Differentiation 100 (2018) 26–30

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

Review article

Telomeres and telomerase in heart regeneration

Esther Aix, Alex Gallinat, Ignacio Flores*

Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Melchor Fernandez Almagro 3, Madrid E-28029, Spain

A B S T R A C T

Although recent advances have overturned the old view of the human heart as an inert postmitotic organ, it is clear that the adult heart´s capacity to regenerate after an ischemic episode is very limited. Unlike humans, zebrafish and other lower vertebrates vigorously regenerate damaged myocardium after cardiac injury. Understanding how the zebrafish is able to conserve life-long cardiac regeneration capacity while mammals lose it soon after birth is crucial for the development of new treatments for myocardial infarction. Mammals and lower vertebrates differ markedly in their rates of cardiomyocyte proliferation and levels of telomerase activity. Here, we review recent discoveries identifying lack of telomerase activity and concomitant telomere dysfunction as natural barriers to cardiomyocyte proliferation and cardiac regeneration.

1. Introduction

Cardiovascular disease is currently the leading cause of death, responsible for one out of every three deaths worldwide. The most frequent form of cardiovascular disease is coronary heart disease, in particular myocardial infarction (MI). During MI, a portion of the heart is deprived of blood flow, resulting in ischemia and ultimately in the death of approximately one billion cardiomyocytes. Adult human cardiomyocytes have a very limited capacity to proliferate, and the surviving cardiomyocytes therefore cannot replace the lost myocardium through cell division. The lost myocardium is instead replaced by a non-contractile fibrotic scar, resulting in a decreased cardiac output that can eventually provoke adverse consequences such as heart failure, arrhythmia, and free-wall rupture. Despite years of intense research into regenerative medicine, heart transplantation remains the only effective treatment for heart failure after severe MI. The failure of cardiac regenerative therapies reflects gaps in our understanding of cardiac repair processes and their temporal and spatial regulation. In this review, we summarize current knowledge on the function of telomerase and telomeres in heart regeneration.

2. Heart regeneration is age-dependent in mammals and age-independent in lower vertebrates

The hearts of newborn human infants can fully recover function after MI (Haubner et al., 2016), and this regenerative capacity coincides with a period of active cardiomyocyte proliferation (Bergmann et al., 2015; Mollova et al., 2013). To understand how the hearts of newborn mammals recover after MI, researchers have investigated the underlying mechanisms in mice. The hearts of newborn mice (1-day-old) recover completely after ventricular resection or coronary artery ligation, and this recovery is dependent on a substantial proliferation of endogenous cardiomyocytes to replace those lost after injury (Porrello et al., 2011, 2013). Injury-induced heart recovery in neonatal mice also involves accelerated cardiomyocyte differentiation (Zebrowski et al., 2017). However, this cardiac repair potential is rapidly lost, and similar cardiac injury from postnatal day 7 onward results in fibrosis instead of regeneration (Porrello et al., 2011, 2013). This switch coincides with the transition of most cardiomyocytes to permanent cell-cycle arrest, rendering them unable to proliferate in response to injury (Porrello et al., 2011, 2013). Postnatal cardiomyocyte cell-cycle arrest is in turn associated with changes in DNA content: extensive binucleation in mouse cardiomyocytes (Paradis et al., 2014; Soonpaa et al., 1996), and increased ploidy in human cardiomyocytes (Adler and Costabel, 1975; Bergmann et al., 2015).

Unlike mammals, other vertebrate species such as urodele amphibians and the zebrafish maintain the ability to efficiently repair damaged myocardium throughout life (Foglia and Poss, 2016; Vivien et al., 2016). Zebrafish heart regeneration is driven by preexisting cardiomyocytes that are able to dedifferentiate and proliferate, generating new cardiomyocytes that replace those lost at the injury site (Jopling et al., 2010; Kikuchi et al., 2010). Post-injury generation of zebrafish cardiomyocytes is possible because they retain proliferative ability throughout life (Jopling et al., 2010; Kikuchi et al., 2010; Wills et al., 2008). Almost all zebrafish cardiomyocytes are mononuclear and diploid (Jopling et al., 2010; Kikuchi et al., 2010; Kikuchi and Poss,

* Corresponding author.
E-mail address: iflores@cnic.es (I. Flores).

https://doi.org/10.1016/j.diff.2018.01.003
Received 28 October 2017; Received in revised form 19 January 2018; Accepted 23 January 2018
Available online 03 February 2018

2012; Wills et al., 2008). In line with this, recent research in inbred mouse strains indicates that the frequency of mononuclear diploid cardiomyocytes is a variable trait that correlates with cardiomyocyte proliferation and heart function recovery (Patterson et al., 2017), providing further evidence that only diploid cardiomyocytes are able to proliferate.

The development of regenerative therapy for MI patients will thus benefit from understanding why urodele amphibians and zebrafish maintain a diploid cardiomyocyte genome and the capacity for cardiomyocyte proliferation and regeneration throughout life, whereas these features are lost early in the postnatal life of mammals.

3. Telomerase and telomeres

Complete replication of genetic information is possible because of the presence at chromosomes ends of nucleoprotein structures called telomeres. Telomeres are made of repeated DNA sequences and the multiprotein complex shelterin (Palm and de Lange, 2008). Due to the end replication problem and other degradative activities, telomeres get shorter with each cell division (Saretzki and Von Zglinicki, 2002). Telomere shortening can be reversed by the action of an enzyme called telomerase, which contains a catalytic subunit (Tert) and an RNA template (Terc). Telomerase acts as an RNA-dependent DNA polymerase, using the RNA template to add DNA repeats to telomeres after cell division (Greider and Blackburn, 1985, 1987, 1989).

Telomerase activity can be regulated at multiple levels. The Tert subunit is subject to transcriptional and posttranslational regulation, including phosphorylation and ubiquitination, affecting its structure, stability, subcellular localization, and enzyme activity; moreover, different Tert variants can be generated by alternative processing. Terc is similarly subject to transcriptional and posttranscriptional regulation. Telomerase subnuclear localization is controlled during the cell cycle and contributes to the regulation of enzyme activity, and there is also regulation at the level of transport and assembly of telomerase components, accessibility and binding to the telomere, and interaction with accessory proteins (Cifuentes-Rojas and Shippen, 2012; Flores et al., 2006; Pfeiffer and Lingner, 2013).

Genomic integrity must be preserved during DNA replication in order to avoid senescence, cell-cycle arrest, and apoptosis (Artandi and Attardi, 2005). Telomere status therefore likely constitutes a prominent variable determining the proliferative and regenerative nature of tissues during aging. Here, we review recent discoveries that have identified telomere dysfunction and the lack of telomerase as natural barriers to cardiac regeneration and cardiomyocyte proliferation (Aix et al., 2016; Bednarek et al., 2015).

4. Telomerase and telomeres in mouse heart regeneration

Telomerase activity is readily detectable in the hearts of rodents during embryonic development, but is rapidly inactivated postnatally (Aix et al., 2016; Borges and Liew, 1997; Oh et al., 2001). One of the limiting factors for telomerase activation is *Tert* transcription (Cifuentes-Rojas and Shippen, 2012; Meyerson et al., 1997; Ramlee et al., 2016). In mouse hearts, the decline in telomerase activity coincides with a decrease in *Tert* expression, likely reflecting a drastic decrease in the number of *Tert*-positive cardiac cells (Aix et al., 2016; Richardson et al., 2012). There is currently scarce information regarding the mechanism by which *Tert* expression is downregulated in most cardiac cells after birth, and this presents an interesting question for future research.

Postnatal telomerase inhibition in mouse cardiomyocytes is paralled by rapid telomere shortening during the first two weeks after birth (Aix et al., 2016). To analyze the effect of telomere shortening on neonatal cardiomyocyte proliferation, investigators have studied hearts from mice lacking *Terc*, the RNA template component of telomerase, and therefore lacking the main mechanism for telomere-maintenance. Premature telomere dysfunction in late-generation G3 $Terc^{-/-}$ newborns causes a decrease in cardiomyocyte proliferation (Aix et al., 2016). Prolonged, exacerbated telomere shortening results in dilated cardiac myopathy, linking attenuated cardiomyocyte proliferation in postnatal states to heart failure in adults (Leri et al., 2003). This study found that the presence of short telomeres (< 12 kb) in $Terc^{-/-}$ cardiac cells coincided with DNA-damage-induced stabilization of p53. The impaired cardiac performance was observed in fifth generation $Terc^{-/-}$ mice (G5 $Terc^{-/-}$), in which a high proportion of cardiac cells showed short telomeres and p53 stabilization; in contrast, heart function was normal in G2 $Terc^{-/-}$ mice, in which there were relatively few p53-positive cardiac cells with short-telomeres. These results thus suggest that functional abnormalities in telomerase-deficient adult mice are linked to telomere damage rather than telomerase deficiency *per se* (Leri et al., 2003). Conversely, conservation of telomere length in mice by forced expression of human Tert from the αMHC promoter increased cardiomyocyte proliferation, ultimately causing hypertrophy (Oh et al., 2001). Similarly, telomere length and cardiomyocyte proliferation were increased by gene-therapy-mediated overexpression of Tert in all cardiac cell types of the adult mouse heart (Bar et al., 2014). These observations support the hypothesis that telomerase is necessary for cardiomyocyte proliferation and indicate that the maintenance of telomerase expression can delay cell-cycle exit in neonatal cardiomyocytes.

To examine the role of telomerase and dysfunctional telomeres in heart regeneration, we recently developed a cryoinjury protocol to damage 1-day-old neonatal hearts. Unlike wild-type cryoinjured hearts, G3 $Terc^{-/-}$ hearts are unable to mount a cardiomyocyte proliferative response to cryoinjury. Instead of increasing proliferation, G3 $Terc^{-/-}$ cardiomyocytes in the cryoinjured heart grow by hypertrophy (Aix et al., 2016). In the absence of a proliferative response, cardiac regeneration is severely impaired in G3 $Terc^{-/-}$ animals (Aix et al., 2016; Lund et al., 2009). These results indicate that ample telomere reserves are necessary for cardiomyocyte proliferation and for efficient heart regeneration after cardiac injury in neonatal mice (Fig. 1). Consistent with results in newborns, forced expression of telomerase in adults reduces apoptosis, cardiac dilation, and scar size and improves post-MI heart function and survival (Oh et al., 2001). Moreover, telomerase therapy after acute MI in adult mice is cardioprotective (Bar et al., 2014). These findings in adults thus indicate the potential of artificial telomerase expression to ameliorate heart failure after cardiac injury. However, the effect of forced telomerase expression on neonatal heart regeneration remains unknown.

5. Telomerase and telomeres in zebrafish heart regeneration

The zebrafish is a “telomerase-positive” animal model, since its telomerase is constitutively active from embryonic stages to adulthood (Anchelin et al., 2011; Lund et al., 2009), contrasting with mammals, in which telomerase is rapidly downregulated after birth (Aix et al., 2016; Borges and Liew, 1997; Oh et al., 2001). Zebrafish are also considered “champions of regeneration” because of their remarkable ability to regenerate several organs after injury, including fins, kidney, liver, pancreas, brain, spinal cord, and heart muscle (Gemberling et al., 2013; Gonzalez-Rosa et al., 2017) . It is thus an attractive hypothesis that the lifelong regenerative ability of zebrafish is dependent on sustained telomerase activity.

To investigate the role of telomerase in cardiac regeneration, we recently studied the consequences of cryoinjury in zebrafish. Cryoinjury in adult wild-type hearts induces heart regeneration and transiently activates telomerase and elongates telomeres (Bednarek et al., 2015). In contrast, cryoinjury in telomerase knockout ($Tert^{-/-}$) zebrafish results in telomere shortening and there is no regeneration, with the result that the initial fibrotic scar persists instead of being replaced by regenerated tissue. The lack of fibrosis regression impedes the recovery of ventricular function, indicating that telomerase is essential for heart regeneration (Bednarek et al., 2015).