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Short Telomeres — A Hallmark of Heritable Cardiomyopathies

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Abstract

Cardiovascular diseases are the leading cause of death worldwide and the incidence increases with age. Genetic testing has taught us much about the pathogenic pathways that drive heritable cardiomyopathies. Here we discuss an unexpected link between shortened telomeres, a molecular marker of aging, and genetic cardiomyopathy. Positioned at the ends of chromosomes, telomeres are DNA repeats which serve as protective caps that shorten with each cell division in proliferative tissues. Cardiomyocytes are an anomaly, as they are largely non-proliferative post-birth and retain relatively stable telomere lengths throughout life in healthy individuals. However, there is mounting evidence that in disease states, cardiomyocyte telomeres significantly shorten. Moreover, this shortening may play an active role in the development of mitochondrial dysfunction central to the etiology of dilated and hypertrophic cardiomyopathies. Elucidation of the mechanisms that underlie the telomere-mitochondrial signaling axis in the heart will provide fresh insights into our understanding of genetic cardiomyopathies, and could lead to the identification of previously uncharacterized modes of therapeutic intervention.

Keywords

telomere shortening; cardiomyopathy; telomerase; TRF2; TERT; mitochondria

1. Introduction

More people die each year throughout the world from cardiovascular disease than from any other cause (Roth et al., 2017). Cardiomyopathy constitutes a subset and refers to diseases of heart muscle that are driven by gene mutations (genetic cardiomyopathy) or result from unknown causes (idiopathic cardiomyopathy) (McKenna et al., 2017). Clinically,

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Author contributions

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cardiomyopathies are commonly classified by the phenotype presented. Dilated Cardiomyopathy (DCM) is defined by a loss of cardiomyocytes leading to dilation of the heart due to weakened heart walls whereas Hypertrophy Cardiomyopathy (HCM) is defined by an increase in cardiomyocyte volume resulting in an enlarged, thick and rigid heart that fails to pump. In both DCM and HCM hearts, cardiomyocytes undergo apoptosis and are replaced by scar tissue (Akyürek O et al., 2001; Narula et al., 1996; 1999; Olivetti et al., 1997). Functionally, due to loss of cardiomyocytes, the supply of blood to the body becomes inadequate leading to heart failure or arrhythmias, a degenerative and irreversible disease state. Patient outcomes range from reduced quality of life due to heart failure to sudden cardiac death (Hershberger and Siegfried, 2011). Major limitations in studying the underlying mechanisms of cardiomyopathies have been the relative inaccessibility of the heart and primary human cardiomyocytes, as well as the lack of adequate *in vitro* or *in vivo* disease models. Consequently, the molecular mechanisms underlying cardiomyopathies remain poorly understood and pharmacological interventions are largely palliative. Here we discuss recent studies implicating telomere shortening in cardiomyopathies suggest cardiomyopathies are diseases of accelerated aging (Chang et al., 2016; Mourkioti et al., 2013; Sharifi-Sanjani et al., 2017; Terai et al., 2013).

Telomeres are non-coding TTAGGG DNA repeats at chromosomal ends that shorten with cell division and aging (Blackburn et al., 2015; Booth and Charchar, 2017a). Telomeres are bound by shelterin protein complexes which act as protective caps to prevent genomic instability (de Lange, 2005; Palm and de Lange, 2008). In proliferating eukaryotic cells, telomeres shorten due to replication insufficiency (Sakabe and Okazaki, 1966). Critically short telomeres induce cellular senescence and cell cycle arrest which limits cell proliferation capacity, termed the Hayflick limit (Hayflick and Moorhead, 1961). Uncapped telomeric ends activate DNA damage repair pathways and unresolved ends can result in chromosomal instability (Dench and de Lange, 2007; Dimitrova et al., 2008; Sfeir et al., 2010; Takai et al., 2011). Telomerase, the enzyme that restores telomere length, is comprised of a protein (Tert) and an RNA (Terc; TR) component. Since Tert is not expressed in cardiac tissues postnatally (Oh et al., 2003), there is no mechanism for elongating telomeres. Shortened telomeres have long been correlated with diseases of proliferative tissues, such as cancer (Hanahan and Weinberg, 2011), but evidence is accumulating that telomere shortening also occurs in diseases of non-proliferative tissues, such as the heart and brain (Blackburn et al., 2015; Oh et al., 2003; Terai et al., 2013).

In this review we present an unconventional view that is gaining traction and suggest that telomere shortening is a hallmark of genetically induced cardiomyopathy, including DCM and HCM (Chang et al., 2016; Mourkioti et al., 2013; Sharifi-Sanjani et al., 2017; Terai et al., 2013). In this scenario, a synergy in cardiomyocytes of shortened telomeres and heritable defects causing contractile dysfunction leads to cell death and culminates in cardiomyopathies.

2. Telomere shortening in failing hearts

Aging is the biggest risk factor known for cardiovascular disease; however, how cellular aging results in cardiovascular disease remains unclear. Although in an early report Tabuko

et al. did not find significant differences in telomere length with age in the myocardium of 168 autopsied individuals (Takubo et al., 2002), the same group recently reported telomere reduction of 20 base pairs per year in cardiac tissue when sample number was increased to 530 individuals (Terai et al., 2013). Moreover, this shortening is unlikely to be due to cell division, as human adult cardiomyocytes remain largely non-proliferative as measured by ^{14}C dating (Bergmann et al., 2009; 2015).

Despite significantly longer human life-span, human telomeres (~10–15kb) are significantly shorter than mouse telomeres (~20–50 kb) (Blasco, 2005; Palm and de Lange, 2008). This is not due to a difference in proliferation, as murine cardiomyocytes like human cardiomyocytes are relatively non-proliferative after birth and growth is due largely to increases in cell volume, or hypertrophic growth (Heineke and Molkentin, 2006; Porrello et al., 2011; Soonpaa et al., 1996). Regeneration is potentiated by cardiomyocyte proliferation, but only during a brief time window immediately after birth (Porrello et al., 2011). Replacement of injured cardiomyocytes is negligible in adulthood.

Genetic HCM and DCM are caused by mutations in proteins with a diverse range of functions that impact contraction (Booth and Charchar, 2017b; Parvari and Levitas, 2012). HCM is the most common inherited heart defect, affecting 1 in 500 people worldwide. It is heritable in 50–75% of cases, is commonly caused by autosomal dominant mutations in genes encoding sarcomeric proteins, and is characterized by ventricular wall thickening. On the other hand, DCM affects 1 in 2500 people, is heritable in only 30–35% of cases, and is characterized by dilation of the ventricular chamber and decreased systolic contractile function (Hershberger and Siegfried, 2011). DCM is the leading indication for heart transplantation. Patient outcomes for both HCM and DCM range from reduced quality of life due to heart failure to sudden cardiac death between the ages of 20 and 60 (Force et al., 2010; Hershberger and Siegfried, 2011).

dePinho and colleagues provided early evidence implicating telomere length in cardiac function. They showed that mice lacking *Terc*, the RNA component of telomerase (TR^{KO}), succumb due to dilated cardiomyopathy. Telomere shortening occurs in the germline and heart failure was first manifested by the fourth generation (mTR^{G4}), when telomeres reached a critically short length. Heart failure was mediated by p53 activation and a consequent block in mitochondrial biogenesis via inhibition of PGC1alpha and beta, which led to respiratory chain deficiency and elevated reactive oxygen species (ROS), culminating in metabolic failure (Sahin et al., 2011). A limitation of this study is that telomeres were ubiquitously shortened and multiple tissue defects were evident, eg., in the liver and in hematopoietic stem cells. Although telomeres have been reported to shorten somewhat in murine cardiac tissues with aging (Boon et al., 2013), this may be due in part to other cell types in the heart, as single cardiomyocyte telomere lengths were not examined. Notably, in the healthy human heart, the average telomere length of cardiomyocytes remains relatively stable (Terai et al., 2013) and shortening is pathologic. Accordingly, when telomere lengths become critically short, mitochondrial dysfunction ensues, and cardiac function is compromised.

3. Evidence of telomere shortening in genetic DCM

We first implicated telomere lengths in DCM in studies of Duchenne Muscular Dystrophy (DMD) (Chang et al., 2016; Mourkioti et al., 2013). A major conundrum in understanding DMD has been that although the mdx transgenic mouse lacks dystrophin, similar to patients, it does not exhibit cardiac symptoms typical of DCM. We postulated that mice are protected from the disease by the lengths of their telomeres, which are substantially greater than in humans. In support of this hypothesis, we found that ‘humanized’ mdx mice with shortened telomeres due to breeding with mTR^{KO} knockout mice lacking telomerase (mdx^{4cv}/mTR^{G2}), developed the severe skeletal muscle phenotype and heart failure due to DCM seen in Duchenne patients (Chang et al., 2016; Mourkioti et al., 2013; Sacco et al., 2010). Notably, defects were specific to the tissues requiring dystrophin for function at the second generation (G2), when systemic evidence of telomere shortening is not apparent in mTR^{KO} mice, even in high turnover tissues such as the germline.

We corroborated the findings in our mouse model in human DCM. Duchenne patients typically succumb due to cardiac failure in the third decade of life. Cardiomyocytes of Duchenne patients’ hearts exhibited ~50% decrease in average telomere lengths (Chang et al., 2016; Mourkioti et al., 2013). Importantly, in contrast to cardiomyocytes, vascular smooth muscle cells in the same DMD patient or murine cardiac tissues had telomere lengths similar to unaffected controls, indicating that the observed telomere shortening was cardiomyocyte-specific and due to the absence of the structural protein, dystrophin (Chang et al., 2016; Mourkioti et al., 2013). More recently, cardiomyocyte-specific telomere shortening was also detected in HCM patient hearts (Sharifi-Sanjani et al., 2017). These results suggest that the genetic absence of a key structural contractile protein synergizes with the loss of telomerase activity to accelerate cardiac disease progression.

4. Telomere length, homeostasis, and function

In aging and diseased hearts, telomere homeostasis appears to be dysregulated. Molecularly, one of the six shelterin proteins, TRF2, appears to play a central role. Cardiac tissues from heart failure patients exhibited a loss of the TRF2 shelterin protein, which correlated with short telomere lengths (Oh et al., 2003). Notably, in cancer cells TRF2 has been shown to function in a non-canonical fashion to repress p21 gene expression, a marker of senescence (Hussain et al., 2017). Conversely, critically short telomeres marked by γ H2AX, a biomarker for DNA double strand breaks, lead to p21 induction and cardiomyocyte cell-cycle arrest in newborn *mTR^{G3}* mice (Aix et al., 2016). In accordance with these findings, we observed activation of the p53 mediated DNA damage response pathway, 53BP1 and p21, in isolated mdx^{4cv}/mTR^{G2} cardiomyocytes (Chang et al., 2016). That this pathway could also operate in post-mitotic cells was unanticipated. Biomechanical stress induced by partial aortic constriction caused a loss of TRF2, activation of the DNA damage checkpoint, Chk2, and a significant reduction in telomere length in mouse cardiomyocytes within 1 week, similar to that seen in failing human hearts (Oh et al., 2003). Further, the telomere erosion, oxidative stress, and apoptosis could be rescued by ectopic expression of telomerase in mice (Oh et al., 2003; 2001). In addition to inducing a DNA damage response, uncapped telomeric ends lacking TRF2 recruit TAZAP, a non-shelterin telomere binding protein that

results in telomere trimming (J. S. Z. Li et al., 2017). TZAP appears to bind preferentially to long chromosomes. Whether TZAP plays a role in telomere shortening in diseased cardiomyocytes remains to be determined.

Induction of telomerase activity or shelterin expression confers protection in mouse hearts subjected to myocardial infarction (Bär et al., 2014; Oh et al., 2003; 2001). Notably, repetitive hyperthermia, which induces telomerase activity and mimics the beneficial effects of hot springs, also provided cardioprotection in hypertensive rats (Oyama et al., 2012). In aged human hearts, increased expression of microRNA-34a was detected, which targets phosphatase nuclear-targeting subunit-1 (Pnuts), a protein that facilitates the binding of the shelterin TRF2 to telomeric ends. The consequent reduction in TRF2 binding results in the loss of telomere length, but can be countered by Pnuts overexpression (Boon et al., 2013). Together, these studies strongly suggest that telomere length and maintenance are tightly regulated in post-natal cardiomyocytes. Further, interventions that lead to telomere protection or elongation can have beneficial effects on cardiomyocyte function.

The de-protection of telomeres instigates a DNA damage response with consequent metabolic sequelae. In isolated *mdx*^{4cv}/*mTR*^{G2} cardiomyocytes, this was manifested by activation of p53 signaling and consequent repression of expression of mitochondrial master regulators, *Pgc1a* and *b*. This was followed by a drastic loss of mitochondrial biogenesis, respiratory failure, and a rapid increase in ROS levels (Chang et al., 2016). These findings are similar to those obtained with cardiomyocytes from the *mTR*^{G4} telomerase deficient mice (Sahin et al., 2011). Treatment with an antioxidant specific to mitochondria prior to the onset of DCM in these *mdx*^{4cv}/*mTR*^{G2} mice rescued the metabolic defects and prolonged survival (Chang et al., 2016; Mourkioti et al., 2013). Together, these findings suggest a molecular link between short telomere length and mitochondrial compromise in the etiology of dilated cardiomyopathy.

5. Direct effects of telomeres on gene expression

Although telomere length is generally thought of in its proliferative context and function as a tumor suppressor, it is also associated with gene regulation, coined “telomere position effect” (TPE). This mechanism is independent of its effects on mitochondrial gene expression via p53 activation, as described above. Recent studies from Shay and Wright’s laboratories demonstrated that “telomere position effect” can translate to gene expression changes as telomeres shorten, by changing the proximity of a telomeric end to a promoter of gene transcription (Kim et al., 2016; Robin et al., 2014). Perhaps the most striking example to date is the *DUX4* homeobox gene which increases in expression more than 10-fold in myoblasts and myotubes of Facioscapulohumeral muscular dystrophy (FSHD) patients. The expression of *DUX4* is inversely proportional to telomere length (Stadler et al., 2013). This may explain the age-related progressive manifestation of FSHD. Our understanding of the nature of TPE effects is in its infancy, especially with respect to cardiovascular disease, and these effects warrant further investigation.

Another example of TPE derives from studies of Notch regulation in cardiac valve disease. *Notch*^{+/-} mice, in contrast to humans with bicuspid valve disease, exhibit no phenotype. Due

to our results with the mdx mouse that suggested that mice are protected from certain human diseases by the length of their telomeres, this mouse model was bred with the *terc* deficient mice to generation two yielding *Notch^{+/-}mTR^{G2}*. Similar to our observations in the *mdx^{4cv}/mTR^{G2}* mice, ‘humanizing’ telomere length faithfully unmasked the premature calcification typical of the bicuspid valve phenotype in *Notch^{+/-}mTR^{G2}* mice, as seen in patients (Theodoris et al., 2017). *Notch^{+/-}mTR^{G2}* exhibited dysregulation of pro-osteoblast and pro-inflammatory genes in endothelial cells of the aortic valve. The affected gene networks were enriched for telomere-contacting promoters (Theodoris et al., 2017). These findings suggest that telomere shortening may be a more general approach to modeling human diseases in mice. Further, they highlight a potential direct role for telomere length in gene regulation in the cardiac vasculature.

6. Mitochondrial dysfunction, ROS, telomeres, and cardiovascular disease

Mitochondrial dysfunction is often correlated with elevated levels of ROS in cardiomyopathies (Moslehi et al., 2012). Although the *mTR^{G4}* (Sahin et al., 2011) and the *mdx^{4cv}/mTR^{G2}* (Chang et al., 2016) studies seem to suggest that short telomeres drive the onset of mitochondrial dysfunction and ROS through inhibition of PGC1a and b expression, the reverse order of pathogenic events where mitochondrial dysfunction drives premature aging may also be true.

Mitochondrial gene mutations have been shown to play a major role in driving the onset of DCM in patients (Arbustini et al., 1998; Y. Y. Li et al., 1997). Pim-1 is a conserved serine/threonine protein kinase that has been shown to be cardioprotective by regulating mitochondrial biogenesis, mitochondrial integrity, and homeostasis (Borillo et al., 2010; Din et al., 2013). Using a triple knockout mouse model that encompasses Pim-1, 2, and 3 (*PTKO*), Din *et al.* showed that metabolic dysfunction due to mitochondrial dysfunction was the underlying cause of myocardial senescence and premature cardiac aging (Din et al., 2014). Conversely, overexpression of Pim-1 in isolated murine cardiac progenitor cells led to induction of telomerase expression and activity (Cottage et al., 2012). Conditional disruption of mitochondrial fission and fusion led to mitochondrial fragmentation and cardiomyocyte respiratory dysfunction culminating in dilated cardiomyopathy and heart failure in mice (Chen et al., 2011; Song et al., 2015). These studies suggest telomeres may sit upstream of mitochondrial dysfunction.

However, several reports suggest the telomere-mitochondria axis is a two-way street. High oxygen levels are capable of inducing ROS production and telomere shortening in confluent non-proliferative fibroblasts (Sitte et al., 1998; Zglinicki et al., 1995). Conversely, an association of the antioxidant enzyme PRDX1 with telomeric ends was identified (Aeby et al., 2016). Upon loss of PRDX1 protein, oxidized telomeric ends were not recognized by telomerase protein, providing the first molecular evidence that an antioxidant enzyme can cause an alteration in telomere length (Aeby et al., 2016). Clinically, obesity induced mitochondrial dysfunction resulted in increased oxidative stress and telomere shortening up to 30% (Niemann et al., 2011). Together, these findings suggest mitochondrial dysfunction, through generation of ROS, can drive telomere shortening and increase risk for

cardiovascular disease. Conversely, antioxidant enzymes such as PRDX1 can protect telomeres from acute oxidative damage.

7. Perspectives

As described here, mutations in genes central to contraction, like dystrophin, drive telomere shortening and mitochondrial dysfunction, thereby playing a critical role in the development of dilated cardiomyopathy. To date, cardiovascular diseases remain the leading cause of morbidity and age is the biggest risk factor. Telomere attrition is correlated with cardiovascular disease, but the cause and effect relationship remains an enigma. Does the diseased heart induce telomere shortening? Do shortened telomeres lead to heart failure? Clearly a telomere-mitochondrial-oxidative stress axis is at play, but which comes first and is the chicken or the egg remains unknown in cardiomyopathy pathogenesis. To address this, better in vitro model systems are needed that are amenable to molecular and biochemical analyses of perturbations and their consequences.

Therapeutically, a number of approaches have been proposed for treating heart failure. The existence of a cardiac stem cell capable of regeneration appears unlikely (Eschenhagen et al., 2017). Alternative strategies for fixing a broken heart include inducing endogenous cardiomyocyte proliferation (Bassat et al., 2017; Wei et al., 2015). However, neither of these approaches addresses the need for repair when there is a genetic defect driving cardiomyopathy. One obvious potent strategy for such disorders is to fix the mutant gene by specific CRISPR targeted correction (Amoasii et al., 2017; Zhang et al., 2017). We propose an alternate strategy that entails targeting the telomere-mitochondria pathway as a generalizable approach to ameliorating cardiomyopathies. This orthogonal, mutation-independent strategy, could serve as a fresh therapeutic regimen for attenuating disease progression. This approach may not only be of relevance to cardiac disease. A better understanding of the underlying signaling pathways may define a broader and more critical role for telomere shortening beyond the heart in a diversity of aging-associated diseases.

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Table 1

Telomere-mitochondrial axis in cardiomyopathies

Decreased cardiomyocyte function	DCM (Chang et al., 2016; Mourkioti et al., 2013); HCM (Sharifi-Sanjani et al., 2017);
	Decreased Telomere length	Aging (Sahin et al., 2011); Loss of telomere protection (Oh et al., 2003; 2001); Loss of telomere homeostasis in aging (Boon et al., 2013); Long telomeres protecting the heart (Bär et al., 2014; Oh et al., 2003); Telomerase protects the heart (Oh et al., 2003; 2001; Oyama et al., 2012)
	Mitochondrial Dysfunction	Metabolic dysfunction (Din et al., 2014; Sahin et al., 2011); Obesity (Niemann et al., 2011);