SHORT COMMUNICATION

EX VIVO GENE THERAPY OF SEVERE INFANTILE HYPOPHOSPHATASIA MODEL MICE USING LENTIVIRAL TRANSDUCED BONE MARROW CELLS

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Hypophosphatasia (HPP), caused by a genetic defect of tissue-nonspecific alkaline phosphatase (TNALP), is characterized by hypomineralization of systemic bones and teeth, epileptic seizure and respiratory insufficiency. The clinical severity of HPP varies widely; the most severe to the mildest forms are prenatal, infantile, childhood, adult, and odonto-HPP. Clinical trials of enzyme replacement therapy (ERT) for HPP using a bone-targeted TNALP in which deca-aspartate sequence is linked to the C terminus of soluble TNALP (TNALP-D10) have been initiated. However, ERT requires repeated administration of large amounts of TNALP-D10 for long-term correction. Recently, we reported that HPP model mice ($Akp2^{-/-}$ mice), phenotypically mimic to severe infantile HPP, could be treated by a single systemic injection of either lentiviral vector or AAV vector expressing TNALP-D10. We found that a single injection of the vector is sufficient for continuous secretion of TNALP-D10 from transduced systemic organs to the circulation and partial localization of TNALP-D10 in the bone tissues. In this study, instead of in vivo direct injection of viral vector, we examined the feasibility of ex vivo gene therapy using genetically modified bone marrow cells (BMC). BMC were extracted from wild type ($Akp2^{+/+}$) mice and were incubated with lentiviral vector expressing TNALP-D10 for 20 hrs at an moi of 50 with mSCF, mIL3 and rhIL6. Transduced BMC (1 x 10^6 cells) were transplanted through the jugular vein into sub-lethally irradiated (4Gy) neonatal $Akp2^{-/-}$ mice. The treated mice survived for more than 3 months with normal physical activity and healthy appearance, although the untreated mice died by 20 days due to severe skeletal hypomineralization and epileptic seizure. Transplantation of non-transduced BMC resulted in only limited prolongation of survival. The ALP activities in plasma of treated mice were kept high levels (more than tenfold compared to $Akp2^{+/+}$ mice), and X-ray evaluation showed that mineralization in bones was markedly improved by the treatment. These results suggest that lentiviral transduced BMC can serve as a reservoir for continuous supply of TNALP-D10. The neonatal ex vivo gene therapy using BMC would be an effective and practical approach to treat severe infantile HPP.