Gene Therapy (2000) 7, 1969–1970

www.nature.com/gt

Editorial

'Mommy, why can't I have a hamburger like the other kids?'

The family of a child with phenylketonuria (PKU) is forced to grapple with this question every day. The scientific answer, all too advanced for a young child to comprehend, is that individuals with recessively inherited deficiency of the liver enzyme phenylalanine hydroxylase (PAH) cannot metabolize the phenylalanine present in dietary protein. Dietary protein intake must be severely restricted to reduce phenylalanine accumulation in the body and to prevent the PKU-associated phenotype of growth failure, microcephaly, seizures and mental retardation. Such an extreme protein restriction can only be accomplished through the use of synthetic medical foods devoid of phenylalanine to supply the energy and amino acids that are required for the maintenance of normal growth and development. Fortunately, newborn screening for hyperphenylalaninemia, instituted in parts of the USA almost 40 years ago and practised now throughout the Western world, has provided the opportunity to diagnose PKU in the neonate and to initiate dietary therapy before the onset of severe and irreversible clinical manifestations. When counseling the family of a newborn with PKU, I can provide a reassuring prognosis for the health and development of their baby but in the same breath must consign their child to the lifelong consumption of an unpalatable, rigid, and complicated diet. The necessity of lifelong dietary therapy for PKU has recently been affirmed in the USA through a consensus statement issued by the National Institutes of Health (available for download at http://consensus.nih.gov/). Despite the general effectiveness of contemporary dietary therapy, nonadherence to the diet is common, particularly in adolescence; adults with PKU off diet are at risk for new onset neurological deficits, and maternal hyperphenylalaninemia during pregnancy very frequently causes microcephaly, mental retardation, and structural birth defects in the fetus (the so-called maternal PKU syndrome). A permanent cure that would eliminate sole dependence upon dietary therapy is the fervent dream of every individual with PKU, their families, and health care providers. For this reason, gene therapy is an attractive novel approach to the treatment of PKU.

Phenylalanine hydroxylase is an iron-containing cytoplasmic homotetramer expressed in liver that catalyzes the irreversible hydroxylation of phenylalanine to tyrosine. This reaction requires the presence of molecular oxygen and (6*R*)-L-*erythro*-5,6,7,8-tetrahydrobiopterin (BH_4) as cofactors. Restoration of liver PAH activity would cure PKU, so liver is the natural target for gene therapy of PKU. However, difficulties with producing stable, physiologically significant levels of gene expression in liver using contemporary gene transfer methods as well as safety concerns regarding the *in vivo* use of recombinant viral vectors have led my laboratory and others to consider targeting tissues other than liver for gene therapy of metabolic disorders such as PKU. The disease-associated pathology of PKU is caused by toxic effects of circulating phenylalanine and not by local effects of PAH deficiency in the liver. Therefore, any method that effectively removes phenylalanine from the body can favorably influence the PKU phenotype. The central hypothesis of heterologous gene therapy for PKU is that a tissue other than liver can be metabolically engineered to metabolize circulating phenylalanine. In this issue of *Gene Therapy*, Christensen and coworkers[1] report on the development of cultured primary keratinocytes as a metabolic sink for phenylalanine and propose the use of PAH-expressing and BH_4-producing skin grafts as therapy for PKU.

The requirement for BH_4 in the PAH reaction is a major obstacle for the success of heterologous gene therapy of PKU. The synthesis of BH_4 from GTP is catalyzed by a series of three enzymes; the first reaction catalyzed by GTP cyclohydrolase I (GTP-CH) is the rate limiting step in BH_4 synthesis. GTP-CH activity and BH_4 production are abundant in liver and several other tissues, but not in primary keratinocytes. The authors have addressed this limitation by coinfecting cultured human primary keratinocytes with two independent recombinant retroviral vectors expressing PAH and GTP-CH. Although BH_4 production from these cells was not directly assessed (other investigators have previously demonstrated BH_4 production in fibroblasts following infection with GTP-CH encoding recombinant retrovirus),[2] keratinocyte cultures expressing both PAH and GTP-CH were capable of metabolizing phenylalanine. This was demonstrated by the time-dependent decrease in the concentration of phenylalanine in the culture medium and more convincingly by the production of radiolabeled tyrosine from [U-C^{14}]-phenylalanine. Coexpression of PAH and GTP-CH within the same cells was not required for this effect, suggesting that BH_4 synthesized in GTP-CH expressing cells could penetrate adjacent PAH positive cells and support phenylalanine hydroxylation. Metabolic engineering of skin to metabolize phenylalanine is a potentially viable approach to the treatment of PKU.

Two potential limitations of skin-based gene therapy for PKU must be further addressed before it will be clinically applicable. First, the phenylalanine clearance rate of metabolically engineered keratinocytes must be increased

to higher physiologically relevant levels. Based upon a calculated phenylalanine clearance rate of 370 nmol phenylalanine per 24 h per 10^6 cells, the authors estimate that a 20×20 cm epidermal graft containing 2×10^9 PAH and GTP-CH expressing cells could remove about 0.74 mmol phenylalanine per day from the circulation. This compares with a phenylalanine tolerance of 1.2–3.0 mmol/24 h in the typical adult individual with PKU, so a graft of this size would provide only a modest increase in the patient's phenylalanine tolerance and would not eliminate the need for a protein-restricted diet. Second, even if cellular phenylalanine clearance rates can be improved, the rate of phenylalanine flux from the circulation into the skin graft might further limit the rate of phenylalanine clearance from the body. Evaluation of this latter concern and indeed of the overall efficacy of skin-based gene therapy for PKU must await application of this approach to a whole organism, such as the hyperphenylalaninemic *Pah*^{enu2} mouse model.

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