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REVIEW

Developmental origins and lineage descendants of endogenous adult cardiac progenitor cells

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Abstract Mammalian hearts carry a number of primitive stem cell-like populations, although the magnitude of their contribution to tissue homeostasis and repair remains controversial. Recent CRE recombinase-based lineage tracing experiments suggest only a minor contribution to the formation of new cardiomyocytes from such cells, albeit one that might be augmented therapeutically. As the field explores clinical translation of cardiac stem cells, it will be important to understand the biology of these cells in great detail. In this review we document the various reported stem and progenitor cell populations in mammalian hearts and discuss the current state of knowledge on their origins and lineage capabilities.

Contents

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Introduction

In this review we focus on documenting and evaluating the origins and fate of primitive cell populations (cardiac progenitor cells; CPCs) that have been identified in post-natal mammalian hearts and credited with possessing stem cell-like properties in vivo or after ex vivo expansion. Characterization of CPCs is driving forward a new era in CV biology and stem and progenitor cell therapies that regenerate myocardium lost in the wake of ischemic or other injurious stimuli would have enormous benefit to humanity. Clinical data acquired thus far from cell therapy trials using bone marrow (BM) and cardiac-derived cell fractions (Abdel-Latif et al., 2007; Bolli et al., 2011; Clifford et al., 2012; Makkar et al., 2012) appear to be safe, although the therapeutic effect is modest at best (Nowbar et al., 2014) and in most cases the observed benefits likely involve paracrine effects on endogenous repair mechanisms rather than exogenous stem cell deployment (Laflamme and Murry, 2011). Thus, there is much to learn about the biology and therapeutic potential of these cells.

Lineage tracing tools for investigating stem cell biology

The investigation of cell lineage involves labeling cells of interest, then tracing the destiny of their progeny. The reliance on the presence of cell markers alone to infer the origin or fate of a cell population is fallible, as marker expression may change as cellular context changes; for example, markers on BM cells can be down-regulated after their relocation to solid organs (Rota et al., 2007; Spees et al., 2008). Evolving techniques for lineage tracking have used "vital" dyes, stable isotopes, radioactive compounds, inter-species chimeras and lineage-specific molecular markers in normal or genetically modified organisms (Steinhauser et al., 2012; Stern and Fraser, 2001). Transgenic animals expressing indelible genetic lineage tracers have allowed the fate of specific cell populations to be followed over very long periods during homeostasis, aging and under various disease conditions such as myocardial infarction (MI). A useful type of surrogate tracing is achieved if a genetic tracer protein, such as GFP, is more stable than the endogenous protein it replaces, the latter often down-regulated during differentiation (Kikuchi et al., 2010; Lepilina et al., 2006).

Genetic CRE-Lox lineage tracing: strengths and limitations

Indelible genetic cell tagging using DNA recombinase-based technology has been the mainstay for investigating progenitor cell origins in the current era. CRE recombinase catalyzes site-specific DNA deletion between short recognition sequences termed *loxP* sites (Branda and Dymecki, 2004). Specificity of cell labeling is achieved by genetically combining a CRE gene cassette, expressed under control of an appropriate cis-regulatory element, and a "reporter" cassette (Fig. 1A). The reporter may be a "two color" system in which *loxP* sites flank one marker gene cassette ("STOP"), which blocks expression of a second marker cassette until the first one is deleted via CRE recombination (Lobe et al., 1999; Novak et al., 2000; Soriano, 1999) Among the several additional transgenic platforms used for lineage tracing are the tetracycline-sensitive Tet-Off/Tet-On systems (Gossen and Bujard, 1992; Jaisser, 2000) (Fig. 1B). In Tet-OFF, tetracycline or an analog (e.g. doxycycline: Dox) inhibits the activity of a tetracycline-sensitive transcriptional activator, tTA, which otherwise activates expression of transgenes via a synthetic tetracycline response cis-regulatory element (*TRE*) positioned upstream of the transgenic cassette. In Tet-On, a mutant version of tTA (rtTA) can only activate the transgene (via the *TRE*) if it is itself bound by a tetracycline.

There are a number of important issues to consider in interpreting CRE-based lineage data. Although CRE-mediated genetic recombination is a binary and permanent event in each cell, it is influenced by the threshold levels of CRE required to induce recombination, which in turn is influenced by the accessibility of CRE to chromatin. As a consequence, different reporter mice vary in their sensitivity to CRE (Ma et al., 2008).

CRE efficacy can also depend on the genetic background of CRE or reporter alleles (Nakamura et al., 2006). Furthermore, transgenes integrate into the host genome approximately randomly and, since most cloned cis-elements are incomplete, they can fall under the influence of endogenous chromosomal regulatory elements that positively or negatively impact the penetrance, expressivity or specificity of transgene expression. In knock-in mice, the insertion site may unintentionally disrupt regulatory elements including intronic enhancers, non-coding RNA binding sites, or domains important for chromosome architecture. Thus, the absence of