ET-1 receptor blockade in engineered 3D high-grade serous ovarian cancer tumoroids

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Laura Rosanò[1], Judith Pape[2], Umber Cheema[2], Marilena Loizidou[3] and Anna Bagnato[1]

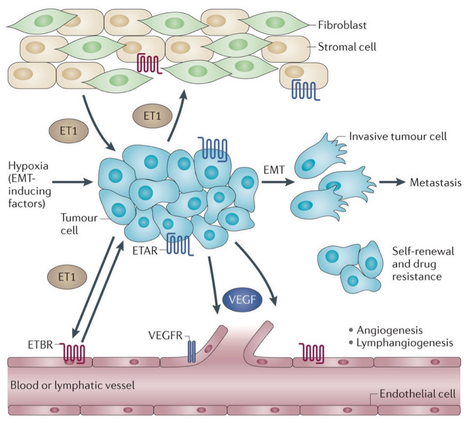
[1]Unit of Preclinical Models and New Therapeutic Agents, IRCCS, Regina Elena National Cancer Institute, Rome Italy; [2] UCL Institute of Orthopaedics and Musculoskeletal Sciences, UCL Division of Surgery and Interventional Science, Stanmore Campus, and [3]UCL Division of Surgery and Interventional Science, Royal Free Campus, London.

BACKGROUND

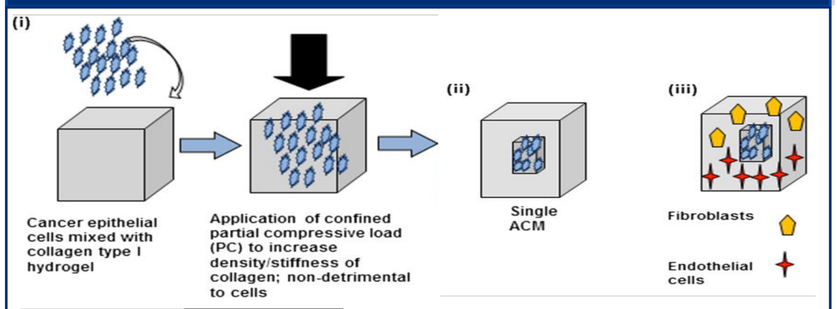
- ✓ High-grade serous ovarian cancer (HG-SOC) is responsible of 70% of deaths due to late diagnosis with detectable metastatic spread in abdominopelvic cavity (1).
- ✓ The metastatic process is heavily influenced by the extracellular matrix (ECM) density and composition of the surrounding tumour microenvironment (TME), and cancer cells respond to signals provided by the TME, such as growth factors, to facilitate their metastatic spread.
- ✓ In the G-protein coupled receptor (GPCR) family, the endothelin-1 receptor (ET-1R) has a critical role in SOC progression, by controlling different tumor promoting effects, including invasion (2). Many pathways involved in ET-1R-driven cancer invasion and metastasis are mediated by β-arrestin-1 (β-arr1), acting as cytoplasmic/nuclear molecular hub to organize complex signalings (3).
- ✓ 3D models of cancer ("tumouroids"), engineered with stromal surrounds comprising a range of ECM densities, composition and stromal cell populations, are increasingly being used as a platform to study microenvironment clues of tumour progression, such as invasion and vascular remodeling occurring during tumour growth and treatment (4-6).
- ✓ We developed a 3D HG-SOC models recapitulating ECM and stromal cell composition that represent a more physiological opportunity to study tumour progression driven by ET-1R and the effects of its pharmacological manipulation.

AIMS

1. Development of a new tissue-engineered biomimetic tumoroid model of HG-SOC.
2. Effect of ET-1 on invasive and metastatic properties of ovarian cancer cells in a complex tumouroid environment.
3. Effect of ET-1R antagonist, macitentan, in tumouroids.

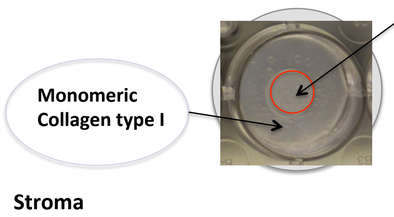


1. Methodology- Making a Tumouroid



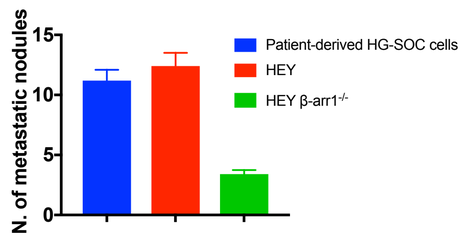
(i) Formation of an artificial cancer mass (ACM). Partial compression of the cancer cell populated collagen hydrogel to increase the collagen and cell density. (ii) Insertion of the ACM into acellular collagen hydrogel. (iii) Insertion of the ACM into a cellular collagen hydrogel populated with fibroblasts and/or endothelial cells.

2. Making ovarian cancer tumouroids



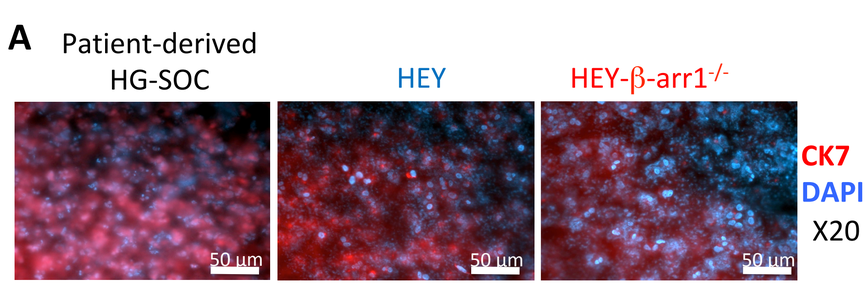
Artificial Cancer Mass (ACM): (50X10^3 cells)
Patient-derived HG-SOC cells (High metastatic)
HEY cells (High metastatic)
HEY-β-arr1$^{-/-}$ cells (Low metastatic)

Stroma
- fibroblasts (HDF) (25X10^3)
- endothelial cells (HUVEC) (100x10^3)
- laminin (50 µg/ml)
- collagen type I (2.05 mg/ml)

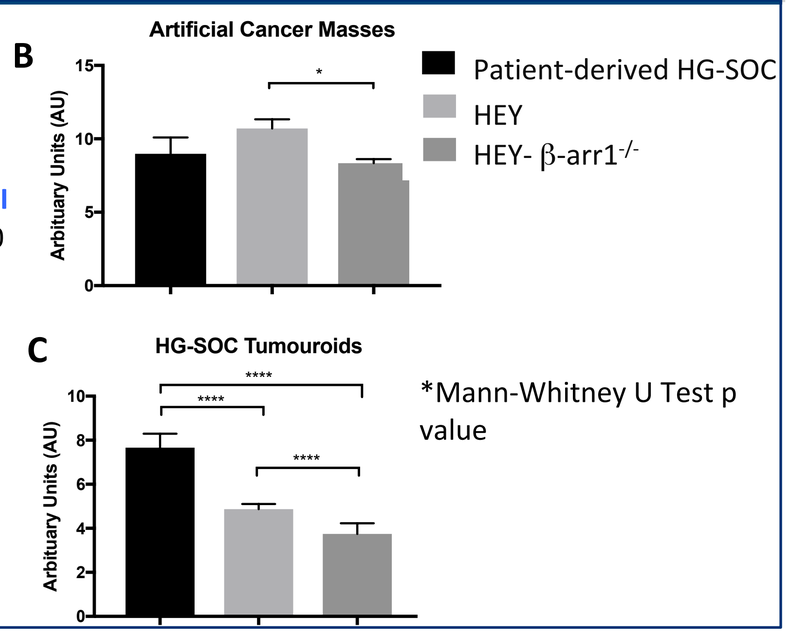


Schematic representation of HG-SOC tumouroids. The ACMs were prepared using the RAFT™ 3D cell culture system, in which different ovarian cancer cells were added to collagen type I. Each ACM was incorporate in cellular stroma reaching a 10% matrix cultures by interstitial fluid removal using hydrophilic RAFT absorbers.
Histograms indicate the metastatic potential of indicated cells intraperitoneally injected in female nude mice and examined for visible metastases after 5 weeks. Values represent the average ±s.e.m. of 10 mice for group; n=3.

3. Patient-derived HG-SOC show higher metabolic activity



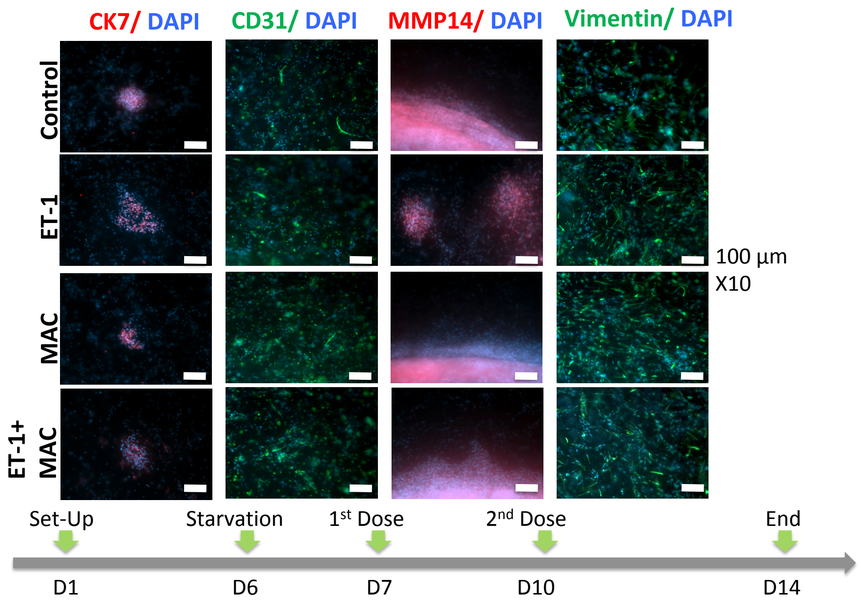
Artificial Cancer Masses (ACM)



*Mann-Whitney U Test p value

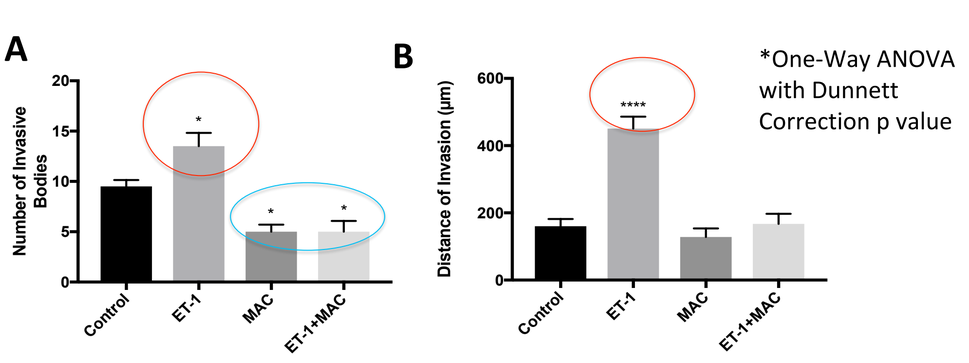
Metabolic activity of cells at day 14 in the ACMs (**B**) and tumoroids (**C**) evaluated by PrestoBlu assay, in which resazurin is used as an oxidation-reduction indicator that undergoes colorimetric change in response to cellular metabolic reduction, to quantitatively measure cell viability and cytotoxicity.

4. Ovarian cancer cells invaded the stroma in tumouroids

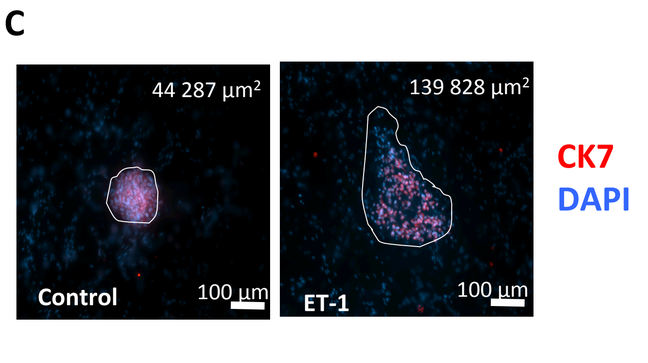


Representative invasive bodies, endothelial structures and invasive front of HEY biomimetic tumouroids. Engineered ACMs of 10% (w/v) collagen HEY cells embedded in 10% matrix density stroma and treated with ET-1 (100 nM) and/or ET-1R antagonist macitentan (MAC, 1µM). Morphology was assed using immunofluorescence of CK7 or MMP14 (red) and DAPI (blue), HUVEC morphology using immunofluorescence of CD31 (green)/ DAPI and HDFs using vimentin (green)/DAPI. HEY cells invaded as cellular aggregates in 10% matrix tumouroids forming a network of budding glandular structures resembling tumour budding observed in vivo.

5. ET-1R activation enhances the number, size and distance of invasive bodies



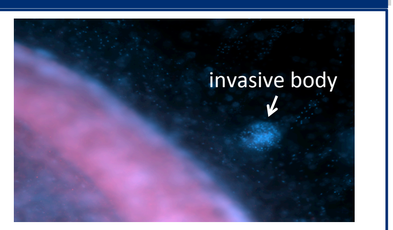
*One-Way ANOVA with Dunnett Correction p value



Number of invasive bodies within the stromal compartment at day 14 (**A**) and distance of invasion (**B**) by these invasive bodies into each respective stromal surround after treatment with ET-1 (100nM) and/or MAC (1 µM) by day 14. (**C**) Example of surface area of an invasive body for ET-1 group, showing 3-fold upregulation as compared to control group. The white dotted lines denote the boundary of the ACM and the stromal surround.

Conclusions

- ➢ **All cell types were able to form spheroids within the novel 3D collagen matrix.**
- ➢ **In a complex tumouroid and in the presence of stromal cells, the patient-derived HG-SOC tumouroids had the highest metabolic activity.**
- ➢ **Silencing of β-arr1 significantly inhibited the metabolic activity of HG-SOC tumouroids.**
- ➢ **In HEY tumouroids, ET-1 treatment significantly increased the number of budding invasive bodies, as well as their size and the distance from the ACM.**
- ➢ **ET-1R blockade with macitentan significantly reverted ET-1 effects.**



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(1) Kurman RJ. **Ann Oncol. 2013**; S10:x16-21; (2) Rosano L, et al. **Nat Rev Cancer 2013**; 13:637-51; (3) Rosanò L, Bagnato A. **J Exp Clin Cancer Res. 2016** 29;35:121; (4) Nyga A, Loizidou M, Emberton M, Cheema U. **Acta Biomater. 2013**; 9:7917-26; (5) Magdeldin T, López-Dávila V, Villemant C, Cameron G, Drake R, Cheema U, Loizidou M. **J Tissue Eng.** 2014;5:2041731414544183; (6) Magdeldin T, López-Dávila V, Pape J, Cameron GW, Emberton M, Loizidou M, Cheema U. **Sci Rep. 2017** Mar 9;7:44045.

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15th Nov 2018

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