RESEARCH ARTICLE

β-arrestin Promotes c-Jun N-terminal Kinase Mediated Apoptosis via a GABA_BR·β-arrestin·JNK Signaling Module

Jin-Xia Wu[1,2], Feng-Xiao Shan[2], Jun-Nian Zheng[1,2,3]*, Dong-Sheng Pei[1,2]*

Abstract

Evidence is growing that the GABA_B receptor, which belongs to the G protein-coupled receptor (GPCR) superfamily, is involved in tumorigenesis. Recent studies have shown that β-arrestin can serve as a scaffold to recruit signaling protein c-Jun N-terminal knase (JNK) to GPCR. Here we investigated whether β-arrestin recruits JNK to the GABA_B receptor and facilitates its activation to affect the growth of cancer cells. Our results showed that β-arrestin expression is decreased in breast cancer cells in comparison with controls. β-arrestin could enhance interactions of the GABA_BR·β-arrestin·JNK signaling module in MCF-7 and T-47D cells. Further studies revealed that increased expression of β-arrestin enhances the phosphorylation of JNK and induces cancer cells apoptosis. Collectively, these results indicate that β-arrestin promotes JNK mediated apoptosis via a GABA_BR·β-arrestin·JNK signaling module.

Keywords: β-arrestin - GABA_B receptor - JNK - apoptosis

Introduction

As one of the most common types of cancer, breast cancer represents the most prevalent and lethal malignancy among female. Its incidence in China has gradually increased accompanied with the changes in people's lifestyle (Wang et al., 2013). Despite developments in surgery and the increased number of drugs for radio- and chemotherapy, post-operative morbidity and other health problems significantly reduced the life quality of patients (Li et al., 2013). Thus, a better understanding of the molecular mechanism that drives breast cancer growth and invasion is required to promote development of more effective therapies for breast cancer patients.

GABA, also known as γ-aminobutyric acid, was originally identified as a principal inhibitory neurotransmitter which widely distributes in the adult mammalian brain (Watanabe et al., 2002). There are three different types of GABA receptors including ionotropic receptor families GABA_AR and GABA_CR. GABA_AR is commonly used as a drug target in clinical practice, while GABA_CR is found exclusively in the retina. As G protein-coupled receptors (GPCR), GABA_B receptors are seven trans-membrane receptors which are coupled to G-proteins to counteract the activity of beta-adrenoreceptors (β-ARs) in the central nervous system via GABA_B receptor-mediated inhibition of adenylyl cyclase (Chebib et al., 1999; Gladkevich et al., 2006). However, researchers recently have discovered GABA and GABA

receptors also exist in many peripheral tissues and play a pivotal role in many physiological and pathological processes outside the nervous system (Watanabe et al., 2002). Moreover, several reports recently showed that GABA and GABA_BR can reduce the migratory activity in colon carcinoma cells and breast cancer cells (Joseph et al., 2002; Drell et al., 2003). Tatsuta M et al. discovered that the GABA_B receptor agonist baclofen inhibited the growth of colon tumors via the GABA_B receptor (Tatsuta et al., 1992). Consistent with its anti-cancer effect, Schuller HM et al. revealed that GABA_BR inhibited β-ARs signaling in PDAC and pancreatic duct epithelial cells and blocked driving forces of cancer progression (Schuller et al., 2008). These findings raise new questions regarding understanding the function of GABA and GABA receptors and the prevention and treatment of cancer.

As key regulators of GPCR signaling, β-arrestins carry out many important roles in G protein-regulated biological functions (Ferguson, 2001; Lefkowitz et al., 2005). The β-arrestins (β-arrestin 1 and β-arrestin 2) are ubiquitously expressed proteins that are instrumental in negative regulation of GPCR signaling and have been shown to regulate receptor desensitization and internalization (Pfister et al., 1985; Benovic et al., 1987; Goodman et al., 1996; Krupnick et al., 1997; Laporte et al., 2002). β-arrestins also function as signaling scaffolds for the organization of signaling complexes, including Src family members, mitogen-activated protein kinases (Luttrell et al., 1999; DeFea et al., 2000a; DeFea et al.,

[1]*The First Clinical Medical College, Nanjing Medical University, Nanjing,* [2]*Jiangsu Key Laboratory of Biological Cancer Therapy, Xuzhou Medical College,* [3]*Center of Clinical Oncology, Affiliated Hospital of Xuzhou Medical College, Xuzhou, China *For correspondence: jnzheng@xzmc.edu.cn; dspei@xzmc.edu.cn*

2000b). Together with Luttrell and McDonald' study showing that overexpression of ASK1 or activation of angiotensin II type 1α receptor (AT1aR) has led to a significant increase of phosphorylated JNK in the presence of β-arrestin2 and β-arrestin2 and JNK are all distributed in endosomal vesicle compartment, in turn, there is raised the possibility that β-arrestins may mediate the activation of JNK (McDonald et al., 2000; Luttrell et al., 2001).

c-Jun N-terminal kinases (JNKs), as a distant member of the superfamily of the mitogen activated protein kinase (MAPK), is encoded by three distinct genes, namely JNK_1, JNK_2 and JNK_3 (Gupta et al., 1996; Dhanasekaran et al., 1998; Dhanasekaran et al., 2007). Recent studies have shown that JNKs are indispensable for cell apoptosis in mitochondrial intrinsic as well as death receptor-initiated extrinsic apoptotic pathways (Dhanasekaran et al., 2008). Activated JNKs initiate apoptotic pathways by the upregulation pro-apoptotic genes via the transactivation of specific transcription factors or by directly modulating the activities of mitochondrial pro- and anti-apoptotic proteins through distinct phosphorylation events (Fan et al., 2001; Aoki et al., 2002). For example, activated JNK can phosphorylate the transcription factors c-Jun at the N-terminal Ser-63 and Ser-73 residues to up-regulate the pro-apoptotic genes (Hibi et al., 1993; Derijard et al., 1994). Moreover, it can also phosphorylate 14-3-3 and promote Bax translocation to mitochondria, increase the release of cytochrome c and increase caspase-3 activation to induce apoptosis (Tournier et al., 2000; Yuan et al., 2000). Taken together, there may exist the triplicate complex $GABA_BR·β$-arrestin·JNK, which could form a signaling module to involve in the determining cell survival or apoptosis fate.

Despite the fact that β-arrestins participate in the regulation of cell proliferation and apoptosis have been found (Ma et al., 2007), the molecular mechanisms mediating these effects remain at best only partially understood. Furthermore, β-arrestin mediate JNK-induced apoptosis via $GABA_BR·β$-arrestin·JNK signal module has not been investigated. Therefore, we investigated the interaction of β-arrestin with $GABA_BR$ and JNK and consequent proliferation and apoptosis of the breast cancer cells. Our results may indicate that β-arrestin is a potential candidate for a new clinical marker, which should be of great value clinically.

Materials and Methods

Cell lines and transient transfection

MCF-7 (the human breast cancer cell lines), T-47D (the human ductal breast carcinoma cell lines) and HK-2 (the human proximal tubular epithelial cells) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM (Gibco-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Invitrogen, Shanghai, China) and maintained in incubators at 37°C, 5% CO_2. Cells were grown to 90% confluence before being transiently transfected with β-arrestin plasmid using Lipofectamine 2000 (Invitrogen, Shanghai, China) according to the

manufacturer's instructions. Six hours after transfection, the medium containing transfection reagents was removed and incubated in fresh medium. Twenty-four hours after transfection, cells were lysed for Western blotting, and subjected to CCK-8 cell proliferation assay, Annexin V/Propidium Iodide apoptosis assay.

Western blotting

Cells were harvested and washed twice with PBS. Cell lysates were centrifuged at 15,000 g at 4°C for 15 min, and the concentration of protein was determined using the bicinchoninic acid (BCA) Kit (Pierce, USA) according to the manufacturer's instructions. Finally, the protein samples were denatured, electrophoresed on SDS-polyacrylamide gels, transferred to nitrocellulose membranes and incubated overnight at 4°C with the following antibodies: anti-β-arrestin, anti-JNK, anti-p-JNK, anti-$GABA_BR$, anti-β-actin (Santa Cruze, CA, USA). Membranes were then washed and incubated with secondary antibodies (goat anti-mouse IgG and goat anti-rabbit IgG) for 2 h, stained by coloration fluid which contains 10 ml alkaline phosphatase buffer, 66 μl NBT, and 33 μl BCIP, and finally, the membrane is scanned.

Immunoprecipitation

Cells were harvested and lysates (400 μg of protein) were prepared in HEPES buffer (pH7.4), containing 50mM HEPES, 10% glycerol, 1% Triton X-100, 150mM NaCl and 1mM each of EGTA, PMSF, EDTA, Na_3VO_4). Samples were preincubated for 1h with 20 μl protein A agarose at 4°C and then centrifuged to remove proteins adhered non-specifically to protein A. The supernatants were incubated with 1-2 μg antibodies overnight at 4°C. They were then incubated with protein A agarose at 4 °C for 2h before three washes with HEPES buffer. Immunoprecipitates were suspended in sodium dodecyl sulfate (SDS) sample buffer and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and western blotting.

Cell proliferation assay

Cells ($1×10^4$ per well) were seeded in a 96-well culture plate and treated with transfection of β-arrestin. Cell survival rate was detected using 3- (4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay at various time points 24 h, 48 h, 72 h and 96 h according to the manufacturer's instructions. The optical density at 490nm was measured on ELX-800 spectrometer reader (Bio-Tek Instruments, Winooski, USA). Four replicate wells were tested per assay and each experiment was repeated three times.

Annexin V-FITC binding assay

Cell culture and treatment were performed as described above. Cells were washed twice with ice-cold PBS, then incubated with 200 μl 1×binding buffer containing 5 μl Annexin V-FITC, and then in 300 μl 1×binding buffer containing 5 μl Propidium iodide (PI) for 5 minutes at room temperature in the dark. After incubation, cells were visualized under a fluorescence microscope. Under microscopy, 5 fields were randomly selected from every
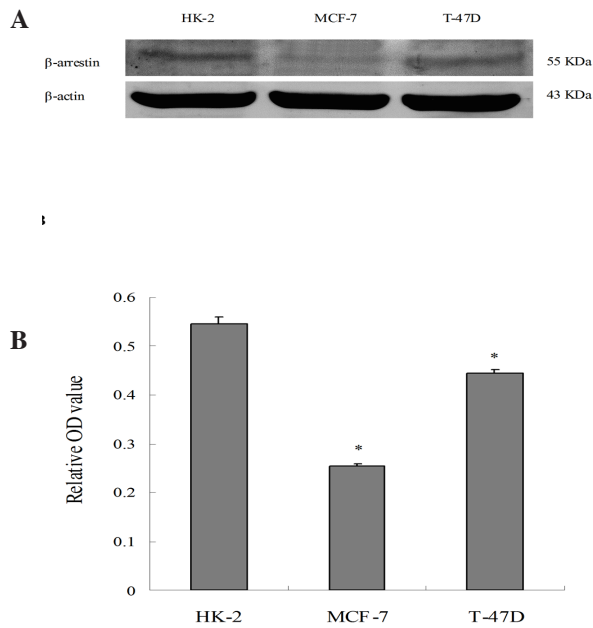
Figure 1. Protein Expression Level of β-arrestin in HK-2, MCF-7 and T-47D Cells. (A) Western blot analysis to evaluate the protein levels of β-arrestin with β-arrestin antibody, β-actin served as a loading control. Results showed that expression of β-arrestin was decreased in breast cancer cells by comparison with normal controls. (B) Densitometric analysis of β-arrestin. The intensity of β-arrestin was quantified by densitometry (software: Image J, NIH). Data are presented as mean ± SD (n =3). *$P < 0.05$

sample and independent observers performed cell counting in a blind fashion.

Statistical analysis

All values are expressed as means ± SD. Student's t-tests or one-way analysis of the variance (ANOVA) was performed for determination of *P* values with SPSS 16.0 software (SPSS). All experiments were performed at least three times unless otherwise indicated. *P*-value<0.05 was considered as statistically significant.

Results

β-arrestin expression is decreased in breast cancer cells

Western blot analysis was used to detect the expression of β-arrestin. β-arrestin was substantially expressed in different cells, while MCF-7 and T-47D cells displayed lower level of β-arrestin expression compared with HK-2 cells (Figure 1A-B) indicating that β-arrestin was commonly expressed in the cells above whereas the expression in breast cancer cells was lower.

Overexpression of β-arrestin facilitates the assembly of GABA_BR·β-arrestin·JNK signaling module and induces the activation of JNK

To look further into the function of β-arrestin, pcDNA3.1-control or pcDNA3.1-β-arrestin plasmids were transiently transfected into MCF-7, T-47D or HK-2 cells. Twenty-four hours after transfection, β-arrestin protein was significantly over-expressed (Figure 2A-B). Transfected cells were subjected to immunoprecipitation (IP) and immunoblotting (IB). To test the hypothesis
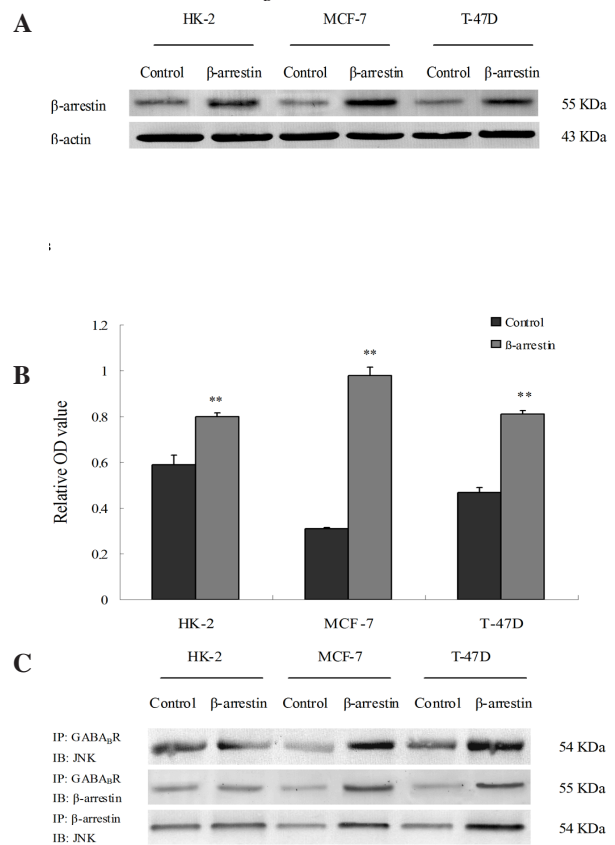

Figure 2. Overexpression of β-arrestin Facilitates the Assembly of GABA_BR·β-arrestin·JNK Signaling Module and Induces the Activation of JNK. (A) Efficient expression of β-arrestin is shown by western blot. Cells were transfected with β-arrestin plasmid. One day after transfection, western blot was used to analyse the prepared cell lysates. Results showed that β-arrestin was significantly over-expressed in HK-2, MCF-7 and T-47D cells. (B) Densitometric analysis of β-arrestin. The intensity of β-arrestin was quantified by densitometry (software: Image J, NIH). (C) After transfection with β-arrestin plasmid, cell lysates was subjected to immunoprecipitation with anti-GABA_BR or anti-β-arrestin antibody. Immunoblot stained with anti-JNK, or anti-β-arrestin antibody. Results showed that overexpression of β-arrestin can significantly increased the levels of GABA_BR binding with JNK or β-arrestin compared with controls. Meanwhile, the amount of β-arrestin combined with JNK has been increased. Data are presented as mean ± SD (n =3). **$P < 0.01$

mentioned previously that the triplicate complex GABA_BR • β-arrestin • JNK may exist so as to facilitate the activation of JNK signaling pathway, we detected the association of JNK and GABA_BR by using GABA_BR antibody following with JNK antibody for immunoprecipitate and immunoblotting, respectively. Correspondingly, the interactions of GABA_BR and JNK with β-arrestin were also detected by the above co-immunoprecipitation. As shown in Figure 2C, the interactions of GABA_BR and JNK with β-arrestin were enhanced in MCF-7 and T-47D cells. However, no coprecipitation of β-arrestin with JNK or GABA_BR was observed in HK-2 cells. As JNK can function as a pro-apoptotic kinase and is involved in other stimuli-induced apoptosis in non-neuronal cells (Davis, 2000; Lin, 2003). We investigated whether overexpression of β-arrestin could enhance the phosphorylation of JNK. Interestingly, we found that in all three cell lines, β-arrestin
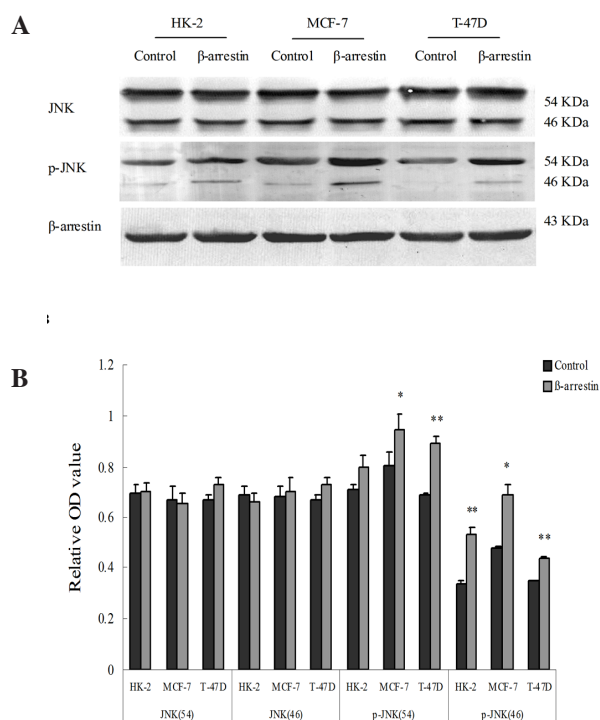
A

HK-2   MCF-7   T-47D

Control β-arrestin Control β-arrestin Control β-arrestin

JNK — 54 KDa / 46 KDa

p-JNK — 54 KDa / 46 KDa

β-arrestin — 43 KDa

B

Figure 3. β-arrestin Overexpression Induces Phosphorylation of JNK. JNK protein levels were determined by immunoblot after overexpression of β-arrestin. (A) The phosphorylation of JNK is elevated in HK-2, MCF-7 and T-47D cells after β-arrestin overexpression. (B) Densitometric analysis of JNK and p-JNK. The intensity of JNK and p-JNK were quantified by densitometry (software: Image J, NIH). Data are presented as mean ± SD (n =3). *$p<0.05$, **$p<0.01$

overexpression could phosphorylate JNK whereas JNK protein level stayed invariable, suggesting that JNK activation was attributed to phosphorylation induced by overexpression of β-arrestin treatment (Figure 3A-B). The data above indicated that β-arrestin overexpression enhanced the activation of JNK via GABA$_B$R·β-arrestin·JNK signaling module in MCF-7 and T-47D cells.

Overexpression of β-arrestin inhibits cell proliferation

To investigate whether β-arrestin-mediated JNK activation could affect cancer cell growth, cell proliferation assay was performed after β-arrestin overexpression in HK-2, MCF-7 and T-47D cells with the absorbance values detected at 24, 48, 72 and 96 h respectively. Results showed that the cell proliferation were similar between the control group and the treatment group after β-arrestin overexpression in MCF-7 and T-47D cells within 24 h ($P> 0.05$), while within 48h, 72h and 96h, the difference were statistically significant in two groups (Figure 4A-B). In contrast, there was no significant difference in HK-2 cells (Figure 4C). Results showed that breast cancer cells proliferation after overexpression of β-arrestin were dramatically inhibited.

Overexpression of β-arrestin induces apoptosis

Since β-arrestin overexpression could promote JNK phosphorylation, we next demonstrated whether JNK activation could affect cell apoptosis in MCF-7 and T-47D cells. Annexin V-FITC binding assay was used to observe apoptotic cells by fluorescence microscope.
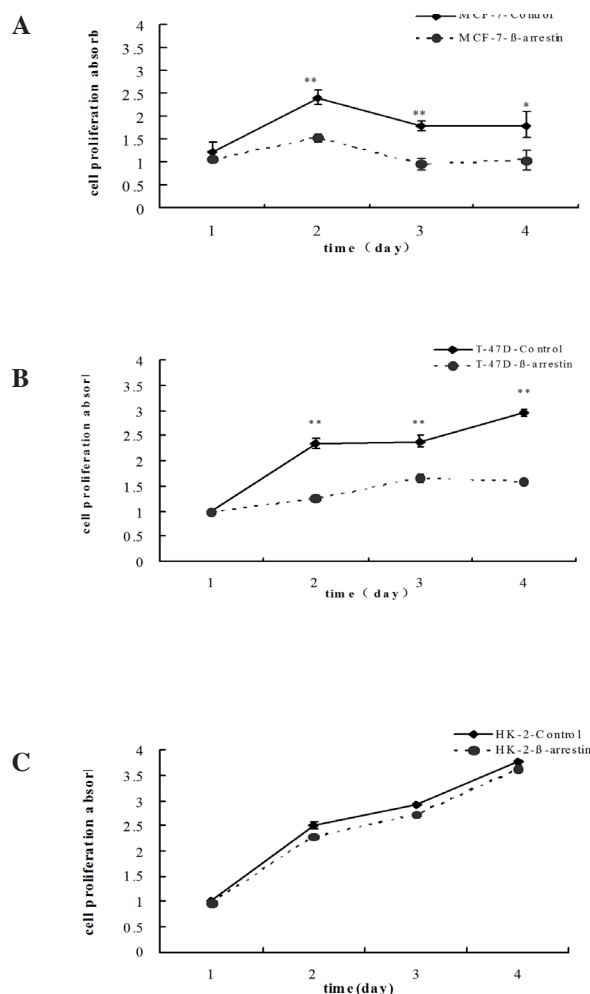
A

B

C

Figure 4. Effects of β-arrestin Overexpression on the Proliferation of Cells. (A-B) Overexpression of β-arrestin could markedly inhibit the survival of cancer cells at 24, 48 and 72 hours. However, there was no significant difference observed in HK-2 cells (C). Data are presented as mean ± SD (n =3). *$p<0.05$, **$p<0.01$

Our results showed that high levels of Annexin V-FITC positive cells existed in the treatment groups after β-arrestin overexpression when compared with control groups in MCF-7 and T-47D cells at prophase (Figure 5A-B) and anaphase (Figure 5C-D). In contrast, HK-2 cells after overexpression of β-arrestin did not show any change in the number of apoptotic cells (Figure 5A-D). Together, these data demonstrated that β-arrestin is critically important for cell survival and overexpression of β-arrestin could promote cell apoptosis in MCF-7 and T-47D cells.

Discussion

The discovery of β-arrestins resulted from the observation that β adrenergic receptor kinase progressively lost the ability to desensitize G protein activation in a reconstituted β-2 adrenergic receptor system (Benovic et al., 1987). The β-arrestins are ubiquitously expressed proteins that play an important role in regulating signal transduction by numerous G-protein-coupled receptors (Lefkowitz et al., 2005; Reiter et al., 2006). Here we demonstrated that the expression of β-arrestins was
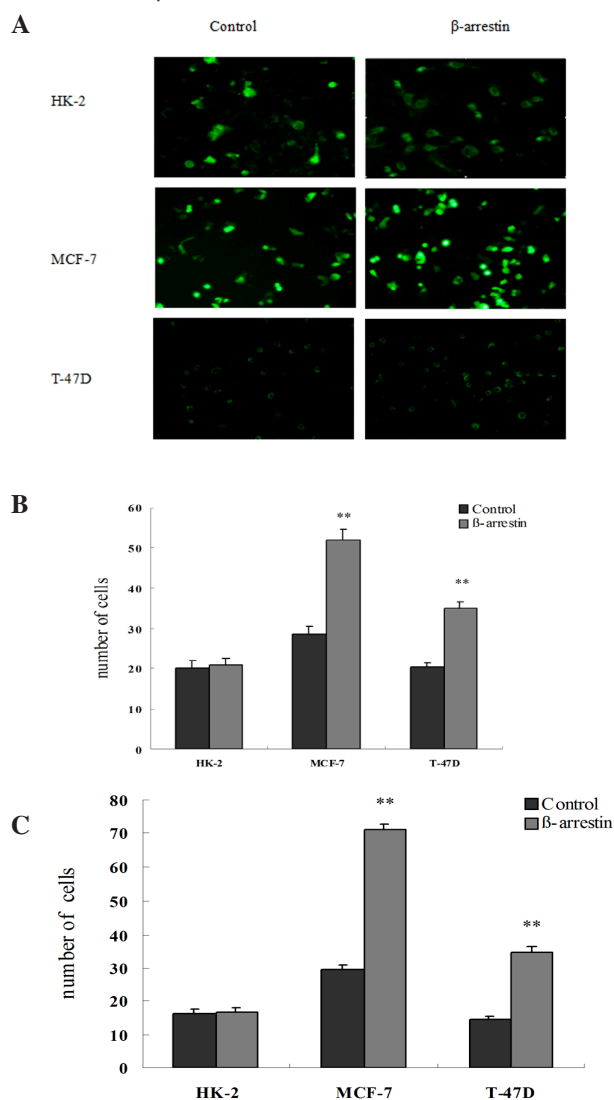
A

B

C

Figure 5. Effects of β-arrestin Overexpression on Cellular Apoptosis. Cells were analyzed for apoptosis by Annexin V-FITC binding assay after overexpression of β-arrestin and five fields were randomly selected to count the number of apoptotic cell. (A-B) Prophase apoptotic cells were recognized by binding with FITC on the membrane (cell membrane displays green). (C-D) Anaphase apoptotic cells were recognized by binding with FITC and PI on the nuclei (nuclei displays red). Data are presented as mean ± SD (n =3). **$p<0.01$

significantly decreased in MCF-7 and T-47D cells than in HK-2 cells, which was consistent with studies by Wu and colleagues indicated that the serum level of β-arrestin2 in non-small-cell lung cancer patients were all significantly lower than those in healthy controls (Wu et al., 2011).

Recent evidence shows that β-arrestins can also serve as a scaffold to recruit endocytic proteins and a variety of signaling molecules to the receptors, thus connecting GPCR to JNK$_3$ and p38 of MAPK family subsequently (McDonald et al., 2000; Bruchas et al., 2006). In addition, GABA$_B$ receptor is a member of G protein-coupled receptor family and is therefore related to chemokine receptors and catecholaminergic receptors, which both have been shown to be involved in the regulation of tumor cell migration (Entschladen et al., 2002). Thus, it is closely linked GABA$_B$ receptor to β-arrestins and JNK. Results of reciprocal co-IP and IB showed that

β-arrestin overexpression in MCF-7 and T-47D cells not only increased the interactions of GABA$_B$R with JNK and β-arrestin, but also increased the interaction of β-arrestin and JNK. At the same time, results from western blot provided strong evidence that overexpression of β-arrestin induced JNK activation. These results suggested that β-arrestin recruited JNK to GABA$_B$R to promote the activation of JNK in breast cancer cells. In contrast to β-arrestin-mediated JNK activation, there does not exist GABA$_B$R·β-arrestin·JNK signaling module in HK-2 cells.

Deregulation of apoptosis has been implicated in cancer (Rinkenberger et al., 1997). The initiation and execution of apoptosis is regulated by many intracellular signaling pathways, including the JNK pathway (Davis, 2000). JNKs belong to the superfamily of MAP-kinases and are indispensable for both cell proliferation and apoptosis. Whether JNKs activation leads to cell proliferation or apoptosis is dependent on the cell type, nature of the death stimulus (Lin et al., 2002; Liu et al., 2005). Current study showed that the activation of JNK inhibited breast cancer cells proliferation and resulted in the apoptosis of tumor cells. Notably, β-arrestin overexpression promoted the phosphorylation of JNK in HK-2 cells, while JNK activation did not modulate the apoptosis of HK-2 cells. The possible explanation about the discrepancy may be attributed to different ways in gene expression regulation of β-arrestin and nature of the death stimulus. These data also suggested the reduction of β-arrestin may contribute to the development of breast cancer. Therapeutic administration of β-arrestins selective agonist may thus provide an effective novel tool for the treatment and prevention of breast cancer.

In conclusion, our data provides evidence that the expression of β-arrestin is decreased in breast cancer cells, and overexpression of β-arrestin recruits JNK to GABA$_B$R to promote the activation of JNK. The subsequent activation of JNK can induce the apoptosis of breast cancer cells. It suggests that β-arrestin overexpression promotes JNK mediated apoptosis via GABA$_B$R·β-arrestin·JNK signaling module. Thus, β-arrestin may provide a promising therapeutic approach for breast cancer.

Acknowledgements

References

Aoki H, Kang PM, Hampe J, et al (2002). Direct activation of mitochondrial apoptosis machinery by c-Jun N-terminal kinase in adult cardiac myocytes. *J Biol Chem*, **277**, 10244-50.

Benovic JL, Kuhn H, Weyand I, et al (1987). Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). *Proc Natl Acad Sci U S A*, **84**, 8879-82.

Bruchas MR, Macey TA, Lowe JD, et al (2006). Kappa opioid receptor activation of p38 MAPK is GRK3- and arrestin-dependent in neurons and astrocytes. *J Biol Chem*, **281**, 18081-9.

Chebib M, Johnston GA (1999). The 'ABC' of GABA receptors: a brief review. *Clin Exp Pharmacol Physiol*, **26**, 937-40.

Davis RJ (2000). Signal transduction by the JNK group of MAP kinases. *Cell*, **103**, 239-52.

DeFea KA, Vaughn ZD, O'Bryan EM, et al (2000a). The proliferative and antiapoptotic effects of substance P are facilitated by formation of a beta -arrestin-dependent scaffolding complex. *Proc Natl Acad Sci U S A*, **97**, 11086-91.

DeFea KA, Zalevsky J, Thoma MS, et al (2000b). beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated ERK1/2. *J Cell Biol*, **148**, 1267-81.

Derijard B, Hibi M, Wu IH, et al (1994). JNK_1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell*, **76**, 1025-37.

Dhanasekaran DN, Kashef K, Lee CM, et al (2007). Scaffold proteins of MAP-kinase modules. *Oncogene*, **26**, 3185-202.

Dhanasekaran DN, Reddy EP (2008). JNK signaling in apoptosis. *Oncogene*, **27**, 6245-51.

Dhanasekaran N, Premkumar Reddy E (1998). Signaling by dual specificity kinases. *Oncogene*, **17**, 1447-55.

Drell TL, Joseph J, Lang K, et al (2003). Effects of neurotransmitters on the chemokinesis and chemotaxis of MDA-MB-468 human breast carcinoma cells. *Breast Cancer Res Treat*, **80**, 63-70.

Entschladen F, Lang K, Drell TL, et al (2002). Neurotransmitters are regulators for the migration of tumor cells and leukocytes. *Cancer Immunol Immunother*, **51**, 467-82.

Fan M, Chambers TC (2001). Role of mitogen-activated protein kinases in the response of tumor cells to chemotherapy. *Drug Resist Updat*, **4**, 253-67.

Ferguson SS (2001). Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev*, **53**, 1-24.

Gladkevich A, Korf J, Hakobyan VP (2006). The peripheral GABAergic system as a target in endocrine disorders. *Auton Neurosci*, **124**, 1-8.

Goodman OB, Jr, Krupnick JG, et al (1996). Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. *Nature*, **383**, 447-50.

Gupta S, Barrett T, Whitmarsh AJ, et al (1996). Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J*, **15**, 2760-70.

Hibi M, Lin A, Smeal T, et al (1993). Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev*, **7**, 2135-48.

Joseph J, Niggemann B, Zaenker KS, et al (2002). The neurotransmitter gamma-aminobutyric acid is an inhibitory regulator for the migration of SW 480 colon carcinoma cells. *Cancer Res*, **62**, 6467-9.

Krupnick JG, Goodman OB, Jr, Keen JH, Benovic JL (1997). Arrestin/clathrin interaction. Localization of the clathrin binding domain of nonvisual arrestins to the carboxy terminus. *J Biol Chem*, **272**, 15011-6.

Laporte SA, Miller WE, Kim KM, Caron MG (2002). beta-Arrestin/AP-2 interaction in G protein-coupled receptor internalization: identification of a beta-arrestin binging site in beta 2-adaptin. *J Biol Chem*, **277**, 9247-54.

Lefkowitz RJ, Shenoy SK (2005). Transduction of receptor signals by beta-arrestins. *Science*, **308**, 512-7.

Li J, Jia S, Zhang W, et al (2013). Survival Analysis Based on Clinicopathological Data from a Single Institution: Chemotherapy Intensity Would Be Enhanced in Patients with Positive Hormone Receptors and Positive HER2 in China Who Cannot Afford the Target Therapy. *ISRN Oncol*, **2013**, 606398.

Lin A (2003). Activation of the JNK signaling pathway: breaking the brake on apoptosis. *Bioessays*, **25**, 17-24.

Lin A, Dibling B (2002). The true face of JNK activation in apoptosis. *Aging Cell*, **1**, 112-6.

Liu J, Lin A (2005). Role of JNK activation in apoptosis: a double-edged sword. *Cell Res*, **15**, 36-42.

Luttrell LM, Ferguson SS, Daaka Y, et al (1999). Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science*, **283**, 655-61.

Luttrell LM, Roudabush FL, Choy EW, et al (2001). Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc Natl Acad Sci U S A*, **98**, 2449-54.

Ma L, Pei G (2007). Beta-arrestin signaling and regulation of transcription. *J Cell Sci*, **120**, 213-8.

McDonald PH, Chow CW, Miller WE, et al (2000). Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK_3. *Science*, **290**, 1574-7.

Pfister C, Chabre M, Plouet J, et al (1985). Retinal S antigen identified as the 48K protein regulating light-dependent phosphodiesterase in rods. *Science*, **228**, 891-3.

Reiter E, Lefkowitz RJ (2006). GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab*, **17**, 159-65.

Rinkenberger, J. L., and Korsmeyer, S. J. (1997). Errors of homeostasis and deregulated apoptosis. *Curr Opin Genet Dev*, **7**, 589-96.

Schuller, H. M., Al-Wadei, H. A., and Majidi, M. (2008). GABA B receptor is a novel drug target for pancreatic cancer. *Cancer*, **112**, 767-78.

Tatsuta, M., Iishi, H., Baba, M., and Taniguchi, H. (1992). Attenuation by the GABA receptor agonist baclofen of experimental carcinogenesis in rat colon by azoxymethane. *Oncology*, **49**, 241-5.

Tournier, C., Hess, P., Yang, D. D., Xu, J., Turner, T. K., Nimnual, A., Bar-Sagi, D., Jones, S. N., Flavell, R. A., and Davis, R. J. (2000). Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science*, **288**, 870-4.

Wang, X., Song, Z. F., Xie, R. M., Pei, J., Xiang, M. F., and Wang, H. (2013). Analysis of death causes of in-patients with malignant tumors in Sichuan Cancer Hospital of China from 2002 to 2012. *Asian Pac J Cancer Prev*, **14**, 4399-402.

Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., and Hayasaki, H. (2002). GABA and GABA receptors in the central nervous system and other organs. *Int Rev Cytol*, **213**, 1-47.

Wu, Z., Tong, W., Tan, Z., Wang, S., and Lin, P. (2011). [The clinical significance of beta-arrestin 2 expression in the serum of non-small cell lung cancer patients]. *Zhongguo Fei Ai Za Zhi*, **14**, 497-501.

Yuan, J., and Yankner, B. A. (2000). Apoptosis in the nervous system. *Nature*, **407**, 802-9.