Cancer Stem Cell Oxidative Phosphorylation: Target for Cancer Therapy

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Abstract

Cancer is one of the most important leading causes of death across the globe. Cancer cells that divide uncontrollably are killed by chemotherapy, radiation therapy, surgery, or a combination of these. Though these conventional therapies are recognized to be highly effective, a group of heterogeneous cells called "cancer stem cells" (CSCs) which comprise about 0.001–0.1% of the tumor cell population are believed to escape the treatment and contribute to the relapse of tumors by inducing metastasis. These CSCs have stem cell-like properties, that is, they can self-renew and differentiate into multiple lineages. They were first identified in leukemia in 1994 and can be isolated by their surface antigens such as CD44 and

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CD133 and intracellular proteins such as ALDH1. As they are highly proliferative like cancer cells, they produce energy (ATP) by both glycolysis and oxidative phosphorylation. Hence, inhibition of these mechanisms can be a potential therapy for eradication of CSCs. Several studies have shown that these cells are more dependent on oxidative phosphorylation than on glycolysis. Hence, there are several strategies to inhibit mitochondrial function at different levels. Natural compounds such as vitamin C and silibinin are used occasionally, whereas FDA-approved drugs, for example; doxycycline, metformin, phenformin, are most commonly used while each one uniquely targets OXPHOS (i.e., mechanism of action), thus intervening with any possible relapse of cancer.

Introduction

Cancer Stem Cells

The term cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide at an uncontrollable rate and spread into surrounding tissues. This harmful disease can start almost anywhere in the human body, which is made up of trillions of cells (Roy and Saikia 2016). Under normal conditions, human cells grow and divide to form new cells as the body needs them, discarding and secreting "old" cells in order to maintain homeostasis in a biological environment. So, when cells grow old or become damaged, there are certain mechanisms that will deal with the disposal of these "old" cells; hence, new cells take their place (Hanahan and Weinberg 2011).

When a biological system is presenting signs of cancer, the orderly process that maintains the homeostasis will be interrupted or it may break down. As cells progressively become more abnormal, old, or damaged, these cells survive when they should be replaced or eliminated. These altered cells can divide without stopping and may form abnormal uncontrollable groups of cells called tumors. Tumors are in summary a huge mass of cells that divide continuously without showing any signs of stopping; this mass can be analyzed in tissue (Bray et al. 2018). Cancers of the blood, such as leukemia, generally do not form solid tumors. In some cases, few cancer cells present in tumor growth can break off and travel to distant places in the body through the blood or the lymph system, and form new tumors far from the original tumor, which is one of the many mechanisms that can be seen in this aggressive disease (Ferlay et al. 2015).

Cancer is one of the leading causes of death in both developed and developing countries. The number of cases are increasing due to risk factors such as tobacco use (lung, colorectal, stomach, and liver cancer), overweight/obesity and physical inactivity (breast and colorectal cancer), and infection (liver, stomach, and cervical

cancer) (Kwon et al. 2015). According to GLOBOCAN, there were 18.1 million new cancer cases and 9.6 million deaths in the year 2018. Among the various types of cancer, lung cancer is commonly diagnosed (18.6%) followed by breast (11.6%), prostate (7.1%), and colorectal (6.1%) for the incidence rate and colorectal (9.2%) and stomach and liver cancers (8.2%) for mortality rate (Khazir et al. 2014) (Fig. 1a).

Chemotherapy, radiotherapy, surgery, and immunotherapy, or a combination of these are the different forms of treatment for cancer, of which chemotherapy being the most common one. Although chemotherapeutic drugs have been recognized to be highly effective in treating cancer, they lead to multi-drug-resistance and have low bio-availability in in-vivo systems. Hence, there is a need for potent drugs with lesser side effects and higher efficacy (Prakash et al. 2013). Their phenotypic (size, morphology, membrane composition) and functional heterogeneity (antigen expression and biochemical nature) are the root causes of treatment failure. This is due to varied responses in aspects such as proliferation, metastasis, and sensitivity to chemotherapy. "Clonal evolution model" and "cancer stem cell hypothesis" are two proposed models to explain this complex organization (Fig. 1b). In the first model, cancer is considered as evolutionary and is believed to be accompanied by natural selection but this fails to explain the ways to eradicate the disease, whereas the latter explains about a small group of cells that drives the origin of cancer called cancer stems cells (CSCs) or tumor initiating cells (TICSs)are the reason for the failure of treatment (De Francesco et al. 2018).

Cells that have the ability to self-renew and mature into specific cell/tissue type by differentiation are known as stem cells. They can be totipotent; each cell can develop into a new individual (example, 1–8 cell stage embryos), pluripotent; cells that can form over 200 different cell types (which includes embryonic stem cells and induced pluripotent stem cells); and multipotent; cells that can differentiate into a number of other tissues (e.g., adult stem cells). These cells have wide clinical applications due to their ability to differentiate and are thus used for therapy (Zakrzewski et al. 2019). Tumor cells and stem cells are related in characteristics of the ability to regulate their self-renewal, tumor cells could arise from normal stem cells, and it is believed that tumors contain "cancer stem cells." A heterogeneous group of cells (0.001–0.1%) in the tumor cell population having stem like properties are called cancer stem cells (CSCs). These subset of cells within the tumor are self-renewing and have the ability to differentiate to multiple lineages (Neuzil et al. 2007). Although their origin is not well understood, CSCs remain in the quiescent G0 phase. Milestones of various work done that led to the identification of CSCs is presented in Fig. 2. CSCs were first identified in leukemia in 1994 followed by breast cancer, colorectal cancer, prostate cancer, lung cancer, melanoma, and brain tumor (Nguyen et al. 2012). They are identified by cell-surface antigens (Table 1) that are also expressed by normal stem cells such as CD44 and CD133. Apart from cell surface markers, intracellular proteins such as aldehyde dehydrogenase 1(ALDH1) have been used to identify CSCs in various carcinomas (Neuzil et al. 2007). There are evidences that CSCs undergo metabolic alteration that are critical for their function such as mitochondrial expansion, lipid metabolism, and glycolytic activities. These metabolic mechanisms
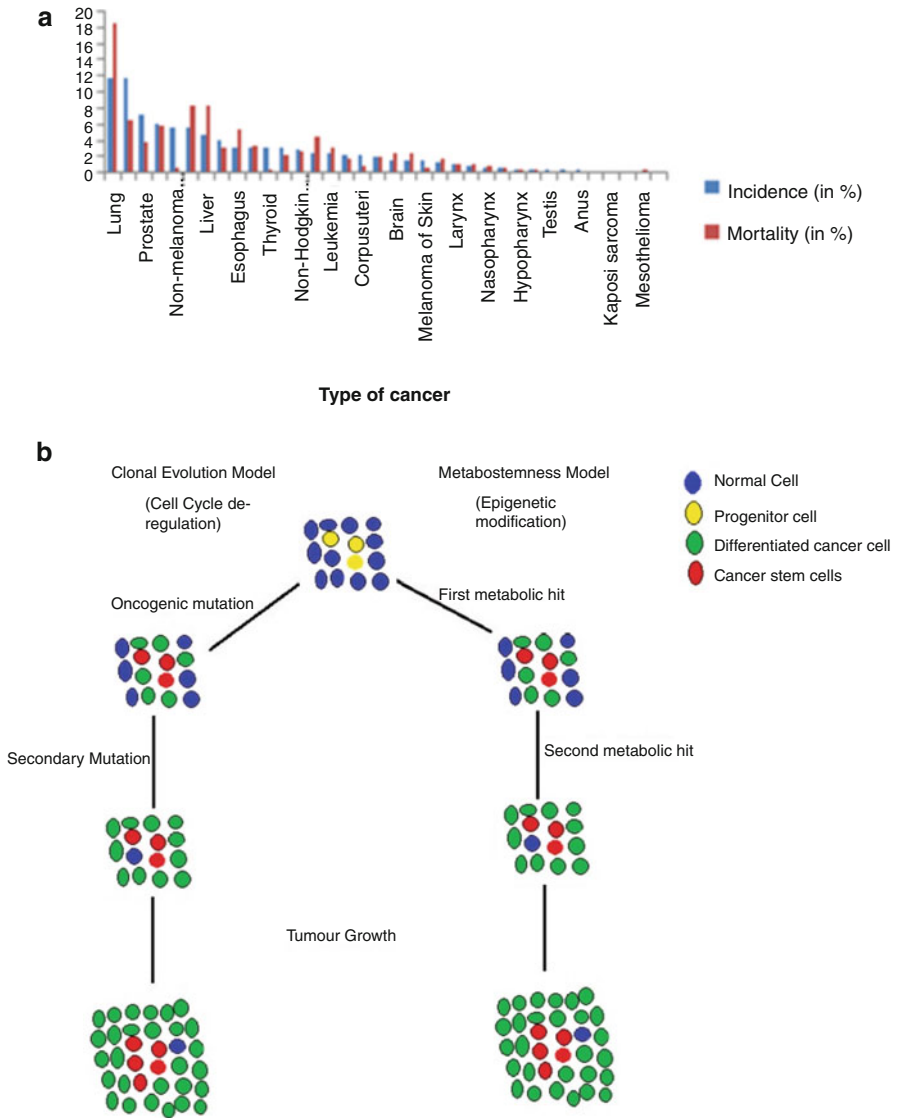
a

Type of cancer

b

Fig. 1 Global Statistics 2018 and models for evolution of cancer cells. (**a**) Incidence and mortality for 36 cancers estimated by Globocan in 2018 for both male and female. It can be observed that lung cancer is the most common in case of both incidence and mortality (Khazir et al. 2014). (**b**) Two models to explain genetic and functional heterogeneity of cancer. According to the first model proposed by Peter Nowell, cancer is the result of oncogenic mutations that lead to differentiation, uncontrolled proliferation, and inability to activate cell death pathways. In the case of the second model (also called cancer stem cell hypothesis model), it recognises a rare subset of cells (CSCs) that are self-renewing and tumorigenic, responsible for the generation of cancer cells due to metabolic events (De Francesco et al. 2018)
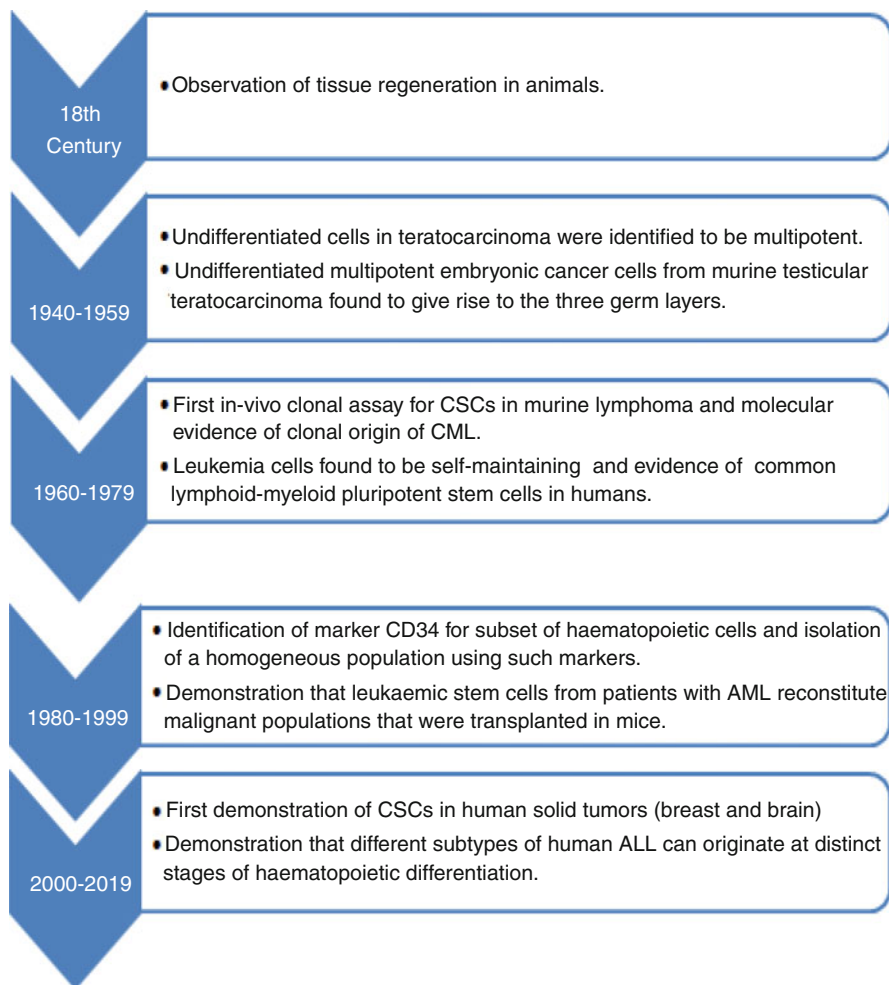
Fig. 2 Identification of CSCs. The flowchart depicts the milestones contributing to the understanding of the development of cancer stem cells (CSCs) and their role in cancer relapse after treatment (Nguyen et al. 2012). *CML* chronic myeloid leukemia, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia

can be exploited to provide effective therapies and reduce the risk of recurrence of cancer and metastasis (Pattabiraman and Weinberg 2014).

While conventional therapy reduces tumor by killing differentiated cancer cells, these CSCs escape from the treatment and cause reoccurring tumors and induce metastasis. Hence, use of specific CSC inhibitors will result in the reduction of resistance to therapy, relapse of tumor, and loss of stem cell-like properties (Chae and Kim 2018).

Table 1 Markers to identify CSCs

Marker	Expression in cancer cells
CD34	Hematopoietic malignancies along with CD38 (Lapidot et al. 1994)
CD38	Hematopoietic malignancies along with CD34 (Lapidot et al. 1994)
CD44	Breast (Al-Hajj et al. 2003), colon (Ricci-Vitiani et al. 2007), gastric (Takaishi et al. 2009), neck and head (Prince et al. 2007), liver (Yang et al. 2008), ovarian (Yang et al. 2008), pancreatic (Li et al. 2007) and prostate (Collins et al. 2005)
CD24	Breast (Al-Hajj et al. 2003), gastric (Zhang et al. 2011) and pancreatic (Li et al. 2007)
CD90	Brain (Singh et al. 2004), liver (Ma et al. 2007) and lung (Donnenberg et al. 2007)
CD133	Brain (Singh et al. 2004), colon (Ricci-Vitiani et al. 2007), endometrial (Rutella et al. 2009), liver (Ma et al. 2007), lung (Bertolini et al. 2009), ovarian (Ferrandina et al. 2008) and prostate (Collins et al. 2005)
ALDH	Breast (Al-Hajj et al. 2003), colon (Ricci-Vitiani et al. 2007), head and neck (Prince et al. 2007), liver (Ma et al. 2007), melanoma (Boonyaratanakornkit et al. 2010) and pancreatic (Li et al. 2007)
Hoechst 33342 dye exclusion	Astrocytomas (Chua et al. 2008), gastrointestinal (Haraguchi et al. 2006), gliomas (Bleau et al. 2009), hepatocellular (Chiba et al. 2006), lung (Bertolini et al. 2009) and thyroid (Mitsutake et al. 2007).

The following are commonly used markers to identify CSCs along with their expression in normal cells and cancer cells. *ALDH* Aldehyde dehydrogenase

Metabolism

Glycolysis and Cancer

Since cancer cells are highly proliferative, a great multitude of ATP molecules are constantly generated to fulfill their energy demands. Similar to normal stem cells, they require glucose for survival. A total of about 64% cancer cells are dependent on glycolysis (Pattabiraman and Weinberg 2014). Expression of certain genes such as c-Myc, Glut-1, HK-1, HK-2, and PDK-1 in CSCs is induced by glucose, and studies revealed that inhibition of glycolysis resulted in the decline in their population. For instance, CSC population from ovarian cancer, breast cancer, glioblastoma, lung cancer, and colon cancer was dependent on glycolysis, and it was found to be the preferred state to resist radiotherapy. It has been identified that tumor cells alter the metabolic machinery of normal cells to meet their demand. Hence, they mostly produce ATP by glycolysis (from glucose by pentose phosphate pathway) rather than OXPHOS as it is more rapid and efficient. However, there exists an epigenetic diversity within respective tumors, and it is believed CSCs depend on both glycolysis and OXPHOS based on the cancer/tumor type (Sancho et al. 2016). Multipotent stem cells are found to be completely glycolytic, whereas the differentiated somatic cells are mostly considered to be dependent on OXPHOS for metabolism. Hence, it is believed that metabolism has a control over stem-like properties of cells. For

instance, a switch to glycolysis from OXPHOS was seen in breast cancer CSCs for better functionality and lower ROS levels. In the case of hepatocellular carcinoma and nasopharyngeal carcinoma, the increased expression of MYC (oncogenic factor) indicated stemness properties and glycolysis (Zheng 2012).

Oxidative Phosphorylation and Cancer

CSCs are also identified to be reliant on mitochondrial metabolism to generate ATP. Certain CSCs prefer mitochondrial oxidative metabolism in tumor types including lung cancer, breast cancer, glioblastoma, and pancreatic cancers. It was also identified that they have elevated mitochondrial mass and high oxygen consumption rate (Chae and Kim 2018). It is also known to help them survive chemotherapy. Mitochondria play a major role in the production of energy required for cellular activities (such as metabolism) and influence cell fate decisions (by interfering in cell signal pathways). The human mitochondria has its own DNA (called mitochondrial DNA or mtDNA) that encodes for 12S and 16S RNAs, 22 tRNAs, and 13 polypeptides required for its function (Pattabiraman and Weinberg 2014). The synthesis of adenosine triphosphate (ATP) is through oxidative phosphorylation OXPHOS coupled with tricarboxylic acid (TCA) cycle. Nutrients such as glutamine, pyruvate, and fatty acids are reduced to nicotinamide adenine dinucleotide phosphate (NADH) and flavin adenine dinucleotide (FADH2), that act as electron donors for the electron transport chain (ETC). The transport of electrons across OXPHOS complexes generates a force (called as proton motive force) leading to the production of ATP from energy released from the oxygen molecule that acts as electron acceptor (Solaini et al. 2011).

Electron Transport Chain

The electron transport chain localized to cristae consists of respiratory complexes (I, II, III, IV, and V) where protons are transported by proton pumps to create a proton gradient (Fig. 3). Complex I or nicotinamide adenine dinucleotide dehydrogenase catalyzes the regeneration of nicotinamide adenine dinucleotide from its reduced form by oxidation. This is a crucial point of respiration because the oxidized product is for tricarboxylic acid cycle and also reduces coenzyme ubiquinone (Q10) to ubiquinol. A total of four protons are pumped into the intermembrane space from the matrix and two electrons pass to complex II. Complex II or succinate dehydrogenase is a membrane-bound lipoprotein attached covalently to cofactor flavin adenine dinucleotide that is directly involved in the tricarboxylic cycle by oxidizing succinate to fumarate and reducing flavin adenine dinucleotide to hydroquinone. The electrons generated from the oxidation of this reduced molecule results in the release of electrons transferred to complex III by ubiquinone (Q10). Complex III or ubiquinone-cytochrome c reductase has two centers, Qi facing to the matrix and Qo facing the intermembrane space. The reaction mechanism consists of two steps
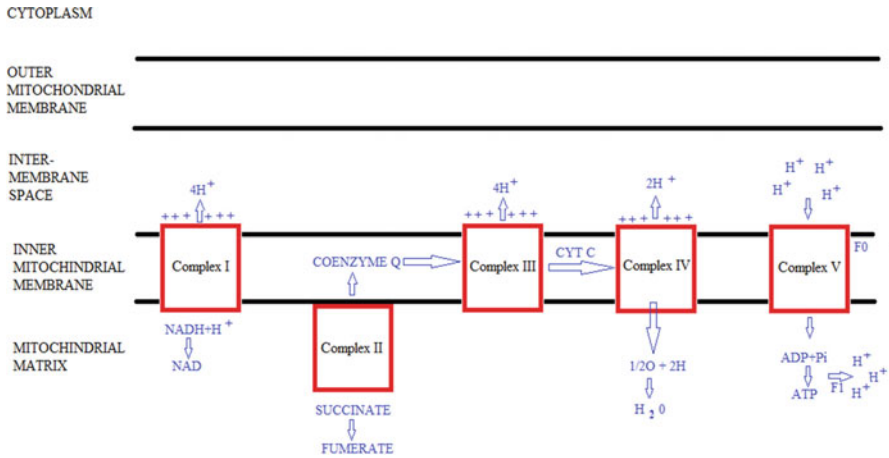
CYTOPLASM

OUTER
MITOCHONDRIAL
MEMBRANE

INTER-
MEMBRANE
SPACE

INNER
MITOCHINDRIAL
MEMBRANE

MITOCHINDRIAL
MATRIX

Fig. 3 Schematic representation of electron transport chain. The process by which ATP is formed as a result of electron transfer through various complexes of the mitochondrial membrane is known as oxidative phosphorylation. Each complex has its vital role in the process and has various molecules involved. Since cancer cells are highly proliferative and require a lot of energy, other than glycolysis, they are also dependent on mitochondria and thus on oxidative phosphorylation (Zhao et al. 2019; Guo et al. 2017)

and is called Q cycle, where four protons are released into the intermembrane space. Electrons are continuously transported to complex III which is involved in catalyzing the oxidation of ubiquinol and reduction of another important cofactor cytochrome C. Cytochrome C transfers electrons from complex III to IV. Complex IV or cytochrome C reductase catalyzes the final reduction of oxygen molecule to water and also mediates the pumping of four protons to complex V. Complex V or adenosine-5-triphosphate synthase has two domains; F1-soluble domain localized to inner membrane connected to F0-domain which spans the membrane from inner to outer side. This formation converts electrochemical energy to chemical energy as protons pass through the membrane and forces the synthesis of Adenosine triphosphate (ATP). This also results in the production of reactive oxygen species (ROS) as a by-product (Zhao et al. 2019).

Oxidative phosphorylation is regulated at various levels: by direct modulation of electron transport chain parameters, regulation of intrinsic efficiency, alteration of mitochondrial network dynamics, mitochondrial degradation and biogenesis, and changes in the cellular and mitochondrial micro-environment (Sancho et al. 2016).

Other Roles of Mitochondria

The self-renewal property of hematopoietic cells and leukemia-initiating cells are due to their mitochondrial metabolism. NANOG, a pluripotency marker, was found to be highly expressed when genes of fatty acid oxidation were induced in

hepatocellular carcinoma cells. In pancreatic CSCs, PGC-1alpha, regulator of biogenesis of mitochondria, was expressed, and it played a major role in the function and contribution to self-renewal in in-vivo. Hence, these factors can be used to target OXPHOS. Further, OXPHOS enables CSCs to have high resistance to nutrient deprivation. Though it operates at a low rate, it has selective advantages. Also, lactate secreted by cancer cells that are surviving on glycolysis acts as an extra fuel for OXPHOS metabolism (Chae and Kim 2018).

Other than OXPHOS, the mitochondria has other roles such as regulating stemness, release of cytochrome C resulting in apoptosis, release of ROS, and release of metabolites. Increased mitochondrial mass and increased telomerase activity (hTERT) are important markers for increased mitochondrial biogenesis. This could be tracked by staining with Mito-Tracker (non-toxic fluoroprobes). In case of MCF7 cell line, two different fractions were observed after staining such as "Mito-high" and "Mito-low." The Mito-high population had increased mitochondrial mass, increased 3D spheroids, and tumor initiation in in-vivo condition (Pavlova and Thompson 2016; Sotgia et al. 2018).

Thus, in most cancers, CSCs have shown distinct metabolic patterns and also rely on OXPHOS because glycolysis alone is insufficient, as glucose availability becomes less and its metabolism is restricted. These metabolic patterns can be targeted to eliminate CSCs and thus reduce the possibility of recurrence of cancer. The levels to which this can be exploited still needs clarity. In few cases, mitochondrial respiration is restricted by blocking the agents that drive the electron transport chain (ETC) resulting in cell death by apoptosis. This is proven to block glycolytic pathway in breast cancer or nasopharyngeal carcinoma. Some delocalized lipophilic cations (e.g., triphenylphosphonium) accumulate in the matrix along with Mitochromanol (analog of Vitamin E), inducing cell death by inhibiting OXPHOS in breast cancer. CSCs also depict high mitochondrial membrane potential and produce ROS that aids in maintaining their stemness and causing high fidelity. Many drugs are also used to target these metabolic pathways and their compounds are used at different levels to inhibit OXPHOS, other mitochondrial activities in these cancer stem cells and eliminate them completely (Bonuccelli et al. 2017).

Lipid accumulation, which is the result of an imbalance between fatty acid synthesis and oxidation, has been observed in many different types of cancer such as breast cancer stem cells, glioma stem cells, colorectal cancer stem cells, and pancreatic cancer stems cell (Yadav et al. 2020; Yi et al. 2018; Brandi et al. 2017). Cells generate energy by breaking down fatty acid by a process known as fatty acid oxidation (FAO) or β-oxidation (Kuo and Ann 2018). Lipid droplets (LD) have free fatty acids which sustain ATP production through FAO. During limiting conditions of glucose metabolism, CSC survival depends on increased FAO. Tumor spheres possessing intrinsic properties of cancer stem cells exhibited elevated expression of enzymes involved in fatty acid β-oxidation pathway such as ACS1, CPT1/2, HADC1, ECH1, and ACAA1 (Wang et al. 2019). Pharmacological inhibition of fatty acid oxidation with irreversible CPT1 inhibitor, etomoxir reduced the ATP levels significantly in breast CSCs but not in non-cancer stem cells (Wang et al. 2018). CSCs have more LD as compared to non-cancer stem cells. Colorectal cancer

stem cells with high levels of LD show increased tumorigenesis potential compared to low LD colorectal cancer stem cells (Tirinato et al. 2015). Understanding the molecular mechanism and therapeutic targeting of lipid metabolism may be used to obliterate cancer stem cells more effectively.

Inhibition of Oxidative Phosphorylation

There are a growing number of pharmacological drugs that can be repurposed to suppress mitochondrial function at different levels. For example, natural compounds such as vitamin C and silibinin and a mitochondria inhibitor called actinonin derived from honeybee propolis can be used as inhibitors of glycolysis (Fiorillo et al. 2018). Also, brutieridin and melitidin (bergamot) are the latest naturally occurring molecules that inhibit mevalonate metabolism and eradicate CSCs (Lamb et al. 2015). The FDA-approved drugs broadly belong to four classes such as tetracycline family (doxycycline/tigecycline), erythromycin family (azithromycin), anti-parasitic (pyrvinium pamoate and atovaquone), and antimicrobial (bedaquiline). These drugs were found to be effective against many CSCs originating from DCIS, ovarian, prostate, breast, lung, melanoma, glioblastoma, and pancreatic cancers (Fiorillo et al. 2016). In addition, there are four new therapeutic drug types, namely, mitoriboscins (targets mitochondrial ribosome involved in the synthesis of protein), mitoketoscins (targets OXCT1 and ACAT1 involved in ketone metabolism), mitoflavivoscins (targets electron transport complexes I/II involved in respiratory process), and TPP* (targets mitochondrial matrix involved in signaling) (Sica et al. 2020). There are direct targeting methods such as inhibiting mitochondrial transfer, inhibiting mitochondrial protein synthesis, blocking electron transport chain, and targeting cationic lipophils. The indirect methods involve inhibiting OXPHOS and other combinational methods using tyrosine kinase inhibitors and metabolic manipulation along with the process of OXPHOS (Kuntz et al. 2017).

Inhibition of Mitochondrial Function

One of the major functions of mitochondria is to produce proteins needed for its ATP production. A total of 60 nuclear envelope proteins were found in CSCs as a result of their increased biogenesis. Drugs are used to target these processes or their products and thus disrupt its function. One such group of antibiotics includes tetracyclines. They inhibit mitochondrial function due to their prokaryotic origin. For example, tigecycline eradicates chronic myeloid leukemia stem cells in in-vitro, in-vivo, and xenografts. Doxycycline and azithromycin also inhibit protein translation in early breast cancer. A decrease in stemness markers such as aldehyde dehydrogenase and CD44 was also noted (De Francesco et al. 2007).

The problem with doxycycline is the development of drug resistance. A shift from OXPHOS to glycolysis was observed in CSCs of MCF7 cells. There was a 35-fold loss of mitochondrial DNA-encoded proteins such as MT-ND3, MT-CO2, and

MT-ATP6/8 necessary for OXPHOS. A decrease in proliferation, migration and no spheroid formation was observed. Hence, to improve efficacy, doxycycline was conjugated with nine other drugs (or nutraceuticals) to eradicate DoxyR CSCs. These include atovaquone, irinotecan, sorafenib, niclosamide, and berberine (natural compound) to inhibit OXPHOS; 2-deoxyglucose (2-DG), vitamin C (natural supplement), and stiripentol to inhibit glycolysis. Chloroquine is another drug sensitive to autophagy inhibitors (Ashton et al. 2018).

Inhibition of Mitochondrial Transfer

CSCs generate new mitochondria that are easier to target and mitochondrial DNA are often mutated. This is to alter mitochondrial biogenesis and thus improve their chances of survival. It was observed in in-vivo conditions that, cells without mitochondria form delayed tumors and acquire mitochondria by horizontal transfer. A movement of mitochondria from normal stromal cells to affected cells has been observed in various disease conditions, especially cancer. In case of acute myeloid, NADPH oxidase 2 prevents mitochondrial transfer from bone marrow stromal cells to tumor cells. This helped immune-deficient mice to survive that were inoculated with AML human cells. An intracellular mitochondrial transfer from normal cells was also observed in case of multiple myeloma cells in order to maintain OXPHOS (Ellinghaus et al. 2013).

Inhibiting Respiratory Chain Complexes

Every complex has specific inhibitors, or one drug can inhibit one or more complexes. To inhibit complex I, biguanide anti-diabetics such as metformin and phenformin are being used. Metformin is needed in millimolar concentration to be effective in in-vivo. Also, carboxyamidotriazole orate in combination with fenofibrate is used to inhibit non-voltage-dependent calcium channels. High-affinity compounds like BAY87-2243, IACS-010759, and ME-344 are highly specific to this complex but have nausea or vomiting as side-effects (Ashton et al. 2018). Vitamin E analog "alpha-tocopherol succinate" and ionidamine are low-affinity compounds used to inhibit complex II (Ferrandina et al. 2008; Boonyaratanakornkit et al. 2010). Anti-microbial agents atovaquone and arsenic trioxide were used to treat acute promyelocytic leukemia by inhibiting complex III and IV. Atovaquone along with another compound pyrvinium pamoate inhibits complex II and III. VLX600 inhibits more than two complexes (I, II and IV) (Molina et al. 2018).

Inhibition Based on Membrane Potential

Cationic lipophils enrich in the mitochondrial matrix due to the changes in the membrane potential. Triphenylphosphonium (TPP+) conjugated with a conjugate of metformin called "mito-met10" and 3-carboxyl-proxyl targets mitochondrial

matrix. This inhibits OXPHOS, disrupts structure of mitochondria, abolishes changes in membrane potential, and increases mitophagy. Another combination of TPP with tamoxifen, which is called "MitoTam," inhibits complex 1, disrupts other respiratory complexes, increases reactive oxygen species (ROS), and dissipates changes in membrane potential. In glioblastoma xenografts in mice, Gboxin accumulated in the matrix driven by changes in membrane potential and inhibits FOF1 ATP synthase in complex V resulting in hyperpolarization and thus creates energy crisis (Reily et al. 2013; Boyle et al. 2018).

Indirect Inhibition of OXPHOS

Venetoclax (a BCL2 antagonist) inhibits glycolysis and glutaminolysis to kill cancer cells in-vitro. It works in combination with an epigenetic modifier called azacytidine to inhibit complex II through reduced glutathionylation of succinate dehydrogenase, that affected acute myeloid leukemia stem cells (Hubackova et al. 2019; Marzo et al. 1998).

Combination with Tyrosine Kinase Inhibitors and Metabolic Activities

To overcome the resistance acquired by CSCs to OXPHOS suppression, inhibitors of OXPHOS are conjugated with other inhibitors related to mitochondrial functions (Table 2). In case of melanoma, phenformin and BRAF inhibitor lead to a decreased melanoma progression. In mouse models of gastrointestinal stromal tumors, VLX600 was used with cKIT inhibitor to control manifestation (Vitiello et al. 2018). Ibrutinib and IACS-1010759 were effective in the treatment of mantle cell lymphoma. Sometimes, these compounds are conjugated with receptors of tyrosine kinase, as they regulate glycolysis (Zhang et al. 2019).

Inhibiting glycolysis restores higher rate of OXPHOS as it alternates metabolism. In case of hereditary leiomyomatosis renal carcinoma, a loss of function mutation in gene related to OXPHOS makes it automatically dependent on glycolysis. Hence, the process of OXPHOS must be inhibited as it is also known to inhibit glycolysis. However, simultaneously inhibiting both these processes would completely disrupt bioenergetics and thus kill all the cancer cells. For example, inhibiting phosphogluconate dehydrogenase using RNAi interference alters the NADPH generation and redox homeostasis that also blocks glycolysis. Further, when they are conjugated with complex I inhibitor IACS-010759, they result in lethality though they are resistant to OXPHOS (Sun et al. 2019). In hepatocellular carcinoma, hexokinase 2 expressed only in case of cancer makes cells more sensitive to metformin. Dimethyl alpha-KG, a cell-permeable precursor of alpha-KG in conjugation with BAY87-2243, was used on multiple human cancer cell lines in in vitro and in vivo (Sica et al. 2019).

Table 2 Compounds and their mechanism of action

Mechanism	Compounds (and combinations)
Inhibition of mitochondrial function (i.e., protein biogenesis)	Tetracycline family Tigecycline (Fiorillo et al. 2016) (combined with imatinib) Doxycycline (Fiorillo et al. 2016)
	Erythromycin family Azithromycin (Fiorillo et al. 2016)
	Mitoriboscins (Sica et al. 2020)
	Mitoketoscins (Sica et al. 2020)
Inhibition of respiratory chain complexes	Complex I Biguanide antidiabetics Metformin and phenformin and carboxyamidotriazole orotate;BAY87-2243,IACS-010759, and ME-344 (Ashton et al. 2018)
	Complex II Antimalarial agent Pyrvinium pamoate (Molina et al. 2018)
	Complex III Antimalarial agent Atovaquone (Molina et al. 2018)
	Complex IV VLX-600 (Molina et al. 2018)
	Complex V Bedaquiline (Molina et al. 2018)
Inhibition based on membrane potential	Mito-Met (metformin along with TPP) (Reily et al. 2013; Boyle et al. 2018); Mito-Tam (tamoxifen along with TPP) (Reily et al. 2013; Boyle et al. 2018); Gboxin (Reily et al. 2013; Boyle et al. 2018)
Direct and indirect inhibition of OXPHOS	Direct Atovaquone, irinotecan, sorafenib, niclosamide, and berberine (Sotgia et al. 2018)
	Indirect Venetoclax combined with azacytidine, 2-deoxyglucose, vitamin C, stiripentol (Boyle et al. 2018; Hubackova et al. 2019).
Inhibition with tyrosine kinases	Phenformin VLX600 combined with imatinib (Vitiello et al. 2018); IACS-010759 along with ibrutinib (Zhang et al. 2019)
Inhibition of metabolic activities	Metformin (after inhibition of hexokinase-2) (Sun et al. 2019); IACS 010759(followed by phosphogluconate dehydrogenase inhibitor) (Zhang et al. 2019); chloroquine (Sotgia et al. 2018).

The table contains various compounds used to inhibit oxidative phosphorylation at various levels
It can be seen that few of the compounds work in combinations for better efficacy

Summary and Conclusion

Cancer stem cells (CSCs) have been proved to be the reason for the alteration of metabolic reprogramming in cancer cells that lead to metastasis, tumorigenesis, resistance to chemotherapy, and tumor relapse. These cells are known to inherit both stem cell-like and tumorigenic properties. They also require a lot of energy to maintain their metabolic needs. Thus, these cells possess unique metabolic patterns especially in the mitochondrial function, structure, and dynamics. Also, mitochondrial ROS production in CSCs maintains its stemness and high fidelity. Hence, targeting oxidative phosphorylation (OXPHOS) in these cells is a novel approach for the eradication of progression of various cancer cells. Drugs such as tigecycline, doxycycline, azithromycin, metformin, phenformin, pyrvinium pamoate, atovaquone, gboxin, vitamin and stiripentol, and VLX600, or a combination of these, are used to target mitochondria at various levels. Although it is a better approach compared to the traditional therapies, challenges related to the cancer heterogeneity in its metabolic patterns need to be addressed. Thus, efficient metabolic targeting of all CSCs may eventually support the elimination of cancer stems cells without damaging the normal cells.

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