Divergent Autocrine Roles for the Endothelial SASP

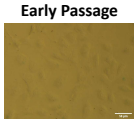
QUEEN'S UNIVERSITY BELFAST
WELLCOME-WOLFSON INSTITUTE FOR EXPERIMENTAL MEDICINE

Shannon McDonnell, Jasenka Guduric- Fuchs, Pietro Maria Bertelli, Edoardo Pedrini, Reinhold Medina
The Wellcome-Wolfson Institute for Experimental Medicine
Belfast BT7 1NN, Northern Ireland, UK

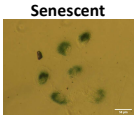
INTRODUCTION

- Senescent Cells appear large with flattened morphology and positive staining for Beta-Galactosidase. The senescence associated secretory phenotype (SASP) is a characteristic feature of senescent cells.
- The secretion of SASP cytokines has been suggested to promote age related disease (Annual Review of Pathology 2010; 5: 99-118).
- Human Cord Blood derived Endothelial Colony forming Cells (ECFCs) can be harnessed for cell therapies that promote vascular repair; however, when expanded in vitro, they undergo replicative senescence (Frontiers in Medicine, 2018; 5: 273).
- This study investigated the autocrine effects of endothelial SASP on early passage (EP) endothelial cells.

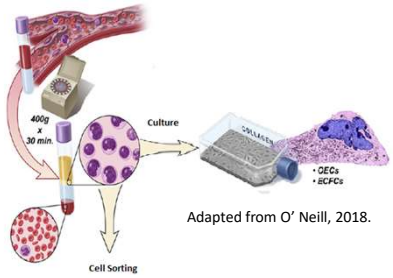
Early Passage



Senescent

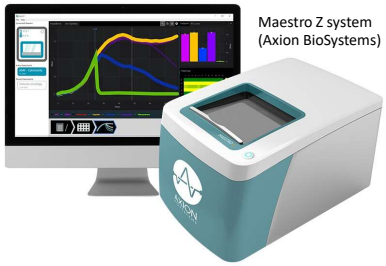


METHOD



Adapted from O' Neill, 2018.

- Human Endothelial Colony Forming Cells were isolated from umbilical cord blood.
- ECFCs were grown under normal conditions until they reached their Hayflick Limit.
- Conditioned Media was collected at early (p1-p7) and late (P22-P29) passages.
- Colony forming assays were performed using 50:50 fresh and conditioned media.
- 3D Matrigel angiogenesis assay was conducted to measure tube forming capacity.
- TEER Measurements were taken at 3 frequencies using Maestro Z system (Axion BioSystems).



Maestro Z system (Axion BioSystems)

RESULTS

Figure 1 Effect of SASP on proliferative and tubulogenic capacity of ECFCs.

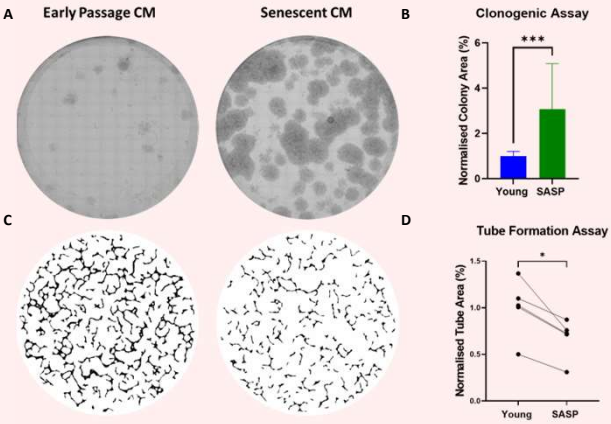


Figure 1. Clonogenic assay to assess the affect of SASP on ECFC proliferation following low density seeding. EP ECFCs (n=5) were plated in 50:50 EP or Senescent conditioned media (CM). **(A)** Representative EVOS images of colony forming assay after 7 days in culture. **(B)** The senescent CM significantly increased the colony forming ability of the ECFCs when compared with early passage CM. **(C)** Representative mask of EVOS images of tube formation after 48 hours. **(D)** Senescent CM significantly decreases the tube forming ability of the ECFCs when compared with EP CM. (*p≤.05, *** p<.001).

Figure 2 Senescent Cells have decreased barrier and are susceptible to disruption.

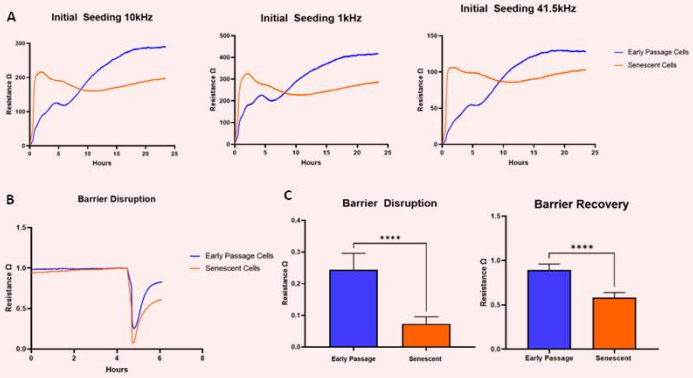


Figure 2. **(A)** Initial seeding of early and late passage ECFC's in Maestro Z system (Axion BioSystems). Cells were seeded and allowed to form a barrier overnight. **(B)** On day 2, cells were treated with thrombin to simulate barrier disruption and their recovery was measured. **(C)** Graphs illustrate the barrier disruption and recovery of the cells. Senescent Cells are more susceptible to barrier disruption and it hinders their recovery. (**** p<.0001).

Figure 3 SASP protected ECFCs from thrombin-induced barrier disruption.

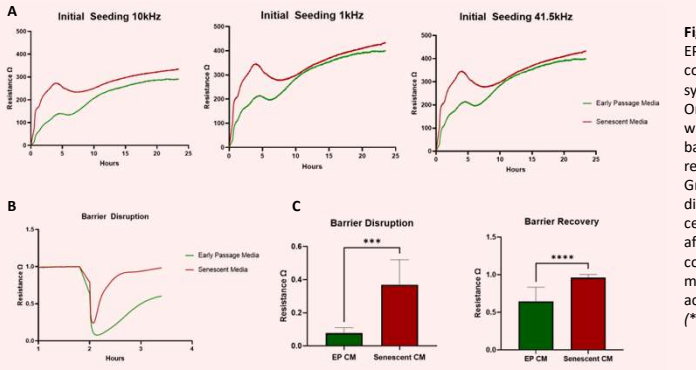


Figure 3. **(A)** Initial seeding of EP ECFC's treated with conditioned media in Maestro Z system (Axion BioSystems). **(B)** On day 2, cells were treated with thrombin to simulate barrier disruption and their recovery was measured. **(C)**. Graphs illustrate the barrier disruption and recovery of the cells. SASP has a protective affect against barrier disruption compared with EP conditioned media. The SASP promotes active recovery of the barrier. (*** p<.001,**** p<.0001).

CONCLUSION

SASP significantly increased the clonogenic capacity of ECFC's but significantly decreased their 3D tube forming ability compared to EP media.

Senescent Cells have decreased barrier forming capability but CM from senescent cells exhibited a significant protective effect on thrombin induced barrier disruption and promoted an effective recovery of the barrier.

AXION BIOSYSTEMS
explore life's circuitry™

BBSRC
bioscience for the future

Email s.mcdonnell@qub.ac.uk
Webpage http://medinalab.atwebpages.com/