β-Arrestin 1 is required for endothelin-1-induced NF-κB activation in ovarian cancer cells

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ABSTRACT

Aims: In epithelial ovarian cancer (EOC), activation of endothelin-1 (ET-1)/endothelin A receptor (ET$_A$R) signalling is linked to many tumor promoting effects, such as proliferation, angiogenesis, invasion and metastasis. These effects are dependent by the activation of critical signalling pathways, such as MAPK, Akt, and β-catenin, through specific cytosolic and nuclear scaffolding functions of β-arrestin 1 (β-arr1). Here, we have assessed the potential role of ET-1/ET$_A$R in promoting NF-κB signalling in EOC cells through β-arr-1 recruitment.

Main methods: We used cultured HEY EOC cells cultured in the presence or absence of ET-1 and the ET$_A$R antagonist BQ123. The phosphorylation of p65 and Iκ-Bα was evaluated by immunoblotting analysis. The interaction between p65 and β-arr1 was evaluated by immunoprecipitation experiments in nuclear extracts. NF-κB promoter activity was evaluated by transfection with NF-κB-driven luciferase reporter construct. Assessment of the function of β-arr1 was achieved by β-arr1 silencing with shRNA and expression of β-arr1-FLAG expression vector.

Key findings: In EOC cells, ET-1 promotes the phosphorylation of p65 subunit and the cytoplasmic inhibitor IκB that in turn led to increased NF-κB transcriptional activity. These effects were inhibited by the use of BQ123, as well as by β-arr-1 silencing, suggesting that ET-1 through ET$_A$R promotes the recruitment of β-arr1 to regulate NF-κB signalling. Moreover, the nuclear physical interaction between p65 and β-arr1 indicates a nuclear function of β-arr-1 in ET$_A$R-driven NF-κB transcriptional activity.

Significance: Altogether these findings reveal a previously unrecognized pathway that depends on β-arr1 to sustain NF-κB signalling in response to ET$_A$R activation in ovarian cancer.

Introduction

It has been shown that endothelin-1 (ET-1)/endothelin A receptor (ETAR) system plays an important role in tumorigenesis and progression in many types of human cancers, including epithelial ovarian cancer (EOC), representing a key driver in promoting cell proliferation, escape from apoptosis, angiogenesis, invasion, and metastasis (Rosanò et al., 2013a). Moreover, ET receptors are the first family of G protein-coupled receptors (GPCRs) that have the prospect of being targeted for cancer therapy (Bagnato et al., 2011). Stimulation of EOC cells with

ET-1 initiates a series of signalling cascades and leads to activation of several transcription factors and intracellular pathways, including MAPK, Akt, and β-catenin (Rosanò et al., 2013a). Although ET-1 activates these signalling cascades by binding to its GPCRs, ET$_A$R and ET$_B$R, on the cell surface, and by association with the trimeric G protein complex, emerging evidences indicate that ET-1 receptors also associate with β-arrestin (β-arr) upon stimulation of ET-1 (Shenoy and Lefkowitz, 2011; Rosanò et al., 2009, 2013b; Spinella et al., 2013). β-arr-1 and β-arr-2 regulate the function of most GPCRs and are expressed ubiquitously (Shukla et al., 2011). It has been reported that β-arrs are involved in various signalling pathways, leading to the activation of MAPK, PI3K and Akt (Shenoy and Lefkowitz, 2011; Shukla et al., 2011). In particular, it has been demonstrated that β-arr-1 and -2 are scaffold proteins that bind to ET$_A$R and ET$_B$R and that serve as molecular 'hubs' to organize complex signalling networks, leading to activation of β-catenin signalling and to the transactivation of receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 3 (VEGFR3),

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and downstream MAPK and Akt (Rosanò et al., 2009; Spinella et al., 2013; Cianfrocca et al., 2010).

In different cancer cells, the binding of ligands, such as ET-1, with several GPCR can induce the activation of nuclear factor κB (NF-κB), a family of transcription factors that exist as various homo- and heterodimers (Morinelli et al., 2013; Gupta et al., 2012; Delekta et al., 2010 Dec 31; Sun and Lin, 2008; Kizaki et al., 2009; Yang et al., 2009). As for many activators of NF-κB, this occurs through the so-called canonical pathway, whereby activation depends upon stimulation of the IκB kinase (IKK) complex (DiDonato et al., 2012). In unstimulated cells, NF-κB is associated with a family of inhibitor protein, IκB, which masks the nuclear localization signal of NF-κB, thereby sequestering NF-κB (particularly in the form of RelA/p65) in the cytoplasm. Various stimulation signals activate the IKK complex, which phosphorylates IκBα and rapidly triggers a ubiquitination-mediated degradation of IκBα. Furthermore, phosphorylation of the p65 subunit in one of two of its transactivation domains has been shown to be essential for NF-κB transcriptional activation (Naumann and Scheidereit, 1994). The degradation of IκBα releases NF-κB, which translocates into the nucleus and binds to the cognate sequences in the promoter of its target genes (Moreno et al., 2010), leading to the modulation of various biological responses in cancer, including cell survival and proliferation, immunity, and inflammation (Elinav et al., 2013). ET-1 induces NF-κB p65 via ETₐR in various tumor cell lines (von Brandenstein et al., 2008). A pioneer study provides evidence that in prostate cancer cells ET-1 activates NF-κB through autocrine loop and that an ETₐR antagonist abrogates NF-κB activation favoring proapoptotic environment, leading to greater killing when combined with chemotherapeutic agents (Banerjee et al., 2007; Mangelus et al., 2001). Moreover, it has been demonstrated that activation of NF-κB might be involved in the induction of migration activity caused by ET-1 stimulation in cancer cells (Wu et al., 2012). However, the molecular mechanism of ET-1-induced NF-κB activation is not fully defined. In this work, we have investigated the role of β-arr-1 in NF-κB activation induced by ET-1. We found that β-arr-1 functions as a positive regulator for ET-1-induced NF-κB activation in ovarian cancer cells. Moreover, we demonstrated that the specific inhibition of ETₐR by the receptor antagonist BQ123, by blocking the interaction of ETₐR with β-arr-1, controls the transcriptional activation of NF-κB.

Materials and methods

Cell lines and reagents

The established human ovarian serous adenocarcinoma cell line, HEY, obtained by Prof. Giovanni Scambia (Catholic University School of Medicine, Rome, Italy), and cultured as previously described (Rosanò et al., 2009), was passaged in our laboratory for fewer than 3 months after resuscitation. All cells were tested routinely for cell proliferation (^{3}H-thymidine incorporation) as well as mycoplasma contamination, and they showed similar growth rate and negative mycoplasma during the experiments. Cells were serum starved by incubation 24 h in serum-free medium prior to each experiment. ET-1 (100 nM) and BQ123, Cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu) (1 μM) were purchased from Bachem (Switzerland). NFκB inhibitory ligand (36 μM) was purchased from Upstate (Millipore).

Quantitative real-time-PCR

Total RNA was isolated using the TRIzol (Invitrogen) according to the manufacturer's protocol. 5 μg of RNA was reversed transcribed using the SuperScript® VILO™ cDNA synthesis kit (Invitrogen). Quantitative real-time-PCR was performed by using the LightCycler rapid thermal cycler system (Roche Diagnostics) according to the manufacturer's instructions. The number of each gene-amplified product was normalized to the number of cyclophilin-A amplified product. Each qPCR analysis was done twice separately.

The primer sets used were as follows:

ETₐR F: GTCTGCTGTGGGCAATAGTTG

ETₐR R: GCTTCCTGGTTACCACTCATCAA

ET_BR F: TCAACACGGTGGTGTCCTGC

ET_BR R: ACTGAATAGCCACCAATCTT

Bcl-2 F: CTGCACCTGACGCCCTTCACC

Bcl-2 R: CACATGACCCCACCGAACTCAAAGA

MMP-2 F: GGATGATGCCTTTGCTCG

MMP-2 R: ATAGGATGTGCCCTGGAA

Cyclophilin F: TTCATCTGCACTGCCAAGAC

Cyclophilin R: TCGAGTTGTCCACAGTCAGC

RNA silencing

The lentiviral-based short hairpin RNAs (shRNA) plasmids (pLKO.1 plasmids) used to knockdown β-arr1 were purchased from Sigma. Five plasmid-clones were tested for their knockdown efficiency (TRCN0000230149; TRCN0000230147; TRCN0000005160; TRCN0000005161; TRCN0000230150). Non-target shRNA control vector (SRC) was used as negative control. Transient transfection was performed by adding 2 μl/well of shRNA plasmid along with 5 μl/well LipofectAMINE 2000 (Invitrogen) in 6-well plates for 48 h.

β-Arr1 plasmid and transfection constructs

For exogenous expression of β-arr1, we used pcDNA3-β-arr1-FLAG (wild type) plasmid construct, a "wobble" mutant construct encoding rat β-arr1 sequences resistant to siRNA targeting kindly provided by Dr. Robert Lefkowitz (Howard Hughes Medical Institute, Duke University). For transient expression of β-arr1-FLAG, 2 μg of each construct was transfected in cells using LipofectAMINE 2000 reagent (Invitrogen) following the manufacturer's instructions. Cells transfected with the empty vector pCDNA3 were used as control (Mock transfected).

Immunoblotting and immunoprecipitation

Cells were lysed in lysis buffer [250 mM NaCl, 50 mM HEPES (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Nonidet P-40, protease inhibitors]. NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology) were used to separate cytoplasmic and nuclear fractions. Protein content of the extracts was determined using the Bio-Rad protein assay kit. Cell lysates were resolved by SDS-PAGE and transferred to 0.45 μm nitrocellulose membranes (Bio-Rad Laboratories), and the interacting proteins were detected by immunoblotting (IB) with the following antibodies (Abs): NF-κB p65, phospho-NF-κB p65 (Ser536), IκB-α, phospho-IκB-α (Ser32/36) (Cell Signalling) and α-tubulin (Santa Cruz Biotechnology). For the IP assays, the nuclear extracts were treated with 15 μ/ml of DNase I (Invitrogen) and precleared cell lysates were incubated with β-arr-1 Abs (Santa Cruz Biotechnology) and protein A-agarose beads (Amersham Pharmacia Biotech) at 4 °C overnight. The IP and input (3% of the total nuclear extracts) samples were boiled for 5 min in SDS loading buffer, loaded onto 10% SDS/PAGE, and transferred to nitrocellulose membrane and IB with different Abs as before. To obtain clean and specific IB signals of β-arr1 which run very close to heavy chain of IgG, we used HRP-conjugated protein A (Pierce) instead of HRP-conjugated secondary Ab. Blots were developed with the enhanced chemiluminescence detection system (ECL; GE Healthcare Life Sciences).

Luciferase reporter gene assay

To measure the NF-κB promoter activity, cells were transiently cotransfected with NFκB-luc vector that contains multiple copies of the NF-κB consensus sequence fused to a TATA-like promoter (P_TAL)

or with the negative control, the pTAL-Luc vector and with pCMV-β-galactosidase vector (Promega) using LipofectAMINE 2000 reagent (Invitrogen). Reporter activity was measured using the Luciferase assay system (Promega) and normalized to β-galactosidase activity. The mean of three independent experiments performed in sextuplicate was reported.

Statistical analysis

Statistical analysis was performed using Student's *t* test and Fisher's exact test as appropriate. All statistical tests were carried out using the SPSS software (SPSS 11, SPSS Inc. Chicago, IL). A two-sided probability value of <0.05 was considered statistically significant.

Results

ET-1 activates NF-κB pathway in human ovarian cancer cells through ET_AR

To perform our study, we used the human ovarian cancer cell line HEY, which expresses ET_AR at higher levels compared to ET_BR, as shown by real-time-PCR analysis (Fig. 1A). In order to evaluate the ability of ET-1 to activate the NF-κB pathway, we transiently transfected HEY cells with a luciferase reporter plasmid under the control of a synthetic promoter that contains direct repeats of the transcription recognition sequences for NF-κB (pNF-κB-Luc) or in parallel to a negative control (pTAL-Luc). As shown in Fig. 1B, ET-1 treatment (100 nM), a dose capable to induce maximum agonist response, for 24 h was able to increase NF-κB-driven luciferase expression (1.5-fold). Then, we found that the addition of 1 μM BQ123, a peptide ET_AR antagonist, inhibited the ability of ET-1 to induce NF-κB activity (Fig. 1B, indicating that ET_AR mediates the ET-1 response of the NF-κB pathway in these cells. NF-κB is a complex of proteins, including p50 and p65 subunits. Given that the phosphorylation of p65 is required for NF-κB transcriptional activity (Moreno et al., 2010), we used a specific antibody against phosphorylated p65 (Ser536). We found that treatment of HEY cells with ET-1 resulted in the p65 phosphorylation, starting from 5 min until 60 min (Fig. 1C) and, according to the above results, BQ123 inhibited the ability

of ET-1 to induce the p65 Ser536 phosphorylation (Fig. 1D). A key event in the activation of the NF-κB pathway is the phosphorylation of the cytoplasmic inhibitor IκB that drives ubiquitination and degradation, leading to the release of p50/p65 heterodimer, nuclear translocation, and transcriptional activity (Yaron et al., 1997; Mercurio et al., 1997). Stimulation of cells with ET-1 induced IκB-►αphosphorylation (Ser 32/36) and the addition of BQ123 prevented this phosphorylation (Fig. 1D), confirming that ET-1 through ET_AR causes rapid activation of the canonical NF-κB pathway in ovarian cancer cells.

Specific requirement of β-arrestin-1 for ET-1-induced NF-κB activation

Earlier studies indicate that β-arrs associate with GPCRs, which mediates receptor endocytosis, and also function as adaptors to form "signalosomes" facilitating GPCR signalling pathways (Shenoy and Lefkowitz, 2011). More recently, it has been shown that β-arr can bind to IκBα, leading to suppression of NF-κB activation induced by TNFα, IL-1β, and UV light (Wang et al., 2006; Luan et al., 2005; Witherow et al., 2004; Gao et al., 2004). To address whether ET-1 might recruit β-arr-1 to induce NF-κB activation, we stimulated cells silenced for β-arr-1 or overexpressing β-arr-1 with ET-1 and then examined NF-κB luciferase activity. Interestingly, we found that ET-1 stimulation failed to induce NF-κB activation in β-arr1 silenced cells, whereas the overexpression of β-arr1 rescued this effect (Fig. 2A and B), suggesting that β-arr-1 might function as a positive regulator of ET-1-dependent NF-κB signalling. To confirm the results, we evaluated by real-time-PCR analysis the expression of genes known as NF-κB target genes, such as bcl-2 and matrix metalloprotease-2 (MMP-2) in cells silenced for β-arr-1 or treated with a specific NF-κB inhibitor, which inhibits translocation of NF-kappa B to the nucleus. According to the above results, the silencing of β-arr1 led to reduced expression of bcl-2 and MMP-2 in the same extent of the NF-κB inhibitor (Fig. 2B). Next, we sought to determine whether β-arr1 links also complexes with p65. Cytosolic and nuclear fractions were made from unstimulated and ET-1-stimulated HEY cells, incubated with an anti-p65 antibody for IP, and analyzed by Western blot for the co-IP of the β-arr1. Following the stimulation there was an increase in the
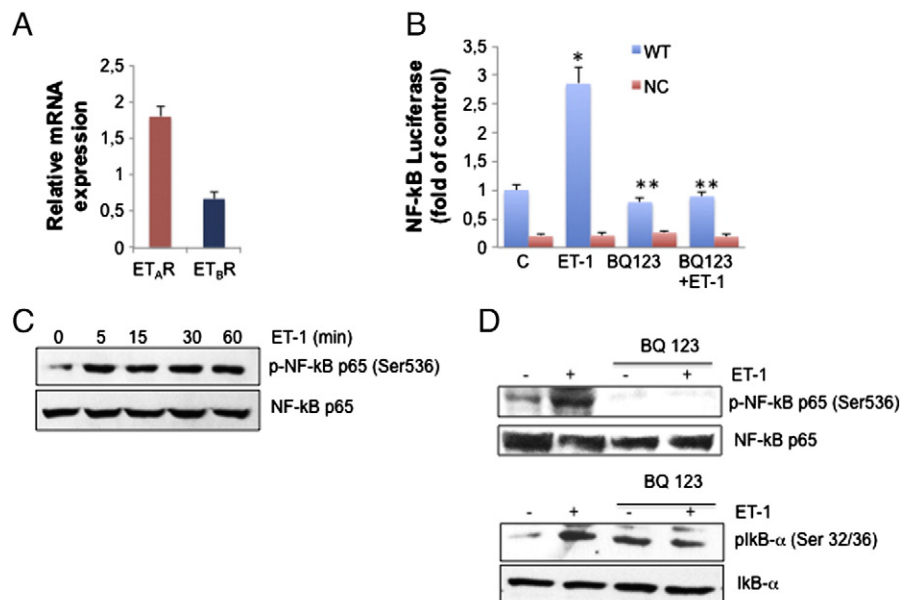

Fig. 1. A. qPCR for ET_AR and ET_BR expression in HEY cells. Values are mean ± SD of 3 measurements. B. NF-κB reporter activity in HEY cells treated with ET-1 (100 nM) and/or with BQ123 (1 μM), transiently cotransfected with NFκB-luciferase (WT)reporter construct or with negative control(NC) pTAL-Luc construct, together with pCMV-β-galactosidase vector. Values are mean of 6 measurements ± SD. *, p < 0.002 vs control (C); **, p < 0.05 vs ET-1. C. Immunoblotting (IB) for phospho-NF-κB p65 (Ser536) and NF-κB p65 expression in total extracts of HEY cells treated with ET-1 (100 nM) at different times. D. Protein expression of HEY cells, treated for 5 min with ET-1 (100 nM) in the presence or in the absence of BQ123 (1 μM), was evaluated by IB using the following Abs: NF-κB p65, phospho-NF-κB p65 (Ser536), IκB-α, phospho-IκB-α (Ser32/36).
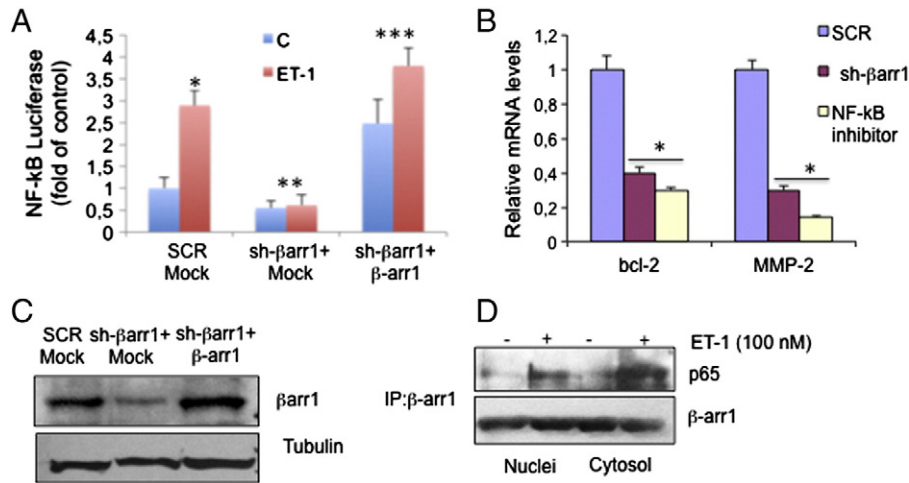
Fig. 2. A. NF-κB reporter activity in HEY cells treated with ET-1 (100 nM) and transfected with non-target shRNA control vector (SCR) or silenced for β-arr1 (sh-β-arr1) and/or rescued with β-arr1 or with the empty vector pCDNA3 (Mock). Values are mean of 6 measurements ± SD. *, p < 0.002 vs control of SCR-Mock transfected cells; **p < 0.05 vs ET-1-treated SCR-Mock transfected cells; ***, p < 0.001 vs sh-βarr1-Mock transfected cells. B. qPCR for bcl-2 and MMP-2 expression in HEY cells transfected with non-target shRNA control vector (SCR) or silenced for β-arr1 (sh-β-arr1) or treated with a specific NF-κB inhibitor. Values are mean ± SD of 3 measurements. *p < 0.02 vs SCR-transfected cells C. Lysates of HEY cells treated as in A were evaluated by IB using anti-β-arr-1. Anti-α-tubulin was used as loading control. D. Cytosolic and nuclear extracts of HEY cells treated for 24 h with ET-1 (100 nM) were IP with anti-β-arr1 and IB with anti-NF-κB p65 and anti-β-arr1 Abs.

association between β-arr1 and p65 in both compartments (Fig. 2C), along with increased β-arr1 nuclear accumulation.

Discussion

Among cancer-specific targets in human cancers, several studies have reported that increased ET_AR expression in human tumor tissues is related to cancer progression (Rosanò et al., 2013a). The binding of ET1 to its cognate receptor triggers the activation of a diverse network of signalling pathways, including MAPK, NF-κB, PI3K-AKT, β-catenin, and hypoxia inducible factor (HIF)1α, to drive cell growth, angiogenesis, invasion and metastasis. Although many of these signalling pathways are well studied in human cancer cells, the role of ET-1 to

activate NF-κB signalling and molecular mechanism underlying this activation in ovarian cancer remains to be explored. Based on our recent data showing that ET_AR/β-arr-1 interaction is critical in cancer progression, here we examined whether NF-κB is a downstream signal of the ET-1/ET_AR pathway and the involvement of β-arr1. We found that, in EOC cells expressing high levels of β-arr-1 (Rosanò et al., 2013b), ET-1 treatment promotes a significant NF-κB activation, that can be controlled by ET_AR blockade with the use of a specific antagonist. Moreover, our results suggest that β-arr1 forms a complex with p65 in the nucleus following ET-1 stimulation, that might be critical for its ability to potentiate NF-κB transcriptional activity (Fig. 3).

Previous studies have been reported that ET-1 is capable of activating the NF-κB signalling cascade via ET_AR not only in normal cells but also in
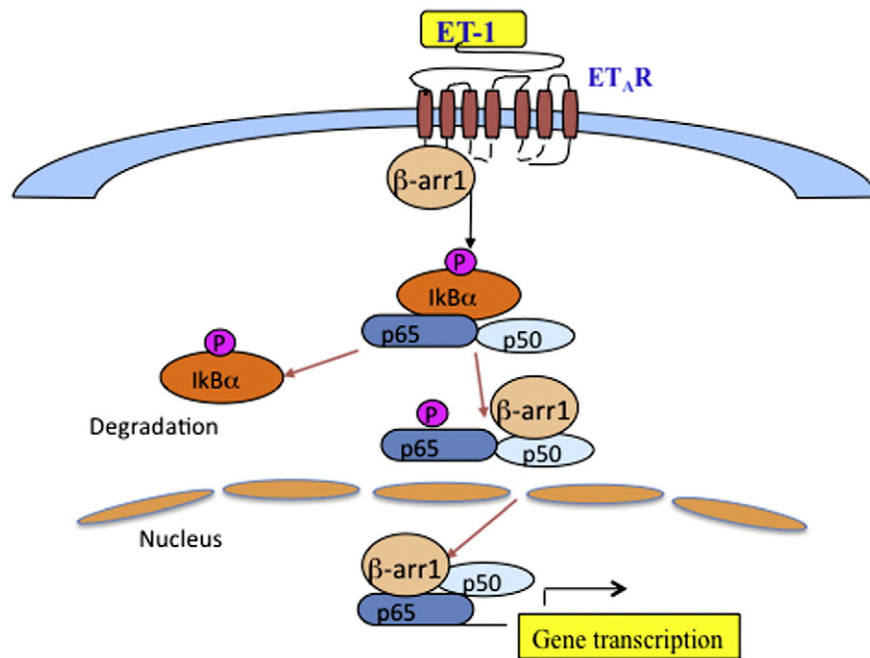

Fig. 3. Proposed mechanism by which β-arr1 regulates ET-1-induced NF-κB activity in epithelial ovarian cancer cells. ET-1 binding on ET_AR induces β-arr1 recruitment. In the cytosol, ET-1 induces the concomitant phosphorylation of p65 (Ser536) and IκB-α (Ser 32/36), key events in the activation of the NF-κB pathway. In ET-1-dependent manner, β-arr-1 links p65 subunits and together translocates in the nucleus, promoting NF-κB p65 transcriptional activity.

several cancer cell lines (von Brandenstein et al., 2008; Banerjee et al., 2007; Mangelus et al., 2001; Wu et al., 2012; Gerstung et al., 2007). Our findings indicate that ET-1 activates NF-κB transcriptional activity in EOC cells, by enhancing phosphorylation of the p65 and IκBα subunit thus favoring that the free NF-κB might translocate to the nucleus, where it activates the responsive genes. One of the mechanisms by which ET-1 might regulate NF-κB activation and transcriptional response involves the recruitment of β-arr1 and p65 in the nucleus and scaffolding them together (von Brandenstein et al., 2008). Transcriptional regulation by β-arr1 was reported in other studies, contributing in the regulation of GPCR-dependent expression of different genes, including p27 and c-fos, IFN-γ, VEGF, and ET-1 (Rosanò et al., 2013b; Wang et al., 2006; Kang et al., 2005; Shenoy et al., 2012). Indeed, previous works have shown that β-arr-1 is actively imported into the nucleus, and that an intact N domain (amino acids 1–185) is required for this import (Scott et al., 2002; Wang et al., 2003). A more recent paper reported the identification of a novel nuclear localization sequence in β-arr1, mediating active transport of β-arr1 into the nucleus, independent of its membrane and cytosolic functions (Hoeppner et al., 2012). Moreover, previous reports indicated that in the cytosolic compartment, β-arrs bind to several NF-κB proteins, including p105 and the inhibitory protein IκBα, capable of functioning as negative or positive regulators of NF-κB signalling in a different cell type and in a GPCR-dependent manner (Wang et al., 2006; Luan et al., 2005; Witherow et al., 2004; Gao et al., 2004; Gerstung et al., 2007; Parameswaran et al., 2006). Furthermore, GPCRs associate with β-arrs upon stimulation by their ligands such as lysophosphatidic acid (LPA) (Kizaki et al., 2009; Gesty-Palmer et al., 2005) or neurotensin (Law et al., 2012) to activate NF-κB signalling. In particular, β-arr2 is required for LPA-induced NF-κB activation through recruiting CARMA3 to its receptor, functioning as a positive regulator for LPA-induced NF-κB activation and cytokine production (Kizaki et al., 2009). Moreover GPCR-induced nuclear translocation of β-arr1 increases NF-κB transcriptional responsiveness (Hoeppner et al., 2012). In line with these findings, our results support an important function of β-arr1 in ETₐR-driven NF-κB transcriptional regulation in tumor cells. However, the underlying mechanisms are to be explored. In particular, how exactly β-arr1 and members of the NF-κB complex allows nuclear translocation has yet to be determined. Study from Hoeppner et al. (2012) indicates that β-arrestin-1 is not involved in shuttling the members of the NF-κB pathway into and out of the nucleus, and that p65 is able to translocate into the nucleus independent of the translocation of β-arrestin-1, but the accumulation of β-arrestin-1 in the nucleus contributes significantly to NF-κB activation. They also demonstrated that one of the mechanisms by which β-arrestin-1 modulates NF-κB transcriptional activity involves the recruitment of CBP/p300 and a protein kinase to p65 in the nucleus and scaffolding them together, contributing to the phosphorylation and acetylation of p65 and increased transcriptional responsiveness (Hoeppner et al., 2012). In this regard, our previous studies demonstrated that, in an ET-1/ETₐR-dependent manner β-arr1, but not β-arr2, is able to shuttle from the cytoplasm to the nucleus in a time- and dose-dependent manner, indicating that ET-1 controls the nuclear trafficking of β-arr1 most likely to modulate nuclear signalling of ETₐR in EOC cells. Thus, in response to ETₐR activation, β-arr1 increases its nuclear translocation and form a transcriptional complex, promoting histone acetylation, and gene transcription, required for cell migration, and invasion (Rosanò et al., 2013b), suggesting that also epigenetic modification-driven by nuclear function of β-arr1 might control NF-κB transcriptional activity. According to these findings, other studies demonstrated that β-arr-1 controls the recruitment of specific transcription factors, such as E2F and HIF-1, or epigenetic modifiers, such as histone acetyltransferase p300, and gene transcription (Kang et al., 2005; Shenoy et al., 2012; Hoeppner et al., 2012). Although these findings ascribe the regulation of gene transcription to β-arr-1 nuclear function, the majority of underlying molecular mechanisms and pathophysiological significance remain to be elucidated. NF-κB can transcriptionally regulate a diverse array of genes encoding proteins and microRNAs that regulate a wide range of biological effects. These include cytokines, chemokines and their respective receptors, which are traditionally associated with the important role of NF-κB in the inflammatory response, together with genes regulating cell survival, proliferation, cell adhesion and the cellular microenviroment (Perkins, 2012). Therefore, understanding how ETₐR induce NF-κB signalling through nuclear β-arr1 function is of interest in uncovering additional pathophysiological functions of β-arr1 during ovarian tumor progression, not only promoting cancer cell survival, cell proliferation or angiogenesis, but also by regulating the expression of MMPs associated with the metastatic potential of EOC cells. Recent studies determined the ability of ET peptides and related agonists to recruit β-arrestin via ETₐ and ET_B receptors and to compare the ability of well characterized ET-1 receptor selective and non-selective antagonists to block these responses (Maguire et al., 2012). Therefore further understanding of the potential contribution of β-arrestin mediated signalling to endothelin receptor function as well as the differences in the pharmacology of ET-1 receptor antagonists in neoplastic diseases is required.

Conclusions

In conclusion, we identify a novel role of β-arr-1 as a nuclear platform in mediating ET-1-induced NF-κB responses. Hence, our study, revealing a previously unrecognized pathway for regulating cellular responsiveness to ET-1, provides further mechanistic insights by which β-arr-1 represents the initial scaffold on which transcriptional regulatory complexes could be built to regulate different biological outcomes. Distinct β-arr1-complexes can recruit with factors that regulate the transcription of specific target genes, orchestrating the network that regulates tumor progression. Our findings indicating that nuclear β-arr-1-NF-κB nuclear complexes are essential for ETₐR signalling transduction provide further insight into the role of β-arr-1 in tumor progression. Further investigation is needed to address the functional effects of β-arr1-driven NF-κB signalling in EOC cells, such as cell survival and invasiveness.

Conflict of interest statement

None.

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