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INTRODUCTION.

Ischemic heart disease is the leading cause of mortality worldwide. Its most frequent and serious complication is acute myocardial infarction, after which the remaining contractile tissue undergoes a process characterized by myocyte hypertrophy and death of the remaining myocardium, and its progressive replacement by fibrosis (Sutton and Sharpe 2000). This process, termed remodeling, eventually leads to left ventricular dilation and heart failure.

The extent of remodeling is largely dependent upon infarct size (Lenderink et al. 1995). Small infarcts do not induce significant remodeling. Large ones, contrarily, provoke substantial remodeling and therefore evolve towards contractile failure.

This explains why regenerating the contractile tissue, a process named cardiomyogenesis, has become a major objective in biomedical research.

THE STEM CELL APPROACH TO CARDIOMYOGENESIS.

At present, the most prevalent approach amongst basic and clinical investigators is the implantation of stem cells of diverse origin in the myocardium. Since this is not the subject of our mini-review, the interested reader is referred to excellent recent reviews (Kajstura et al. 2008, Strauer et al. 2008, Segers and Lee 2008). We would like to make notice that one of the main problems with stem cells is that in addition to differentiating into adult cardiomyocytes and proliferating, they must establish electromechanical connections with the resident cells in order to be functionally integrated to the syncytium, a fact that has not yet been convincingly shown. It has been claimed that cardiomyocytes differentiated from human embryonic stem cells can replace rat myocardium in a xenogenic model of myocardial infarction (Laflamme et al. 2005). However, to increase our optimism with the stem cell approach, we need more evidence on issues such as proportion and time of survival of the implanted cells, and, most importantly, we need experiments performed in animal models closer to the human. Recently, a population of resident stem cells that may differentiate into myoblasts and eventually into adult myocytes has been described in the adult heart of humans and other mammals. These cells can be isolated from heart biopsies, grown in culture, and eventually used for myocardial regeneration (see Lyngbaek et al. 2007 for review). However, recent reviews insist on the need for a better understanding on the biology and fate of these cells following pathological insults (Kajstura et al. 2008).
THE ADULT MAMMALIAN HEART IS NOT A TERMINALLY DIFFERENTIATED ORGAN.

More than thirty years ago, Linzbach postulated that the size increase of the failing heart could not be explained by only hypertrophic growth (Linzbach 1976). Although he could not demonstrate cardiomyocyte mitosis, he definitely contributed extinguishing the dogma of the heart being a fully post-mitotic organ. At present, there is plenty of evidence that the adult cardiomyocyte can re-enter the cell cycle and even progress into mitosis (Anversa and Kajstura 1998, Beltrami et al. 2001) (Figure 1A). However, most of the times human adult cardiomyocytes, rather than progressing towards cell division, become polyploid by endomitosis, as observed in our laboratory on hearts of patients who died after acute myocardial infarction (Cabeza Meckert et al. 2005) (Figure 1B).

Taken together, these findings indicate that adult mammalian cardiac myocytes preserve the capacity, although to a limited extent, of dividing into daughter cells in response to injury.

The unequivocal evidence against the post-mitotic nature of the cardiac myocyte has encouraged the investigation of strategies aimed to stimulate the adult cardiomyocyte to re-enter the cell cycle and advance into cell division through gene-mediated interventions targeting the cell cycle regulators, or by transferring genes encoding mitotic cytokines.

Figure 1. Panel A: human adult cardiomyocyte undergoing mitosis in the border zone of an acute myocardial infarction. Panel B: Polyploid human adult cardiomyocytes (both images belong to Dr. Laguens’s archives). Bars: 20 µm.

GENETIC MANIPULATION OF THE CARDIOMYOCYTE CELL CYCLE.

The mammalian cardiomyocyte cell cycle is a tightly regulated process. Progression between consecutive phases requires overcoming checkpoints that assure completion of all necessary steps. This provides a mechanism to detect defective cells. Transition through the various checkpoints is regulated in part by the activity of a family of protein kinases (the cyclin-dependent kinases or CDKs) and their activating partners (the cyclins). The G2/M checkpoint is the most difficult to overcome, a fact that prevents the adult cardiac myocyte to advance into mitosis. Other genes and their protein products are engaged in initiating or suppressing cell cycle progression. Cell cycle stimulators (cyclins, the cyclin-
dependent kinases [CDKs], protooncogenes) are highly expressed in embryonic and newborn hearts, and are downregulated in the adult heart.

Various studies have used the approach of genetically targeting cell cycle regulators to encourage the cardiomyocyte to re-enter the cell cycle and progress into mitosis and cytokinesis.

A number of studies have targeted transcription factors like E2F-1 (Kirschenbaum et al. 1996, von Harsdorf et al. 1999), protooncogenes (Jackson et al. 1991, Xiao et al. 2001) and, more recently, beta-catenin (Hahn et al. 2006).

Rather than overexpressing transcription factors, other authors have stimulated cell cycle promoters. In mice overexpressing cyclin D1, Soonpaa et al observed increased DNA synthesis, yet not G2/M progression (Soonpaa et al. 1997). They later showed that mice overexpressing cyclin D2 (though not those overexpressing cyclin D1 or D3) exhibited newly formed myocardium, infarct size regression and improved function (Pasumarthi et al. 2005, Hassink et al. 2008). Other cell cycle promoters that have been targeted are bcl-2 (Limana et al. 2002), CDK2 (Liao et al. 2001), and Cyclin A2 (Chaudhry et al. 2004), with diverse degree of success regarding progression into mitosis and cell division.

Finally, some studies have tested interfering with cell cycle inhibitors, either by transfecting E1A, which binds pocket proteins or p300 (Kirshenbaum and Schneider 1995), or by antagonizing proteins like p27KIP1 (Poolman et al. 1999), p193 and p53 (Nakajima et al. 2004), and p38 (Engel et al. 2005).

All these studies may help designing future strategies to promote mitosis and division of the adult cardiomyocyte. However, their limitation is that they have been done in laboratory rodents, whose differences in heart size, gestation time and life span with respect to human, make them inappropriate models for direct extrapolation of the results to the clinical setting.

In large mammals, on the other hand, despite the lack of fundamental knowledge regarding many aspects of myocyte division, some authors have described mitosis and even hyperplasia of adult cardiomyocytes by transferring genes coding for mitotic cytokines.

**GROWTH FACTOR GENE TRANSFER AND CARDIOMYOGENESIS.**

We and others have used the transfer of genes encoding growth factors into the heart to induce the adult cardiomyocyte to re-enter the cell cycle and advance into mitosis.

In pigs with Ameroid-induced experimental chronic myocardial ischemia, the intramyocardial injection of a plasmid encoding VEGF165 induced angio-arteriogenesis (Crottogini et al. 2003), adult cardiomyocytes mitosis (Laguens et al. 2002) and hyperplasia (Laguens et al. 2003) (Figure 2).

We later showed that the plasmid provoked infarct size reduction in sheep with left anterior descending artery occlusion (Figure 3) by a combination of effects including an early angiogenic response (7 days after gene transfer), profuse arteriolar proliferation, decreased collagen deposition in the risk area, decreased myofibroblast proliferation, cardiomyocytes mitosis and proliferation of circulating or resident myocyte precursors (myoblasts) (Vera Janavel et al. 2006).

The reasons for these effects were not established, but it might be speculated that VEGF upregulated the synthesis of cytokines acting on the myocyte cell cycle (such as TGF-β and FGF), or, since VEGF receptors have been described in rat cardiomyocytes, it directly activated the myocyte mitogen-activated protein kinase cascade.
VEGF<sub>165</sub> gene transfer-induced cardiomyogenesis was later confirmed in the rat (Liu et al. 2007), and very recently in pigs with reperfused myocardial infarction (Guerrero et al. 2009).

In pigs with hibernating myocardium, Suzuki et al showed that intracoronary adenoviral FGF-5 induced, at 2 weeks after transfer, a nearly 30% increase in LV mass, significant hypertrophy, a 7-fold increase in cycling myocytes and a 4-fold increase in mitotic myocyte nuclei (Suzuki et al. 2005). Regional wall thickening in the hibernating zone, yet not global left ventricular function, increased as a result of hypertrophy.

Improved left ventricular function along with increased regional myocardial blood flow in the same animal model was in fact observed in a later study by that group, (Lynch et al. 2007) after 4 weeks of intracoronary Ad-FGF-5 transfer. In this study, the higher cardiomyocyte nuclear density of treated pigs suggested that FGF-5 favorably affected the balance between cell death and cell growth/regeneration, and the smaller myocyte size of transfected animals could be taken as an indication that cytokinesis and/or mobilization of stem cells had occurred.
CONCLUDING REMARKS.

Repairing the injured heart is a central goal of medicine. In acute myocardial infarction, the possibility of limiting its size would reduce the incidence of heart failure; and in dilated cardiomyopathy, replacing fibrosis by contracting tissue would ameliorate the symptoms and reduce the severity of left ventricular failure.

The approach that has gained more adepts in the last 10 years is the implantation of stem cells of diverse origin, with the expectation that they may differentiate into a myocyte phenotype, and even proliferate. The contrast between the positive results reported in isolated cases or open-label studies with small number of patients, and the negative ones from the few randomized, placebo-controlled trials so far available (Abdel-Latif et al. 2007) has raised controversy. Moreover, in our opinion this strategy has moved much too fast into the clinical arena, especially considering that most of the preclinical data stems from mice and rats rather than from large mammals, and that fundamental issues, such as the ultimate fate of the implanted cells, are still unknown.

The nowadays unequivocal evidence that the adult human cardiomyocyte is not a terminally differentiated cell, able, though to a very little extent, to progress into mitosis, has encouraged the alternative approach of promoting this process by gene-based manipulation of the cell cycle or by transferring genes coding for diverse growth factors.

Theoretically, this is a more physiological approach to cardiomyogenesis, because it would preserve the electromechanical connection between cells needed for adequate myocardial function.

Unfortunately, our knowledge about the signalling pathways and cascade of biochemical events leading to cardiomyocyte division is still very limited as to expect prompt results. Likely, a bulk of biotechnological research is still needed in terms of vectors and delivery methods. However, given the physiological nature of its underlying rationale, the gene therapy approach to cardiomyogenesis should not be discouraged. A slow, though sustained, progress is to be expected inasmuch basic science will certainly provide us with new, necessary insights on cell cycle regulation and mechanisms of cardiomyocyte division.

Last, but not least, all new strategies should be investigated in large mammals before proposing its testing in clinical trials of heart diseases characterized by loss of contractile tissue.

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REFERENCES.


