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Molecular Pathways: Sterols and receptor signaling in cancer

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Abstract

Accelerated cholesterol and lipid metabolism are the hallmarks of cancer and contribute to malignant transformation due to the obligatory requirement for cholesterol for the function of eukaryotic membranes. To build new membranes and maintain active signaling, cancer cells depend on high intensity of endogenous cholesterol biosynthesis and uptake of lipid particles. This metabolic dependency of cancer cells on cholesterol and other lipids is tightly regulated by the cholesterol homeostasis network including: 1) sterol response element binding proteins (SREBP), master transcriptional regulators of cholesterol and fatty acid pathway genes; 2) nuclear sterol receptors (liver X receptors, LXR) which coordinate growth with the availability of cholesterol; 3) lipid particle receptors such as LDL receptor providing exogenous sterols and lipids to cancer cells. In addition, activity of oncogenic receptors such as MUC1 or EGFR, accelerates sterols uptake and biosynthesis. Therefore, a general strategy of reducing the cholesterol pool in cancer cell is challenged by the highly efficient feedback loops compensating for a blockade at a single point in the cholesterol homeostatic network.

Besides the well-established structural role of cholesterol in membranes, recent studies uncovered potent biological activities of certain cholesterol metabolic precursors and its oxidized derivatives, oxysterols. The former, meiosis activating sterols, exert effects on trafficking and signaling of oncogenic epidermal growth factor receptor (EGFR). Cholesterol epoxides, the highly active products of cholesterol oxidation, are being neutralized by the distal sterol pathway enzymes, EBP and DHCR7. These recently discovered “moonlighting” activities of the cholesterol pathway genes and metabolites expand our understanding of the uniquely conserved roles these sterol molecules play in the regulation of cellular proliferation and in cancer.

Background

Synthesis of cholesterol and its intermediates

Cholesterol is a crucial component of cell membranes and its homeostasis is critical for normal cell functioning (1). Cholesterol biosynthesis is highly conserved in all the eukaryotes with a minimal difference between the end-products, human cholesterol and fungal ergosterol, arising at the level of zymosterol conversion (2). A series of elongation reactions of the non-aromatic fatty acid produces farnesyl pyrophosphate, which is

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converted to squalene – the first four-ring sterol precursor in the pathway (3). The pre-squalene steps of the cholesterol pathway produce isoprenoids, farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are critical for membrane anchoring of multiple signaling oncogenic proteins such as RAS (4), phosphoinositie-3-kinase (PI3K) (5) and AKT (6). Squalene epoxidase (SQLE) and lanosterol synthase (LSS) catalyze the conversion of squalene to a relatively inert sterol, lanosterol, which is highly abundant in skin appendages such as hair (from *lanus*, Latin, wool) (3). The subsequent steps produce a series of precursors possessing various biological activities. For instance, highly biologically active C4-methylated sterols are also known as *meiosis-activating sterols* (7) for their unique role in regulating the second division of meiosis in the gonads. The final product of the pathway, cholesterol, is subjected to a series of oxidative conversions in the molecule's "tail" and the "B" ring, to produce bile acids, steroid hormones and vitamin D (8, 9). Metabolic arrest of the pre-squalene steps of cholesterol pathway during normal development is universally lethal in all eukaryotes due to the disruption of critical membrane-based signaling. Contrastingly, mutations in the distal cholesterol pathway genes are viable but produce severe developmental defects (10). Therapeutic trials of cholesterol supplementation have led to only modest improvements (11, 12), thus suggesting unique biological activities for the accumulating intermediate sterol metabolites specific for each genetic lesion.

Maintenance of high sterol levels in cancer cells

More than hundred years ago an association between lipid metabolism and tumor progression was first investigated by John Holden Webb, who suggested that cancer was due to crystallization of cholesterol from living cells (13). Since that time the involvement of lipid metabolism in tumorigenesis has been thoroughly investigated. Cholesterol composition of cellular membranes has been established as an essential metabolic requirement for cell divisions (14, 15) and it was shown that proliferating cells increase cholesterol uptake (16, 17). Cancer cells adapt to maintain high intracellular cholesterol through different mechanisms (Fig. 2) including accelerated endogenous production of cholesterol and fatty acids (18) regulated by the sterol response element binding proteins (SREBP) (19), or by reducing cholesterol efflux trough ATP-binding cassette (ABC) class A transporters such as ABCA1 reported in prostate cancer (20), or by increasing the uptake of low density lipid particles (LDL) (21). Deregulation of cholesterol homeostasis can be a powerful tool to suppress cancer growth and signaling of oncogenic receptors such as epidermal growth factor receptor (EGFR) family (16, 22, 23). Cholesterol promotes cancer signaling, in part, due to the assembly of cholesterol-rich membrane microdomains called lipid rafts (24) (Fig. 1). The overall dependency of rapidly dividing cells on lipid and sterol metabolism makes deregulation of these metabolic pathways a promising anti-cancer target. In this review, we will focus on the recent discoveries in the function of sterol metabolites and cholesterol pathway enzymes in cancer.

The endocytic matrix

As a major component of lipid rafts, cholesterol is the main critical component of lipid rafts activating multiple membrane-bound receptor signaling pathways (1, 24) (Fig. 1). Lipid rafts regulate endocytosis and catalytic activities of surface receptors, including signaling

duration, receptor recycling and receptor stability (1). Any essential biological process involving non-diffusible protein complexes is dependent on highly coordinated budding and fusion of the cellular membranes, thus forming the so-called “endocytic matrix” within the cell (25). The function of the EGFR in the context of this “matrix” has been thoroughly investigated. Endocytosis is one of the major mechanisms of attenuation of EGFR (and other receptor tyrosine kinases) signaling as it permits the removal of receptor from the cell surface and its delivery to the sites of inactivating endoplasmic reticulum-based phosphatases (26, 27). Sigismund et al. revealed that EGFR signaling might be regulated by two different ways of internalization. They found that low EGF cell stimulation causes clathrin-mediated endocytosis (CME) predominantly, which promotes mostly recycling of the EGFR to the cell surface and this, in turn, allows for prolonged CME-dependent signaling. Stimulation with high EGF level activates non-clathrin endocytosis (NCE) that sends a sizeable pool of receptors to degradation thereby protecting the cell from overstimulation. Detailed kinetic analysis of the EGFR endocytosis and signaling showed marked dependency of NCE on the membrane cholesterol contents (28).

Participation of cholesterol metabolites in endocytic trafficking of signaling molecules has been discovered in our recent studies (23, 29): depletion of the C4-demethylating enzymes (SC4MOL and NSDHL) markedly sensitizes cancer cell lines and tumor xenografts to EGFR-targeting drugs via accelerated shuttling of the internalized EGFR endosomes towards late endosomes and lysosomes for degradation. The analysis of the highly evolutionary conserved (30, 31) interactions for these sterol pathway genes demonstrated significant enrichment for multiple components of the vesicular trafficking apparatus in the cell (23). Furthermore, genetic deficiency of NSDHL in heterozygous bare patches (*Bpa*$^{1H/+}$) mice (32) consistently revealed a direct effect on EGFR signaling in keratinocytes. The skin areas expressing the mutant X-linked *Bpa*^{1H} allele (NSDHL-null areas) showed a marked downregulation of the EGFR signaling coincident with reduced proliferation (23).

The regulation of receptor endocytosis via cholesterol homeostasis has been shown for multiple receptors, and thus may represent a general mechanism: e.g. melanocortin-4 receptor (MC4R) endocytosis to maintain MC4R responsiveness to its agonist α-MSH is highly sensitive to depletion of membrane cholesterol (33).

Membrane cholesterol concentration also regulates the extracellular LDL cholesterol uptake via binding with the LDL receptor (LDLR) and its following internalization. The “classical” way of LDLR internalization is based on clathrin-dependent endocytosis. Acidification of early endocytic vesicles liberates LDL from the receptor and allows the cargo to be delivered to lysosomes where the lipoprotein particle is degraded and cholesterol is salvaged for cellular use (34) (Fig. 1). The endocytosis of LDLR is tightly regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), a serine endoproteinase which binds to the extracellular domain of LDLR and induces its internalization (35). Endocytosis of LDLR via the clathrin-mediated pathway is accelerated in cells with low intracellular sterol level. The uptake of LDL cholesterol is highly responsive to even small changes of cholesterol concentration in the ER membranes since deprivation of cholesterol leads to SREBP

cleavage-activating protein (SCAP)-mediated induction of the SREBP transcription factors activation (36), which in turn regulates LDLR transcription (37).

Conversely, increased sterol concentrations activate the sterol-sensing nuclear receptors LXRα and LXRβ (Fig. 2). Activated LXRs, as heterodimers with retinoid X receptors (RXR), generally reduce intracellular cholesterol through the expression of cholesterol efflux proteins such as ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, ADP-ribosylation factor-like 7 (ARL7) and apolipoprotein E (38). LXR also reduce uptake of the LDL cholesterol via the transcription of an E3 ubiquitin ligase, inducible degrader of LDLR (IDOL) (39). IDOL promotes internalization and degradation of LDLR in a clathrin-independent manner (34). An LXRα binding site in the proximal promoter region of the rat 7α-hydroxylase CYP7A also promotes the removal of cholesterol by increasing its conversion to bile acids (40). Under physiological conditions, oxysterols such as 22(R)-hydroxycholesterol, 24(S),25-epoxycholesterol and 24(S)-hydroxycholesterol, strongly induce LXRα and LXRβ transcriptional targets (40), but not in response to other sterols (lanosterol, desmosterol, steroid hormone precursors, testosterone, progesterone or bile acids) (41).

“Moonlighting” functions of cholesterol pathway

While cholesterol composition of the cellular membranes has been the focus of research, recent data highlight the unique biological properties of the sterol pathway metabolites. Among the signaling pathways that are critically dependent on the intact cholesterol biosynthesis, sonic hedgehog is arguably the best studied (42). Oncoprotein Smoothened (Smo), one of the principal signaling effectors of hedgehog signaling, contains a so-called sterol sensing domain capable of binding sterols and other lipid molecules. While the structural details of the sterol sensing domain interaction with individual sterol species remain unclear, certain naturally occurring products of cholesterol oxidation, oxysterols such as 20(S)-hydroxycholesterol, directly bind and activate SMO causing SMO translocation to the primary cilium and interaction with GLI (43).

Another example of sterol pathway metabolites involvement in cancer is meiosis activating sterols (MAS). MAS are the cholesterol pathway intermediaries downstream of lanosterol, and they play a unique physiological role to activate meiotic resumption in mouse oocytes *in vitro* (7). FF-MAS (4,4-dimethyl-5a-cholesta-8, 14, 24-triene-3b-ol) was extracted from human preovulatory follicular fluid and T-MAS (4,4-dimethyl-5a-cholest-8,24-diene-3b-ol) from the bull testicular tissue (44). In addition, MAS have been identified as natural ligands for liver X receptors (LXR) alpha and beta (41). We have recently reported that sterol C4-methyl oxidase-like (SC4MOL) and NADP-dependent steroid dehydrogenase-like (NSDHL), catalyzing oxidative demethylation of the MAS cholesterol precursors at C4 position, also regulate the endocytic traffic of EGFR in normal and cancer cells (23).

One step below SC4MOL and NSDHL, emopamyl binding protein (EBP) in a complex with dihydrocholesterol-7 reductase (DHCR7) catalyzes isomerization of the double-bond between C7 and C8 in the second cholesterol ring (45). Besides binding of different structural classes of ligands such as ring B oxysterols, there appears to be a new “moonlighting” function for this protein complex. As it turns out, this complex also

mediates a previously enigmatic activity of cholesterol epoxide hydrolase (46). Furthermore, the biological activities of the cholesterol epoxides regulated by EBP/DHCR7 extend far beyond the canonical cholesterol. A naturally occurring steroidal alkaloid, 5alpha-Hydroxy-6beta-[2-(1H-imidazol-4-yl)ethylamino]cholestan-3beta-ol, otherwise known as dendrogenin A, is produced in normal, but not in cancer cells, via an enzymatic conjugation of 5,6alpha-epoxy-cholesterol and histamine (47, 48). This sterol conjugate has been shown to suppress cancer cell growth and to induce differentiation *in vitro* in various tumor cell lines of different types of cancers (48). It also inhibited tumor growth in melanoma xenografts studies *in vivo* and prolonged animal survival: 40% survivors at day 50 compared with none in the control (47). The activity of another sterol synthesizing enzyme, DHCR24, has been implicated in regulation of KRAS-induced senescence via deregulation of P53 (49, 50).

Clinical-Translational Advances

Opportunities for targeting cholesterol homeostasis in cancer

Dependency of cholesterol reveals a range of therapeutic opportunities to target cholesterol homeostasis in cancer.

SREBPs and LXR are considered as potent targets, since coordinated activities of these proteins control cellular proliferation (51). Effective and orally bioavailable synthetic LXR agonists such as GW3965 may offer an opportunity to antagonize the G1 to S cell cycle progression in cancer cells via the active form of Rb and deregulation of cyclin-dependent kinases as well as induction of the negative regulator p15 (52). Anti-proliferative effect of the LXR agonist T0901317 was demonstrated to suppress β-catenin transcriptional activity by direct interaction in colon cancer HCT116 cell line *in vitro* (53). Activation of LXR *in vivo* in the azoxymethane/dextran and ApcMin/+ animal models of colon cancer resulted in blocking cells at the G1 phase of the cell cycle and activating an apoptosis program, that led to the 70% reduction in tumor number and approximately 40% in tumor size (54).

Another possibility to target cholesterol homeostasis is based on the ability of cancer cells to develop mechanisms to accumulate cholesterol. For example, prostate cancer cells showed high expression of HMG-CoA reductase (HMGCR) but failed to express the major cholesterol exporter ABCA1 (55). Statins block the *de novo* synthesis of cholesterol at its rate-limiting step (16, 56). The efficacy of this approach however is limited by toxicities arising from the impairment of mitochondrial metabolism and of small GTPases (e.g. Rho and Ras) dependent on isoprenoids for membrane anchoring (56). Moreover, cancer cells can bypass the effects of statins by unrestrained cholesterol importation via the LDLR pathway (16) and increased expression of the pathway genes via the SREBPs (57).

To overcome such redundancy of cholesterol regulation, blocking multiple compensatory targets may potentially yield synthetic lethal interactions (29). In cancer cells, cholesterol uptake and endogenous production are stimulated by the oncogenic signaling. For example, glioblastoma multiforme, an aggressive form of brain tumor, a constitutively active mutated EGFR-variant III promotes LDLR expression and tumor growth (16). Such a dependency

relationship between the EGFR pathway and the lipid biosynthesis is regulated via transcriptional activity of SREBP1 (16).

Another mTOR-dependent mechanism of lipid biosynthesis activation has been discovered recently involving lipin-1, a conserved negative regulator of nuclear translocation of SREBP1 (58). Lipin-1 is highly homologous to the *C.elegans* LPIN-1, and has been shown to regulate nuclear envelope and nuclear shape (58). Torin-1, an mTOR kinase inhibitor, has been effective in preventing lipin-1 phosphorylation by mTOR and SREBP1 nuclear translocation. Thus, mTOR kinase inhibitors may be effective in suppressing mTOR pathway-induced sterol and lipid biosynthesis in cancer. Finally, direct inhibitors of SREBP have been developed. One such compound, betulin, a natural pentacyclic triterpene, specifically inhibits SREBP proteolysis by facilitating the interaction of the SREBP cleavage activating protein (SCAP) and a negative SREBP regulator, INSIG. As the result, the cholesterol biosynthesis is inactivated both *in vitro* and *in vivo* (59).

Conclusions

In summary, high dependency of cancer cells on accelerated biogenesis and uptake of lipids and cholesterol lends itself as a therapeutic opportunity for metabolic targeting of cancer growth. Most previous attempts to target cholesterol metabolism in cancer have utilized HMGCR inhibitors, statins, or farnesyl transferase inhibitors acting at the level of non-aromatic steps of the cholesterol pathway. Multiple redundancies and feedback mechanisms highly abundant in the mammalian metabolic systems (60), often preclude successful pathway targeting at a single gene level. Instead, combinations of signaling inhibitors of the EGFR and/or mTOR pathways with a growing number of inhibitors of various regulatory molecules in the cholesterol homeostasis may prove to be the winning anti-cancer strategy. Emerging data on unprecedented biological activities of certain sterol metabolites (23, 47), and opportunities to activate LXRs (16), or direct inhibition of SREBPs (59, 61) may be fruitful strategies to win over cancer by cholesterol starvation.

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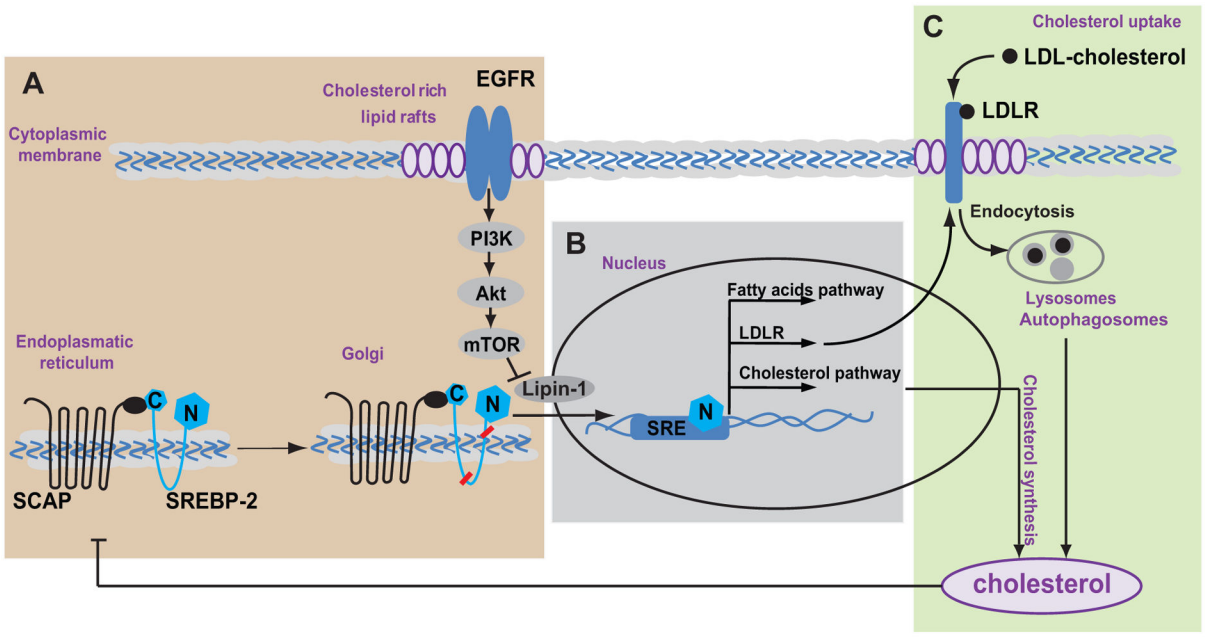

Figure 1. Cholesterol homeostasis regulation

A. Activated EGFR and mTOR signaling promotes SREBP-SCAP complex to move to the Golgi where it undergoes cleavage to form an active nuclear SREBP fragment (N, b*lue diamonds*). **B.** In the nucleus, the nuclear fragment of SREBP (N, b*lue diamonds*) binds to the sterol regulatory element DNA sequences (56) and induces expression of fatty acid and cholesterol pathway genes and the LDL receptor (LDLR). **C.** Uptake of exogenous LDL cholesterol is via its binding to LDL receptor. LDLR internalization and degradation in lysosomes and autophagosomes releases free cholesterol (34). Under the conditions of excess, cholesterol acts as a negative regulator by binding to SCAP and blocking SREBP processing.

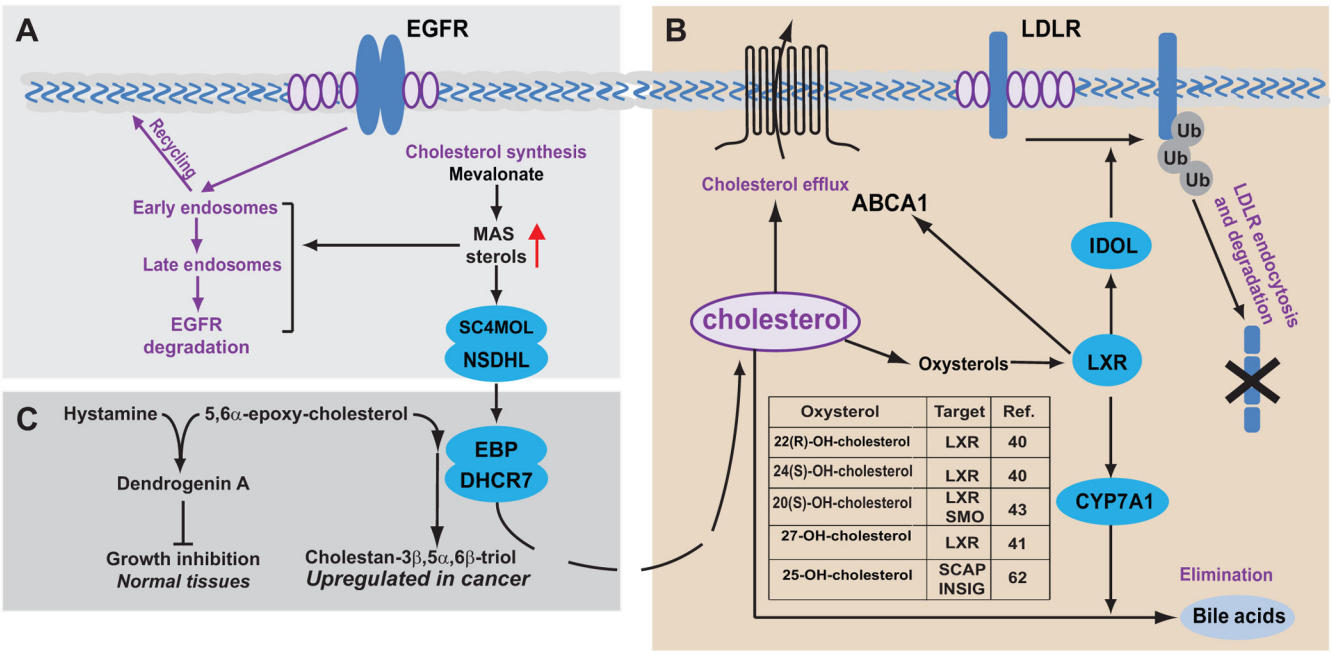

Figure 2. "Moonlighting" functions of cholesterol pathway enzymes and metabolites

A. Depletion of the C4-demethylating enzymes (SC4MOL and NSDHL) results in accumulation of meiosis activating sterols (MAS) abundantly present in gonads. MAS influences endocytosis of surface receptors (e.g. EGFR) by promoting shuttling of the internalized EGFR to late endosomes for degradation which may be important for EGFR targeting in cancer and developmental defects in the hereditary cholesterol pathway gene deficiencies (23). B. Some endogenously generated oxysterols (e.g. 22-R-hydroxycholesterol, 25-hydroxycholesterol) bind to LXR/RXR heterodimeric nuclear receptors. LXR induces expression of ABCA1 and ABCG1 efflux pumps, and cholesterol conversion to bile acids via CYP7A1 (40, 62). LXR also promotes LDL receptor degradation via increased endocytosis and ubiquitin ligation by IDOL E3 ligase (50). C. A complex of EBP and DHCR7 known as ChEH, cholesterol epoxide hydrolase, disposes of toxic cholesterol epoxides and promotes growth of tumors cells. Contrastingly, in normal tissues, cholesterol epoxides react with histamine to produce dendrogenin A which promotes cell differentiation and suppresses cell growth (18, 26, 27).