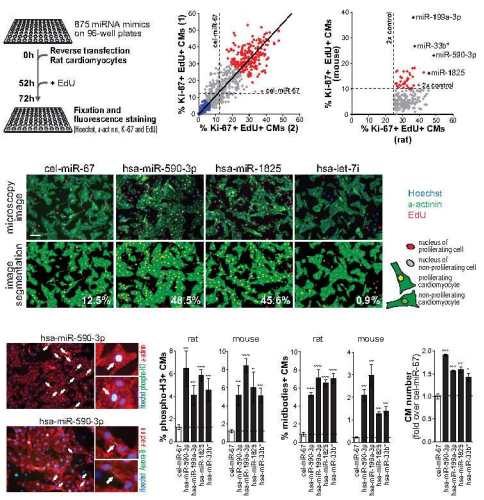
Searching for novel genes and miRNAs inducing myocardial protection and regeneration

A. Eulalio, G. Ruozi, M. Mano, S. Zacchigna, F. Bortolotti, M. Dal Ferro, L. Zentilin, A. Falcione, L. Braga, M. Sturnega and M. Giacca

Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

High-throughput functional screening identifies microRNAs inducing cardiac regeneration

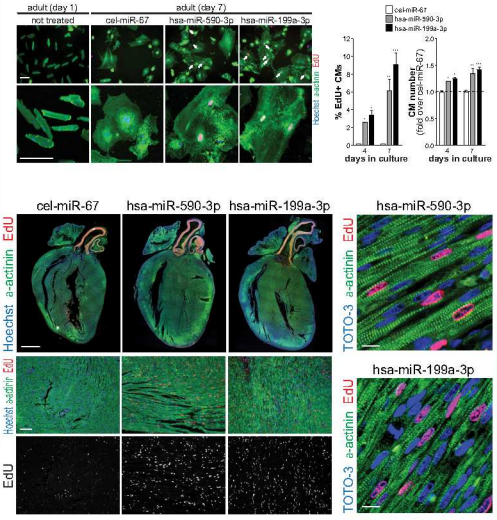
microRNAs can induce proliferation of neonatal rat and mouse cardiomyocytes



A high-content, fluorescence microscopy-based screening in neonatal rat cardiomyocytes using a library of 875 microRNA mimics (miRBase 13.0) identified 204 microRNAs that significantly increase cardiomyocyte proliferation by more than 2-fold. Of these, 40 microRNAs also enhanced proliferation of mouse cardiomyocytes by at least 2-fold.

Increase in Histone H3 phosphorylation on serine 10 (a marker of late G2/mitosis) and Aurora B kinase localization in midbodies (transient structures formed during cytokinesis) demonstrated effective cell cycle progression of the microRNA-treated cells.

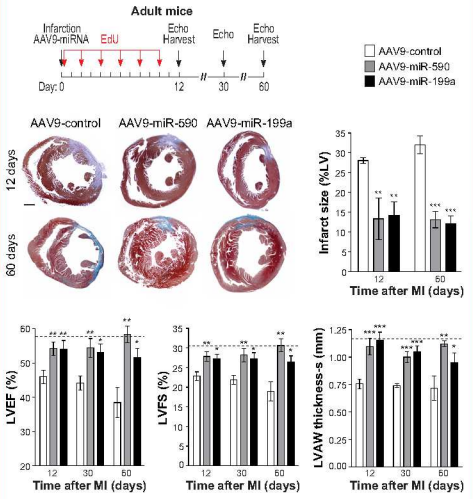
hsa-miR-590 and hsa-miR-199a increase proliferation of adult cardiomyocytes and are also effective in vivo



Treatment of fully differentiated cardiomyocytes isolated from adult (2-month old) rats with hsa-miR-590-3p and hsa-miR-199a-3p determined a time-dependent re-entry of the cells in the cell cycle, eventually leading to an increase of the number of cells.

In vivo, administration of hsa-miR-590-3p and hsa-miR-199a-3p led to a significant increase in the number of EdU-positive cells in both neonatal and adult animals.

hsa-miR-590 and hsa-miR-199a preserve cardiac function after myocardial infarction

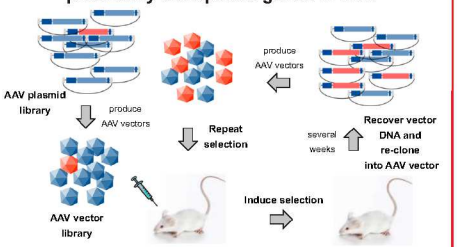


To explore the long term effects of the microRNAs in vivo, vectors based on the adeno-associated virus serotype 9 were generated. After myocardial infarction in mice, induced by descending coronary artery ligation, hsa-miR-590 and hsa-miR-199 stimulated marked cardiac regeneration and an almost complete recovery of all cardiac functional parameters.

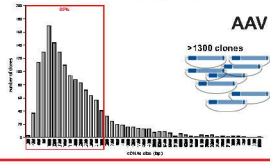
Eulalio et al. (2012), Nature 492:376-381

FunSel: Identification of novel cardioprotective factors by in vivo functional selection using AAV vector libraries

Functional selection of AAV vectors expressing potentially therapeutic genes in vivo



AAV arrayed library



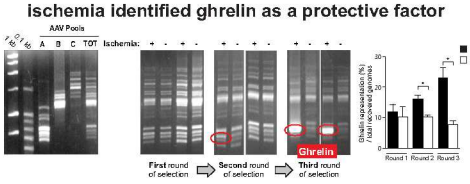
>1300 clones

The **Secretome** gene collection: all cDNAs coding for mouse secreted proteins

Currently ongoing projects exploiting the AAV-based FunSel strategy

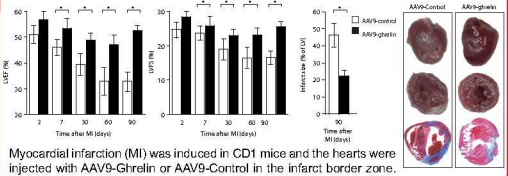
- **Diabetes Mellitus:** Identification of secreted factors conferring β-cells protection into streptozotocin-injected diabetic mice *(in collaboration with F. Bosch - UAB Barcelona and P.Halban - Geneva University)*.
- **Retinal Degeneration:** Identification of genes able to prevent photoreceptors degeneration in a mice model of light-induced retinal damage *(in collaboration with P. Leon - Ospedali Riuniti Trieste)*.
- **Viral Myocarditis:** A subset of AAV vectors composed by cytokines and interleukins has been delivered and screened in a model of viral myocarditis induced by Coxsackie Virus B3 *(in collaboration with S. Heymans - Maastricht University)*.
- **Mesenchymal Stem Cells (MSC) homing and retention in vivo:** Identification of cytokines/IL improving bone marrow derived MSCs retention after injection into ischemic heart.
- **Skeletal Muscle Degeneration:** The AAV library has been delivered and screened in a model of hind limb ischemia.
- **Myocardial Infarction and Heart Failure:** Screening of the AAV library in mouse models of myocardial infarction and isoproterenol-induced heart failure.

In vivo functional selection in a model of hind limb ischemia identified ghrelin as a protective factor



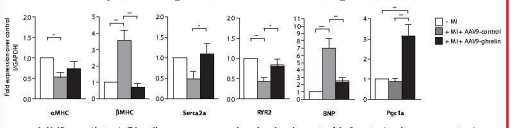
After intramuscolar injection of the vector pool, CD1 mice were subjected to hind limb ischemia by femoral artery ligation, and, after 15 days, persisting vector DNA was recovered, re-cloned into AAV and used for subsequent cycles of in vivo selection. At the third round a marked enrichment for surviving myocytes transduced with the AAV vector expressing **Ghrelin** was observed.

AAV9-Ghrelin preserves cardiac function after myocardial infarction and reduces infarct size



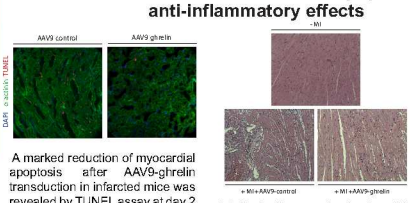
Myocardial infarction (MI) was induced in CD1 mice and the hearts were injected with AAV9-Ghrelin or AAV9-Control in the infarct border zone. Heart function was evaluated by echocardiography for 3 months. AAV-Ghrelin markedly preserved cardiac function and reduced infarct size.

AAV9-Ghrelin prevents the deregulated expression of pathological LV remodelling markers



AAV9 mediated Ghrelin overexpression in the heart of infarcted mice prevented the deregulated expression of markers of pathological left ventricle remodelling.

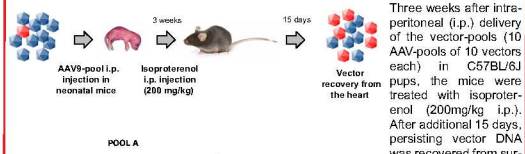
AAV9-Ghrelin exerts marked anti-apopotic and anti-inflammatory effects



A marked reduction of myocardial apoptosis after AAV9-ghrelin transduction in infarcted mice was revealed by TUNEL assay at day 2 after MI.

AAV9-ghrelin treated animals exibited a clear inflammatory cells infiltration reduction in the ischemic area.

In vivo selection in a model of isoproterenol-induced myocardial damage identified INSL6 as a protective factor



Three weeks after intraperitoneal (i.p.) delivery of the vector-pools (10 AAV-pools of 10 vectors each) in C57BL/6J pups, the mice were treated with isoproterenol (200mg/kg i.p.). After additional 15 days, persisting vector DNA was recovered from surviving tissues.

After a single FunSel round for POOL A, a marked enrichment for surviving cardiomyocytes transduced with the AAV vector expressing **INSL6** was observed.

Transduction with AAV9-INSL6 preserves cardiac function after myocardial infarction