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Canonical wnt signaling reverses the ‘aged/senescent’ human endogenous cardiac stem cell phenotype

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Background: The adult human myocardium harbors endogenous, multi-potent cardiac stem cells (eCSCs). Manipulation of eCSCs ex-vivo and in situ has opened new therapeutic avenues for functional myocardial regeneration. However as aging/senescence of eCSCs determines their function and regenerative capacity, regulation of this parameter will impact the efficacy of these therapies, considering the advanced age of the majority of patients in need of regenerative therapy.

Objectives: Our aim is to determine the main factor(s) that determine the ‘aged’ human eCSC phenotype and investigate its potential reversibility.

Methods: c-kitpos CD45neg eCSCs were isolated from the right atria appendage (~200mg) of different aged patients (32 to 85 years) by enzymatic digestion followed by MACS (Miltenyi). eCSCs were characterised for co-expression of ageing/senescence markers (p16INK4a, p53, p21, senescence-associated β-galactosidase) with known stemness/multipotency (Oct-4, Nanog, Bmi-1, TERT, Sox-2) and proliferation (Ki67) markers. Telomere length of eCSCs was determined using Q-FISH analysis. DNA damage was assessed using γ-H2AX. The growth (BrdU labelling), clonogenicity and differentiation potential of young and old eCSCs were also evaluated.

Results: The number of eCSCs isolated was similar regardless of age, gender and pathology (~45,000/gram of tissue). eCSCs isolated from young and old hearts showed age-correlated increased expression of ageing/senescence markers and decreased expression of stemness/multipotency and proliferation markers. Single cell expression analyses revealed heterogeneity within the eCSC population with eCSCs isolated from old hearts harboring a greater proportion of eCSCs with critically short telomeres and increased DNA damage. ‘Aged-senescent’ eCSCs showed limited cloning and growth capacity and impaired cardiac differentiation capacity. Moreover, ‘aged-senescent’ eCSCs expressed increased senescence-associated secretory phenotype (SASP) factors relative to their younger counterparts. Treatment with the canonical Wnt ligand, Wnt3a significantly increased the proliferation of ‘aged-senescence’ eCSCs to levels observed in younger eCSCs. Conversely a switch to non-canonical Wnt signaling imparted a negative ‘ageing’ effect on eCSCs. Importantly, although the cloning efficiency was inversely age-related, single-cell derived eCSC clones obtained from young and old hearts were indistinguishable by their gene expression and differentiation potential, strongly suggesting that eCSC aging is a stochastic process.

Conclusion: eCSCs stochastically develop a senescent phenotype with age impacting their growth and differentiation potential. Manipulation of canonical and non-canonical Wnt signaling pathways reversed the ‘aged/senescent’ phenotype.