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17β-Hydroxysteroid dehydrogenase type 7 (Hsd17b7) reverts cholesterol auxotrophy in NS0 cells

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Abstract

NS0 is a host cell line widely used for the production of recombinant therapeutic proteins. In this work, we investigated the cholesterol-dependent phenotype of NS0 cells. Growth response to different precursors and comparative transcript analyses pointed to deficiency of 17β-hydroxysteroid dehydrogenase type 7 (*Hsd17b7*) in NS0 cells. *Hsd17b7* was previously shown to encode for an enzyme involved in estrogenic steroid biosynthesis. Its recent cloning into a yeast mutant deficient in ERG27 led to its functional characterization as the 3-ketoreductase of the cholesterol biosynthesis pathway. To ascertain that its cholesterol biosynthesis is blocked at the reduction reaction catalyzed by Hsd17b7, we genetically engineered NS0 cells to over express Hsd17b7. The stable transfectants of Hsd17b7 were able to grow independent of cholesterol. The results affirm the role of Hsd17b7 in the cholesterol biosynthesis pathway in mammals. Further, the findings allow for rational engineering of this industrially important cell line to alleviate their cholesterol dependence.

Keywords: Cholesterol biosynthesis; 17β-Hydroxysteroid dehydrogenase type 7; Hsd17b7; NS0; 3-Ketoreductase; Mouse myeloma; Cholesterol auxotrophy; Cell engineering; Mammalian cell culture; Gene expression

1. Introduction

NS0 is one of the primary host cell lines used for the production of recombinant therapeutics (Chu and Robinson, 2001; Zhou et al., 1997). Human therapeutics produced in NS0 cells include Zenapax™, Remicade™ and Synagis™. It is a murine myeloma cell line that was established from mineral oil induced plasmacytomas in 1960s (Horibata and Harris, 1970, Barnes, 2000). NS0 cells require an exogenous supply of cholesterol for their survival and growth. Due to its low solubility, cholesterol is often supplied as a conjugate with serum albumin, or as complexes with cyclodextrin (Keen and Steward, 1995; Ohmori, 1988).

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In pursuing chemically defined protein-free culture conditions, it is highly desirable to eliminate cholesterol from the culture medium to simplify its formulation as well as to facilitate downstream purification of the expressed product (Sinacore et al., 2000; Gorfien et al., 2000). There have been published reports demonstrating slow adaptation of NS0 cells to cholesterol-free (Keen and Steward, 1995) and protein-free culture conditions (Gorfien et al., 2000).

Cholesterol fulfills many key physiological functions in higher organisms. Biological manifestation of cholesterol, its metabolites and biosynthetic intermediates span various cellular and developmental processes, ranging from cellular membrane transport, intracellular signaling, stress response, cancer and reproductive biology (Simons and Toomre, 2000; Liscum and Munn, 1999; Tabas, 2002). The synthesis of cholesterol begins with condensation of three carbon units of acetyl-CoA by 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase (HMGCS) to form HMG-CoA as shown in Fig. 1 (Step 1). HMG-CoA is converted to mevalonate by HMG-CoA reductase (HMGCR). Mevalonate is further metabolized to farnesyldiphosphate by a series of peroxisomal enzymes. These earlier steps in the cholesterol biosynthetic pathway that lead to the formation of isoprene units are shared for the biosynthesis of many other compounds, such as heme A, dolichol, isopentenyl-tRNA, etc. The first committed step towards the synthesis of cholesterol occurs in the endoplasmic reticulum and is marked by the formation of squalene (Fig. 1, Step 7). Squalene epoxidase and oxidosqualene cyclase convert linear squalene into cyclic lanosterol. Lanosterol contains a four-ring nucleus characteristic of the steroids, but with three additional methyl groups: 4α, 4β and 14α. The removal of those three methyl groups via oxidative demethylation, combined with destauration and isomerisation reactions, converts lanosterol to lathosterol and finally to cholesterol in mammalian cells.

Conversion of lanosterol to lathosterol (Steps 10–19) is a complex pathway in itself. First, the 14α methyl group is removed from lanosterol through an oxidative demethylation reaction catalyzed by lanosterol 14α-demethylase (Cyp51). Subsequent reduction of the C14 double bond by steroid-14-reductase (Tm7sf2) allows for the removal of the second methyl group (4α). Oxidation at C4 by 4 methyl sterol oxidase is followed by oxidative decarboxylation by C3 sterol dehydrogenase (also known as C4 decarboxylase, Sc4mol). Removal of the last methyl group at C4 is catalyzed by the same set of enzymes responsible for removing the 4α methyl group (4 methyl sterol oxidase and C3 sterol dehydrogenase) (Risley, 2002; Gaylor, 2002), but not until the 3-keto group has been reduced to a hydroxyl group by 3-ketoreductase (Hsd17b7, Step 14). As the last methyl group is removed, the 3-hydroxyl group is again reduced to a keto group. Reduction of this 3-keto group to the β-hydroxy sterol by the same 3-ketoreductase (Step 17) completes the conversion of lanosterol to zymosterol.

Reactions downstream of zymosterol that convert it to cholesterol proceed via two alternative pathways (Fig. 1). The two pathways differ mainly in the point at which Δ24 is reduced. One pathway (Steps 18–21) involves reduction of the Δ24 double bond in zymosterol first, followed by Δ8, Δ7 isomerisation to give lathosterol and then cholesterol. The alternative pathway (Steps 18a–21a) involves isomerization and desaturation of zymosterol first to give 7-dehydrodesmosterol which is further metabolized to cholesterol. Given the presence of both lathosterol and desmosterol in mammals, the exact sequence of steps downstream of zymosterol have been speculated to be tissue specific (Liscum, 2002).

Cloning of genes encoding enzymes in cholesterol biosynthesis has greatly facilitated elucidation of the pathway as well as its regulation (Bach and Benveniste, 1997). 17β-Hydroxysteroid dehydrogenase type 7 (*Hsd17b7*) was the last gene of the mammalian cholesterol biosynthesis pathway to be cloned and characterized. Hsd17b7 had originally been described as an estrogenic hydroxysteroid dehydrogenase regulating the biological potency of steroids in mammals. It was not until recently that its role as the 3-ketoreductase of the cholesterol biosynthesis pathway was reported via functional complementation of the mammalian gene in a yeast strain deficient of 3-ketoreductase in its ergosterol biosynthetic pathway (Marijanovic et al., 2003). In this study, we demonstrate the role of *Hsd17b7* in the inherently cholesterol auxotrophic mammalian cell line, NS0. Identification of the metabolic block leading to cholesterol auxotrophy in NS0 cells and its alleviation by over expression of *Hsd17b7* is described in this report.