# Dissecting the signaling and assembly pathways of invadopodia triggered by endothelin-1 receptor/β-arrestin1 axis

(hrs)

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MAC/ET-1

CTR

ET-1

## BACKGROUND

- $\succ$  To metastasize, tumor cells must be able to degrade and remodel the extracellular matrix and the underlying vasculature, which can be accomplished through activity of invadopodia, actin-rich adhesive membrane protrusions with a complex molecular structures, hotspots for secretion of matrix-degrading metalloproteinases (MMPs).
- $\succ$  The endothelin-1 receptor (ET-1R) has a critical role in ovarian cancer (SOC) progression, by controlling different tumor promoting effects, including invasion and metastasis, which are mediated by  $\beta$ -arrestin1 ( $\beta$ -arr1), acting as molecular hub that orchestrates active signaling complexes.
- $\succ$  We recently reported that upon ET-1R activation,  $\beta$ -arr1 regulates actin regulators to form invadopodia.

The aim of this study is to investigate whether ET-1R/ $\beta$ -arr1 axis might operate through a cross-talk with invadopodia regulators, such as IQGAP1, to promote invadopodia assembly.

### 1. ET-1 regulates IQGAP1 in SOC cells

ET-1

IQGAP1

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IQGAP

#### **IQGAP1/DAPI**

## 2. ET-1R activation promotes association of IQGAP1 with $\beta$ -arr1 to activate Rho A/C GTPases

3. ET-1R/ $\beta$ -arr1 network promotes Rac1 deactivation through RacGAP1

MAC



IP: β-arr1



(A) Lysates of SKOV-3 cells incubated with ET-1 (100 nM) for the indicated times were immunoprecipitated with anti- $\beta$ -arr1 Ab or control IgG. (B) Lysates of HEY cells incubated with ET-1 and/or MAC (1 $\mu$ M) for 60 min were immunoprecipitated with anti-IQGAP1, anti- $\beta$ -arr1 Ab or control IgG. (C) Proximity ligation assay (PLA) of protein complexes containing IQGAP1 and  $\beta$ -arr1 in HEY cells stimulated with ET-1 and/or MAC for 60 min. Scale bar, 50 µm. (D) Confocal laser scanner microscopy examination in HEY cells stimulated with ET-1 and MAC for 60 min and stained for IQGAP1 (green) or  $\beta$ -arr1 (red). Nuclei are reported in blue (DAPI). Scale bar= 30  $\mu$ m. (E) RhoA and C-GTP pull down assay from si-SCR, si-IQGAP1 and si- $\beta$ -arr1 transfected SKOV-3 cells stimulated with ET-1 and/or MAC for 5 min.





(A) Rac1-GTP pull down assay from si-SCR, si-IQGAP1 and si- $\beta$ -arr1 transfected HEY cells stimulated with ET-1 and/or MAC for 5 min. (B) Lysates of HEY cells stimulated with ET-1 for 5 min were immunoprecipitated with anti- $\beta$ -arr1 Ab. (C) Rac1-GTP pull down assay from si-SCR, si-RacGAP1 transfected HEY cells stimulated with ET-1 for 5 min.

### 5. IQGAP1/β-arr1 network is required in the ET-1Rinduced MMP activation and cell invasion







6. ET-1 receptor blockade impairs metastatic

behaviour and interferes with IQGAP1 expression

**IQGAP1** 

Vinculir

Tubulin

MT1-MMP

CTR MAC

2.

the of invadopodia activity of HEY cells plated on Alexa-488 gelatin and treated with ET-1 for 48 hrs. Orthogonal views (*y*–*z* plane: red box; *x*– black box) are Black arrows indicate areas of gelatin degradation and actin are colocalized. Scale bar=30 μm.

CTR MAC

p-cortactin

cortactin

Tubulin

(A) 3D spheroid invasion assay using si-SCR, si-IQGAP1 or si-β-arr1 transfected SKOV-3 cells using ET-1/MAC as chemoattractant. Right, Quantification of 3D invasion relative to cumulative sprout length and invasion area. Scale bar = 100  $\mu$ m. (B) Transendothelial migration assay of SCR, IQGAP1 or  $\beta$ -arr1-siRNA transfected HEY cells, stimulated with ET-1. Scale bar: 30μm. (C) Conditioned media from HEY cells transfected with si-SCR, si-IQGAP1 or si-β-arr1 and treated with ET-1 and/or MAC were analysed by gelatin zymography. Arrows denote active forms of MMP-9 and MMP-

### 7. IQGAP1/ARRB1/EDNRA expression as prognostic gene signature in **HG-SOC** patients



Kaplan Meier analysis and logrank test using a TCGA cohort of Progression Free Survival (PFS) ovarian cancer patients with low or high tumor expression of IQGAP1 or IQGAP1/ARRB1 or IQGAP1/ARRB1/EDNRA.

(A) Female nude mice i.p. injected with SKOV-3 cells were treated after two weeks with vehicle control (CTR) or macitentan (MAC, 30 mg/kg) for 5 weeks. At the end of the treatment, all mice were euthanized and intraperitoneal organ were examined for visible metastasis. Representative intraperitoneal nodules are indicated by white arrowheads. (B) Expression of IQGAP1, Cortactin, phospho-Cortactin, Vinculin and MT1-MMP was evaluated by IB from metastatic nodules of xenografts treated as in A.

CTR MAC

# Conclusions

• Engagement of IQGAP1 by ET-1R/ $\beta$ -arr1 favours invadopodia function and aggressive behaviour of SOC cells.

• Interruption of ET-1R/IQGAP1/ $\beta$ -arr1 network impairs invadopodial function and metastatic behaviour of SOC cells, indicating the therapeutic efficacy of MAC.

• High expression level of EDNRA/IQGAP1/ $\beta$ -arr1 correlates with poor prognosis suggesting a clinical implication of these factors in SOC progression.



Α



