The Prognostic Significance and Tumor-promoting Roles of MED30 in Pancreatic Cancer

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Research

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Abstract

Background: MED30 is an evolutionarily new member of the mediator complex that can bridge transcription factors and preinitiation complex for the transcription. Pancreatic cancer is highly chemoresistant and is the fourth leading cause of cancer-related deaths. Rapid progression and poor prognosis of pancreatic cancer result in shortened survival rate following diagnosis.

Methods: Expression of MED30 was investigated by the analysis of TCGA database and immunohistochemistry of patients tissues. Roles of MED30 were investigated by gain-of-function and loss-of-function studies.

Results: Analysis of TCGA database and immunohistochemistry proved the frequent amplification and overexpression of MED30 in the pancreatic cancer tissues compared with the normal pancreatic tissues at the chromosome, mRNA and protein levels. Notably its overexpression was associated with poor prognosis of pancreatic cancer patients in the Kaplan-Meier curve analysis ($P=0.00072$) and multivariate analysis ($P=0.027$). Uno's C-index values in the time-dependent Area Under the Curve (AUC) analysis and AUC value in the receiver operating characteristics (ROC) curves showed its good performance as a prognostic marker. To reveal the functional roles of MED30 during the progression of pancreatic cancer, we overexpressed or knocked down MED30 using cDNA or siRNA, respectively. Gain-of-function and loss-of-function studies showed that MED30 can regulate proliferation, migration, and invasion of pancreatic cancer cells. Furthermore overexpression of MED30 promoted tumorigenicity in SCID mice significantly.

Conclusions: MED30 is frequently amplified and overexpressed and its overexpression is associated with poor prognosis of pancreatic cancer patients. Moreover it has tumor-promoting roles in proliferation, migration, invasion and in vivo tumorigenicity of pancreatic cancer cells. Thus, MED30 could be a potent diagnostic and therapeutic target in pancreatic cancer.

Background

Transcriptional regulation is a crucial step for cellular gene expression programs and characteristics of cellular behaviors [1, 2]. Eukaryotic transcription is carried out in the nucleus and proceeds in three sequential stages: initiation, elongation, and termination [3-5]. The transcription factors first bind to the specific DNA elements and to coactivators, and then help recruit general transcriptional factors and RNA polymerase (Pol) II around the transcription start site (TSS), which is also known as the core promoter. The completed assembly of transcription factors, RNA polymerase, and cofactors around the TSS constitutes the transcription pre-initiation complex (PIC) [6, 7]. RNA Pol II molecules begin transcription from the initiation site, generally transcribing 20–50 bp, and then pause control factors cause it stall some tens of base pair downstream. Various transcription factors and cofactors recruit elongation factors to release the pause factors, thereby initiating the elongation stage. After the elongation in a bubble of the unwound DNA, RNA Pol II terminates transcription at the end of the gene being transcribed.

Critical roles of mediators during the transcription initiation and the switch to elongation have been extensively examined [1, 8, 9]. It works as a key coactivator, which facilitates the ability of enhancer-bound transcription factors to recruit RNA Pol II to the promoters of the target genes. Moreover it can stimulate TFIIF kinase-dependent phosphorylation of the Pol II C-terminal domain at the end of preinitiation complex formation. The mediator complex may also regulate Pol II transcription at the stages of post-initiation [9-11]. It promotes efficient transcript elongation by recruiting elongation factors. The mediator complex is composed of up to 30 subunits in mammals. All subunits in the mediator complex can be classified into three distinct structural submodules: head, middle, tail, and kinase module. The head module directly interacts with Pol II, whereas the elongated tail module interacts with trans-activating domains of transcription factors. The middle module acts in regulatory signal transfer at a post-binding stage.

Due to the late onset of symptoms and its high aggressiveness, pancreatic cancer is one of the leading causes of cancer deaths. Because patients usually show metastasis by the time of diagnosis, their five year survival is around 5%. If patients don't have metastatic disease, the surgery will improve the 5-year survival rate to 25% [12]. Clearly, personalized treatment based on new etiological, prognostic, or therapeutic targets will be needed to improve the survival rate of pancreatic cancer.

MED30, an evolutionarily new member of the mediator complex, is a component of head subunit of mediator complex. However, its roles and expression in normal physiology and diseases have been poorly characterized. MED30 homozygous mice showed its roles in regulating mitochondrial functions and integrity [13]. Recently, MED30 has been reported to regulate the proliferation and motility of gastric cancer cells [14]. In the present study, we examined the expression and roles of MED30 in pancreatic cancer.

Materials And Methods

Data analysis

The expression of MED30 in the pancreatic cancer and normal tissues were analyzed using the cBioPortal (<http://www.cbioportal.org/>) and GEPIA (<http://gepia.cancer-pku.cn/>) online platform which includes the cancer datasets from The Cancer Genome Atlas (TCGA) database. Survival analyses were performed to predict the overall survival (OS). Kaplan-Meier survival curves were used to evaluate the discrimination. In the Kaplan-Meier analyses, the median cut-off value was used. Two values were used for the examination of biomarker performance; Uno's C-index in the time-dependent area under the curve (AUC) analysis and AUC values in receiver operating characteristics (ROC) curves at the 5 year mark. These values were obtained in ESurv online platform (<https://easysurv.net/>). We used univariate and multivariate Cox regression analyses to compare the effect of MED30 on prognosis along with other clinical variables. In multivariate analysis, we included clinical variables that are not related to survival in the univariate analysis for considering the confounders. Statistical analyses were performed using SPSS (IBM, ver 25). cBioPortal also shows genomic data including DNA copy-number alteration (CNAs) and

amplification. We used the analytical platform for the automatic calculation of MED30 gene amplification based on the GISTIC2 algorithm. We plotted MED30 gene amplification frequency in pancreatic cancer. Univariate and multivariate Cox regression analysis were performed SPSS (IBM, ver 25) using TCGA dataset.

Cell culture

Pancreatic cancer cell lines (PANC-1, MIA PACA-2, AsPC-1, SNU-213, and Capan-1) were purchased from the Korean Cell Line Bank (Seoul). Cells were cultured and maintained as instructed from the Bank.

Immunohistochemistry

Tissue microarray sections containing pancreatic cancer tissues from patients were obtained from SuperBiochip (Seoul). Histopathologic diagnoses were performed as described before [14]. All patients had histologically confirmed pancreatic cancer, and tumor samples were checked to ensure that tumor tissue composed more than 80% of samples. Remaining procedures were performed as described before [14].

siRNA transfection

SCR and MED30 siRNAs were purchased from Dharmacon (Lafayette, CO, USA) and Bioneer (Daejeon, Korea), respectively. Pancreatic cancer cells were seeded at 2000 cells/well in 96-well plates, or 8×10^4 cells/well in 6-well plates. Cell transfection with MED30 siRNA was performed as described before [14]

Plasmid DNA construction and generation of stable cell lines

To construct the MED30-overexpression vector, the coding sequence of MED30 (cDNA of MED30) was inserted into the 3×-FLAG-CMV10 vector. The MED30 cDNA was purchased from Ultimate™ ORF Clones (Invitrogen). To generate the MED30 overexpressing stable cell line, PANC-1 and MIA PACA-2 were plated at 8×10^4 cells/well in 6-well plates and transfected with the empty vector or the MED30-expressing vector using Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions. After 48 h incubation, G418 (PANC-1, 500 ug/mL; MIA PACA-2, 500 ug/mL; Roche Diagnostics GmbH, Mannheim, Germany) was added to select the transfected cells.

Real-time PCR

To measure the efficiency of MED30 knockdown or overexpression, the MED30 mRNA level was detected by real-time PCR. The total RNA was extracted from cells transfected with siRNA or overexpressing the MED30 vector cells using the RNeasy Mini kit (Quiagen, Valencia, CA, USA). Next, 1 µg of total RNA was used to synthesize cDNA with oligo-dT, dNTP, RNase inhibitor, and MMLV reversetranscriptase (Promega, Madison, WI, USA). For thereal-time PCR, the cDNA was mixed with FastStartEssnetial DNA Green Master (Roche Diagnostics GmbH, Mannheim, Germany)to determine MED30 mRNA levels. Real-time PCR was performed on the LightCycler TM 96 Real-time PCR system (Roche, Nonnenwald, Germany) according to the manufacturer's instructions. Each experiment was performed three times, and GAPDH was used as the internal control. The primer sequences for real-time PCR were as follows: GAPDH forward primer, 5'-TGG TGA CCA GGC GCC CAA TAC G-3'; GAPDH reverse primer, 5'-GCA GCC TCC CGC TTC GCT CT -3'; MED30 forward primer, 5'-ACC GGT TAA CAA AGC TAC AGG A-3'; reverse primer, 5'- TAA GTT GCT CGA CTG GAA TGG G-3'.

Western blotting

The total proteins were extracted from cells lysed with RIPA buffer (Thermo Scientific, Rockford, IL, USA) containing a protease inhibitor cocktail. Protein concentrations were measured using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). Equal amounts of protein samples (30 µg) were separated by SDS-PAGE on 10% acrylamide gel and then transferred to PVDF membranes at 4°C overnight. After transferring, membranes were blocked with 5% BSA in TBS-T buffer for 2 or 3 h, and then incubated with antibodies diluted in TBS-T buffer containing 5% BSA. The antibodies used for western blotting were as follows: anti-MED30 (1:1000;Abcam, Cambridge, MA, USA), anti-FLAG (1:1000; Sigma Aldrich St. Louis, MO, USA), anti-β-actin (1:10,000; Santa Cruz Biotechnology, Dallas, TX, USA).

Boyden chamber assay

The bottom chamber of a transwell chamber was filled with 50 µL of 10% FBS-containing media. The bottom chamber then was covered with collagen-coated membranes (Neuro Probe, Gaithersburg, MD, USA). For collagen coating, 5 mL of PBS containing 0.02 µg/µL of collagen was treated for 30 min. Cells (5×10^4 cells/well) in 50 µL of 0.5% FBS-containing media were seeded in the upper chambers. After 6 or 8 h, the cells that migrated through the membrane were fixed, and the membranes were stained by Diff-quick solution (Sysmex, Kobe, Japan). The stained membranes were rinsed and attached on micro slide glasses (Matsunami glass, Osaka, Japan). The total number of migrated cells was counted using Adobe Photoshop CS6 software. SCR or MOCK values were used as controls to calculate the relative cell migration.

Matrigel invasion assay

To examine the FBS-induced cell invasion, we used 24-well BioCoat™ Matrigel chamber inserts (BD Bioscience, San Jose, CA, USA). The remaining procedures of Matrigel invasion assay were performed as described before [14]

Soft-agar assay

Knockdown or MED30-overexpressing PANC-1 cells were suspended in media containing 0.25% agar and overlaid on 0.5% agar in 6-well plates. After 2 weeks, colonies were stained with 0.005% crystal violet in 10% methanol and photographed.

Xenograft assay

Xenograft assay using the PANC-1 cells stably overexpressing MED30 (MED30-over) and empty control vector-transfected (MOCK) cells were performed as described before [14]. This experiment was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health. The Pusan National University Institutional Animal Care and Use Committee (PNUIACUC) approved the experimental protocol.

Statistical analysis

The Mann-Whitney U-test or the Student's t-test was used to determine the significance of differences between the mean values of the two groups. * and ** indicate a P value of <0.05 and <0.01, respectively. Data were analyzed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA). Results are presented as means ± SEs.

Results

Expression and Prognostic Significance of MED30 in pancreatic cancer

To examine the expression level of MED30 in patients with pancreatic cancer, we analyzed a public database (TCGA, n=179, Table 1) and performed immunohistochemistry (n=20). We compared the expression level of MED30 of pancreatic cancer tissues with normal pancreatic tissues (GTEx Project, n=171) using GEPIA online platform. The comparison showed that MED30 was overexpressed by 2.87 fold in the pancreatic cancer tissues compared with the normal tissues (Fig. 1a. $P < 0.001$).

Immunohistochemistry confirmed the overexpression of MED30 in the pancreatic cancer tissues (Fig. 1f-1h) compared with the normal pancreatic tissues (Fig. 1e) at the protein level.

Table 1
Patients' information used in the current research. * indicates data are missing.

Pancreatic Cancer Cohort (TCGA)			
Patients' information (n=179)	Gender	Male	99
		Female	80
	Age	≤60	59
		60<, ≤70	58
		70<	62
	Stage	Stage I	21
		Stage II	147
		Stage III~IV	9
		StageX*	2

Notably the overexpression of MED30 was significantly associated with the prognosis of pancreatic cancer patients in the Kaplan-Meier curve analysis (Fig. 1b). Higher expression of MED30 showed shorter survival rates in TCGA cohort (Fig. 1b, $P=0.0072$). Its prognostic significance was further validated by the multivariate analysis (Table 2, $P=0.027$).

Table 2
Univariate and multivariate analysis of overall survival in TCGA cohort. * indicates the statistical significance.

Univariate analysis					Multivariate analysis			
Parameters	P	HR	95 CI		P	HR	95 CI	
MED30	0.024*	1.606	1.065	2.422	0.027*	1.590	1.054	2.399
Stage	0.319	1.204	0.836	1.735	0.408	1.176	0.801	1.728
Gender	0.319	1.230	0.818	1.849	0.346	1.218	0.808	1.834
Age	0.022*	1.346	1.043	1.737	0.042*	1.310	1.009	1.699

In addition, the analysis of MED30 in the genetic status of pancreatic cancer patients (TCGA) showed the frequent amplification of MED30 (8.57%, cBioportal) and its association with poor prognosis of pancreatic cancer patients (Data Not Shown). Consistently, the positive correlation between its amplification and mRNA expression was observed (Fig. 1c).

Next we assessed the performance of MED30 as a biomarker by examining the Uno's C-index values in the time-dependent AUC analysis and AUC values in ROC curves (Fig. 1d, Table 3). MED30 showed a high

C-index value for 5 years. Interestingly, its AUC value was more significant in the female group (Table 3).

Table 3
Performance of MED30 as a biomarker in pancreatic cancer patients. AUC(20) indicates AUC value at the 20th month

		AUC(20)	C-index
All		0.62	0.62
Gender	Male	0.53	0.53
	Female	0.73	0.66
Stage	Stage I~II	0.62	0.62
	Stage III~IV	0.86	0.59

Effect of MED30 on the proliferation, migration, and invasion of pancreatic cancer cells

To study functional roles of MED30 in pancreatic carcinogenesis, we performed loss-of-function and gain-of-function experiments on the proliferation, migration, and invasion of five pancreatic cancer cells (PANC-1, MIA PACA-2, AsPC-1, SNU-213, Capan-1). To confirm the efficiency of the knockdown and overexpression of MED30, levels of the mRNA and the protein were examined by real-time PCR or western blotting (Fig. 2a–2b). After these cells were transfected with MED30 siRNA (100 nM), MED30 expression was diminished by about 90% after 2 days versus scrambled (SCR) control siRNA. Furthermore, MED30 overexpression by cDNA transfection in PANC-1 and MIA-PACA1 cells increased MED30 mRNA levels versus the empty control vector-transfected cells (MOCK cells) by more than 20- and 9-fold, respectively. We next investigated the role of MED30 in the proliferation of cancer cells. Proliferation assays were performed 5 days after the transfection with SCR or MED30 siRNA (100 nM). Knockdown of MED30 inhibited the proliferations of pancreatic cancer cells tested (PANC-1, MIA PACA-2, and Capan-1) versus SCR by 22%, 31% and 41%, respectively (Fig. 2c). Furthermore, MED30 overexpression enhanced the growths of PANC-1 and MIA PACA-2 cells by 60% and 25%, respectively, versus MOCK cells.

To reveal roles of MED30 in the migration of pancreatic cancer cells, we used a Boyden chamber assay. MED30 siRNA treatment decreased FBS-induced migrations of AsPC-1 and SNU-213 cells by 70% and 30%, respectively, compared with SCR siRNA (Fig. 3a and 3c). Moreover, the stable overexpression of MED30 increased the migration of PANC-1 and MIA PACA-2 cells by 140% and 150%, respectively, versus MOCK cells (Fig. 3b and 3c). In a Matrigel invasion assay to examine roles of MED30 in the invasion of pancreatic cancer cells, its siRNA decreased FBS-induced invasions of AsPC-1 and SNU-213 cells versus SCR siRNA by 76% and 73%, respectively (Fig. 4a and 4c), and its stable overexpression increased the invasions of PANC-1 cells compared with MOCK cells by 180% (Fig. 4b and 4c).

Effect of MED30 on the colony formation of pancreatic cancer cells

To examine whether MED30 had an effect on colony formation, a soft agar assay was performed using PANC-1 cells. MED30-knocked-down or MED30-overexpressing PANC-1 cells were suspended in media containing 0.25% agar and overlaid on 0.5% agar. Colonies were stained with 0.005% crystal violet and photographed after two weeks of incubation. These results indicated that the number and size of the colonies in soft agar assay formed from MED30-overexpressing cells were increased by 50%, when compared with the MOCK cells. In contrast, knockdown of MED30 decreased the number of colonies by 70% compared to SCR siRNA-treated cells. (Fig. 5a–5c).

Effect of MED30 on the regulation of *in vivo* tumorigenicity

To examine the effect of MED30 on *in vivo* tumorigenicity, we subcutaneously injected MED30-overexpressing PANC-1 cells into SCID mice and observed tumor growth for several weeks. Xenograft tumors were detected within three weeks in both MOCK and MED30-overexpressing groups. However, MED30-overexpressing PANC-1 cells showed faster growth than MOCK cells. Five weeks after the injection, mice were sacrificed, tumors were isolated, and their volumes and weights were examined (Fig. 5e and 5f). The results showed that MED30 overexpression in PANC-1 cells increased tumor volumes and weights.

Discussion

Pancreatic cancer is one of the most aggressive malignancies worldwide. To improve patient outcome in pancreatic cancer, new etiologic, prognostic, or therapeutic targets need to be developed. In the present study, we evaluated the expression status of MED30 and its pathophysiological roles during the progression of pancreatic cancer. We found MED30 regulates the proliferation, migration, and invasion of pancreatic cancer cells and their *in vivo* tumorigenicity. Furthermore, we also found the frequent amplification and overexpression of MED30 and its association with poor prognosis of pancreatic cancer patients (Fig. 1). These results suggested that MED30 might be a good diagnostic and therapeutic target in pancreatic cancer.

Association of MED subunits with cancer progression has been reported in various cancers. Sequencing studies have shown mutations or alterations in various MED subunits (MED1, MED12, MED19, MED23, MED28, CDK8, and cyclin C) [8]. Tumor-promoting roles of MED19 in growth and/or metastasis have been reported in ovarian cancer, gastric cancer, pancreatic cancer, osteosarcoma, and liver cancer [15]. Tumor-promoting roles of CDK8 has been reported in colon cancer via its CDK8 kinase activity [16]. MED23 was associated with lung cancers with hyperactive Ras signaling, and its expression levels was correlated with poor prognosis [17]. Many studies have suggested mediator complex subunits as oncogenic functional components on carcinogenic progress [18, 19], although mechanisms related to

these phenotypes are unknown. Since the mediator complex has been described as the end point of the cell signaling pathway, different transcription factors bind specific mediator subunits to activate transcriptional regulation. In the present study, we found new roles of MED30 in pancreatic cancer. However, its underlying mechanism need to be further investigated in the future.

Several mediator complex-associated signaling pathways have been reported to promote the tumorigenicity of pancreatic cancer [20-22]. The aberrant activation of the hedgehog signaling pathway has been demonstrated during the progression of human pancreatic cancer. Hedgehog signaling pathway-related molecules (IHH, PTCH, SMO, and GLI1) were overexpressed in the pancreatic cancer tissues and pancreatic cancer cells. Inhibition of the hedgehog pathway decreased cell proliferation and EMT, and induced apoptosis[23]. The physical and functional interaction between MED12 and Gli3 was reported. Another pathway important in the progression of pancreatic cancer is the WNT signaling pathway. Knockdown of β -catenin decreased proliferation of pancreatic cancer cells via accelerated apoptosis. The mediator complex has been associated with the WNT pathway (Silencing Med12 Gene Reduces Proliferation of Human Leiomyoma Cells Mediated via Wnt/ β -Catenin Signaling Pathway[24, 25]. Whether MED30 triggers any molecules or signaling pathways involved in pancreatic cancer needs to be further investigated.

Conclusions

MED30 is frequently amplified and overexpressed, and its amplification is associated with poor prognosis of pancreatic cancer patients. Moreover it has tumor-promoting roles in proliferation, migration, invasion and in vivo tumorigenicity of pancreatic cancer cells. Thus, MED30 could be a potent diagnostic and therapeutic target in pancreatic cancer.

List Of Abbreviations

AUC; Area Under the Curve, ROC; receiver operating characteristics, Pol; polymerase, TSS; transcription start site, PIC; pre-initiation complex, TCGA; The Cancer Genome Atlas, OS; overall survival, SCR; scrambled

Declarations

Ethics approval and consent to participate:

animal experiments were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health. The Pusan National University Institutional Animal Care and Use Committee (PNUIACUC) approved the experimental protocol.

Consent for publication:

not applicable

Availability of data and materials:

the data that support the present study are available from the corresponding author upon the reasonable request

Competing interests:

the authors declare no competing financial and non-financial interests.

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Authors' contributions:

conception and design: SOO, LL, MEH. acquisition of data: LL, MEH, GHK, SYP, JYK. analysis and interpretation of data: SOO, YHK, MEH. writing, review of the manuscript: SOO, YHK, MEH, LL. study supervision: SOO. All authors have read and approved the final version of this manuscript.

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Figures

Figure 1

MED30 was overexpressed in pancreatic cancer tissues. (a-c) Immunohistochemical staining showing the MED30 overexpression in pancreatic cancer tissues (b, c) versus normal pancreas tissue (a). dUsing the cBioPortal online platform, the overall survival of pancreatic cancer patients in TCGA was examined by MED30 amplification status. e Using the cBioPortal online platform, the correlation between MED30 gene amplification and the mRNA expression status was plotted. The level of mRNA was presented as the RSEM value normalized from Illumina HiSeq RNA SeqV2. fUsing the Easy Survial online platform, overall survival in TCGA was examined by MED30 expression status.

Figure 2

MED30 regulated the proliferation of pancreatic cancer cells. The level of MED30 expression after the transfection with siRNA or in MED30-overexpressing stable cells, was examined by real-time PCR (a) and western blotting (b). c Effect of MED30 knockdown or overexpression on the proliferation of pancreatic cancer cells (PANC-1, MIA PaCa-2, and Capan-1). Cell viability assays were performed, 5 days after the transfection with 100 nM MED30 siRNA or SCR siRNA, or after 3 days of incubation of the MED30-

overexpressing stable cells. Bar graphs show the means $\pm$ SEs of three independent experiments. *, $P < 0.05$; **, $P < 0.01$ versus SCR or MOCK.

Figure 3

MED30 accelerated the migration of pancreatic cancer cells. Cell migration was evaluated using a Boyden chamber assay. a-b MED30 knockdown with MED30 siRNA significantly inhibited the FBS-induced migration of AsPC-1 and SNU-213 cells compared with those transfected with the scrambled (SCR) siRNA. Scale bar, 100 μ m. c-d MED30 overexpression (MED30-over) significantly increased the migration of PANC-1 and MIA PACA-2 cells compared to the MOCK control. Scale bar, 100 μ m. (b, d) The number of migrated cells was counted, and the MOCK/SCR siRNA-treated cells were used as controls to calculate the percentage of cell migration in the MED30-overexpression/knockdown cells, respectively. Results are presented as a bar graph. Values are the means $\pm$ SDs of three independent experiments performed in triplicate. *, $p < 0.01$ versus SCR or MOCK.

Figure 4

MED30 induced the invasion of pancreatic cancer cells. A Matrigel invasion assay was used to examine the invasive ability of pancreatic cancer cells. a-b Knockdown of MED30 significantly inhibited the FBS-induced invasions of AsPC-1 and SNU-213 cells as compared with the scrambled (SCR) siRNA. Scale bar, 100 μ m. c-d Overexpression of MED30 significantly enhanced cell invasion of PANC-1 cells compared with the MOCK control. Scale bar, 100 μ m. (b, d) The number of invading cells were counted, and the MOCK/SCR siRNA-treated cells were used as controls to calculate the percentage of cell invasion. Results are presented as a bar graph. Values are the means $\pm$ SDs of three independent experiments performed in triplicate. *, $p < 0.01$ versus SCR or MOCK.

Figure 5

MED30 increased colony formation of pancreatic cancer cells and tumor growth in SCID mice. a-d To examine the effects of MED30 overexpression or knockdown on the colony formation of pancreatic cancer cells, PANC-1 cells were transfected with scrambled (SCR) control or MED30 siRNA (a, b), and PANC-1 cells stably expressed the MOCK control or MED30 cDNA (c, d). Obvious effects were observed on the colony forming ability of the pancreatic cancer cells after overexpression or knockdown of MED30. Bar graphs show the means $\pm$ SEs of three independent experiments. *, $P < 0.01$ versus SCR or MOCK. Bar, 100 μ m. e-h To examine the effects of MED30 on the in-vivo tumor growth, MED30-overexpressing or MOCK PANC-1 cells were subcutaneously injected into two sites per SCID mouse separately (left site, MOCK cells; right site, MED30-overexpressing cells, e). The growth of tumor volumes was measured weekly from three to five weeks post-injection (f). Five weeks later, the mice were sacrificed and tumor sizes were measured (g-h).