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Fetal progenitor cell transplantation treats methylmalonic aciduria in a mouse model

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ABSTRACT

Methylmalonic aciduria is a rare disorder caused by an inborn error of organic acid metabolism. Current treatment options are limited and generally focus on disease management. We aimed to investigate the use of fetal progenitor cells to treat this disorder using a mouse model with an intermediate form of methylmalonic aciduria.

Fetal liver cells were isolated from healthy fetuses at embryonic day 15–17 and intravenously transplanted into sub-lethally irradiated mice. Liver donor cell engraftment was determined by PCR. Disease correction was monitored by urine and blood methylmalonic acid concentration and weight change.

Initial studies indicated that pre-transplantation sub-lethal irradiation followed by transplantation with 5 million cells were suitable. We found that a double dose of 5 million cells (1 week apart) provided a more effective treatment. Donor cell liver engraftment of up to 5% was measured. Disease correction, as defined by a decrease in blood methylmalonic acid concentration, was effected in methylmalonic acid mice transplanted with a double dose of cells and who showed donor cell liver engraftment. Mean plasma methylmalonic acid concentration decreased from 810 ± 156 (sham transplanted) to 338 ± 157 μmol/L (double dose of 5 million cells) while mean blood C3 carnitine concentration decreased from 20.5 ± 4 (sham transplanted) to 5.3 ± 1.9 μmol/L (double dose of 5 million cells).

In conclusion, higher levels of engraftment may be required for greater disease correction; however these studies show promising results for cell transplantation biochemical correction of a metabolic disorder.

1. Introduction

Methylmalonic aciduria (MMA) is a rare disorder caused by an inborn error of organic acid metabolism [1]. While patients with MMA due to no or minimal methylmalonyl-CoA mutase activity (mut^0) most commonly present in the neonatal period with severe metabolic encephalopathy, those with residual activity (mut^-) may also present in infancy with failure to thrive and developmental delay.

Novel therapies for MMA focus on organ transplantation to stabilize plasma methylmalonic acid levels by enabling metabolism of circulating metabolites [2]. Liver transplantation appears successful in reducing plasma methylmalonic acid levels [3,4], whilst combined liver and kidney transplantation shows a trend toward even lower levels [5]. Despite this, it is unclear whether continuing metabolic damage to the kidney may still occur in the long term due to the continued presence of methylmalonic acid [1,3,4,6,7]. Additionally, accumulation of methylmalonic acid in the brain does not appear to be affected by liver transplantation [1,3,4,6–8], and continued susceptibility to neurological complications have been reported [6,9,10]. While it is generally agreed that organ transplantation cannot provide a cure, partial correction has been achieved, allowing liberalization of dietary restrictions, prevention of episodes of metabolic decompensation and overall better quality of life [5]. The shortage of donors, coupled with the rarity of the disease, prevents adequate study of the effectiveness of whole organ transplantation, thus cell therapy can be considered [11].

Adult hepatocytes are more readily available than whole organs for transplantation and have been shown to provide the best engraftment and disease correcting ability of the different cell types trialled; however they tend to be fragile making isolation and preservation difficult. Adult bone marrow (BM) cells have been shown to contribute to liver regeneration [11] as have cord blood cells [12,13]. Fetal hepatic progenitor cells [14] may also be an alternative cell therapy.

Abbreviations: BM, bone marrow; MMA, methylmalonic aciduria.

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During gestational development, the fetal liver is initially a site of hematopoiesis, and only later exhibits hepatic functions. Human fetal liver cells are effective in treating severe immunodeficiencies, hematological disorders and inborn errors of metabolism following *in utero* transplantation in humans [15–17]. The use of syngeneic fetal hepatocytes has also been beneficial in the treatment of fulminant hepatic failure [18]. In comparison to adult cells, fetal liver cells have demonstrated a much greater proliferative ability [19], increasing the likelihood of liver repopulation, as opposed to just cell engraftment without cell number expansion.

To create a mouse model of MMA, a knockout mouse with a null phenotype was initially developed [20]. Homozygous knockout pups ($Mut^{-/-}$) appeared normal when born, but became unwell and died by 24 h. Transgenic mice lines were therefore produced [21]. Mice with two copies of the human transgene on one allele on a homozygous knockout background ($Mut^{-/-}MUT^{2h/-}$) [21] showed a phenotype similar to mut^{-} MMA patients. These partially rescued mice were significantly smaller than control littermates (mice with mouse methylmalonyl-CoA mutase gene). Biochemically, these partial rescue mice exhibited elevated methylmalonic acid levels in urine, plasma, kidney, liver and brain tissue. We utilized these mice (MMA mice), with an intermediate phenotype of MMA, as our model for this study.

This project involved transplanting fluorescently tagged fetal progenitor cells into mice with an intermediate form of the metabolic disorder MMA to determine whether the cells could correct the elevated metabolite levels. The use of cell transplantation has the potential of not only treating MMA, but translating to medical therapies for patients with other metabolic disorders.

2. Materials and methods

2.1. Mouse husbandry

All procedures involving treatment of mice were approved by the Murdoch Childrens Research Institute Animal Ethics Committee.

The donor Frataxin-Green Fluorescent Protein (FGFPc) mice expressed normal human Frataxin protein tagged with EGFP, causing ubiquitous mitochondrial expression of enhanced green fluorescent protein (EGFP) [22]. Thus the FGFPc mouse model produces EGFP in all cells allowing identification of the donor cells following transplantation. MMA mice and FGFPc mice were modifications of a C57BL/6 mouse background, so donor cells were congenic eliminating concerns about cell rejection and elimination via the immune system.

2.2. Isolation of donor cells

Embryonic day 15–17 fetal livers from FGFPc mouse pups were collected then digested in 10% fetal bovine serum in RPMI-1640 media (referred hereafter as RPMI), to which 3500 U collagenase (Sigma–Aldrich Company, St Louis, MO) was added and incubated at 37 °C to release the cells. The mixture was filtered, centrifuged (5 min, 100*g*) and the cell pellet erythrocytes lyzed. Fetal progenitor cells were washed, resuspended and the cell viability determined. Cell suspensions with >95% cell viability were used in all transplants. The cell population expressed EGFP and at least 7% of the main cell population expressed Sca-1 and are thus potential stem cells.

2.3. Cell transplantation

Experimental mice were randomly allocated into groups (Table 1). All mice were culled between 8 and 10 weeks of age (4 weeks post-transplantation). Mice were sub-lethally irradiated (4 Gy) the day before transplantation. Mice were injected via the tail vein with 100 μL of cell suspension (1–5 million cells). Mice receiving a double dose of cells were transplanted again 1 week later. A control sham transplanted group was injected with RPMI media alone. MMA mice had no mouse *Mut* gene and were heterozygous for the human *MUT* gene ($Mut^{-/-}MUT^{2h/-}$). Control/normal mice were litter mates of MMA mice which were heterozygous or homozygous for the mouse *Mut* gene and were null or heterozygous for the human *MUT* gene ($Mut^{+/-}MUT^{2h/-}$, $Mut^{+/+}MUT^{2h/-}$, $Mut^{+/-}$ or $Mut^{+/+}$).

2.4. Metabolite analysis

Urine samples were collected weekly. Urine methylmalonic acid levels were measured by direct injection electrospray MSMS as previously described [20]. Creatinine levels were measured for urine diluteness normalization using a standard Jaffe method.

A blood spot from each mouse was collected on card (Whatman 903 paper) at 3 weeks of age, at transplant, and at 2- and 4-weeks post-transplant and analyzed for plasma acyl-carnitine [23].

Plasma from whole blood was collected pre-transplant, at 2-weeks post-transplant and at cull, then stored at −20 °C until analyzed for methylmalonic acid concentration [21].

2.5. Flow cytometry

BM and splenocytes were analyzed using flow cytometry. Samples with >0.5% proportion of cells expressing EGFP were considered positive for donor cell engraftment. Flow cytometry was performed using a LSRII machine (Becton–Dickinson, USA) and analyzed using the FACSDiva Software Package, Version 1.4 (Becton–Dickinson).

2.6. DNA analysis

Genotyping was performed using PCR to amplify the EGFP and murine Myogenin (internal control) genes [24]. Real-time PCR was used to quantify engraftment levels of donor cells in the liver, BM and spleen. A *SYBR-Green PCR Master Mix* kit (Applied Biosystems) was used for the real-time PCR (according to the supplier's protocol) with primers for the human frataxin gene [22] used to identify donor cell DNA and primers for the murine beta-actin gene used as internal controls. A 7300 Real-Time PCR System (Applied Biosystems), with the supplied Sequence Detection Software (version 1.2.2) was used. Samples with >0.5% proportion of donor cells were considered positive for cell engraftment.

2.7. Data analysis

Experimental groups were combined for the purposes of analysis of disease correction. Data expressed as mean (± standard error mean, SEM). Analyses were performed using independent samples *t*-test. Statistical significance was accepted at $p < 0.05$.

Table 1
Number of mice in each experimental group.

Cell dose	Control mice	Methylmalonic acid mice
0 (Sham)	4 (2 Male, 2 female)	10 (6 Male, 4 female)
1×10^6	5 (3 Male, 2 female)	1 (0 Male, 1 female)
2.5×10^6	7 (3 Male, 4 female)	5 (5 Male, 0 female)
5×10^6	16 (6 Male, 10 female)	19 (6 Male, 13 female)
2 Doses of 5×10^6	11 (4 Male, 7 female)	12 (4 Male, 8 female)

3. Results

Mice from all groups were assessed for BM and spleen engraftment using both PCR and flow cytometric analysis; with donor cell tissue engraftment confirmed by real-time PCR if both methods were positive. Liver engraftment was assessed by PCR and real-time PCR of DNA. Initial studies showed that up to 20% of mice transplanted with a dose of 5 million cells were able to show donor cell engraftment in the liver without irradiation (lower cell doses failed to show liver engraftment).

Of mice which were sub-lethally irradiated 60% showed donor cell engraftment in the liver (with cell doses of 2.5 million, 5 million and double dose of 5 million cells). The splenic results mirrored the liver engraftment results, whilst the BM was more likely to be engrafted with up to 80% of recipient mice with donor cells. The dose of cells did not alter the probability of a mouse being engrafted with donor cells after sub-lethal irradiation, however the percentage of donor cells detected was increased in the liver and BM with increasing cell dosage (Fig. 1). Brain and kidney engraftment was assessed using real-time PCR, however no donor cells were detected at these sites.

Experimental groups were combined for the purposes of analysis of disease correction. Transplanted MMA mice were subdivided into two groups: those which demonstrated donor cell engraftment in the liver by real-time PCR and those which did not. Control mice were not divided for analysis of disease correction. The two MMA mouse experimental groups were compared with each other, with corresponding transplanted control mice, and with sham transplanted MMA mice for indicators of disease correction including weight, urine methylmalonic acid concentration and plasma methylmalonic acid concentration.

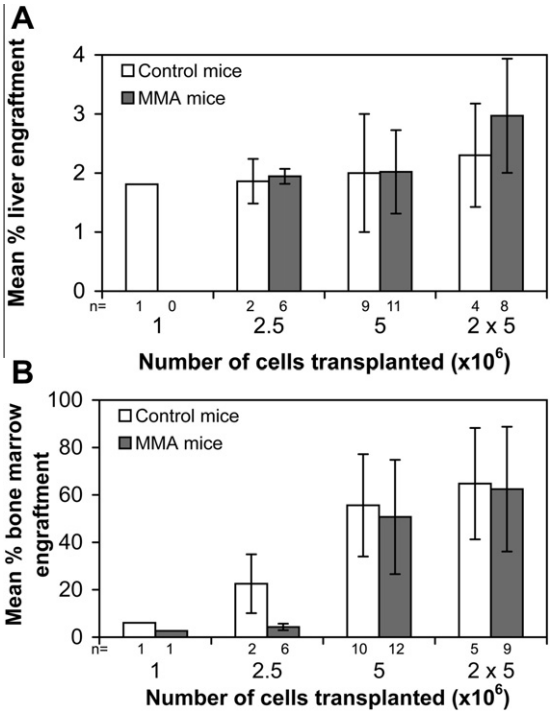

Fig. 1. Sub-lethally irradiated mice transplanted with different doses of fetal progenitor cells showed varying amounts of donor cell engraftment 4 weeks post-transplant. Donor cell engraftment levels were determined by real-time PCR of liver and bone marrow samples and compared to a standard curve of donor cell DNA (100% EGFP). **A.** Mean% engraftment of donor cells in the liver. **B.** Mean% engraftment of donor cells in the bone marrow. Results presented as mean ± SEM.

Plasma methylmalonic acid concentration at 2- and 4-weeks post-transplantation was significantly higher in sham transplanted MMA mice than age-matched control mice (Fig. 2). At 4 weeks post-transplant mice with liver engraftment of donor cells have a lower mean plasma methylmalonic acid concentration than sham transplanted mice (810 ± 156 μmol/L), which is highlighted by the double cell dose group which have a decrease of 60% (338 ± 157 μmol/L), however this is still higher than the control mice (1.6 ± 1.1 μmol/L). The 2- and 4-week post-transplantation plasma methylmalonic acid concentration results for liver engrafted MMA mice show a similar pattern, with only a slight decrease in concentration detected over the 2 week period.

The MMA mouse model had a dried blood spot plasma propionylcarnitine (C3 carnitine) value of 7.3 ± 1.5 μmol/L at 6 weeks of age compared to 1.6 ± 1 μmol/L in control mice. The C3 carnitine levels at transplant and at 2- and 4-weeks post-transplant were significantly elevated in all MMA mice compared to age-matched control mice (Fig. 3). Without cell treatment (sham transplantation) there was a significant increase in C3 carnitine levels at 8 and 10 weeks of age in MMA mice (2- and 4-weeks post-transplant).

At 4 weeks post-transplant the double cell dose (2 × 5 million cells) decreased the mean C3 carnitine levels in MMA mice with donor cell engraftment in the liver by more than 70% (from 20.5 ± 4 μmol/L found in sham transplanted mice down to 5.3 ± 2 μmol/L) (Fig. 3). MMA mice with no donor cell liver engraftment (who may have BM engraftment) still had decreased C3 carnitine levels of almost 60% (down to 8.9 ± 3 μmol/L) suggesting that extra-hepatic sites may be aiding in disease correction.

The mean weekly weight of transplanted male and female MMA mice were generally higher than sham transplanted mice, however they were not considerably different from their sham transplanted counterparts at any period of time prior to or following transplantation, regardless of whether or not donor cell engraftment was detected (data not presented).

Levels of urine methylmalonic acid in treated and untreated MMA mice were always significantly higher than age-matched controls (260 ± 230 μmol/L methylmalonic acid/mmol/L creatinine, $n = 63$). At 4-weeks post-transplant there was no difference between transplanted MMA mice with (27,800 ± 13,000 μmol/L methylmalonic acid/mmol/L creatinine, $n = 18$) or without (27,900 ± 10,500 μmol/L methylmalonic acid/mmol/L creatinine, $n = 23$) donor cell engraftment. At 4-weeks post-transplant there was a decrease compared to sham transplanted controls (50,500 ± 10,000 μmol/L methylmalonic acid/mmol/L creatinine, $n = 11$), however the was no clear pattern over time.

4. Discussion

Donor fetal progenitor cell engraftment in the liver of MMA mice of between 0.5% and 5% was found to ameliorate blood methylmalonic acid levels. Sub-lethal irradiation preconditioning and transplantation with one or two doses of 5 million fetal progenitor cells (rather than 2.5 million or less cells) was required to cause substantial donor cell liver engraftment. BM and spleen were also found to be engrafted under these conditions suggesting disease correction may be produced via extra-hepatic engraftment sites.

At the age used in this study, the fetal liver is undergoing transition from its early function as a haematopoietic organ to its mature hepatic function, and thus fetal hepatic progenitor cells are expected to contain a combination of cells of both haematopoietic and hepatic lineage [25–27]. This study was designed to use 16 and 17 days gestation embryos to enrich for the higher proportion of cells of hepatocytic stem cell lineage [27]. The diameter of fetal hepatic progenitor cells is less than that of adult hepatocytes, which should theoretically allow systemic circulation to optimize

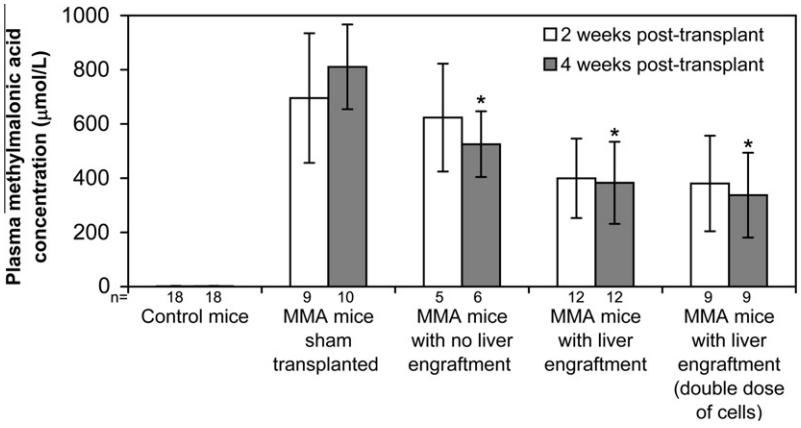

Fig. 2. Comparison of plasma methylmalonic acid concentration of transplanted MMA mice with and without liver engraftment and sham transplanted controls at 2- and 4-weeks post-transplant. Results presented as mean ± SEM. $^{*}p < 0.05$ (when comparing sham transplanted MMA mice and MMA mice transplanted with cells). Comparison of plasma methylmalonic acid concentration between mice given a single dose of 5 million donor cells (data not presented) and double dose of 5 million donor cells, showed there was no significant difference. Control mice have a plasma methylmalonic acid concentration below 2 µmol/L.

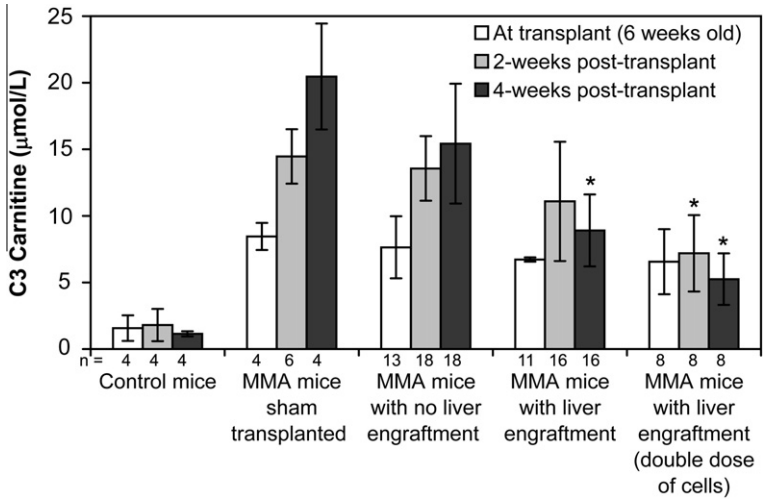

Fig. 3. Comparison of C3 carnitine levels (µmol/L) of transplanted MMA mice with and without liver engraftment and sham transplanted controls at transplant and 2- and 4-weeks post-transplantation. Results presented as mean ± SEM. $^{*}p < 0.05$ (when comparing sham transplanted MMA mice and MMA mice transplanted with cells). There was also a significant difference in C3 carnitine between a single (data not shown) and double dose of 5 million cells at 2-weeks ($p = 0.003$) and 4-weeks ($p = 0.03$) post-transplant.

engraftment in various organs following intravenous delivery. Most metabolic liver disorders have little or no liver injury so cell engraftment without cell expansion (or repopulation) is the goal for cell therapies. In the non-injurious forms of metabolic liver disorders there is unlikely to be a drive for donor stem cells to become hepatocyte competent and therefore adult or fetal hepatic progenitor cell transplants are likely to remain the preferred form of cellular therapy.

Total-body or lethal irradiation is often used in murine transplantation studies as a method of increasing donor cell engraftment prior to BM transplantation. Pre-transplant irradiation of the recipient is known to induce ablation of endogenous cells especially rapidly dividing cells [28], creating 'space' in which the donor cells can potentially engraft. This study confirmed that sub-lethal irradiation can be reliably used to increase donor cell engraftment in the liver, BM and spleen of mice. While donor cell engraftment in BM and spleen was observed in mice receiving 5 million fetal progenitor cells without irradiation, engraftment efficiency was considerably lower.

In mice showing donor cell engraftment, cells appeared in the liver, BM and spleen for the majority of cases. The frequency of donor cell engraftment in the BM and spleen would be due to the presence of haematopoietic lineage cells in the cells transplanted, as well as those of hepatocytic lineage. Although liver engraftment was the aim, BM and spleen engraftment may prove beneficial by enabling disease correction in a number of ways, depending on cell fate. Donor cells engrafting anywhere in the body should possess methylmalonyl-CoA mutase activity and have the potential to correct MMA. However, the high rate of blood flow through the liver makes this organ the target for engraftment. Donor cells present in the liver are granted greatest exposure to circulating metabolites, which they can metabolize and effectively 'filter' from the bloodstream.

Fetal cell therapy used in this study attempted to achieve repopulation of the liver by donor hepatocytes, as this is the most prevalent cell population in the liver, enabling greatest potential for disease correction. However, donor cell engraftment of the BM could provide a potential source of hepatocyte activity, either through differentiation into hepatocyte-like cells or cell fusion. It had been hypothesized in earlier studies of BM transplantation in a mouse model of Wilson's disease that donor cell engraftment of the liver is necessitated by BM engraftment [24,29]. The high frequency of co-engraftment of donor cells in the BM, liver and spleen of recipient mice seen in this study supports this hypothesis.

An alternate explanation for the disease correction detected, which may be more likely, is that donor cells engrafted in the liver are not hepatocytes, but are comprised of cells of other lineages. Repopulation of the BM may provide stem cells which later differentiate into Kupffer cells, enabling long term engraftment of donor cells in the liver and disease correction. However, as Kupffer cells only represent a small proportion of hepatic cells, expansion of donor cells is limited, which in turn limits disease correction achievable. Donor cells of the cholangiocyte lineage present in the liver are similarly limited in their ability to achieve disease correction.

In mice that received a double dose of 5 million cells (and had donor cell liver engraftment) plasma methylmalonic acid concentration levels were stable and were significantly decreased compared to sham-transplanted mice. The decrease suggests donor cells are metabolically active and that significant metabolic correction has been initiated. Transplantation into younger mice may result in disease stabilization at lower levels, and be a more effective treatment.

C3 carnitine levels in MMA mice were significantly corrected by treatment with a double dose of 5 million cells, showing that the metabolic pathway is functional with reduced substrate accumulation. C3 carnitine levels in these mice were maintained over the post-transplant time period, where sham-transplanted controls showed increasing levels over the same period. This suggests that donor cells were able to maintain C3 carnitine levels, but not reduce the levels below pre-transplant sample levels. Whole liver transplantation of MMA patients has been shown to stabilize C3 carnitine levels; however, there may be a threshold for blood metabolite clearance by the donor liver [3].

Whilst weight and urine methylmalonic acid concentration were investigated, the transplanted MMA mice were not shown to be significantly different from sham transplanted controls, suggesting that transplantation did not effect metabolic correction enough to show total body metabolic recovery. Alternatively the kidney may be producing a higher level of MMA which is spilling over into the urine. Correction of weight would be best observed by allowing longer periods following transplantation, thus gradual increases in weight could be observed. Furthermore whilst mice are indistinguishable size wise at birth, by 3 weeks of age there is an easily detectable difference. Transplanting the mice at birth prior to the increase in metabolite levels would also aid in the treatment and provide a better opportunity to prevent the already established size difference.

Future studies should ensure the use of sub-lethal irradiation and cell dosage of at least 5 million fetal progenitor cells to maximize engraftment in recipient tissues. Another method to maximize donor cell engraftment of the liver may be considered, such as partial hepatectomy, in order to increase disease correction. Alternatively, future studies may utilize *ex vivo* gene therapy techniques (either viral or non-viral) to increase the level of methylmalonyl-CoA mutase expression prior to transplantation, in order to increase the potential of engrafted cells to correct disease.

This study concludes that transplantation of a double dose of 5 million fetal progenitor cells (with pre-transplantation sub-lethal irradiation) is effective in attaining donor cell engraftment in the liver, BM and spleen. Reduced plasma methylmalonic acid and C3 carnitine levels were demonstrated, showing the donor cells were active and able to correct the metabolites to some degree in the MMA mouse model.

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References

[1] F. Deodato, S. Boenzi, F.M. Santorelli, C. Dionisi-Vici, Methylmalonic and propionic aciduria, Am. J. Med. Genet. 142 (2006) 104–112.
[2] S. Nagarajan, G.M. Enns, M.T. Millan, S. Winter, M.M. Sarwal, Management of methylmalonic acidaemia by combined liver-kidney transplantation, J. Inherit. Metab. Dis. 28 (2005) 517–524.
[3] P.W. Chen, W.L. Hwu, M.C. Ho, N.C. Lee, Y.H. Chien, Y.H. Ni, P.H. Lee, Stabilization of blood methylmalonic acid level in methylmalonic acidemia after liver transplantation, Pediatr. Transplant. 14 (2010) 337–341.
[4] M. Kasahara, R. Horikawa, M. Tagawa, S. Uemoto, S. Yokoyama, Y. Shibata, T. Kawano, T. Kuroda, T. Honna, K. Tanaka, M. Saeki, Current role of liver transplantation for methylmalonic acidemia: a review of the literature, Pediatr. Transplant. 10 (2006) 943–947.
[5] P.J. Mc Guire, E. Lim-Melia, G.A. Diaz, K. Raymond, A. Larkin, M.P. Wasserstein, C. Sansaricq, Combined liver-kidney transplant for the management of methylmalonic aciduria: a case report and review of the literature, Mol. Genet. Metab. 93 (2008) 22–29.
[6] J.V. Leonard, J.H. Walter, P.J. McKiernan, The management of organic acidaemias: the role of transplantation, J. Inherit. Metab. Dis. 24 (2001) 309–311.
[7] W.G. van't Hoff, M. Dixon, J. Taylor, P. Mistry, K. Rolles, L. Rees, J.V. Leonard, Combined liver-kidney transplantation in methylmalonic acidemia, J. Pediatr. 132 (1998) 1043–1044.
[8] P. Kaplan, C. Ficicioglu, A.T. Mazur, M.J. Palmieri, G.T. Berry, Liver transplantation is not curative for methylmalonic acidopathy caused by methylmalonyl-CoA mutase deficiency, Mol. Genet. Metab. 88 (2006) 322–326.
[9] A. Chakrapani, P. Sivakumar, P.J. McKiernan, J.V. Leonard, Metabolic stroke in methylmalonic acidemia five years after liver transplantation, J. Pediatr. 140 (2002) 261–263.
[10] W.L. Nyhan, J.J. Gargus, K. Boyle, R. Selby, R. Koch, Progressive neurologic disability in methylmalonic acidemia despite transplantation of the liver, Eur. J. Pediatr. 161 (2002) 377–379.
[11] K.J. Allen, N.E. Buck, R. Williamson, Stem cells for the treatment of liver disease, Transpl. Immunol. 15 (2005) 99–112.
[12] Y. Sun, D. Xiao, R.S. Zhang, G.H. Cui, X.H. Wang, X.G. Chen, Formation of human hepatocyte-like cells with different cellular phenotypes by human umbilical cord blood-derived cells in the human-rat chimeras, Biochem. Biophys. Res. Commun. 357 (2007) 1160–1165.
[13] X.P. Tang, M. Zhang, X. Yang, L.M. Chen, Y. Zeng, Differentiation of human umbilical cord blood stem cells into hepatocytes in vivo and in vitro, World J. Gastroenterol. 12 (2006) 4014–4019.
[14] M.H. Walkup, D.A. Gerber, Hepatic stem cells: in search of, Stem Cells 24 (2006) 1833–1840.
[15] H. Pschera, Stem cell therapy in utero, J. Perinat. Med. 28 (2000) 346–354.
[16] J.L. Touraine, Transplantation of fetal liver stem cells into patients and into human fetuses, with induction of immunologic tolerance, Transplant. Proc. 25 (1993) 1012–1013.
[17] J.L. Touraine, M.G. Roncarolo, R. Bacchetta, D. Raudrant, A. Rebaud, S. Laplace, P. Cesbron, L. Gebuhrer, M.T. Zabot, F. Touraine, et al., Fetal liver transplantation: biology and clinical results, Bone Marrow Transplant. 11 (Suppl. 1) (1993) 119–122.
[18] C.M. Habibullah, I.H. Syed, A. Qamar, Z. Taher-Uz, Human fetal hepatocyte transplantation in patients with fulminant hepatic failure, Transplantation 58 (1994) 951–952.
[19] H.C. Fiegel, C. Lange, U. Kneser, W. Lambrecht, A.R. Zander, X. Rogiers, D. Kluth, Fetal and adult liver stem cells for liver regeneration and tissue engineering, J. Cell. Mol. Med. 10 (2006) 577–587.
[20] H. Peters, M. Nefedov, J. Sarsero, J. Pitt, K.J. Fowler, S. Gazeas, S.G. Kahler, P.A. Ioannou, A knock-out mouse model for methylmalonic aciduria resulting in neonatal lethality, J. Biol. Chem. 278 (2003) 52909–52913.
[21] H.L. Peters, J.J. Pitt, L.R. Wood, N.J. Hamilton, J.P. Sarsero, N.E. Buck, Mouse models for methylmalonic aciduria, PLoS ONE 7 (2012) e40609.
[22] J.P. Sarsero, T.P. Holloway, L. Li, S. McLenachan, K.J. Fowler, I. Bertoncello, L. Voullaire, S. Gazeas, P.A. Ioannou, Evaluation of an FRDA-EGFP genomic reporter assay in transgenic mice, Mamm. Genome 16 (2005) 228–241.
[23] M.S. Rashed, M.P. Bucknall, D. Little, A. Awad, M. Jacob, M. Alamoudi, M. Alwattar, P.T. Ozand, Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles, Clin. Chem. 43 (1997) 1129–1141.
[24] N.E. Buck, D.M. Cheah, N.J. Elwood, P.F. Wright, K.J. Allen, Correction of copper metabolism is not sustained long term in Wilson's disease mice post bone marrow transplantation, Hepatol. Int. 2 (2008) 72–79.
[25] D.E. Harrison, C.M. Astle, C. Lerner, Ultimate erythropoietic repopulating abilities of fetal, young adult, and old adult cells compared using repeated irradiation, J. Exp. Med. 160 (1984) 759–771.

[26] T. Kinoshita, T. Sekiguchi, M.j. Xu, Y. Ito, A. Kamiya, K.i. Tsuji, T. Nakahata, A. Miyajima, Hepatic differentiation induced by oncostatin M attenuates fetal liver hematopoiesis, Proc. Natl. Acad. Sci. USA 96 (1999) 7265–7270.
[27] D.A. Shafritz, M. Oertel, A. Menthena, D. Nierhoff, M.D. Dabeva, Liver stem cells and prospects for liver reconstitution by transplanted cells, Hepatology 43 (2006) 89–98.
[28] R.S. Cotran, V. Kumar, T. Collins, Robbins Pathologic Basis of Disease, W. B. Saunders Company, USA, 1999.
[29] K.J. Allen, D.M. Cheah, X.L. Lee, N.E. Pettigrew-Buck, J. Vadolas, J.F. Mercer, P.A. Ioannou, R. Williamson, The potential of bone marrow stem cells to correct liver dysfunction in a mouse model of Wilson's disease, Cell Transplant. 13 (2004) 765–773.