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Cholesterol metabolism in cancer: mechanisms and therapeutic opportunities

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Cholesterol metabolism produces essential membrane components as well as metabolites with a variety of biological functions. In the tumour microenvironment, cell-intrinsic and cell-extrinsic cues reprogram cholesterol metabolism and consequently promote tumourigenesis. Cholesterol-derived metabolites play complex roles in supporting cancer progression and suppressing immune responses. Preclinical and clinical studies have shown that manipulating cholesterol metabolism inhibits tumour growth, reshapes the immunological landscape and reinvigorates anti-tumour immunity. Here, we review cholesterol metabolism in cancer cells, its role in cancer progression and the mechanisms through which cholesterol metabolites affect immune cells in the tumour microenvironment. We also discuss therapeutic strategies aimed at interfering with cholesterol metabolism, and how the combination of such approaches with existing anti-cancer therapies can have synergistic effects, thus offering new therapeutic opportunities.

Cholesterol is vital for the survival and growth of mammalian cells. More than a membrane constituent, cholesterol is a precursor to bile acids and steroid hormones, which can initiate or promote colon, breast and prostate cancers[1–3]. Cholesterol can also modulate signalling pathways involved in tumourigenesis and cancer progression by covalently modifying proteins including hedgehog and smoothened[4,5], and by facilitating the formation of specialized membrane microdomains[6,7].

Brief overview of cholesterol metabolism

Every mammalian cell can synthesize cholesterol through the mevalonate pathway (Fig. 1a). Two acetyl-CoA molecules in the cytosol condense, thus forming acetoacetyl-CoA, which reacts with the third acetyl-CoA and yields 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA is reduced to mevalonate by HMG-CoA reductase (HMGCR), the primary rate-limiting enzyme in cholesterol biosynthesis. A series of enzymatic reactions convert mevalonate to farnesyl pyrophosphate (FPP), a precursor of sterols and all non-sterol isoprenoids. The condensation of two FPP molecules to squalene commits the process to sterol production. FPP also gives rise to geranylgeranyl pyrophosphate (GGPP), and both FPP and GGPP can prenylate and activate several oncogenic proteins such as Ras[8]. Squalene is then oxidized by squalene epoxidase (SQLE) to 2,3-epoxysqualene, which is cyclized to lanosterol. In the next steps, lanosterol follows the Bloch pathway, the Kandutsch-Russell pathway or a hybrid pathway before it is finally converted to cholesterol.

Beyond de novo cholesterol biosynthesis, most cells acquire cholesterol from low-density lipoprotein (LDL) taken up from the circulation via LDL receptor (LDLR)-mediated endocytosis[9]. Enterocytes absorb dietary cholesterol from the intestinal lumen in a process involving the cholesterol transporter NPC1L1, the clathrin adaptor NUMB and the adaptor protein LIMA1 (refs. [10–12]). Cholesterol within the cell is dynamically transported, reaching the destined membranes for structural and functional needs[13]. Cholesterol in excess of the current cellular demand is either exported from the cell by ATP-binding cassette (ABC) transporters, including ABCA1, ABCG1, ABCG5 and ABCG8, or converted to less toxic cholesteryl esters (CEs) by acyl-coenzyme A:cholesterol acyltransferases (ACATs; also known as SOATs) and then stored in lipid droplets or secreted within lipoproteins[14,15].

Cholesterol concentrations at both the cellular and systemic levels are subject to stringent and fine-tuned regulations. The master transcriptional regulators governing cholesterol homeostasis include sterol regulatory element–binding protein-2 (SREBP-2), liver X receptors (LXRs) and nuclear factor erythroid 2 related factor-1 (NRF1). Accumulation of cholesterol and cholesterol-derived oxysterols inactivates the SREBP-2 pathway by inducing insulin-induced gene (INSIG)-mediated retention of the SREBP-cleavage activating protein (SCAP)–SREBP-2 complex in the endoplasmic reticulum (ER), thereby downregulating cholesterol biosynthesis and uptake[16]. Meanwhile, desmosterol, the immediate precursor of cholesterol in the Bloch biosynthetic pathway, and oxysterols bind and activate LXRs, thereby enhancing the expression of genes involved in cholesterol efflux such as ATP-binding cassette subfamily A member 1 (*ABCA1*) and others such as myosin regulatory light chain interacting protein (*MYLIP*, also known as *IDOL*)[17]. High cholesterol concentrations also prevent nuclear translocation of NRF1 and block its inhibition of the LXR pathway[18]. Under conditions of cholesterol deficiency, the three regulatory pathways function in a coordinated and opposing manner, thereby ensuring an increase in cholesterol biosynthesis and uptake as well as a decline in cholesterol efflux and esterification.

Reprogrammed cholesterol metabolism in cancer cells

Hallmark features of cancer cholesterol metabolism. As fast-proliferating cells, cancer cells require high levels of cholesterol for membrane biogenesis and other functional needs. For example, the cholesterol-derived oncometabolite 6-oxo-cholestan-3β,5α-diol, which is enriched in patients with breast cancer, binds glucocorticoid receptors and subsequently promotes tumour growth[19]. In general, cholesterol metabolism substantially contributes to cancer progression, including cell proliferation, migration and invasion[20–23].

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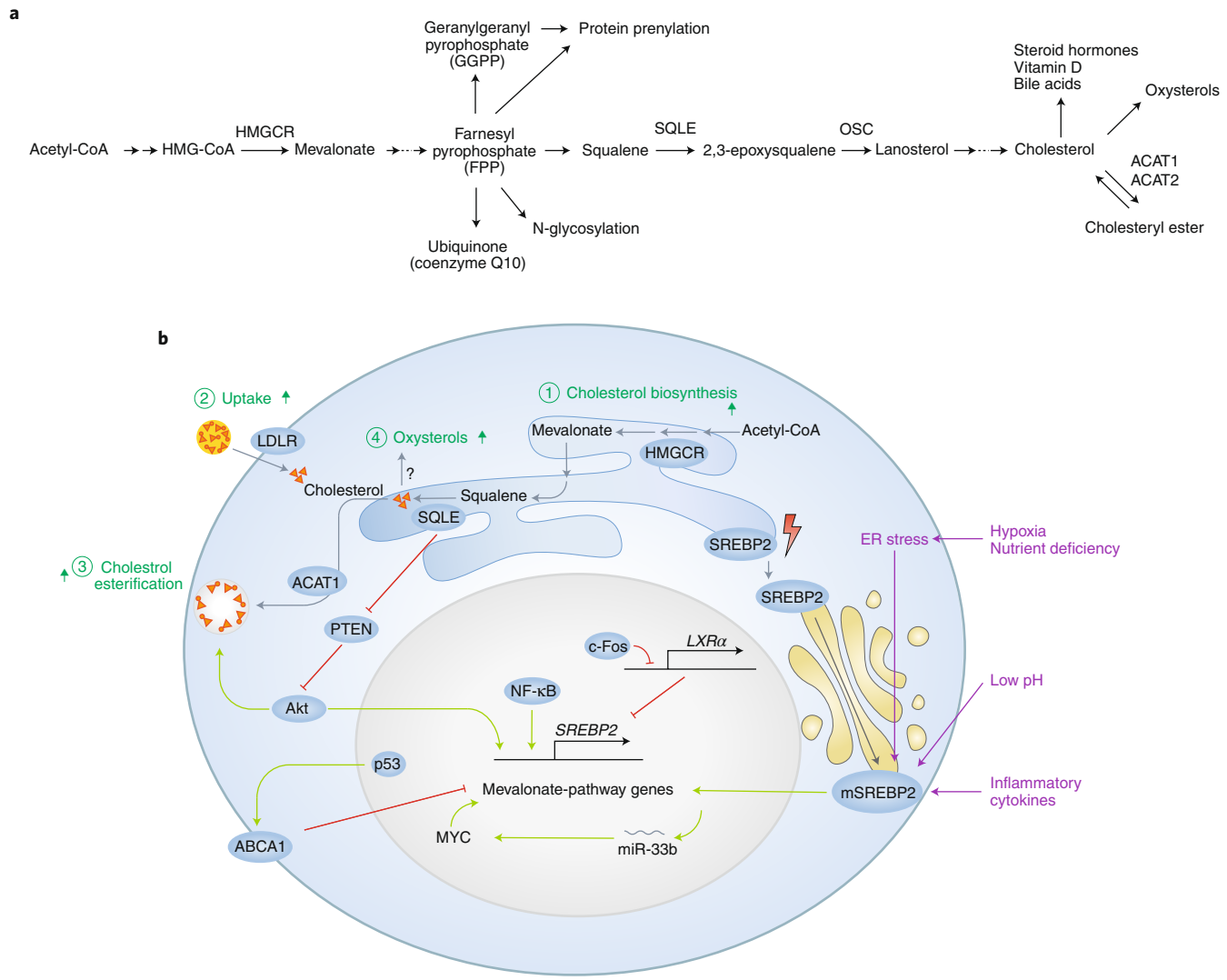

Fig. 1 | Hallmarks and key drivers of cholesterol metabolism in the TME. a, Cholesterol-biosynthesis pathway. Starting from acetyl-CoA, HMG-CoA is generated and further synthesizes mevalonate. This process is mediated by HMGCR, the first rate-limiting enzyme in cholesterol biosynthesis. Mevalonate is then converted to FPP, which has several uses: (1) production of GGPP for protein prenylation; (2) contribution to N-glycosylation; (3) production of ubiquinone and (4) providing squalene for cholesterol synthesis. Squalene is then converted to 2,3-epoxysqualene by SQLE and further to lanosterol by OSC. Cholesterol is used as a precursor to generate: (1) CE by ACAT1 or ACAT2, (2) oxysterols by enzymatic or non-enzymatic conversion, (3) vitamin D, (4) bile acids and (5) steroid hormones. **b**, In the TME, cholesterol metabolism is generally enhanced, thus supporting cancer progression, as evidenced by four aspects as shown: (1) enhanced cholesterol biosynthesis, (2) increased exogenous cholesterol uptake by LDLR, (3) elevated cholesterol esterification by ACAT1 and (4) increased oxysterol production. Intrinsic drivers include: (1) activation of oncogenes such as MYC, thus leading to mevalonate-pathway activation and further upregulating miR-33b expression, and consequently increasing MYC expression through positive feedback; (2) c-Fos-mediated suppression of transcription of *LXRα* (official symbol *NR1H3*), thus decreasing LXRα signalling and promoting cholesterol biosynthesis; (3) loss of p53-mediated repression of the mevalonate pathway, in a process relying on the target ABCA1; (4) SQLE-mediated inhibition of PTEN expression and activation of Akt, thus leading to CE accumulation. Extrinsic cues comprise ER stress, low pH and inflammatory regulators, thus directing mature SREBP2 (mSREBP2) translocation into the nucleus for activation.

As a consequence, cholesterol depletion or trafficking blockade hinders tumour growth and invasion in a variety of cancers[24–27].

Increased cholesterol biosynthesis and uptake. Increased cholesterol biosynthesis is a hallmark of many cancers. In situations in which lipids and/or oxygen is limited, such as in the glioblastoma microenvironment, the master transcription factor SREBP2 and its downstream targets, including mevalonate-pathway enzymes, are significantly upregulated in tumors[28]. Beyond SREBP2, another transcription factor, RORγ, activates the cholesterol-biosynthesis program and facilitates progression of triple-negative breast cancer[29].

Cholesterol biosynthesis also has a critical role in maintaining cancer stem cells by activating cellular signalling pathways downstream of sonic hedgehog, Notch and receptor tyrosine kinases[30]. Furthermore, elevated cholesterol biosynthesis is observed in patient-derived cancer stem cells under mammosphere culture conditions[31]. These lines of evidence suggest that ensuring a sufficient cholesterol supply for the cancer stem cell population may be essential to support cancer progression, thus highlighting a fundamental role of cholesterol in cancer cells.

In addition, the upstream mevalonate pathway is oncogenic in a variety of cancers[32]. Besides producing cholesterol, the mevalonate pathway has other fundamental consequences on cells (Fig. 1a).

For example, the mevalonate pathway is required for protein prenylation, which directly regulates the function of Ras-family small GTPases[33]. This pathway also promotes the production of ubiquinone (also known as coenzyme Q10 or CoQ10), thereby supporting mitochondrial electron transport in p53-deficient cancer cells[34]. Moreover, ferroptosis suppressor protein 1 (FSP1) uses ubiquinone to limit excess lipid peroxidation, thus protecting cells from ferroptosis[35,36]. Hence, upregulation of the mevalonate pathway promotes tumourigenesis in various ways.

Compared with time- and energy- consuming de novo cholesterol synthesis, increasing cholesterol uptake might be more efficient for cancer cells. An extreme example is that of some anaplastic large cell lymphoma cells, which fully rely on cholesterol uptake to acquire cholesterol, owing to loss of SQLE, a rate-limiting enzyme in the cholesterol-biosynthesis pathway[37]. These cancer cells actively upregulate the LDLR, which takes up exogenous cholesterol as an alternative strategy to support proliferation. In addition, loss of SQLE leads to accumulation of the upstream precursor squalene, which protects cells against ferroptosis by maintaining the adequate composition of membrane polyunsaturated fatty acids.

Unexpectedly, lower levels of LDLR but higher levels of SQLE are expressed in advanced-stage prostate cancer, thus indicating a greater reliance on cholesterol synthesis than uptake[38]. In contrast to the LDLR, the high-density-lipoprotein receptor (also known as SR-BI) is upregulated in some prostate cancer samples[39]. In the case of intestinal tumourigenesis, both cholesterol synthesis and uptake contribute[40]. Although cholesterol uptake is an important source of cholesterol for cancer cells, how cells orchestrate cholesterol biosynthesis and uptake during cancer progression is complex and remains to be clarified.

Enriched cholesterol derivatives: cholesteryl esters and oxysterols. Accumulation of CE is another common signature in cancer. CE is usually stored in lipid droplets, ubiquitous cytosolic organelles that serve as a reservoir for neutral lipids such as CEs and triacylglycerols[41]. ACAT1 is the key enzyme that converts cholesterol to CE in most tissues and appears to exert a pro-tumour function, for example in pancreatic cancer and lymphocytic leukemia[42,43]. Inhibition of ACAT1 significantly impedes prostate cancer progression[44]. Treatment with the ACAT1 inhibitor CP-113818 significantly decreases breast cancer cell migration capability[45]. In glioma patient samples, ACAT1 knockdown dramatically impedes tumour progression in a nude-mouse xenograft model[46]. In patients with hepatocellular carcinoma, proteomic and phospho-proteomic analyses have indicated elevation of ACAT1 as a prominent feature. Application of the ACAT1 inhibitor avasimibe markedly represses tumour growth in an ACAT1-high-expression patient-derived-xenograft model[47]. In line with these findings, another CE-metabolic enzyme, lysosomal acid lipase, is upregulated and subsequently promotes cell proliferation in clear cell renal cell carcinoma, thus suggesting active CE metabolism in these cells[48].

Therefore, CE appears to serve as a reservoir of cholesterol that cancer cells can tap under increased demand. This function also explains the cancer-associated upregulation of the relevant enzymes such as ACAT1 and lipase, which ensures rapid conversion between esterified and free cholesterol. Importantly, this CE-as-reservoir strategy provides substantial benefits when cancer cells are challenged with unfavourable stimulation such as drug treatment, as discussed in the last section of this Review.

Beyond CEs, oxysterols are another group of cholesterol metabolites enriched in the tumour microenvironment (TME)[49]. With cholesterol as a precursor, oxysterols can be generated by enzymatic or non-enzymatic oxidative processes. Although this oxidation leads to chirality of oxysterols, which might affect their functions, only several oxysterols have been precisely defined with *R*/*S* nomenclature to date. For example, 24*S*-hydroxycholesterol is abundant in the brain, and 22*R*-hydroxycholesterol is the first metabolite in the steroid-hormone-biosynthesis pathway[50]. Functional oxysterols, including 25-hydroxycholesterol (25HC), 27-hydroxycholesterol (27HC), 24-hydroxycholesterol (24HC), 22-hydroxycholesterol (22HC), 7α/β-hydroxycholesterol and 7-ketocholesterol, exert broad functions by binding nuclear receptors such as LXR and ROR[49,51].

Extensive studies have been performed to elucidate the effects of 27HC on cancer cells. In patients with oestrogen-receptor-positive breast cancer, 27HC is elevated in both breast tissues and tumours[52]. Treatment to elevate 27HC promotes cell proliferation and tumour growth by modulating a series of genes including oestrogen-receptor signalling genes such as *ARMT1* and *PARD6B* as well as genes involved in the GDFN–RET signalling pathway. A similar function of 27HC facilitates breast cancer metastasis to the lung[53]. Additional functions of 27HC in breast cancer progression include: (1) a decrease in p53 activation by enhancing p53 E3 ligase MDM2 function and promoting cell proliferation[54], (2) activation of STAT3–VEGF signalling, thus facilitating angiogenesis[55], and (3) induction of epithelial–mesenchymal transition and matrix metallopeptidase 9 expression, thus promoting cell migration and invasion[56].

Because oxysterols are known LXR ligands that repress SREBP signalling, they are likely to inhibit cell proliferation. Indeed, 27HC together with 24(*R*/*S*), 25-epoxycholesterol dampens gastric cancer cell proliferation and migration via modulation of LXR signaling[57]. Similarly, 27HC treatment impedes cell proliferation in colorectal cancer cells, but this effect is mediated by decreased activating phosphorylation of the kinase Akt rather than LXR activation[58]. In addition to 27HC, other oxysterols such as 7-ketocholesterol, cholestane-3β-5α-6β-triol and 5α-cholestane-3β,6β-diol can impede cell-cycle progression and trigger apoptosis in multiple human and mouse cancer cell lines[59]. Collectively, oxysterols can be both friend and foe for cancer cells, depending on the particular tumour context. Oxysterols also have multiple functions in shaping the immunological landscape, as discussed in the next section.

Because active cholesterol metabolism has an essential role in promoting cancer progression, understanding the key factors driving altered cholesterol pathways in cancer cells is critical, as summarized in Fig. 1b.

Intrinsic drivers of cholesterol metabolism. Gain of oncogenes and loss of tumour suppressors are key characteristics of cancer cells. Interestingly, these features correspond well with fluctuations in cholesterol metabolism. In normal retinal pigment epithelium cells, activation of Akt leads to upregulation of SREBP and its target genes in the cholesterol pathway[60], thus suggesting a positive correlation between oncogene activation and cholesterol metabolism. The oncogene *MYC* is required for upregulation of the mevalonate pathway in patient-derived brain-tumour-initiating cells[61]. This upregulation of the mevalonate pathway further upregulates the microRNA miR-33b, thus increasing *MYC* expression and establishing a positive feedback loop facilitating tumour growth. In hepatocytes, transgenic expression of the oncogene c-Fos represses LXRα signalling and elevates the production of cholesterol and cholesterol-derived metabolites such as oxysterols and bile acids[62], which in turn is associated with increased inflammation and hepatocellular carcinogenesis. Whereas oncogene activity promotes cholesterol upregulation, tumour suppressors have the opposite effect. For example, the well-known tumour suppressor p53 upregulates the cholesterol-efflux transporter ABCA1, thereby restricting SREBP2 maturation and subsequently repressing the mevalonate pathway[63]. Furthermore, suppression of the mevalonate pathway by statins appears to effectively retard tumourigenesis in p53 loss-of-function cancer.

Beyond de novo cholesterol biosynthesis, tumour-suppressor activity restricts cholesterol uptake and esterification. In prostate cancer, loss of the tumour suppressor phosphatase and tensin

homolog (PTEN) activates PI3K–Akt signalling and leads to accumulation of CE by increasing cholesterol uptake and causing further esterification[44]. Consistently, in hepatocellular carcinoma induced by non-alcoholic fatty liver disease, SQLE expression is dramatically elevated. Enhanced levels of SQLE cause downregulation of PTEN expression and subsequent activation of Akt signalling, thus increasing CE levels and facilitating cancer development[64]. In summary, whereas oncogenes promote active cholesterol biosynthesis and facilitate tumour growth, tumour suppressors antagonize this overactivated state and maintain cholesterol homeostasis (Fig. 1b). Therefore, loss of tumour suppressors during tumourigenesis leads to dysregulation of cellular cholesterol metabolism.

Extrinsic cues affecting cholesterol metabolism. *Acidification of the TME.* Secretion of protons and CO_2 from cells as a result of glycolysis is well known to contribute to acidification of the TME[65]. These low-pH conditions increase cholesterol biosynthesis. In pancreatic cancer, when the extracellular pH decreases to 6.8, SREBP2 translocates to the nucleus and activates expression of downstream target genes[66]. Importantly, 12 low-pH-responsive genes are associated with the SREBP2 pathway and are inversely correlated with patient survival[66].

The inflammatory TME. Inflammation is closely associated with cancer development through multiple mechanisms[67]. In hepatocellular carcinoma cells, treatment with the pro-inflammatory factor lipopolysaccharide activates both cholesterol biosynthesis and uptake[68]. This effect is mediated by two components of the NF-κB signalling pathway, IKKα and TAB3, which are negatively regulated by miR-195. Interestingly, enhanced cholesterol levels further strengthen NF-κB signalling in a positive feedback loop. Furthermore, long-term (24-hour) stimulation by the cytokine TNF promotes SREBP2 activation and facilitates macrophage polarization[69]. Although this process occurs in macrophages, TNF in the TME is likely to also influence cholesterol metabolism in cancer cells.

TME-induced ER stress. An accumulation of unfolded or misfolded proteins in the ER triggers the unfolded-protein response, in which release of the ER chaperone Grp78 leads to activation of ER signalling proteins such as PERK, IRE1 and ATF6. This induction of the unfolded-protein response results in either removal of misfolded proteins or cell death, depending on the degree of accumulation of misfolded proteins[70]. In the harsh conditions of the TME, hypoxia and low nutrient concentrations induce ER stress and the unfolded-protein response in an adaptive response[71]. The ER is the primary location of cholesterol biosynthesis, esterification and oxidation, and ER cholesterol levels have a predominant role in determining SREBP2 activation. Therefore, ER stress might be expected to affect cholesterol metabolism. Accordingly, docosahexaenoic acid treatment induces ER stress in the colorectal cancer cell line SW620 and is accompanied by upregulation of key genes in cholesterol metabolism, such as *HMGCR*, *SREBF2* and *NPC1* (ref. [72]). Although the effect of docosahexaenoic acid on SREBP2 activation is independent of ER stress, ER stress markedly increases SREBP2 levels[73]. However, understanding of how ER stress regulates cholesterol synthesis is preliminary, and further investigation is warranted.

In summary, cancer cells respond to unfavourable extrinsic environments by activating cholesterol biosynthesis, thus allowing for cellular adaptation and better survival (Fig. 1b).

Functions of cholesterol and cholesterol-derived metabolites in the TME

In addition to containing cancer cells, tumours contain a variety of immune-effector cells and immunosuppressive cells—collectively referred to as tumour-infiltrating immune cells (TIIs), which have diverse anti-tumour or pro-tumour functions, depending on cancer type and stage[74]. TIIs include T lymphocytes, B lymphocytes, tumour-associated macrophages (TAMs), dendritic cells (DCs), myeloid derived suppressor cells (MDSCs), neutrophils and natural killer cells[75]. In this section, we summarize the diverse effects of cancer-derived cholesterol metabolites, especially oxysterols, on TII functions (Fig. 2).

Enrichment in immunosuppressive cells. Neutrophils are emerging as an important immunosuppressive population in the TME[76,77], thus prompting the question as to which molecular factors guide neutrophils into the TME. Specifically accumulating in the conditioned medium of various cancer cells, 22HC can recruit $CD11b^{high}Gr1^{high}$ neutrophils[78]. Unexpectedly, in this context, 22HC binds and activates the G-protein-coupled receptor C-X-C motif chemokine receptor 2 (CXCR2) instead of LXR to recruit neutrophils. In addition, 24HC and 27HC can recruit neutrophils in other cancer types. In pancreatic neuroendocrine tumours, elevation of 24*S*-hydroxycholesterol by hypoxia-inducible factor-1a (HIF1α) attracts neutrophils, thus promoting angiogenesis[79]. Moreover, in a breast cancer model based on high-cholesterol-diet feeding, 27HC has been found to attract polymorphonuclear neutrophils and γδ T cells but to deplete cytotoxic CD8 T cells, thereby promoting tumour metastasis[80].

MDSCs share high similarity with neutrophils but also display unique features[77]. A recent discovery has characterized lectin-type oxidized LDL receptor-1 (LOX-1) as a prominent marker distinguishing polymorphonuclear myeloid-derived suppressor cells from neutrophils[81]. LOX-1 overexpression has been confirmed in different human cancers and correlated with poorer survival rates. Because LOX-1 is an LDL receptor, these observations hint at reprogramming of cholesterol metabolism in MDSCs in the TME, thus prompting the question of how LOX-1 overexpression influences MDSC function in establishing an immunosuppressive microenvironment.

Beyond neutrophils and MDSCs, TAMs can also be reprogrammed as a result of alterations in cholesterol metabolism. Cancer cells secrete hyaluronic acid oligomers, thereby increasing cholesterol efflux in TAMs and directing TAMs towards an M2-like phenotype that accelerates tumour progression[82]. Moreover, 25HC interacts with G-protein-coupled receptor 183 and triggers migration of both macrophages and human blood monocytes by reorganizing the cytoskeletal protein vimentin[83]. In addition to these effects on TAMs, 25HC can advance gastric cancer cell metastasis by promoting matrix metallopeptidase expression, without affecting cell proliferation and apoptosis[84]. Together, these lines of evidence indicate that oxysterols affect immunosuppressive cells and consequently facilitate cancer development.

Inhibiting immune-effector cells. Similarly to cancer cells, activated T cells also undergo rapid proliferation and therefore depend on elevated cholesterol metabolism to supply enough cholesterol to be used as building blocks. Whereas SREBP2 signalling has been shown to be essential for CD8 T cell proliferation and effector function[85], LXR signalling negatively regulates T cell activation[86]. Therefore, oxysterols enriched in the TME might inhibit T cell anti-tumour immunity via LXR activation, whereas upregulation of intrinsic cholesterol biosynthesis or uptake in T cells could augment T cell anti-tumour function.

Inhibition of the cholesterol-esterification enzyme ACAT1 reprograms cholesterol metabolism in CD8 T cells, thus leading to accumulation of free cholesterol at the plasma membrane[87]. Cholesterol then directly binds T cell receptors and facilitates nanoclustering, which promotes antigen-induced signalling and the consequent upregulation of cholesterol biosynthesis and uptake[88]. Moreover, cholesterol aids in establishing a mature immunological synapse for targeted killing of cancer cells.

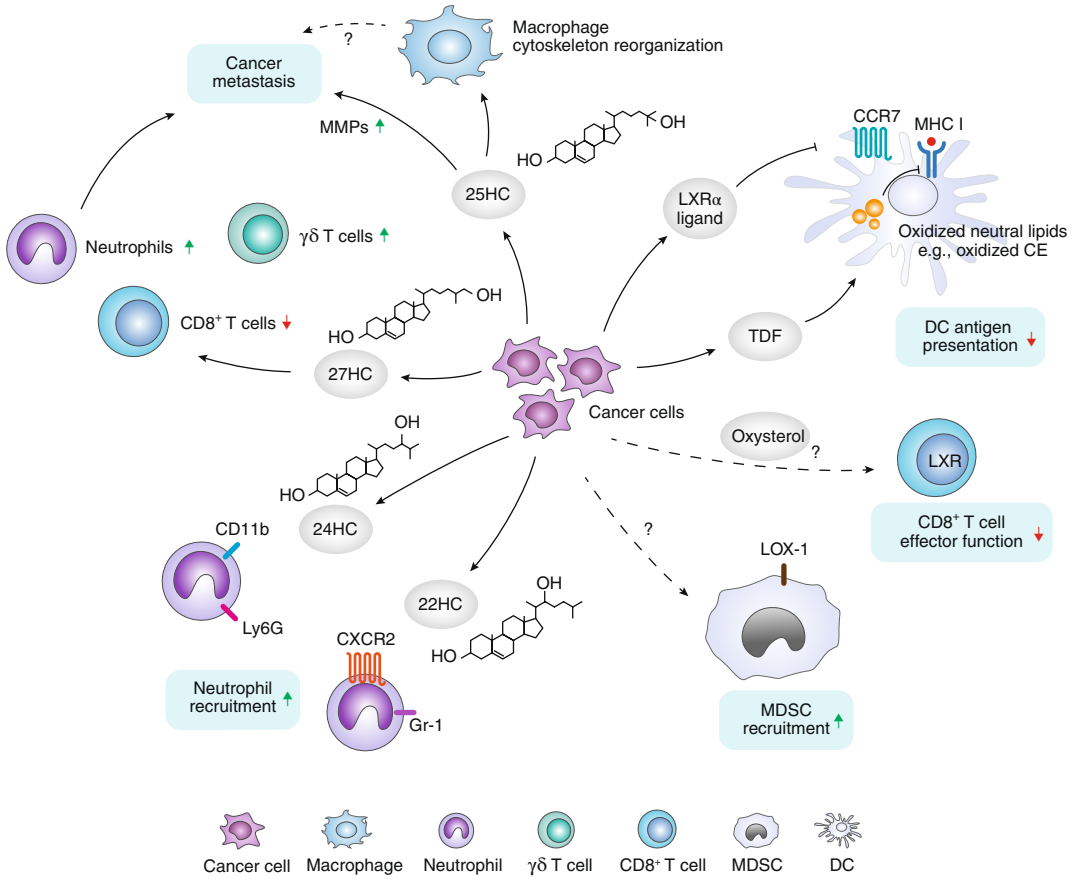

Fig. 2 | Immune-modulation functions of cancer-derived oxysterols. Various oxysterols are secreted in the TME and regulate immune cell functions. For neutrophils, 22HC binds CXCR2 and recruits Gr-1-high neutrophils to cancer cells; 24HC attracts Ly6G- and CD11b-positive neutrophils; 27HC increases neutrophils and γδT cells but decreases CD8 T cells, thus promoting breast cancer metastasis. For macrophages, 25HC attracts macrophages by directing cytoskeletal reorganization, thus probably contributing to cancer metastasis, and also promotes cancer metastasis by upregulating the expression of matrix metallopeptidases (MMPs). For DCs, potential oxysterols that activate LXRα inhibit CCR7 expression, thereby suppressing DC function. An unknown tumour-derived factor (TDF) leads to accumulation of oxidized neutral lipids such as CE and decreases MHC-I peptide expression on the cell surface, thus dampening DC antigen-presentation ability. For CD8 T cells, some oxysterols presumably activate LXR signalling and suppress the effector function. For MDSCs, yet-undefined factors recruit LOX-1-positive MDSCs, which exert pro-tumour functions.

In contrast, cholesterol accumulation in the TME has been shown to trigger ER stress and further increase T cell exhaustion in a study using several well-known surface markers, such as PD-1, TIM-3 and LAG-3, to examine T cell exhaustion[89]. However, these markers can also reflect T cell activation. Furthermore, the cause of T cell ER stress could be cholesterol metabolites such as oxysterols rather than cholesterol itself. In an in vitro polarized CD8 T cell subset, Tc9, much lower levels of cholesterol are needed to produce the signature cytokine IL-9 than in the Tc1 CD8 T cell subset, thus reflecting the heterogeneity of cholesterol metabolism in different T cell subsets[90]. Therefore, it will be important to carefully compare cholesterol metabolism in different tumour-infiltrating T cell subsets, such as effector versus memory, functional versus dysfunctional, and helper versus killer. In addition, the functions of extrinsic and intrinsic cholesterol might differ. Upregulation of intrinsic cholesterol biosynthesis and uptake might be beneficial, whereas too much or too little extrinsic cholesterol might cause T cell dysfunction.

Inhibition of antigen presentation. Conditioned medium from several cancer cells can activate LXR-α signalling in DCs, thus decreasing expression of CC chemokine receptor-7 (CCR7) on the DC surface[91]. In agreement with a role of CCR7 in mediating lymphoid homing of DCs, CCR7 inhibition dampens DC migration from tumour beds to draining lymph nodes and consequently suppresses the presentation of tumour antigens to T cells. Blockade of cholesterol synthesis in tumour-bearing mice or inactivation of LXR-α ligand by expression of SULT2B1b, a cholesterol sulfotransferase that converts oxysterol to inactive sulfated oxysterol, restores DC functionality as well as the anti-tumour response[91]. However, the exact oxysterol that exerts these effects remains to be identified. Additionally, tumour-derived factors have been shown to directly cause accumulation of oxidized neutral lipids including CE, triacylglycerols and fatty acids in DCs, thus decreasing levels of peptide–MHC class I complexes on the cell surface and the presentation of exogenous antigens[92].

Targeting cholesterol metabolism for cancer therapy

Targeting cholesterol synthesis. Because of the vital and varied functions of cholesterol metabolism in cancer progression, impeding active cholesterol metabolism, for example via inhibition of the mevalonate pathway, has been demonstrated to be a feasible anti-tumour strategy[93,94] (Table 1). To date, statins, HMGCR inhibitors, have been the most widely used cholesterol-metabolism-targeting drugs in clinical studies for patients with cancer. In general, statins are safe at standard doses, showing only mild adverse effects on the muscle and liver[95]. Side effects vary depending on the exact statin used, as well as the dosage and combination with other drugs.

Table 1 | Anti-cancer therapies that target cholesterol metabolism

	Reagent	Target	Mechanism	Cancer type	References
Targeting cholesterol biosynthesis	Statins	HMGCR	Decreased cancer mortality and longer survival, according to retrospective clinical analysis	CRC, prostate cancer, multiple myeloma and other cancers	97–101
	Lipophilic statins		Decreased small-GTPase geranylgeranylation, increased antigen presentation and T cell activation	B16-Ova melanoma and TC-1 papillomavirus-associated tumour models	103
	R048-8071	OSC	Dampened cell proliferation, increased apoptosis and decreased cell migration	HCT116 CRC and HPAF-II pancreatic adenocarcinoma models	106
	Zaragonic acids	Squalene synthase	Decreased neutrophil infiltration and enhanced T cell function	RMA lymphoma and Lewis lung carcinoma models	107
Targeting cholesterol esterification	Avasimibe	ACAT1	Impaired Wnt–β-catenin signalling through decreased Wnt3a secretion; decreased metastasis	PC-3M prostate cancer model	111
			Increased T cell receptor clustering and immune synapse formation; elevated CD8 T cell effector function	B16 melanoma model	87
	Avasimin		Increased cell apoptosis with no clear cytotoxicity	PC3 prostate and HCT116 CRC models	112
	Bitter-melon extract		Decreased ACAT1 expression and SREBP activity	MDM-MB-231 breast cancer model	113
Targeting LXR signalling	RGX-104	LXR	MDSC depletion and increased T cell activation	B16F10 melanoma and Lewis lung carcinoma models	119
	LXR623		Decreased cellular cholesterol of cancer cells	Clear cell renal cell carcinoma model	121
	SR9243		Repression of lipogenesis and glycolysis of cancer cells; induction of cell apoptosis	CRC, prostate and lung cancer models	120,121
Combination therapy	Lapatinib + lovastatin	HER2 + HMGCR	Restricted surface receptor signalling, thus further sensitizing blockade of HER2 or androgen-receptor	Breast cancer model	122
	Enzolutamie + simvastatin	Androgen receptor + HMGCR		Prostate cancer model	123
	Metaformin or aspirin + fluvastatin	AMPK +HMGCR	Suppression of AMPK or MEK, thus disrupting the feedback response triggered by statin-mediated inhibition of cholesterol biosynthesis	MCF10A-based model	124
	AZD6244 + simvastatin	MEK +HMGCR		Pancreatic ductal adenocarcinoma model	125
	Gemcitabine, paclitaxel, doxorubicin + avasimibe	Chemotherapy + ACAT1	Modulation of cancer metabolism by avasimibe, which synergizes with conventional chemotherapies and increases anti-tumour efficacy	Pancreatic ductal adenocarcinoma, melanoma, lung and breast cancer models	126–128
	Kras peptide vaccine, DC vaccine, anti-PD-1 + avasimibe	Immunotheray + ACAT1	Enhancement of CD8 T cell function and inhibition of the regulatory T cell population by avasimibe via modulation of T cell metabolism, thus strengthening cancer-vaccine or checkpoint-blockade immunotherapy	Lung, head and neck cancer, melanoma models	87,115,129

HER2, receptor tyrosine-protein kinase ErbB-2; CRC, colorectal cancer.

Nevertheless, even for patients with cardiovascular disease and abnormal liver function, statins remain safe to use and improve the results of liver tests (concentrations of serum alanine aminotransferase or aspartate aminotransferase)[96].

For patients with cancer, numerous clinical studies have suggested benefits of statin usage on patient survival. Patients receiving statins for at least 5 years have been found to have a 47% lower risk of colorectal cancer than non-statin users, after adjustment for other known risk factors[97]. In another study, statins decreased patient mortality across various cancer types, regardless of whether they were taken before or after cancer diagnosis[98–101]. Experiments on leiomyoma have shown that simvastatin treatment not only inhibits cell proliferation and enhances cell apoptosis, but also suppresses levels of extracellular matrix proteins[102].

Unexpectedly, statins and other mevalonate-pathway inhibitors have been found to act as vaccine adjuvants[103]. Lipophilic statin can effectively decrease geranylgeranylation of the small GTPase Rab5, which is involved in endosomal trafficking, thus eventually enabling better antigen presentation and tumour-suppressive responses. A synergistic effect has been observed when statins are combined with anti-PD1 therapy. Notably, although statins are well-known cholesterol-pathway inhibitors, they might target other pathways as well. For instance, statin treatment in various cancer cells leads to

a striking elevation of mitochondrial membrane potential without affecting cholesterol levels[104].

Other enzymes in the cholesterol-biosynthesis pathway can also be targeted pharmacologically (Table 1). SQLE, which catalyses squalene oxidization, is considered to be an oncogene and has been evaluated as an anti-tumour target[105]. Several drugs against SQLE have been clinically approved as antifungals, and whether they can be repurposed as anti-tumour drugs should be investigated. Ro 48-8071, an inhibitor of oxidosqualene cyclase (OSC), an enzyme that converts 2,3-oxidosqualene to lanosterol, significantly suppresses growth and metastasis of both colorectal cancer and pancreatic cancer[106]. Application of this inhibitor decreases cell proliferation, increases cell apoptosis and impairs cancer cell migration. More importantly, Ro 48-8071 synergizes with 5-fluorouracil, thus eliciting an enhanced anti-tumour effect; these results suggest that potential benefits might arise from combination therapy. Zaragozic acids, which inhibit squalene production, effectively repress both lymphoma and Lewis lung carcinoma growth without causing clear toxic side effects[107]. These inhibitors could potentially be used together with anti-cancer vaccination or adoptive immunotherapy for increased anti-tumour efficacy.

Cancer cells should not be assumed to always require high cholesterol levels, nor should any anti-tumour strategy be assumed to necessarily limit cholesterol biosynthesis: exceptions can exist. For instance, inhibition of Ephrin type-A receptor 2 impairs ABCA1 function and decreases cholesterol efflux, thus resulting in membrane rigidity and cell apoptosis in triple-negative breast cancer cells[108]. Similarly, in a mouse model of hepatocellular carcinoma, a cholesterol-rich diet raises membrane cholesterol levels, and consequently increases CD44 relocation into membrane lipid rafts and decreases the interaction between CD44 and the actin-binding protein Ezrin, thus restricting metastasis[109]. Collectively, whereas inhibiting cholesterol biosynthesis is a plausible and promising therapeutic strategy to treat cancer, modulating cholesterol levels at the plasma membrane might provide a novel alternative approach.

Targeting cholesterol esterification. In the previous section, we outlined the positive roles of CE in cancer progression. Developing inhibitors against CE is of high clinical interest (Table 1). In an imatinib-resistant chronic myelogenous leukaemia cell line, inhibition of CE by the ACAT1 inhibitor avasimibe suppresses tumour growth and subsequently restores imatinib sensitivity by downregulating MAPK signaling[110]. In prostate cancer, avasimibe treatment impairs the Wnt–β-catenin pathway and thus suppresses metastasis[111]. Application of avasimin, a nanomedicine version of avasimibe encapsulated with human serum albumin[112], specifically induces cancer cell apoptosis and is effective in tumour xenograft models.

Bitter-melon extract has also been reported to downregulate CE accumulation by inhibiting ACAT1 expression in triple-negative breast cancer cells[113]. Bitter-melon-extract treatment also effectively decreases mammosphere xenograft growth.

In addition to having direct effects on cancer cells, CE inhibition affects human chimeric antigen receptor–modified T cells. Administration of avasimibe augments the in vitro cytotoxic effect of these cells, an effect that can be partly explained by an increased ratio of cytotoxic CD8 T cells[114]. This observation is consistent with findings showing that avasimibe treatment effectively promotes CD8 effector T cell function[87,115]. Thus, targeting CE has dual benefits, by repressing cancer cells on the one hand and augmenting CD8 T cell anti-tumour function on the other hand.

Targeting LXR signalling. LXR agonists have shown promising results for the treatment of a variety of cancers, mainly by inhibiting cancer cell proliferation and inducing apoptosis[116–118]. However, modulating LXR signalling affects not only cancer cells but also immune cells (Table 1). The LXR agonist RGX-104, which efficiently represses growth of a broad range of mouse and human tumors[119], depletes MDSCs through upregulation of the LXR transcriptional target *APOE* and subsequently increases T cell activation. Importantly, this observation has been further validated in patients with cancer in a phase I clinical trial. Moreover, LXR activation can augment other immunotherapies such as adoptive T cell transfer and checkpoint-blockade therapy in mouse models[119].

Beyond efforts to exploit LXR activation to suppress cancer cell growth and boost anti-tumour immune responses, the effects of LXR repression on cancer progression have been tested. The LXR inverse agonist SR9243 recruits an LXR corepressor that represses LXR activity, thus inducing massive apoptosis in cancer cells[120]. In this context, LXR inhibition effectively impairs tumour growth by suppressing lipogenesis and glycolysis. Furthermore, the effects of both the LXR agonist LXR623 and the inverse agonist SR9243 on clear cell renal cell carcinoma have been examined[121]. Both drugs effectively decrease cancer cell proliferation and induce apoptosis. Whereas SR9243 predominantly suppresses lipogenesis by inhibiting enzymes such as acetyl-CoA carboxylase, fatty acid synthase and stearoyl-CoA desaturase 1, LXR623 significantly decreases cellular cholesterol content by promoting cholesterol efflux and limiting cholesterol uptake. Together, this evidence suggests that both excessive LXR activity and insufficient LXR activity are unfavourable for cancer cells.

Combination strategies. Accumulating evidence indicates that targeting cholesterol metabolism sensitizes cancer cells to other anti-tumour therapies (Table 1). Here, we summarize two different benefits of combination therapy.

First, in some cancer cells, a combination strategy limits cell-surface-receptor-mediated oncogenic activation more effectively than single therapies. For instance, in breast cancer cells positive for Erb-b2 receptor tyrosine kinase (ErbB2, also known as HER2), suppression of cholesterol biosynthesis by inhibitors such as lovastatin can trigger ErbB2 internalization and degradation[122]. In this scenario, use of ErbB2 inhibitors such as lapatinib and neratinib together with lovastatin dramatically represses tumour growth. In prostate cancer cells, administration of the anti-androgen drug enzalutamide upregulates enzymes such as HMGCR in the mevalonate pathway. HMGCR inhibition by simvastatin further suppresses androgen signalling by lowering androgen-receptor levels via repression of signalling by mammalian target of rapamycin. A combination therapy of enzalutamide and simvastatin has been shown to have a significant synergistic effect on tumour suppression[123].

Second, in other cancer cells, cholesterol-metabolism-blockade therapy causes feedback responses that decrease drug efficacy. Therefore, inhibiting feedback responses with another therapy might enhance anti-tumour efficacy. For example, although fluvastatin treatment can repress colony formation in breast cancer cells, HMGCR expression is induced as a compensatory mechanism. Because AMP-activated protein kinase (AMPK) activation by agonists, such as aspirin or metformin, blocks this feedback on HMGCR, a combination treatment combining aspirin or metaformin with fluvastatin has been found to almost completely abrogate the colonization capability of breast cancer cells[124]. In another recent study, statin treatment has been found to significantly decrease levels of the mevalonate-pathway product coenzyme Q in cancer cells, thus leading to excessive oxidative stress[125]. Given that cancer cells can upregulate antioxidant pathways by enhancing cystine import and consequently decrease oxidative stress, the use of AZD6244—an inhibitor of mitogen-activated protein kinase kinase (MEK), which limits cystine import—in combination with statins has been shown to be highly cytotoxic in cancer cells[125].

Other than inhibiting cholesterol biosynthesis, inhibition of cholesterol esterification is also an option in combination strategies. One possible strategy is a combination of cholesterol-esterification

inhibitors with traditional chemotherapy drugs. For example, the combination of avasimibe with well-known chemotherapy drugs such as gemcitabine[126], paclitaxel[127] or doxorubicin[128] increases anti-tumour effects in tumour models. Another strategy is combining cholesterol-esterification inhibitors with immunotherapies, such as anti-cancer vaccines and anti-PD-1 therapy. Although avasimibe treatment effectively inhibits regulatory T cell populations and increases CD8 T cell infiltration in a lung tumour model, a combination of avasimibe with a Kras peptide vaccine has stronger tumour inhibitory effects[115]. Avasimibe can also be combined with a DC vaccine to boost adaptive anti-tumour immunity, as shown in a head and neck cancer model[129]. Finally, a combination of avasimibe with anti-PD-1 therapy effectively controls melanoma growth[87].

Perspective. Numerous lines of evidence support the concept that cholesterol metabolism is critical for cancer progression. Intrinsic and extrinsic cues are now understood to drive reprogramming of cholesterol metabolism in the TME, and cancer-derived cholesterol metabolites are understood to exert immunomodulatory functions. Therapeutic targeting of cholesterol metabolism in both cancer cells and immune cells is likely to move towards clinical application.

Despite exciting progress in the field, many fundamental questions remain to be addressed, such as: How does cholesterol metabolism preferentially help cancer cells but harm immune cells? Do other cholesterol metabolites contribute to cancer progression and immunosuppression apart from the well-studied oxysterols? Can a specific cholesterol pathway be modulated to achieve both anti-tumour and pro-immune effects? What are the most effective combination strategies that attack cancer cells with different approaches? Could some drugs that are currently used for the treatment of metabolic diseases be repurposed as anti-tumour drugs? These outstanding questions reflect the urgent need for more mechanistic studies of cholesterol metabolism in cancer, which might pave the way for next-generation therapies.

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Author contributions

C. X. designed the framework. B.-L.S. wrote the first section, and B.H. wrote the rest of the manuscript. C.X. revised the manuscript.

Competing interests

C.X. is a scientific co-founder of Hangzhou MetMed Therapeutics. The other authors declare no competing interests.

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