A mammalian cell model for classic galactosemia using RNA interference (RNAi) as a setup tool

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Classic galactosemia is an inborn error of metabolism that is inherited in a recessive pattern (1). A defect in any of the enzymes in the Leloir pathway can cause galactosemia but the usually severe defect resultant from galactose-1-phosphate uridilyltransferase (GALT) malfunction is referred to as classic galactosemia. The symptoms of transferase galactosemia are usually attributed to the toxic accumulation of galactose-1-phosphate and galactitol in patient tissues (2). The lack of a human cellular model for galactosemia hinders direct investigation of the pathobiology of this disorder. The critical role of development of a human model for simulation of galactosemia was confirmed by the lack of complications reminiscent of galactosemia when GALT-knockout mice were used as models for galactosemia (3). In classic galactosemia, the enzyme activity of the GALT protein has been used to evaluate the molecular defects on the GALT gene (4). Thus, the incapacity of the GALT gene to produce residual RNA and consequently functional protein due to RNAi can be evaluated by quantifying the respective enzyme activity (5). Evaluation of effective RNAi usually involves quantification of the respective RNA or protein product of the targeted gene. The methods employed in this study concern the standardisation of different biochemical assays to aid in understanding the specific effects of RNAi on the targeted GALT gene causative of classic galactosemia. Three different analyses were performed, firstly, the determination of the neomycin lethal dose on the three different test cell types, with a view of using the neomycin-resistant gene as a marker for RNAi transfection. Secondly, the GALT gene enzyme product was assayed to evaluate its activity for comparison with the transfected counterpart in order to determine the efficiency of RNAi. Finally, the metabolites of the Leloir pathway were quantified to verify the validity of the anticipated metabolic block.

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