HIF-1α links β-adrenoceptor agonists and pancreatic cancer cells under normoxic condition

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Aim: To examine whether β-adrenoceptor (β-AR) agonists can induce hypoxia-inducible factor (HIF)-1α accumulation which then up-regulate the expression of its target genes in pancreatic cancer cells at normoxia, and to further elucidate the mechanism involved.

Methods: Pulse-chase assay, RT-PCR, and Western blot were employed to detect the effects of β-AR agonists and antagonists, siRNA as well as several inhibitors of signal transduction pathways on MIA PaCa2 and BxPC-3 pancreatic cancer cells.

Results: Treatment of pancreatic cancer cell lines with β-AR agonists led to accumulation of HIF-1α and then up-regulated expression of its target genes independently of oxygen levels. The induction was partly or completely inhibited not only by β-AR antagonists but also by inhibitors of PKA transduction pathways and by siHIF-1α. Both β1-AR and β2-AR agonists produced the above-mentioned effects, but β2-AR agonist was more potent.

Conclusion: Activation of β-AR receptor transactivates epidermal growth factor receptor (EGFR) and then elicits Akt and ERK1/2 in a PKA-dependent manner, which together up-regulate levels of HIF-1α and downstream target genes independently of oxygen level. Our data suggest a novel mechanism in pancreatic cancer cells that links β-AR and HIF-1α signaling under normoxic conditions, with implications for the control of glucose transport, angiogenesis and metastasis.

Keywords: β-adrenergic receptor; epidermal growth factor receptor; hypoxia-inducible factor-1α; protein kinase A; ERK1/2; Akt; hypothalamic-pituitary-adrenal axis; pancreatic cancer

Introduction

Stress can alter immune, neurochemical, and endocrine functions, as well as tumor development, but the underlying biological mechanisms are not understood[1]. In response to stressors, activation of the hypothalamic-pituitary-adrenal (HPA) axis leads to release of catecholamines, glucocorticoids and other stress hormones from the adrenal gland as well as from brain and sympathetic nerve terminals. Chronic exposure to these hormones can promote tumor cell growth, migration and invasive capacity, and can stimulate angiogenesis via the induction of pro-angiogenic cytokines.

The effects of catecholamines are primarily mediated by β-adrenergic receptors (β-ARs). These receptors are known to modulate diverse cellular processes including the growth and differentiation of tumor cells. Three β-ARs subtypes have been reported (β1-AR, β2-AR, and β3-AR) though β2-AR appears to be the major mediator of the biological effects of catecholamines[2, 3]. Recent studies in human cancer cell lines and in animal models have shown that adenocarcinoma growth in lung, pancreas and colon is under β-adrenergic control. The β-ARs are widely expressed in adipose tissue, blood, brain, heart, lung, nose, pancreas, skeletal muscle, skin, and vessel. A normal pancreatic duct epithelial cell line and several pancreatic cancer cell lines express β1 and/or β2-ARs as well as epidermal growth factor receptor (EGFR) including Panc-1, BxPC-3, PC-2, PC-3, and HPDE6-c7. Moreover, the EGFR is frequently over-expressed in pancreatic cancer[4-6].

HIF-1 is a heterodimer composed of an inducible HIF-1α subunit and a constitutively-expressed HIF-1β subunit. HIF-1α levels and activity are regulated by cellular oxygen concentration. In human cancer cells, both intratumor hypoxia and genetic alterations affecting signal transduction pathways lead to increased HIF-1 activity, which in turn promotes angiogenesis, metabolic adaptation, and other critical
aspects of tumor progression[7]. Moreover, pancreatic cancer is thought to have a poor blood supply and to be able to survive and proliferate under severe hypoxia. This has been ascribed to hypoxia-induced over-expression of HIF-1α in pancreatic cancer[9], indeed malfunction of the HIF-1α signaling network has been associated with several types of cancer.

HIF-1α protein degradation is regulated by O2-dependent prolyl hydroxylation, which targets the protein for ubiquitylation by E3 ubiquitin-protein ligases. These ligases contain the von Hippel–Lindau tumour-suppressor protein (VHL), which binds specifically to hydroxylated HIF-1α. Ubiquitylated HIF-1α is rapidly degraded by the proteasome at normoxia[9]. However, most pancreatic cancer cell lines (75%) have been shown to weakly accumulate HIF-1α protein under normoxic conditions[10]. It reveals that the existence of HIF-1α protein in the presence of oxygen is not unimaginable.

Agonist binding to the β-AR receptor can transactivate EGFR leading to phosphorylation of Akt and ERK1/2, which are known to promote tumor progression[11, 12]. Coincidently, HIF-1α expression and activity are regulated by major signal transduction pathways including PI3K/Akt/mTOR and Ras/Raf/ERK1/2[7]. We therefore surmised that β-AR activation might lead to HIF-1α accumulation in pancreatic cancer cells via above-mentioned pathways.

To investigate this possibility, we analyzed HIF-1α levels in pancreatic cancer cells exposed to β-AR ligands or hypoxia and elucidate the underlying mechanism involved. As a result, we report that β-AR activation leads to up-regulation of HIF-1α independently of oxygen levels by Akt and ERK1/2. The both pathways are elicited from EGFR, whose transactivation requires PKA. All of above-mentioned pathways further promote the expression of downstream target genes which are responsible for glucose transport, angiogenesis, and metastasis through the heart of this regulatory system-HIF-1α.

Materials and methods

Cell cultures and treatments

The MIA PaCa2 (with K-ras mutation) and BxPC-3 (without K-ras mutation) human pancreatic cancer cell lines (obtained from the American Tissue Type Collection, USA) were maintained in Dulbecco’s modified Eagle’s medium (DMEM, GibCO, USA) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), 0.1 mmol/L nonessential amino acids, 0.2 mmol/L glutamine, 1 mmol/L pyruvate, and 10% fetal bovine serum (FBS) and incubated in a 95% air/5% CO2 humidified atmosphere at 37 ºC. Cells were grown to 80% confluence prior to treatment. For exposure to hypoxia experiment, cells were incubated at 37 ºC in a sealed chamber flushed with 3% O2, 5% CO2 and 92% N2. To strengthen the effect, “serum free hypoxic medium” (less than 0.1% O2) was added to cells during hypoxia. The special medium was achieved using an anaerobic jar equipped with Anaero Pack (O2 absorbing and CO2 generating agent, Mitsubishi Gas Chemical, Tokyo, Japan). As normoxic control, serum free medium was added to cells in normoxic condition (21% O2).

For studies of β-AR agonists, antagonists, other signal transduction pathway inhibitors and hypoxia, media was replaced with basal medium 6 h prior to drug addition and hypoxia treatment. All these drugs were obtained from the Sigma Chemical Co (USA). Inhibitor concentrations employed were LY294002 (10 μmol/L, PI3K inhibitor), PD98059 (10 μmol/L, MAPKK inhibitor), SQ22536 (10 μmol/L, adenyl cyclase inhibitor), H-89 (10 μmol/L, PKA inhibitor), AG1478 (10 μmol/L, selective inhibitor of EGFR-specific tyrosine kinase), metprolol (10 μmol/L, selective β1-AR antagonist), butaxamine (10 μmol/L, selective β2-AR antagonist) or propranolol (10 μmol/L, nonselective β-AR antagonist). These drugs were dissolved in dimethylsulfoxide (DMSO) and added to the cells 30 min prior to treatment with β-AR agonists xamoterol (1 μmol/L, selective β1-AR agonist), salbutamol (1 μmol/L, selective β2-AR agonist), isoproterenol (1 μmol/L, β-ARs agonist) or with the adeny cyclase activator forskolin (1 μmol/L). Epidermal Growth Factor (EGF, 100 ng/mL) was a positive control.

Measurements of cyclic AMP levels

MIA PaCa2 and BxPC-3 cells were seeded in complete medium to reach 80% confluence. The media were replaced with basal medium for 6 h; cells were then pre-incubated for 30 min with 1 mmol/L 3-isobutyl-methylxanthine (IBMX) (Sigma). After 3 washes with basal medium, cells were treated with metprolol (10 μmol/L), butaxamine (10 μmol/L), propranolol (10 μmol/L) or SQ22536 (10 μmol/L). Inhibitors were dissolved in dimethylsulfoxide (DMSO) and added to the cells in fresh basal medium containing 1 mmol/L IBMX 30 min before treatment with xamoterol (1 μmol/L), salbutamol (1 μmol/L) or isoproterenol (1 μmol/L) for 10 min. Forskolin (1 μmol/L) was used as a positive control. After three washes in cold PBS, the concrete operation of cAMP assay was performed following the manufacturer’s instructions.

siRNA Assay

MIA PaCa2 and BxPC-3 cells 2×106 were transfected with siRNA targeted against HIF-1α (100 nm/L) or control siRNA (Qiagen) using Lipofectamine 2000 (Invitrogen). Cells were recovered overnight before starvation, followed by treatment with xamoterol (1 μmol/L) or salbutamol (1 μmol/L) for 12 h, and then harvested for real-time PCR.

Pulse-chase assay

After starvation, both cells were treated with β1-AR or β2-AR agonist for 12 h, hypoxia (3% oxygen, 12 h) or normoxia (12 h), and then washed and incubated in methionine/cysteine-free DMEM supplemented with 0.5% FBS and 50 μCi/mL of [3S] methionine/cysteine Promix (Amersham Biosciences, Buckinghamshire, UK) for 15 min, chased with cold methionine and cysteine, lysed and immunoprecipitated using antibodies against HIF-1α.

RT-PCR (Reverse transcription-polymerase chain reaction) and Real-time PCR

Total RNA from MIA PaCa2 and BxPC-3 cells was iso-
lated using TRIzol reagent (GIBCO BRL). First-strand cDNA was synthesized from 2 μg of total RNA using the RevertAid Kit (Fermentas MBI, USA). The PCR primers designed for β1-AR (236 bp) were forward 5'-CGCCTCTTCGTCTTCTTCAACTG-3' and reverse 5'-ACATCTCCGTGTCGGTCGTAGC-3'; for β2-AR (526 bp) were forward 5'-TGCCAATGAGACCTGCTGTGAC-3' and reverse 5'-TGTCGTCGGTCGGTCGGGAGTTAG-3'; for HIF-1α (179 bp) were forward 5'-CGCAAGTCCTCAAAGCACAGTTAC-3' and reverse 5'-GCAGTGGTAGTGGTGGCATTAGC-3'; for VEGF (140 bp) were forward CTGGGCTGTTCTCGCTTCG-3' and reverse 5'-CTCTTCCTCTCTCTCTCTCTCC-3'; for MMP-9 (111 bp) were forward 5'-TGTCGTCGGTCGGTCGGGTG-3' and reverse 5'-GCCTCCTGTGGAGATTGGG-3'; for GLUT-1 (124 bp) were forward 5'-CCGCTTCCTGCTCATCAACC-3' and reverse 5'-CATCATCTGCGACTCTCTTCC-3'; for CXCR-4 (180 bp) were forward 5'-ACGCCCAACACGTCAGG-3' and reverse 5'-GGAAAACACACACACACACAGT-3'; for β-actin (179 bp) were forward 5'-ATCGGCTGTCGATCAGG-3' and reverse 5'-AGGAAGAAGGGCTGGAAGAT-3'. The concrete operation of RT-PCR and real-time PCR assay was performed following the manufacturer's instructions. The housekeeping gene β-actin was used as an internal reference.

Western blotting

For immunodetection, the primary antibody preparations were as following: anti-HIF-1α (120 kDa, 1:1000, CHEMICON, USA); β1-AR (51 kDa, 1:500, ABCAM, UK); β2-AR (60 kDa, 1:500, ABCAM, UK); p-EGFR (Tyr1173, Tyr1608, Tyr992, 175 kDa, 1:1000, CST, USA); EGFR (175 kDa, 1:1000, CST, USA); p-ERK1/2 (Tyr1173, Tyr1608, 42/44 kDa, 1:1000, CST, USA); ERK1/2 (42/44 kDa, 1:1000, CST, USA); p-Akt (Ser473, 60 kDa, 1:1000, CST, USA); Akt (60 kDa, 1:1000, CST, USA); β-actin (43 kDa, 1:1000, SANTA CRUZ, USA); and the secondary antibody preparation was either anti-rabbit or anti-mouse (1:2000, PIERCE, USA). After drug treatments, the concrete operation of Western blot assay was performed following the manufacturer’s instructions. And the results were visualized using the ECL Western blotting substrate (Pierce) and photographed by GeneBox (SynGene).

Statistics and graphics

Data from at least 3 independent experiments with duplicate determinations are expressed as means±SEM. One-way ANOVA and Tukey-Kramer multiple comparison tests were applied. Statistical significance was set at $P<0.05$.

Results

β1-AR and β2-AR expression in pancreatic cancer cells

We first addressed whether pancreatic cancer cells express the β-ARs. RT-PCR and Western blot analysis confirmed that β1-AR and β2-AR were expressed in both MIA PaCa2 and BxPC-3 cells. The levels of β2-AR mRNA and protein in both cell lines were significantly higher than of β1-AR, indicating that β2-AR may be the predominant β-adrenergic receptor in these pancreatic cancer cells (Figure 1).

Figure 1. Expression of β1-AR and β2-AR at mRNA and protein levels in MIA PaCa2 and BxPC-3 cells. (A) Expression of mRNA for β1-AR, β2-AR, and β-actin in MIA PaCa2 and BxPC-3 cells. Total RNA was isolated and subjected to RT-PCR. The β1-AR primers amplified a 236 bp fragment, the β2-AR primers amplified a 526 bp fragment and the β-actin amplified a 179 bp fragment. Lane 1 MIA PaCa2, β2-AR primers; Lane 2 MIA PaCa2, β2-AR and β-actin primers; Lane 3 MIA PaCa2, β-actin primers; Lane 5 BxPC-3, β2-AR primers; Lane 6 BxPC-3, β2-AR and β-actin primers; Lane 7 BxPC-3, β-actin primers; Lane 9 MIA PaCa2, β1-AR primers; Lane 10 MIA PaCa2, β1-AR and β-actin primers; Lane 11 MIA PaCa2, β-actin primers; Lane 13 BxPC-3, β1-AR primers; Lane 14 BxPC-3, β1-AR and β-actin primers; Lane 15 BxPC-3, β-actin primers; Lane 4, 8, 12, and 16 are negative controls without MMLV reverse transcriptase. (B) Expression of protein for β1-AR, β2-AR and β-actin in MIA PaCa2 and BxPC-3 cells. Total lysate from untreated cells was subjected to Western blot with anti-β1-AR and anti-β2-AR antibody. The housekeeping protein β-actin was used as a control to ensure equal loading of the protein.
β-AR agonists and hypoxia lead to HIF-1α protein other than mRNA accumulation in pancreatic cancer cells

Both kinds of cells were treated with xamoterol, salbutamol or isoproterenol for 0, 4, 8, 12, and 24 h. 3% Oxygen was used as a positive control. Some previous studies have reported stimulatory, inhibitory or no effects of hypoxia on HIF-1α mRNA in different cells\[^{13-15}\]. But in the present study, the mRNA levels of HIF-1α were not significantly affected by either hypoxia or β-AR agonists using real-time PCR assay (Data not shown), indicating that HIF-1α protein accumulation was not due to enhanced mRNA transcription, but rather to later events.

In the absence of β-AR agonists and hypoxia (t=0 time-point) HIF-1α protein was barely detectable in either cell line by means of Western blot. Unlike the mRNA level, exposure (0−24 h) to either β-AR agonists or hypoxia (3% oxygen) led to HIF-1α protein accumulation in both cell lines. After 12 h of exposure to hypoxia and β-AR agonists, HIF-1α levels reached the peak and then decreased. The elevations were statistically significant in all cases (P<0.05). And the induction efficiencies were 3% oxygen>isoproterenol>salbutamol>xamoterol in both cell types (Figure 2). Because induction by the selective β2-AR agonist salbutamol was greater than with β1-AR-specific xamoterol, these results confirm that β2-AR activation is the predominant mediator of HIF-1α, although simultaneous activation of both β1-AR and β2-AR produced greater accumulation than either alone.

HIF-1α protein stability was analyzed by pulse-chase assay. Newly synthesized HIF-1α protein declined after 20 min and was hardly detectable after 60 min in the presence of β1-AR or β2-AR agonist and hypoxia. The normoxia control showed HIF-1α protein half-life was merely 5 min. In order to examining the effects of β-AR agonists on HIF-1α protein synthesis, 10 μg/mL cycloheximide (Chx, the protein translation inhibitor) was used. As a result, HIF-1α protein expression was reduced by cycloheximide indicating that HIF-1α accumulation is also dependent on ongoing protein synthesis (Figure 3).

β-AR agonists and hypoxia induce the expression of HIF-1α target genes

To investigate whether β-AR agonists and hypoxia modulate the expression of known HIF-1α target genes, both kinds of cells were treated with drugs either activating or antagonizing β-adrenergic receptors for 12 h and we also explored drugs targeting associated signaling pathways and siRNA targeting HIF-1α. These treatments were applied alone or in combinations. Hypoxia (3% oxygen, 12 h) was as a positive control.

3% Oxygen and the β-AR agonists xamoterol, salbutamol and isoproterenol all increased mRNA levels of VEGF, MMP-9, GLUT-1, and CXCR4 compared to controls (P<0.05). The relative efficiencies of inducing effect were isoproterenol>salbutamol>xamoterol, again indicating that β2-AR is the main mediator of effect. Interestingly, β-AR agonists increased

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**Figure 2.** Time course of HIF-1α protein levels following treatment with β-AR agonists. (A) MIA PaCa2 and BxPC-3 cells were treated with xamoterol, salbutamol and isoproterenol; 3% oxygen provided a positive control. Protein levels were determined using Western blotting. (B) Quantitation of Western blotting data. Data from at least 3 independent experiments with duplicate determinations are expressed as means±SEM versus controls. \(^{\dagger} P<0.05 \) vs control.
GLUT-1 mRNA independently of oxygen levels: the encoded enzyme is thought to be a key mediator of glycolysis triggered by HIF-1α at hypoxia\[16\].

When applied alone, the inhibitors failed to exert detectable effects on HIF-1α target gene expression; nevertheless, they partly or completely blocked the effects of β-AR agonists. These results indicate that the expression of these downstream genes may be under controls of pathways including PKA, P13K, and ERK1/2. Of all these inhibitors, the blocking effects of PD98059 and LY294002 were weaker than AG1478, indicating that P13K and ERK1/2 together take part in this process, because the two pathways can be activated by EGFR signaling\[17\]. To investigate whether the up-regulation of these genes in the presence of hypoxia or β-AR agonists was dependent on HIF-1α transcriptional activity, siRNA targeting HIF-1α was used. For ruling out the off-target effect, two siRNA sequences (Qiagen, Ng 1 SI00436338 and Ng 2 SI02778090) were used. Both siRNAs efficiently blocked β-AR-agonists-induced enhancement of HIF-1α protein expression in both kinds of cells, whose inhibition rate ranged similarly from 47% to 61%. The Ng 2 sequence was selected to carry out the whole study subsequently (Figure 4). siHIF-1α attenuated the effects of β-AR agonists and led to decrease of these genes (Figure 5).

β-AR agonists and forskolin increase cAMP accumulation
To determine whether β-AR agonists modulate cAMP levels in pancreatic cancer cells, both kinds of cells were treated with different drugs alone or in combinations for 10 min. Forskolin provided a positive control. β-AR antagonists were added to the cells 30 min prior to addition of agonists.

Adeny1 cyclase activator forskolin or with β-AR agonists (xamoterol, salbutamol or isoproterenol) significantly increased the accumulation of cAMP: levels were increased 16.77, 7.06, 9.97, and 14.90-fold in MIA PaCa2 and 14.85, 6.34, 8.03, and 13.40-fold in BxPC-3 compared to controls (P<0.05). The relative efficiencies of inducing cAMP accumulation were forskolin>isoproterenol>salbutamol>xamoterol. Alone, β-AR antagonists and SQ22536 failed to influence cAMP accumulation but could completely block the effects of β-AR agonists (Figure 6). β2-AR still mainly mediated the effect in this assay.

β-AR agonists and EGF increase EGFR phosphorylation
EGFR transactivation was caused by binding of β-AR agonist to its receptor\[5\]. Both cells were treated with β-AR ligands alone or in combinations for 10 min. The β-AR antagonists and H-89 were added to the cells for 30 min before the addition of agonists; EGF (100 ng/mL) was as a positive control.

β-AR agonists failed to increase EGFR expression levels. However, increased phosphorylation was recorded at 3 EGFR sites subjected to tyrosine phosphorylation. All of β-AR agonists increased phosphorylation at Tyr1173, Tyr1608 and Tyr992 to varying degrees (P<0.05). The relative efficiencies of inducing effect were EGF>isoproterenol>salbutamol>xamoterol, which were completely blocked by β-AR antagonists and H-89 (Figure 7). It indicated that β-AR agonists elicit transactivation of EGFR reflected by phosphorylation at Ty1173, Tyr1608, and Tyr992 and this transactivation process requires the key regulatory site-PKA.
β-AR agonists and hypoxia increase the expression of p-ERK1/2, p-Akt, and HIF-1α

Hypoxia can up-regulate both p-ERK1/2 and p-Akt [18–20]. To investigate whether β-AR agonists also activate ERK and Akt pathways, both kinds of cells were treated with different drugs or their combinations for 10 min and then levels of p-ERK1/2 and p-Akt were measured, whereas levels of HIF-1α were determined at 12 h after treatment. β-AR antagonists and other inhibitors were added to the cells 30 min prior to addition of agonists. Hypoxia (3% oxygen) provided a positive control.

While hypoxia and β-AR agonists failed to increase total ERK1/2 and Akt expression levels, they increased p-ERK1/2, p-Akt and HIF-1α. The increases were statistically significant (P<0.05) in all cases. The order of efficiency was 3% oxygen>isoproterenol>salbutamol>xamoterol, again supporting the contention that these effects are mediated principally by β2-AR.

Alone, LY294002 and PD98059 failed to completely block the increases of HIF-1α in response to β-AR agonists (P<0.05), indicating that HIF-1α is simultaneously under control of both PI3K and ERK1/2 pathways. As reported by Ratushny et al that the two pathways can be activated by EGFR signaling[17], AG1478 showed a complete inhibitory effect on p-ERK1/2, p-Akt and HIF-1α in the presence of β-AR agonists, so did H89 (Figure 8). In addition, we have demonstrated that β-AR

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**Figure 5.** Changes in VEGF, MMP9, GLUT-1, and CXCR4 mRNA levels in (A) MIA PaCa2 and (B) BxPC-3 cells following several treatments as indicated below the panel and 3% oxygen provided a positive control. Cells were treated with the drugs alone or in combinations as indicated below the histogram and mRNA levels were quantified by Real-time PCR. Data from at least 3 independent experiments with duplicate determinations were expressed as means±SEM vs controls. bP<0.05 vs control.

**Figure 6.** Intracellular cAMP levels following treatment with β-AR agonists and antagonists. MIA PaCa2 and BxPC-3 cells were treated with a range of activators and inhibitors as indicated below the histogram; forskolin provided the positive control. Data from at least 3 independent experiments with duplicate determinations were expressed as means±SEM vs controls. bP<0.05 vs control.
agonists-induced EGFR phosphorylation is dependent on PKA. Altogether, these findings suggest that β-AR agonists can transactivate EGFR and then elicit Akt and ERK1/2 in a PKA-dependent manner, which together up-regulate levels of HIF-1α and downstream target genes independently of oxygen.

Discussion
The transcriptional regulator HIF-1α is crucial for solid tumor growth and survival, and over-expression has been reported in many human tumors. Indeed, HIF-1α over-expression has been linked to poor patient outcome in several kinds of carcinoma[21]. We report here that hypoxia up-regulates the accumulation of HIF-1α in MIA PaCa2 and BxPC-3 pancreatic cancer cells through posttranscriptional mechanisms. Expression of its known target genes was also increased in response to hypoxia, as was the phosphorylation of ERK1/2 and Akt, indicating that hypoxia may lead to HIF-1α accumulation and then up-regulate its downstream target genes to promote pancreatic cancer progression through the both pathways.

Stress was defined physiologically as the state in which the autonomic nervous system (ANS) and the HPA axis are co-activated[22]. The fight-or-flight stress responses in the ANS or the defeat/withdrawal responses associated with HPA activation result in the secretion of catecholamines (norepinephrine and epinephrine) from sympathetic neurons and the adrenal medulla and of cortisol from the adrenal cortex[23, 24]. Most of the effects of catecholamines are mediated by β-ARs. In addition, it has been suggested that β-ARs play a prominent role in pancreatic cancer, and other studies have implicated β-ARs as important mediators of cancer growth and/or invasiveness in adenocarcinoma of lungs, prostate, colon, stomach, breast, and ovary[25]. In the present study, we showed that β-AR receptor occupancy could up-regulate downstream genes in pancreatic cancer cells, which are responsible for glucose transport, angiogenesis, and metastasis. Of all β-ARs, β2-AR is the main mediator of the effect.

Carie and Sebti demonstrated that inhibition of the Raf-1/Mek-1/ERK1/2 pathway by a β-AR agonist can result in growth inhibition of MDA-MB-231 breast cancer cells in vivo[26]. Shin et al reported that the catecholamines stimulate β2-AR while carcinogens like nicotine can interact with β2-AR to activate the downstream protein kinase C/ERK1/2/cyclooxygenase 2 pathway, leading to the proliferation of gas-
tragic cancer cells\(^{[27]}\). Agonists binding to β-ARs activate signaling by receptor-coupled GTP binding proteins (G-proteins); downstream effectors include adenylyl cyclase, cyclic AMP, and c-SRC. And c-SRC participates in signal transduction mediated by the epidermal growth factor receptor (EGFR) tyrosine kinase and then leads to activation of the Akt and ERK1/2 pathways\(^{[28, 29]}\). Moreover, HIF-1α expression and activity are regulated by major signal transduction pathways including Akt and ERK1/2\(^{[27]}\). It is also showed in the present study that the increase in HIF-1α levels following treatment with β-AR agonists is dependent on ERK1/2 and Akt by a posttranscriptional mechanism. The both pathways are elicited from EGFR, whose transactivation requires PKA.

Our results suggest that HIF-1α, as a potential target for the prevention and therapy, is an interesting and missing link between β-AR and pancreatic cancer development independently of oxygen level. A cohort study of cardiovascular patients receiving β-AR antagonists found, remarkably, that the cancer risk declined by 49% in these patients compared with control patients who did not receive the drugs; there was a 6% decrease in risk for every additional year of β-AR antagonist use\(^{[30]}\). In accordance with this, our data also indicate that the use of β-AR antagonists significantly attenuate the effects of β-AR agonists on increased expression of HIF-1α and its target genes which are related to glucose transport, angiogenesis and metastasis in pancreatic cancer cells. However, it must be recognized that cancer is a multifactorial disease and management may require multimodal therapy. The potential of β-AR antagonists as anticancer therapeutics may be best realized in combination with other treatment modalities including surgery, chemotherapy and radiotherapy.

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Author contribution
Heng-tong HU, Su-gang SHEN, Qing-yong MA, and Ke-ping XIE designed research; Heng-tong HU, Dong ZHANG, Liang HAN, Ya-dong MA, and Ruo-fei LI performed research; Ya-dong MA and Ruo-fei LI contributed part reagents; Heng-tong HU analyzed data; Heng-tong HU wrote the paper.

Abbreviations
HPA axis, Hypothalamic–pituitary–adrenal axis; β-AR, β-adrenergic receptor; EGFR, Epidermal growth factor receptor; HIF-1, Hypoxia-inducible factor 1; IBMX, 3-isobutyl-1-methylxanthine; ERK, Extracellular signal-regulated kinase; Akt, Proteïn kinase B; PI3K, phosphatidylinositol 3-kinase; cAMP: cyclic adenosine monophosphate; PKA, Protein Kinase A; MEK, mitogen activated protein (MAP) kinase; mTOR, mammalian target of rapamycin; Ras, protocogene G protein; Raf, protooncogene serine/threonine kinase; AG1478, 4,6-dimethoxyquinazoline; LY294002, 4-(4-Morpholino)-8-phenyl-1(4H)-benzopyran-4-one hydrochloride; P98,059, 2-(Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; SQ22536, 9-(Tetrahydro-2-furanyl)-9H-purin-6-amine, 9-THF-Adc-H-89, N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide 2HCl hydrate

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