Small-for-size liver graft injury—impact on tumor behavior

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Abstract

The success of liver transplantation has led to an ever-increasing demand for liver grafts. Since the first successful living donor liver transplantation, this surgical innovation has been well established in children and has significantly relieved the crisis of donor organ shortage for children. However, the extension of living donor liver transplantation to adult recipients is limited by the graft volume. The major concern of adult-to-adult living donor liver transplantation is the adequate graft that can be harvested from a living donor. Small-for-size graft injury is frequently observed. To develop novel effective treatments attenuating small-for-size liver graft injury during living donor liver transplantation, it is important to explore the precise mechanism of acute phase small-for-size graft damage. Recently, a number of clinical studies and animal experiments have been conducted to investigate the possible key issues on acute phase small-for-size liver graft injury, such as mechanical injury from shear stress, subsequent inflammatory responses, and imbalance of vasoregulatory factors. This review focuses on the mechanism of small-for-size liver graft injury based on the number of clinical and experimental studies. The latest research findings of the significance of acute phase liver graft injury on late phase tumor recurrence and metastasis are also addressed.

1. Background

The emergence of adult-to-adult living donor liver transplantation (ALDLT) over the last decade has led to a dramatic increase in the number of liver grafts available. With a continued shortage of cadaveric donor livers, ALDLT currently offers a significant survival advantage to patients listed for liver transplantation [1]. In single-center series, candidates with potential living related donors are twice as likely to undergo transplantation than those awaiting cadaveric donor grafts, reducing waiting list mortality by half [2,3]. Furthermore, a liver graft from a living related donor allows earlier intervention and lowers dropouts from disease progression, leading to a higher life expectancy [4-6].

A major limitation of ALDLT is the size of the graft that can be safely harvested from the living donor. In an adult recipient, the liver graft from a living related donor is almost always small-for-size. Owing to the reduced liver volume, a small-for-size graft is less effective in coping with the portal flow of an adult recipient. The shear stress from transient portal hypertension causes acute phase mechanical injury [7-9], which subsequently induces severe inflammatory responses and then has a deleterious effect on the posttransplantation outcome. In spite of rapid hepatic regeneration, small-for-size liver grafts are associated with worse graft function and survival [7-9]. More recently, studies have reported an association between small-for-size liver grafts and higher tumor recurrence after transplantation for hepatocellular carcinoma (HCC) [10-12]. Despite its limitations, the small-for-size graft remains the most promising solution to the severe shortage of liver donors, and the pursuit of its interests should be continued.

Recent evidence has shed new light on the understanding of small-for-size liver graft injury. This review examines the pathogenesis of small-for-size liver graft injury as well as its effect on graft and patient survival. The recent link between small-for-size liver graft injury and tumor recurrence after transplantation for HCC, albeit controversial, will also be discussed.

2. The issue of liver graft size

The size of a liver graft selected varies according to the patient, the donor, and the transplant center [13]. In essence, the choice is a delicate balance between donor safety and recipient graft survival. However, as living donors are...
3. Definition of the small-for-size graft

An accepted definition of the small-for-size graft has not been established within the transplantation community. There are currently 2 schools of thought.

Small-for-size liver grafts can be classified as those with graft weight to estimated standard liver weight ratio of less than 40% [22-24]. Grafts below the critical size are associated with poor early graft function and a marked reduction in graft and patient survival after transplantation [13]. Alternatively, the definition of small-for-size liver grafts can be extended to include all grafts smaller than the standard liver volume (calculated using the formula by Urata et al [25]) [26].

The former definition focuses on grafts with unacceptable survival, whereas the latter classifies virtually all liver grafts from living related donors as small-for-size [27]. Because of the increasing evidence that size-related injury extends beyond grafts less than 40% of the standard liver weight, the latter definition is preferred because it represents a more complete picture of the pathologic process. The cohort described by the former will be referred to as marginal grafts.

4. Small-for-size liver graft injury

Small-for-size liver graft injury refers to the insult related to the small size of the graft in addition to ischemia/reperfusion injury [27]. The initiating event is transient portal hypertension after reperfusion due to graft size mismatch [7,28]. High portal pressure causes mechanical damage to the hepatic sinusoids (Fig. 1), which in turn leads to tissue ischemia, imbalance of vasoregulatory factors, increase in free radical formation, and exaggerated inflammatory response. The consequence of small-for-size liver graft injury is dependent on the extent of damage, which is inversely proportional to the graft size. The possible mechanism of small-for-size liver graft injury was summarized in Fig. 2.

4.1. Transient portal hypertension

The liver has 2 major sites of vascular inflow: the portal vein and the hepatic artery. Although portal blood flow undergoes very little autoregulation within the liver, a buffer system is in place to prevent excessive blood flow from damaging the liver. An increase in portal pressure can be countered by the hepatic arterial buffer response, where a change in portal flow induces reciprocal effects on arterial flow [29]. Through this buffer system, a liver can tolerate a maximum of 20% increase in blood flow [30]. Such a buffer system is present after transplantation [31] but is not enough to compensate for the mismatched portal flow in small-for-size grafts [32]. Because the portal flow destined for a whole liver is directed through a partial liver, excessive portal pressure is built up in small-for-size grafts, resulting in portal hypertension. The increase in portal pressure due to graft size mismatch is transient in nature and lasts for 30 minutes after reperfusion.

Shear stress from the transient portal hypertension inflicts mechanical damage upon the hepatic sinusoidal endothelium. Using a novel pig liver transplantation model, Kelly et al [33] demonstrated an inverse relationship between the graft size and the extent of hepatic sinusoidal injury. The presence of severe sinusoidal injury was reported in grafts with an estimated standard liver volume (ESLV) of 20% or less. The damage was less severe in grafts with an ESLV of 30% and was further lessened in grafts with an ESLV of 60%. Damage was virtually nonexistent in whole grafts [28].

4.2. Severer ischemia injury

Being the principle vessels involved in transvascular exchange between the blood and the liver parenchymal cells, the sinusoids are critical in the maintenance of hepatic functions [28]. A healthy microcirculatory environment is vital to the recovery of graft function after reperfusion [34,35]. Injury to the sinusoidal cells results in sinusoidal stasis and congestion [36], and a lack of functional microcirculation exposes the liver tissue to ischemia [37]. Although the hemodynamic changes are transient, tissue ischemia secondary to sinusoidal insult causes progressive cellular damage. The histologic hallmarks of prolonged ischemia include swelling of mitochondria and hepatocytes as well as presence of apoptosis [28].

4.3. Imbalance of vasoregulatory peptides

In the normal liver, the maintenance of hepatic microcirculation relies heavily on the delicate balance of vasoconstriction and vasodilatation. Prolonged ischemia from sinusoidal damage tips the balance in favor of vasoconstriction...
The gene for endothelin 1 is overexpressed as a result of ischemia, resulting in its overproduction \[38\]. Endothelin 1 is the most potent constrictor peptide of vascular smooth muscle \[39\] and increases intrahepatic resistance by directly constricting the remaining hepatic sinusoids. On the other hand, the gene for endothelial nitric oxide synthase is underexpressed.

Fig. 1. Hepatic ultrastructural changes after liver transplantation in animal (A) and human (B) studies. A. Tremendous mitochondrial swelling with unvisualized cristae (*) was found in the hepatocytes. Collapse of the Disse space (arrow) and an irregular large gap (arrow head) between the sinusoidal lining cells also occurred. Total disruption of hepatic sinusoidal lining cells (arrows) was presented at 24 hours after reperfusion in animal study (animal study from Man et al \[28\]; human study from Man et al \[7\]).

Fig. 2. Pathways to small-for-size liver graft injury.
Endothelial nitric oxide synthase is involved in the maintenance of vasodilatation through the production of endogenous nitric oxide [40]. Endogenous nitric oxide in turn is vital in the physiological regulation of blood flow [41]. This results in a further shift of balance toward vasoconstriction.

Furthermore, genes for heat shock protein 70 and heme oxygenase 1, which are important for tissue repair [42] and protection against vascular constriction [43], respectively, are also underexpressed in small-for-size grafts. A shift toward vasoconstriction results in further ischemic damage.

### 4.4. Increase in free radical formation

The production of free radicals is markedly increased in small-for-size grafts shortly after reperfusion [44]. Zhong et al [44] suggested 2 possible mechanisms of free radical formation in small-for-size grafts. First, a reduction in graft size increases the metabolic burden on the graft. As metabolism increases, mitochondria produce more oxygen radicals. At the same time, reduced glutathione, a potent antioxidant, is consumed more rapidly in smaller grafts. The result is a net increase in free radicals and more severe graft injury [45]. A second explanation involves Kupffer cells. Each individual Kupffer cell in a small-for-size graft is exposed to higher amounts of endotoxin than that in a whole liver graft [46]. In turn, the exposure to endotoxin triggers Kupffer cells to release a large quantity of free radicals. Regardless of the method of release, the free radicals cause direct oxidative damage to the remaining hepatocytes and sinusoids. An increase in free radical formation is associated with increased graft injury and worse graft function, as indicated by increased serum alanine transferase and bilirubin levels as well as histologic signs of necrosis and leukocyte infiltration.

### 4.5. Exaggerated inflammatory response

A common finding in liver grafts after transplantation is a cellular infiltrate secondary to ischemia/reperfusion injury. The cellular infiltrate predominantly consists of macrophages. As a result of transient portal hypertension, the macrophage cell number and activity are substantially increased in small-for-size grafts. Using a rat liver transplantation model, Yang et al [47] demonstrated a significantly higher macrophage infiltrate in the portal area of small-for-size grafts compared with that of whole grafts during early phase after reperfusion. The number of macrophages expanded to reach a peak at 72 hours after reperfusion. In addition to an increase in the cell number, the activity of the macrophages was also enhanced in small-for-size liver grafts. This was represented by exaggerated expression of inducible nitric oxide synthase by the macrophages in small-for-size grafts.

The invading macrophages in small-for-size grafts secrete high levels of cytokines, specifically interleukin 1β and interleukin 2, during early phase after reperfusion. In the in vitro setting, Yang et al [47] demonstrated that interleukin 1β transforms macrophages into antigen-presenting cells, as evidenced by the expression of CD80 and CD86. These 2 molecules provide vital stimuli to prime T cells against antigens presented by antigen-presenting cells. The presence of a larger number of antigen-presenting cells in small-for-size grafts enhances alloantigen recognition and subsequently results in accelerated graft rejection.

In a separate study by Yang et al [48], vascular endothelial growth factor (VEGF) was put forward as the vital link between transient portal hypertension and macrophage activation in small-for-size grafts. Microcirculatory injury after reperfusion triggers VEGF secretion from hepatocytes, a vital physiological process needed for rapid hepatic regeneration. However, VEGF is also an important mediator of monocyte and activities, and excessive VEGF is produced in small-for-size grafts because of more profound microcirculatory damage. Subsequently, an increase in production of intragraft VEGF results in the enhancement of migratory activities of monocytes in small-for-size grafts [48].

### 5. Clinical consequence of small-for-size liver graft injury

The most severe consequence of small-for-size liver graft injury is small-for-size syndrome, defined as graft dysfunction or graft failure during the first postoperative week after the exclusion of other causes [49]. This syndrome is almost exclusive to marginal grafts. Several notable studies have reported different cutoff points for marginal grafts. Kawasaki et al [50] set the limit of safe liver graft size at 30% of ESLV. This threshold point is shared by Nishizaki et al [51]. Lo et al [52] reported that graft sizes below 40% of ESLV have low success rates. Lee et al [72] used a graft-recipient weight ratio (GRWR) of less than 0.8% as the limit, whereas Ben-Haim et al [53] reported that a GRWR as low as 0.6% is suitable for patients with less severe end-stage liver disease. It is important to note, however, that these limits are mainly arbitrary.

Small-for-size syndrome is attributable solely to size of the graft because not every marginal graft results in dysfunction or failure. Small-for-size syndrome is a multifactorial disease. Other factors involved in small-for-size syndrome include graft-related causes (a lack of liver regeneration [54], high portal inflow [55], low venous outflow [56], preexisting steatosis in the donor [57]), and recipient-related causes (severe preoperative end-stage liver disease [53], presence of cirrhosis [58], and poor preoperative health status).

For small-for-size liver grafts with GRWR of more than 0.8%, a difference in survival compared with whole liver grafts is less convincing. Earlier reports on short-term survival after liver transplantation suggested poorer graft survival in ALDLT recipients. Using the United Network for Organ Sharing data in a retrospective analysis, Abt et al [8] discovered a significantly higher rate of allograft failure in ALDLT recipients compared with deceased donor liver transplantation (DDLT) recipients (hazards ratio, 1.66;
with the same database, Thuluvath and Yoo [9] reported a 2-year graft survival of 64.4% in ALDLT recipients compared with 73.3% in the DDLT recipients. The difference in graft survival between the 2 groups was statistically significant. However, because of the availability of retransplantation for recipients who developed allograft failure, patient survival after ALDLT remained similar to that of DDLT. A crucial limitation of the United Network for Organ Sharing database is omission of data on graft size. It is impossible to confirm whether size plays a role in the poorer graft survival.

A more recent study on long-term survival after liver transplantation refutes previous evidence of poorer survival in ALDLT recipients. Maluf et al [59] found no statistical differences between ALDLT and DDLT in the patient or graft survival rates at 5 years after transplantation. A possible reason for the discrepancy in results is an improvement of surgical technique at a high volume center. Results from our center also demonstrated a lack of significant difference in patient and graft survival between the 2 groups. With a median follow-up time of 27 months, the graft and patient survival rates were 88% and 90%, respectively, in the ALDLT group, whereas the survival rates were both 84% in the DDLT group. In this study, the ALDLT grafts had a significantly lower median graft weight to estimated standard liver weight ratio (48.9% vs 98.2%). Liu et al [46] cited that their regular inclusion of the middle hepatic vein in partial liver grafts, which allows good venous drainage to meet the high metabolic demand of recipients, might have resulted in the more favorable survival outcomes in ALDLT.

6. Small-for-size liver graft injury on late phase tumor recurrence

6.1. Background

Although ALDLT confers a substantial survival advantage for patients with HCC awaiting transplantation, the preference of ALDLT to DDLT is based primarily on hypothetical studies using decision analysis models [4,5]. Such studies assume comparable tumor recurrence rates and patient survival after ALDLT and DDLT. With the adoption of the Milan criteria, DDLT offers a tumor recurrence rate of less than 10% [60]. This in turn confers a patient survival rate comparable to patients undergoing DDLT without HCC (75% survival at 4 years) [61,62]. In comparison, the tumor recurrence rate after ALDLT remains controversial.

6.2. Clinical evidence of inferior oncologic outcome

Recently, a number of centers have compared the outcomes of ALDLT and DDLT using retrospective clinical data. The inability to conduct a randomized controlled trial has led to conflicting results in different centers. However, there is increasing evidence that partial liver grafts lead to higher tumor recurrence after ALDLT for HCC.

On one hand, Hwang et al [63] reported no significant difference in the HCC recurrence rates between the 2 cohorts. Instead, tumor size, gross major vessel invasion, and histologic differentiation were cited as the major risk factors for tumor recurrence. Gondolesi et al [64] also observed comparable tumor recurrence rates between ALDLT and DDLT.

On the other hand, an increasing amount of evidence points to a higher tumor recurrence rate after transplantation using ALDLT. In 2004, Kulik et al [12] reported higher HCC tumor recurrence rates in the “fast tracked” ALDLT cohort (22%) compared with the DDLT cohort (3%). They reasoned that the shorter waiting time did not allow an adequate period to assess the tumor’s biological behavior. Hence, the ALDLT group included more aggressive tumors, which may have accounted for the higher tumor recurrence rate. However, the team also hypothesized that, by virtue of its relatively small size, the liver graft from a living donor could potentially promote tumor growth and metastasis independently and, thus, result in a higher tumor recurrence rate. This theory has become the focus of more recent studies on the topic.

In 2007, Lo et al [11] reported a 5-year recurrence rate of 22% in the ALDLT cohort and a 5-year recurrence rate of 0% in the DDLT cohort. In the study, tumor recurrence was the major cause of death after transplantation, with 6 of the 10 deaths attributable to tumor recurrence [11]. Furthermore, the 5-year patient survival rate was substantially lower in the ALDLT cohort (58% vs 94%), which, however, without statistical significance, was most likely because of the small sample size. In the same year, the multicenter Adult-to-Adult Living Donor Liver Transplantation Cohort Study reported a 3-year higher tumor recurrence rate in the ALDLT cohort (29% vs 0%) [10]. This time, however, there was little difference in the overall patient survival of the 2 groups. A striking similarity in the 2 studies was the lack of tumor recurrence in the DDLT cohort. The finding that even transplantation for tumors outside the Milan and University of California, San Francisco, criteria lacked recurrence suggested that factors other than tumor aggressiveness before transplantation were responsible for the findings. Again, both studies proposed that the small size of the living donor liver graft was potentially responsible for the higher recurrence rate. However, the hypothesis yet remains unproven.

Most recently, Hwang et al [65] tested the effect of GRWR on tumor recurrence with a retrospective study on past transplantations. The study found no statistical differences in the overall patient survival (P = .105) and recurrence-free survival (P = .406) among grafts with GRWR less than .8 (small grafts), GRWR of .8–1.0 (mid sized), GRWR greater than 1.0 (large sized), and those with GRWR greater than 1.5 (whole grafts). However, because of a high amount of sample stratification, the sample size of each group and, hence, the power were not large enough to detect a statistical difference. Table 1 shows the results of the aforementioned studies. In the face of such controversy, an
6.3. Molecular pathways linking small-for-size liver graft injury to tumor recurrence after transplantation for HCC

Crucially, there is an overlap of processes in small-for-size graft injury with those involved in tumor invasion. Such factors may provide a favorable environment for tumor recurrence in small-for-size grafts.

The main pathway in which the outgrowth of preexisting micrometastases can be promoted in small-for-size grafts is through tissue ischemia. It is present in small-for-size grafts secondary to portal hyperperfusion injury. The ischemia is exacerbated by the hepatic arterial buffer response because it leads to a decrease in hepatic arterial flow. In a study by Doi et al.,

Table 1: Tumor recurrence after liver transplantation using DDLT and LDLT

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size</th>
<th>Date</th>
<th>Tumor recurrence rate</th>
<th>Patient survival rate</th>
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<tr>
<td></td>
<td>DDLT vs LDLT</td>
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<td>DDLT</td>
<td>LDLT</td>
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<tr>
<td>Kulik</td>
<td>41 vs 33</td>
<td>2004</td>
<td>0%/9 mo</td>
<td>15%/8 mo</td>
</tr>
<tr>
<td>Gondolesi</td>
<td>36 vs 165</td>
<td>2004</td>
<td>83%/2 yrs</td>
<td>74%/2 y</td>
</tr>
<tr>
<td>Hwang</td>
<td>237 vs 75</td>
<td>2005</td>
<td>82%/2 y</td>
<td>79.7%/2 y</td>
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<tr>
<td>Lo</td>
<td>43 vs 17</td>
<td>2007</td>
<td>0%/5 y</td>
<td>29%/5 y</td>
</tr>
<tr>
<td>Fisher</td>
<td>58 vs 34</td>
<td>2007</td>
<td>0%/3 y</td>
<td>29%/y</td>
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Fig. 3. A, Liver tumor development after liver transplantation using whole or small-for-size liver grafts at day 14 and day 21. B, The liver occupied by tumor was compared at different time points. C, Liver tumor growth in nude mice at week 4 and week 6 after tumor implantation from group W or group S. D, The volume of tumors from nude mice was also compared. *P < .05 group W vs group S. (From Man et al., Ann Surg, in press).
et al [66], prolonged liver ischemia induced higher secretion of inflammatory cytokines, increased free radical formation, and subsequently increased liver metastasis of colon cancer. van der Bilt et al [67] reported that ischemic lobes in murine livers had a 5-fold to 6-fold increase in outgrowth of micrometastases compared with nonischemic lobes. The same study also discovered that a decrease in ischemic time would drastically decrease the incidence of metastasis.

6.4. Liver graft injury and tumor recurrence

Recently, we have demonstrated the significance of surgical stress on tumor behavior in a rat liver tumor model undergoing ischemia/reperfusion injury and major hepatectomy. The surgical stress resulting from hepatic ischemia/reperfusion injury and/or major hepatectomy did not only make the hepatic microenvironment (“soil”) favorable for tumor cell growth, migration, and invasion through stimulation of acute phase inflammatory response and disturbance of microcirculatory barrier function but also made the tumor cells ("seeds") more aggressive for local and distant metastases by directly activating cell migration and invasion pathways in the tumor cell itself [68]. We also demonstrated the significance of graft size in tumor growth and invasiveness after liver transplantation in a rat model. More rapid and invasive tumor development in small-for-size liver grafts was evident in morphological examination and was supported by the signaling linking to angiogenesis and tumor invasiveness. We further confirmed the invasiveness of tumors developed from small-for-size grafts in a novel orthotopic nude mice implantation model (Fig. 3) [69]. It was found that acute phase small-for-size liver graft injury does not only provide a microenvironment that favors tumor development but also promotes the invasiveness of tumor cells.

The small liver remnants and small-for-size liver graft expressed significantly high levels of early overexpression of early growth response 1, focal adhesion kinase, and VEGF [68,69]. Early growth response 1 switches on several cascades of inflammatory response as well as angiogenesis and cell adhesion [70]. The activation of focal adhesion kinase is related to microvascular barrier dysfunction and has been demonstrated to promote an invasive tumor cell phenotype [71]. Vascular endothelial growth factor is a major promoter of angiogenesis [72].

From the studies, there are several possible mechanisms in which small-for-size liver graft injury can increase the incidence of tumor recurrence. They may include increase in cell adhesion factors, liver parenchyma damage/microvascular barrier dysfunction, and angiogenesis.

6.5. Cell adhesion

Prolonged ischemia is associated with higher expression of E-selectin and, consequently, a higher incidence of tumor metastasis [73]. E-selectin is a molecule that is important in inflammatory responses [74] because it facilitates the adhesion of leukocytes to endothelial cells [75]. It is also reportedly involved in liver cancer growth and metastasis [76] by facilitating the adhesion of cancer cells to the endothelium [77]. Expression of E-selectin increases with the length of ischemic period. This is because E-selectin is up-regulated by tumor necrosis factor α and interleukin 1 [78], whose expression is in turn promoted by liver ischemia.

**Liver transplantation using small-for-size grafts**

Fig. 4. Possible mechanism of invasive tumor growth after transplantation using small-for-size grafts.
Affect short-term graft survival but may also contribute to late phase tumor recurrence and metastasis in liver transplantation for liver cancer (Fig. 4). An improved understanding of small-for-size liver graft injury will facilitate preventive and therapeutic measures not only for early graft dysfunction but also for late phase tumor recurrence and metastasis.

References

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