β-arrestin1/YAP/mutant p53 protein complex orchestrates endothelin A receptor response in high-grade serous ovarian cancer

72

Piera Tocci[1], Roberta Cianfrocca[1], Valeriana Di Castro[1], Laura Rosanò[1], Andrea Sacconi[2], Sara Donzelli[2], Silvia Bonfiglio[3], Gabriele Bucci[3], Enrico Vizza[4], Gabriella Ferrandina[5], Giovanni Tonon[3,6], Giovanni Blandino[2], and Anna Bagnato[1]

[1]Preclinical Models and New Therapeutic Agents Unit, IRCCS, Regina Elena National Cancer Institute, Rome, Italy; [2]Oncogenomic and Epigenetic Unit, IRCCS, Regina Elena National Cancer Institute, Rome, Italy; [3]Center for Translational Genomics and Bioinformatics, IRCCS, San Raffaele Scientific Institute, Milan, Italy; [4]Gynecologic Oncology Unit, IRCCS, Regina Elena National Cancer Institute, Rome, Italy; [5]Gynecologic Unit, Catholic University of Rome, Italy; [6]Functional Genomics of Cancer Unit, IRCCS, San Raffaele Scientific Institute, Milan, Italy.

BACKGROUND

High-grade serous ovarian cancer (HG-SOC), harboring high frequency of *TP53* mutations, is unresponsive to conventional chemotherapy. The transcriptional co-activators YAP (Yes-associated protein) and its ortholog TAZ, key components of the Hippo pathway, are pervasively activated in many human malignancies, including ovarian cancer, trough different inputs, comprising those acting on G-protein coupled receptors (GPCR) (1-4). Moreover, among the YAP partners, it has been recently identified mutant p53 (mutp53) (5-7). Understanding the mechanisms leading to YAP/TAZ activation during the acquisition of drug resistance is essential to develop more effective strategies, or reawaken drug sensitivity. In HG-SOC, the autocrine and paracrine loop mediated by the aberrant activation of endothelin-1 receptors (ET-1R), elicits pleiotropic effects, including cell proliferation, stem-cell like maintenance and chemoresistance. Of relevance, ET-1 modulates different pathways acting on tumor cells, which express the ETA receptor (ET_AR), and on the microenvironmental cells, which express the ETB receptor (ET_BR) (8-10). Despite these findings, there are still open questions regarding how YAP activity is regulated by GPCR, what are the molecular determinants triggering it, and what are the functional consequence of the input blockade. Therefore, in the present study we investigate whether the molecular interaction between β-arrestin1 (β-arr1) and YAP may allow transcriptional responses to ET-1 in a G-protein independent manner. In addition, we value the potential therapeutic approach of ET-1R blunting by the FDA approved dual receptor antagonist macitentan to target the oncogenic interplay between β-arr1 and YAP/mutp53.

1. ET-1/ET_AR axis promotes YAP/TAZ nuclear accumulation in chemoresistant ovarian cancer cells

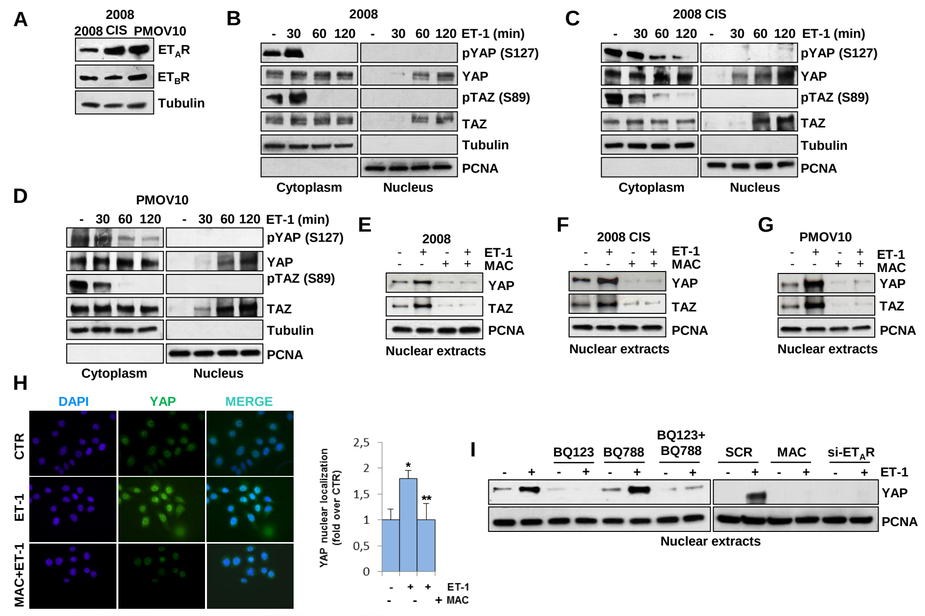


Figure 1. (A) IB analysis of ET_AR and ET_BR in total extracts of 2008 sensitive and cisplatinum (CIS) resistant cells and in patient-derived HG-SOC cells (PMOV10). **(B-D)** IB analysis of the indicated proteins in 2008 (B), 2008 CIS (C) and PMOV10 (D). **(E-G)** IB analysis of YAP and TAZ in 2008 (E), 2008 CIS (F) and PMOV10 cells (G) **(H)** YAP localization evaluated by immunofluorescence (IF) (Scale bar: 20μm, magnification 64X) in 2008 CIS cells. Graph (*Right*) represents the quantification of YAP nuclear localization. Bars are means ± SD (*, p<0.001 vs CTR; **, p<0.01 vs ET-1). **(I)** IB analysis of YAP protein levels in PMOV10 cells treated with the selective ET_AR antagonist BQ123, or with the ET_BR antagonist BQ788, or with MAC, or silenced for ET_AR, and stimulated or not with ET-1.

2. β-arr1 mediates the ET-1/ET_AR-dependent YAP cytoplasmic-nuclear shuttling

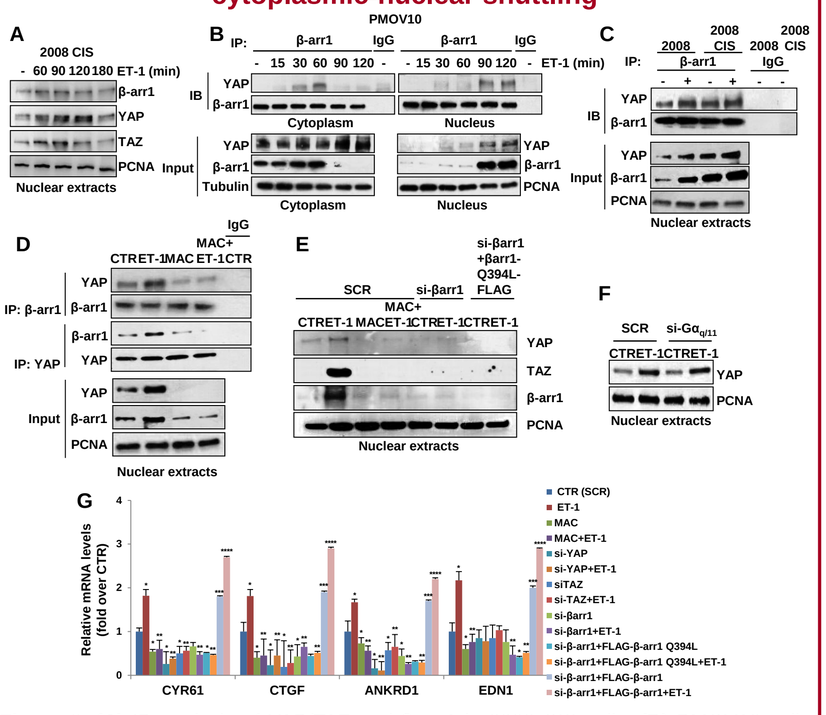


Figure 2. (A) IB analysis of YAP/TAZ and β-arr1 in 2008 CIS cells. **(B)** PMOV10 cells were IP for β-arr1 and IB for β-arr1 and YAP. **(C, D)** 2008 and 2008 CIS cells stimulated with ET-1 (C) and PMOV10 cells stimulated with ET-1 and/or MAC (D) were IP for β-arr1 or YAP and IB for β-arr1 and YAP. **(E)** IB analysis for YAP/TAZ and β-arr1 in PMOV10 cells transfected with the indicated siRNA or with mutant β-arr1. **(F)** IB analysis for YAP in PMOV10 cells silenced for $G\alpha_{q/11}$. **(G)** Expression analysis of the indicated YAP target genes in PMOV10 cells stimulated with ET-1 and treated with MAC or transfected with the indicated si-RNA or with mutant β-arr1, and then rescued with β-arr1. Bars are means ± SD (*, p<0.01 vs CTR; **, p<0.01 vs ET-1; ***, p<0.03 vs β-arr1 silenced cells; ****, p<0.05 vs β-arr1 silenced cells treated with ET-1).

3. ET-1R/β-arr1 activates YAP signaling through LATS, Trio, RhoA and actin cytoskeleton

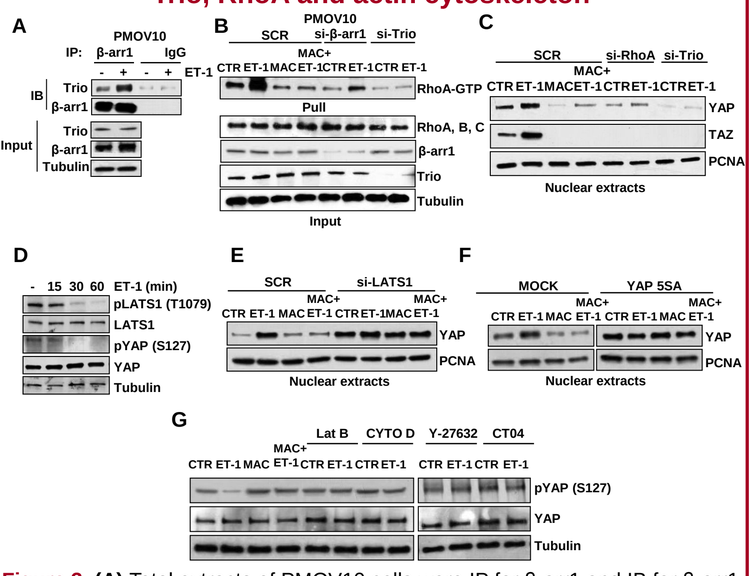


Figure 3. (A) Total extracts of PMOV10 cells were IP for β-arr1 and IB for β-arr1 and anti-Trio. **(B)** Rhotekin was used to pull down RhoA-GTP from total lysates of PMOV10 cells transfected with the indicated siRNA. **(C)** IB analysis for YAP/TAZ in PMOV10 cells transfected with the indicated siRNA, or treated with MAC, and stimulated with ET-1. **(D)** PMOV10 cells were IB for the indicated proteins. **(E, F)** PMOV10 cells transfected with SCR or si-LATS1 (E), or with an empty vector (MOCK) or with a vector encoding for YAP constitutively active (YAP 5SA-Myc) (F) were IB for YAP. Bars are means ± SD of densitometric measurements of YAP normalized to PCNA (*Bottom graphs*; *, p<0.001 vs CTR; **, p<0.001 vs SCR ET-1) (E), (*Bottom graphs* *, p<0.001 vs CTR; **, p<0.001 vs MOCK ET-1) (F). **(G)** PMOV10 cells treated with the indicated disruptors of actin cytoskeleton filaments were IB for the indicated proteins

4. β-arr1, forming a nuclear complex with YAP, mutp53 and TEAD, mediates ET-1R-induced YAP/TEAD gene transcription

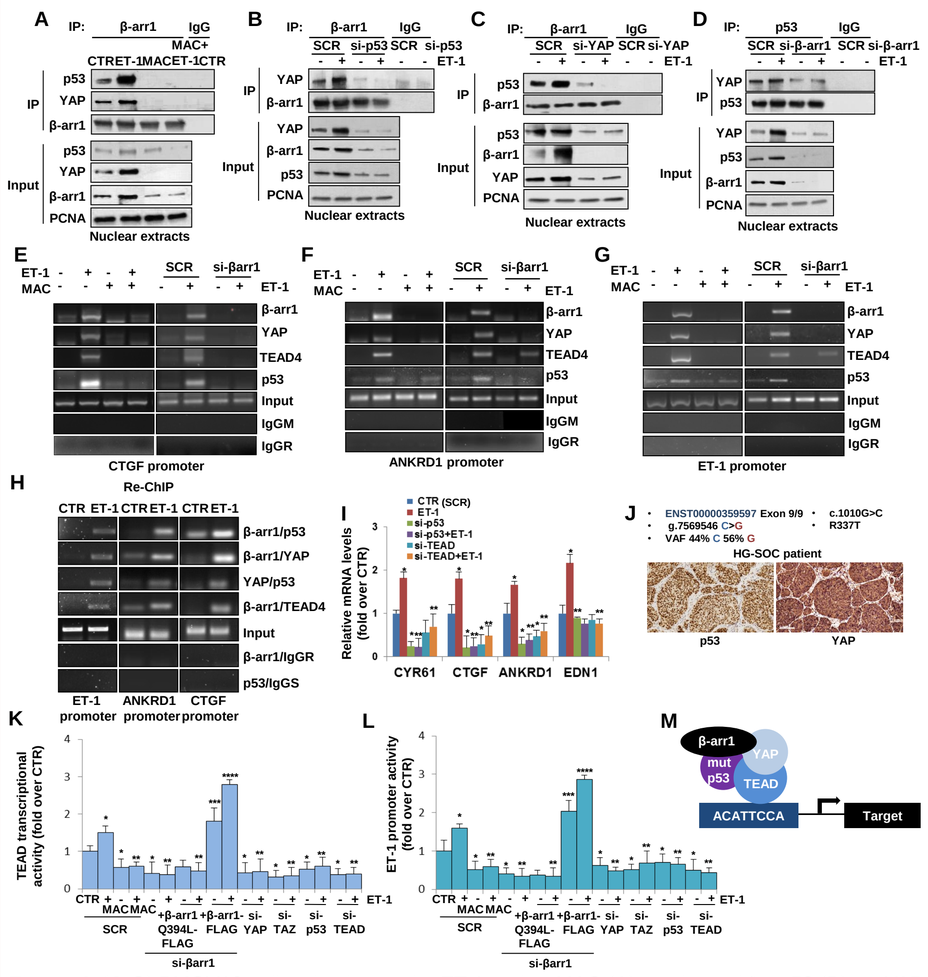


Figure 4. (A-C) PMOV10 cells stimulated with ET-1 and/or MAC (A) or silenced for p53 (B), or YAP (C) were IP for β-arr1, p53 and YAP. **(D)** PMOV10 cells silenced for β-arr1 were IP for p53 and IB for p53 and YAP. **(E-G)** In PMOV10 cells the binding of the indicated proteins on *CTGF* promoter (E), *ANKRD1* promoter (F) and *ET-1* promoter (G) was measured by ChIP analysis. **(H)** In PMOV10 cells the co-occupancy of β-arr1/YAP/TEAD/p53 to the indicated promoters was measured by ChIP-re-ChIP assays. **(I)** Expression analysis of the indicated YAP/TEAD target genes in PMOV10 cells Bars are means ± SD (*, p<0.01 vs CTR; **, p<0.01 vs ET-1). **(J)** *TP53* gene sequencing; p53 and YAP staining in the HG-SOC patient from which PMOV10 cells were derived. **(K, L)** TEAD transcriptional activity (K) and ET-1 promoter activity (L) in PMOV10 cells Bars are means ± SD (*, p<0.01 vs CTR; **, p<0.001 vs SCR ET-1; ***, p<0.002 vs β-arr1 silenced cells; ****<0.05 vs β-arr1 silenced cells treated with ET-1). **(M)** Schematic representation of the β-arr1/YAP/mutp53/TEAD complex bound to the specific ACATTCCA-box sequences on the target promoters.

5. ET-1R blockade by macitentan impairs apoptosis protection promoted by ET-1R/β-arr1/YAP pathway and sensitize to platinum

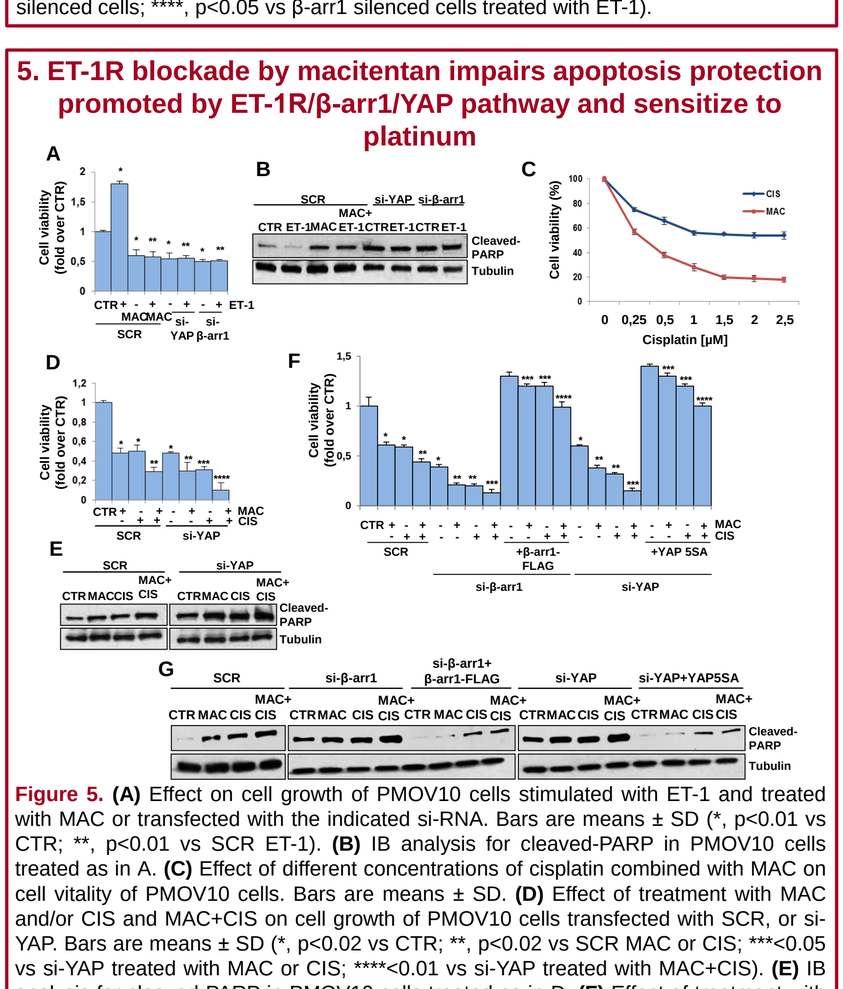


Figure 5. (A) Effect on cell growth of PMOV10 cells stimulated with ET-1 and treated with MAC or transfected with the indicated si-RNA. Bars are means ± SD (*, p<0.01 vs CTR; **, p<0.01 vs SCR ET-1). **(B)** IB analysis for cleaved-PARP in PMOV10 cells treated as in A. **(C)** Effect of different concentrations of cisplatin combined with MAC on cell vitality of PMOV10 cells. Bars are means ± SD. **(D)** Effect of treatment with MAC and/or CIS and MAC+CIS on cell growth of PMOV10 cells transfected with SCR, or si-YAP. Bars are means ± SD (*, p<0.02 vs CTR; **, p<0.02 vs SCR MAC or CIS; ***<0.05 vs si-YAP treated with MAC or CIS; ****<0.01 vs si-YAP treated with MAC+CIS). **(E)** IB analysis for cleaved-PARP in PMOV10 cells treated as in D. **(F)** Effect of treatment with MAC and/or CIS and MAC+CIS on cell growth of PMOV10 cells transfected with the indicated siRNA or plasmids. Bars are means ± SD (*, p<0.03 vs CTR; **, p<0.01 vs SCR MAC or CIS; ***, p<0.02 vs β-arr1 or YAP silenced cells treated with MAC or CIS; ****, p<0.01 vs β-arr1 or YAP silenced cells treated with MAC+CIS). **(G)** IB analysis for cleaved-PARP in PMOV10 cells treated as in F.

6. Combined expression of ET_AR, β-arr1 and YAP as a prognostic gene signature in HG-SOC patients

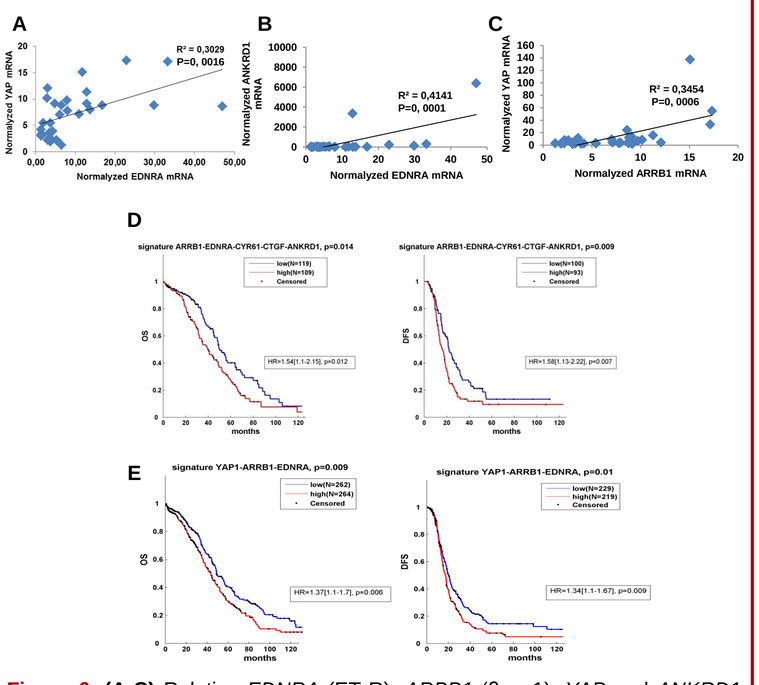


Figure 6. (A-C) Relative *EDNRA* (ET_AR), *ARBB1* (β-arr1), *YAP* and *ANKRD1* mRNA expression levels in 30 HG-SOC human specimens normalized for *CYPA* mRNA expression, were analyzed for their correlation: (A) *EDNRA* and *YAP* correlation, (B) *EDNRA* and *ANKRD1* correlation, (C) *ARRB1* and *YAP*. **(D, E)** (D) Overall survival (OS) and disease free survival (DFS) of HG-SOC patients with high (z score > 0.5) and low (z score < 0.5) combined expression levels of ET_AR, β-arr1 correlated with YAP gene signature (E) OS and DFS of HG-SOC patients with high (z score > 0) and low (z score < 0) combined signature of ET_AR, β-arr1, and YAP.

7. ET-1R blockade, targeting YAP/mutp53 activity, inhibits tumor growth and metastasis and enhances cisplatinum efficacy in HG-SOC PDX

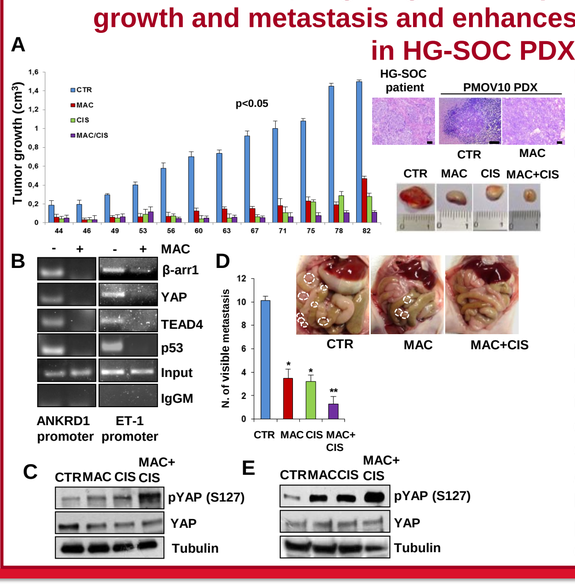
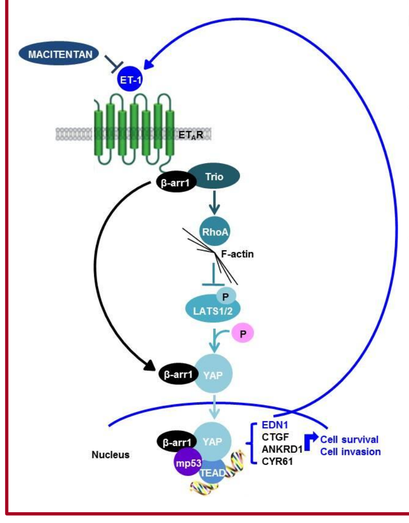


Figure 7. (A) PDX obtained by s.c. injection of PMOV10 cells, treated as indicated. *Right.* Representative tumors of PMOV10 PDX and H&E staining of HG-SOC patient section from which PMOV10 cells were derived. **(B)** The binding of the indicated proteins on *ANKRD1* and *ET-1* promoters was analyzed in PDX tumors by ChIP assays. **(C)** IB analysis for pYAP (S127) and YAP in PMOV10 PDX. **(D)** Nude mice ip injected with PMOV10 cells and treated as in A. Bars are means ±SD of ten mice for group. (*, p<0.001 vs CTR; **, p<0.01 vs MAC or CIS). *Right*, representative ip nodules of PMOV10 PDX **(E)** IB analysis for pYAP (S127) and YAP on ip nodules extracts of PMOV10 PDX.

CONCLUSIONS



In patient derived HG-SOC cells we observed that:

- **β-arrestin1, that controls ET-1R signaling, interacts with YAP triggering its cytoplasmic-nuclear shuttling;**
- **β-arrestin1/YAP interaction allows the recruitment of mutp53 to the YAP/TEAD transcriptional complex, leading to the transcription of target genes, including EDN1 (ET-1) that ensures persistent signals sustaining aggressive traits;**
- **The combined expression of YAP gene signature, β-arrestin1 and ET_AR is associated with poor clinical outcome, suggesting that this network might be especially valuable for the prognosis of recurrent HG-SOC.**

Our findings uncover the oncogenic and poor prognostic role of β-arrestin1/YAP/mutp53 network in HG-SOC. Moreover, ET-1R represents a promising target to hamper this network, warranting clinical trials of the FDA approved dual receptor antagonist macitentan for precision medicine of HG-SOC

References:

1. Zanconato F, et al. Cancer Cell. 2016; 29:783-803; **2.** F-X Yu, et al. Cell 2012; 150: 780-791; **3.** Wang L, et al. Cancer Res. 2017; 77: 2413-2423; **4.** Haemmerle M, et al. Nat. Commun. 2017; 8:310; **5.** Di Agostino S, et al. EMBO Rep. 2016; 17: 188-201; **6.** Ferraiuolo M, et al. Int. J. Mol. Sci. 2017; 18: 5; **7.** Lo Sardo F, et al. Cancers (Basel) 2018; 10; **8.** Rosanò L, et al. Nat. Rev. Cancer 2013; 13: 637-651; **9.** Rosanò L, et al. Cancer Res. 2014; 74: 7453-7464; **10.** Semprucci E, et al. Oncogene 2016; 35 : 3432-3442.

IRE ISTITUTO NAZIONALE TUMORI REGINA ELENA

15th Nov 2018

ISAB IOVS