Can we achieve cardiac regeneration in humans?

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There is an impelling need to develop novel therapeutic strategies aimed at inducing cardiac repair and regeneration in patients with myocardial infarction and heart failure. In contrast to other species that regenerate the heart during the whole life, in mammals post-natal damage to the myocardium is repaired through formation of a scar. Multiple evidence, however, indicates that a limited capacity of myocardial renewal might also exist in adult individuals. Recent information shows that, over a life-time, approximately half of the cardiac myocytes are generated *de novo*. In addition, several investigators have observed that, after myocardial infarction, there is a significant increase in the number of proliferating cardiomyocytes in the infarct border zone. Finally, novel, exciting evidence shows that complete healing of the fetal and neonatal heart is normally achieved through true cardiac regeneration, a property that is, however, lost approximately one week after birth. Taken together, this variegate information indicates that *de novo* cardiac myocytes formation can actually occur in the mammalian, however this process is blunted after birth and largely ineffectual during the adult life.

Over the last several years, my laboratory has become deeply interested in developing methods to search for factors able to foster cardiac regenerative capacity and that might be turned into effective therapeutics. Two parallel approaches are currently followed, both based on the unbiased screening for novel factors either directly *in vivo* or in *ex vivo* cultured cardiomyocytes. A first approach entails direct myocardial gene transfer using viral vectors based on the Adeno-Associated Virus (AAV). These vectors recently have gained popularity for *in vivo* gene transfer due to their safety profile, lack of inflammatory response, prolonged transgene expression, relative genetic simplicity and high efficiency of transduction of post-mitotic tissues, such as muscle, heart, brain and retina (Lovric *et al.*, 2012).

We have developed two AAV-based libraries, one corresponding to the mouse secretome (~1500 factors) and the other to the miRNome (~800 clones) and developed a novel procedure (FunSel) for their use *in vivo*, for the unbiased identification of novel factors providing therapeutic benefit against myocardial disease. FunSel entails the delivery of AAV vector pools from the two arrayed libraries in various mouse models of cardiac damage (including myocardial infarction), where the degenerative condition drives the selection for molecules putatively preventing cell death, enhancing cell function or promoting tissue regeneration. Subsequent rounds of delivery and selection progressively enrich for potentially therapeutic genes.

FunSel has so far generated a number of novel, and in some cases unexpected, molecules able to protect the heart after myocardial infarction. In particular, three round of selection have generated a marked enrichment for surviving myocytes transduced with ghrelin, a neurohormone involved in endocrine metabolism. Cardiac gene delivery of AAV9-ghrelin after myocardial infarction determined a marked improvement of cardiac function, while significantly reducing infarct size. These effects were paralleled by a reduced apoptotic rate at early times after infarction. Moreover, ghrelin counteracted the induction of markers of cardiac damage (in particular, miR21 and MMP-2) and prevented the deregulated expression of pathological left ventricle remodelling markers, such ad bMHC, BNP and others.

A second approach to identify novel factors providing benefit after myocardial infarction and, possibly, inducing cardiac regeneration, entails the *ex vivo*, high throughput screening for microRNAs promoting primary cardiomyocyte proliferation. By a high-content, fluorescence microscopy-based high-throughput screening in rat neonatal cardiomyocytes using a library of microRNA mimics corresponding to all the annotated microRNAs (~1000 microRNAs), we have identified 40 microRNAs able to increase cardiomyocyte proliferation, as evaluated by analyzing EdU incorporation (DNA synthesis), G2/M phase of the cell cycle (phospho-H3 positivity) and karyokinesis (Aurora B staining in midbodies). Deep sequencing of endogenous microRNAs revealed that several of the identified microRNAs were expressed in neonatal, replicating cardiomyocytes but not in adult cardiomyocytes. Two of these microRNAs were tested *in vivo* by injecting either the synthetic microRNA intracardially or by delivering their coding sequence using AAV9 vectors into the heart of newborn animals. Both microRNAs induced marked proliferation of cardiomyocytes. The same microRNAs, administered after myocardial infarction in adult mice, were also effective in reducing infarct areas and improving cardiac function (Eulalio *et al.*, 2012). *In vivo* experiments using transgenic animals, genetically labelled to overexpress the GFP gene selectively in adult, MHC+ cardiomyocytes, indicate that the selected miRNAs directly act on differentiated cardiac cells. Indeed, their administration to adult rat cardiomyocytes triggers their proliferation up to 2 weeks. The selected microRNAs target the mRNAs for several cytoskeletal proteins; accordingly, siRNA-mediated silencing of these proteins, individually or in combination, mimics the proliferative effect of miRNAs.

Overall, our results indicate it should be possible to develop innovative biological therapies based on

cytokine or miRNA administration and able to stimulate cardiac repair *in vivo* by directly activating the proliferative potential of differentiated cardiomyocytes, thus bypassing the requirement of stem cell expansion and differentiation.

Lovric J, Mano M, Zentilin L, Eulalio A, Zacchigna S, Giacca M. (2012) *Molecular Therapeutics* **20**, 2087-97.
Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M. (2012) *Nature* **492**, 376-81.