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Regulation of cholesterol biosynthesis and cancer signaling

Andrey Gorin, **Linara Gabitova**, and **Igor Astsaturov**[†]
Program in Developmental Therapeutics, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA, 19111

Abstract

Cellular growth is highly dependent on sustained production of lipids. Sterol composition of cellular membranes determines multiple biochemical and biophysical properties of membrane-based processes including vesicle traffic, receptor signaling and assembly of protein complexes. Lipid biogenesis has become an attractive biochemical target in cancer given the high level of dependency on sterols and lipids in a cancer cell. This review summarized the current knowledge of mechanisms of interaction between the metabolism of sterols and receptor signaling.

Introduction

Emerging data from profiling of cancer tissues [1] and in vitro cell line models [2] demonstrate critical contribution of cholesterol metabolism to cancer origins and drug resistance. The early steps in the cholesterol biosynthesis provide cells with several compounds that are essential for cell growth and division, such as mevalonic acid, farnesyl pyrophosphate and geranylgeranyl pyrophosphate essential for PI3K, Akt, Ras and other GTPases signaling. Because of the marked dependency of cancer cells on isoprenylated molecules for cell growth, targeting HMG-CoA-reductase with statins and prenylation enzymes with farnesyl transferase inhibitors has been extensively explored in the laboratory but so far produced modest results in the clinic, apparently due to significant pathway redundancies [3].

The distal steps of cholesterol biosynthesis below the level of first aromatic precursor, squalene, have been relatively understudied in the context of cancer signling (Figure 1). The genetic syndromes arising from mutations in the genes of distal cholesterol biosynthesis show aberrations in sonic hedgehog signaling pathway, and now been proposed as targets for cancer therapy. The azole antifungal compounds, such as ketoconazole, block the function of several cytochrome P450 enzymes involved in the steroid hormone biosynthesis. Its activity against CYP51A1 (which catalyzes demethylation of lanosterol) and CYP17A1 (which mediates a step in the synthesis of androgens) has been utilized clinically to treat hormone refractory prostate cancer, and has recently been surpassed by abiraterone, a CYP17A1 antagonist[4]. Itraconazole via its inhibitory effects on Smoothened in the hedgehog pathway has shown activity against medulloblastoma [5]. Another unsuspected effect of itraconazole is suppression of angiogenesis via its interference with lysosomal cholesterol trafficking [6], thus offering a new clinical applications for this class of agents.

[†]To whom correspondence should be addressed: igor.astsaturov@fccc.edu, Address: Igor Astsaturov, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111., Phone (215) 728-3135, (443) 465-5212; Fax (215) 728-3616.

In this review, we attempted to explore future therapeutic opportunities based on interactions between cholesterol metabolism and signaling in cancer cells.

Cholesterol pathway in cancer biology and therapy

Active sterol biosynthesis remains an essential metabolic component of cancer, and changes in the function of this pathway are thought to contribute to treatment resistance. Analysis of microarray transcriptional profiling demonstrated that refractory cancers exhibit significant overexpression of a number of cholesterol pathway genes. As some examples, a sterol pathway transcriptional signature has been identified for refractory MUC1-positive breast cancer and NSCLC [7] associated with tamoxifen resistance and poorer survival. The functional significance and the mechanism by which cholesterol pathway genes contribute to resistance are yet unclear.

In normal cells lipogenesis processes are at low level via transcriptional regualtion of the key genes involved in lipid biosynthesis (i.e. ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid) [8]. In cotrast, in tumor cells, increased signaling activity of growth factor or steroid hormone receptors via PI3K/AKT and MAPK/ERK1/2 [9], HIF-1α, p53 [10], and SHH [11] pathways modulate and activate SREBP-1, the main regulatory component of lipogenesis. Overexpression of lipogenic enzymes was observed in a number of carcinomas [12] and was described to correlate with disease severity, increased risk of recurrence, and a lower chance of survival [13,14].

Besides increased synthesis, the level of intracellular cholesterol can be regulated by coordination of import/export machinery. The low-density lipoprotein (LDL) receptors import cholesterol from the milieu and are, in turn, regulated by the SREBP transcriptional factors [15]. The excess of cholesterol or of its oxidized products, oxysterols, activate liver X receptors (LXR) and retinoid X receptor (RXR) heterodimeric transcriptional factors. Subsequent induction of expression of ABC-family transporters results in efflux of cholesterol. LXR also suppresses LDL receptor levels via its ubquitin-mediated degradation [16]. In the clinical trials or cancer cell lines models, EGFR-driven glioblastoma tumors are dependent on lipid synthesis so that inhibition of lipogenesis shows promising activity [17]. Activation of LXR with an agonist [18] or inhibition of lipogenic enzymes [19] may be potential therapeutic avenues in these tumors.

Accelerated synthesis of lipids and sterols is also an essential mechanistic component of malignant transformation: OLR1, oxidized LDL receptor 1, is required for Src kinase transformation of immortalized MCF10A cells [2]. Besides the significant induction of OLR1 during transformation it has been shown that depletion of OLR1 by siRNA blocks morphological transformation and inhibits cell migration and invasion and results in reduction of tumor growth in vivo [2]. Conversely, overexpression of ORL1 protein in MCF10A and HCC1143 cells leads to significant upregulation of *BCL2*, a negative regulator of apoptosis [20].

Cholesterol in cellular membranes and receptor signaling

Lipid rafts are small domains (10–200 nm) in plasma membrane with specific composition and properties. Lipid rafts are enriched in cholesterol and sphingolipids and present in liquid-ordered phase that is more rigid and less liquid comparing to surrounding membrane [21]. Two major types of rafts have been distinguished: planar non-caveolar and caveolae that form tube-like invaginations of plasma membrane [22]. Rafts may act as signaling platforms to harbor various proteins including receptors and signaling molecules. The recruitment of proteins to lipid rafts is mediated through the assembly of protein complexes where caveolin-1, flotillins and other scaffolding factors attract Src family kinases and a

variety of cytoskeleton proteins to regulate signaling and endocytosis of surface molecules [23]. Later work from the Di Fiore laboratory [24] implicated endocytosis as the mechanism for cholesterol regulation of EGFR signaling and endocytic turnover. Clathrin-independent endocytosis was associated with shortened EGFR signaling output and increased degradation, while the clathrin-mediated endocytic EGFR trafficking promoted EGFR recycling.

After the initial engagement of the EGFR on the cell surface with its ligands, the receptor signaling activity, and the EGFR stability and functional availability is governed by its endocytic routes [25]. EGFR can be internalized through two major pathways: clathrin-dependent and clathrin-independent (reviewed in [26]). The dynamic choice between these two pathways is susceptible to regulation which *in vitro* can be modeled by altering the ligand concentration. Under low EGF stimulation, the EGFR is predominantly internalized to the clathrin-coated vesicles, whereas high, albeit within the range of physiological level, EGF concentration leads to equal distribution of receptor between clathrin and non-clathrin vesicles [24]. The way of internalization determines the fate of the receptor. After clathrin-dependent endocytosis EGFR mostly recycles back to the plasma membrane, whereas clathrin-independent pathway moves EGFR towards degradation. Clathrin-independent internalization can be inhibited by lipophilic cholesterol-binding agents (nystatin, filipin) which shift the EGFR internalization towards clathrin-dependent mechanism and, as a consequence, leads to decreased level of EGFR degradation [24].

Sterol composition of cellular membranes directly regulates internalization of GPI-anchored membrane proteins (e.g. folate receptor, bacterial shiga toxin and cholera toxin B) and viruses via a clathrin and dynamin-independent mechanism, clathrin-independent carriers/GPI-enriched early endosomal compartments (CLIC/GEEC) [27]. This mechanism of endocytosis appears to be highly sensitive to cholesterol depletion as spontaneous formation of invaginating tubular structures from the surface membranes is preceded by cholesterol-dependent membrane reorganization. Modeling of the lipid bilayers in cell-free systems shows changes in the membrane curvatures caused by changes in the tilt of polarized GM1 lipids when bound to cholera toxin B ligand [28]. Experiments in energy-depleted cells demonstrated that CLIC/GEEC tubular structures could spontaneously form after protein binding to the lipids due to shifts in the physical properties of membranes [29]. Subsequent polimerization of cortical actin regulated by Arp2/3 complex finishes the job of pinching off the endocytic vesicle [29].

A highly regulated sequential engagement and disengagement of specific Rab-family small GTPases with EGFR-containing vesicles specifies the unidirectional trafficking of the vesicular cargo (reviewed in [30]). Importantly, the association of Rab proteins with intracellular membranes is sensitive to even minor fluctuations in the sterol composition of the intracellular membranes [31]. Rab4, Rab7 and Rab9 are stabilized in the membrane-bound state by increased cholesterol in endosomal membranes, and defects in sterol membrane composition are associated with Niemann-Pick disease [32]. Of particular interest, Rab4 has been shown to regulate rapid recycling of αvβ3-integrins during cell adhesion and spreading [33]. Rab-coupling protein, RCP (also known as RAB11FIP1), which controls recycling of both integrins and EGFR, is a frequently amplified in breast cancer locus (8p11–12), and promotes motility, anchorage-independent growth and metastatic behavior [34].

Internalized endosomal EGFR undergoes an activation-dependent conjugation of ubiquitin to its intracellular tail, which serves as a molecular tag to direct the vesicle-attached EGFR cargo to its final destination [35]. The process of EGFR degradation in lysosomes and late endocytic vacuoles is an important regulatory mechanism of signaling [36]. We have

combined network analysis and siRNA library screening to identify genes that modulate the sensitivity of cells to EGFR inhibitors that are widely used in the clinic [37]. As a result of this screen, we first discovered that knockdown of the SC4MOL protein, best known as a component of the sterol pathway (Figure 1), potently sensitizes tumor cells to EGFR inhibitors. SC4MOL catalyzes an initial conversion step of 4,4-dimethyl-5α-cholesta-8,24-trien-3β-ol (also known as testicular meiosis activating sterol, or T-MAS) to zymosterol in the distal sterol biosynthesis pathway. Suggestively, recent comprehensive analyses of genetic interactions in yeast showed that the genes regulating ergosterol and fatty acid biosynthesis exhibit the strongest interactions with genes involved in vesicular trafficking and Golgi and vacuole endosome sorting [38].

Regulation of vesicular trafficking: possible role for sterol-binding proteins

Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORP or OSBP-like proteins) are a highly conserved eukaryotic family of lipid binding proteins. OSBPLs were originally isolated because of their ability to bind cholesterol and its oxysterol derivatives. Recently, OSBP and ORPs have been shown to mediate a number of cellular processes including signal transduction [39], lipid metabolism [40], vesicular trafficking and non-vesicular sterol transport [41]. A direct role for OSBP in signaling was demonstrated in the regulation of ERK1/2 dephosphorylation [39] through direct physical interaction with PP2A and HePTP phosphatases. OSBP family members contain a core OSBP-related domain, and many also contain an N-terminal pleckstrin homology domain, the endoplasmic-reticulum-targeting FFAT (two phenylalanines in an acidic tract) motifs, GOLD (Golgi dynamics) domains and/or ankyrin repeats. The sterol-binding (SBD) domain is composed of an arrangement of antiparallel beta-sheets forming a barrel-like sterol binding cavity capable of binding various sterol species including cholesterol and oxysterols among tested. The first 1–29 N-terminal residues form a lid that covers the tunnel opening. When SBD is sterol-bound, the lid closed conformation is stabilized and is resistant to trypsin digestion. Depleting cells of cholesterol or addition of 25-OH-cholesterol causes the OSBP to change its conformation and the complex to disassembly. Thus, OSBP functions as a sterol sensor that regulates ERK activity in response to binding sterols by functioning as a “scaffold” for the assembly of a signaling protein complex [39]. The PH domain allows for some OSBP proteins to bind phosphoinositides (PI4P) and targets it to the Golgi membranes. The OSBP family SBD resembles the sterol-binding domains of steroidogenic acute regulatory protein (StAR) transport (START) proteins (MLN64, StarD4) [42].

Cholesterol composition is critical for the function of late endosomes and multivesicular bodies. Kobuna et al. [43] generated deletion mutants of all four ORP genes in *C. elegans*. Single mutant worms with deletions in *obr-1*, *obr-2*, *obr-3*, *obr-4* were viable and fertile, while the quadruple mutants that lacked all *obr* genes exhibited embryonic lethality (11%) and slow growth during larval development (18%). These data indicated that four *C. elegans* ORP proteins act redundantly during embryonic and larval development. An RNAi modifier screen in *obr* quadruple mutants identified the ESCRT complex genes as strong genetic interactions with *obr* genes. In *obrs* mutants, or in OPR1L siRNA-treated HeLa cells, degradation of membrane proteins, such as an EGF receptor and caveolin, was delayed and the late endosomes/lysosomes were enlarged. In Drosophila, another auxotrophic organism whose genome contains only 4 OSBP proteins, OSBP and cholesterol are required for spermatogenesis. The separation of individual sperm cells in flies involves significant membrane remodelling and vesicular trafficking during which OSBP is directly interacting with FAN, an ER-specific vesicle coat VAP protein [44].

The importance for the sterol-binding genes in cancer has recently become even more apparent after an elegant biochemical identification of OSBP and OSBPL4 as targets for

several naturally occurring cytotoxic agents [45] with selective activity in multiple cancer cell lines characterized by loss of NF1, or p21/WAF1[46]. This discovery has not only provided the field with new chemical tools to study the function of sterol transporters but will also undoubtedly spur development of a completely new class of anti-cancer agents.

The detailed mechanisms by which the sterol pathway genes modulate receptor trafficking and signaling are not yet clear. However, studies in yeast show that highly conserved sterol pathway genes regulate physiological activity of cell surface receptors. In yeast, *erg3/erg6* and *erg2/erg6* double mutants demonstrate abnormal signaling by the pheromone receptor STE2, and influence STE2 endocytosis [47]. Mutations of the *hydra*/EBP sterol pathway gene in *Arabidopsis* results in grossly abnormal growth receptor signaling [48]. Furthermore, a specific association between EGFR signaling and specific methylsterols relevant to our screening results has been demonstrated in reproductive physiology, where EGFR plays an important role. The C4-methylsterols known as follicular meiosis activating sterol and testicular meiosis activating sterol (F-MAS and T-MAS) are abundant in the follicular fluid in ovaries and in testes, respectively. Their accumulation is regulated by SC4MOL/ERG25 and another functional partner, DHCR14/ERG24 (hydroxysterol delta 14-reductase). These methylsterols are important in regulating germinal vesicle breakdown, meiosis resumption by oocytes, and dispersal of cumulus cells, processes also regulated by the EGFR-MAPK pathway [49]. These data suggest that sterol metabolism and the sterol composition of cellular membranes plays an important conserved role in the functionality of growth factor receptors.

The regulatory effects of sterol metabolites on receptor signaling present an opportunity to exploit cholesterol pathway inhibitors for the treatment of human cancers. This potential should be however considered in the context of multifunctionality of the sterol pathway enzymes. For example, EBP in a complex with DHCR7 serve as a cholesterol epoxide hydrolase for toxic cholesterol-5,6-epoxide metabolites [50]. Cholestan-5α,6α-epoxy-3β-ol (αCE), cholestan-5β,6β-epoxy-3β-ol (β-CE), and 7-ketocholesterol are oxidized on the ring B of cholesterol and are the major autoxidation products of cholesterol. Cholesterol epoxides (α-CE and β-CE) are the only known substrates of ChEH, and 7-ketocholesterol is an inhibitor of ChEH. Of note, the side-chain oxysterols had no binding activity towards cholesterol epoxide hydrolase. While no information is available on the effect of naturally occurring cholesterol epoxides on Shh and other signaling pathways, loss of DHCR7 function (gene mutated in Smith-Lemli-Opitz syndrome) may mediate its inhibitory effect via these cholesterol autooxidation products or their derivatives.

Concluding remarks

Maintenance of cholesterol homeostasis is one of the fundamental requirements for growth in eukaryotic cells. This metabolic pathway underwent very little changes from yeast to humans with nearly identical principal metabolites (especially from mevalonate to zymosterol) and highly homologous enzymes. Challenges in organic chemistry of sterol derivatives and methodological limitations for their direct detection in vivo, especially in the context of sterol-protein interactions, made this field relatively less explored. Recent breakthroughs, however, with few striking examples of critical importance of the sterol derivatives in cancer biology and in signaling pathways [45] reinvigorated the field. Based on these examples, we anticipate that sterol-binding binding proteins and sterol pathway genes, especially the enzymes downstream of squalene, will emerge as novel pharmacologically amenable regulators of membrane-based cellular processes. Modulating the activity of these proteins indirectly, or through a direct modification of sterol composition of cell membranes will have a profound effect on vesicular trafficking and signaling with potential far-reaching implications for cancer biology and therapy.

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Highlights

- Cholesterol biosynthesis is a highly conserved, from yeast to humans, metabolic pathway that is the essential for cell growth
- Alterations in the cellular sterol pools have profound effects on endosomes trafficking
- And also on the signaling activity of most cellular receptor systems in cancer cells
- Specific targeting of sterol pathway enzymes or sterol binding proteins may be a powerful anticancer strategy
- Especially given the absolute linearity and non-redundancy of the pathway

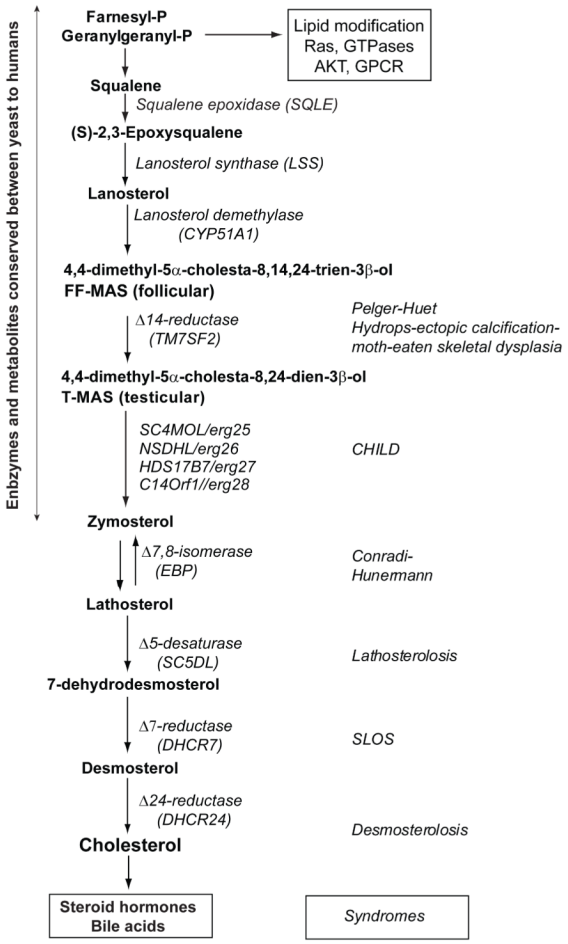

Figure 1.

Schema of the cholesterol pathway. The parallel structures arising from the activity of “tail” reductase DHCR24 on every level of the pathway are not shown for simplicity. Relevant enzymes and genetic syndromes arising from enzyme mutations are italicized. Some biological functions are shown in boxed text.

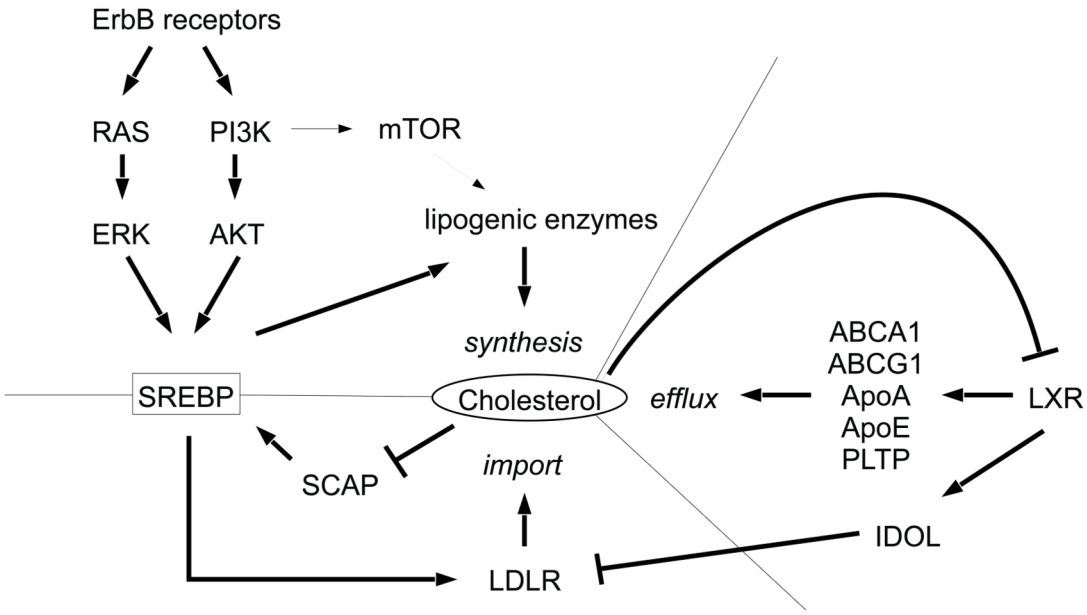

Figure 2.

Regulation of cholesterol homeostasis. Increased demands for de novo cholesterol biosynthesis is regulated via signaling inputs from cell surface growth factor receptors (e.g. ErbB family shown) which induce transcriptional activity SREBP-1 and -2. Excess of cholesterol will suppress SREBP via its inhibitory interaction with SCAP. The alternative to de novo biosynthesis is cholesterol import via LDL-receptor. LDL-R has to undergo lysosomal degradation in order to liberate cholesterol from the endocytosed lipid particles. Oxidized cholesterol species bind to LXR which is a heterodimeric transcriptional factor for various regulatory proteins including cholesterol efflux pumps, ABC-A1 and -G1. A ubiquitin ligase IDOL is involved in LXR-dependent LDL-R degradation.