Hallmarks of cancer stem cell metabolism

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Cancer cells adapt cellular metabolism to cope with their high proliferation rate. Instead of primarily using oxidative phosphorylation (OXPHOS), cancer cells use less efficient glycolysis for the production of ATP and building blocks (Warburg effect). However, tumours are not uniform, but rather functionally heterogeneous and harbour a subset of cancer cells with stemness features. Such cancer cells have the ability to repopulate the entire tumour and thus have been termed cancer stem cells (CSCs) or tumour-initiating cells (TICs). As opposed to differentiated bulk tumour cells relying on glycolysis, CSCs show a distinct metabolic phenotype that, depending on the cancer type, can be highly glycolytic or OXPHOS dependent. In either case, mitochondrial function is critical and takes centre stage in CSC functionality. Remaining controversies in this young and emerging research field may be related to CSC isolation techniques and/or the use of less suitable model systems. Still, the apparent dependence of CSCs on mitochondrial function, regardless of their primary metabolic phenotype, represents a previously unrecognised Achilles heel amendable for therapeutic intervention. Elimination of highly chemoresistant CSCs as the root of many cancers via inhibition of mitochondrial function bears the potential to prevent relapse from disease and thus improve patients' long-term outcome.

Cellular Metabolism. In non-transformed, mostly slowly proliferating or even quiescent somatic cells, mitochondria represent the main source of energy production through the tricarboxylic acid (TCA) cycle coupled to oxidative phosphorylation (OXPHOS), which takes place in the mitochondrial matrix. Several carbon fuels such as pyruvate, glutamine and fatty acids can feed into the cycle to produce reducing equivalents (nicotinamide adenine dinucleotide phosphate, NADH; Flavin adenine dinucleotide, $FADH_2$) that are subsequently used as electron donors for the electron transport chain (ETC). The transport of electrons across the different complexes of the ETC is coupled to the generation of a proton motive force, used by ATP synthase (complex V) to generate ATP (Chandel, 2014).

Cancer cells, however, are characterised by high proliferation and thus need to adapt their cellular metabolism in order to provide constant support for the increased division rate: rapid ATP generation to maintain energy status, increased biosynthesis of macromolecules and tight regulation of the cellular redox status (Vander Heiden et al, 2009). Moreover, tumour cells must evade the checkpoint controls that under physiological conditions inhibit proliferation in the challenging metabolic conditions regularly found in the tumour microenvironment. Levels of glucose, glutamine and oxygen are spatially and temporally heterogeneous and frequently sparse as compared with conditions in well-perfused organs.

Accordingly, tumour cells reprogramme their metabolic machinery to meet their needs during tumour growth, but also during the changing conditions of the metastatic process.

For this purpose, cancer cells shift from ATP generation via OXPHOS to ATP generation via glycolysis, despite having still sufficient oxygen concentrations in the tumour microenvironment (Warburg effect). As a result, many transformed cells derive a substantial amount of their energy via aerobic glycolysis, which is more rapid than OXPHOS, but also far less efficient in terms of ATP generated per unit of glucose consumed, resulting in an abnormally high rate of glucose uptake. Under these circumstances, glucose is also metabolised through the pentose phosphate pathway (PPP) and other alternative pathways (Vander Heiden et al, 2009), which produce large quantities of reduced NADPH and other macromolecules to generate the necessary building blocks required for sustaining high rates of cellular division.

Cancer stem cells. Over the past decade it has been conclusively demonstrated for leukaemia and various solid cancers that not all malignant cells are functionally equivalent. Convincing evidence now demonstrates that substantial (epi-)genetic heterogeneity exists within each individual tumour. First, multiple subclonal populations of cancer cells are assumed to foster tumour adaptation and therapeutic failure through Darwinian selection.

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Second, cancer heterogeneity also exists within each of these subclones, despite their identical genetic background, via the acquisition of stemness features in a subset of cells. The resulting hierarchical organisation of the tumour is vaguely reminiscent of that found in many normal tissues (Figure 1) (Hermann *et al*, 2007; Malanchi *et al*, 2012; Visvader and Lindeman, 2012; Garcia-Silva *et al*, 2013; Miranda-Lorenzo *et al*, 2014; Chen *et al*, 2015). At the apex of this hierarchy are populations of cancer stem cells (CSCs) or tumour-initiating cells (TICS), capable of self-renewal, bearing long-term *in vivo* tumourigenicity as well as generating more differentiated progenies constituting the epigenetically defined intraclonal bulk tumour cells (Hermann *et al*, 2007).

This new view of intraclonal functional heterogeneity bears the potential to fundamentally change the way we should analyse and treat cancer. To aid this, there will be a need to understand the mechanisms underlying this close relationship between stem cells and their malignant counterparts, which includes their metabolic features. Although CSCs do not necessarily arise from tissue stem cells, these cells have acquired stemness features allowing them to indefinitely self-renew and give rise to their respective differentiated progenies. Epigenetic regulation mimicking, at least in part, normal differentiation contributes to the generation of these hierarchically organised clones that, although sharing common mutation profiles, bear diverse gene expression patterns and functions (Visvader and Lindeman, 2012). Accumulating evidence also suggests striking parallels between mechanisms orchestrating normal embryogenesis and those that invoke tumourigenesis and CSCs in particular (Lonardo *et al*, 2011). One important feature of (embryonic) stem cells relies in their

distinct metabolic phenotype when compared with their differentiated progenies (Folmes *et al*, 2013) and arising evidence now suggests that CSCs are no exception.

METABOLIC PHENOTYPE OF CSCS

The metabolic phenotype of CSCs has been the subject of intense investigation over the past years. Interestingly, CSCs have been described as primarily glycolytic or preferentially relying on OXPHOS in a tumour type-dependent manner, but contradictory results for the same tumour entity have also been reported (Table 1). The following paragraphs summarise the key findings for each category.

Evidence for primarily glycolytic CSCs. Originally, it was hypothesised that CSCs should bear a metabolic phenotype reminiscent of normal tissue hierarchy where multipotent stem cells are fundamentally glycolytic, while differentiated somatic cells rely on OXPHOS (Folmes *et al*, 2012). Similar patterns have been reported for induced pluripotent stem cells (iPS cells), where the reprogramming process is associated with a switch from OXPHOS to a glycolytic programme, which is indeed essential for effective acquisition of a pluripotent state. These findings did not only suggest that the metabolic phenotype and stemness are intrinsically linked, but rather that the cellular metabolism actually controls stemness properties. Thus, it was postulated that activation of the glycolytic programme favours stemness via different mechanism, including enhanced antioxidative capacity, with PPP being most relevant.
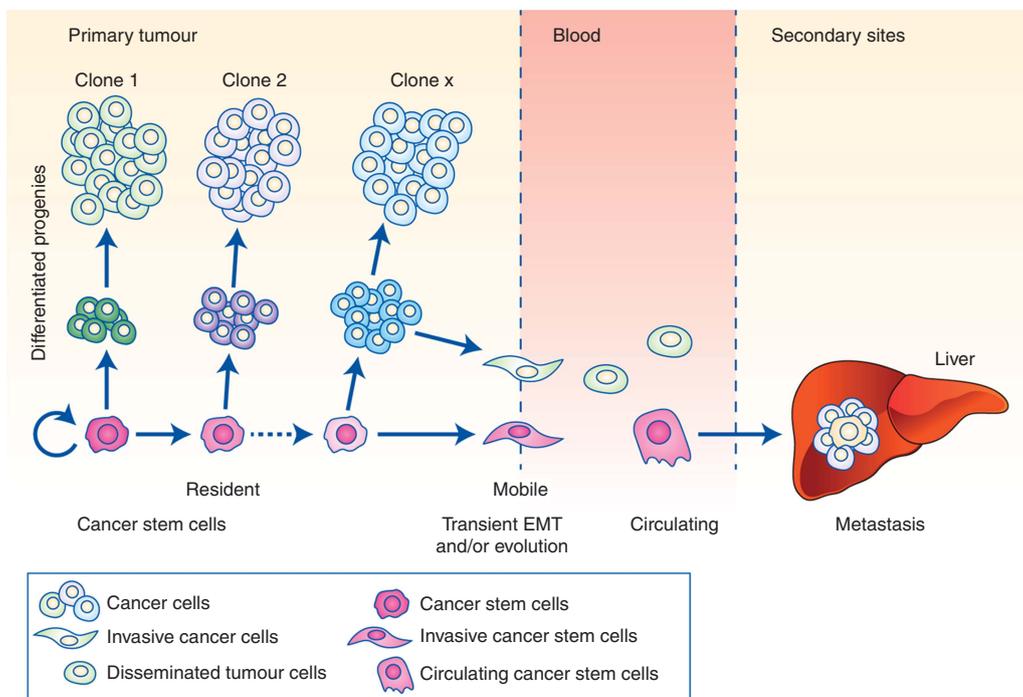
Figure 1. The cancer stem cell concept in cancer progression and metastasis. For various solid cancers it has been shown that intraclonal heterogeneity is formed by CSCs and their differentiated progenies (left) (Visvader and Lindeman, 2012; Miranda-Lorenzo *et al*, 2014). CSCs are capable of undergoing unlimited cell division while retaining their stem cell identity (self-renewal) and giving rise to more differentiated cells with limited or no tumour-initiating and metastatic capacity, despite their high proliferative capacity. CSCs evolve as the tumour progresses via (epi-) genetic alterations, but also in response to interactions with their niche, leading to diverse CSC subclones with distinct functionality (Sainz *et al*, 2014, 2015). While both CSCs and differentiated cancer may acquire enhanced mobility, for example, via epithelial–mesenchymal transition (EMT), to date only arising metastatic CSCs have been shown to initiate secondary lesions (Hermann *et al*, 2007; Malanchi *et al*, 2012) and are tractable as circulating CSCs in the blood (Clausell-Tormos *et al*, 2014; Yang *et al*, 2015) (centre). Importantly, these cells must survive the hostile environment of the blood stream, evade immune surveillance and extravasate at a distant location to form metastatic lesions, rendering the process extremely inefficient (right).

Table 1. Identified metabolic phenotype for various cancer types (in chronological order)

Cancer type	Year	Model/material	Experimental setting	Phenotype	Sensitivity to mitochondrial targeting	References
Breast	2010	Cell lines	CD44$^+$CD24^{low}	ND	Metformin	(Vazquez-Martin et al, 2010)
	2013	Cell lines	SP$^+$ sphere-forming cells	ND	Niclosamide	(Wang et al, 2013)
	2013	Cell lines	CD44$^+$CD24^{low}EPCAM$^+$ cells	Glycolytic	ND	(Dong et al, 2013)
Lung	2011	Cell line	SP$^+$ cells, Sphere-forming cells	OXPHOS	ND	(Ye et al, 2011)
Glioblastoma	2012	Fresh human tumours, PDX	CD133$^+$ cells, Gliomaspheres	OXPHOS	ND	(Janiszewska et al, 2012)
Ovarian	2012	Cell lines	SP$^+$ cells	ND	Niclosamide	(Yo et al, 2012)
	2014	Mouse Ovarian Surface Epithelium cells (MOSE)	Serial in vivo passaging	Glycolytic	Oligomycin, Antimycin, Rotenone, Metformin	(Anderson et al, 2014)
	2014	Fresh human samples	CD44$^+$CD117$^+$ cells	OXPHOS	Oligomycin, Antimycin, Rotenone, Metformin	(Pasto et al, 2014)
AML	2013	Primary cultures from human samples	Quiescent ROS^{low} cells	OXPHOS	ABT-263	(Lagadinou et al, 2013)
Nasopharyngeal	2013	Cell lines	Radioresistant sphere-forming cells	Glycolytic	Resveratrol	(Shen et al, 2013)
	2015	Cell lines	Radioresistant sphere-forming cells	Glycolytic	FCCP	(Shen et al, 2015)
Pancreas	2015	Primary cultures, fresh PDX tumours	CD133$^+$ cells, Sphere-forming cells	OXPHOS	Metformin, Resveratrol, Rotenone, Menadione	(Sancho et al, 2015)
Liver	2015	Fresh tumours (murine/human)	CD133$^+$CD49f$^+$ cells	Glycolytic	Paraquat	(Chen et al, 2015)

Abbreviations: ND = not determined; OXPHOS = oxidative phosphorylation; PDX = patient-derived xenografts.

Subsequently, a number of investigations aimed to expand the concept of glycolysis-driven stemness to CSCs and experiments in breast cancer cell lines did support this hypothesis. For example, Dong et al (2013), demonstrated that the metabolic switch from OXPHOS to aerobic glycolysis was essential for the functionality of breast CD44$^+$CD24^{low}EPCAM$^+$ CSCs, due to decreased ROS levels. Moreover, glycolysis was also found to be the preferred metabolic programme in radioresistant sphere-forming cells in nasopharyngeal carcinoma (Shen et al, 2015) and CD133$^+$CD49f$^+$ TICS in hepatocellular carcinoma (Chen et al, 2015). Interestingly, elevated expression of oncogenic MYC was identified as the main driver of stemness for these three cancer types (Gabay et al, 2014), which is in line with findings for iPS cells. While MYC levels did not determine the metabolic wiring of iPS cells, their tumorigenic potential as evidenced by teratoma formation was intrinsically linked to a MYC-driven glycolytic programme (Folmes et al, 2013). Therefore, MYC is a likely candidate for linking glycolysis and stemness, which is intimately related to the tumorigenic potential of iPS cells, and the cancer types listed above.

Evidence for OXPHOS-dependent CSCs. Surprisingly, CSCs in other cancer types have demonstrated OXPHOS as the preferred energy production process. To date, this has been shown convincingly for side population cells in lung cancer (Ye et al, 2011), sphere-forming and CD133$^+$ cells for both glioblastoma (Janiszewska et al, 2012) and pancreatic ductal adenocarcinoma (PDAC) (Sancho et al, 2015), and ROS^{low} quiescent leukaemia stem cells (Lagadinou et al, 2013). Besides glucose, CSCs may also rely on mitochondrial fatty acid oxidation (FAO) for ATP and NADPH generation. Self-renewal in both hematopoietic stem cells and leukaemia-initiating cells appears to be dependent on FAO (Samudio et al, 2010; Ito et al, 2012), a metabolic process that also sustains ATP production and survival in epithelial cancer cells following loss of matrix attachment (Schafer et al, 2009; Carracedo et al, 2012). Interestingly, the pluripotency factor NANOG promoted a highly tumourigenic and chemoresistant metabolic programme in CD133$^+$CD49f$^+$ hepatocellular carcinoma cells by directly inducing the expression of FAO genes (Chen et al, 2016).

Therefore, FAO inhibition offers an additional pharmacological strategy to target mitochondrial metabolism in CSCs (Carracedo et al, 2013).

Although the mechanisms determining the observed OXPHOS phenotype have not yet been fully characterised for all aforementioned tumour types, regulatory proteins of mitochondrial biogenesis and structure could have a crucial role in maintaining stemness properties and functionality (Janiszewska et al, 2012; Sancho et al, 2015). Indeed, findings for PDAC clearly demonstrated that expression of the transcription factor PPARGC1A (PGC-1α), a master regulator of mitochondrial biogenesis, in pancreatic CD133$^+$ CSCs was essential for their OXPHOS functionality and, most importantly, self-renewal and maximal in vivo tumorigenic capacity (Sancho et al, 2015). Intriguingly, a MYC-driven glycolytic programme could only be found in more differentiated PDAC cells and overexpression of MYC actually counteracted stemness via negatively controlling PGC-1α expression. These data seem to challenge the concept for MYC favouring stemness via activation of glycolysis, as demonstrated for iPS cells and other cancer types. However, those apparently contradictory findings could be reconciled in a concept where MYC serves as a general modulator of differentiation, promoting either stemness or differentiation in a context and cell type-dependent manner.

Interestingly, the metabolic rewiring to OXPHOS rendered CSCs in these tumour types resistant to inhibition of glycolysis, which may provide the cells with a higher degree of independency from microenviromental nutrient supply. Indeed, OXPHOS equips CSCs with increased resistance to nutrient deprivation and, in general, to the metabolic austerity characterising many solid tumours. Although OXPHOS operates at a significantly lower rate, it constitutes a far more efficient source for energy generation. Thus, OXPHOS-dependent CSCs may acquire a selective advantage in the context of specific tumour microenvironments, as they make better use of the limited nutrients. In addition, lactate excreted by more differentiated cancer cells that are preferentially running on glycolysis may in return serve as additional fuel for oxidative respiration in cellular subsets that depend on mitochondrial metabolism, that is, CSCs, constituting a metabolic symbiosis system (Nakajima and Van Houten, 2013).

Controversies and the importance of the utilised model systems. In addition to the above differences in the metabolic phenotype of CSCs derived from various tumour types, contradictory results regarding the CSC metabolic phenotype have also been reported for individual cancer types. Several reasons may account for these unexpected differences. Most importantly, the definition and isolation techniques for CSCs were not uniform across these studies and thus different cellular entities with diverse properties may have been studied. For example, in murine models of ovarian cancer, TICs were obtained following serial *in vivo* passages (MOSE-L$_{FFv}$) and were found to be primarily glycolytic (Anderson *et al*, 2014) as compared with respective parental cells (MOSE-E). On the other hand, CD44$^+$CD117$^+$ cells isolated from primary human cultures, either directly from patients with ovarian cancer or following *in vivo* expansion in immunocompromised mice (PDX models), were OXPHOS-dependent (Pasto *et al*, 2014). Apart from the potential differences in species, isolated CD44$^+$CD117$^+$ cells are unlikely to be comparable to MOSE-L$_{FFv}$ cells. The latter may represent the *in vivo* selection of clones with enhanced aggressiveness, not necessarily presenting stem-related properties.

Even more importantly, first studies on the metabolic phenotypes of CSCs were based on established cancer cell lines. The findings in these studies that the selected putative CSCs are highly glycolytic remains at least debatable as cell lines represent rather homogeneous phenotypes, often with no clear CSC subset based on functional assays (Miranda-Lorenzo *et al*, 2014) and also lack a suitable tumour microenvironment. Indeed, very recently in lung cancer the metabolic dependencies were found to be different between *in vitro* and *in vivo* settings (Davidson *et al*, 2016), highlighting the importance of the microenvironment for the metabolic phenotype. In line with these observations, certain end products of glycolysis such as high-energy lactanes and ketones released by strongly glycolytic stromal cells promoted the expression of stemness-associated genes and shifted cancer cells towards OXPHOS (Martinez-Outschoorn *et al*, 2011). Thus, the metabolic phenotype of cancer (stem) cells may heavily depend on microenvironmental conditions and should be studied in fresh tumour samples or, at least, in early passage primary (co-)cultures, where tumour cells are more likely to still present a phenotype reminiscent of that within the tumour. It remains to be determined whether the phenotype of CSCs originally reported for some cancer types will eventually be validated in clinically more relevant models.

Mitochondrial function is crucial for CSC phenotypes. In addition to constituting a major source of ATP for cancer cells, mitochondria participate in controlling multiple signalling pathways, including the release of Cytochrome C to initiate apoptosis, the release of bioactive ROS and the production of metabolites such as acetyl-CoA regulating protein acetylation (Chandel, 2014). As such, mitochondria also appear to regulate stemness properties, irrespective of the underlying metabolic phenotype in individual cells (Diehn *et al*, 2009; Lamb *et al*, 2014; Sancho *et al*, 2015; Lamb *et al*, 2015c). Indeed, enhanced mitochondrial biogenesis appears to represent a key factor for CSC functionality in both glycolytic and OXPHOS-dependent CSCS (Janiszewska *et al*, 2012; De Luca *et al*, 2015; Sancho *et al*, 2015). Increased mitochondrial mass, a surrogate marker for elevated mitochondrial biogenesis, can be easily tracked and may identify cells with enhanced self-renewal capacity and chemoresistance (De Luca *et al*, 2015; Farnie *et al*, 2015; Lamb *et al*, 2015a; Sancho *et al*, 2015) independently of the cancer type. Specifically, in PDAC, the use of this metabolic biomarker allowed to identify a subpopulation of CD133$^+$ cells with low mitochondrial mass, but increased metabolic plasticity driven by MYC within the CSC compartment (CD133$^+$Mito^{low}) (Sancho *et al*, 2015). Consequently, these cells showed increased

resistance to mitochondrial targeting. However, the metabolic plasticity came at the expense of a reduced self-renewal capacity and *in vivo* tumorigenic potential, suggesting a delicate balance between stemness and metabolism, thus adding another level of complexity to the metabolic features of CSCs. Whether the state of these two different CSC subpopulations with differential metabolic features and tumorigenic potential is dynamic and cells are able to transition between states, or is hard wired due to distinct genetic backgrounds remains to be elucidated.

TARGETING CELLULAR METABOLISM

The apparent OXPHOS dependence of CSCs in various tumours and the recently identified role of mitochondria in the regulation of stemness properties (Diehn *et al*, 2009) suggest that targeting mitochondrial metabolism could be an effective pharmacological strategy for the elimination of CSCs. Moreover, as mitochondria in cancer cells are often altered by mutations in their vulnerable DNA, the pharmacological disruption of certain mitochondrial processes could damage CSCs without affecting healthy tissues also relying on OXPHOS. Pharmacological agents targeting OXPHOS at various levels are currently being explored in preclinical and clinical studies for cancer treatment (Figure 2).

Specifically, targeting mitochondrial OXPHOS could be an effective strategy to eliminate cancer cells, which cannot fully meet their energetic demands by glycolysis, either due to limited availability of glucose in poorly vascularised tumours, glycolysis inhibition by current therapies or restricted metabolic plasticity as observed in CD133$^+$ CSCs (Janiszewska *et al*, 2012; Sancho *et al*, 2015). Inhibition of mitochondrial respiration by agents blocking ETC complexes selectively induced apoptosis in CD133$^+$ versus CD133$^-$ cells (Sancho *et al*, 2015). The fact that such agents are also effective in eliminating primarily glycolytic CD44$^+$CD24^{low} cells in breast cancer or side population cells in nasopharyngeal carcinoma (Vazquez-Martin *et al*, 2010; Shen *et al*, 2013), highlights the importance of mitochondria for CSCs beyond energy production. In fact, tumour cells displaying mutations impairing TCA cycle or ETC, thus predominantly relying on glycolysis for ATP production, still require active mitochondria for the generation of metabolites from glutamine via reductive carboxylation (Mullen *et al*, 2012).

Drug screens aiming for the identification of compounds that selectively eliminate CSCs resulted in the selection of several FDA-approved compounds that inhibit mitochondrial activity. For example, the antibiotic salinomycin, which inhibits OXPHOS, was identified in a screen of breast cancer-initiating cells displaying an EMT phenotype and eliminated the CSC gene expression signature in subsequent *in vivo* studies (Gupta *et al*, 2009). Remarkably, salinomycin was also selected in an independent screen aimed at targeting colorectal cancer cells in glucose-deprived multicellular tumour spheroids with inner hypoxia (Senkowski *et al*, 2015), which may more closely reflect the *in vivo* microenvironment of CSCs in solid tumours. Besides salinomycin, four other compounds were identified (nitazoxanide, niclosamide, closantel and pyrvinium pamoate), all of them inhibiting mitochondrial respiration. Niclosamide, an anti-helmitic drug that uncouples mitochondrial OXPHOS, was also among the selected compounds in two independent screens using side population cells derived from breast and ovarian cancers, respectively (Yo *et al*, 2012; Wang *et al*, 2013). Apart from the direct inhibition of mitochondrial complexes, OXPHOS can also be suppressed by inhibitors of mitochondrial translation, for example, using the antibiotic tigecycline, which was identified in a screen using OXPHOS-dependent leukaemia cells (Skrtic *et al*, 2011).
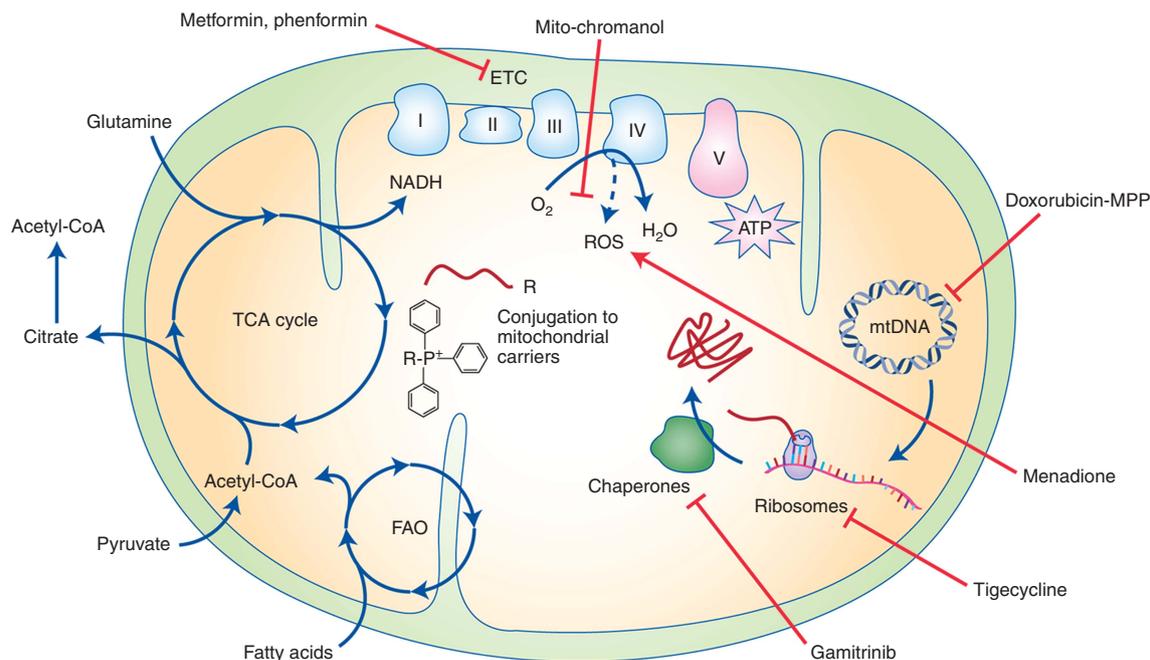
Figure 2. Targeting cancer stem cells through inhibition of mitochondrial function. CSCs dependent of OXPHOS can be eliminated by various strategies impairing mitochondrial energy metabolism. Direct inhibition of OXPHOS can be achieved with small molecules such as the antidiabetic agents metformin and phenformin, which inhibit the ETC Complex I and cause cell death by energy crisis in CSCs. Conjugation of pharmacologic agents to mitochondrial carriers such as TPP or mitochondria-penetrating peptides (MPPs) may allow their selective delivery and accumulation in mitochondria. This strategy has already been used for chemotherapeutic agents such as doxorubicin to selectively disrupt mtDNA integrity and the expression of ETC proteins. Mitochondrial protein biosynthesis can also be blocked by the inhibition of mitochondrial ribosomes using tigecycline and other FDA-approved antibiotics, which impair OXPHOS and bear toxicity against CSCs. Similarly, the functionality of ETC components can also be targeted by mitochondrial delivery of the chaperone inhibitor gamitrinib. Cell signalling by OXPHOS-generated mitochondrial ROS is crucial for cancer cell proliferation and can be targeted by the mitochondrial accumulation of antioxidants such as mito-chromanol. Conversely, CSCs can be eliminated by inducing toxic levels of ROS in mitochondria with the ROS-inducer menadione. Finally, OXPHOS can also be impaired at the level of mitochondrial carbon metabolism, either by altering the enzymes involved in the TCA cycle or fatty acid oxidation (FAO) or by interfering with the supply of mitochondrial fuels.

As mitochondria originally evolved from bacteria, it is not surprising that multiple antibiotics can disrupt mitochondrial function. Indeed, a recent study suggested that the self-renewal capacity in multiple tumour types could be targeted by treatment with certain widely prescribed antibiotics via disrupting mitochondrial respiration, either by the inhibition of mitochondrial ribosomes or by the direct targeting of OXPHOS (Lamb *et al*, 2015b, c).

The antidiabetic agent metformin has also emerged as a promising candidate for targeting OXPHOS in pancreatic sphere-forming and CD133^{+} cells, respectively (Sancho *et al*, 2015). Interest in metformin emerged after retrospective studies suggested a lower incidence of cancer in diabetic patients treated with metformin compared with other antidiabetic regimens. Although the reduced insulin levels may also contribute to the reported effects, compelling evidence demonstrated that the anti-tumoural activity of metformin involved the impairment of OXPHOS via direct inhibition of mitochondrial Complex I (Wheaton *et al*, 2014). Interestingly, metformin selectively induced apoptosis in pancreatic CD133^{+} cells as a result of their inability to switch to glycolysis and subsequent energy crisis (Sancho *et al*, 2015). Still, a minor subset of CD133^{+} cells displaying low mitochondrial mass and a predominantly glycolytic metabolism were inherently resistant to metformin. The presence of these cells in PDAC appeared to account for the observed uniform relapse of tumours in mice treated with metformin and may provide an alternative rational for the negative outcome of the first clinical trials testing the effects of metformin in PDAC patients (Kordes *et al*, 2015), while,

based on recent pharmacokinetics data (Chandel *et al*, 2016; Dowling *et al*, 2016; Sivalingam *et al*, 2016), the originally quoted insufficient bioavailability of metformin seems less likely to be a major contributing factor.

These data suggest that metformin and other drugs targeting mitochondrial ATP production need to be combined with agents counteracting the mechanism of resistance allowing some CSCs to overcome OXPHOS inhibition, for example, via modulation of the PGC1-α/MYC ratio. Intriguingly, metformin resistance in pancreatic CD133^{+} cells could be prevented/reversed by the knockdown of MYC expression using shRNA or indirectly via bromodomain and extra-terminal motif (BET) inhibition, suggesting the therapeutic potential of MYC inhibition in combination with mitochondrial targeting for PDAC treatment (Sancho *et al*, 2015). Alternatively, agents interfering with mitochondrial function at various levels may be more effective in targeting all CSCs. In fact, while drug resistance was observed with most OXPHOS inhibitors, such as rotenone or resveratrol, resistance was not observed for menadione, a drug which acts via dual mechanism – inhibition of Complex I and induction of mitochondrial ROS. On the other hand, the efficacy of metformin may also be limited by its requirement of organic cation transporters (OCTs) for cellular uptake, which restricts its effects in healthy tissues but also limits its potential use to tumour cells expressing OCTs. Phenformin, another biguadine formerly used in diabetes, could overcome this limitation as it is more hydrophobic and is delivered to mitochondria more efficiently than metformin. Phenformin also promotes cancer cell death by inhibiting Complex I and has offered promising

preclinical results in certain cancers such as non-small-cell lung carcinoma (Shackelford *et al*, 2013).

Therefore, an important factor concerning the design of novel pharmacological strategies targeting mitochondria is to ensure the efficient and preferably selective delivery of the drug to the mitochondria in cancer cells. CSCs relying on OXPHOS show an elevated mitochondrial membrane potential ($\Delta\psi$m), which can be exploited for selectively increasing drug delivery to the mitochondria of these cells. Delocalised lipophilic cations such as triphenylphosphonium (TPP) accumulate in the mitochondrial matrix and can be conjugated to small compounds for selective drug delivery to mitochondria (Murphy, 2008). The mitochondrial accumulation of mito-chromanol, a vitamin E analogue conjugated to TPP, induced cell death by inhibiting OXPHOS in breast cancer cells without affecting non-transformed cells (Cheng *et al*, 2013). Conjugation with TPP has also been utilised to selectively deliver the chaperone inhibitor gamitrinib to active mitochondria and disrupt energy production in cancer cells by impairing protein folding in mitochondria (Chae *et al*, 2012). However, mitochondria-penetrating peptides might be preferred for the treatment of certain tumours, as they can deliver cargo molecules irrespective of $\Delta\psi$m. Their conjugation to chemotherapeutic agents, such as doxorubicin, directs their activity towards mitochondrial DNA (mtDNA), promoting drug selectivity for cancer cells with reduced mtDNA integrity while their stable mitochondrial localisation prevents the acquisition of resistance by drug efflux (Chamberlain *et al*, 2013).

CONCLUSIONS

In various cancers, CSCs have now been shown to bear a distinct metabolic phenotype and are highly dependent on OXPHOS and/or mitochondrial function. The reasons are likely multifactorial. Apart from gaining greater independence from the sparse nutritional support in the tumour microenvironment, increasing evidence now also suggests that the tight control of mitochondrial ROS production in CSCs is a prerequisite for maintaining their stemness and high fidelity. This apparent metabolic vulnerability provides vast new therapeutic opportunities to more efficiently eliminate these highly tumourigenic cells, even though resistance may arise in some instances (Sancho *et al*, 2015). The latter could be related either to the acquisition of metabolic plasticity in a subset of CSCs or to the pre-existence of a small subset of CSCs with a higher degree of metabolic plasticity. As the latter bear reduced stemness properties, they are less likely to significantly contribute to cancer progression in treatment naive tumours, but metabolic targeting may provide these cells with a selective advantage so that they eventually take over and drive relapse of the disease. Thus, a more effective strategy could be to combine distinct targeting strategies or to use mitochondria-targeting agents with dual mechanism of action. Efficient metabolic targeting of all CSCs may help to eventually improve the still poor outcome of many cancer patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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