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Review

Developmental processes regulated by the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) pathway: Highlights from animal studies

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ABSTRACT

The 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) is the rate-limiting enzyme in the biosynthesis of cholesterol and isoprenoids, which are substrates required for post-translational modification of signalling proteins that can potentially regulate various aspects of embryonic development. The HMGCR transcripts are detectable during early embryogenesis in both invertebrates and vertebrates, which suggests a conserved developmental requirement for mevalonate derivatives. Consistently, recent animal and *in vitro* studies have yielded valuable insights into potential morphogenic parameters that are modulated by HMGCR activity. These developmental end-points include brain and craniofacial morphogenesis, PGC migration and survival, myocardial epithelial migration and fusion, EC migration and survival, and vascular stabilization. By providing a synthesis of these studies, we hope that this review will highlight the need to comprehensively examine the entire suite of developmental processes regulated by HMGCR.

Contents

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1. Introduction

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR; E.C. 1.1.1.88) is an endoplasmic reticulum-bound, cytoplasmic protein [1]. Although HMGCR transcripts are highly enriched in liver cells (hepatocytes) [2,3], where cholesterol is converted into bile salts and where lipoproteins involved in transporting cholesterol are synthesized and exported [4,5], the transcripts are also detected in extra-hepatic tissues, including the heart [6], blood vessels [7], and the brain [8,9]. HMGCR converts HMG-CoA to mevalonate, the key precursor for the synthesis of cholesterol and isoprenoids. These mevalonate-derived metabolites coordinate diverse cellular and molecular processes, with various developmental and pathological implications. As a result of the deterministic effects of HMGCR pathway on endogenous synthesis of cholesterol, feedback-mediated regulation of enzyme activity is exploited by pharmaceuticals (statins) that selectively bind part of the C-terminal domain of the enzyme and render it inactive [10]. Statins bind specific residues on the active site of the enzyme [10] and efficiently inhibit *de novo* cholesterol synthesis, in addition to lowering plasma LDL cholesterol levels [11] (Fig. 1). The high efficacy of statin use in the treatment of hypercholesterolemia has been extensively demonstrated through the use of animal models and based upon the spectrum of clinical studies [12–14].

However, the potential developmental implications of impaired HMGCR activity have hitherto not been fully addressed in a comprehensive review. A major metabolite of HMGCR pathway, Cholesterol, a major metabolite of HMGCR pathway, is an essential developmental metabolite [15]. In addition to being a major constituent of cell membranes and lipid rafts [16]; cholesterol serves as a precursor for the biosynthesis of steroid hormones [17]. Statins also curtail the route of generation of other metabolites downstream of mevalonate, namely isoprenoid intermediates including geranyl- and farnesyl-related metabolites (Fig. 1). GGPP is a long hydrocarbon chain ($C_{20}H_{36}O_7P_2$) that serves as a lipid attachment for heterotrimeric G proteins and the Rho/Ras GTPase family of proteins, which harbour the CAAX plasma membrane targeting signal [18]. This post-translational modification enables the prenylated complexes to be localized to the plasma membranes, which is followed by their activation [19] (Fig. 1). In light of the diverse developmental roles of Rho GTPases, which range from cell survival [20] to cytoskeletal organization [21], it is highly conceivable that inhibiting HMGCR function could adversely affect a wide range of Rho GTPase-dependent parameters, particularly during early development. Some of these Rho-dependent developmental processes regulated by HMGCR function have only recently been uncovered in genetically tractable model organisms, including *Drosophila melanogaster* and the zebrafish (*Danio reriro*). The purpose of this review is to synthesize the information provided by these diverse animal and *in vitro* studies in order to highlight the need to more comprehensively study the potential outcomes of impaired HMGCR function on embryonic development.

2. HMGCR and cholesterol-dependent developmental processes

Cholesterol is one of the metabolites of the HMGCR pathway (Fig. 1). It has been shown that mice harbouring a deletion of squalene synthase (SQS; knockout), the first committed enzyme in the synthesis of squalene, the cholesterol precursor derived from farnesyl diphosphate and downstream of HMGCR, show developmental delay and defective neural tube closure [22]. Further lending support to this finding, a null mutation in *dhcr7*, the gene encoding 7-dehydrocholesterol reductase which catalyzes the conversion of 7-dehydrocholesterol to cholesterol, is associated with severe birth-defects, typified by mental retardation, facial dysmorphism and cleft palate, all of which are pathophysiological outcomes associated with the Smith–Lemli–Opitz syndrome (SLOS) [23]. Interestingly, the surge in the cellular 7-dehydrocholesterol levels, which is the hallmark of SLOS, exacerbates the cholesterol deficiency by accelerating the proteolysis of the HMGCR enzyme, thereby severely depriving the developing organism of mevalonate-derived metabolites [24,25].

At the molecular level, cholesterol is a major component of cell membranes, regulating membrane fluidity and permeability [16], and it is the precursor molecule of other steroids, including bile salts, steroid hormones, and vitamin D [17]. An additional developmental role for cholesterol is through its covalent modification of the morphogenic protein sonic hedgehog (SHH) specifically at the carboxyl end of the immature protein. SHH signalling plays a vital role in craniofacial development, limb and digit morphogenesis, brain development, as well as several regenerative processes [26–29]. Lending support to embryonic requirement for cholesterol, exposure of zebrafish embryos to ethanol during gastrulation reduces total cholesterol content, resulting in the inhibition of cholesterol-mediated covalent modification of SHH [30]. This is shown to induce dose-dependent teratogenicity, cyclopia, craniofacial defects, failure of the heart tube to loop and pericardial oedema [30].

More direct evidence linking the disruption of cholesterol metabolism to abnormal SHH signal transduction was recently shown in mice harbouring a mutation in *Hsd17b7* [31]. The *Hsd17b7* gene encodes an enzyme in cholesterol biosynthesis and its impaired function results in disrupted SHH signalling, defective growth/patterning of craniofacial structures, and limb defects [31]. What is more, depletion of endogenous cholesterol synthesis through pharmacological inhibition of HMGCR inhibits SHH signalling *in vitro* [32]. On balance, it is highly conceivable that modulation of HMGCR activity can concomitantly affect cholesterol-mediated covalent modification of SHH processing and the transcription of target genes, which further highlights a developmental requirement for both maternal and embryonic HMGCR.

3. HMGCR expression is detected in early development

The HMGCR transcripts are shown to be expressed in invertebrate models of developmental research, such as the sea urchin, a marine invertebrate deuterostome, and *Drosophila melanogaster*, a highly popular fly model in genetic research [33,34]. Gene expression analyses in vertebrate model organisms like the chick, zebrafish, rats, and mice also demonstrate that HMGCR transcripts are highly enriched during early stages of embryogenesis [35–38]. Consistently, HMGCR transcripts are detected in human first trimester foetal tissues [39], suggestive of a conserved requirement for HMGCR expression during early developmental processes in both invertebrates and vertebrates.

Toxicology studies in pregnant rats reported that exposure to high dose mevinolin (>60 mg/kg/day), a fungal-derived inhibitor of HMGCR, resulted in foetuses with malformed vertebrae and ribs, and the failure of the abdominal wall to close, resulting in protrusion of the stomach and intestine to the outside (e.g. gastroschisis) [40]. Experiments using rats and rabbits to assess the teratogenicity of HMGCR inhibitors, reported that at maternally toxic doses, statin treatment can negatively impact on parameters such as maternal body weight and levels of food consumption [41]. More recently, studies in rats and rabbits have shown maternal and embryonic toxicity associated with higher than physiologically relevant doses of statins [42].

In addition to the pharmacological approach, targeted disruption of the HMGCR pathway in mice was achieved through