Yes-associated protein (YAP65) in relation to Smad7 expression in human pancreatic ductal adenocarcinoma

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) is characterized by multiple alterations in the TGF-ß signaling pathway. Yes-associated protein (YAP65) interacts with Smad7 thereby influencing TGF-ß signaling. In the present study, the expression of YAP65 in PDAC was analyzed in order to elucidate the potential role of this molecule in the pathogenesis of pancreatic cancer. YAP65 mRNA expression levels in human pancreatic tissue samples and cell lines were analyzed by Northern blotting and quantitative RT-PCR. Immunohistochemistry was carried out to localize and quantify YAP65 expression in relation to Smad7 expression and Smad4 mutations. The effects of TGF-ß1 on Smad7 and YAP65 mRNA expression were analyzed by quantitative RT-PCR. Enhanced expression of YAP65 mRNA was identified by Northern blotting and quantitative RT-PCR in PDAC in comparison to the normal pancreas (2.5-fold increase) and to chronic pancreatitis (1.3-fold increase). In the normal pancreas, YAP65 was absent in acinar cells, large ducts and islet cells, but exhibited moderate to strong immunoreactivity in centroacinar cells and ductules. Tubular complexes in CP and CP-like lesions in PDAC also exhibited strong staining. In contrast, weak to moderate YAP65 immunoreactivity was present in the cancer cells. There was no correlation between YAP65 immunostaining and Smad7 staining or Smad4 mutations in the cancer samples. TGF-ß1 strongly induced Smad7 mRNA in Colo-357 and in Panc-1 cells, but only slightly induced YAP65 mRNA in Colo-357 cells. In conclusion, YAP65 is expressed mainly in centroacinar and small ductal cells in the normal pancreas. In PDAC, YAP65 is present in tubular complexes and to a lesser extent in cancer cells. Together with the known function of YAP65 in different growth and differentiation regulating pathways, it is suggested that this gene plays a role in the normal and diseased pancreas.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in the US according to the latest statistical data (1). Pancreatic ductal adenocarcinoma is characterized by late diagnosis, early recurrence after potential curative resection and relative unresponsiveness to standard oncological therapies such as radiotherapy and chemotherapy (2). The overall five-year survival rate is <1%, and >85% of the tumors are diagnosed when the tumor has infiltrated into adjacent organs or when distant metastases are present (3).

Numerous studies have analyzed different genetic and epigenetic alterations in PDAC (4-6). A histologic-genetic progression model for pancreatic cancer was proposed suggesting series of alterations from normal to pancreatic intraepithelial neoplasia (PanIN) and cancer, including mutation of the proto-oncogene K-ras, and tumor suppressor gene mutations such as p53, Smad4/DPC4 and p16/CDKN2A mutations (7). Nevertheless, the initial event that sets the cells along this tumorigenic pathway is still unknown (8). Oncogene activation, loss of tumor suppressor gene function and over-expression of receptor-ligand systems are all involved in this process (4-6). These changes contribute to give PDAC cells a distinct growth advantage.

Yes-associated protein 65 (YAP65, also named YAP1), a 65 kDa proline-rich phosphoprotein, was originally identified due to its interaction with the SH3 domain of the c-Yes proto-oncogene product which belongs to non-receptor tyrosine kinases of the Src family (9). The SH3 domains mediate non-covalent protein-protein interactions essential for cellular and intercellular signaling (10), and YAP65 was also shown to bind to other signaling molecules that contain SH3 domains including Nck, Crk, and v-Yes (9,10), implicating YAP65 in important signaling pathways. Upon alternative splicing, two isoforms of YAP65, which possess only one or two WW

domains have been isolated (10). In addition, there is a PDZ domain-binding motif at the carboxyl-terminal end of YAP65 that allows for interaction with the submembranous scaffold protein, EBP50, as well as a coiled-coil (CC) domain (11).

As an adaptor-type signaling protein, the exact molecular function of YAP65 remains largely unknown. YAP65 has been reported to bind to p53 binding protein-2 (p53BP-2), an important regulator of the apoptotic activity of p53 through its WW and SH3 domain interactions (12). In addition, YAP65 also interacts with p73 and p63, two p53 homologues that can bind to p53 DNA binding sites to activate transcription from p53-responsive promoters, and overexpression of p73 and p63 can induce apoptosis, growth arrest, and differentiation (12,13). YAP65 is considered as a novel transcriptional co-activator, which binds and activates Runx/PEBP2α (14) and TEAD/TEF (15-17) transcription factors. Moreover, other transcription factors with similar motifs that could potentially be co-activated by YAP65 include AP2, C/EBPα, c-Jun, Krox-20, Krox-24, MEF2B, NF-E2, Oct-4 and p73α (14-17). YAP65 shares 45% homologues with TAZ, a novel transcriptional co-activator regulated by the interaction with 14-3-3 and PDZ domain containing proteins (16). The expression of YAP65 is ubiquitous in normal adult tissues, but relatively high in placenta, prostate, ovary, and testis. In contrast, its expression is absent in peripheral blood leukocytes (10). It was shown recently that YAP65 can also interact with the transforming growth factor-beta (TGF-ß)/Smad signaling pathway (18).

TGF-ßs are multifunctional polypeptides that inhibit the growth of epithelial cells and exert multiple effects on the extracellular matrix (19). TGF-ß1 signals through a family of transmembrane receptors that have intrinsic serine/threonine kinase activity (19). The inhibitory Smads, Smad6 and Smad7 associate with the activated TGF-ß receptor type I, thereby blocking access and phosphorylation of Smad2 and possibly Smad3 (20,21). This prevents their interaction with Smad4, and blocks their subsequent nuclear translocation (20,21). Smad7 contains a highly conserved PY motif which interacts with WW domains of YAP65. YAP65 therefore enhances the inhibitory activity of Smad7 on TGF-ß signaling (18). Since overexpression of Smad7 contributes to enhanced tumorigenicity in pancreatic cancer (22), which is mediated, in part, through the functional inactivation of the retinoblastoma (Rb) protein (23), in the present study we investigated the expression of YAP65 in PDAC in order to elucidate the role of this protein in this malignancy.

Materials and methods

Patients and tissue samples. Fifty human primary PDAC (31 male, 19 female; median age 66 years; range 38-84 years) and 16 chronic pancreatitis samples (12 male, 4 female; median age 54 years; range 34 -68 years) were collected from patients who underwent pancreatic resection in the Department of Visceral and Transplantation Surgery Inselspital (University of Bern, Switzerland) and the Department of General Surgery (University of Heidelberg, Germany). Twenty-four normal human pancreatic tissue samples (14 male, 10 female; median age 42 years; range 14-73 years) were obtained from previously healthy organ donors through an organ donor program. Freshly removed samples were snap-frozen in liquid nitrogen and stored at -80˚C until analysis. In addition, a portion of the tissue samples were fixed in 5% paraformaldehyde solution for 12-24 h and paraffin-embedded for histological analysis. The study was approved by the Ethics Committees of the University of Bern and University of Heidelberg. Written informed consent was obtained from all patients prior to analysis.

Real-time quantitative polymerase chain reaction (qRT-PCR). All reagents and equipment for mRNA/cDNA preparation were supplied by Roche Applied Science (Mannheim, Germany). mRNA was prepared by automated isolation using the MagNA pure LC instrument and isolation kit I (for cells) and kit II (for tissues). cDNA was prepared using the first strand cDNA synthesis kit for RT-PCR (AMV) according to the manufacturer's instructions. Target sequences were amplified using the light cycler primer sets (Search-LC, Heidelberg, Germany) with the LightCycler FastStar DNA SYBR Green I kit (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's protocol. RNA input was normalized to the average expression of the two housekeeping genes HPRT and cyclophilin B. The transcript number was calculated from a virtual standard curve, obtained by plotting a known input concentration of a plasmid to the PCR-cycle number at which the detected fluorescence intensity reaches a fixed value. The data of two independent analyses for each sample and parameter were averaged and presented as adjusted transcripts/μl cDNA (24).

RNA extraction and Northern blot analysis. Total RNA from human pancreatic tissues and pancreatic cancer cell lines was isolated by the single-step guanidinium method as described previously (22). A 401-bp RT-PCR fragment of the human YAP65 gene (GenBank: NM 006106) was subcloned into the pGEMT-easy vector (Promega Biotechnology, Madison, WI, USA) using standard conditions (24). Authenticity was confirmed by sequencing (Qiagen Genomic services, Hilden, Germany). RNA (15 μg/lane) was size fractionated on 1.2% agarose/1.8 M formaldehyde gels. Gels were stained with ethidium bromide for verification of RNA integrity and loading equivalency. Fractionated RNA was transferred onto Genescreen membranes (NEN™ Life Science Products, Boston, MA, USA) and cross-linked by UV irradiation. Blots were prehybridized for 12 h at 42˚C in 50% formamide, 1% SDS, 5X Denhardt's, 100 μg/ml salmon sperm DNA, 50 mM Na_2PO_4, pH 7.4, 10% dextran, 75 mM NaCl and 5 mM EDTA. Blots were hybridized for 24 h at 42˚C in the presence of α^{32}P-dCTP (Amersham Pharmacia Biotech, Freiburg, Germany) labeled cDNA probe for YAP65 (10^6 cpm/ml) or 7S (10^5 cpm/ml), rinsed twice with 2X SSC, and washed twice with 0.2X SSC/2% SDS at 50˚C and 55˚C for 20 min, respectively. All blots were exposed at -80˚C to Kodak BiomaxMS film with Kodak intensifying screens.

Immunohistochemistry. Consecutive paraffin-embedded tissue sections (3 μm thick) were deparaffinized and rehydrated. Slides were covered with 3% H_2O_2 for 10 min to quench endogenous peroxidase activity and then with 10% normal goat serum for 30 min at room temperature before incubation overnight with a rabbit anti-YAP65 (Santa Cruz Inc., Santa

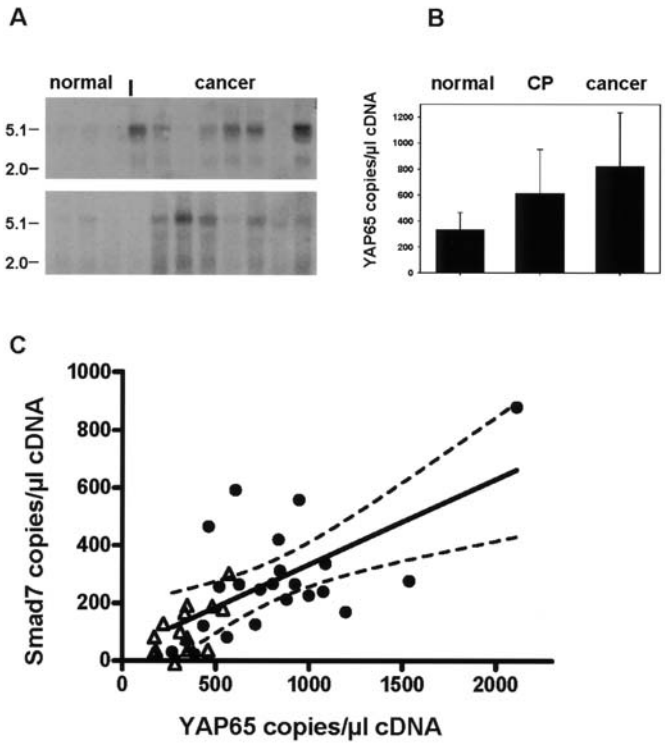

Figure 1. YAP65 mRNA expression analysis in pancreatic tissues. (A) Total RNA (15 μg/lane) isolated from the indicated pancreatic tissues was subjected to Northern blot analysis and hybridized with a ^{32}P-labeled YAP65 cDNA probe. Size markers (in kb) are indicated on the left. (B) Quantitative RT-PCR in the normal pancreas (normal), chronic pancreatitis (CP) and PDAC (cancer) tissues as described in Materials and methods. Data are presented as mean ± SD. (C) Expression level of YAP65 mRNA in pancreatic cancer tissues (black dots) and in the normal pancreas (white triangles) in correlation with Smad7 mRNA levels as determined by qRT-PCR. Linear regression line (solid line) and 95% confidence band (dotted line) for the cancer cases.

Cruz, CA, USA), rabbit anti-Smad7 (Santa Cruz Inc.) or mouse anti-Smad4 (Santa Cruz Inc.) antibodies at 4˚C. Slides were incubated with polymer of HRP-linked anti-rabbit secondary antibody (Dako envision kit; Dako, USA) for 45 min, followed by incubation with a buffered substrate for liquid DAB/liquid DAB chromogen mixture for 20 sec. Finally, sections were counterstained with Mayer's hematoxylin and mounted in permanent mounting medium.

Cell culture. Human pancreatic cancer cells were grown routinely in Dulbecco's modified Eagle medium (PANC-1, MIA-PaCa-2, and COLO-357) or RPMI-1640 (ASPC-1, BxPc-2, Capan-1 and T3M4) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mmol/l L-glutamine. Cells were maintained at 37˚C in a humid chamber with 5% CO_2 and 95% air atmosphere.

Protein extraction. Pancreatic cancer cells were lysed in 0.5 ml lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA (pH 8.0) and 1% SDS with proteinase inhibitors (1 tablet/10 ml, Roche Molecular Biochemicals, Mannheim, Germany). Protein concentration was determined by the BCA protein assay (Pierce Chemical Co, Rockford, IL, USA).

Western blot analysis. Total protein (20 μg) were size-fractionated by SDS-polyacrylamide gel electrophoresis (SDS/PAGE) and transferred onto a nitrocellulose membrane (BioRad Laboratories, Hercules, CA, USA). Blots were blocked in TBS with Tween-20 (0.1%) at pH 7.5 containing 5% non-fat milk for 1 h and then incubated overnight with the rabbit anti-YAP65 antibody. Following three washes with TBS-T, membranes were incubated with the secondary antibody for 1 h at room temperature. Antibody detection was performed with an enhanced chemiluminescence (ECL) reaction system (Amersham Life Sciences, Amersham, UK).

TGF-ß1 induction. Subconfluent COLO-357 and Panc-1 cells were incubated overnight in serum-free medium and subsequently incubated in the absence or presence of 200 pM TGF-ß 1 (R&D Systems Inc., Minneapolis, USA) for the indicated time points (0, 0.5, 1, 6, 12, 24, 48 h). RNA was extracted and qRT-PCR was carried out as described (24).

Statistics. Patient data are expressed as median and range. Results are expressed as mean ± SEM of at least three independent experiments unless indicated otherwise. For statistical analysis, the Mann-Whitney U test was used. $p<0.05$ was taken as the level of significance.

Results

YAP65 expression in human pancreatic cancer. Northern blot analysis of total RNA isolated from 48 pancreatic cancers and 24 normal pancreatic tissues showed absent to weak expression of the approximately 5.1 kb YAP65 mRNA transcript (10) in normal pancreatic samples. In contrast, in 36 of 48 (75%) pancreatic cancer samples, the YAP65 mRNA transcript was clearly detectable. In 21 (44%) of these samples YAP65 mRNA was expressed at a relatively high level, whereas in the remaining 15 samples there was low to moderate expression of this mRNA moiety. An additional transcript of 2.4 kb mRNA was detectable in 16 of 48 (31%) cancer samples but not in the normal pancreas. The significance of this additional transcript is currently not known. A representative Northern blot of 16 cancerous and 8 normal tissue samples is shown in Fig. 1A.

To validate the Northern blot analysis and to better quantify changes in YAP65 mRNA levels, quantitative RT-PCR was carried out next. In pancreatic cancer samples (n=23), the median expression level of YAP65 mRNA was 2.5-fold ($p<0.01$) and 1.3-fold ($p=0.1$) higher than in the normal (n=14) and chronic pancreatitis (n=16) tissue samples, respectively. Moreover, the median expression level of YAP65 in chronic pancreatitis was 1.8-fold ($p<0.01$) higher than in the normal pancreas (Fig. 1B). We also analyzed a possible correlation between YAP65 and Smad7 mRNA expression levels in normal and cancerous tissues by quantitative RT-PCR (Fig. 1C). A positive correlation of YAP65 and Smad7 mRNA expression levels was observed in pancreatic cancer tissues (black dots) ($r2=0.38$; $p=0.0018$), yet not in the normal pancreas (white triangles).

Localization of YAP65 in human pancreatic tissues. To further delineate the significance of YAP65 overexpression in PDAC,

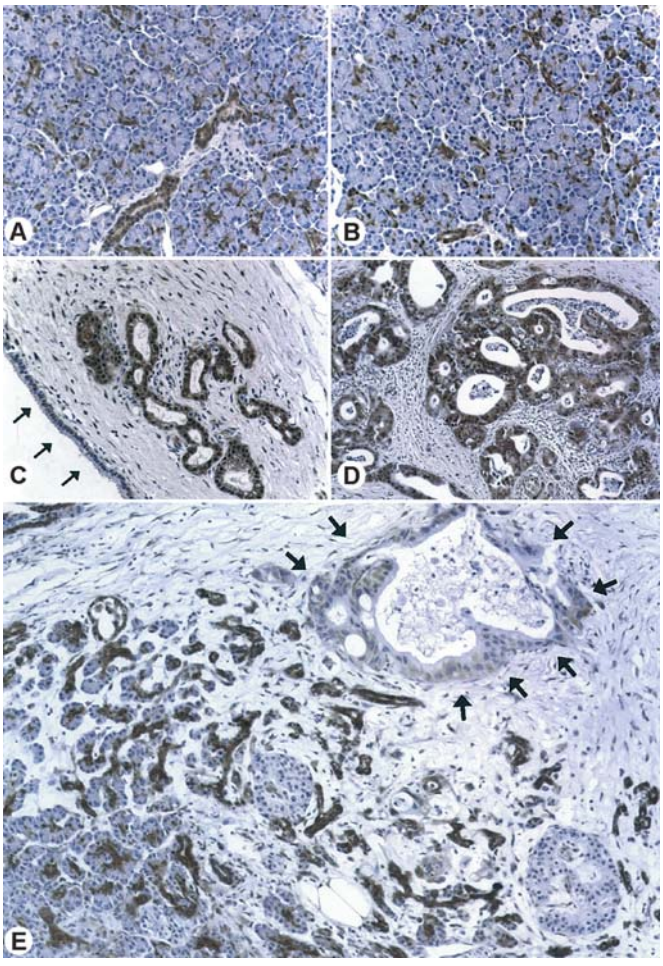

Figure 2. Localization of YAP65 in the normal pancreas and PDAC tissues. Immunohistochemistry was carried out using a YAP65-specific antibody as described in Materials and methods. (A and B) Normal pancreas shows strong staining in centroacinar and small ductal cells. (C) In an area next to PDAC, strong staining in tubular complexes and moderate staining in cells forming a large duct (arrows) is shown. (D) PDAC depicting moderate YAP65 immunostaining in the cancer cells. (E) PDAC depicting weak staining in the cancer cells (arrows) and strong staining in the surrounding tubular complex.

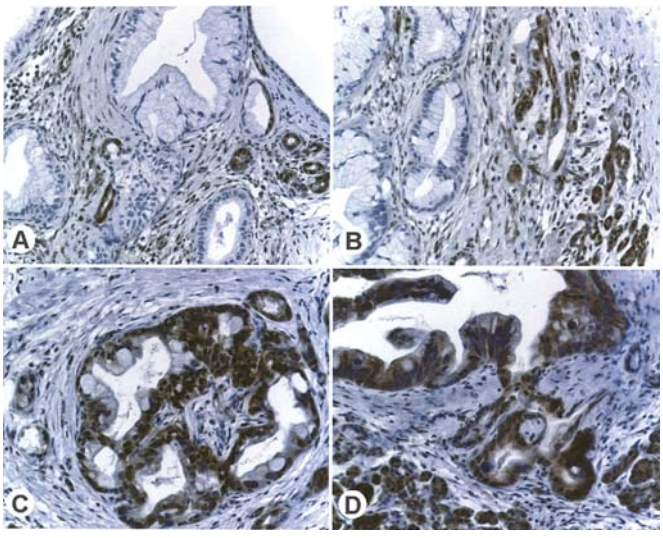

Figure 3. Smad4 immunohistochemistry in PDAC tissues. Smad4 immunohistochemistry analysis was carried out as described in Materials and methods. (A and B) PDAC samples that were devoid of Smad4 immunoreactivity in the cancer cells, while showing Smad4 expression in non-neoplastic ducts. (C and D) PDAC samples showing Smad4 positivity in the cancer cells.

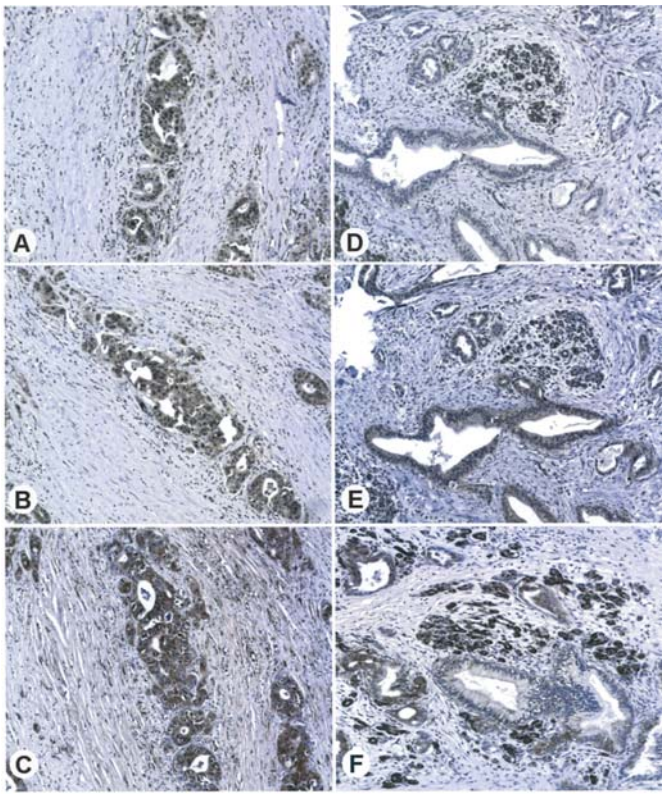

Figure 4. YAP65, Smad4 and Smad7 immunohistochemistry in consecutive PDAC sections. Immunohistochemistry of YAP65 (A and D), Smad4 (B and E) and Smad7 (C and F) was carried out as described in Materials and methods. PDAC tissue 1 (left row, A-C) shows high YAP 65 and Smad7 levels and Smad4 expression (no mutation). PDAC tissue 2 (right row, D-E) shows low YAP 65 and Smad7 levels and Smad4 expression (no mutation).

immunohistochemistry was carried out. YAP65 localized mainly in the cytoplasm, as well as occasionally in the nuclei in some cells. In the normal pancreas, YAP65 immunoreactivity was absent in acinar cells, large duct and islet cells (Fig. 2A and B). Moderate to strong YAP 65 staining was observed in centroacinar cells and in cells forming small ducts (Fig. 2A and B). In areas surrounding the tumor, strong cytoplasmic staining was observed in tubular complexes (Fig. 2C), whereas the cells forming large ducts displayed only moderate YAP65 immunoreactivity (Fig. 2C, arrows). In contrast, weak to moderate YAP65 immunoreactivity was present in the cancer cells within the pancreatic tumor mass in 48 of 50 (96%) cancer samples (Fig. 2D and E, arrows). In most cases, there was a marked reduction of YAP65 staining in the cancer cells (Fig. 2E, arrows) by comparison with the surrounding tubular complexes and centroacinar cells (Fig. 2E).

Correlation between YAP65 and Smad7 expression, as well as Smad4 mutations in PDAC. Next, we analyzed the correlation between YAP65 expression and Smad4 and Smad7 expression in PDAC. Previously, it has been shown that loss of Smad4 expression correlates with Smad4 mutations in PDAC (25). Loss of Smad4 expression was observed in 26 of 50 (52%)

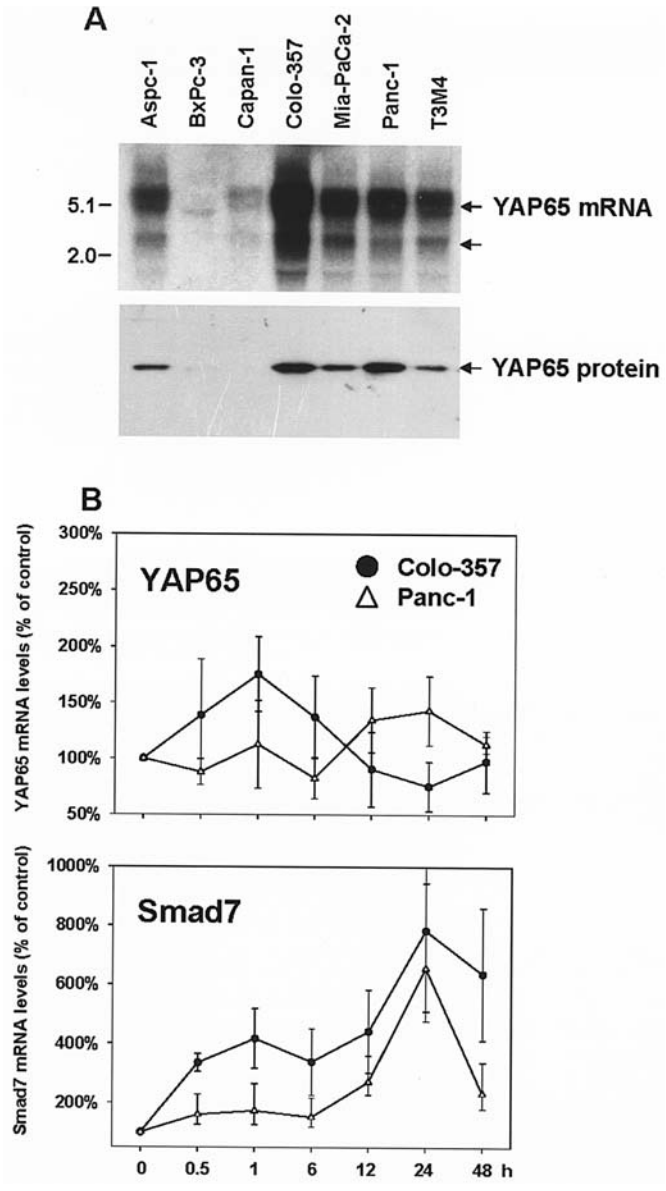

Figure 5. YAP65 mRNA and protein expression and regulation in pancreatic cells. (A, upper panel) Total RNA (15 μg) isolated from the indicated cell lines was subjected to Northern blot analysis and hybridized with a ^{32}P-labeled YAP65 cDNA probe. Size markers are indicated on the left. (A, lower panel) Protein lysates (20 μg) of the indicated pancreatic cancer cell lines were subjected to Western blot analysis using an anti-YAP65 antibody as described in Materials and methods. (B) COLO-357 (black dot) and Panc-1 (white triangle) cells were incubated with 200 pM TGF-ß1 for the indicated time. RNA was extracted and quantitative RT-PCR was carried out as described in Materials and methods. Results are presented as mean ± SEM of three separate experiments.

tumor samples (Fig. 3A and B). In contrast, 18 of 50 (36%) pancreatic cancer samples exhibited moderate to strong Smad4 expression (Fig. 3C and D), whereas the remaining 6 (12%) cancer tissues exhibited areas in which the cancer cells were Smad4 positive and areas where no Smad4 immunostaining in the cancer cells was observed. Absent/weak expression of Smad7 was observed in 30 of 50 (60%) consecutive PDAC sections, in comparison with 20 of 50 (40%) PDAC tissue samples that exhibited moderate to strong Smad7 expression.

Correlation analysis between expression of YAP65, Smad4 and Smad7 in consecutive PDAC sections (n=50) was carried out next. There was no correlation between YAP65 expression and either Smad7 or Smad4 expression in pancreatic cancer cells. In addition, there was no correlation between Smad7 and Smad4 expression. Representative consecutive sections of YAP65, Smad7 and Smad4 immunostaining are shown in Fig. 4.

YAP65 mRNA and protein expression in pancreatic cancer cell lines. In order to further investigate the role of YAP65 in pancreatic cancer, 7 human pancreatic cancer cell lines were analyzed by Northern blot and Western blot analysis (Fig. 5A). Five pancreatic cancer cell lines (Aspc-1, Colo-357, Mia-PaCa-2, Panc-1 and T3M4) exhibited high levels of the 5.1 kb YAP65 mRNA transcript, but low levels of the 2.1 kb mRNA transcript. In contrast, in BxPc-3 and Capan-1 pancreatic cancer cells only low expression levels of YAP65 mRNA were detectable for both transcripts (Fig. 5A). Western blot analysis revealed that there was an excellent correlation between YAP65 protein levels in the various cell lines and YAP65 mRNA levels (Fig. 5A).

To analyze whether YAP65 expression is regulated by TGF-ß, cells were incubated with TGF-ß1 (200 pM) for the indicated time. TGF-ß1 transiently induced YAP65 mRNA levels between 0.5 and 6 h, with maximal effects of 1.75-fold up-regulation occurring after 1 h in Colo-357 (Fig. 5C). However, there was no significant effect on YAP65 mRNA levels in Panc-1 cells (Fig. 5C). In contrast, TGF-ß1 induced a sustained up-regulation of Smad7 mRNA levels with maximal effects of 6.6-7.8-fold after 24 h in both cell lines (Fig. 5C).

Discussion

YAP65 is the first protein in which a WW domain was identified to interact with the SH3 domain of c-Yes, as well as to bind to other Src family members such as Crk, and v-Yes (10). WW domains are broadly distributed among natural proteins and these modules play a key role in bringing specific proteins together (26). In addition, YAP65 contains a PDZ domain-binding motif and 14-3-3 binding motif at the carboxyl-terminal end, as well as a coiled-coil (CC) domain (16). YAP65 is a transcriptional co-activator which is involved in binding and activating several transcription factors such as Runx, TEAD/TEF, ErbB-4 and others (14,17,27,28). Via interaction with p53 family member p73 and p53 binding protein-2 (p53BP-2), YAP65 can regulate p53 biological function in physiological and pathological cellular responses. It has been reported that EGF can induce phosphorylation of YAP65 by Akt/PKB, resulting in the attenuation of p73-mediated apoptosis (27). YAP65 may also be involved in other apoptosis signaling pathways. Smad family proteins, except Smad4 contain the well conserved PY motif which interacts with WW domain-containing protein such as YAP65 (29). YAP65 binds and enhances the Smad7 inhibitory potential in TGF-ß signaling pathway through WW domain and PY motif interaction.

In the present study, we have investigated the expression of YAP65 in PDAC and its relation to TGF-ß signaling molecules. YAP65 mRNA levels were increased in PDAC in

comparison to levels observed in chronic pancreatitis samples or in normal pancreatic tissues. YAP65 mRNA levels positively correlated with Smad7 mRNA levels in cancer tissue but not in the normal pancreas, where YAP65 expression was detected in the centroacinar cells and in the cells of intralobular ducts. In the tumor tissues, strong YAP65 staining was observed in the tubular complexes, whereas in the cancer cells, only a weak immunohistochemical signal was found.

It has been shown previously that YAP65 is a novel Smad7 partner that is involved in regulating the activity of Smad proteins (18). Aside from its inhibitory activities on TGF-ß signaling, Smad7 has also been shown to induce apoptosis (30), and to activate JNK kinases (31). Approximately 50% of pancreatic cancer exhibited increased Smad7 expression (22). Enhanced levels of Smad7 and YAP65 in pancreatic cancer may contribute to resistance towards TGF-ß signaling thereby providing a potential growth advantage. However, there was no correlation between YAP65 and Smad7 protein expression in the cancer cells, which is discordant with the results obtained in bulk tissues at the mRNA level. This discrepancy may be due to the different content of e.g. tubular complexes in the bulk cancer specimens, or due to different stability of the respective mRNA and/or protein.

In the present study, 42% of the PDAC samples exhibited loss of Smad4 expression in the cancer cells, which has been shown to correlate with the Smad4 mutation status. No correlation was evident between YAP65 and Smad4 expression, suggesting that both alterations may interfere at different points in the TGF-ß signaling pathway and/or influence other pathways. In line with this argument, there was only a slight up-regulation of YAP65 expression in response to TGF-ß1 in 1 out of 7 examined pancreatic cancer cell lines.

Another interesting aspect was the different subcellular localization of YAP65 in the cancer cells, which might indicate a different functional status. In the nucleus, the de-phosphorylated form of YAP65 can function as a transcriptional co-activator of several transcription factors such as Runx, c-Jun and others (17). Phosphorylation of YAP65 by akt/PKB mediates its translocation to the cytosol, possibly as a result of interaction with 14-3-3 proteins, thereby negatively regulating its activity as a transcriptional co-activator (27). In the present study, both nuclear and cytoplasmic localization was observed, which suggests different roles of YAP65 in signaling transduction and processing. A number of genes that have the potential to interact with YAP65-like members of EGF receptor family, the 14-3-3 family and the family of Runx transcription factor are known to be de-regulated in pancreatic cancer (32-34), suggesting important potential interactions.

In conclusion, i) YAP65 is expressed in centroacinar and ductal cells in the normal pancreas, implying a physiological role of this gene in the normal pancreas. ii) YAP65 is highly expressed in tubular complexes compared to the weak to moderate expression in pancreatic cancer cells. iii) YAP65 expression does not correlate with Smad7 levels or Smad4 mutations in pancreatic cancer cells. iv) The potential interaction with a number of genes that display deregulated expression in PDAC point to an important role of YAP65 in modulating aberrant signaling pathways that contribute to the pathogenesis of this disease.

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References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ and Thun MJ: Cancer statistics, 2005. CA Cancer J Clin 55: 10-30, 2005.
2. DiMagno EP, Reber HA and Tempero MA: AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. American Gastroenterological Association. Gastroenterology 117: 1464-1484, 1999.
3. Gudjonsson B: Cancer of the pancreas. 50 years of surgery. Cancer 60: 2284-2303, 1987.
4. Bardeesy N and De Pinho RA: Pancreatic cancer biology and genetics. Nat Rev Cancer 2: 897-909, 2002.
5. Li D, Xie K, Wolff R and Abbruzzese JL: Pancreatic cancer. Lancet 363: 1049-1057, 2004.
6. Ozawa F, Friess H, Tempia-Caliera A, Kleeff J and Buchler MW: Growth factors and their receptors in pancreatic cancer. Teratog Carcinog Mutagen 21: 27-44, 2001.
7. Hruban RH, Adsay NV, Bores-Saavedra J, Compton C, Garrett ES, Goodman SN, Kern SE, Klimstra DS, Kloppel G, Longnecker DS, Luttges J and Offerhaus GJ: Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol 25: 579-586, 2001.
8. Bockman DE, Guo J, Buchler P, Muller MW, Bergmann F and Friess H: Origin and development of the precursor lesions in experimental pancreatic cancer in rats. Lab Invest 83: 853-859, 2003.
9. Sudol M: Yes-associated protein (YAP65) is a proline-rich phosphoprotein that binds to the SH3 domain of the Yes proto-oncogene product. Oncogene 9: 2145-2152, 1994.
10. Sudol M, Bork P, Einbond A, Kastury K, Druck T, Negrini M, Huebner K and Lehman D: Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain. J Biol Chem 270: 14733-14741, 1995.
11. Mohler PJ, Kreda SM, Boucher RC, Sudol M, Stutts MJ and Milgram SL: Yes-associated protein 65 localizes p62(c-Yes) to the apical compartment of airway epithelia by association with EBP50. J Cell Biol 147: 879-890, 1999.
12. Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, Oren M, Sudol M, Cesareni G and Blandino G: Physical interaction with Yes-associated protein enhances p73 transcriptional activity. J Biol Chem 276: 15164-15173, 2001.
13. Yagi R, Chen LF, Shigesada K, Murakami Y and Ito Y: A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator. EMBO J 18: 2551-2562, 1999.
14. Vassilev A, Kaneko KJ, Shu H, Zhao Y and De Pamphilis ML: TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes Dev 15: 1229-1241, 2001.
15. Fossdal R, Jonasson F, Kristjansdottir GT, Kong A, Stefansson H, Gosh S, Gulcher JR and Stefansson K: A novel TEAD1 mutation is the causative allele in Sveinsson's chorioretinal atrophy (helicoid peripapillary chorioretinal degeneration). Hum Mol Genet 13: 975-981, 2004.
16. Kanai F, Marignani PA, Sarbassova D, Yagi R, Hall RA, Donowitz M, Hisaminato A, Fujiwara T, Ito Y, Cantley LC and Yaffe MB: TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. EMBO J 19: 6778-6791, 2000.
17. Mahoney WM Jr, Hong JH, Yaffe MB and Farrance IK: The transcriptional co-activator TAZ interacts differentially with transcriptional enhancer factor-1 (TEF-1) family members. Biochem J 388: 217-225, 2005.
18. Ferrigno O, Lallemand F, Verrecchia F, L'Hoste S, Camonis J, Atfi A and Mauviel A: Yes-associated protein (YAP65) interacts with Smad7 and potentiates its inhibitory activity against TGF-beta/Smad signaling. Oncogene 21: 4879-4884, 2002.
19. Massague J: TGF-beta signal transduction. Annu Rev Biochem 67: 753-791, 1998.

20. Hayashi H, Abdollah S, Qiu Y, Cai J, Xu YY, Grinnell BW, Richardson MA, Topper JN, Gimbrone MA Jr, Wrana JL and Falb D: The MAD-related protein Smad7 associates with the TGFbeta receptor and functions as an antagonist of TGFbeta signaling. Cell 89: 1165-1173, 1997.
21. Imamura T, Takase M, Nishihara A, Oeda E, Kawabata M, Hanai J and Miyazono K: Smad6 inhibits signalling by the TGF-beta superfamily. Nature 389: 622-626, 1997.
22. Kleeff J, Ishiwata T, Maruyama H, Friess H, Truong P, Falb D, Buchler MW and Korc M: The TGF-beta signaling inhibitor Smad7 enhances tumorigenicity in pancreatic cancer. Oncogene 18: 5363-5372, 1999.
23. Arnold NB and Korc M: SMAD7 abrogates TGF-beta 1 mediated growth inhibition in colo-357 cells through functional inactivation of the retinoblastoma protein. J Biol Chem 280: 21858-21866, 2005.
24. Guo J, Kleeff J, Li J, Ding J, Hammer J, Zhao Y, Giese T, Korc M, Buchler MW and Friess H: Expression and functional significance of CDC25B in human pancreatic ductal adenocarcinoma. Oncogene 23: 71-81, 2004.
25. Wilentz RE, Su GH, Dai JL, Sparks AB, Argani P, Sohn TA, Yeo CJ, Kern SE and Hruban RH: Immunohistochemical labeling for dpc4 mirrors genetic status in pancreatic adenocarcinomas: a new marker of DPC4 inactivation. Am J Pathol 156: 37-43, 2000.
26. Espinosa JF, Syud FA and Gellman SH: An autonomously folding beta-hairpin derived from the human YAP65 WW domain: attempts to define a minimum ligand-binding motif. Biopolymers 80: 303-311, 2005.
27. Basu S, Totty NF, Irwin MS, Sudol M and Downward J: Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. Mol Cell 11: 11-23, 2003.
28. Komuro A, Nagai M, Navin NE and 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 278: 33334-33341, 2003.
29. Rotin D: WW (WWP) domains: from structure to function. Curr Top Microbiol Immunol 228: 115-133, 1998.
30. Lallemand F, Mazars A, Prunier C, Bertrand F, Kornprost M, Gallea S, Roman-Roman S, Cherqui G and Atfi A: Smad7 inhibits the survival nuclear factor kappaB and potentiates apoptosis in epithelial cells. Oncogene 20: 879-884, 2001.
31. Mazars A, Lallemand F, Prunier C, Marais J, Ferrand N, Pessah M, Cherqui G and Atfi A: Evidence for a role of the JNK cascade in Smad7-mediated apoptosis. J Biol Chem 276: 36797-36803, 2001.
32. Graber HU, Friess H, Kaufmann B, Willi D, Zimmermann A, Korc M and Buchler MW: ErbB-4 mRNA expression is decreased in non-metastatic pancreatic cancer. Int J Cancer 84: 24-27, 1999.
33. Guweidhi A, Kleeff J, Giese N, Fitori JE, Ketterer K, Giese T, Buchler MW, Korc M and Friess H: Enhanced expression of 14-3-3sigma in pancreatic cancer and its role in cell cycle regulation and apoptosis. Carcinogenesis 25: 1575-1585, 2004.
34. Li J, Kleeff J, Guweidhi A, Esposito I, Berberat PO, Giese T, Buchler MW and Friess H: RUNX3 expression in primary and metastatic pancreatic cancer. J Clin Pathol 57: 294-299, 2004.