REVIEW

Mitochondria as therapeutic targets for cancer stem cells

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Abstract

Cancer stem cells (CSCs) are maintained by their somatic stem cells and are responsible for tumor initiation, chemoresistance, and metastasis. Evidence for the CSCs existence has been reported for a number of human cancers. The CSC mitochondria have been shown recently to be an important target for cancer treatment, but clinical significance of CSCs and their mitochondria properties remain unclear. Mitochondria-targeted agents are considerably more effective compared to other agents in triggering apoptosis of CSCs, as well as general cancer cells, *via* mitochondrial dysfunction. Mitochondrial metabolism is altered in cancer cells because of their reliance on glycolytic intermediates, which are normally destined for oxidative phosphorylation. Therefore, inhibiting cancer-specific modifications in mitochondrial metabolism, increasing reactive oxygen species production, or stimulating mitochondrial permeabilization transition could be promising new therapeutic strategies to activate cell death in CSCs as well, as in general cancer cells. This review analyzed mitochondrial function and its potential as a therapeutic target to induce cell death in CSCs. Furthermore, combined treatment with mitochondria-targeted drugs will be a promising strategy for the treatment of relapsed and refractory cancer.

Key words: Cancer stem cells; Mitochondria; Relapsed and refractory cancer; Therapeutic target; Mitochondrial energy metabolism

Core tip: This review is devoted to the analysis of mitochondrial function as a therapeutic target to induce cell death in cancer stem cells (CSCs). In particular, we focused on the differences in energy metabolism and features between CSC and non-CSC mitochondria, and between CSCs and normal stem cells. We described the roles of mitochondria that may make CSCs more susceptible to anti-cancer treatment and apoptosis, and how these may be useful to develop novel strategies for cancer treatment, such as through combined therapy

with specific mitochondrial-targeting drugs.

INTRODUCTION

Over the last decade, cancer therapies have improved the quality of life of cancer patients. However, although almost all developed anti-cancer drugs are apparently successful following initial therapy, secondary tumors development and disease relapse is common. The limitation of classical anti-cancer therapies has been attributed recently to the existence of cancer stem cells (CSCs), which are quiescent, have relatively small population, and highly drug-resistant cells. CSCs act like stem cells (SCs) and are responsible for cancer growth and metastasis[1]. Through the continued effort of many researchers, CSCs features have been revealed, such as anti-cancer drug resistance, metastasis, proliferation, hypoxic tolerance, and the capacity for neovessel induction[2,3].

Mitochondria-targeted drugs may overcome potentially the drug-resistance mechanisms that have progressed toward conventional chemo-therapeutics in cancer[4-7]. Mitochondria produce ATP, but they also mediate cell death and produce reactive oxygen species (ROS). Although ROS are affected in the regulation of various cellular responses, excessive production may be harmful to the cell[8]. Cancer cells also exhibit extensive metabolic rearrangement that makes them more susceptible to alteration of mitochondria than normal cells[9,10]. However, mitochondrial properties of CSCs in tumors remain unknown.

This review analyzed the potential role of mito-chondria as a therapeutic target for inducing cell death in CSCs. In particular, we focused on the differences in energy metabolism and mitochondrial features between CSCs and non-CSCs, as well as between CSCs and normal SCs, and how these unique features of CSCs may increase the susceptibility of CSCs to anti-cancer treatment and apoptosis induction. We described how CSC mitochondria may be useful targets for the development of novel cancer treatment strategies, such as targeting CSCs *via* combination therapy with specific mitochondrial-targeting drugs.

CURRENT STATUS OF CSCs

History

The concept of CSCs is many decades old[11]. In the middle of 1800s, the embryonal rest theory of cancer introduced the idea that cancer arises from SCs, but the existence of CSCs in tumors could not be verified due to a lack of techniques. Furth *et al*[12] first alluded to CSCs in 1937 when they showed that a single cell within a tumor initiates the generation of new tumor in a recipient mouse[12]. This finding was defined in the 1960s and 1970s by the development of quantitative methods to measure the tumorigenic ability able to sustain tumor growth *in vivo*. In the middle of 1900s, Radiolabeling permitted the measurements of cellular phenotype such as cell proliferation, lifespan, and hierarchical organizations within normal tissues[13]. Al-Hajj *et al*[14] and Singh *et al*[15] represented that a small subset of cells within breast and brain tumors can be isolated prospectively and can generate phenotypically heterogeneous tumor *in vivo*. Thus, these various evidences represent that diverse solid tumors are organized hierarchically and sustained by a distinct subpopulation of CSCs.

Identification of CSCs

CSCs are classified according to several properties such as the presence of cell surface markers and their occupancy in the Fluorescence Activated Cell Sorting (FACS) analysis. Flow cytometry with antibodies against cell surface antigens has been the preferred method for characterizing and sorting normal stem cells. However, differences between CSC and normal SC markers are not well defined, and CSCs and normal SCs share some surface markers.

Most of CSCs studies isolate CSCs marker or a combination of markers, which is expressed hetero-geneously in a certain tumor type. Based on this marker heterogeneity, subpopulations including CSCs are isolated from original tumors and injected into immuno-deficient mice, after which tumor growth is assessed several weeks or months later. Table 1 shows current CSC markers according to cancer types, as FACS markers allow for consistent sorting according to marker expression. For example, Al-Hajj *et al*[14] used a marker combination of the CD24 and CD44 as an indicator of breast CSC, and the CD133 marker has been shown to be both normal SC and CSC marker[16-20].

Stem cells and CSCs

The first embryonic SC lines were developed from the inner cell mass of early embryos in 1998[21]. In 1999 and 2000, it was discovered that it could produce different cell types through manipulating adult mouse tissues, indicating that stem cell differentiation and proliferation could be controlled externally. Both somatic SCs and CSCs generate numerous daughter cells, differentiate into a variety of cell types, actively express telomerase, activate anti-apoptotic pathways, increase active membrane transports, and metastasize[22]. Moreover, SCs are induced to differentiate by niche signaling and outer environmental stimuli. Niche signaling keeps the undifferentiation of SCs until they are stimulated to

Table 1 Markers used to identify stem cells and cancer stem cells

Marker	Cancer origin	Marker properties	Ref.
ALDH1	Breast	Catalyzes the oxidation of aliphatic and aromatic aldehydes Converts retinol to retinoic acid AdSC	[81]
ABC135	Melanomas	ATP binding cassette family Involved in transport of sterol and other lipids	[82]
Bmi-1	Breast, prostate, leukemias, neuroblastomas	HSC, NSC, and AdSC marker	[83,84]
CD20	Metastatic melanomas	Hematopoietic marker	[85]
CD29	Breast, colon	AdSC marker	[86,87]
CD34	Leukemias, sarcomas	HSC, MSC marker	[88-91]
CD44	Breast, pancreas, colon, head and neck, prostate	Adhesion molecule related to metastasis HSC and pluripotent stem cell marker Normal prostate epithelial stem cell marker	[91-96]
CD49f	Prostate	Adhesion to extracellular matrix	[97]
CD90	Liver, breast, glioblastomas	Glycoprotein, role in stem cell differentiation MSC marker	[98-100]
CD113	Lung, pancreas, colon, glioblastoma, melanomas, *etc.*	HSC, NSC AdSC (colon) marker	[16-18,101-104]
CD117	Breast, ovarian, lung, glioblastoma	Progenitor cell marker	[105,106]
Oct4	Many carcinomas	Embryonic stem cell and induced pluripotent stem cell marker	[107,108]
Sca-1	Lung	Skin epithelial stem cell and HSC marker	[109]

AdSC: Adult stem cell marker; HSC: Hematopoietic stem cell; NSC: Neuronal stem cell; MSC: Mesenchymal stem cell.

generate new cells, suggesting a similarity with signaling pathways that govern normal SC proliferation. Local environment signaling can initiate CSC proliferation, and thus, trigger tumor initiation and growth[23]. Therefore, SC markers and features may not be effective therapeutic targets for inhibiting CSC growth.

MITOCHONDRIA AND CANCER

Roles of mitochondria

As the main energy producers, mitochondria produce ATP using the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). However, they also generate ROS during this process, which are harmful to the cell if produced excessively. In addition, mitochondria play a crucial role for the regulation of cell death pathways and intracellular Ca^{2+} homeostasis. Mitochondria activate apoptosis by regulating the releasement of proapoptotic proteins space to the cytosol from the mitochondrial intermembrane[7], and they also play a crucial role in non-apoptotic cell death[24].

Key regulators related to cell death and other cellular processes in the mitochondria are frequently altered in cancer cells[8], as cancer cell mitochondria differ functionally and structurally compare with that of normal cells[25]. Fast growing tumors result in hypoxia because of an inadequate amount of oxygen from the local vasculature. In addition, cancer cells include the DNA mutation of mitochondria and nucleus, which affect the OXPHOS components and result in ROS overproduction, wasteful ATP production, and mitochondrial oxidative damage[25]. Warburg[26] pioneered research on the cancer-related alterations in mitochondrial respiration and suggested a mechanism to explain how they progress during the tumorigenesis.

The proposed mechanism differs from that in non-malignant cells utilizing OXPHOS. Although aerobic glycolysis has been corroborated in cancer cells, the function of mitochondria has been controversial[27]. In cancer cells, the aerobic glycolysis generate glycolytic intermediates to the pentose phosphate pathway. Moreover, the glycolytic ATP generation is important for survival in hypoxic conditions[28]. In OXPHOS, the ATP synthesis requires much oxygen, which leads to continuous the ROS production such as superoxide anion, organic peroxide, and hydrogen peroxide[29]. If the generated ROS are not eliminated by redox regulating system, they may cause cellular damage.

Mitochondrial antioxidant system

Mitochondria have a multi-level network of redox-defense systems for the elimination of hydrogen peroxide (Figure 1). Glutathione and glutathione peroxidases require nicotinamide adenine dinucleotide phosphate (NADPH) for the reduction of H_2O_2 and other peroxides generated in the mitochondria. Mitochondrial redox balances are also regulated by the mitochondrial inner membrane electrochemical gradient, which mitochondrial Complex V (ATP synthase) uses to produce ATP from ADP and inorganic phosphate (Pi).

Moreover, the physiological significance of mitochondrial redox balance has been highlighted by the antioxidant genes-deletion and over-expression. As antioxidant defense system, Peroxiredoxin (Prx) 3, Prx5, superoxide dismutase 2, and thioredoxin 2 eliminates ROS produced in mitochondria[30,31]. Knockout (KO) of Prx3 mice result in induction of oxidative damage[32], KO of thioredoxin 2 mice showed an embryonic lethal phenotype[33] and KO of superoxide dismutase 2 mice die within 3 wk of birth because of mitochondrial
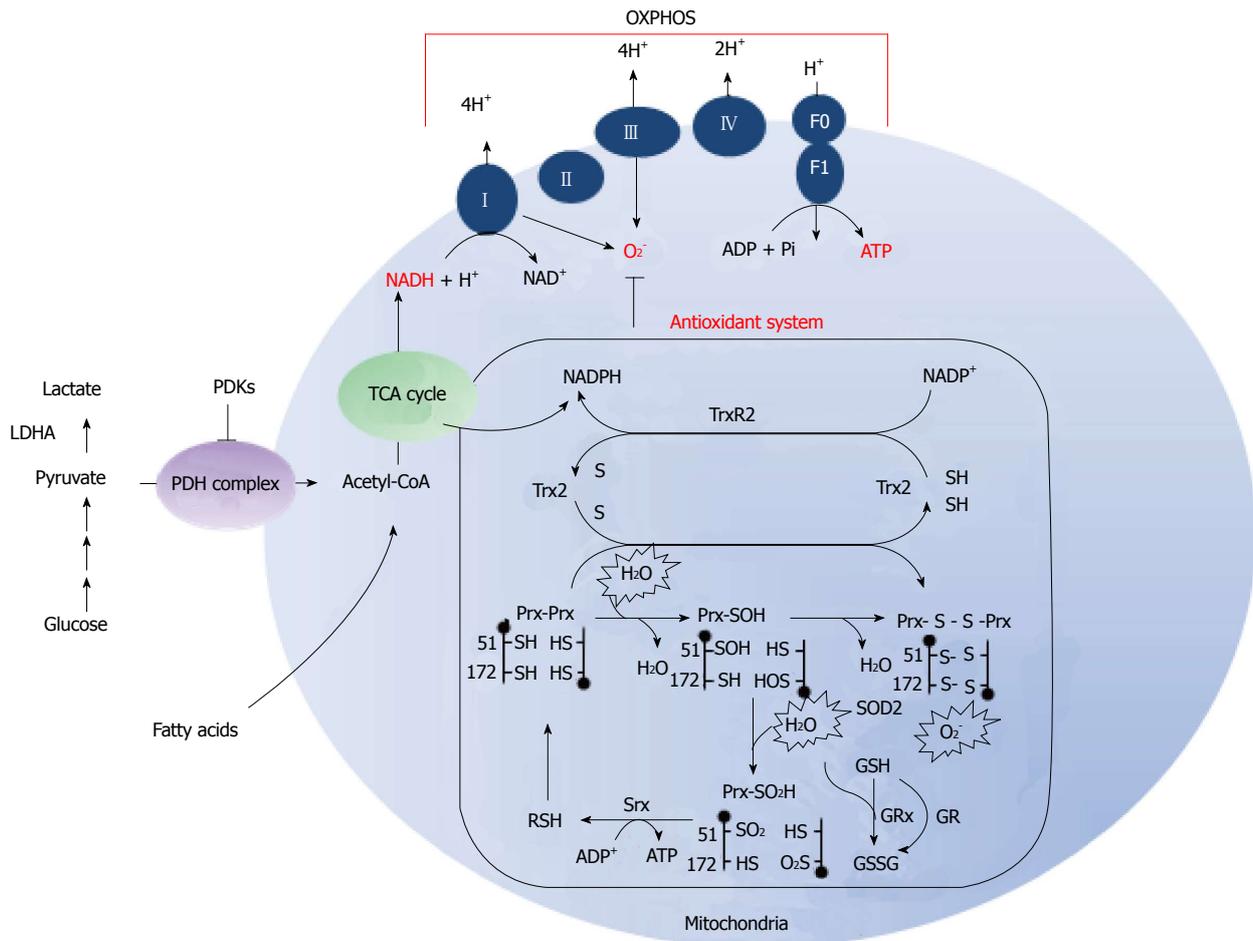
Figure 1 Antioxidant and oxidative phosphorylation systems in mitochondria. Under normal conditions, normal cells rely primarily on oxidative phosphorylation for ATP synthesis, whereas cancer cells rely more on glycolysis. Pyruvate from glycolysis is converted to acetyl-CoA, CO_2, and NADH by pyruvate dehydrogenase (PDH). Acetyl-CoA enters the TCA cycle by the citrate synthase-mediated reaction with oxaloacetate to generate citrate. NADH is oxidized first by Complex I in the electron transport chain (OXPHOS). Electrons from Complex I and II are transferred to coenzyme Q10, then passed on to Complex III, cytochrome c, Complex IV, and finally to O_2 to generate H_2O. O_2^- is converted to H_2O_2 through the action of SOD2 and/or spontaneous dismutation. H_2O_2 is eliminated by three mechanisms: (1) glutathione (GSH) peroxidase (GPx) coupled to GSH and GSH reductase (GR); (2) Prx3 coupled to Trx2 and Trx reductase (TrxR) 2; and (3) non-enzymatic eliminating by redox compounds. The H_2O_2 selectively oxidized cysteine Cys-SH to Cys-SOH, which then reacts with the resolving cysteine Cys-SH of the other subunit in the homodimer to form an intermolecular disulfide bond. The disulfide bond is reduced by Trx2. Moreover, the generated Cys-SOH is oxidized to Cys-SO$_2$H. Reactivation of the enzyme is achieved by reduction of the Cys-SO$_2$H moiety and is catalyzed by sulfiredoxin (Srx). Nicotinamide adenine dinucleotide phosphate (NADPH) is utilized by the reductases in the peroxidase system (GR and TrxR) to reduce disulfide bonds formed in proteins during the elimination of H_2O_2. TCA: Tricarboxylic acid; ATP: Adenosine triphosphatase; ADP: Adenosine diphosphate.

oxidative damage and severe neurodegeneration[34,35]. Therefore, the inhibition of antioxidant systems may provide a targeted therapy that leads to mitochondrial dysfunction and cell death.

Mitochondrial membrane potential

Mitochondria harbor a robust mitochondrial transmembrane potential ($\Delta\Psi m$), and the exchange of small metabolites between the mitochondrial matrix and the cytosol is induced by the low conductance of permeability transition pore complex (PTPC)[36]. The rupture of mitochondrial membranes leading to functional impairment result in the release of toxic mitochondrial intermembrane space proteins, such as apoptosis-inducing factor and cytochrome *c*, into the cytosol[37]. Under apoptotic conditions, including ROS and Ca^{2+} overload, the PTPC presumes a high conductance state allowing uncontrolled influx of small solutes into the matrix of mitochondria. This mitochondrial permeability transition (MPT) leads to osmotic swelling of the mitochondrial matrix and dissipation of the $\Delta\Psi m$[38,39], and eventually cell death occurs due to mitochondrial outer membrane permeabilization[40]. The MPT is triggered by reagents increasing ROS generation, cytosolic Ca^{2+} concentrations, or acting on the PTPC. Therefore, the induction of mitochondrial membrane permeabilization are attractive targets to develop drug for cancer therapy.

Mitochondria-targeted cancer therapy

As mentioned above, mitochondria play important role in apoptosis, but also trigger cell death through various mechanisms[41-43]. Various mitochondria-targeted strategies for cancer treatment have been developed over the last decade[6,44] that focused on the development of agents that regulate the MPT,

Bcl-2 family proteins, and ROS production in cancer[6]. Numerous molecules, acting on mitochondria, are currently used or being tested in clinical trials[45]. Several experimental anti-cancer drugs, such as ceramide[46], CD437[47], and MKT077[48], and clinically approved anti-cancer drugs, such as etoposide[49], paclitaxel[50], and vinorelbine[51], induce apoptosis *via* mitochondria dysfunction. Furthermore, determining of pathophysiological differences of mitochondria between cancer cells and normal cells, will improve the selectivity of mitochondria-targeted anti-cancer agents.

MITOCHONDRIA OF CSCs

Because mitochondria play a key role in the alteration of oxidative stress, energy status, and apoptotic stimuli, scientists have assumed that they are also involved in the regulation of stemness and differentiation in SCs. Researchers have attempted to employ mitochondrial properties in the selection of SCs[52]. Lonergan *et al*[53] and Bavister[54] suggested that functional mitochondrial characteristics, such as subcellular localization and metabolic activity could verify stemness, SC stability, and pluripotency. Mitochondria are localized in perinuclear sites in embryonic stem cells (ESCs) and have a more scattered distribution throughout the cytoplasm after differentiation and senescence[55].

Mitochondrial metabolic activity is also related to cell differentiation, as early passages of an adult primate stromal cell line have a higher oxygen consumption rate (OCR) and a low ATP/ mitochondrial DNA content compared with long-term cultured cells[53]. In CD34+ hematopoietic SCs, a low mitochondrial OCR and mitochondrial mass result in a predominantly perinuclear mitochondrial arrangement[56].

Antioxidant enzyme expression also shows a dramatic change during differentiation[57]. Moreover, ROS play an agonistic role in the differentiation of ESCs. Enhanced intracellular ROS as the differentiation stimulus may act on transplanted SCs into the cardiovascular lineage[58], indicating that mitochondrial redox metabolism act as a crucial regulator in cardiac differentiation of SCs. Furthermore, Plotnikov *et al*[59] suggest a correlation of the mitochondrial function and the status of neural SCs.

SC mitochondria play important roles in maintaining stemness and differentiation. However, whether the roles of CSC mitochondria are similar to SC mitochondria or cancer cells in general is uncertain. Two hypotheses on the origin of CSCs, both of which contribute to acute myeloid leukemia[1,60], have been proposed. One hypothesis of the origin of CSCs is that they are derivatives of SCs residing in various organs. Genetic mutations and epigenetic changes, which are crucial for initiation and progression of tumor growth, accumulate in long-lived stem cells, and the transformation of SCs into CSCs initiates carcinogenesis.

CSCs may also have a greater differentiation potential than other SCs. (SCs can be divided into the following groups based on differentiation potential: the totipotent, pluripotent, multipotent, and unipotent group). Another hypothesis assumes the existence of ESC-like cells that convert into CSCs when they are exposed to damaging environmental factors. Additional differentiation and mutation of these cells may also contribute to development of CSCs[61]. Based on these reports, the CSCs may be more differentiated than normal SCs and likewise, the mitochondrial properties of CSCs are different from those of SCs or general cancer cells.

Recently, Ye *et al*[62] determined the mitochondrial features between lung CSCs and non-CSCs. As a results, it is showed a lower mtDNA contents, lower OCR, glucose consumption, intracellular ATP and ROS level in the lung CSCs compared to non-CSCs. Leukemia CSCs showed a low ROS level and reduced OXPHOS compared with that of non-CSCs[63]. However, Pastò *et al*[64] reported that CSCs exhibited over-expressed genes related to glucose uptake, oxidative phosphorylation, and fatty acid β-oxidation, indicating higher ability to direct pyruvate towards the TCA cycle. As reported, ovarian CSCs showed higher mitochondrial ROS production and ΔΨm than non-CSCs. In addition, targeting mitochondrial biogenetics induced caspase-independent cell death in ovarian CSCs[65]. In glioma CSCs, a higher mitochondrial reserve capacity was measured as compared to the differentiated cells[66]. Glioblastoma CSCs also depend on OXPHOS for their energy production and survival[67]. Besides, breast CSCs have higher ATP content compared to their differentiated progeny[68]. Based on these studies, CSCs mitochondria showed the different roles and features according to the cancer type. A summary of the mitochondrial features between CSCs and non-CSCs according to cancer origin is highlighted in Table 2. Although the mitochondrial features of CSCs in several cancers are not identical, CSCs mitochondria obviously differ from those of non-CSCs. Moreover, mitochondrial features of CSCs have not been clearly defined in other cancer types. Most importantly, little has been known about the mitochondrial features related to energy metabolism and the ROS/antioxidant enzyme system of CSCs in colon, stomach, liver, bone, and prostate cancer. Therefore, defining these features will be essential for developing a mitochondria-targeted therapeutic drug that induces death of CSCs, and therefore, reduces the risk of relapsed or refractory cancer.

CLINICAL IMPLICATION AND THERAPEUTIC TARGETS OF CSCs

Despite the recent surge of published studies on CSCs, the clinical significance of this population remains unclear and has been slow in progression of the development of clinical agents to eliminate CSCs. However, most experts agree that effective

Table 2 Mitochondrial features of cancer stem cells according to cancer origin

Cancer origin	Mitochondria features			Energy metabolism of CSC	Target/drug for CSCs	Ref.
	Feature	CSC	Non-CSC			
Breast	Glucose uptake	High	Low	OXPHOS		[68]
	ATP contents	High	Low			
	OCR	High	Low			
	Lactate production	Low	High			
	Membrane potential	High	Low			
Glioma	Glucose consumption	Low	High	OXPHOS		[66]
	ATP contents	High	Low			
	Lactate production	Low	High			
	OCR	High	Low	OXPHOS	IMP-2	[67]
	ATP contents	High	Low			
Leukemia	ROS	Low	High	Low glycolysis	Bcl-2/	[63]
	Proliferation rate	Slow	Fast	Low OXPHOS	ABT263	
	OCR	Low	High			
	Lactate production	Low	High			
	ATP contents	Low	High			
Lung	Glucose consumption	Low	High			[62]
	OCR	Low	High			
	ROS level	Low	High			
	ATP contents	Low	High			
	Membrane potential	High	Low			
	Mitochondrial DNA	Low	High			
Ovarian					NV-128	[65]
	ROS	High	Low	OXPHOS		[64]
	Membrane potential	High	Low			
	ATP contents	High	Low			
	Glucose deprivation	Resist	Sensitive			

CSC: Cancer stem cell; OCR: Oxygen consumption rate; ROS: Reactive oxygen species; OXPHOS: Oxidative phosphorylation; ABT263: Bcl-2 inhibitor; NV-128: Isoflavone derivative (play a role as inhibitor of mitochondrial function); IMP-2: Insulin-like growth factor 2 mRNA-binding protein 2.

anti-cancer drugs should be targeted toward CSCs in addition to non-CSCs. Current cancer treatments such as conventional chemotherapy and radiotherapy target rapidly proliferating cells that make up the bulk of the tumor, but do not specifically target CSCs. Thus, the hypotheses on the origin of CSCs may explain the development of relapsed and metastatic cancer. In cancer therapy, the new paradigm requires development of novel anti-cancer drug molecules and drug targets to assess drug responses of CSCs.

Altered expression of genes involved in apoptosis, survival, and DNA repair machinery are among the multiple mechanisms responsible for the chemo-resistance of leukemic[69], brain[70], pancreatic[71], breast[72], melanoma[73,74], and colon cancer[75] CSCs. Liu *et al*[23] reports that CD133+ glioblastoma cells isolated from patients have a high expression of genes in the Bcl-2 and inhibitor of apoptosis (IAP) families. Moreover, several types of CSCs have upregulated ATP binding cassette (ABC) pumps that make them resistant to various chemotherapeutics[73,74]. Therefore, finding targets that efficiently promote CSC cell death is important and a focus of intensive research. Dong and colleagues demonstrate that loss of fructose-1,6-biphosphatase in breast CSCs induces glycolysis, as well as inhibiting oxygen consumption and ROS generation, through the suppression of mitochondrial Complex I activity[76]. The report implies

that overproduction of ROS and reduction in glucose metabolism may be effective against breast CSCs. Hirsch *et al*[77] showed that metformin, an AMPK activator and Complex I inhibitor often used as the first-line drug for treating diabetes, and selectively kills CSCs in breast cancer cell lines. The novel isoflavone derivative NV-128 significantly decreased mitochondrial function, as shown by a decreases in ATP, Complex I, and Complex IV levels, and induced cell death in ovarian CSCs[65]. These results demonstrate that specific mitochondrial targeted compounds can induce cell death in chemoresistant CSCs and may be a new venue for treating ovarian cancer patients with relapsed or metastatic cancer. The new-generation taxoid SB-T-1214 significantly inhibited stemness gene expression profiles and induced cell death in both CSCs and general cancer cells, indicating its promise in overcoming relapsed and refractory cancer due to CSCs[78]. Finally, mitochondria-targeted vitamin E succinate (MitoVES), which includes the positively charged triphenylphosphonium group, may be the most well-characterized toxic agent in its ability to induce apoptosis in breast CSCs[79]. Meanwhile, it was reported that a drug which inhibits the self-renewal of CSCs by targeting of Notch and Hedgehog pathway has been developed[80]. It was also reported that has been developed a drugs, which can eliminate CSCs by targeting cell surface markers such as CD133 and EpCAM. However, the use of these drugs increases

the exposure to side effects due to the sharing of signaling pathway and cell surface marker with normal SCs. Thus, it is important to understand how CSCs differ from normal SCs and differentiated cells. Moreover, a full understanding of the mitochondrial function and energy metabolism in CSCs contributes to the development of the agents targeting mitochondrial functions (such as ROS overproduction, energy metabolism inhibition, and antioxidant protein inhibition), and presents a need to develop new strategies to target CSCs in the clinical field[80].

CONCLUSION

In summary, the mitochondria are an important tool to investigate CSCs properties and to develop anti-cancer drugs. However, the properties and clinical significance of mitochondria in CSCs have not been verified. Because mitochondria-targeted therapy may open new strategies for the treatment of relapsed and refractory cancer, mitochondrial properties unique to CSCs need to be defined. Furthermore, combined treatment with mitochondrial-targeted and anti-cancer drugs may specifically induce the death of both CSCs and general cancer cells and promises to be a novel cancer therapy.

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