of complete differentiation in cardiac phenotype during these few days.

Conclusions: In this study the addition of retinoic acid during serum reduction favours a cardiac phenotype at the expense of skeletal muscle trans-differentiation, confirmed by NP and ET system expression. Moreover, the lacking of a complete differentiation of cells on CNT-Sc highlights the need of more day of culture to realize this process. Nevertheless further analysis on novel biomaterials to enhance cell growth/proliferation and to support the damaged heart will be needed to bring heart tissue engineering into clinical application, these results are a useful starting point to develop new Sc-based biomaterials.

doi:10.1016/j.vph.2015.11.014

Pre-conditioning cardioprotection mediated by the estrogen receptors in spontaneously hypertensive female rats

T. Angelone[a], C. Penna[b], E.M. De Francesco[c], T. Pasqua[a], C. Rocca[a], P. Cantafio[a], S. Femminò[b], E. Filice[a], P. Pagliaro[b], M. Maggiolini[c], M.C. Cerra[a]
[a]*Department of Biology, Ecology and E.S., University of Calabria, Arcavacata di Rende (CS), Italy*
[b]*Department of Biological and Clinical Sciences University of Turin, Turin, Italy*
[c]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Arcavacata di Rende (CS), Italy*

Objectives: Endogenous estrogens are important regulators of cardiovascular homeostasis in premenopausal women and interfere with the development of hypertension and coronary artery disease. Estrogens mediate profound effects throughout the body, and regulate physiological and pathological processes in both woman and men. These hormones act via three different estrogen receptors (ERs), ERα and ERβ, and GPER. GPER is implicated in both rapid signaling and transcriptional regulation and is expressed in the cardiovascular system. Ischemic and pharmacological pre-conditioning are therapeutic interventions that limit reperfusion injury via RISK pathway. However, these cardioprotective interventions are blunted in pathological animal models. Whether GPER plays a role in preconditioning cardioprotection has not been fully investigated yet. The aim of this study was to evaluate the potential cardioprotective role mediated by GPER and ERs in spontaneously hypertensive female rats in a preconditioning context.

Materials and methods: The effects of the selective GPR30 agonist, G1, or of the selective GPR30 antagonist, G15, both given 20 min after ischemia, were evaluated on ischemia/reperfusion (I/R) injury. Specific inhibitors of the RISK pathway (AkT, L-NIO and MitoKATP channels) were co-infused with G1, just before I/R. For comparative purposes, the same protocol was used to study the role of the ER antagonist, ICI. Infarct size and cardiac function were assessed by using the isolated and Langendorff perfused heart technique.

Results: Pre-treatment with G1 limited I/R injury at the lowest concentration tested (10 nM), while the cardioprotective effect was blunted at higher doses (100 nM). Administration of G15 (100 nM) induced an increase of myocardial damage which was greater than that induced by ICI (100 nM). Co-infusion with specific RISK inhibitors blocked the G1 protective effects.

Conclusions: These results suggest that GPERs play a major role in mediating cardioprotection in preconditioning. Our data pave the way to evaluate whether, in female, G1 can be regarded as a potential therapeutic agent in the presence of hypertension, being able to target and attenuate reperfusion-induced cell death and to overcome hypertension-induced heart failure.

doi:10.1016/j.vph.2015.11.015

C-kit/Creert2 knock-in allele minimally tags c-kit positive resident endogenous cardiac stem cells and its cardiomyocyte progeny in the adult life

E. Cianflone[a], C. Vicinanza[a], I. Aquila[a], M. Scalise[a], F. Marino[a], T. Mancuso[a], E.D. Giovannone[a], C. Indolfi[a], G.M. Ellison[b], B. Nadal-Ginard[b], D. Torella[a]
[a]*Laboratory of Molecular and Cellular Cardiology, Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy*
[b]*Centre of Human & Aerospace Physiological Sciences and Centre for Stem Cells & Regenerative Medicine, Faculty of Medicine & Life Science, King's College London, London, UK*

Objectives: Recently, we have demonstrated that resident tissue-specific c-kit + endogenous cardiac stem cells (eCSCs) are essential agents for myocyte replenishment in adult rodents. However, the first mouse model for c-kit genetic fate mapping using a Cre knock-in the ckit Exon1 concluded that the c-kitpos cells minimally produce cardiomyocytes (CMs) either during development as well as in healthy or damaged adult hearts. PURPOSE: We addressed whether a tamoxifen (TAM)-inducible Cre knock-in the c-kit Exon1 efficiently recombine c-kit + eCSCs in the adult myocardium for reliable cell fate mapping.

Materials and methods: Heterozygous c-kitCreER(T2)/+ mice were crossed with the global double-fluorescent Cre reporter mice (R26mT/mG) that express in the ROSA (R26) locus a membrane-targeted tandem Tomato dimer (mT) prior to Cre excision and membrane-targeted green fluorescent protein (mG or GFP) after Cre recombination.

Results: CreER(T2) Knock-in in the c-kit Exon 1 produces a null c-kit allele with mice that are hemizygous c-kit hypomorphs. Indeed, c-kitCreER(T2) knock-in downregulates c-kit expression in all c-kit expressing cells of the body. When double mutant c-kitCreER(T2)/+: R26mT-mG/+ mice were treated with standard TAM diet for 3 months, ~80% of total c-kitpos bone marrow cells were recombined to express eGFP. The same high level of recombination was shown in c-kit + cells of the skin, spleen, lung and testis. When we isolated c-kit + HSCs, only a fraction of them, about 15%, were recombined by TAM. Thus, the c-kitCreER(T2)/+ mouse, exactly like the recently reported c-kitmER-CremER/+ mouse, correctly labels the known c-kit expressing cells even though at an expected different rate proportional to c-kit expression. Importantly, TAM labeled ~80% of all c-kit + cardiac cells. However, these recombined c-kit + cardiac cells were all lineage-committed cells as they were all either CD45 + or CD31 + cells. When gating the low expressing c-kit (c-kit-low) CD45neg/CD31neg cells, that are enriched for true eCSCs, only ~8% of them were recombined to express GFP. Consequently, only rare GFP+ CMs were detected. Finally, the c-kitCreER(T2)/+ null allele reduced clonogenicity and sphere formation of eCSCs when compared to WT c-kit+/+ counterpart.

Conclusions: Cre recombination in c-kitCreER(T2)/+ mice is dependent on c-kit expression. Cells that express high levels of c-kit are efficiently recombined. However, true c-kit-low eCSCs are only minimally recombined. Thus, using this fate map strategy, it impossible to appropriately quantify the cardiac cell progeny, and CMs in particular, of c-kitlow eCSCs in vivo.

doi:10.1016/j.vph.2015.11.016

The protective effects of Grk5 gene removal in cathecolamine-induced cardiomyopathy

M. De Rosa[a], L. Napolitano[a], J. Gambardella[b], A. Fiordelisi[a], B. Trimarco[a], G. Iaccarino[b], D. Sorriento[c], M. Ciccarelli[b]
[a]*"Federico II" University of Naples, Italy*
[b]*University of Salerno, Italy*
[c]*IBB-CNR, Italy*