REVIEW ARTICLE

What determines ageing of the transplanted liver?

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Abstract

Background: Liver transplantation is used to treat patients with irreversible liver failure from a variety of causes. Long-term survival has been reported, particularly in the paediatric population, with graft survival longer than 20 years now possible. The goal for paediatric liver transplantation is to increase the longevity of grafts to match the normal life expectancy of the child. This paper reviews the literature on the current understanding of ageing of the liver and biomarkers that may predict long-term survival or aid in utilization of organs.

Methods: Scientific papers published from 1950 to 2013 were sought and extracted from the MEDLINE, PubMed and University of Melbourne databases.

Results: Hepatocytes appear resistant to the ageing process, but are affected by both replicative senescence and stress-related senescence. These processes may be exacerbated by the act of transplantation. The most studied biomarkers are telomeres and SMP-30.

Conclusion: There are many factors that play a role in the ageing of the liver. Further studies into biomarkers of ageing and their relationship to the chronological age of the liver are required to aid in predicting long-term graft survival and utilization of organs.

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Introduction

Excellent 10-year survival rates of up to 82% can be achieved in paediatric liver transplantation,[1] however, the goal is for paediatric transplant recipients to live for decades longer than this.[2] Paediatric liver transplantation is often performed with adult donor organs either with a living related donor or with a deceased reduced liver segment. Do these grafts continue to age at the same rate, and what are the factors involved in this ageing process?

Cellular senescence

Cellular senescence occurs when a cell does not divide further but retains the majority of the other abilities of the cell. Telomeres are a nucleoprotein complex, encoded by DNA segments (TTAGGG) that are repeated up to thousands of times.[3] These repeated segments are shortened by cell division and thus may confer a maximal lifespan on a particular cell lineage with cells entering into a stable state with an arrested cell cycle. The loss of telomere length to a critical level renders the cell unable to divide as it enters a replicative senescence phase, a phenomenon known as the Hayflick limit.[4,5] Little is known about the cellular senescence of transplanted livers, which ideally are required to survive at least as long as the recipient's other organs.

There is also the suggestion that the stress of transplantation accelerates cellular senescence.[6–8] In the only study that analysed long-term liver allografts in the paediatric population, telomere signal intensity was measured to be significantly lower in the transplanted organs compared with predicted normal ageing.[6] Most of the decline appeared in the first year although there was no correlation with acute cellular rejection or idiopathic post-transplant hepatitis. The authors suggest that ischaemia-reperfusion injury was the likely causative factor.[6]

With the ageing of transplanted livers, histological damage increases with up to 43% of asymptomatic patients at 5 years and 64% at 10 years developing chronic hepatitis.[9] Up to 97% of grafts older than 3 years old exhibited some form of fibrosis.[10] While most of this damage appears to be immunologically mediated,

there is a percentage of patients in whom no cause is identified and may be a reflection of age-related damage.[9–11] This is supported by evidence suggesting that fibrosis is increased where there is a high donor-to-recipient age ratio.[11]In addition, it has been hypothesized that cellular senescence may be responsible for some of the long-term deterioration and damage of transplanted organs.[8,12]

It is of no surprise that outcomes from older donors are clearly worse,[13,14] but this is not universal. The search is on to find markers that are predictive of function and longevity of transplanted organs. One such marker is that of telomere length, but not all replicative cellular senescence is related to telomeres and other factors may play a role in the ageing of cells.[15]

With the expansion of gene array analysis, literally hundreds of gene expression profiles change with age, but unfortunately none so far have been found to be sensitive or specific for cellular senescence.[15–17] The predominant genetic factors and markers that have been studied are telomere length, senescence marker protein-30 (SMP-30) and CDKN2A ($p16^{INK4a}$).

Telomeres

Cells with critically short telomeres have impaired regenerative capacity, a function required after the transplantation process as well as for continued longevity of the organ.[18,19] Of importance, transplantation-related ischaemia has been found to decrease telomere length.[20] Telomeres may shorten through the advancement of age, as well as other processes that damage the cell.[21,22] Telomerase activity may help to abrogate this change by adding telomeric repeats on to the end of chromosomes, but it appears that this only occurs in foetal livers.[23] Four groups have studied the relationship between telomere length and ageing in humans.[6,24–26]

In one of the first studies of telomeres in human liver samples derived from liver resections, Aikata *et al.* reported a reduction rate of 120 bp in 23 individuals (aged 17–81 years) with telomere length reducing to 10 kbp at the age of 80.[24] In a separate group of chronic liver disease patients, they found a minimum telomere length of around 5 kbp which they suggested may be indicative of the Hayflick limit. Additionally, this demonstrated that cellular damage significantly shortened telomeres irrespective of age.

In a study of 73 deceased donor livers (age 5–79 years) performed by Verma *et al.*, age-related telomere shortening was restricted to Kupffer cells and stellate cells, with cholangiocytes and hepatocytes being spared of age-related shortening.[26] Significantly, the authors took great care to highly select these donors to exclude those with any evidence of senescence-related diseases (only 8% of the donor population were included).

Takubo *et al.* studied liver specimens from 94 individuals aged 0–101 years with telomere shortening with age found to be significant (55 bp per year).[25] The mean telomere length in five neonates was 12.9 kbp, with an estimated telomere length of approximately 7 kbp at the age of 100. Their data suggested that telomere length shortened faster before the age of 40 years and shortened slower in subsequent years, although more data are required to support this.

Aini *et al.* attempted to directly answer the question of ageing in paediatric liver transplants, measuring telomere signal intensity in 17 patients who received a living donor liver transplant.[6] Compared with predicted normal ageing, telomere signal intensity was measured to be significantly lower.[6]

It must be remembered that these studies have a paucity of young tissue with which to work and this confounds results.[27] The studies also differ in their calculation of telomere shortening and this could additionally confound results. While there is little evidence, it appears that telomere shortening is more predominant in the early years during high tissue turnover and growth, with possibly only minimal if any telomere shortening in the aged.[25,27] This is supported by studies in other tissues types such as haematopoietic cells and skeletal muscle.[28] Thus, a lack of data in the early years may partly explain the difference in results in between these highlighted studies.

SMP-30

SMP-30 was originally discovered by Fujita *et al.* as a senescence marker, with expression decreasing by up to 40% in rat livers.[29] It was later found to be identical to regucalcin, a protein that regulates intracellular Ca^{2+} homeostasis.[30–33] A lack of SMP-30 may also lead to liver fibrosis owing to its secondary effect on vitamin C production.[34–36] SMP-30 has also been found to be markedly reduced in aged zebrafish livers as well as models of hepatectomy and liver tumours.[30]

Unfortunately, while there is an abundance of SMP-30 within the livers of young children, Eguchi *et al.* have shown quite convincingly that there is minimal if any SMP-30 within the livers of young human adults which drastically reduces SMP-30s usefulness as a biomarker for use in clinical transplantation.[37] Additionally, there was no increase in SMP-30 in adult-to-child grafts suggesting that any senescence or rejuvenation of these grafts would be SMP-30 independent.

CDKN2A/$p16^{INK4a}$

CDKN2A ($p16^{INK4a}$) is a key senescence marker, and has shown to be associated with ageing as well as transplant-related injury in kidney transplantation.[8,12,38] CDKN2A is a cell-cycle inhibitor and while is often observed with telomere shortening, is thought to be independent of this process and is linked to stress-induced senescence.[20,39] Most of the literature on CDKN2A in transplantation has been performed on renal grafts, and the corollary with liver transplantation is uncertain.

Recently, two independent studies have confirmed CDKN2A to be a more important predictor of long-term graft function than donor age and telomere length, with best results using both $p16^{INK4a}$ and donor age to predict long-term renal transplant outcome.[12,39] Koppelstaetter *et al.* found telomere length to significantly correlate with donor age pre-transplant, however, donor