A novel coculture system reveals the onset of pro-tumorigenic crosstalk between adipose-derived mesenchymal stem cells (AD-MSC s) and endometrial cancer cells

Antona, A.[a]; Garro, G.[a]; Cracas, S.V.[a]; Varalda, M. [a]; Venetucci, J. [a]; Tortorelli, M.F. [a]; Sseguya, P. [a]; Surico, D. [a,b], Valente, G.[a,c]; Capello, D. [a]

[a] Department of Translational Medicine, Centre of Excellence in Aging Sciences, Università del Piemonte Orientale, Novara, Italy

[b] Gynecology and Obstetrics, Maggiore della Carità Hospital, Novara, Italy

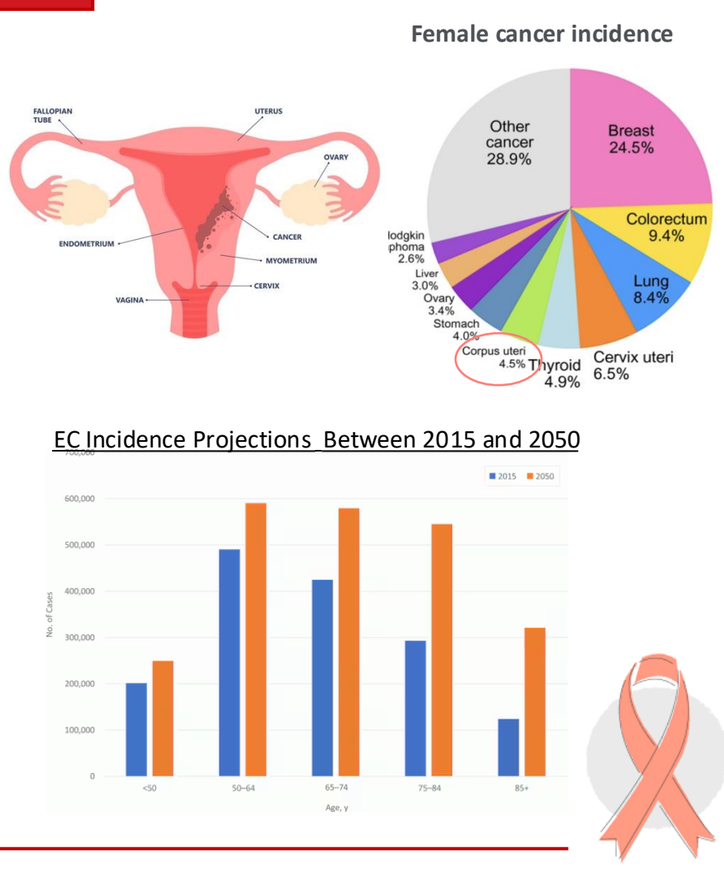
[c] Pathology Unit, Ospedale Sant'Andrea, Vercelli, Italy

annamaria.antona@uniupo.it

BACKGROUND

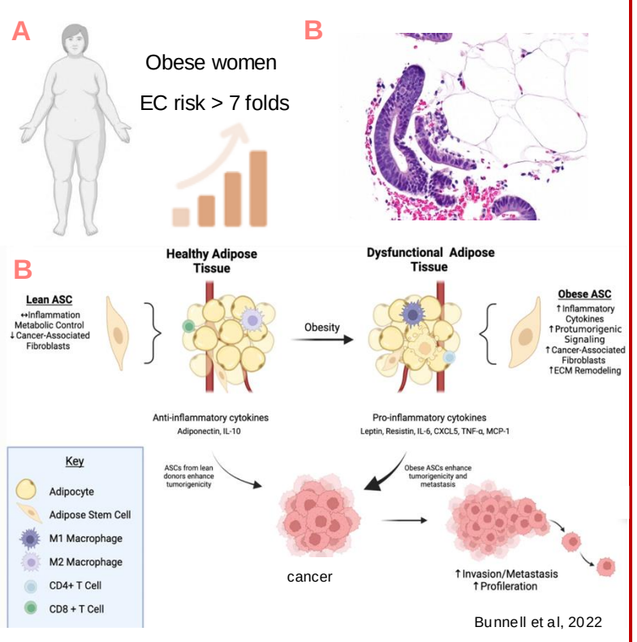
Obesity prevalence has increased significantly among women of all ages, particularly in those under 45, correlating with a rising incidence of endometrial cancer (EC), the most common gynecologic cancer in developed countries. **This trend is more pronounced in women aged 45 and older, highlighting the need to understand the interactions between obesity, aging, and EC progression**. The molecular and metabolic interactions between adipose-derived mesenchymal stem cells (AD-MSCs), adipocytes, and EC cells remain poorly investigated. **Herein, we characterized AD-MSCs from patients undergoing surgical procedures and we set up a new co-culture system enabling the characterization of the paracrine interaction between AD-MSCs and EC cells.**

Estrogen-dependent (Ishikawa) and -independent (HEC1A) EC cells were cocultured (CoC) with AD-MSCs for 14 days. Notably, CoC EC cells displayed a 1.5-fold increase in cell number compared to control cells highlighting the role of AD-MSCs in supporting EC cells proliferation. Spheroids formation and migration assay disclosed enhanced 3D growth and migration potential in CoC EC cells. Also, CoC HEC1A cells displayed a 3- fold increase in chemoresistance (20 hours of exposure to 17 nmol/L paclitaxel) over a timeframe of 7 days. In CoC AD-MSCs and EC cells, the morphological analysis revealed a significant accumulation of vesicular structures, identified as lipid droplets by Nile red staining, suggesting adipogenic differentiation of AD-MSCs. HEC1A displayed increased expression of two genes involved in lipid metabolism, which were reported as adverse EC prognostic markers: diacylglycerol O-acyltransferase 2 (DGAT2) and CD36. Lastly, increased gene expression of peroxisome proliferator-activated receptor γ coactivator1α (PGC-1α) and increased mitochondrial membrane potential suggested a link between lipid metabolism and mitochondrial function in EC cancer progression.



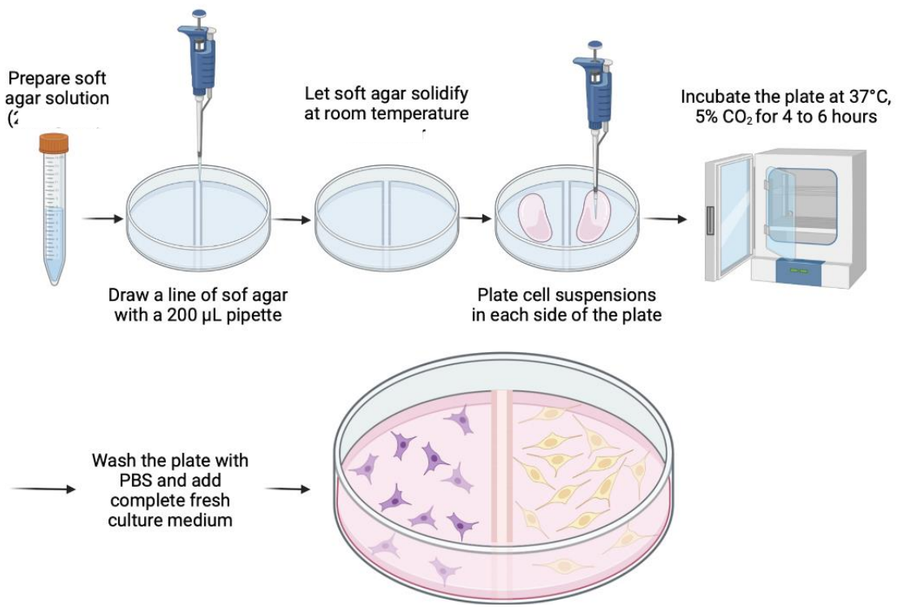
RESULTS

EC, adipose tissue and tumor microenvironment



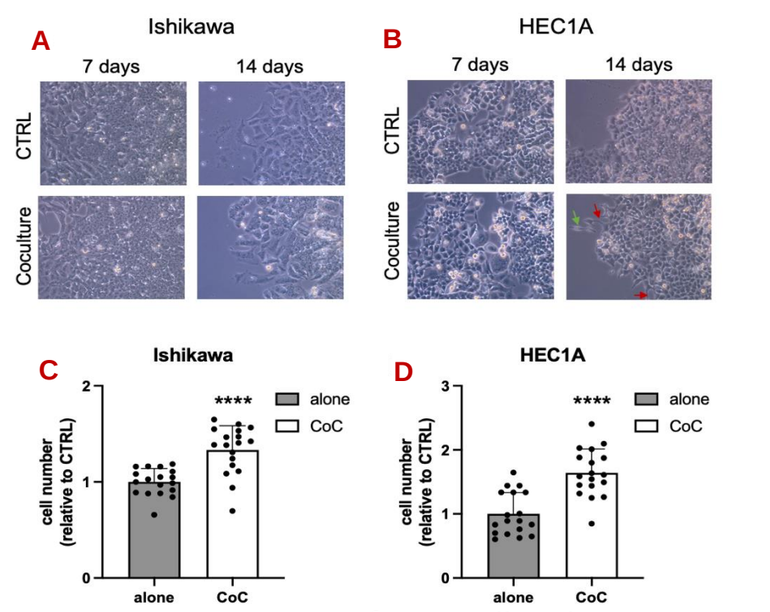
A EC risk increase in obese women. **B** Micrograph of an endometrial biopsy with adipose tissue. H&E stain. **C** Role of AD-MSCs in tumor microenvironment

Establishment of a fast, easy, and economic coculture system



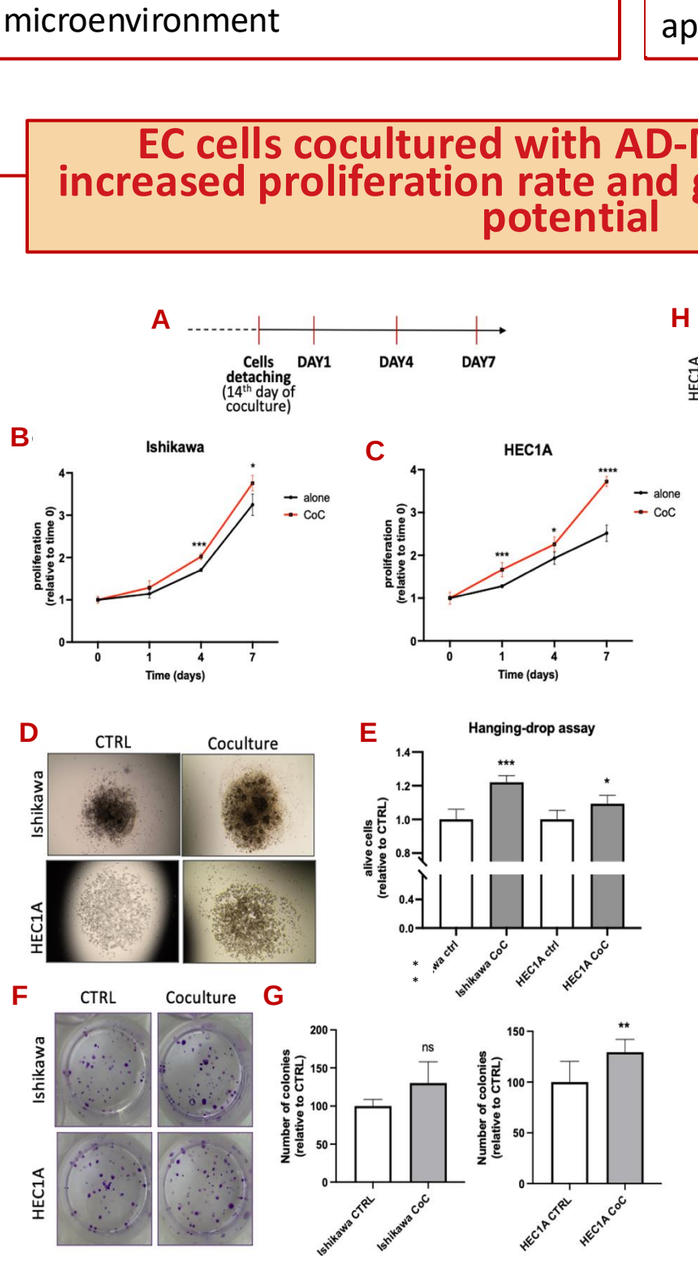
Schematic representation of the establishment of a coculture system overcoming major limits of existing coculture techniques: 1. Characterization and the subculturing of only one cell type taking part in the interaction (e.g., trans well inserts), 2. Fixed number of cells, 3. Elevated costs not applicable for a preliminary investigation.

AD-MSCs sustain EC cells proliferation in coculture



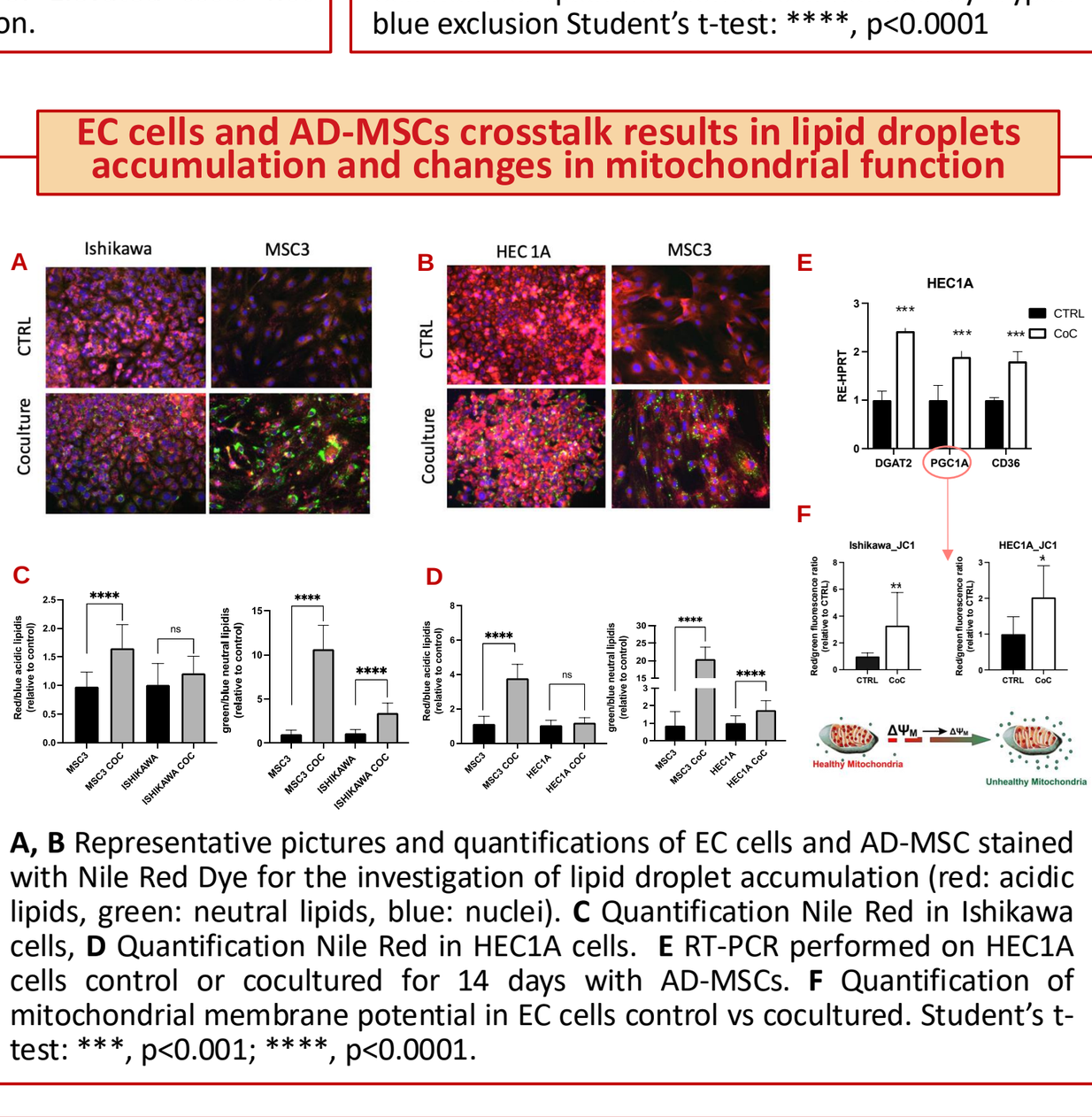
Phase-contrast pictures of EC cells alone or co-cultured for 7 and 14 days. **A** Ishikawa snd **B** HEC1A cells cocultured for 7- and 14- days display an increase in the vesicular bodies (black spots). **C**, **D** Cell number quantification of cell number by trypan blue exclusion Student's t-test: ****, p<0.0001

EC cells cocultured with AD-MSCs display an increased proliferation rate and greater tumorigenic potential



A Scheme of the experiments. **B,C** Ishikawa and HEC1A cells proliferation were monitored at different time points after 14 days of coculture. **D** Representative phase-contrast pictures of hanging drop assay. **E** Quantification of viable cells in the hanging drop assay. **F,G** Representative pictures and quantification of colony formation assay of EC cells. **H,I** Representatives pictures and quantification Wound healing assay. EC cells cocultured with AD-MSCs for 2 weeks were plated in the inserts, wounds area was analyzed at several time points. **L,M** Drug sensibility analysis in EC cells, 20 hours of exposure to 17 nmol/L paclitaxel, after washout viability was monitored at days 1, 4, 7. Student's t-test: *, p<0.05; **, p<0.01 ***, p<0.001 ****, p<0.0001

EC cells and AD-MSCs crosstalk results in lipid droplets accumulation and changes in mitochondrial function



A, B Representative pictures and quantifications of EC cells and AD-MSC stained with Nile Red Dye for the investigation of lipid droplet accumulation (red: acidic lipids, green: neutral lipids, blue: nuclei). **C** Quantification Nile Red in Ishikawa cells, **D** Quantification Nile Red in HEC1A cells. **E** RT-PCR performed on HEC1A cells control or cocultured for 14 days with AD-MSCs. **F** Quantification of mitochondrial membrane potential in EC cells control vs cocultured. Student's t-test: ***, p<0.001; ****, p<0.0001.

CONCLUSIONS

Overall, our data support the efficacy of our coculture system in reproducing the paracrine interaction between AD-MSCs and EC cells. Our results support the hypothesis that AD-MSCs sustain EC progression by promoting migration, clonogenicity potential, and chemoresistance.

Further studies are needed to dissect the molecular pathways involved in the crosstalk during ageing to identify new therapeutic targets for EC prevention and treatment.