COMMENTARY

β-Arrestin1 mediates hMENA expression and ovarian cancer metastasis

Hamsa Thayele Purayil[a] and Yehia Daaka[a,1]

Ubiquitously expressed β-arrestin1 (β-arr1) and β-arrestin2 (β-arr2) proteins were originally identified and characterized based on their function to desensitize activated G protein-coupled receptors (GPCRs) with respect to heterotrimeric G protein signaling, and to mediate GPCR endocytosis. Work over the past decade has shown that β-arrestins also function as molecular scaffolds that control spatiotemporal distribution and mitogenic activity of partner proteins involved in a myriad of fundamental cellular functions, ranging from metabolism to growth and migration (1, 2). For example, β-arr1 binds to and activates the tyrosine kinase c-Src (3) that, in turn, transactivates EGF receptor to promote ERK protumorigenic activity in colorectal cancer (4). β-arr1 was also shown to promote prostate tumor growth by regulating androgen receptor activity (5), and transgenic mice with overexpressed β-arr1 allowed the faster growth rate of xenograft hepatic tumors in comparison with control animals (6). Similarly, β-arr2 was reported to mediate the initiation and progression of myeloid leukemia through activation of Wnt signaling (7), and to induce ovarian tumor cell invasion and metastasis by nuclear β-catenin (8). Hence, β-arrestins regulate specific cellular functions as a result of interacting with defined partner proteins. In PNAS, Di Modugno et al. (9) identify hMENA, an actin binding protein of the ENA/VASP family, as a novel interacting partner of β-arr1 critical for endothelin-1 (ET-1) and its cognate receptor $ET_{1A}R$ signaling in ovarian cancer cell invasion and metastasis (Fig. 1). Using elegant biochemical and cellular imaging approaches, the authors convincingly show a required role for β-arr1 in ET-1–regulated assembly of an $ET_{1A}R$/β-arr1/hMENA complex, invadopodia maturation, and ovarian tumor invasion and metastasis. Clinical relevance of the cell- and animal-based conclusions are established with bioinformatics analysis of human patient datasets. Using Kaplan–Meier analysis and the log-ranked test showed that expression level of EDRNA (the gene encoding $ET_{1A}R$) was predictive of overall survival and progression-free survival in patients diagnosed with high-grade serous carcinoma (HGSC). Remarkably, the high *EDRNA*, ARRB1 (gene encoding β-arr1), and ENAH (gene encoding hMENA) expression signature correlated with worse overall survival and progression-free survival, in comparison with the expression level of each individual biomarker. These results imply the potential use of this gene signature as prognostic for serious ovarian cancer and $ET_{1A}R$/β-arr1/hMENA signal axis as a druggable target to treat the so far incurable disease.

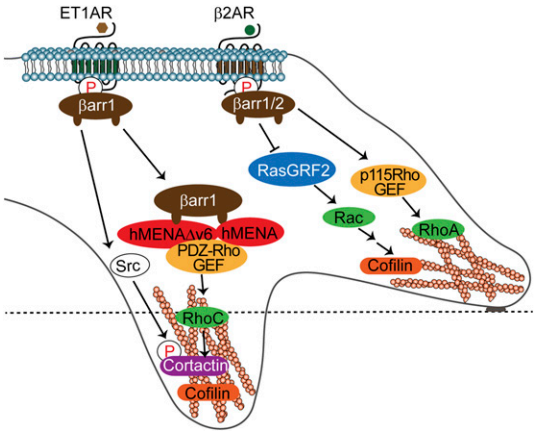

Fig. 1. Model for β-arrestin regulation of invadopodia maturation and cytoplasmic protrusions. Activated $ET_{1A}R$ promotes the β-arr1/hMENA/PDZ-RhoGEF complex formation and activation of RhoC. The β-arr1/hMENA also activates Src to phosphorylate cortactin that is required for invadopodia maturation. In kidney cancer cells, β-arr1 regulates cell migration through the inhibition of the rasgrf2 gene. RasGRF2, a dual Ras/Rac GEF, activates Rac to promote actin polymerization through dephosphorylation and activation of cofilin. This leads to the formation of multiple protrusions and increased circularity of cells with a consequent decrease of directional cell migration. Activation of β2AR also promotes β-arr2–dependent activation of p115RhoGEF and RhoA, leading to focal adhesion remodeling in lamellipodia and directed cell migration.

Ovarian cancer ranks fifth in cancer deaths among women in the United States, and this year alone 22,240 American women will be diagnosed with ovarian cancer and 14,070 will die from the disease (10). Worldwide,

[a]Department of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, FL 32610
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[1]To whom correspondence should be addressed. Email: ydaaka@ufl.edu.
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ovarian cancer is diagnosed in about 250,000 women annually and causes more than 140,000 deaths each year. The majority (85–90%) of ovarian cancers are classified histopathologically as epithelial ovarian carcinomas with the HGSC type being the most common. HGSC accounts for 70–80% of ovarian cancer deaths and associates with aggressive metastatic behavior (11). The standard of care for patients newly diagnosed with organ-confined ovarian cancer is surgical resection and debulking of the tumor mass, sometimes together with peritoneal organs, including both ovaries. Often, patients present with advanced disease and the most active therapeutic agents are platinum analogs and a taxane. Recurrence of cancer after initial platinum-based therapy is common and patients invariably develop platinum-resistant disease. Recurrent ovarian cancers exhibit a spectrum of genetic mutations and the available therapies target blood vessel formation (e.g., pazopanib) or DNA repair and genomic stability (e.g., olaparib). Nonetheless, only minimal improvement in the mortality has been observed over the past decade, pointing to the molecular complexity of ovarian cancer and resultant obstacles in developing effective therapies (11). Di Modugno et al. (9) used complementary strategies of gene overexpression and knockdown, together with pharmacologic activators and inhibitors, to credibly implicate $ET_{1A}R$, β-arr1, and hMENA as mediators of HGSC invasion and metastasis. To implicate $ET_{1A}R$, the authors used macitentan, a dual $ET_{1A}R/ET_{1B}R$ ligand antagonist, and showed that treatment with macitentan obliterated the ET-1–induced expression of hMENA and proinvasive isoform hMENAΔv6, but decreased expression of the antiinvasive $hMENA^{11a}$ isoform. Moreover, Di Modugno et al. show that macitentan inhibited the ET-1–induced hMENA/hMENAΔv6 protein complex formation with β-arr1. Macitentan was isolated based on its ability to block Gαq signaling and mobilization of intracellular Ca^{+2} following stimulation with ET-1 (12), and the present results (9) clearly show that it can also inhibit signaling through β-arr1 as well. Considering the emerging field of biased ligands on GPCRs (13) that favor (or inhibit) signaling by a given GPCR through heterotrimeric G proteins or β-arrestins, these findings provide a platform and opportunity to discover a more selective β-arr1–biased ligand antagonist on $ET_{1A}R$ (and not $ET_{1B}R$, which is known to be expressed on ovarian cancer). Identification of such biased antagonist would be expected to have a more optimal clinical profile than macitentan to interfere with ovarian cancer metastasis. This could be especially relevant, as macitentan was reported to cause unwanted side effects, like dizziness, anemia, and embryo-fetal toxicity.

Although β-arr1 and β-arr2 exhibit a high degree of sequence homology and functional overlap, they have distinct subcellular distribution (14). Whereas β-arr2 is detected strictly in the cytosol (as a result of encoding a nuclear export signal), β-arr1 is found in both the cytosolic and nuclear compartments. The nuclear expression of β-arr1 implies a potential role in transcription regulation, and previous work has shown that nuclear β-arr1 interacts with transcription regulators, including p300 (15), E2F (16), β-catenin (8), and HIF-1α (17) to regulate expression of their target genes. In PNAS, Di Modugno et al. (9) report that stimulation with ET-1 promotes ENAH gene expression in a manner that is dependent upon expression of β-arr1, but not β-arr2. Moreover, the authors show that the ET-1/$ET_{1A}R$/β-arr1 axis differentially regulates expression of hMENA isoforms: stimulation of ovarian cancer cells with ET-1 increases expression of the wild-type and proinvasive hMENAΔv6 isoform but decreases expression of antiinvasive $hMENA^{11a}$. $ET_{1A}R$ is a class A GPCR (18) and, as such, it is likely that β-arr1 subcellular distribution following ET-1 stimulation is independent of the receptor trafficking. It remains to be determined whether ET-1 promotes the nuclear distribution of β-arr1. Nonetheless, these observations imply that β-arr1 controls invasion and metastasis of ovarian cancer cells, at least in part, through its nuclear activity that regulates *ENAH* expression.

In addition to invadopodia, productive cancer cell invasion involves cell polarization and the formation of leading and trailing edges that require actin cytoskeletal remodeling. The best-studied mediators of actin cytoskeletal remodeling are Rho GTPases, whose expression and activities are regulated by β-arrestins. Rho GTPases include RhoA, RhoB, and RhoC. In particular, RhoC plays a critical role during tumor metastasis and has been identified as a biomarker for invasive breast cancer (19). Work in renal cell carcinoma has shown that β-arrestins regulate cell migration and invasion downstream of β2-adrenergic receptors (β2AR) (Fig. 1). Here, activation of β2AR induces translocation of β-arr2 and p115RhoGEF from cytosol to the plasma membrane and subsequent activation of RhoA, leading to focal adhesion remodeling for lamellipodia formation and cell migration (20). Furthermore, activated β2AR induced the β-arr1–mediated expression of the tumor-suppressor *RASGRF2*, with consequent effect on Rac and cofilin activities, actin remodeling, and cell migration (21). Di Modugno et al. (9) report that ET-1 promotes relocalization of β-arr1/hMENA/PDZ-RhoGEF from the periphery in the tips of actin stress fiber-like structures to the cytosol to colocalize with F-actin/cortactin-enriched microdomains, leading to the activation of RhoC and invadopodia maturation. Hence, engaged β-arr1 forms complex with a particular RhoGEF to activate a Rho GTPase that is required for actin cytoskeleton rearrangement and cell invasion. It remains possible that ET-1–induced activation of the $ET_{1A}T$/β-arr1/hMENA signaling axis is involved in the directional movement of ovarian cancer cells by regulating cytoplasmic protrusions other than invadopodia.

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In summary, the work by Di Modugno et al. (9) establishes a role for $ET_{1A}R$, β-arr1 and hMENA in HGSC invasion and metastasis. Use of the *EDRNA/ARRB1/ENAH*-expression signature may be especially valuable for prognosis of recurrent and therapy-resistant disease. Moreover, the $ET_{1A}R$/β-arr1/hMENA axis may prove to be an effective drug target to treat the so far incurable HGSC that affects the lives of too many women and their families.

1 Smith JS, Rajagopal S (2016) The β-arrestins: Multifunctional regulators of G protein-coupled receptors. *J Biol Chem* 291:8969–8977.
2 DeWire SM, Ahn S, Lefkowitz RJ, Shenoy SK (2007) β-arrestins and cell signaling. *Annu Rev Physiol* 69:483–510.
3 Luttrell LM, et al. (1999) β-arrestin-dependent formation of β2 adrenergic receptor-Src protein kinase complexes. *Science* 283:655–661.

4 Buchanan FG, et al. (2006) Role of β-arrestin1 in the metastatic progression of colorectal cancer. *Proc Natl Acad Sci USA* 103:1492–1497.
5 Purayil HT, et al. (2015) Arrestin2 modulates androgen receptor activation. *Oncogene* 34:3144–3151.
6 Zou L, Yang R, Chai J, Pei G (2008) Rapid xenograft tumor progression in β-arrestin1 transgenic mice due to enhanced tumor angiogenesis. *FASEB J* 22:355–364.
7 Fereshteh M, et al. (2012) β-arrestin2 mediates the initiation and progression of myeloid leukemia. *Proc Natl Acad Sci USA* 109:12532–12537.
8 Rosanò L, et al. (2009) β-arrestin links endothelin A receptor to β-catenin signaling to induce ovarian cancer cell invasion and metastasis. *Proc Natl Acad Sci USA* 106:2806–2811.
9 Di Modugno F, et al. (2018) hMENA is a key regulator in endothelin-1/β-arrestin1–induced invadopodial function and metastatic process. *Proc Natl Acad Sci USA* 115:3132–3137.
10 Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. *CA Cancer J Clin* 68:7–30.
11 Matulonis UA, et al. (2016) Ovarian cancer. *Nat Rev Dis Primers* 2:16061.
12 Monaco TJ, Davila CD (2016) Safety, efficacy, and clinical utility of macitentan in the treatment of pulmonary arterial hypertension. *Drug Des Devel Ther* 10:1675–1682.
13 Smith JS, Lefkowitz RJ, Rajagopal S (2018) Biased signalling: From simple switches to allosteric microprocessors. *Nat Rev Drug Discov*, 10.1038/nrd.2017.229.
14 Scott MG, et al. (2002) Differential nucleocytoplasmic shuttling of β-arrestins: Characterization of a leucine-rich nuclear export signal in β-arrestin2. *J Biol Chem* 277:37693–37701.
15 Kang J, et al. (2005) A nuclear function of β-arrestin1 in GPCR signaling: Regulation of histone acetylation and gene transcription. *Cell* 123:833–847.
16 Pillai S, et al. (2015) β-arrestin1 mediates nicotine-induced metastasis through E2F1 target genes that modulate epithelial-mesenchymal transition. *Cancer Res* 75:1009–1020.
17 Shenoy SK, et al. (2012) β-arrestin1 mediates metastatic growth of breast cancer cells by facilitating HIF-1-dependent VEGF expression. *Oncogene* 31:282–292.
18 Oakley RH, Laporte SA, Holt JA, Caron MG, Barak LS (2000) Differential affinities of visual arrestin, β-arrestin1, and β-arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *J Biol Chem* 275:17201–17210.
19 Kleer CG, et al. (2005) RhoC-GTPase is a novel tissue biomarker associated with biologically aggressive carcinomas of the breast. *Breast Cancer Res Treat* 93:101–110.
20 Ma X, Zhao Y, Daaka Y, Nie Z (2012) Acute activation of β2-adrenergic receptor regulates focal adhesions through β-arrestin2- and p115RhoGEF protein-mediated activation of RhoA. *J Biol Chem* 287:18925–18936.
21 Ma X, et al. (2014) β-arrestin1 regulates the guanine nucleotide exchange factor RasGRF2 expression and the small GTPase Rac-mediated formation of membrane protrusion and cell motility. *J Biol Chem* 289:13638–13650.