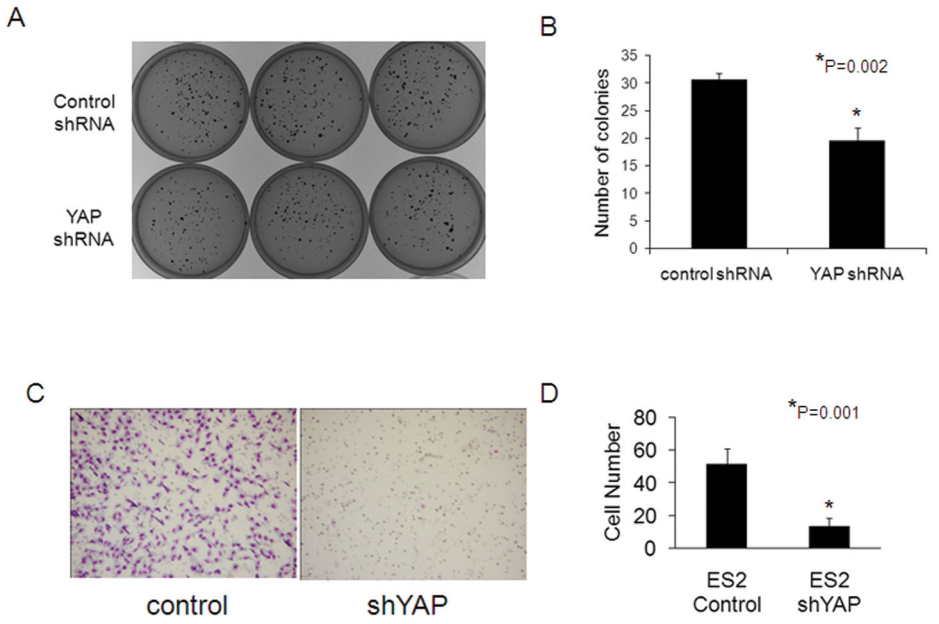

Figure 4. Effect of YAP knockdown on anchorage-independent growth and cell invasion

(A) ES2 cells stably expressing YAP-specific shRNA (shYAP) or control shRNA (control) were cultured in soft agar for 3 weeks. The number of colonies larger than 0.5 mm was counted and the results are shown in (B). P<0.002; n=3; Student's *t* test.

(C) ES2 cells stably expressing YAP-specific shRNA (shYAP) or control shRNA (control) were analyzed using the BD BioCoat™ Matrigel™ Invasion Chamber. Representative images of cells that penetrated the Matrigel are shown.

(D) Statistical analysis of cells that migrated through Matrigel. P<0.001; n=3; Student's *t* test.

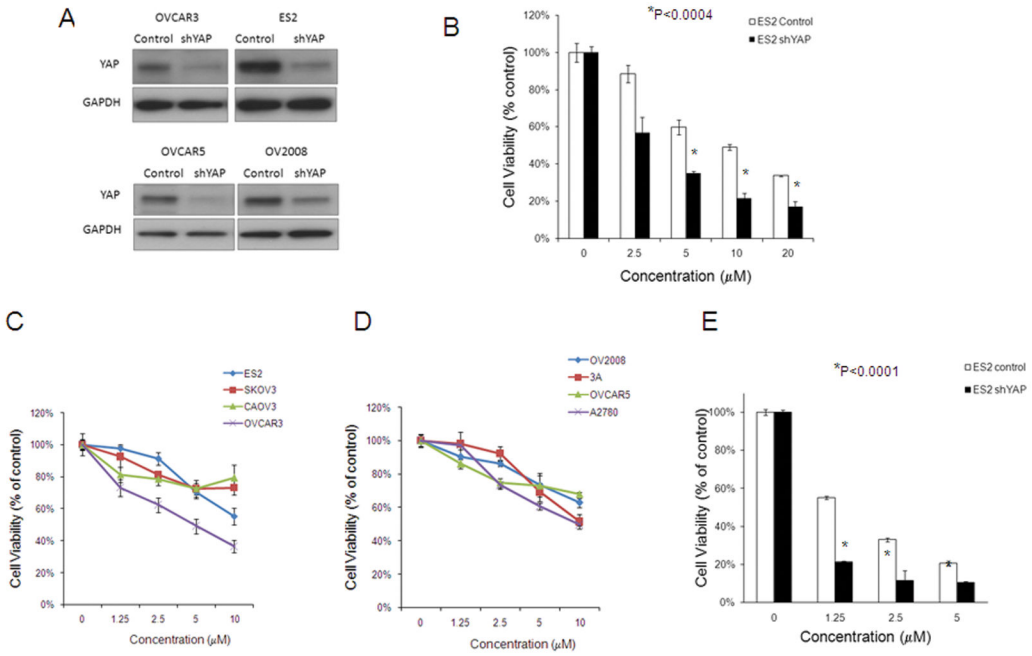

Figure 5. Knockdown of YAP sensitizes ovarian cancer cells to EGFR inhibitor erlotinib

(A) Western blot analysis of cells stably expressing YAP-specific shRNA (shYAP) or control shRNA (control).

(B) Cells were treated with erlotinib for 48 hours. Cell viability was examined by CellTiter-Glo luminescent assay.

(C) Cells stably expressing YAP-specific shRNA (shYAP) or control shRNA (control) were treated with erlotinib for 48 hours. Cell viability was examined by CellTiter-Glo luminescent assay. Student's *t* test was used for statistical analysis (n=3).

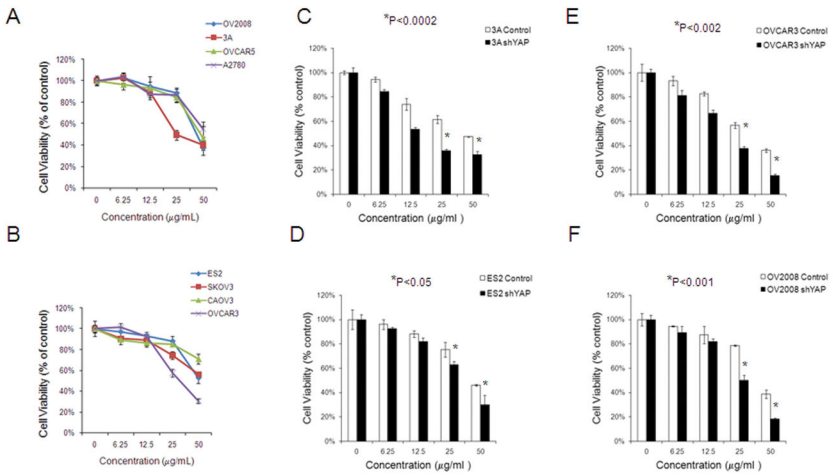

Figure 6. Knockdown of YAP sensitizes ovarian cancer cells to survivin antagonist S12

(A and B) Cells were treated with S12 for 48 hours. Cell viability was examined by CellTiter-Glo luminescent assay.

(C-D) Cells stably expressing YAP-specific shRNA (shYAP) or control shRNA (control) were treated with S12 for 48 hours. Cell viability was examined by CellTiter-Glo luminescent assay. Student's *t* test was used for statistical analysis (n=3).

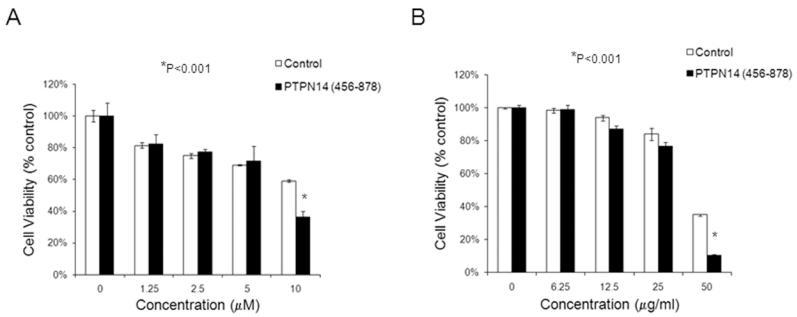

Figure 7. A deletion mutant form of PTPN14 sensitizes ovarian cancer cells to therapeutic agents

(A and B) OV2008 cells stably expressing FLAG PTPN14 (456-878) or control were treated with erlotinib (A) or S12 (B) for 48 hours. Cell viability was examined by CellTiter-Glo luminescent assay. Student's *t* test was used for statistical analysis (n=3).

Table 1

YAP-associated protein identified by mass spectrometry from NIH-3T3 cells

Protein	Function
AMOTL2 (Angiomotin like 2)	Cell adhesion, migration
14-3-3 gamma	Chaperone
TEAD3 (TEA domain family member 3)	Transcriptional factor
14-3-3 theta	Chaperone
14-3-3 epsilon	Chaperone
PTPN14 (Tyrosine – protein phosphatase non-receptor type 14)	Cell adhesion
14-3-3 zeta	Chaperone
14-3-3 beta	Chaperone
MUPP1 (Multiple PDZ domain protein 1)	Cell adhesion
AMOT (Angiomotin)	Cell adhesion, migration
14-3-3 eta	Chaperone
TEAD2 (TEA domain family member 2)	Transcriptional factor
LATS1 (Large tumor suppressor homolog 1)	Kinase, tumor suppressor