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Structure and function of β-arrestins, their emerging role in breast cancer, and potential opportunities for therapeutic manipulation

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Abstract

β-Arrestins (βarrs) are multifunctional intracellular proteins with an ability to directly interact with a large number of cellular partners including the G protein-coupled receptors (GPCRs). βarrs contribute to multiple aspects of GPCR signaling, trafficking and downregulation. Considering the central involvement of GPCR signaling in the onset and progression of diverse types of cancers, βarrs have also emerged as key players in the context of investigating cancer phenotypes, and as potential therapeutic targets. In this chapter, we first provide a brief account of structure and function of βarrs and then highlight recent discoveries unfolding novel functional attributes of βarrs in breast cancer. We also underscore the recent paradigms of modulating βarr functions in cellular context and potential therapeutic opportunities going forward.

1 Introduction

β-Arrestins (βarrs) are cytoplasmic proteins with ubiquitous expression throughout the body, and due to their critical role in regulating GPCRs, they have emerged as one of the important nodes in cellular signaling pathways (Lefkowitz & Shenoy, 2005). In the classical paradigm of GPCR signaling and regulation, βarrs are used as one of the primary mechanisms to terminate heterotrimeric G-protein coupling to agonist-bound GPCRs through steric hindrance based mechanism (DeWire, Ahn, Lefkowitz, & Shenoy, 2007). Their roles, however, have broadened significantly over the last two decades with uncovering of an ever-increasing number of interactions that they are involved in, and fundamental cellular processes that they directly or indirectly regulate downstream of GPCRs (Gurevich & Gurevich, 2019; Kang, Tian, & Benovic, 2014; Shenoy & Lefkowitz, 2011) (Fig. 1).

For example, βarrs interact with various components of clathrin coated endocytosis machinery to regulate agonist-induced receptor trafficking (Kang et al., 2014). Not only it serves as a mechanism for downregulating receptor density at the cell surface and thereby downstream signaling response but it also drives receptor compartmentalization to influence functional outcomes (Calebiro, Godbole, Lyga, & Lohse, 2015; Lobingier & von Zastrow, 2019; Vilardaga, Jean-Alphonse, & Gardella, 2014). Similarly, βarrs can also interact with

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E3 ubiquitin ligases to mediate receptor ubiquitination and degradation (Shenoy & Lefkowitz, 2011), and a diverse set of kinases and phosphatases to contribute to downstream signaling (DeWire et al., 2007; Peterson & Luttrell, 2017). More recently, formation of GPCR-G-protein-βarr complexes have also been described as a potential mechanism for endosomal signaling by GPCRs (Thomsen et al., 2016).

A number of studies across different GPCRs have established a contribution of βarrs in various cellular processes including cell cycle regulation, cellular proliferation and migration which are directly linked with the onset and development of different types of cancer (Bagnato & Rosano, 2019). As a result, investigating the role of βarrs in multiple aspects of carcinogenesis and cancer metastasis has come to the forefront, especially in the context of GPCR signaling. In addition to βarrs, related proteins referred to as Arrestin Domain Containing Proteins (ARRDCs) (Aubry & Klein, 2013) have also been implicated in different aspects of cancer phenotypes in *ex vivo* and *in vivo* model systems.

2 Structure and function of β-arrestins

As mentioned above, βarrs mediate a broad spectrum of functional outcomes in the context of GPCR signaling and regulation. In terms of their relevance in breast cancer, and cancer in general, the ability of βarrs to influence receptor trafficking and contribute to downstream signaling are most critical. The subfamily of arrestins includes four different members (named as arrestin 1–4) of which, two (i.e., arrestin 1 and 4) are primarily restricted to visual system, and they are typically referred to as visual-arrestins. The role of visual arrestins is limited primarily to the visual receptor, rhodopsin. The other two subtypes of arrestins, more commonly known as β-arrestin 1 (arrestin-2) and β-arrestin 2 (arrestin-3) are ubiquitously distributed and they typically interact with, and modulate the functions of, the majority of GPCRs.

βarrs have a two-domain structure (i.e., the N- and the C-domain), which primarily consists of anti-parallel β-strands linked with small loop regions (Gurevich & Gurevich, 2019). The carboxyl-terminus of βarrs is folded back onto the N-domain and contributes in maintaining βarrs in the basal state. The basal conformation of βarrs is stabilized by two different intramolecular interactions referred to as the "polar core" and the "three-element interaction" which are disrupted upon their interaction with activated and phosphorylated GPCRs (Gurevich & Gurevich, 2019). Multiple structures of βarrs are now described in literature including an active conformation of βarr1 in complex with a phosphorylated peptide corresponding to the carboxyl-terminus of the vasopressin V2 receptor (Shukla et al., 2013). In addition, a low-resolution architecture of a chimeric β2 adrenergic receptor with βarr1 (Shukla, Westfield, et al., 2014) and a cryo-EM structure of the neurotensin receptor-βarr1 fusion protein (Yin et al., 2019) have also been described. These studies have started to provide direct structural insights into GPCR-βarr interaction and activation mechanisms although high-resolution structural details on GPCR-βarr complexes are still awaited.

The two isoforms of βarrs are structurally similar but often display functional divergence in the context of GPCR signaling and regulation (Srivastava, Gupta, Gupta, & Shukla, 2015). A recent study has demonstrated distinct conformations of receptor-bound βarr1 and 2 as a

mechanism for their functional divergence (Ghosh et al., 2019). In addition, there are also emerging indications of preferential recruitment of one isoform over the other for different receptor systems. Furthermore, the conformational diversity sampled by βarrs upon their interaction with differentially phosphorylated receptors adds an additional level of functional fine-tuning which is conceptualized in terms of a bar code hypothesis (Nobles et al., 2011; Reiter & Lefkowitz, 2006; Shukla et al., 2008) and a flute model (Yang et al., 2017). Finally, distinct binding modalities in receptor-βarr complexes have also been linked to different functional outcomes, which further broaden their functional capabilities (Cahill et al., 2017; Kumari et al., 2016, 2017; Sente et al., 2018).

3 β-Arrestin interactome: Clues into their multi-functionality

A key feature of βarrs is their ability to bind a large number of cellular proteins and facilitate the formation of multi-protein signalosomes in cellular context (DeWire et al., 2007). The interaction network of βarrs are responsible for driving their broad functional capabilities to a large extent including their highly conserved role in GPCR internalization and signaling. In addition to focused studies on specific βarr interactions, a global proteomics analysis has identified a large number of interaction partners of βarrs upon stimulation of the angiotensin II type 1a receptor (Xiao et al., 2007). Interestingly, this study has identified a number of cellular partners of βarrs which are critical players in cell cycle progression, cellular proliferation and migration, further corroborating the potential role of βarrs in different types of cancer phenotypes (Xiao et al., 2007). This interactome of βarrs has paved the way for characterization of several novel interactions including those which are directly implicated in the context of carcinogenesis and metastasis relevant signaling pathways (Hara et al., 2011; Kovacs et al., 2008). An interesting study identified βarr interactome upon activation of PAR-2 in MCF-7 cells using mass spectrometry based proteomics approach (Parisis, Metodieva, & Metodiev, 2013). Similar to the βarr interactome mentioned above, this study also discovered a large number of βarr interaction partners, and subsequent bioinformatics analysis revealed that networks involved in interleukin signaling, actin cytoskeleton and PI3K/AKT pathways are significantly enriched (Parisis et al., 2013). This comprehensive study offers an extremely useful platform to probe the contribution of βarr interaction network in different aspects of breast cancer.

The latest addition to the list of βarr binding proteins is the interaction between βarr2 and MELK (Maternal embryonic leucine zipper kinase) (Perry et al., 2019). MELK expression is upregulated in a range of cancer cells including breast cancer cells. This study uncovers, using *in vitro* binding assay with purified proteins, that βarr2 directly interacts with the kinase domain of MELK, and moreover, also validates their interaction in cellular system (Perry et al., 2019). Most interestingly, co-expression of βarr2 and MELK decreases the number of cells in the S-phase of cell cycle suggesting a potential role of this interaction in cellular proliferation (Perry et al., 2019).

In addition to GPCRs, βarrs have also been found to impart regulatory effect on other membrane proteins including non-GPCR receptors, ion-channels and transporters (Chen et al., 2016; Kashihara, Nakada, Kojima, Takeshita, & Yamada, 2017; Lefkowitz, Rajagopal, & Whalen, 2006; Liu et al., 2017; Shenoy & Lefkowitz, 2011). For example, βarr1 plays a

critical role in ubiquitination and downregulation of TRPV4 (Transient Receptor Potential cation channel subfamily V member 4) by bringing an E3 ubiquitin ligase to close proximity of AT1aR-TRPV4 heterodimer in the membrane (Shukla et al., 2010). Thus, it is plausible that βarrs play a significantly broader role in mediating the permeation of ions, and transport of substrates, than currently appreciated, which may have a significant bearing on various attributes of cellular outcomes relevant to cancer.

4 Emerging role of β-arrestins in cancer phenotype

There are a large number of reports suggesting a direct contribution of βarrs in diverse types of cancer phenotypes and they appear to be involved in nearly every stage including initiation, promotion and progression. While some of these involve GPCRs, others are mediated through non-GPCR mechanisms as well. A number of recent reviews have summarized an overview of various studies implicating βarrs in cancer (Bagnato & Rosano, 2019; Crudden et al., 2019; Sobolesky & Moussa, 2013; Tocci, Rosano, & Bagnato, 2019) and therefore, we will not describe those examples here. Instead, we have specifically focused the following subsection on the emerging contribution of βarrs in breast cancer with a particular emphasis on studies described in the last 5 years or so.

5 Role of β-arrestins in breast cancer

Several independent studies have reported a change in βarr expression pattern in breast cancer cells and tissues, and also identified critical roles of βarrs in signaling pathways and cellular outcomes implicated in breast cancer phenotype (Table 1). Interestingly, majority of cell line based studies are carried out in TNBC (Triple Negative Breast Cancer) cells, which are relatively more aggressive with poorer outcomes and higher recurrence. For example, βarrs are required for PAR-2 (Protease Activated Receptor subtype 2) mediated migration of MDA MB-231 breast cancer cell lines potentially via an ERK1/2 MAP kinase pathway (Ge et al., 2004). In MCF-7 and MDA MB-231 cells, βarr2 appears to play an inhibitory role in morphine-induced apoptosis via an Akt and caspase-8 pathway (Zhao et al., 2009). Similarly, βarrs have been implicated in the migration and invasion of HS578T and MDA-MB-231 cell lines in context of the lysophosphatidic acid (LPA) receptor through a member of the Ras GTPase family referred to as Ral (Li et al., 2009). Moreover, several studies have implicated the so-called non-canonical GPCRs, also referred to as atypical chemokine receptors (ACKRs), in breast cancer phenotype (Massara, Bonavita, Mantovani, Locati, & Bonecchi, 2016; Mollica Poeta, Massara, Capucetti, & Bonecchi, 2019; Wang, Chen, & Shen, 2018). This is particularly relevant considering that these receptors do not couple to heterotrimeric G-proteins but robustly recruit and activate βarrs (Bachelerie, Ben-Baruch, et al., 2014; Bachelerie, Graham, et al., 2014). We discuss some of the recent studies providing novel aspects of βarr function in breast cancer and emerging mechanistic insights in the following section (Table 1).

Key signaling networks that βarrs appear to regulate involve ERK1/2 MAP kinase activation (DeWire et al., 2007), EGFR transactivation (Barki-Harrington & Rockman, 2008) and cytoskeletal rearrangements (Xiao et al., 2010). Interestingly, this arm of βarr function appears to be one of the key mechanisms through which they play a role in breast cancer,

and other cancer types as well. Similar to PAR-2 example mentioned above, βarr2 is involved in kisspeptin receptor dependent formation of invadopodia in breast cancer cells via an EGFR and ERK1/2 activation pathway (Goertzen et al., 2016). The kisspeptin 1 receptor (KISS1R), also known as GPR54, is a prototypical GPCR that is activated by a peptide hormone called kisspeptin, previously known as metastin, that was originally identified as a human metastasis suppressor gene (Kirby, Maguire, Colledge, & Davenport, 2010). Invadopodia are membrane protrusions rich in actin that are often formed by cancer cells and they are involved in invasion properties and metastasis potential of these cells (Yamaguchi, 2012). Goertzen et al. observed that silencing βarr2 in MDA-MB-231 and Hs578T cell lines significantly inhibits kisspeptin-induced invadopodia formation, suggesting a potential involvement of βarr2 in invasion and metastatic potential of these cells.

miRNAs are single stranded, non-protein coding RNAs of 19–25 nucleotides, and they target mRNAs through a RISC (RNA-induced silencing complex) dependent mechanism (O'Brien, Hayder, Zayed, & Peng, 2018). Emerging data positions them as one of the major contributing factors in diverse types of cancer phenotypes and make them an attractive target for therapeutics (To et al., 2020). A recent study has identified miRNA based control of βarr1 expression in TNBC samples (Son et al., 2019). In a microarray screen, the authors observed a significant upregulation of miR-374a-5p in samples derived from TNBC patients (Son et al., 2019). In line with this observation, targeted inhibition of miR-374a-5p in MDM-MB-231, MDM-MB-468 and MDA-MB-157 cells impairs their growth and migration (Son et al., 2019). Interestingly, suppression of miR-374a-5p enhances βarr1 expression at mRNA and protein levels in these cell lines as well as in xenograft mouse model (Son et al., 2019). Exogenous overexpression of βarr1 in these cells decreases their survival, proliferation and migration potentially via an AMPK (AMP-activated protein kinase) dependent mechanism. Furthermore, the analysis of TCGA (The Cancer Genome Atlas) and METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) datasets reveal that βarr1 expression is downregulated in TNBC patient samples, further underscoring its role as a potential tumor suppressor in breast cancer (Son et al., 2019).

Wu et al. have reported that in MCF-7 and T-47D breast cancer cell lines, the levels of βarr2 is lower compared to corresponding control cell lines, and βarr2 overexpression inhibits proliferation of these cells and triggers apoptosis (Wu et al., 2014). The contribution of βarr2 in this context involves GABA-B receptor and activation of JNK kinase (Wu et al., 2014). Another study has observed overexpression of an orphan GPCR, Gpr161, in MDA-MB-361 and BT-474 cells (Feigin et al., 2014). Knocking down Gpr161 decreases the proliferation of mammary epithelial cells while overexpression promotes proliferation and migration (Feigin et al., 2014). Interestingly, βarr2 appears to scaffold a complex of Gpr161 with IQGAP1 (IQ motif containing GTPase activating protein 1), which appears to be an important driving mechanism underlying Gpr161 mediated effects (Feigin et al., 2014).

One of the key problems associated with therapeutic management of breast cancer is the emergence of "Multi Drug Resistance" (MDR) (Martin, Smith, & Tomlinson, 2014). This is attributed, in part, to over-expression of a membrane protein MDR1/p-gp, which is an efflux transporter (also known as the ATP-binding cassette subfamily B member 1; ABCB1 or

cluster of differentiation 243; CD243) (Endicott & Ling, 1989). Jing et al. analyzed approximately one hundred samples from breast cancer tissues in terms of βarr2 and MDR1 expression pattern, and observed a fairly significant correlation (Jing et al., 2015). Interestingly, βarr2 and MDR1 levels appear to be upregulated in MCF-7/ADM, a multidrug resistant cell line, compared to regular MCF-7 and MDA-MB-231 cells (Jing et al., 2015). Corroborating these findings, not only exogenous overexpression of βarr2 enhances MDR1 expression in MCF-7 and MDA-MB-231 cells but also siRNA mediated silencing of βarr2 results in a dramatic reduction in MDR1 expression in MCF-7/ADM cell line (Jing et al., 2015). Most importantly, silencing βarr2 increases doxorubicin sensitivity in these cell lines while its overexpression has an opposite effect (Jing et al., 2015). Taken together, these observations imply an important contribution of βarr2 in breast cancer MDR, and offer a potential avenue for therapeutic consideration. Considering the role of βarrs in fine-tuning surface levels of non-GPCR membrane proteins by mediating their internalization and/or ubiquitination, it is tempting to speculate that they may have a similar role in the context of MDR1 although it remains to be experimentally tested.

In addition to βarrs, Arrestin Domain Containing Proteins (ARRDCs) which are classified under a broad umbrella of arrestin-family of proteins (Aubry & Klein, 2013), have also emerged as key players in various aspects of breast cancer phenotypes (Arakaki, Pan, Lin, & Trejo, 2018; Soung et al., 2017; Soung, Pruitt, & Chung, 2014; Zheng et al., 2017). ARRDC3 in particular appears to be involved at multiple levels, for example, in the reversal of chemo-resistance, invasive potential of extracellular vesicles derived from MCF-7 and MDM-MB-231 cells, PAR-1 induced MDA-MB-231 invasion, and proliferation and migration of MDM-MB-231 cells (Draheim et al., 2010; Soung, Chung, Yan, Ju, & Chung, 2019; Soung, Ford, Yan, & Chung, 2018; Soung et al., 2017, 2014). A comprehensive discussion of ARRDCs and their role in breast cancer requires a chapter of its own, and thus, it is not covered here in detail.

6 Potential strategies to modulate β-arrestin functions

Unlike GPCRs, βarrs are cytosolic proteins and therefore, strategies to target them directly, for example, using small molecules, pose significant challenges (Chaturvedi et al., 2018; Shukla, 2014). Moreover, universal inhibitors of βarrs such as those for enzymes may not be optimal considering their multi-functionality especially with respect to terminating G-protein signaling, receptor trafficking and signaling. Directly targeting βarrs is beginning to emerge as a niche area, and we discuss some of the recent reports in this section (Fig. 2).

As mentioned earlier, one of the key functions of βarrs is to mediate agonist-induced receptor endocytosis via scaffolding various players of the clathrin coated machinery including clathrin itself and β2-adaptin (Goodman et al., 1996, 1998; Laporte et al., 1999). Such a mechanism is important for controlling GPCR signaling from the perspective of receptor desensitization in the classical paradigm, as well as in terms of the emerging concept of GPCR signaling from endosomal compartments (Tsvetanova, Irannejad, & von Zastrow, 2015; Vilardaga et al., 2014). A recent study has used virtual screening approach to identify a small molecule compound named barbadin that is capable of inhibiting βarr-β2-adaptin interaction in cellular context (Beautrait et al., 2017). Although barbadin binds to

β2-adpatin, it robustly inhibits agonist-induced endocytosis of a broad set of GPCRs by interfering with βarr-β2-adaptin interaction without significantly affecting GPCR-βarr interaction (Beautrait et al., 2017). Barbadin also inhibits sustained cAMP response arising from endosomes and agonist-induced ERK1/2 MAP kinase phosphorylation for selected GPCRs. Although not tested directly in the context of cancer phenotype, the discovery and characterization of barbadin provides a proof-of-principle for the conceptual framework of modulating βarr functions by targeting their interaction interfaces (Fig. 2).

A previous study has used a βarr2-targeting RNA aptamer in the context of chronic myelogenous leukemia (CML) (Jonathan et al., 2014) where βarr2 is critically involved in the onset and maintenance of chronic and blast crisis phases (Fereshteha et al., 2012). This study used an *in vitro* platform referred to as "Systematic Evolution of Ligands by Exponential enrichment (SELEX)" (Gold, 1995; Que-Gewirth & Sullenger, 2007) for selecting RNA aptamers against purified βarr2 and identified a number of different aptamers with reasonable binding affinities (Jonathan et al., 2014). Selected aptamers targeting βarr2 inhibited its interaction with ERK2 at submicromolar concentrations in pull-down assays and therefore, offered a possibility of disrupting βarr signaling in cellular context (Jonathan et al., 2014). Subsequently, βarr2 aptamer was linked to a previously described nucleolin targeting DNA aptamer (Kotula et al., 2012) which allowed its delivery to the K562 cell line which are immortalized myelogenous leukemia cells (Jonathan et al., 2014). Interestingly, this approach resulted in the disruption of multiple signaling pathways in these cells, potentially by interfering with βarr interactions, and also led to a significant reduction in cell growth (Jonathan et al., 2014). Although not tested in the context of breast cancer, it may be an attractive avenue to explore in future, especially considering that βarr-ERK axis appears to be a key player in proliferation and migration of breast cancer cells.

More recently, a set of synthetic antibody fragments (FABs) against βarrs were isolated from a phage display library and characterized in terms of their selectivity against βarr isoforms and ability to modulate different interactions of βarrs (Ghosh et al., 2017). One of these FABs targeting βarr2, referred to as FAB5, selectively inhibited the interaction of βarr2 with clathrin terminal domain in *vitro* (Ghosh et al., 2017). Upon expression as an intrabody, this particular FAB, now referred to as intrabody5, significantly inhibited agonist-induced endocytosis of a diverse set of GPCRs (Ghosh et al., 2017). This example of intrabody-based modulation of βarr interaction and its ensuing functional outcome provides a potential framework for targeting βarr signaling in the context of cancer therapeutics. Moreover, new platforms for generating different types of synthetic binders including nanobodies and monobodies have now been designed (Koide, Wojcik, Gilbreth, Hoey, & Koide, 2012; McMahon et al., 2018) which offer additional possibilities of targeting βarrs from a therapeutic standpoint.

An interesting avenue that has not been explored yet in the context of breast cancer, and cancer phenotype in general, is modulating βarr functions through biased agonism at GPCRs (Roy, Getschman, Volkman, & Dwinell, 2017). The concept of biased agonism broadly refers to preferential signaling through a specific transducer pathway over others, and it has emerged as a novel framework for improving GPCR targeted therapeutics (Costa-Neto, Parreiras, & Bouvier, 2016; Shukla, Singh, & Ghosh, 2014; Shukla, Xiao, & Lefkowitz,

2011). It is conceivable that a G-protein biased agonist may have therapeutic advantages over balanced agonists where βarr signaling contributes positively toward the onset and progression of cancer phenotype. Considering multiple studies demonstrating the role of βarrs in cellular proliferation, migration and invasion of breast cancer cells, this aspect of biased agonism may be highly relevant from therapeutic point of view. A recent review article has discussed this particular aspect in the context of chemokine receptors (Roy et al., 2017).

7 Conclusion and future perspective

Here, we have provided a brief overview of structure and function of βarrs and summarized the recent discoveries on the contribution of βarrs in breast cancer phenotype with specific case examples. We have also discussed several proof-of-principle studies that are now described to target βarr function in cellular context, including an aptamer-based strategy in a myeloid leukemia cell line. A key focus going forward should be on designing additional tools and reagents for directly targeting βarrs in cellular systems as well as in animal models. These future studies may provide promising avenues for therapeutic manipulation of βarr signaling in different cancer phenotypes in including breast cancer.

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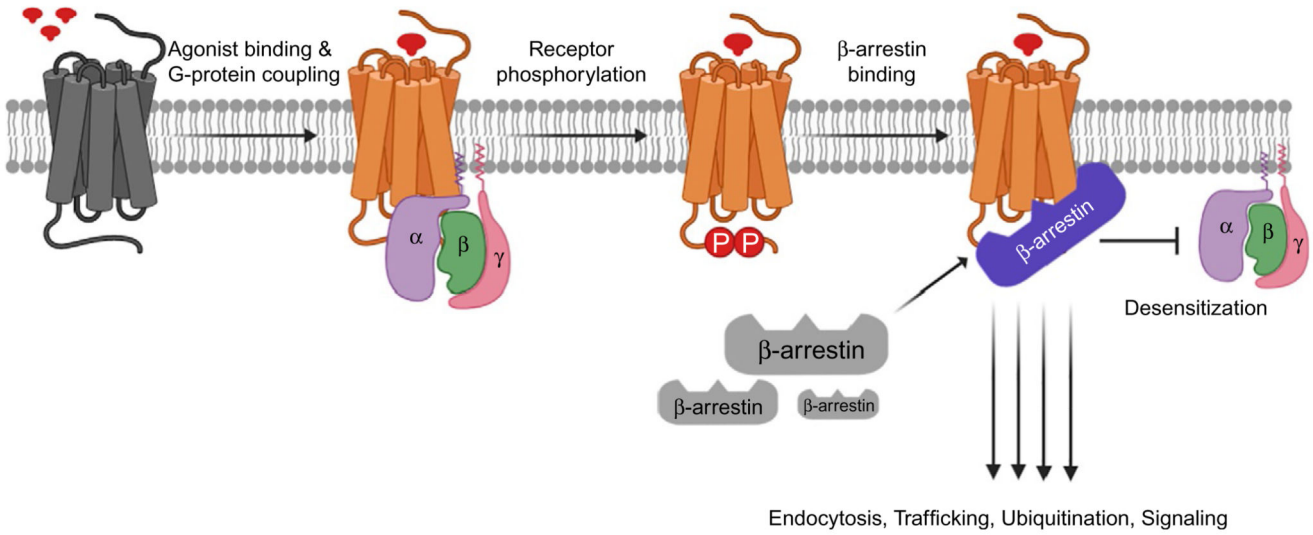

Fig. 1.

A schematic representation showing the multifaceted role of β-arrestins in GPCR signaling and regulation. Agonist-stimulation leads to a conformational change in GPCRs followed by the interaction and activation of heterotrimeric G-proteins. Subsequently, GPCRs are phosphorylated by GPCR kinases (GRKs) that facilitate the binding of β-arrestins. GPCR-β-arrestin interaction terminates further G-protein coupling via steric hindrance mechanism on one hand while on the other, it initiates receptor internalization and β-arrestin mediated signaling.

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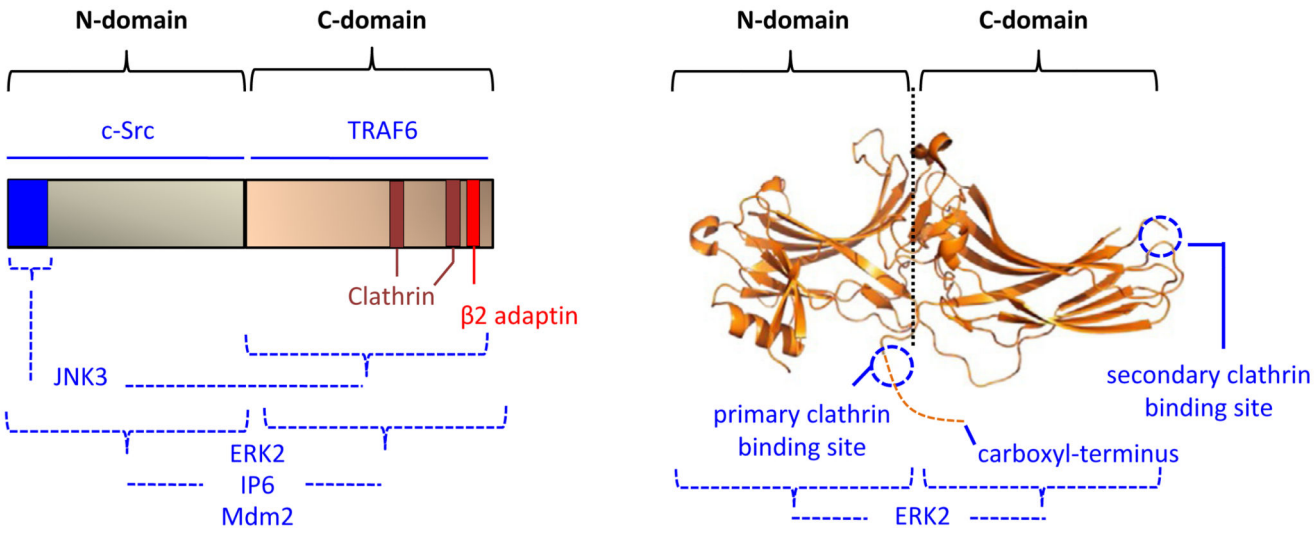

Fig. 2.

Structural representation of β-arrestin indicating some of the interaction interfaces. A potential mechanism underlying the multi-functionality of β-arrestins is their ability to interact with a large number of cellular partners. As some of the interaction interfaces are quite distinct from each other, it offers a framework to modulate selected β-arrestin functions by targeting the protein-protein interaction. *This image is adopted from and modified based on a previous publication from our laboratory Ghosh, E., Srivastava, A., Baidya, M., Kumari, P., Dwivedi, H., Nidhi, K., et al. (2017). A synthetic intrabody-based selective and generic inhibitor of GPCR endocytosis. Nature Nanotechnology, 12, 1190–1198.*

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Table 1

A brief summary of the contribution of β-arrestins in breast cancer phenotype as described in recent studies.

Protein (β-arrestin 1/2)	Expression/functional contribution/localization	References
βarr1 and βarr2	Contribute in PAR-2 mediated migration of MDA MB-231 cells via ERK1/2 MAP kinase activation	Ge, Shenoy, Lefkowitz, and DeFea (2004)
βarr2	Significantly reduces morphine-induced cell death in MCF-7 and MDA-MB231 cells via an Akt and caspase-8 pathways	Zhao et al. (2009)
βarr1 and βarr2	Impair LPA-induced migration and invasion of MDA-MB-231 cells via a Ral GTPase mechanism	Li et al. (2009)
βarr1	Comparatively higher expression in ERα + invasive ductal carcinoma compared to invasive ERα – ductal carcinomas	Rezaul et al. (2010)
βarr1	Inhibits the migration of MDA-MB-468 and MDA-MB-231 cells (2011)	Lundgren et al. (2011)
βarr2	Contributes in Kisspeptin-induced EGFR transactivation, and invasion of MDA-MB-231 cells via MMP-9	Zajac et al. (2011)
βarr2	Overexpression inhibits proliferation and promotes apoptosis of MCF-7 and T-47D cells via $GABA_BR$/JNK pathway	Wu, Shan, Zheng, and Pei (2014)
βarr2	Potentially contributes in GPR161-mediated proliferation and migration of MDA-MB-361 cells via IQGAP1 dependent mechanism	Feigin, Xue, Hammell, and Muthuswamy (2014)
βarr2	βarr2 expression correlates with the levels of MDR1-gp in breast tissue samples and contributes to doxorubicin sensitivity in MDA-MB-231 and MCF-7 cells	Jing et al. (2015)
βarr2	Contributes to Kisspeptin-induced invadopodia formation in MDA-MB-231 via ERK1/2 MAP kinase	Goertzen, Dragan, Turley, Babwah, and Bhattacharya (2016)
βarr1	βarr1 is involved in cellular proliferation and migration in TNBC cells, potentially via AMP kinase activation, and their expression is downregulated in TNBC cells; miR-374a-5p targets and regulates βarr1 expression in TNBC cells	Son et al. (2019)
βarr1 and βarr2	Proteomics-based βarr interactome in MCF-7 cells upon PAR2 activation	Parisis et al. (2013)

Please note that the table is not exhaustive and highlights some of the key examples available in the literature.