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

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

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11

12 **Abstract**

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

25

26 **Key facts:**

- 1 - Cholesterol and its metabolites are actively synthesized by cancer cells and
2 influence initiation, progression and drug resistance.
- 3 - Some of these metabolites are effluxed by cancer cells and modulate the
4 functions of immune cells infiltrating the tumor environment.
- 5 - Pharmacological inhibitors targeting cholesterol metabolism could be
6 repurposed as **immune adjuvant** agents.

7

8 **Keywords:** cholesterol; cancer; **immune modulation**

9

10 **1. Cholesterol metabolism in cancer**

11 Cholesterol is synthesized from acetyl Coenzyme A (AcCoA) in the so called
12 “mevalonate (MVA) pathway”. Most of the enzymes of the MVA pathway are
13 upregulated in tumors (Yang et al., 2020). **This finding explains why tumor tissues**
14 **have higher level of cholesterol than non-transformed tissues.** The pacemaker enzyme
15 3- β -3-hydroxymethylglutaryl Coenzyme A reductase (HMGCR) is a **sterol** sensitive
16 enzyme **and** is transcriptionally induced by the sterol regulatory element binding
17 proteins (SREBPs) (Lee et al., 2020). SREBPs are sequestered within the
18 endoplasmic reticulum by the SREBP cleavage-activating protein/insulin-induced
19 gene-1 (SCAP/INSIG-1) complex if intracellular cholesterol is high; **on the contrary, if**
20 **cholesterol is low, SREBPs are** translocated into the Golgi and cleaved into the
21 transcriptionally active form (Lee et al., 2020). **SREBPs are** often upregulated in
22 cancer cells since the tumoral pyruvate kinase isoform M2 (PKM2) promotes **their**
23 transcription (Zhao et al., 2018). **As a consequence, not** only cholesterol, but also the
24 **upstream** metabolites, such as **MVA**, the isoprenoids isopentenyl pyrophosphate
25 (IPP), geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP), or squalene,

1 are increased. The higher levels of low density lipoprotein receptor (LDLR), which
2 grants a higher uptake of extracellular cholesterol, the altered activity of intracellular
3 sterol transfer proteins and ATP binding cassette transporter A1 (ABCA1), which is
4 the main cholesterol efflux transporter, are additional factors increasing the
5 accumulation of cholesterol and derivatives, such as cholesterol esters (CEs) and
6 oxysterols, within cancer cells (Figure 1).

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

18 Upstream and downstream metabolites play a critical role in cancer initiation and
19 progression as well (Figure 1). IPP is a precursor of the tail of ubiquinone is a key
20 component of the electron transport chain that supports oxidative phosphorylation
21 (OXPHOS), limits lipid peroxidation and prevents ferroptosis (Bersuker et al., 2019).
22 Also squalene protects from ferroptosis, although this effect is tumor-specific and
23 dependent on the levels of endogenous squalene epoxidase and on the resistance to
24 oxidative stress of each tumor (Garcia-Bermudez et al., 2019; Mahoney et al., 2019).
25 FPP and GGPP are essential activators of monomeric G-proteins of Ras, Rho and

1 Rab family that are activated when isoprenylated. Since Ras and Rho mediate cell
2 proliferation, migration, invasion and resistance to chemotherapy, the abundance of
3 FPP and GGPP is a critical factor **controlling** the cell fate of cancer cells (Wang et al.,
4 2016).

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

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

24

25 **2. Cholesterol metabolism regulates the tumor-immune system interaction**

1 If cancer cells have usually a high MVA pathway activity, immune cells display a
2 variable situation, depending on their state. Upon activation, the canonical anti-tumor
3 populations – cytotoxic CD8⁺T lymphocytes, M1 polarized tumor associated
4 macrophages (TAMs) and mature dendritic cells (DCs) – upregulate the
5 Ras/PI3K/mTOR axis that increases SREBP transcriptional activity and MVA pathway.
6 By contrast, quiescent or tumor tolerant immune cells – such as memory T
7 lymphocytes, M2 polarized TAMs, immature DCs – direct AcCoA towards fatty acid
8 oxidation rather than toward cholesterol biosynthesis (Gruenbacher and Thurnher,
9 2017). An active MVA pathway supplies cholesterol to build plasmamembrane: this is
10 critical for rapidly dividing cells as T lymphocytes, but also for DCs and TAMs that must
11 build new plasmamembrane portions during antigen presentation or phagocytosis.
12 Moreover, MVA pathway produces ubiquinone, a critical component of the electron
13 transport chain that supplies activated immune cells with ATP (Thurnher and
14 Gruenbacher, 2015).

15 Neutrophils and TAMs may have anti-tumor or pro-tumor activities, and the cholesterol
16 derived metabolites may switch the activities in these immune infiltrating cells. High
17 levels of cholesterol activate the Toll-like receptor (TLR) present on macrophages,
18 stimulating the assembly of inflammasome. This situation generates a chronic
19 inflammation within the TME that favors tumor progression (Muller et al., 2008). If
20 cholesterol activates the pro-inflammatory phenotype of TAMs, GGPP attenuates TLR
21 signalling by increasing the geranylgeranylation of Ras and its interaction with PI3K:
22 this process blunts the production of inflammatory cytokines (Akula et al., 2016).

23 Contrarily to cholesterol, oxysterols have an immunosuppressive function within the
24 TME. 22-OH-Chol recruits CXCR2-expressing neutrophils able to inhibit the priming
25 and activation of CD8⁺T lymphocytes, and to release pro-metastatic and pro-

1 angiogenic factors (Raccosta et al., 2013). Similarly, 27-OH-Chol favors breast cancer
2 invasion by recruiting polymorphonuclear cells (Baek et al., 2020). 22-OH-Chol also
3 reduces the recruitment of DCs and anti-tumor CD8⁺T lymphocytes by activating a
4 LXR α -dependent transcriptional program in DCs (Villablanca et al., 2009), creating a
5 strongly tumor tolerant TME.

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

9 Intriguingly, the interactions between tumor and immune cells may modulate the MVA
10 pathway in both compartments in a reciprocal way. For instance, ovarian cancer
11 stimulates the efflux of cholesterol from TAMs that in turn is avidly taken up by cancer
12 cells via LDLR or scavenger receptors, promoting tumor growth (Gossens et al., 2019).
13 Moreover, a deregulated MVA pathway in cancer cells affects the activity of different
14 subset of T lymphocytes (Salaroglio et al., 2014; Kopecka et al., 2016). For instance,
15 an active synthesis of FPP promotes the activation of Ras/ERK1/2/STAT3 axis,
16 leading to the transcription of indoleamine dioxygenase (IDO) enzyme. Kynurenine,
17 the product of IDO, is a potent apoptotic stimulus for CD8⁺T lymphocytes and
18 promotes the expansion of tumor tolerant T-regulatory (Treg) cells (Salaroglio et al.,
19 2014; Kopecka et al., 2016). Moreover, Ras/ERK1/2 axis also up-regulates the ABC
20 transporter B1 (ABCB1/P-glycoprotein) that – besides effluxing chemotherapeutic
21 drugs – inhibits the DC-mediated phagocytosis of tumor cells (Kopecka et al., 2016;
22 Kopecka et al., 2020), further contributing to tumor immune resistance. In DCs, the
23 inhibition of GGPP synthesis blocks the geranylgeranylation and activity of Rab5,
24 altering endosome maturation and prolonging the exposure of antigens on the surface,

1 boosting CD8⁺T lymphocytes activity. This event enhances the efficacy of anti-tumor
2 vaccines or immune checkpoint inhibitors (ICPI) (Xia et al., 2018).

3 A correct balance between cholesterol and CEs in CD8⁺T lymphocytes is important to
4 confer the membrane fluidity that is necessary for clustering and signal transduction
5 of T cell receptor (TCR). An increased cholesterol/CE ratio, indeed, creates the optimal
6 conditions for TCR clustering and cytotoxic activity, and enhances the anti-tumor
7 efficacy of ICPI (Yang et al., 2016). MVA pathway is also crucial for Vγ9Vδ2 T cells, a
8 subset of T lymphocytes that have anti-tumor activity against hematological (Castella
9 et al., 2011) and solid (Belisario et al., 2020) tumors. Not only the MVA pathway of
10 tumor cells, but also the MVA pathway of antigen presenting cells such as DCs is
11 critical in activating Vγ9Vδ2 T cells. Both tumor cells and DCs treated with the
12 aminobisphosphonate zoledronic acid, an inhibitor of FPP synthase, accumulate IPP
13 that acts as endogenous ligand of LXRα and upregulates ABCA1. The latter effluxes
14 IPP together with cholesterol and delivers it on apolipoprotein A-I (apo-AI) (Castella et
15 al., 2017). This process, similar to the assembly of high density lipoprotein (HDL)
16 particles, facilitates the activation of Vγ9Vδ2 T cells, since extracellular IPP is the
17 physiological ligand of Vγ9Vδ2 TCR (Castella et al., 2011). ABCA1 cooperates with
18 butirophylin BTN3A1, a surface protein co-localized with ABCA1, critical for
19 presenting IPP to the TCR of Vγ9Vδ2 T cells (Riganti et al, 2018). VγVδ T cells include
20 several subset and their activation is not always beneficial. The recruitment of VγVδ T
21 cells other than Vγ9Vδ2 subset by 27-OH-Chol has a pro-metastatic role in breast
22 cancer: this event, together with the recruitment of polymorphonuclear cells,
23 suppresses the cytotoxic activity of CD8⁺ T lymphocytes (Baek et al., 2017), creating
24 a tumor permissive TME. Moreover, at high concentration of IPP, Vγ9Vδ2 T cells
25 become anergic and exert a tumor tolerant effect (Castella et al., 2015). These findings

1 suggest that quantitative and qualitative differences in the **cholesterol derived**
2 metabolites may produce opposite effects, likely recruiting different subsets of **V γ V δ T**
3 **cells** or suppressing **their anti-tumor functions**.

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

8

9 **3. Therapeutic implications of targeting cholesterol metabolism and future** 10 **perspectives**

11 Despite the high MVA pathway activity in cancer cells, all the regulatory mechanisms
12 are still preserved and sensitive **to pharmacological** inhibitors. Modulating cholesterol
13 metabolism may have a deep impact on the **immune recognition** of cancer cells.
14 Statins and aminobisphosphonates, inhibitors of HMGCR and FPP synthase,
15 respectively, have been already used in various cancer types. The HMG synthase
16 inhibitor dipyrindamole, the squalene epoxidase terfinabine, as well as inhibitors of
17 cholesterol esterification (Avasimibe) and trafficking (**itraconazole**), are under
18 preclinical development as anti-tumor agents. Farnesyl transferase inhibitors, **which**
19 prevent the activation of monomeric farnesylated or geranylgeranylated proteins, have
20 been tested with modest results and high toxicity. Notwithstanding these **negative**
21 **results**, the research on these **compounds** is ongoing, in search of more specific and
22 potent inhibitors (Xu et al., 2020). Except for statins (Sarrabayrouse et al., 2017) and
23 aminobisphosphonates (Salaroglio et al., 2014; Kopecka et al., 2016; Belisario et al.,
24 2020), there are no evidences reporting an **immunesensitizing** effect of the other
25 agents, but – considering the multiple implications of MVA pathway in the immune

1 responses – we cannot exclude that some agents will be effective as
2 **immunesensitizers**. Since MVA pathway is globally **upregulated** in cancer, the most
3 effective strategy **is** the inhibition of the first steps of **the pathway** with statins or the
4 inhibition of the **pathway's controllers**. In this perspective, a great interest has been
5 raised by SCAP/SREBP inhibitors (Lee et al., 2020). One limitation of **cholesterol**
6 **targeting** agents is their low specificity. For instance, statins reduce cholesterol,
7 isoprenylated proteins and ubiquinone in non-transformed tissues, leading sometimes
8 to undesired **side effects**. Tumor-targeting nanocarriers loaded **with MVA** pathway
9 inhibitors may partially overcome this limitation. A second limitation is that both cancer
10 cells and **immune infiltrating** cells have an active MVA pathway: a simultaneous
11 inhibition of cholesterol metabolism in both populations may produce contrasting
12 effects on the anti-tumor **immune activity**. A deeper knowledge of the metabolic **cross**
13 **talks** existing between MVA pathway of cancer cells and immune cells may help to
14 identify the steps and controllers that must be targeted. This knowledge will be
15 translated into novel agents targeting cholesterol metabolism in TME, able to boost
16 the anti-tumor activity of the immune populations.

17

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21

22 **Declarations of interest**

23 None.

24

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24 **Figure legends**

1 **Figure 1. Cholesterol metabolism and effects of cholesterol metabolites in**
2 **cancer cells.** Cholesterol and its derivatives are higher in cancer cells because of the
3 **high *de novo*** synthesis controlled by **INSIG-1/SCAP/SREBP complex** and the higher
4 uptake via LDLR. Cholesterol can be inserted in lipid rafts, critical for proliferation,
5 migration and drug resistance, converted into cholesterol **esters** or oxysterols, which
6 control membrane fluidity and survival, or effluxed via ABCA1. Upstream metabolites
7 of cholesterol have pleiotropic functions: IPP is **a** precursor of ubiquinone of the
8 mitochondrial electron transport chain and an activator of **V γ 9V δ 2 T cells**; FPP and
9 GGPP are necessary for the prenylation and activation of monomeric G-proteins of
10 Ras, Rho and Rab family, controlling proliferation, invasion and antigenicity. LDLR:
11 low density lipoprotein receptor; INSIG-1: insulin-induced gene-1; SCAP: SREBP
12 cleavage-activating protein; SREBP: sterol regulatory element binding protein; ER:
13 endoplasmic reticulum; AcCoA: acetyl **Coenzyme A**; MVA: **mevalonate**; HMGCoA: 3-
14 β -3-hydroxymethylglutaryl **Coenzyme A**; HMGCR: 3- β -3-hydroxymethylglutaryl
15 **Coenzyme A** reductase; IPP: isopentenyl pyrophosphate; GPP: geranyl
16 pyrophosphate; FPP: farnesyl pyrophosphate; UQ: ubiquinone; ABCA1: ATP binding
17 cassette transporter A1; apoA-I: apolipoprotein A-I; HDL: high density lipoprotein.

18 **Figure 2. Cholesterol metabolites modulate the immune environment.** Anti-tumor
19 immune populations, such as **V γ 9V δ 2 T cells**, **CD8⁺ T cells**, M1 macrophages and
20 mature DCs have increased endogenous MVA pathway, contrarily to **tumor tolerant**
21 cells (e.g. memory **T cells**, T-reg cells, M2 macrophages, MDSCs, neutrophils).
22 **Cholesterol derived** metabolites produced by cancer cells also affect the activity of
23 **immune infiltrating** cells. IPP binds LXR α that **upregulates** ABCA1: this transporter
24 effluxes IPP, **which** activates **V γ 9V δ 2 T cells**. FPP increases the tumor activity of
25 Ras/ERK/STAT3/IDO axis that produces kynurenine, an **immunesuppressive**

1 metabolite, **which** induces apoptosis of CD8⁺ **T cells** and expands T-reg cells. At the
2 same time, Ras/ERK/HIF-1 α axis **upregulates** ABCB1, which impairs the tumor cell
3 phagocytosis by DCs. Cholesterol effluxed by tumor cells via ABCA1 activates CD8⁺
4 **T cells** proliferation and boosts the activation of M1 macrophages and DCs. **On** the
5 other hand, cholesterol effluxed by M2 macrophages acts as building block for cancer
6 cells. Oxysterols have a general **immunesuppressive** role: indeed, they inhibit DCs,
7 **blunt CD8⁺ T cells expansion** and activate neutrophils that sustain a metastatic
8 phenotype. MVA: **mevalonate**; IPP: isopentenyl pyrophosphate; GPP: geranyl
9 pyrophosphate; FPP: farnesyl pyrophosphate; LXRA: liver X receptor α ; ABCA1: ATP
10 binding cassette transporter A1; ABCB1: ATP binding cassette transporter B1; IDO:
11 indoleamine dioxygenase; **DCs**: dendritic cells; T-reg: T-regulatory; MDSCs: myeloid-
12 derived suppressor cells.