ENGINEERING OF PROBIOTIC E. COLI TO TREAT METABOLIC DISORDER

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The fields of synthetic biology and microbiome research developed greatly over the last decade. The convergence of these two disciplines is now enabling the development of new therapeutic strategies, using engineered microbes that operate from within the gut as living medicines. Inborn errors of metabolism represent candidate diseases for these therapeutics, particularly those disorders where a toxic metabolite causing a syndrome is also present in the intestinal lumen. Phenylketonuria (PKU), a rare inherited disease caused by a defect in phenylalanine hydroxylase (PAH) activity, is one such disease and is characterized by the accumulation of systemic phenylalanine (Phe) that can lead to severe neurological deficits unless patients are placed on a strict low-Phe diet. As an alternative treatment, Escherichia coli Nissle (EcN), a well-characterized probiotic, was genetically modified to efficiently import and degrade Phe (SYN-PKU). The coupled expression of a Phe transporter with a Phe ammonia lyase (PAL) allows rapid conversion of Phe into trans-cinnamic acid (TCA) in vitro, which is then further metabolized by the host to hippuric acid (HA) and excreted in the urine. Experiments conducted in the enu2−/−PKU mouse model showed that the oral administration of SYN-PKU is able to significantly reduce blood Phe levels triggered by subcutaneous Phe injection. Decreases in circulating Phe levels were associated with proportional increases in urinary HA, confirming that Phe metabolism was caused by the engineered pathway in SYN-PKU. Subsequent studies have shown that SYN-PKU is similarly operative in a non-human primate model, providing a translational link to inform future human clinical studies. In addition to SYN-PKU, a second EcN strain was genetically engineered to rapidly import and degrade branched-chain amino acids (BCAAs) for the treatment of maple syrup urine disease (SYN-MSUD). MSUD, similar to PKU, is a rare genetic disorder caused by a defect in branched-chain ketoacid dehydrogenase activity leading to the toxic accumulation of BCAAs, particularly leucine, and their ketoacid derivatives. The controlled expression in SYN-MSUD of two BCAA transporters, a leucine dehydrogenase, a ketoacid decarboxylase and an alcohol dehydrogenase, result in the efficient degradation of BCAAs into branched-chain alcohols. In a mouse model of MSUD, the oral delivery of SYN-MSUD suppressed the increase in blood BCAAs level induced by a high-protein challenge and prevented the associated moribund phenotype, as measured by locomotor activity. In conclusion, the therapeutic effects observed with SYN-PKU and SYN-MSUD in pre-clinical studies support the further evaluation of engineered microbes as promising approaches for serious inborn errors of metabolism.