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Cholesterol metabolism: at the cross road between cancer cells and immune environment

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Abstract

Mevalonate pathway is a highly conserved pathway that produces isoprenoids and cholesterol, and it is often increased in cancer cells. Cholesterol, upstream metabolites including isoprenoids and cholesterol derivatives such as oxysterols modulate cell proliferation, motility, stemness and drug resistance. Moreover, when produced by cancer cells or immune infiltrating cells, they modulate the activity of immune populations of the tumor microenvironment. In this review, we will focus on the recent findings demonstrating that cholesterol derivatives may regulate tumor immune recognition or immune escape, playing a critical role in the immune surveillance. Since the mevalonate pathway is druggable, a deeper knowledge of the metabolic cross talks existing between the mevalonate pathway of cancer cells and immune cells may help to identify novel agents targeting cholesterol metabolism, able to boost the anti-tumor activity of the immune populations.

Key facts:
Cholesterol and its metabolites are actively synthesized by cancer cells and influence initiation, progression and drug resistance. Some of these metabolites are effluxed by cancer cells and modulate the functions of immune cells infiltrating the tumor environment. Pharmacological inhibitors targeting cholesterol metabolism could be repurposed as immune adjuvant agents.

Keywords: cholesterol; cancer; immune modulation

1. Cholesterol metabolism in cancer

Cholesterol is synthesized from acetyl Coenzyme A (AcCoA) in the so called “mevalonate (MVA) pathway”. Most of the enzymes of the MVA pathway are upregulated in tumors (Yang et al., 2020). This finding explains why tumor tissues have higher level of cholesterol than non-transformed tissues. The pacemaker enzyme 3-β-3-hydroxymethylglutaryl Coenzyme A reductase (HMGCR) is a sterol sensitive enzyme and is transcriptionally induced by the sterol regulatory element binding proteins (SREBPs) (Lee et al., 2020). SREBPs are sequestered within the endoplasmic reticulum by the SREBP cleavage-activating protein/insulin-induced gene-1 (SCAP/INSIG-1) complex if intracellular cholesterol is high; on the contrary, if cholesterol is low, SREBPs are translocated into the Golgi and cleaved into the transcriptionally active form (Lee et al., 2020). SREBPs are often upregulated in cancer cells since the tumoral pyruvate kinase isoform M2 (PKM2) promotes their transcription (Zhao et al., 2018). As a consequence, not only cholesterol, but also the upstream metabolites, such as MVA, the isoprenoids isopentenyl pyrophosphate (IPP), geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP), or squalene,
are increased. The higher levels of low density lipoprotein receptor (LDLR), which grants a higher uptake of extracellular cholesterol, the altered activity of intracellular sterol transfer proteins and ATP binding cassette transporter A1 (ABCA1), which is the main cholesterol efflux transporter, are additional factors increasing the accumulation of cholesterol and derivatives, such as cholesterol esters (CEs) and oxysterols, within cancer cells (Figure 1).

Besides being a building block of cell membranes, necessary for proliferation and migration, cholesterol has been revisited as a signalling molecule in cancer. In plasmamembrane, cholesterol accumulates mainly in lipid rafts, i.e. dynamic platforms rich of growth factor receptors and adhesion molecules, regulating cell proliferation, migration and response to chemotherapy. Mitochondrial cholesterol prevents apoptosis by inhibiting Bax protein, while lysosomal cholesterol activates the pro-survival mTORC1/Akt axis (Xu et al., 2020). Notably, cancer stem cells (CSCs) have the highest levels of de novo synthesis and uptake of cholesterol: cholesterol stimulates stemness pathways supporting proliferation, by activating Notch signalling and Wnt canonical pathway, promoting the maturation of Shh protein and stabilizing YAP/TAZ pathway through GPP (Li et al., 2020).

Upstream and downstream metabolites play a critical role in cancer initiation and progression as well (Figure 1). IPP is a precursor of the tail of ubiquinone is a key component of the electron transport chain that supports oxidative phosphorylation (OXPHOS), limits lipid peroxidation and prevents ferroptosis (Bersuker et al., 2019). Also squalene protects from ferroptosis, although this effect is tumor-specific and dependent on the levels of endogenous squalene epoxidase and on the resistance to oxidative stress of each tumor (Garcia-Bermudez et al., 2019; Mahoney et al., 2019). FPP and GGPP are essential activators of monomeric G-proteins of Ras, Rho and
Rab family that are activated when isoprenylated. Since Ras and Rho mediate cell proliferation, migration, invasion and resistance to chemotherapy, the abundance of FPP and GGPP is a critical factor controlling the cell fate of cancer cells (Wang et al., 2016).

Oxysterols (OH-Chol), such as hydroxycholesterol, 7-ketocholesterol, 22- and 27-hydroxy-cholesterols, generated after enzymatic or non-enzymatic oxidation of cholesterol, have recently emerged as key modulators of membrane fluidity and cell migration (Kloudova et al., 2017). In addition, they are the endogenous ligands of liver X receptors (LXRs) that upregulate ABCA1 and inducible degrader of low-density lipoprotein receptor (IDLr), finely tuning cholesterol homeostasis by controlling either cholesterol efflux or influx. The effects of oxysterols are highly heterogeneous. For instance, 22-OH-Chol has anti-proliferative effects by activating LXR in different solid tumors (Chuu et al., 2010). 27-OH-Chol elicits anti-tumor effects by inhibiting Akt-dependent pathways (Warns et al., 2018) or interfering with lipid rafts stability and STAT3 activation (Dambal et al., 2020), but it has pro-tumorigenic activity in hormone dependent breast cancers being an endogenous ligand of estrogen receptor (Nelson et al., 2013). These discrepancies may be due to the different panel of oxysterol receptors present in different tumors, and/or to the different enzymatic set oxidizing cholesterol, that can be converted in anti-tumor or tumorigenic oxysterol species.

Cholesterols and its metabolites also affect the anti-tumor or the tumor tolerant phenotype of the immune populations infiltrating the tumor microenvironment (TME). Accordingly, new immunesensitizing opportunities may arise from a deeper knowledge of the cholesterol metabolism in tumors.

2. Cholesterol metabolism regulates the tumor-immune system interaction
If cancer cells have usually a high MVA pathway activity, immune cells display a variable situation, depending on their state. Upon activation, the canonical anti-tumor populations – cytotoxic CD8+ T lymphocytes, M1 polarized tumor associated macrophages (TAMs) and mature dendritic cells (DCs) – upregulate the Ras/PI3K/mTOR axis that increases SREBP transcriptional activity and MVA pathway. By contrast, quiescent or tumor tolerant immune cells – such as memory T lymphocytes, M2 polarized TAMs, immature DCs – direct AcCoA towards fatty acid oxidation rather than toward cholesterol biosynthesis (Gruenbacher and Thurnher, 2017). An active MVA pathway supplies cholesterol to build plasmamembrane: this is critical for rapidly dividing cells as T lymphocytes, but also for DCs and TAMs that must build new plasmamembrane portions during antigen presentation or phagocytosis. Moreover, MVA pathway produces ubiquinone, a critical component of the electron transport chain that supplies activated immune cells with ATP (Thurnher and Gruenbacher, 2015).

Neutrophils and TAMs may have anti-tumor or pro-tumor activities, and the cholesterol derived metabolites may switch the activities in these immune infiltrating cells. High levels of cholesterol activate the Toll-like receptor (TLR) present on macrophages, stimulating the assembly of inflammasome. This situation generates a chronic inflammation within the TME that favors tumor progression (Muller et al., 2008). If cholesterol activates the pro-inflammatory phenotype of TAMs, GGPP attenuates TLR signalling by increasing the geranylgeranylation of Ras and its interaction with PI3K: this process blunts the production of inflammatory cytokines (Akula et al., 2016). Contrarily to cholesterol, oxysterols have an immunesuppressive function within the TME. 22-OH-Chol recruits CXCR2-expressing neutrophils able to inhibit the priming and activation of CD8+ T lymphocytes, and to release pro-metastatic and pro-
angiogenic factors (Raccosta et al., 2013). Similarly, 27-OH-Chol favors breast cancer invasion by recruiting polymorphonuclear cells (Baek et al., 2020). 22-OH-Chol also reduces the recruitment of DCs and anti-tumor CD8^+ T lymphocytes by activating a LXRα-dependent transcriptional program in DCs (Villablanca et al., 2009), creating a strongly tumor tolerant TME.

Since cholesterol derived metabolites control multiple circuitries either in cancer cells or in immune cells, it is not surprising that different metabolites of MVA pathway have opposite effects.

Intriguingly, the interactions between tumor and immune cells may modulate the MVA pathway in both compartments in a reciprocal way. For instance, ovarian cancer stimulates the efflux of cholesterol from TAMs that in turn is avidly taken up by cancer cells via LDLR or scavenger receptors, promoting tumor growth (Gossens et al., 2019). Moreover, a deregulated MVA pathway in cancer cells affects the activity of different subset of T lymphocytes (Salaroglio et al., 2014; Kopecka et al., 2016). For instance, an active synthesis of FPP promotes the activation of Ras/ERK1/2/STAT3 axis, leading to the transcription of indoleamine dioxygenase (IDO) enzyme. Kynurenine, the product of IDO, is a potent apoptotic stimulus for CD8^+ T lymphocytes and promotes the expansion of tumor tolerant T-regulatory (Treg) cells (Salaroglio et al., 2014; Kopecka et al., 2016). Moreover, Ras/ERK1/2 axis also up-regulates the ABC transporter B1 (ABCB1/P-glycoprotein) that – besides effluxing chemotherapeutic drugs – inhibits the DC-mediated phagocytosis of tumor cells (Kopecka et al., 2016; Kopecka et al., 2020), further contributing to tumor immune resistance. In DCs, the inhibition of GGPP synthesis blocks the geranylgeranylation and activity of Rab5, altering endosome maturation and prolonging the exposure of antigens on the surface.
boosting CD8+ T lymphocytes activity. This event enhances the efficacy of anti-tumor vaccines or immune checkpoint inhibitors (ICPI) (Xia et al., 2018).

A correct balance between cholesterol and CEs in CD8+ T lymphocytes is important to confer the membrane fluidity that is necessary for clustering and signal transduction of T cell receptor (TCR). An increased cholesterol/CE ratio, indeed, creates the optimal conditions for TCR clustering and cytotoxic activity, and enhances the anti-tumor efficacy of ICPI (Yang et al., 2016). MVA pathway is also crucial for Vy9Vδ2 T cells, a subset of T lymphocytes that have anti-tumor activity against hematological (Castella et al., 2011) and solid (Belisario et al., 2020) tumors. Not only the MVA pathway of tumor cells, but also the MVA pathway of antigen presenting cells such as DCs is critical in activating Vy9Vδ2 T cells. Both tumor cells and DCs treated with the aminobisphosphonate zolendronic acid, an inhibitor of FPP synthase, accumulate IPP that acts as endogenous ligand of LXRα and upregulates ABCA1. The latter effluxes IPP together with cholesterol and delivers it on apolipoprotein A-I (apo-AI) (Castella et al., 2017). This process, similar to the assembly of high density lipoprotein (HDL) particles, facilitates the activation of Vy9Vδ2 T cells, since extracellular IPP is the physiological ligand of Vy9Vδ2 TCR (Castella et al., 2011). ABCA1 cooperates with butirrophylin BTN3A1, a surface protein co-localized with ABCA1, critical for presenting IPP to the TCR of Vy9Vδ2 T cells (Riganti et al., 2018). VyVδ T cells include several subset and their activation is not always beneficial. The recruitment of VyVδ T cells other than Vy9Vδ2 subset by 27-OH-Chol has a pro-metastatic role in breast cancer: this event, together with the recruitment of polymorphonuclear cells, suppresses the cytotoxic activity of CD8+ T lymphocytes (Baek et al., 2017), creating a tumor permissive TME. Moreover, at high concentration of IPP, Vy9Vδ2 T cells become anergic and exert a tumor tolerant effect (Castella et al., 2015). These findings
suggest that quantitative and qualitative differences in the cholesterol derived metabolites may produce opposite effects, likely recruiting different subsets of VγVδ T cells or suppressing their anti-tumor functions.

Overall, since in both myeloid and lymphoid immune infiltrating populations MVA pathway derivatives exert pleiotropic and sometimes contrasting functions (Figure 2), the effect of each metabolite is highly dependent on concentration, origin and target population.

3. Therapeutic implications of targeting cholesterol metabolism and future perspectives

Despite the high MVA pathway activity in cancer cells, all the regulatory mechanisms are still preserved and sensitive to pharmacological inhibitors. Modulating cholesterol metabolism may have a deep impact on the immune recognition of cancer cells. Statins and aminobisphosphonates, inhibitors of HMGCR and FPP synthase, respectively, have been already used in various cancer types. The HMG synthase inhibitor dipyridamole, the squalene epoxidase terfinabine, as well as inhibitors of cholesterol esterification (Avasimibe) and trafficking (itraconazole), are under preclinical development as anti-tumor agents. Farnesyl transferase inhibitors, which prevent the activation of monomeric farnesylated or geranylgeranylated proteins, have been tested with modest results and high toxicity. Notwithstanding these negative results, the research on these compounds is ongoing, in search of more specific and potent inhibitors (Xu et al., 2020). Except for statins (Sarrabayrouse et al., 2017) and aminobisphosphonates (Salaroglio et al., 2014; Kopecka et al., 2016; Belisario et al., 2020), there are no evidences reporting an immunesensitizing effect of the other agents, but – considering the multiple implications of MVA pathway in the immune
responses – we cannot exclude that some agents will be effective as immunesensitizers. Since MVA pathway is globally upregulated in cancer, the most effective strategy is the inhibition of the first steps of the pathway with statins or the inhibition of the pathway’s controllers. In this perspective, a great interest has been raised by SCAP/SREBP inhibitors (Lee et al., 2020). One limitation of cholesterol targeting agents is their low specificity. For instance, statins reduce cholesterol, isoprenylated proteins and ubiquinone in non-transformed tissues, leading sometimes to undesired side effects. Tumor-targeting nanocarriers loaded with MVA pathway inhibitors may partially overcome this limitation. A second limitation is that both cancer cells and immune infiltrating cells have an active MVA pathway: a simultaneous inhibition of cholesterol metabolism in both populations may produce contrasting effects on the anti-tumor immune activity. A deeper knowledge of the metabolic cross talks existing between MVA pathway of cancer cells and immune cells may help to identify the steps and controllers that must be targeted. This knowledge will be translated into novel agents targeting cholesterol metabolism in TME, able to boost the anti-tumor activity of the immune populations.

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**Declarations of interest**

None.

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Figure legends
Figure 1. Cholesterol metabolism and effects of cholesterol metabolites in cancer cells. Cholesterol and its derivatives are higher in cancer cells because of the high de novo synthesis controlled by INSIG-1/SCAP/SREBP complex and the higher uptake via LDLR. Cholesterol can be inserted in lipid rafts, critical for proliferation, migration and drug resistance, converted into cholesterol esters or oxysterols, which control membrane fluidity and survival, or effluxed via ABCA1. Upstream metabolites of cholesterol have pleiotropic functions: IPP is a precursor of ubiquinone of the mitochondrial electron transport chain and an activator of Vγ9Vδ2 T cells; FPP and GGPP are necessary for the prenylation and activation of monomeric G-proteins of Ras, Rho and Rab family, controlling proliferation, invasion and antigenicity. LDLR: low density lipoprotein receptor; INSIG-1: insulin-induced gene-1; SCAP: SREBP cleavage-activating protein; SREBP: sterol regulatory element binding protein; ER: endoplasmic reticulum; AcCoA: acetyl Coenzyme A; MVA: mevalonate; HMGCoA: 3-β-3-hydroxymethylglutaryl Coenzyme A; HMGCR: 3-β-3-hydroxymethylglutaryl Coenzyme A reductase; IPP: isopentenyl pyrophosphate; GPP: geranyl pyrophosphate; FPP: farnesyl pyrophosphate; UQ: ubiquinone; ABCA1: ATP binding cassette transporter A1; apoA-I: apolipoprotein A-I; HDL: high density lipoprotein.

Figure 2. Cholesterol metabolites modulate the immune environment. Anti-tumor immune populations, such as Vγ9Vδ2 T cells, CD8+ T cells, M1 macrophages and mature DCs have increased endogenous MVA pathway, contrarily to tumor tolerant cells (e.g. memory T cells, T-reg cells, M2 macrophages, MDSCs, neutrophils). Cholesterol derived metabolites produced by cancer cells also affect the activity of immune infiltrating cells. IPP binds LXRα that upregulates ABCA1: this transporter effluxes IPP, which activates Vγ9Vδ2 T cells. FPP increases the tumor activity of Ras/ERK/STAT3/IDO axis that produces kynurenine, an immunesuppressive
metabolite, which induces apoptosis of CD8\(^+\) T cells and expands T-reg cells. At the same time, Ras/ERK/HIF-1\(\alpha\) axis upregulates ABCB1, which impairs the tumor cell phagocytosis by DCs. Cholesterol effluxed by tumor cells via ABCA1 activates CD8\(^+\) T cells proliferation and boosts the activation of M1 macrophages and DCs. On the other hand, cholesterol effluxed by M2 macrophages acts as building block for cancer cells. Oxysterols have a general immunesuppressive role: indeed, they inhibit DCs, blunt CD8\(^+\) T cells expansion and activate neutrophils that sustain a metastatic phenotype. MVA: mevalonate; IPP: isopentenyl pyrophosphate; GPP: geranyl pyrophosphate; FPP: farnesyl pyrophosphate; LXR\(\alpha\): liver X receptor \(\alpha\); ABCA1: ATP binding cassette transporter A1; ABCB1: ATP binding cassette transporter B1; IDO: indoleamine dioxygenase; DCs: dendritic cells; T-reg: T-regulatory; MDSCs: myeloid-derived suppressor cells.