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REVIEW ARTICLE

# Metabolic features of cancer stem cells: the emerging role of lipid metabolism

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## Abstract

Cancer stem cells (CSCs) are an uncommon subset of tumor cells capable of self-renewal, differentiating, and recreating the parental tumor when transplanted into the murine background. Over the past two decades, efforts toward understanding CSC biology culminated into identifying a set of signaling pathways sustaining "stemness". Nevertheless, while metabolic rewiring is nowadays considered a hallmark of cancer, no consensus has been reached on the metabolic features underlying the plastic nature of CSCs, which are capable of residing in a dormant state, and able to rapidly proliferate when the need to repopulate the tumor mass arises. An emerging concept in the field of CSC metabolism is that these cells are extremely reliant on the activity of enzymes involved in lipid metabolism, such as stearoyl-CoA desaturase 1 (SCD1) and 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR). Indeed, SCD1 and HMG-CoAR have been described as key factors for the correct function of a number of concatenated pathways involved in CSC fate decision, such as Hippo and Wnt. In the present review, we describe metabolic futures of CSCs with a special focus on lipid metabolism, which until now represents an underappreciated force in maintaining CSCs and an attractive therapeutic target.

## Introduction

Seminal evidence documenting that cancer cells are characterized by metabolic alterations dates back to the first half of the twentieth century, with the pioneering work of the German physiologist Otto Warburg [1]. Central to the original appreciation of metabolic processes exploited by cancer cells to cope with their increased metabolic demands was the increased consumption of glucose in comparison to non-proliferating normal cells. This occurs via a seemingly paradoxical process in terms of ATP production per molecule of glucose, defined as the Warburg effect or aerobic glycolysis. Indeed, while cells generally use oxidative phosphorylation (OXPHOS) as the main pathway to produce energy, cancer cells can produce ATP via glycolysis even under normoxic conditions. Even though aerobic glycolysis is significantly less efficient than OXPHOS, it is more rapid and accounts for the glucose avidity seen in cancer cells. Shortly after, Harry Eagle described that optimal growth of cultured HeLa cells required an excess of glutamine in the medium as compared to other amino acids [2]. Moreover, glutamine was demonstrated to be the most rapidly consumed amino acid by cancer cells, with a consequent depletion of glutamine from the tumor environment [3–6]. Glutamine consumption provides carbon and amino-nitrogen that serve for the biosynthesis of amino acids, nucleotides, and lipids [7]. While cancer metabolism has traditionally been equated with the Warburg effect, a process perceived as inefficient and considered an indirect consequence of neoplastic transformation, the development of more sophisticated biochemical and molecular assays elucidated the bi-directional relationship between genetic derangements that characterize tumors and metabolic reprogramming. While, on the one hand, mutations in

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oncogenes and loss-of-function alterations of oncosuppressors increase the metabolic demands of cancer cells and require an adaptive response to ensure cell fitness, on the other some metabolites possess oncogenic roles, interfering with intracellular signaling, gene expression, and cellular differentiation [8]. On this basis, metabolic reprogramming is nowadays listed among the so-called hallmarks of cancer [9]. In parallel, evidence on deregulated metabolic pathway nodes raised the idea that pharmacological inhibition of metabolic routes may hold therapeutic implications, prompting clinical trials that specifically looked at the antitumor activity of compounds widely prescribed for metabolic disorders such as diabetes and obesity (e.g. metformin and statins) [10–20]. In this review, we describe the origin and evolution of the CSC model. Afterwards, we discuss recent advances in our understanding of the metabolic phenotype of CSCs with special emphasis being placed on an emerging branch of CSC metabolism, namely lipid metabolism.

## Metabolic reprogramming and the tumor microenvironment

Metabolites produced by cancer cells not only influence cancer cell fate, but have the potential to modify cellular dynamics in the tumor microenvironment. It has been widely established that a variety of non-transformed cells such as tumor-associated fibroblasts cancer-associated fibroblasts (CAFs), endothelial cells, and immune cells undergo phenotypic changes when interacting with tumor cells, in a process that further encourages tumor growth [21]. Reprogramming of the metabolic machinery also influences the tumor-stroma crosstalk. For instance, the accumulation of extracellular lactate stemming from the elevated utilization of glucose and glutamine by cancer cells attenuates activation of dendritic and T cells, while stimulating the polarization of resident macrophages to a tumor-promoting state (M2 state or alternatively activated) [22–25]. In such a manner, metabolic reprogramming depresses the antitumor immune response and participates in installing an immune-permissive microenvironment. Next, the excess of lactate increases the production of angiocytokines by endothelial cells, and leads to acidification of the cellular microenvironment [26–29]. In turn, acidification of the extracellular space stimulates the proteolytic activity of matrix metalloproteinases and cathepsins, ultimately enhancing matrix degradation and tumor invasion [28, 29]. Reciprocally, adverse conditions existing within the tumor microenvironment, such as hypoxia and the paucity of nutrients, force cancer cells to evolve metabolic strategies to thrive in a hostile environment [30–41].

## Cancer metabolism: heterogeneous by nature

It has become increasingly clear that a unique model of cancer metabolic program cannot transversally apply to the entire spectrum of cancer types, nor to the various intrinsic subtypes existing within tumors arising in a given body site. This scenario is further complicated when considering that tumors are heterogeneous diseases, composed by different clones and sub-clones that harbor distinct genetic alterations, replicative capabilities, and metabolic requirements [42]. Indeed, levels of nutrient consumption, such as glucose and oxygen, are spatially heterogeneous within the same tumor mass, as routinely observed in clinical practice with positron emission tomography-based imaging. Intratumor heterogeneity was traditionally explained with Darwinian principles of evolution [42]. According to this view, random acquisition of favorable mutations and epigenetic alterations enables some clones to expand and endure perturbations in their microenvironment, ultimately gaining a survival advantage over other clones. Over the past two decades, this model was questioned by the discovery of an uncommon cellular pool endowed with a set of unique properties, such as the ability to self-renew and differentiate, along with tumor-forming ability when transplanted into immunocompromised mice [43]. Commonly defined as cancer stem cells (CSCs), this cellular subset has gained popularity and, with the development of specific in vitro and in vivo assays for purification, characterization, and quantification, CSCs have been defined as the root of metastatic dissemination and therapeutic resistance. An emerging concept is that CSCs rely on a variety of metabolic avenues whose engagement fluctuates over time [44]. Indeed, CSCs can reside in a dormant-like state (quiescence), while rapidly proliferating to fulfill specific needs (i.e. replacing dying cells after cytotoxic therapies). Intuitively, quiescence, self-renewal, and differentiation imply an elevated degree of metabolic plasticity. In turn, some typical features of tumors, such as hypoxia and low pH, have been connected with the acquisition of stem-like traits [45, 46].

## The CSC model

First prospective identification of CSCs was provided in 1997, when a “vertical” architecture was observed in acute myeloid leukemia resulting from the existence of a rare and phenotypically distinct subset of tumor cells able to propagate the tumor in immune-deficient mice [47]. Ever since, a wave of studies relying on a common experimental approach, consisting in flow cytometry-based separation of different cellular subsets (on the basis of the expression of

cell-surface markers) followed by serial transplantation into immunocompromised mice, allowed to separate a rare fraction of tumorigenic cancer cells from the bulk of tumor cells, which failed to recreate a neoplastic lesion when transplanted in the murine background [48–53]. On this basis, a hierarchical model of cancer was postulated, envisioning few CSCs at the top of the pyramid that give rise to progenitors and differentiated cells. Thus, the cardinal property of CSCs is self-renewal, defined as the process through which, upon cell division, a stem cell generates one (asymmetric division) or two (symmetric division) daughter cells that retain stem cell features, thus ensuring the maintenance/expansion of the stem cell compartment. Nevertheless, it is worth mentioning that non-uniform experimental conditions have been exploited in studying CSCs over time (i.e. freshly isolated patient-derived CSCs vs commercial cell lines). On this basis, a great deal of attention nowadays is posed on the experimental workflow for CSCs isolation, purification, and characterization, with particular emphasis on gold-standard in vivo assays. These include serial transplantation experiments to address tumorigenic potential and the ability to recapitulate the histological features of the parental tumor, and limiting-dilution assays for estimating CSC frequency.

As originally formulated, the CSC model postulated the existence of a rigid hierarchy within a tumor. This "fixed" state was in stark contrast with the highly dynamic clonal evolution model, which was shaped on Darwinian principles of evolution. Nevertheless, in more recent years functional studies harmonized the two models, introducing the concept of "dynamic stemness", a process that describes the acquisition of stem-like traits by non-CSCs [54]. Central in elucidating the dynamic nature of the stem cell state was the reprogramming of fibroblasts achieved through the forced expression of a specific set of transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) [55]. With this approach, differentiated cells were reprogrammed into cells harboring the functional properties of embryonic stem cells, defined as induced pluripotent stem cells (iPSCs). Ever since, evidence has accumulated conveying the message that, upon exposure to opportune conditions, non-CSCs can acquire stem-like features. This functional conversion was demonstrated by exposing cancer cells to specific microenvironmental conditions. More specifically, the non-stem to CSC transition was obtained upon exposure to myofibroblast-secreted factors that activate β-catenin-dependent transcription, through cytokines released by tumor-associated cells, via epithelial–mesenchymal transition, and in the presence of specific microenvironmental conditions such as hypoxia and low pH [45, 46, 56–58]. Moreover, multiple evidence side with the hypothesis of clonal evolution in the CSCs pool, spanning from genetic heterogeneity of cancer-propagating cells and the existence of distinct pools of tumor-initiating cells to enrichment of CSCs upon exposure to anticancer therapies or with disease progression [59–65]. Overall, the concept of "dynamic stemness" relies on evolutionary principles, namely the adaption to perturbations arising in the ecosystem. Thus, the CSC concept was refined over the past years, and the two apparently antithetic models nowadays appear not to be mutually exclusive.

## Metabolic features of CSCs

While the metabolic portrait of CSCs has been the focus of intense investigation in recent years, it still remains unclear whether these cells are predominantly glycolytic or they rather exploit OXPHOS.

The idea that CSCs are primarily glycolytic is rooted into the supposed similarities between CSCs and their normal counterparts. Indeed, while multipotent stem cells are glycolytic, their differentiated offspring mostly rely on OXPHOS [66]. Moreover, reprogramming of normal cells into iPSCs is accompanied by a switch from OXPHOS to a glycolytic program, which precedes the acquisition of pluripotency markers [67, 68]. Further supporting this view, mitochondria in iPSCs revert to an immature state, which is characterized by fewer and less mature mitochondria (low content of mitochondrial DNA and low levels of intracellular ATP and reactive oxygen species (ROS)) compared with differentiated cells, whereas differentiation was coupled with mitochondria maturation (functionally active mitochondria with increased levels of ATP and ROS) [69]. In turn, the reduced levels of ROS are essential for maintaining quiescence and self-renewal ability [70]. Regarding studies specifically focusing on CSCs, it has been observed that the side population exhibits higher levels of glycolytic activity compared to the bulk of tumor cells, whereas glucose starvation resulted in a decline of the side population [71]. Consistently, glycolysis inhibition obtained by 3-BrOP hindered tumor-forming ability [71]. The side population assay is a flow cytometry method that exploits the Hoechst dye efflux properties of ATP-binding cassette (ABC) transporters, a transport system deputed to extrude xenobiotics from the cells [72]. Thus, the side population represents a multi-drug resistant cellular fraction enriched in CSCs.

Likewise, basal-like breast cancer cells harboring the CSC phenotype $CD44^{+}CD24^{low}EPCAM^{+}$ are dependent on an OXPHOS to aerobic glycolysis switch [73]. A number of studies further provided hints that CSCs are more glycolytic than their differentiated counterparts: this was observed in nasopharyngeal carcinoma, ovarian cancer, osteosarcoma, glioblastoma (GBM), and colon cancer [74–78].

Other studies reported that CSCs prevalently adopt mitochondrial oxidative metabolism and tolerate glucose deprivation. For instance, clonogenicity of GBM stem cells (GBM-SCs) was abolished upon OXPHOS, but not of glycolysis, inhibition [79]. This metabolic portrait of GBM-SCs was linked to the activity of the oncofetal insulin-like growth factor 2 mRNA-binding protein 2 (IMP2, IGF2BP2), which regulates OXPHOS by participating in the assembly and function of mitochondrial respiratory complexes [79]. Moreover, BCL-2 inhibition efficiently targeted OXPHOS, leading to the selective elimination of $ROS^{low}$ quiescent leukemia stem cells [80]. In pancreatic ductal adenocarcinoma (PDAC), $CD133^{+}$ CSCs rely on the transcription factor PPARGC1A (PGC-1α), a central regulator of mitochondrial biogenesis, for proper OXPHOS function, self-renewal, and in vivo tumor-forming capacity [81]. Consistently, inhibition of mitochondrial respiration triggered apoptosis in CD133+ PDAC cells. Conversely, a MYC-mediated glycolytic program was selectively activated in more differentiated PDAC cells, a process tied to the negative control operated by MYC on PGC-1α [81].

Studies investigating the metabolic liability of tissue-resident stem cells provided some interesting clues that might help dissect the metabolic portrait of CSCs, highlighting the importance of the tumor microenvironment in which stem cell reside (stem cell niche). For instance, Paneth cells, which support intestinal stem cell (ISC) function in the intestinal crypt, exploit glycolysis to produce lactate [82]. This, in turn, is converted into pyruvate in ISCs to support mitochondrial OXPHOS. Likewise, OXPHOS seems to be required for satellite cell activation and proliferation [83]. Conversely, stem cells residing in hypoxic niches (e.g. bone marrow niche), such as hematopoietic stem cells and mesenchymal stem cells, rely on glycolysis to fulfill their metabolic demands [84, 85]. On this ground, it is plausible to speculate that metabolic features of CSCs might be different in relation to the tissue of origin, as well as in the site in which they metastasize.

## Lipid metabolism and CSCs

Given the increasing interest surrounding tumor metabolism, novel evidence is shedding light on alterations in lipid- and cholesterol-associated pathways, which have often been disregarded in the past. Proliferating tumor cells require lipids and cholesterol, a need which is fulfilled by an increased uptake of exogenous lipids and lipoproteins and/or by hyper-activating the metabolic routes deputed to produce lipids (lipogenesis) and cholesterol (mevalonate pathway) [86]. Consistently with the belief that lipid dysfunctions are associated with more aggressive molecular traits, a gene expression study revealed that up-regulation of transcripts related to lipogenesis and cholesterol synthesis pathways is associated with adverse survival outcomes in colorectal cancer patients [87]. Moreover, lipids present in cell membranes, and in particular cholesterol and sphingolipids, form planar microdomains (lipid rafts) that, in cancer cells, contain an array of signaling proteins and receptors involved in pro-oncogenic and apoptotic pathways [88]. Indeed, lipid raft disruption hinders cancer cell proliferation by inhibiting AKT activation [89]. Cancer cells also use lipids coming from adipocytes residing in the microenvironment as their energy source [90, 91]. The co-culture of adipocytes and ovarian cancer cells results in the transfer of lipids from adipocytes to ovarian cancer cells promoting their growth, and a similar strategy is adopted by metastatic bone marrow-derived prostate cancer cells [90, 91]. When specifically looking at the stem cell compartment, it has been demonstrated that both hematopoietic stem cells and leukemia-initiating cells (LICs) rely on fatty acid oxidation (FAO) [92, 93]. Interestingly, LICs co-opt the adipose tissue niche to create a supportive microenvironment that encourages leukemic growth and chemoresistance [93]. According to this model, LICs colonizing the adipose tissue release pro-inflammatory cytokines that elevate lipolysis in the gonadal adipose tissue. This, in turn, leads to the secretion of free fatty acids that fuels FAO in LICs, via the elevated expression of the fatty acid transporter CD36. A similar role for CD36 was also envisioned for oral CSCs [94]. In hepatocellular carcinoma (HCC), the nexus between FAO and stem-like properties was mechanistically identified in the stem cell factor NANOG, which represses OXPHOS genes and activates FAO [95]. This effect might be connected to the uncoupling of FAO from ATP synthesis, as observed in leukemia cells that exploit de novo fatty acid synthesis to support FAO [96].

Next, the excess of lipids in cancer cells is stored into cytoplasmic organelles, probably originating from the endoplasmic reticulum and/or the Golgi apparatus, called lipid droplets (LDs) [86]. Elevated levels of LDs are associated with tumor aggressiveness and accumulation of LDs has been observed in circulating tumor cells whose enumeration, in turn, has been proposed for predicting survival outcomes [97–99]. An increased content of LDs is a distinctive feature of $CD133^{+}$ colorectal CSCs (CR-CSCs), as revealed by Raman spectroscopy imaging [100]. Indeed, a direct correlation was observed between LD content, CD133 expression, and activated Wnt signaling in CR-CSCs. Moreover, upon sorting CR-CSC lines in $LDs^{High}$ and $LDs^{Low}$, it was observed that $LDs^{High}$ cells had greater clonogenic potential, as assessed by in vitro sphere-forming efficiency and in vivo tumor-forming ability.

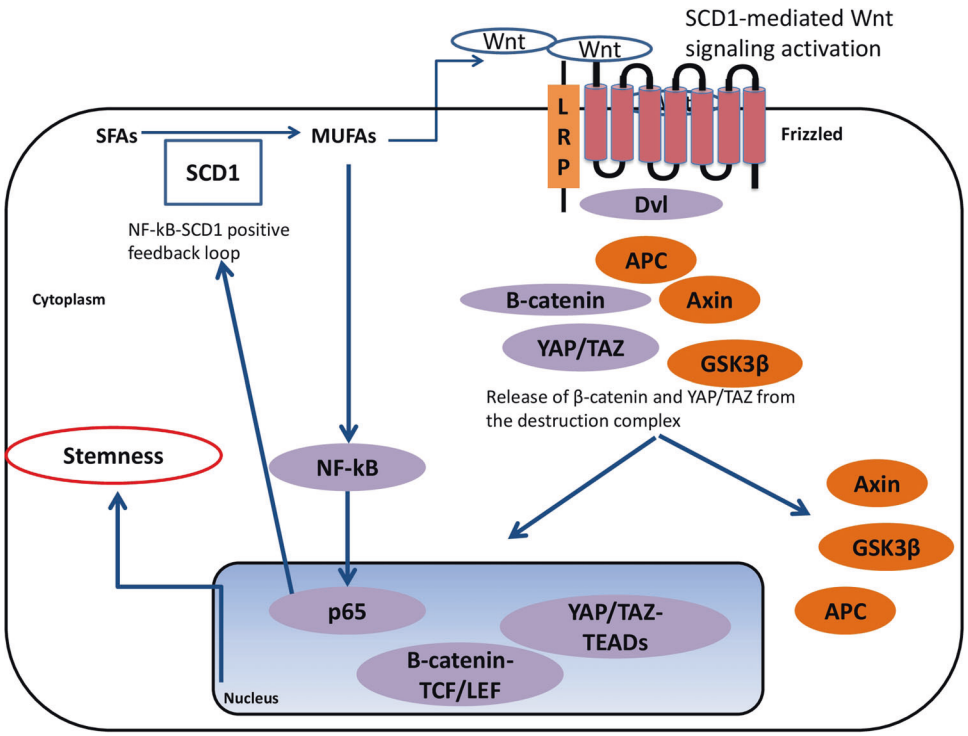


**Fig. 1** SCD1-mediated control of CSCs. SCD1 enhances the production of lipid-modified Wnt proteins that activate the canonical Wnt pathway. Activation of the Wnt pathway leads to the release of both β-catenin and YAP/TAZ from the destruction complex. This enables β-catenin and YAP/TAZ to translocate to the nucleus where, upon interaction with their transcriptional partners, they mediate the reprogramming of cancer cells into CSCs. SCD1 promotes stemness also via the activation of the NF-kB pathway that, in turn, feeds a positive feedback loop by increasing the expression levels of lipid desaturases

## Lipid metabolism and CSCs: the role of SCD1

The connection between lipid metabolism and CSC fate mostly stemmed from studies exploring the activity of stearoyl-CoA desaturase 1 (SCD1) (Fig. 1) [101–104]. SCD1 is a central enzymatic node in the conversion of saturated fatty acids into monounsaturated fatty acids (MUFAs) [105]. MUFAs represent the precursors of a number of lipids essential for plasma membranes, such as triglycerides, cholesterol esters, diacylglycerols, and wax esters [105].

Gene expression profiling carried out to identify genes differentially expressed between pleural effusion-derived lung cancer cells growing as spheroids or under adherent conditions revealed a significant up-regulation of SCD1 in tumor-spheres [101, 106, 107]. SCD1 inhibition, achieved either by RNA interference or the small-molecule inhibitor MF-438, hindered sphere-forming efficiency in a process accompanied by the reduced expression of stem cell markers including ALDH1A1, Nanog, and Oct4. Spheroids treated with the SCD1 inhibitor MF-438 showed many ultrastructural features of cellular damage, such as cytoplasmic vacuolization, mitochondrial swelling, apoptotic nuclei and, in some instances, nuclear fragmentation. Moreover, pharmacological inhibition of SCD1 induced anoikis in ALDH1A1-positive cells, and spheroids generated in the presence of MF-438 had decreased tumorigenic potential upon xenotransplantation in mice as compared to untreated cells. Of note, more differentiated lung cancer cells were unaffected by the abrogation of SCD1 function, thus suggesting that SCD1 activity and the consequent dependence on MUFA generation may be a distinctive trait of CSCs. Moreover, SCD1 inhibition reverted chemoresistance in lung CSCs [108]. These findings are consistent with a high-throughput screen of small molecules carried out to identify compounds capable of killing human pluripotent stem cells, revealing that an SCD1 inhibitor was the most potent compound in inducing endoplasmic reticulum (ER) stress response, attenuation of protein synthesis, and cell death [109]. Mechanistically, SCD1-mediated regulation of CSCs was linked to the control operated by this enzyme on two closely related oncoproteins, namely the Hippo transducers Yes-associated protein (YAP) and its paralogue transcriptional co-activator with PDZ-binding motif (TAZ) [102]. YAP/TAZ represent the transcriptional module of the Hippo signaling, an evolutionary conserved pathway that plays a central role during organ development and tissue repair after injuries [110]. Over the past years, deregulated Hippo pathway and aberrant YAP/TAZ-driven transcription have been observed in multiple tumor types, and tied to the generation of CSCs [110]. By silencing SCD1 in lung cancer spheroid cultures, Noto et al. [102] documented a significant decrease of YAP/TAZ at the protein level together with a decline in their nuclear expression. Furthermore, SCD1 inhibition hampered YAP/TAZ-driven gene transcription, as documented by reduced mRNA levels of the YAP/TAZ target genes birc5 and ctgf. Intriguingly, both SCD1 and YAP/TAZ are molecularly

intertwined with the wnt/β-catenin pathway [111, 112]. Consistently, SCD1 abrogation also led to a preferential cytoplasmic distribution of β-catenin along with a reduction in axin2 levels, a transcriptional target of the β-catenin-TCF/LEF complex [102]. The model proposed for explaining the dual control operated by SCD1 on YAP/TAZ and β-catenin envisioned that SCD1 mediates the release of Wnt ligands. This results in the activation of the canonical Wnt signaling, with the consequent dislocation of β-catenin and YAP/TAZ from the β-catenin destruction complex, their nuclear accumulation and transcription of target genes. As already reported in a study exploring the effects of Wnt signaling and YAP/TAZ on the expansion of intestinal progenitor cells [111], the crosstalk between YAP/TAZ and Wnt occurred independently from the activation of the Hippo regulatory module, the negative controller of YAP/TAZ. In line with the oncogenic role of SCD1, YAP/TAZ, and β-catenin, the combined expression of SCD1/YAP, SCD1/TAZ, and SCD1/β-catenin were associated with inferior survival outcomes [102]. Evidence from breast CSCs (BCSCs) enforced the link between SCD1, the Wnt pathway, and stemness [103]. El Helou et al. [103] carried out a miRNome-wide loss- and gain-of-function screens that led to the identification of micro-RNA (miR)-600 silencing as one of the most potent hits in expanding the BCSC pool. MiR-600-mediated control of BCSCs was correlated with the targeting of SCD1. Indeed, the knockdown of miR-600 de-repressed SCD1 that, in turn, generated oleic acid that is essential for the secretion of lipid-modified Wnt proteins that activate the canonical Wnt pathway. At the molecular level, miR-600 inhibition increased b-catenin transactivator activity, promoted its nuclear accumulation, and increased Wnt3a levels in the medium [103]. Next, Li et al. [104] recently reported that ovarian CSCs (O-CSCs) had significantly higher levels of unsaturated lipids compared with their more differentiated counterparts. Consistently, SCD1 mRNA expression level was significantly higher in ALDH+/CD133+ cells as compared with ovarian cancer cells that did not harbor this repertoire of cell-surface markers. Inhibition of SCD1 obtained with small-molecule inhibitors (CAY10566 and SC-26196) or shRNAs reduced lipid unsaturation levels in spheroids, and led to a drop in the expression of a battery of stem cell markers (ALDH1A1, Sox2, Nanog, and Oct-4). Not surprisingly, SCD1 inhibition hampered sphere-forming efficiency in vitro and suppressed tumor-forming ability. Remarkably, ovarian cancer cells grown as monolayers (containing fewer CSCs) and non-transformed cells (human dermal fibroblasts) were fairly insensitive to desaturase inhibitors, thus further supporting the idea that lipid desaturation represents a distinctive feature of CSC and a potential CSC-directed therapy. Mechanistically, pharmacological inhibitors of SCD1 downregulated a set of stem cell-related signalings in OC spheroids including nuclear factor-kB (NF-kB), Hedgehog, and Notch. In particular, desaturase inhibition suppressed NF-kB transcriptional activity, as denoted by the reduced expression of IL-6 and IL-8. In turn, forced overexpression of p65 significantly up-regulated SCD1, whereas the NF-kB inhibitor DMAPT decreased both lipid unsaturation and SCD1 levels in tumor-spheres [104]. Collectively, these data indicated the existence of a positive feedback loop between SCD1 and NF-kB signaling that sustains "stemness" in ovarian cancer cells. Similarly, the silencing of ferritin heavy chain in ovarian cancer cells increased the expression of both SCD1 and stem cell markers, in a process accompanied by epithelial-to-mesenchymal transition and increased sphere-forming efficiency [113]. Recently, it has been observed that SCD1 regulates liver CSCs (HCC), whereas its inhibition forced these cells to differentiate via the ER stress-induced unfolded protein response (UPR) [114]. Thus, the anti-CSC properties of SCD1 inhibition seems to be correlated with both the inhibition of specific signaling pathways linked to "stemness" and a broader effect represented by ER stress/UPR activation. Finally, the connection between SCD1 and signaling transduction pathways extends beyond stem cell-associated molecular networks. For instance, Zhang et al. [115] provided evidence that EGFR phosphorylates SCD1, thus maintaining SCD1 protein stability and increasing MUFA levels in a process that feeds lung cancer growth.

Overall, enhanced activation of SCD1 and the consequent production of MUFAs appear to be a hallmark of CSCs. On this ground, pharmacological inhibition of SCD1 function may delineate a metabolic vulnerability of CSCs.

## Lipid metabolism and CSCs: the role of HMG-CoAR

3-Hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR) is the rate-limiting enzyme in the mevalonate pathway and the molecular target of statins, the popular cholesterol-lowering agents [116]. The mevalonate pathway represents the metabolic route that leads to the production of steroid-based hormones, cholesterol, and non-sterol isoprenoids. Moreover, this metabolic avenue profoundly affects intracellular signal transduction. Indeed, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), produced in the mevalonate cascade, are required for protein prenylation, a post-translational modification process that enables correct membrane tethering of Ras and Rho family of small guanosine triphosphatases (GTPases). In turn, small GTPases are essential for the function of G-protein-coupled receptors (GPCRs) that, in a neoplastic background, mediate an array of tumor-enhancing functions

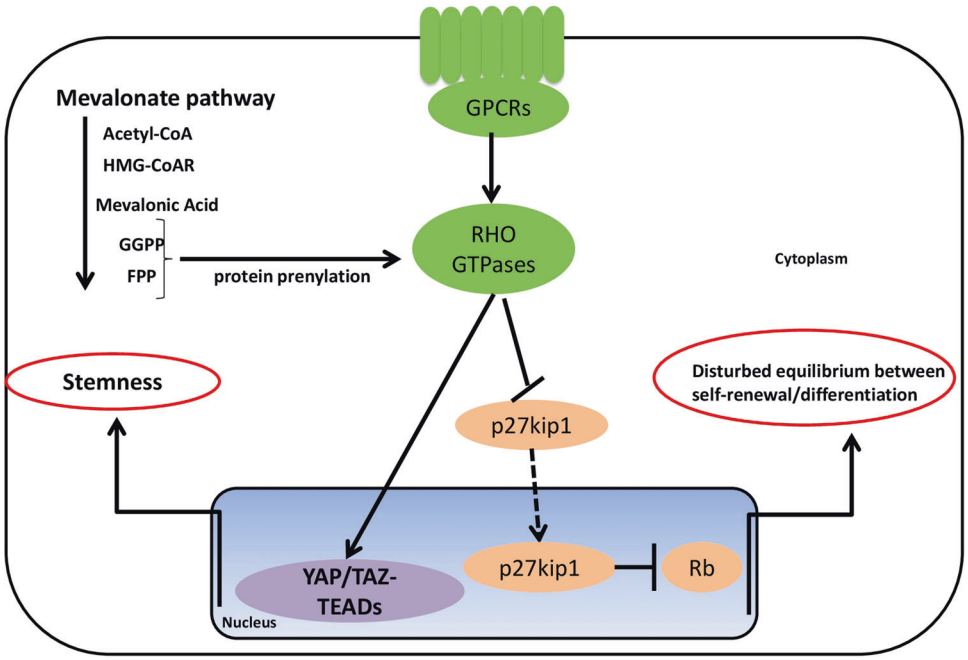


**Fig. 2** HMG-CoAR-mediated control of CSCs. The mevalonate cascade culminates in the production of FPP and GGPP, which are essential for correct membrane anchoring of RHO GTPases. In turn, RHO GTPases maintain "stemness" by activating the Hippo transducers YAP/TAZ and by promoting the degradation of $P27^{kip1}$

ranging from proliferation and survival to invasion and metastasis [116].

A gene expression profile carried out for identifying molecular networks selectively activated in BCSCs revealed that enzymes of the mevalonate pathway were over-represented in basal-like tumor-spheres as compared to matched cells growing under adherent conditions [117]. Inhibition of the mevalonate cascade through simvastatin reduced the CSC population, as assessed by ALDEFLUOR assay, and decreased sphere-forming efficiency. Conversely, addition of mevalonate to the culture medium rescued the ALDEFLUOR-positive population and sphere-forming ability. Mevalonate pathway-mediated maintenance of the BCSC compartment was found to be dependent on protein geranylgeranylation. Indeed, the geranylgeranyl transferase inhibitor GGTI-298 reduced the CSC population by hindering correct membrane anchoring of RHOA. Molecularly, defective activity of RHOA enabled $P27^{kip1}$ to translocate to the nucleus, where it inhibits RB activation ultimately favoring CSC differentiation [117]. Likewise, Sorrentino et al. [118] demonstrated that the mevalonate pathway promotes nuclear localization and activity of YAP/TAZ, whereas the administration of statins led to an accumulation of YAP/TAZ in the cytoplasm. The block imposed by statins on the YAP/TAZ transcriptional program impaired self-renewal ability of BCSCs, as monitored by mammosphere-forming assay, and these effects were recapitulated with other compounds acting at different levels of the mevalonate cascade, including the farnesyl diphosphate synthase inhibitor zoledronic acid and the geranylgeranyl transferase inhibitor GGTI-298. Metabolic control of YAP/TAZ was linked to the production of GGPP with the consequent activation of RHOA, and occurred in a LATS1/2-independent manner. These findings were further corroborated by experiments in *Drosophila*, an established model system for studying the effects of Hippo pathway component manipulation. Indeed, while the forced expression of the YAP/TAZ orthologue *Yorkie* caused a well-known phenotype characterized by a dramatic overgrowth or various organs and appendages, statins or the silencing of the endogenous geranylgeranyl transferase (*ggt-I*) rescued tissue overgrowth and inhibited the Yorkie target genes Diap-1 and expanded (Fig. 2) [118].

Next, it has been recently observed that brain tumor-initiating cells (BTICs) are characterized by elevated mRNA expression levels of mevalonate pathway genes, even including HMG-CoAR, as compared to their differentiated counterparts [119]. Inhibition of HMG-CoAR by shRNAs or statins consistently reduced sphere-forming efficiency. Overexpression of mevalonate pathway genes was linked to MYC-induced gene transcription and, in turn, the mevalonate pathway regulates MYC, thus indicating the existence of a feed-forward regulatory loop between the mevalonate cascade and MYC that sustains BTICs.

Finally, it is interesting to note that both HMG-CoAR and SCD1 have been associated with CSC fate through the activation of oncogenic YAP/TAZ, albeit via different molecular mechanisms. Indeed, the mevalonate cascade promotes YAP/TAZ-driven gene transcription via protein prenylation and Rho GTPases that, in turn, activate YAP/TAZ [118]. Conversely, the connection between SCD1 and CSCs relies on functional and biochemical evidence that

collocate YAP/TAZ in the β-catenin destruction complex [111]. This latter encompasses a set of proteins that, in the absence of Wnt ligand stimulation, retains β-catenin in the cytoplasm and enables its degradation. SCD1 promotes the secretion of lipid-modified Wnt proteins that activate the canonical Wnt pathway, thus disrupting the β-catenin destruction complex and ultimately leading to the nuclear accumulation of both YAP/TAZ and β-catenin [103].

## Therapeutic targeting of SCD1 and HMG-CoAR: challenges and opportunities

Evidence converge on assigning a CSC-promoting function to the excess of MUFAs generated by SCD1. In this regard, two aspects deserve particular mention. First, elevated SCD1 activity seems to be a hallmark of CSCs, given that both non-CSCs and non-transformed cells survived unaffected SCD1 inhibition [101, 104]. This holds promise from a therapeutic perspective, given that SCD1 inhibition might selectively target CSCs, while sparing normal cells, thus minimizing side effects. Second, SCD1 lies at the centerpiece of an intricate molecular network that co-regulates various signaling pathways widely associated with CSC fate, such as Hippo, Wnt, and NF-kB [102–104]. Thus, pharmacological inhibition of SCD1 may hold the potential to shut down multiple crosstalking oncogenic routes controlling CSC functions, which are currently undruggable with specific inhibitors. Nevertheless, it is worth mentioning that the forces driving SCD1 transcriptional and post-transcriptional up-regulation still remain largely unknown. Clarifying the molecular network accountable for the increased SCD1 transcription and enzymatic activity seen in CSCs is a node that should be unraveled to fully appreciate the therapeutic potential of SCD1-directed therapy. Indeed, the existence of positive feedback loops controlling SCD1 expression may represent an adaptive mechanism CSCs evolve in order to adapt to pharmacological inhibition of SCD1.

Similar considerations apply to the mevalonate pathway, with the advantage that enzymes of the mevalonate cascade can be inhibited with compounds already in use in clinical practice, such as statins and nitrogen-containing bisphosphonates. Even though the pharmacological targeting of protein prenylation has not yet yielded convincing proof of efficacy [120], some strategies might provide further evidence encouraging their clinical development. First, pre-surgical window-of-opportunity trials, consisting in the short-term administration of a given compound in the time elapsing between diagnosis and surgery, can provide evidence on whether these agents are actually able to target the CSC compartment [10]. Indeed, availability of pre- and post-treatment tissues offers the possibility to evaluate CSC-related endpoints, spanning from the evaluation of CSC markers and/or CSC gene modules to modification in sphere-forming efficiency. Second, it is unlikely that the administration of mevalonate pathway-targeting agents in the metastatic setting results in a significant improvement of survival outcomes, given that this approach does not configure the targeting of an oncogene addiction capable of producing a rapid tumor shrinkage. Conversely, the delivery of statins or farnesyltransferase inhibitors in conditions characterized by a low tumor burden (adjuvant setting) might efficiently kill residual/disseminated CSCs.

## Conclusions

Over the past years, there has been compelling evidence supporting the view that CSCs account for the maintenance of neoplastic lesions, distant dissemination, and resistance to anticancer therapies. Functional characterization of signaling pathways required for self-renewal was recently paralleled by studies that delineated the central role of metabolic reprogramming in the control of CSC fate.

As anticipated, whether CSCs actually utilize glycolysis or OXPHOS is still a matter of debate, even though the preferential use of OXPHOS may generate a selective advantage in an environment characterized by limited resources. Nevertheless, it is worth noting that a series of hurdles are encountered when attempting to decipher the metabolic portrait of CSCs. First, CSCs are intrinsically plastic and the stem cell state is a dynamic condition. Thus, metabolically plasticity may characterize CSCs, as they can either reside in a quiescent state or proliferate to replenish the original tumor mass. For instance, it has been proposed that, when quiescent, CSCs harbor an oxidative phenotype; in contrast they switch to a combined glycolytic/oxidative metabolic program when forced to proliferate [44]. Second, poor adherence to gold-standard assays for CSC isolation and quantification (long-term clonal growth in functional repopulation experiments and limiting-dilution transplantation assays) generated confusion in the CSC field. This aspect is further remarked when interpreting studies on CSC metabolism, where different experimental conditions have been used, with some reports exploiting commercial cell lines and others freshly isolated, patient-derived CSCs. Third, CSC frequency seems to be higher than hypothesized in the past, as it is highlighted with the use of extremely immunocompromised mice (NOD/SCID vs NOD/SCID interleukin-2 receptor gamma chain null mice) [59]. Fourth, current animal models lack adequate stromal support, thus resulting in the deprivation of paracrine-acting signals involved in “stemness” acquisition. In addition, this leads to underestimating the effects of metabolites released by cancer cells on the various cell types cohabiting the

microenvironment. As aforementioned, lactate affects the function of various non-transformed cell types, even including immune cells, thus participating in installing a pervasive relationship between cancer cells and their neighbor non-transformed cells. Collectively, these observations call for extreme caution when interpreting studies describing metabolic alterations of CSCs, and highlight non-negligible, and still unresolved, issues that need to be addressed to fully appreciate the metabolic repertoire of CSCs. In our opinion, the following points need to be clarified: (i) the link between metabolic plasticity and the transition among the various states characterizing CSCs (quiescence, self-renewal, differentiation), (ii) the way through which CSC metabolism is influenced by microenvironmental conditions and vice versa, and (iii) the elucidation of metabolic changes plausibly occurring at the CSC levels upon administration of anticancer therapies, based on the documented CSC enrichment occurring after conventional anticancer therapies [61, 121].

To sum up, metabolism is no longer viewed as a mere epiphenomenon that accompanies malignant transformation and the transition between the various states that denote the intrinsic plasticity of CSCs. Rather, metabolic plasticity appears to be a central force that enables CSCs to modify their replicative capabilities according to specific needs. Given the considerable interest driven by the development of CSC-directed therapies, dissecting the metabolic features of CSCs may represent a novel therapeutic strategy to achieve the goal of CSC elimination. To this end, adherence to gold-standard assays for CSC characterization along with the use of humanized mouse models able to re-create the supportive infrastructure of human tumors appears necessary steps towards achieving a full appreciation of the metabolic vulnerability of CSCs and its clinical exploitability.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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