Extracellular HSP90 α -LRP1 Signaling Promote Pancreatic Cancer Metastasis and Chemoresistance Via Regulating Epithelial-To-Mesenchymal Transition

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Abstract

Extracellular heat shock protein 90 α (HSP90 α) has been reported to promote cancer cell invasion and migration. However, whether pancreatic cancer (PC) cells expressed membrane-bound or secreted HSP90 α and its underlying mechanism for PC progression were still unclear. Our study pointed out that highly invasive Capan2 cells has a higher level of secreted HSP90 α , rather than membrane HSP90 α , compared with those of less invasive PL45 cells. The conditioned medium of Capan2 cells or recombinant HSP90 α protein was able to stimulate the migration and invasion of PL45 or capan2 cells, which could be prevented by a neutralizing anti-HSP90 α antibody. Furthermore, secreted HSP90 α promoted elements of epithelial-mesenchymal transition (EMT) in PL45 cells, including increases in vimentin and snail expressions, decreases in E-cadherin expression and changes in cell shape towards a mesenchymal phenotype, but these phenomena were reversed by anti-HSP90 α antibody in Capan2 cells. In addition, high levels of low-density lipoprotein receptor-related protein 1 (LRP1) mRNA were associated with worsened patient survival in pancreatic adenocarcinoma. LRP1 as a receptor of eHSP90 α for its stimulatory role of PC cells EMT and metastasis by activating AKT signaling. Down-regulation of LRP1 could promote chemosensitivity to gemcitabine and doxorubicin, but not to topotecan and paclitaxel in Capan2 cells. Therefore, our study reveals a critical role of secreted HSP90 α on EMT events and suggests blocking secreted HSP90 α underlies an aspect of metastasis and chemoresistance.

Introduction

Pancreatic cancer (PC), as one of the most devastating of human malignancies, is reported as the fourth leading cause of cancer death in the developed world. Moreover, PC patients show severe resistance to the current chemotherapeutic regimens and develop local recurrence and/or distant metastases following surgery. These result in the limitation of treatment efficacy. The overall five-year survival rate is less than 5%.¹ The process of epithelial-mesenchymal transition (EMT) is suggested to be vital for tumor metastasis, drug resistance and therapeutic perspectives. As described by Gaianigo N and Zhou PT et al, poor prognosis owing to metastatic dissemination in an early event in PC mainly attributed to EMT.^{2,3} The process of EMT is associated with phenotypic conversion of epithelial cells into mesenchymal-like cells in cell culture conditions, which is characterized by a loss of cell-cell adhesion (with cobblestone-like appearance) and the acquisition of motile polarity (with scattered spindle-shaped morphology). This process is generally accompanied by changes in genome and protein levels, including epithelial markers (N-cadherin and ZO-1) and mesenchymal markers (N-cadherin, vimentin, Twist and Snail, etc.).⁴

Heat shock protein 90 α (HSP90 α), as an essential intracellular molecular chaperone, regulates the protein folding, assembly and maturation to maintain cell survival and growth.⁵ In addition to its intracellular function, HSP90 α also expressed on the cell surface and constitutively secreted by many types of cancer, which is known as extracellular HSP90 α (eHSP90 α) to promote cell motility and invasion in several cancer, such as colorectal cancer, prostate cancer, breast cancer and glioblastoma. Whereas, eHSP90 α does not appear with normal tissue in physiologically.^{6,7} Thereby, selectively target eHSP90 α would be

more effective on tumor cells with less toxic than current inhibitors focused on intracellular HSP90 α . Among all the studies on eHSP90 targets, Low density lipoprotein receptor related protein (LRP1, also known as CD91) as a first-reported surface receptor for eHSP90 α has gone into relatively more details.⁸ Several studies reported that upon binding with LRP1, secreted HSP90 α promotes migration and invasion of various of cancer cells through NF- κ B, PI3K/AKT or MAPK-dependent pathway.^{9,10} With the development of protein-protein interaction technology, some other proteins interacted with eHSP90 α have been identified, such as Toll-like receptor family (TLRs), epidermal growth factor receptor family (EGFRs) and Annexin A.^{11,12} For example, Sidera K, *et al*/reported that surface HSP90/HER-2 interaction leads to cytoskeletal rearrangement and cell motility in breast cancer. And, Thiringer D, *et al*/secreted HSP90 α favors cell migration of glioblastoma cells through TLR4-mediated EGFR activation. However, whether and how eHSP90 α regulates the migration and invasion of PC have not been elucidated.

In this study, we found elevated secreted HSP90 α level is associated with the migration and invasion of PC cells. Upon binding to membrane receptor LRP1, secreted HSP90 α elicits PI3K/AKT-dependent pathway to regulate the mesenchymal-like morphology and marker gene expressions (snail and vimentin), leading to PC cells metastasis. In addition, silencing LRP1 could increase drug sensitivity to gemcitabine and doxorubicin in Capan2 cells. Our findings suggested that blocking secreted HSP90 α present effective therapeutic agents for the treatment of metastasis and chemoresistance of PC.

Materials And Methods

Cell culture and chemical reagents. The human PC cell lines (Capan2, Mia-paca2, SW1990, Bxpc3, PANC1 and PL45) were purchased from the American Type Culture Collection (ATCC, Manassas, VA) or Cell Culture Center at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. These cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco®, Life Technologies TM, Carlsbad, USA) supplied with 10% fetal bovine serum (FBS, Gibco), streptomycin (100 μ g/ml) and penicillin (100 U/ml) at 37°C in a humidified atmosphere containing 5% CO₂. Topotecan (TPT), Doxorubicin (ADM), Gemcitabine (GEM) and Paclitaxel (PTX) were obtained from Selleck (Selleck Chemicals, China). Anti-HSP90 α neutralizing antibody was obtained from Enzo Life Sciences (Plymouth Meeting, PA, USA). E-cadherin, Vimentin, Snail, phospho-AKT_{ser473}, AKT, phospho-ERK1/2_{Thr202/Tyr204}, ERK and β -actin were purchased from Cell Signaling Technologies. Antibody to HSP90 α was obtained from Abcam.

Wound-healing assay. Capan2 and PL45 cells were seeded in 6-well plates and cultured at 37°C. After overnight, a wound was generated using a pipette tip to make a straight scratch. The cells were treated with or without anti-HSP90 α antibody or human recombinant HSP90 α (rHSP90 α) protein for 24 h. The width of the wound was measured under a microscope.

Transwell assays. The invasive assay was performed as described using transwell cell culture chambers (8 μ m pore size polycarbonate membrane; Costar, Cambridge, MA). The membrane was coated with Matrigel (BD Biosciences, Bedford, MA). Capan2 and PL45 cells suspension without FBS (200 μ L,

1×10⁶/mL cells) was placed in the upper chamber, while the bottom chamber was filled with 600 µL of culture medium containing 10% FBS. Anti-HSP90α antibody or rHSP90α protein was added to the FBS-free medium in the upper chamber. Cells that migrated to the lower chamber were fixed with 4% paraformaldehyde for 20 min followed by staining with 0.1% crystal violet (Solarbio, Beijing, China)) for 10 min. The cells were randomly photographed under a light microscope.

Cell survival assays. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was adapted to measure cell viability using Capan-2 and PL45 cells. Cells were treated with different concentrations of GEM (0.03~20 µM), TPT (0.03~20 µM), PTX (0.03~20 µM) or ADM (0.003~3 µM) for 72 h. Then, MTT (0.5 mg/mL) solution was incubated for 4 h. The formazan crystals were solubilized in DMSO and measured by at 570 nm using microplate reader (Biotek Instruments, Inc. USA). IC₅₀ values were calculated using GraphPad Prism 8 software.

Quantitative RT-PCR (qPCR). Total RNA was extracted by the Easypure RNA kit (Tansgen Biotech, Beijing China). cDNA was obtained using the TransScript One-Step gDNA Remove and cDNA Synthesis SuperMix kit (Tansgen, Beijing China). PCR amplifications were performed using a SYBR Green PCR Master Mix kit (Cat. QPK-201, Toyobo, Japan) and an ABI PRISM 7900 Sequence Detection system (Applied Biosystems, Foster City, CA, USA). The primers used for qPCR were as follows: LRP1, F, 5'-GATGAGACACACGCCAACTG-3', R, 5'-CGGCACTGGAACATCATCA-3'; E-cadherin, F, 5'-CAATGCCGCCATCGCTTAC-3', R, 5'-ATGACTCCTGTGTTCTGTTAATG-3'; N-cadherin, F, 5'-GACAATGCCCCCTCAAGTGTT-3', R, 5'-CCATTAAGCCGAGTGATGGT-3'; Vimentin, F, 5'-TCCGCACATTTCGAGCAAAGA-3', R, 5'-ATTCAAGTCTCAGCGGGCTC-3'; Snail, F, 5'-GCTCCACAAGCACCAAGAGT-3', R, 5'-ATTCCATGGCAGTGAGAAGG-3'; GAPDH, F, 5'-GAGTCAACGGATTTGGTCGT-3', R, 5'-TTGATTTTGGAGGGATCTCG-3'. GAPDH was used as an internal control. The indicated gene expression was calculated according to the 2^{-ΔΔCt} method.

Western blot assays. Treated and untreated PC cell lysates (30 µg) were subjected to SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, UK). Membranes were blocked and incubated with specific primary antibody (1:1000) and followed by applying with the corresponding HRP-conjugated secondary antibodies. Bands were visualized by ECL detection reagent using Image Quant LAS 4000 (GE Healthcare, Piscataway, NJ, USA). The relative protein levels were calculated based on β-actin as the loading control and were densitometrically analyzed by ImageJ software (NIH, MD).

Antibodies specific for the following factors were used for western blotting: HSP90α, E-cadherin, Vimentin, Snail, phospho-AKT_{ser473}, AKT, phospho-ERK1/2_{Thr202/Tyr204}, ERK and β-actin.

RNA interference assay. Short hairpin RNAs for LRP1 gene were obtained from GenePharma Co., Ltd. (Shanghai, China). shLRP1 plasmid and control shRNA were transfected using Lipofectamine 3000 reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. The targeting sequences are shown as follow. Transfected cells were selected with puromycin. The LRP1 expression was confirmed by qPCR or western blot with specific antibody.

Data mining and bioinformatic analyses. LRP1 mRNA expression data from the PC dataset of The Cancer Genome Atlas (TCGA; <https://tcga-data.nci.nih.gov>) were analyzed using cBioportal for Cancer Genomics (<http://cbioportal.org>) web resources. RNA sequencing data (FPKM values) of gene expression were downloaded from the Genomic Data Commons (GDC, <https://portal.gdc.cancer.gov/>) using the R package TCGAbiolinks and were transformed into transcripts per kilobase million (TPM) values, which are more similar to those resulting from microarrays and more comparable between samples.¹³ The overall survival was investigated using the Kaplan-Meier method with the log-rank test. We set the high and low gene expression level groups by the median value. The overall survival plot was obtained with the hazard ratios (HR) and the 95% confidence interval information. Pearson's correlation analysis and Spearman's rank correlation analysis were conducted for gene expression level score.

Statistical analysis. The results are expressed as the mean values $\pm$ SD. Statistical significance of obtained data was calculated using Student's t test. $P < 0.05$ was considered statistically significant. We used Kaplan-Meier estimation to analyze survival rates and the log-rank test to determine the significance of the differences between two survival curves. A Cox-proportional hazards model was used for the univariate and multivariate analyses, and hazard ratio was calculated using a 95% confidence interval (CI). Correlations coefficients between LRP1 mRNA expression and snail expression were computed by Spearman and distance correlation analyses.

Results

Secreted HSP90 α is elevated in more aggressive PC cells. Our studies were designed to investigate whether extracellular HSP90 α (eHSP90 α), including the membrane-bound and secreted HSP90 α , were expressed on multiple PC cells. Membrane proteins were effectively isolated from six kinds of human cultured PC cells (Bxpc3, Mia-paca2, Capan2, SW1990, PL45 and PANC1) and surface HSP90 α levels were detected by western blotting analysis. As shown in Figure 1A, there were no significant differences in HSP90 α expression on the cell surface of these PC cells. Flow cytometry data indicated that the surface HSP90 α expressions were almost low without significant difference (Supplementary Figure 1A). However, the amounts of secreted HSP90 α proteins were discrepant on above-mentioned PC cells. Capan2 cells secreted amount of HSP90 α protein, yet PL45 cells almost not products eHSP90 α in supernatant of tumor cells (Figure 1B). And, Capan2 cells were more invasive as compared with PL45 cells (Supplementary Figure 1B). These results suggested that higher invasive PC cells had more secreted HSP90 α , but not membrane-bound HSP90 α level.

Secreted HSP90 α promoted PC cells migration and invasion. The stimulatory role of secreted HSP90 α in PC cells migration was investigated by the wound healing assay. We found that the conditional medium (CM) of Capan2 cells or 10 μ g/ml rHSP90 α protein significantly induce the migration of the PL45 and Capan2 cells. And, the change of CM-induced cell migration activity was inhibited by anti-HSP90 α antibody (Figure 2A). The results confirmed that the secreted HSP90 α is responsible for the induction the PC cells migration. Next, the invasive ability of Capan2 and PL45 cells were examined by the transwell assay. As shown in Figure 2B, Capan2 cells were more invasive than PL45 cells. Treatment with rHSP90 α

protein could promote the invasion of Capan2 and PL45 cells. And, anti-HSP90 α antibody significantly inhibited the invasion activities of Capan2 cells. It is important to note that, the effect of rHSP90 α and anti-HSP90 α antibody used in these assays are directed toward cell invasion migration and invasion, not toward cell proliferation, since the absorbances _{λ 570} were almost no apparent differences in Capan2 or PL45 cultures treated as above (Supplementary Figure 2). These results suggested that secreted HSP90 α became as an inducer of PC cells migration and invasion.

Secreted HSP90 α promoted PC cells EMT. One mechanism of extracellular HSP90 α -induced cell motility is through modulating epithelial EMT.^{10,14} To examine whether secreted HSP90 α functionally regulated PC cells motility consistent with activation of an EMT. We firstly detected the EMT hallmark molecules, such as E-cadherin and Vimentin, in above-mentioned six types of PC cells. Interestingly, these E-cadherin over-expressing cells including SW1990, Bxpc3 and PL45 hardly expressed Vimentin, while Capan2, Mia-paca2 and PANC1 cells with high levels of Vimentin barely expressed E-cadherin, indicating that E-cadherin and Vimentin molecules could represent counteractors of EMT markers (Figure 3A). In view of this, we chose Capan2 and PL45 as the cell pair for monitoring EMT events. In our experiments, we found CM or rHSP90 α could induce Vimentin expression and down-regulate E-cadherin levels in PL45 cells (Figure 3B). And, epithelial-like PL45 or SW1990 cells exhibited mesenchymal phenotype after treatment with CM of capan2 or rHSP90 α , such as tightly packed epithelial cobblestone pattern conversion to scattered spindle-shaped phenotype (Figure 3C, Supplementary Figure 3A). In addition, we also observed that the mRNA levels of mesenchymal molecules (such as N-cadherin, Vimentin and Snail) were significantly increased in rHSP90 α -treated PL45 cells (Figure 3D).

Neutralization of secreted HSP90 α induced PC cells MET. Targeting secreted HSP90 α in mesenchymal-like Capan2 cells by anti-HSP90 α neutralizing antibody resulted in a tight cellular cluster resembling epithelial phenotype (Figure 4A). And, the increase of Vimentin and Snail levels by exogenous HSP90 α protein were repressed by anti-HSP90 α antibody and DMAG-N-oxide (NPGA) treatment, especially by HSP90 α neutralizing antibody (Figure 4B). The snail or vimentin mRNA levels were significantly reduced after exposure of NPGA or anti-HSP90 α antibody (Figure 4C). These data indicated that targeting secreted HSP90 α in mesenchymal phenotype could reverse partially EMT events, resulting in MET conversion.

Secreted HSP90 α acts through LRP1 signaling to regulate PC cells EMT. LRP1 is a first-reported cell membrane receptor for extracellular HSP90 α . And, previous studies reported high LRP1 expression was related to tumor cell migration, invasion or poor prognosis, such as breast cancer, glioblastoma and pancreatic ductal adenocarcinoma.^{15–17} Using TCGA datasets, we found that increased LRP1 expression was significantly correlated with decreased patient survival in pancreatic cancer ($p = 0.018$, $n = 176$) (Figure 5A). And, Correlations of LRP1 mRNA expressions and pro-EMT transcription factors were analyzed in data extracted from the TCGA. In this cohort, correlation analysis revealed that LRP1 mRNA expression was significant correlation with Snail mRNA level (Pearson=0.63, $p < 0.01$) (Figure 5B). In our experiments, we use targeted shRNA to effectively knockdown the mRNA and protein levels of LRP1 in Capan2 cells (Figure 5C). Silencing LRP1 significantly decreased the Snail and Vimentin protein

expressions with slightly increase in E-cadherin in Capan2 cells (Figure 5D.E). These data also proved the good correlation between LRP1 and mesenchymal-like transcripts in PC cells. Furthermore, the mRNA levels of mesenchymal makers (Snail and Vimentin) and the invasion ability of Capan2 cells were remarkably reduced after down-regulation of LRP1 (Figure 5E.F). ERK and AKT are known to be downstream signaling molecules of extracellular HSP90 α . Similarly, we found that ERK and AKT activations after treatment rHSP90 α (Figure 5G). And, silencing LRP1 could strikingly decrease Akt phosphorylation but had no obvious inhibitory effect on phosphorylation of ERK, suggesting that eHSP90 α mainly acts through LRP1-mediated AKT signaling pathway (Figure 5H).

Silencing LRP1 could overcome chemo-resistance in metastatic PC cells. We investigated the sensitivity of Capan2 cells transfected with shLRP1 to chemotherapeutic agent: gemcitabine, doxorubicin, topotecan and paclitaxel. The cell viability was determined by MTT assay. As shown in Figure 6A, GEM, ADM and TPT could dose-dependently decreased the viability of Capan2 cells, with the IC₅₀ value range from about 10⁻⁷ to 10⁻⁶ M. And, silencing of LRP1 was observed to promote increased levels of chemosensitivity of Capan2 cells to GEM and ADM, but not to TPT and PTX (Figure 6B-E). Down-regulated LRP1 of Capan2 cells show an IC₅₀ value of 0.127 μ M and 0.777 μ M treated by ADM or GEM, significantly different than the 0.721 μ M and 1.494 μ M value for the Capan2 control cells.

Discussion

Our study provides several insights into the role and molecular mechanism of extracellular HSP90 α on metastasis and chemo-resistance in PC. In this study, we showed that upon binding to LRP1, secreted HSP90 α promoted migration and invasion of PC cells through regulation of EMT marker proteins (Vimentin and Snail) and cell morphology. Inactivation of LRP1 contributed to the acquisition of drug sensitivity.

Although reports demonstrated the ability of extracellular HSP90 α to promote cell motility in several types of cancers,^{6,18,19} a unifying mechanistic basis for eHSP90 α metastatic function in PC has not yet been defined. Our study showed that secreted HSP90 α , but not surface HSP90 α , was increased in metastatic Capan2 cells comparable to levels observed in less invasive PL45 cells. And, rHSP90 α enhanced the migration and invasion of PC cells, further underscoring that secreted HSP90 α is also a potent driver of metastasis in PC. Activation of the EMT program was considered a major driver of tumor progression from initiation to metastasis.^{20,21} We investigated the EMT initiating activity including several critical molecules and cell morphology consistent with this program to support the regulatory ability of secreted HSP90 α . Generally, epithelial markers contain E-cadherin, γ -catenin and zonula occludens-1 (ZO-1), whereas mesenchymal markers include fibronectin, N-cadherin, β -catenin, vimentin, Snail, Twist and Zeb, etc.^{4,18} According to the level of EMT makers in several PC cell lines in our experiments, highly epithelial cell line as reported,^{22,23} such as Bxpc3, has high levels of E-cadherin, while another group of PC cell (Mia-paca2 and PANC-1) exhibited strong expression of vimentin, indicating that E-cadherin and vimentin could be used to a pair of counteract makers of distinguishing the epithelial-mesenchymal phenotype. We

found secreted or ectopic HSP90 α could facilitate the EMT in PL45 cells, whereas anti-HSP90 α neutralizing antibody could reverse this process in Capan2 cells by regulating cellular E-cadherin and vimentin protein expressions. Taken together, we suggested that secreted HSP90 α -induced EMT is contributed for the high propensity of metastasis in PC.

So far, some other proteins (TLRs and EGFRs) binding toward to eHSP90 α had been reported, but LRP1 as the receptor of eHSP90 α is still mainly studied in cancer metastasis.⁷ LRP1 is a ubiquitously expressed surface receptor with recognizing numerous ligands to regulate a wide range of biological functions.²⁴ LRP1 highly expressed in multiple type of cancers, and its mRNA level was associated with patient survival in bladder urothelial carcinoma.²⁵ Our studies supported that high LRP1 mRNA level was associated with worsened patient survival, and LRP1 expression is significantly more highly correlated to the level of Snail in PC cohort from TCGA database. Snail is a zinc transcription factor that mediates EMT in tumor cells. As a repressive transporter of E-cadherin promoter, Snail inhibition is likely to up-regulate E-cadherin expression, resulting in the reversal of EMT phenotype, the reduction of tumor cell migration and proliferation, and the enhancement of drug sensitivity in PC.^{26–28} Our data showed that silencing LRP1 in PC cells is not rate-limiting for suppressing metastasis but highlights its role in chemosensitization, these findings may be related to reduction of the Snail induced EMT program.

As most reported, through the cell membrane receptor LRP1/CD91, eHSP90 α activated PI3K/AKT, ERK or NF- κ B signaling to promote the cell motility.^{10,29} Consistently, we found secreted HSP90 α stimulated the AKT and ERT activation in PC cells. However, genetic silencing of the LRP1 to attenuate the eHSP90 α signaling decreased the levels of phosphorylated AKT, without effect of phosphorylated ERK level. Thus, we suggested that eHSP90 α induced metastasis through LRP1 is dependent on AKT pathway. Furthermore, administration of rHSP90 α in LRP1-knockdown Capan2 cells still reduced epithelial marker (E-cadherin) and increased mesenchymal makers (Snail and Vimentin) gene expressions, indicated that except for the LRP1-AKT-dependent pathway, other interacted protein-mediated signaling would be participated into the secreted HSP90 α -induced EMT. The detailed mechanism still needs to be further explored.

In all, our study highlights the importance of secreted HSP90 α in the metastasis of PC through regulating EMT process partially in a LRP1-AKT-dependent manner. Accordingly, blocking secreted HSP90 α –LRP1 axis might form the basis of new therapeutic strategies that target metastatic or chemo-resistant PC.

Abbreviations

EMT, epithelial-mesenchymal transition; HSP90 α , heat shock protein 90 α ; LRP1, Low density lipoprotein receptor related protein; PC, Pancreatic cancer; TLR, Toll-like receptor; EGFR, epidermal growth factor receptor; TPT, Topotecan; ADM, Doxorubicin; GEM, Gemcitabine; PTX, Paclitaxel

Declarations

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Disclosure

No potential conflicts of interest are disclosed.

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Figures

A **B**

Figure 1

Elevated secreted HSP90α levels in invasive PC cells. (A), 5×10^6 PC cells were plated overnight, then membrane proteins were isolated by thermo scientific Mem-PER Plus membrane protein extraction Kit and surface HSP90α level were detected by western blot analysis. (B), 5×10^6 PC cells were seed in a 10 cm dish with serum starvation for 24 h, cell supernatants were filtered through $0.45 \mu\text{M}$ filters and concentrated by an Amicon Ultracel-30k centrifugal filter (Millipore) for equal volume. The secreted HSP90α levels were detected by western blot analysis.

Figure 2

Secreted HSP90 α induces migration and invasion of PC cells. (A), exogenous HSP90 α increases the migratory activities of PC cells. Migratory ability of PL45 and Capan2 cells were treated with (10 μ g/ml) rHSP90 α and CM with or without anti-HSP90 α antibody for 16 h and analyzed by wound healing assays. (B), rHSP90 α increases cell invasiveness in PC cells. PL45 and Capan2 cells were treated with PBS, rHSP90 α or anti-HSP90 α antibody for 24 h and allowed to invade through Matrigel for 16 h. Invasive cells on the filters of the Transwell inserts were measured under a microscope.

Figure 3

Secreted HSP90 α induces epithelial-mesenchymal transition (EMT) in PC cells. (A), The protein expressions of EMT maker genes (E-cadherin and Vimentin) were examined from six human PC cell lines. (B), The expressions of E-cadherin and Vimentin in CM-treated or rHSP90 α -treated PL45 cells were detected by western blot. (C), Images of PL45 cells morphology after stimulated by CM of Capan2 for 72 h. (D), Relative mRNA levels of genes encoding E-cadherin, N-cadherin Vimentin and Snail in rHSP90 α -treated PL45 cells were detected by qRT-PCR. Data represent the mean $\pm$ SD. * $P \leq 0.05$; ** $P \leq 0.01$.

Figure 4

Anti-HSP90 α neutralizing antibody reverses epithelial-mesenchymal transition (EMT) in PC cells. (A), Changes of cell morphology after administrated with Anti-HSP90 α antibody for 72 h in Capan2 cells. (B), Immunoblot analysis of mesenchymal proteins Vimentin and Snail after treatment with Anti-HSP90 α antibody or NPGA in Capan2 cells. (C), The E-cadherin, N-cadherin Vimentin and Snail mRNA expressions in Anti-HSP90 α antibody-treated or NPGA-treated Capan2 cells were validated by qRT-PCR.

Figure 5

LRP1-mediated AKT signaling is involved in secreted HSP90α induced metastasis and EMT events in PC cells. (A), Kaplan-Meier curves of overall survival probability for low (red line) and high (blue line) LRP1 expression in 176 PC patients using TCGA datasets (log-rank test, $P < 0.05$). (B), Correlation analysis between LRP1 mRNA expression with Snail mRNA level in data extracted from the TCGA. (C), The LRP1 mRNA and protein level after LRP1 knockdown by vector-mediated RNAi. (D), The invasion ability of Capan2 cells after silencing LRP1 by transwell assay. (E) The LRP1, Vimentin, Snail and E-cadherin

protein expressions in stably transfected shLRP1 Capan2 cells. (F), The mRNA levels of E-cadherin, N-cadherin, Snail and Vimentin in stably transfected shLRP1 Capan2 cells or in presence of rHSP90 α were detected by qRT-PCR. (G, H), The AKT and ERK signaling pathway activation in Capan2 cell after rHSP90 α treatment or LRP1 knockdown were detected by western blot.

Figure 6

Silencing LRP1 could overcome chemo-resistance in PC cells. (A), The cell viability of Capan2 was determined by MTT assay after exposure of different chemotherapeutic drugs for 72 h. (B-E), The Capan2

cells stably transfected control shRNA and targeted shLRP1 were administrated different concentration of gemcitabine, doxorubicin, topotecan or paclitaxel for 72 h, the cell viability was detected by MTT assay.

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