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Abstract

Living-donor liver transplant is a life-saving procedure for people with end-stage liver disease that has increased the number of organs available for people on the liver transplant waiting list. Patients often receive grafts from their relatives in living-donor liver transplantation. Maintenance of long-term graft function is important in liver transplant recipients. Livers from older donors have worse graft survival rates in human liver transplantation, and therefore, accurate evaluation of graft aging and senescence is expected to provide critical data for therapeutic intervention in long-term grafts. Many insults, including rejection, can contribute to post-transplant damage. Late post-transplant biopsies frequently show chronic hepatitis of unknown cause, and this can cause late graft dysfunction leading to cirrhosis. Telomere length in chronic hepatitis or cirrhosis is significantly lower than that in normal livers of the same age. Sustained cellular turnover in chronic liver disease accelerates cellular senescence or a crisis because of telomere shortening. Here, we review the mechanisms involved in post-transplant complications including acute cellular rejection, chronic rejection, and chronic hepatitis of unknown cause by ageing and senescence due to telomere shortening.

Keywords: Transplantation, liver, telomere

1. Introduction

1.1. Summary

Living-donor liver transplant is a life-saving procedure for people with end-stage liver disease that has increased the number of organs available for people on the liver transplant waiting list. Patients often receive grafts from their relatives in living-donor liver transplantation.
Maintenance of long-term graft function is important in liver transplant recipients. Livers from older donors have worse graft survival rates in human liver transplantation, and therefore, accurate evaluation of graft aging and senescence is expected providing critical data for therapeutic intervention in long-term grafts. Many insults, including rejection, can contribute to post-transplant damage. Late post-transplant biopsies frequently show chronic hepatitis of unknown cause, and this can cause late graft dysfunction leading to cirrhosis. Telomere length in chronic hepatitis or cirrhosis is significantly lower than that in normal livers of the same age. Sustained cellular turnover in chronic liver disease accelerates cellular senescence or a crisis because of telomere shortening. Here, we review the mechanisms involved in post-transplant complications including acute cellular rejection, chronic rejection, and chronic hepatitis of unknown cause by ageing and senescence due to telomere shortening.

1.2. Background

Telomeres are comprised of tandem nucleotides repeats (TTAGGG) and their functional role includes protection against the degradation of chromosomes and the maintenance of genome integrity and stability [1]. Telomere shortening relates with the etiology of liver allograft dysfunction and/or graft failure such as acute cellular rejection, chronic rejection, and chronic hepatitis of unknown cause (idiopathic post-transplant hepatitis) after liver transplantation. Older people are more sensitive to most acquired liver disorders and are more indefensibly to the consequences of liver disease. In the chronic hepatic injury and inflammation, cellular senescence functions as an essential stress-response mechanism to restrict the proliferation of damaged cells, but this benefit is at the expense of senescence-related organ dysfunction. The dual role of cell senescence in chronic liver disease will make this an intriguing but challenging area for future clinical interventions. In the setting of chronic liver disease, telomere reduction was more significant than in hepatocytes of normal livers of subjects of the same age [2-4]. In this review, we will discuss mechanism of telomere shortening involved in hepatocyte senescence after liver transplantation by examining the present-day knowledge of telomere structure. Particularly, we discuss mechanisms by which inflammation, acute stress, and oxidative stress accelerate cellular senescence.

2. Human telomere structure

Telomere is nucleoprotein complex at the end of chromosomes that is composed of repeated DNA sequences and interaction binding proteins [5]. Human telomeric DNA is generally in the 5–15 kb length range and contains double-stranded tandem repeats of TTAGGG, followed by single-stranded G-rich overhang [6-7]. The G-rich overhang can form a structure called t-loop [8]. T-loops have been identified by electron microscopy [9]. Moreover, the telomere fold backs on itself, so that the single-stranded G-rich overhang invades into double-stranded DNA to form a D-loop (displacement loop) [5]. The G-rich DNA sequences fold into noncanonical secondary structures called G-quadruplex. G-quadruplex is formed by stacking of several G-tetrads. The G-tetrads are formed by four guanines arranged in a plane by hydrogen bonds. These structures have been identified in human cells by using a highly specific DNA G-quadruplex antibody recently [10]. Protein complex of telomeres, known as shelterin, consists
of TRF1 (telomeric repeat binding factor 1), TRF2 (telomeric repeat binding factor 2), RAP1 (repressor/activator protein 1), TIN2 (TRF1 interacting nuclear factor 2), TPP1 (TIN2-POT1 organizing protein), and POT1 (protection of telomere 1) [5, 11-12]. TRF1 and TRF2 directly bind the telomeric double-strand DNA, while POT1 binds the single-stranded overhang. TIN2 and TPP1 interact with POT1. RAP1 has no obvious effect on protection or length regulation of human telomeres [13]. Additionally, telomere-associated complex has recently been identified in mammalian cells. This complex, called CST, consists of Conserved Telomere Component 1 (CTC1), STN1 and TEN1 [14-15].

2.1. Telomere length regulation
Telomere length is determined by the degree of DNA elongation by telomerase, DNA erosion by incomplete DNA replication, and double-strand breaks caused by DNA damage [16]. Telomerase is a ribonucleoprotein enzyme that synthesizes new telomeric DNA to compensate for replication-associated telomere reduction [17]. Telomerase is composed of the telomerase reverse transcriptase (TERT) and a telomerase RNA component (TERC) that serves as the template for telomere extension [18]. Overexpression of human TERT (hTERT) can lead to rapid telomere elongation while hTERT knockdown or inhibition results in telomere shortening [19]. Telomere binding proteins, such as TRF2, participate in telomere length regulation in humans [20]. Human Pot1 (hPot1) also participate in telomere length regulation by disrupting the DNA binding activity. Knocking down the expression of hPot1 in cells causes apoptosis or senescence [21]. Moreover, STN1, a part of CST complex, plays critical role in regulating telomere lengths and replicative potential of normal human fibroblasts. STN1 knockdown cells displayed a greater increase in telomere erosion and entered cellular senescence as a consequence of telomere dysfunction [22].

2.2. Functions of human telomeres
Human telomeres protect chromosome ends from degradation and DNA double-strand break repair [23-24]. The protective function of telomeres presumably depends on their state, whether they have “capping” or “uncapping” structures. Telomeres achieve their “capping” function by a combination of their higher-order DNA structure and binding proteins. The t-loop and G-quadruplex provide the possible cap status for telomere protection. Telomere loss leads to the disruption of telomere structures, inducing gradual telomere uncapping [25-31]. Binding proteins prevent telomeres from being recognized by the cell as a DNA break and repaired by nonhomologous end joining (NHEJ) or homologous recombination (HR)-mediated repair [5]. TRF1 and TRF2 have a negative regulating function for telomere length and participate in telomere end protection [32]. TIN2 has a role in maintenance of telomere cohesion [33]. TPP1 is essential for both telomere end protection and length regulation, through repressing DNA damage signaling and modulating telomerase-dependent telomere elongation [34-35]. Protection from a DNA damage response (DDR) or unwanted DNA repair, referred to as “capped” [8, 31]. Uncapped telomeres are recognized by the DDR proteins and chromosome ends, which are referred to as telomere dysfunction-induced foci (TIFs) [36]. It has been identified that cell proliferation in vitro is accompanied by telomere shortening [1]. If telomere shortening reaches a limit and DDR foci accumulation reaches 4 or 5 foci, widespread end-to-end fusion of chromosomes and cell death would occur [37].
3. Age-related disease and telomere shortening

Telomere length decreases with age [38-39]. Telomere length, when shorter than the average telomere length, is associated with increased incidence of age-related diseases and/or decreased life span in humans [40-41]. Telomere length is a risk marker for cardiovascular disease [42-43]. The degree of telomere shortening correlated with the severity of heart disease [44]. Chromosomal instability in ulcerative colitis is associated with telomere shortening [45]. Liver cirrhosis correlated with hepatocyte-specific telomere shortening [3]. Type 2 diabetes was associated with reduced telomere length [46-47]. Short telomere lengths are predictors for the development of diabetic nephropathy [48]. Short telomeres are a risk factor for the development of idiopathic pulmonary fibrosis [49]. Moreover, telomere shortening has been involved in the dramatic age-related changes in the immune system as well, and this is one of the main factors believed to influence morbidity and mortality [50]. Telomere shortening promotes genome instability, leading to cancer initiation [51]. Short telomeres are likely to be recognized as double-strand breaks, resulting in induction of DNA damage repair by nonhomologous end joining pathway, leading to end-to-end chromosomal fusions. When cells with fused chromosomes enter mitotic cycles, these chromosomal fusions are likely to break and result in chromosomal abnormalities. Repeated breakage–fusion–bridge cycles cause accumulation of chromosomal instability that leads to final malignant transformation [52-53]. Telomerase activity are well correlated with development of cancer besides telomere shortening. Telomerase activity has been detected in many kinds of human cancers [54]. In addition, obesity and smoking as well as hypertension and lower socioeconomic status are associated with leucocyte telomere reduction [38, 55-57]. Telomeres in liver cells, compared to cells of other major organs, shorten most rapidly with age. The telomere shortening in hepatocytes is especially rapid in infants, and then the rate of shortening slows from adolescence to middle age, while no significant decrease is evident in adults in their forties up to centenarians [58-60].

4. Liver allograft rejection

4.1. Acute cellular rejection

The diagnosis of acute cellular rejection is determined by portal mixed cellular infiltration, bile duct inflammation or damage, and portal or central veins’ endotheliitis [61]. The portal inflammatory cells include lymphocytes, neutrophils, and eosinophils predominantly. Bile duct damage is composed of variation in nuclear size, eosinophilic degeneration, vacuolation of the cytoplasm, and lymphocytic infiltration into the bile duct epithelium. However, acute cellular rejection represents an immune-mediated injury directed toward the bile ducts or vascular endothelium, rather than toward hepatocytes [62]. Sustained cellular turnover in chronic liver disease accelerates cellular senescence [2-4, 63-65]. In liver transplantation, aged donors have worse prognosis [66]. Graft survival for hepatic allografts from aged donors was significantly lower than for allografts from younger donors, suggesting there is an inability of older grafts to expand to meet the functional demands of recipients [67]. Elder donor tissue has reduced ability to withstand stress and repair. Preexisting aging presumably decreases repair and survival capacity, and post-transplant stress (e.g., rejection) further disrupts this
capacity and causes graft failure [68]. Many occurrences including rejection contribute to post-transplant damage. Greater rate of telomere decline with episodes of acute rejection lead to greater telomere reduction during the post-transplant period. Accordingly, the frequency of post-transplant events (e.g., rejection) should be diminished preventing additional cell turnover.

4.2. Chronic rejection

Clinically, chronic rejection is characterized by progressive jaundice, unresponsive to immunosuppression, and, histologically, by obliterative vasculopathy, affecting large and medium-sized muscular arteries. Moreover, chronic rejection is characterized by the loss of small bile ducts [69-71]. Although the incidence of chronic rejection has decreased from 15–20% to 2–3% due to effective immunosuppression and early assessment of liver biopsies [69-70, 72-74], it is still an important cause of liver allograft failure. Our previous study showed that accelerated telomere intensity decline occurred in hepatocytes in chronic rejection within a year of transplantation. This accelerated telomere intensity decline might be a general process occurring in all grafts, since observed soon after liver transplantation. This observed decline may be due to premature aging following the acute stress observed in organ transplants and the high rate of cell turnover that occurs in graft regeneration immediately after transplantation [75]. Our previous data suggest that accelerated graft aging during the early post-transplantation term is inevitable even in tolerated grafts. The limit of proliferative life span by telomere shortening might be determined early after post-transplantation. Chronic rejection patients have one or more episodes of acute cellular rejection within a year of transplantation, and thus it is possible that acute cellular rejection induces a further early telomere intensity decline in hepatocytes [62, 76]. Care in organ preservation and preconditioning of the graft are important to achieve a better prognosis, which in turn is likely a consequence of the prevention of telomere erosion caused by various stressors immediately after transplantation. We have previously reported hepatocyte telomere signal intensity significantly lower than the predicted age-dependent decline observed in chronic rejection, as revealed by quantitative fluorescence in situ hybridization [77].

5. Idiopathic post-transplant hepatitis

Some groups have also called chronic hepatitis of unknown cause, such as idiopathic post-transplant hepatitis, de novo autoimmune hepatitis. This condition is commonly associated with positive autoantibodies, such as antinuclear antibody and elevation of IgG levels, and biochemically and histopathologically resembles autoimmune hepatitis in patients who did not receive transplants [78-79]. Increasing evidence suggests that late acute rejection, de novo autoimmune hepatitis, and idiopathic post-transplant hepatitis are part of an overlapping spectrum of immune-mediated late allograft damage occurring in long-term post-transplant patients [62]. Together with idiopathic post-transplant hepatitis, immune-mediated late allograft damage can cause late graft dysfunction leading to cirrhosis. Telomere length observed in chronic hepatitis or cirrhosis is significantly lower than that in normal livers of the same age. Sustained cellular turnover in chronic liver disease accelerates cellular senescence.
or a severe damage because of telomere shortening. We initially hypothesized that idiopathic post-transplant hepatitis may show more progressive telomere shortening due to higher cell turnover [3-4]. Cellular senescence in the explanted livers of young children was reported to be associated with hepatocyte damage rather than to a corresponding age-dependent phenomenon [80]. However, we observed no significant telomere reduction in hepatocytes taken from patients with idiopathic post-transplant hepatitis at late biopsy. Telomere shortening does not necessarily reflect the long-term graft status in idiopathic post-transplant hepatitis, which differs clinically and histologically. Telomere length in hepatocytes already shortened during the early post-transplant period. Increasing number of senescent cells associated with telomere shortening confirmed in a mouse model of ischemia–reperfusion injury [81]. Therefore, hepatocyte damage related to ischemia–reperfusion injury is likely to be a major factor in the accelerated telomere decline observed in the early post-transplant period. On the other hand, from the standpoint of telomere shortening in the early post-transplantation phase, telomere decline is considered a risk factor for late dysfunction of the graft. This finding is clinically significant in follow-up examinations of high-risk allografts.

6. Tolerance

Tolerance is a condition in which an allograft functions normally and lacks histological evidence of rejection in the absence of immunosuppression [82]. Tolerated grafts are suitable study materials for evaluating the biological organ age of grafts unaffected by inflammation and immunosuppression. We have previously reported a significant reduction in hepatocyte telomere signal intensity compared to the predicted age-dependent decline in the tolerated liver allograft, using quantitative fluorescence in situ hybridization [80,21]. Recently it has been demonstrated that measurement of relative average telomere lengths can be accomplished by real-time polymerase chain reaction (PCR) using a carefully designed pair of oligonucleotide primers [83]. In a larger number of cases, we performed quantitative real-time PCR, and confirmed accelerated telomere shortening relative to the chronological graft age in tolerated grafts. It is possible that a significant proportion of liver transplantation recipients are tolerant [84-86]. Accelerated telomere intensity decline occurred in hepatocytes in tolerated graft within a year of transplantation. The results of the previous study have suggested that even tolerated grafts might undergo a lowering of renewal capacity and a decrease in function as the recipients become older [2]. According to our previous study, the allograft could be older than the predicted age of the allograft even in tolerated grafts, and the telomere length shortened based on the graft age.

7. Oxidative stress after living-donor liver transplantation

Ischemia and reperfusion during transplantation produce a transient increase of reactive oxygen species in the organ, which are potent inducers of DNA breaks. In a rat model, both allogeneic and syngeneic transplants were characterized by shortened telomeres during
ischemia at transplantation [87]. Oxidative stress accelerates telomere shortening [88-89]. Low ambient oxygen conditions can extend the life span of cells in culture [90]. In cell culture protected from oxidative stress through low ambient oxygen tension, the addition of antioxidants, or overexpression of antioxidant enzymes delays telomere shortening [91-93]. Further data have demonstrated the important interaction between telomere-induced senescence and oxidative stress. Senescence leads to the development of oxidative stress that reinforces the senescent state of the cell and causes further oxidative stress. The telomere decline is probably due to premature aging of the graft that might occur during ischemia–reperfusion injury or graft regeneration immediately after transplantation [81]. Thus, telomere shortening in grafts could reflect not only the proliferative history of a cell, but also the accumulation of oxidative damage during the early post-transplant period [94]. Telomere reduction is presumably accelerated by the transplantation process, in both young and old tissues, modification of peri- or post-transplantation environmental stress may probably reverse aging-dependent factors.

8. Telomere length in other organ transplants

Telomere length is associated with kidney function [95]. Ischemia–reperfusion during kidney transplantation is associated with rapid telomere shortening [96]. Cellular senescence in zero hour biopsies predicts outcome in renal transplantation [97]. Telomere length assessed in biopsy specimens collected in the peri-transplant period predicts long-term kidney allograft function. Complications of kidney transplantation, like delayed graft function, acute rejection, and chronic allograft dysfunction are linked with the telomere length and thus, graft ageing [98]. Moreover, telomere length is a predictive marker of transplant outcome [99]. Rapid telomere reduction in the first year after hematopoietic stem cell transplantation were identified in recipients of bone marrow grafts [100-101]. Short telomeres are associated with the presence of chronic graft versus host disease and receiving graft from a female donor [102].

9. Conclusions and future directions

Studies of age-related disease have mostly focused on telomere length, because excessive telomere shortening leads to diseases such as cardiovascular disease, ulcerative colitis, liver cirrhosis, diabetes, and idiopathic pulmonary fibrosis. Mechanisms of complications (e.g., rejection) of organ transplantation and consequent graft failure related with ageing and senescence are due to telomere shortening. Therefore, studying of telomere length is essential in the field of organ transplantation. Telomere length was negatively associated with patient age, male sex, acute rejection, and fatty liver, and was positively associated with time from transplantation [103]. Our previous study confirmed that accelerated telomere decline in hepatocytes in the first year post-liver-transplantation is presumably due to premature ageing following the acute stress of transplantation and the high rate of cell division that occurs during graft regeneration immediately after transplantation [77]. Accelerated telomere shortening and hepatocyte senescence identified even tolerated human liver allografts [104].
problem of older donor tissue is its lower ability to endure stress and repair. Preexisting aging may reduce repair and survival capacity, and post-transplant stress such as rejection exhausts further this capacity, leading to graft failure [68]. Ischemia and reperfusion during transplantation lead to a temporary increase of reactive oxygen species in the organ, which are predominant inducers of DNA breaks. Oxidative DNA damage advances telomere shortening [94]. Furthermore, sustained cellular turnover in chronic liver disease accelerates cellular senescence [2-4, 65-66]. The confluence of acute stress, oxidative stress, ageing, and senescence suggests possible mechanisms leading to graft failure. Avoidance of factors associated with oxidative stress and telomere dysfunction is recommended in association with current liver transplantation techniques. Telomeres in grafted livers may elongate somewhat longer if the grafts are immunologically well controlled [105]. Taken together, telomere length is one of the available indicators for evaluation of liver allograft status (Fig.1).

Figure 1. Allograft failure and telomere dysfunction
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