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REVIEW ARTICLE

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Is heme biosynthesis influenced the mitochondrial function and cell proliferation in cancer?

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ABSTRACT

Heme is known as a compound that consists of an iron (Fe) atom bound to a pyrrole ring forming protoporphyrin IX (PPIX). Protoporphyrin combines with a protein-forming hemoprotein compound that plays an important role in oxygen-binding and transport as well as in the process of energy production in the mitochondria. Increased heme biosynthesis is found in some cancer cells and is thought to be related to an increase in cancer cell proliferation. Inhibition of heme biosynthesis in some cancer cells leads to a decrease in cell proliferation. This review article discusses the synthesis of heme, the role of heme in energy metabolism which is needed for cell proliferation, the inhibition of heme synthesis and its effect on cancer cell proliferation, and the possibility of the inhibition of heme biosynthesis as an approach in therapy of cancer in the future.

Keywords: heme biosynthesis, hemoprotein, inhibition of heme biosynthesis, cancer cell proliferation

Introduction

Uncontrolled cell proliferation is one of the triggering factors for cancer [1,2]. An adequate energy supply for cells is known to play an essential role in cell proliferation in tumor development. Tumor cells require nutrients for the supply of cellular energy and the synthesis of building blocks. Otto Warburg suggested that tumor cells tend to metabolize glucose anaerobically to obtain energy even though there is sufficient oxygen available. However, recent studies have shown that there are also changes in the metabolic pathways of these cancer cells [3].

Kaambre et al (2012) stated that there was an increase in mitochondrial respiration activity in breast cancer cells [4]. In addition, oxidative phosphorylation was maintained in colon cancer cells even though oxygen availability was at a concentration of 1% [5]. This indicates that mitochondrial activity and respiration are critical in the metabolism and bioenergetics of cancer cells [6]. Mitochondrial function as well as aerobic metabolism are known to be related to heme function.

Heme plays an important role in various cellular processes [7]. Heme acts as a prosthetic group of various proteins that function in oxygen transport and storage, respiratory chain complexes, and enzymes that play a role in oxygen detoxification [1,7]. Heme can affect cell proliferation and survival which is associated with its function in various proteins and enzymes involved in various energy-forming processes through the respiratory chain.

Heme synthesis can change under certain pathological conditions. Increased heme synthesis is known to play an essential role in lung [6], colorectal, and pancreatic cancer malignancies [8]. On the other hand, inhibition of heme synthesis can cause a decrease in cell proliferation and growth. Some studies showed that inhibition of heme synthesis using succinyl acetone could subside the proliferation of HeLa [1] and lung cancer cells [6]. Based on those various explanations, this paper has been compiled to discuss the role

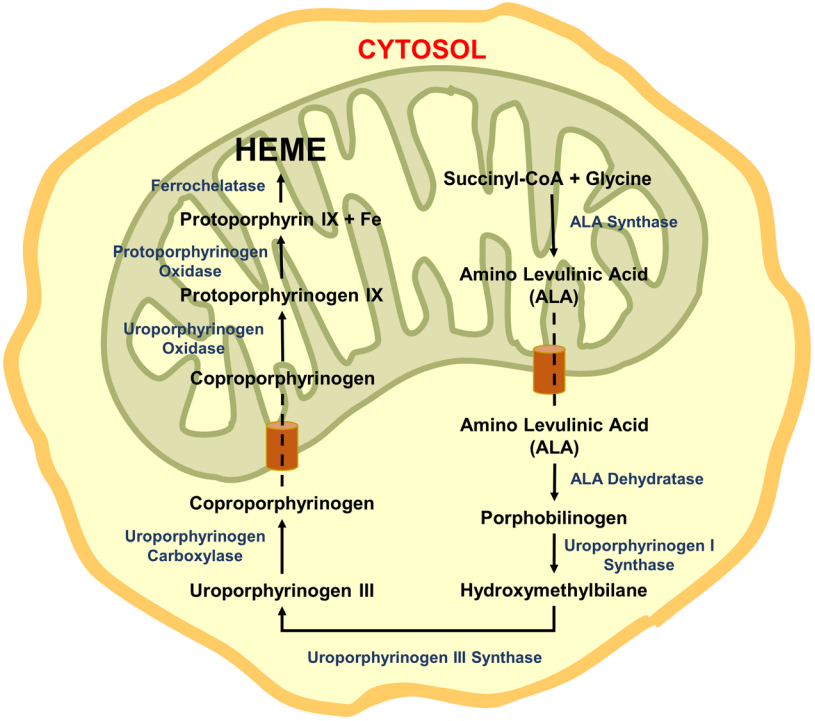

Figure 1. Heme biosynthesis pathway. The heme biosynthesis involves of eight enzymatic steps, four of which are in the mitochondria, and the other four are in the cytosol. The final stage of heme biosynthesis involves the enzyme ferrochelatase which catalyzes the insertion of iron (Fe) ions into protoporphyrin molecules on the inner membrane of the mitochondria.

of heme in cell proliferation and its association with mitochondrial function in cell metabolism to produce energy.

Heme structure

Heme is a compound consisting of iron (Fe) bound to protoporphyrin IX (PPIX) [7,9]. The heme compound is composed of cyclic tetrapyrrole linked by methylene bridges. The structure of heme generally consists of methyl, vinyl, and propionate groups [7]. Heme-containing proteins are known to be widespread in nature [7]. Heme biosynthesis occurs in nearly all mammalian cells, except for mature erythrocytes, without mitochondria. About 85% of heme synthesis occurs in erythroid precursor cells. Meanwhile, the remaining majority occurs in hepatocytes [7,9].

Biosynthesis of heme

Heme biosynthesis involves eight enzymatic steps that occur in the mitochondria and cytosol [10]. Figure 1 shows the biosynthesis of heme which begins with the condensation of succinyl-CoA and glycine in the mitochondrial matrix, forming the amino levulinate acid (ALA). This reaction is catalyzed by the amino-levulinate acid synthase (ALAS) enzyme. The initial product formed is amino adipic acid which undergoes spontaneous decarboxylation to form ALA [7,11]. There are two isozymes of ALA synthase in mammals, namely ALAS1 and ALAS2. ALAS1 is known to be expressed in almost all body cells, meanwhile, ALAS2 is only expressed in erythrocyte precursor cells.

The reaction catalyzed by the enzyme ALAS1 is a reaction that limits the rate of heme biosynthesis in the liver. ALAS1 enzyme activity is known to be strongly influenced by intracellular heme levels. The synthesis of ALA by the ALAS1 enzyme will only occur if the level of intracellular heme is low [7]. From the mitochondrial matrix, ALA is then transported to cytosol [12]. Next, two molecule of ALA are condensed by ALA dehydratase to form porphobilinogen [7,11].

Then, four molecules of porphobilinogen are condensed to form a straight chain tetrapyrrole hydroxymethylbilane, by the urophorphyrinogen I synthase to make uroporphyrinogen III [7].

Uroporphyrinogen III is then converted to coproporphyrinogen by urophorphyrinogen carboxylase. Furthermore, coproporphyrinogen is transported back to the mitochondria from the cytosol. In mitochondria, coproporphyrinogen is converted to protoporphyrinogen IX by coproporphyrinogen oxidase. Protoporphyrinogen IX is further oxidized to form protoporphyrin IX through a reaction catalyzed by protoporphyrinogen oxidase. The last step of heme biosynthesis involves the incorporation of an iron (Fe) into protoporphyrin IX by ferro-chelatase. The rate of heme biosynthesis and degradation in cells is highly regulated by heme availability. This regulation is very important because the balance of intracellular heme is needed [12].

Compounds containing-heme

After being synthesized in the mitochondrial matrix, heme can be used to form heme-containing compounds, such as hemoproteins. The synthesized heme is then transferred and used to synthesize the cytochrome c protein group or hemoprotein which is needed in the mitochondrial respiration process to produce energy. Heme can also be used to synthesize hemoproteins in the matrix or the space between mitochondrial membranes, as well as extra-mitochondrial hemoproteins such as globin proteins [13].

Hemoproteins are proteins that contain heme as their prosthetic group [14,15]. Based on their function, hemoproteins can be classified into three groups. The first group is a protein that can bind to oxygen reversibly. These proteins generally are involved in the transport and storage of oxygen in various tissues [15,16]. Some examples of hemoproteins from this group include hemoglobin, myoglobin, neuroglobin and cytoglobin [15-17]. The second group is a protein that can bind to oxygen and activate it. One example is cytochrome P450 [18]. The third group is proteins that cannot bind to oxygen and act as electron carriers. An example of this group of proteins is cytochrome c [15,19].

Hemoproteins that are involved in oxygen transport and electron carriers are known to support mitochondrial function in cell metabolism to produce energy, which is then related to cell proliferation. Furthermore, the function of mitochondria in the process of energy formation and cell proliferation will be discussed, as well as the role of heme on mitochondrial function related to proliferation and malignancy in cancer.

Mitochondria and cell proliferation

Cell proliferation is an activity that requires energy. It is regulated at checkpoints of the cell cycle [20]. Cells that are about to divide must be metabolically ready to support the energy requirements for proliferation. The metabolic cycle essentially alternates between an oxidative phase and a reductive phase during cell proliferation. The production of various cellular components characterizes the oxidative phase (G1 phase), where energy generated from mitochondrial activity fuels this phase. After that, the reductive phase characterized by DNA replication and mitochondrial biogenesis (S/G2/M phase) will follow the oxidative phase. This phase usually uses energy that is obtained from non-respiration processes [21,22]. Based on this, the mitochondria have an essential role in the cell proliferation process through energy formation.

In terms of energy production, mitochondria play an important part in eukaryotic cell proliferation, which, of course, needs energy. Mitochondria are the main site in the production of ATP to fulfill the cell's bioenergetic demands [23,24]. Carbon compounds, such as pyruvate from glycolysis can be used to produce ATP. The pyruvic acid compound will undergo oxidative decarboxylation, and enter the citric acid cycle (TCA) which takes place in the mitochondrial matrix to produce electron carriers in the form of NADH and FADH2. NADH and FADH2 play a role in electron transfer in the electron transport chain (ETC) and oxidative phosphorylation (OXPHOS) pathways that take place in the inner mitochondrial membrane [7,25].

It is known that most of the cellular ATP are produced in the mitochondria via the OXPHOS. In

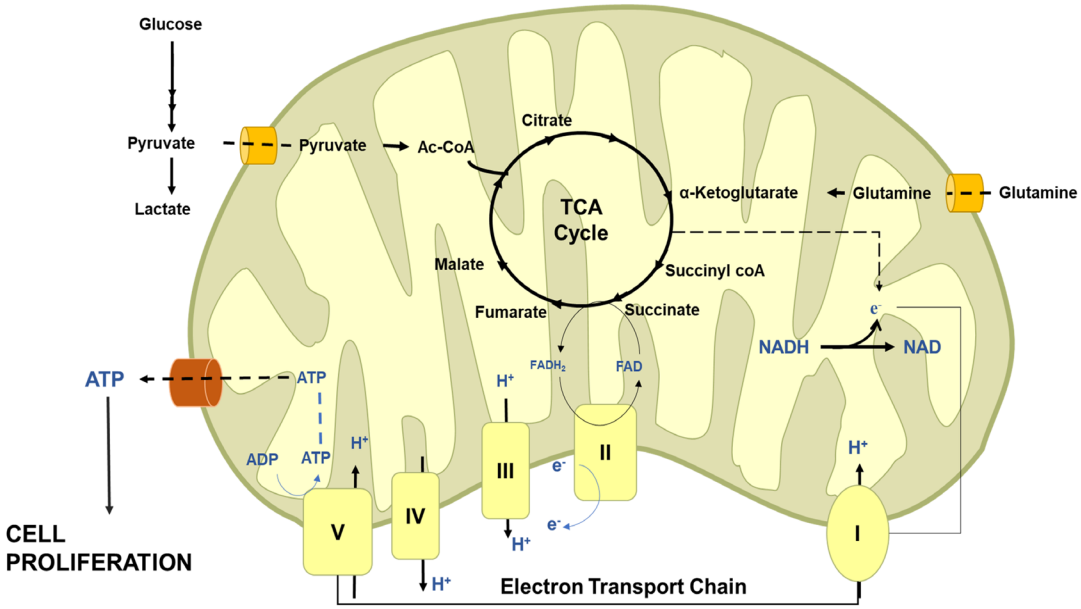

Figure 2. The relation of mitochondria and cell proliferation in cancer

addition to its role in the formation of cell energy, mitochondria also play the center for catabolic and anabolic reactions that enables great metabolic adaptation, especially in cancer cells [26]. The TCA and OXPHOS cycles are a set of interconnecting events that occur during the energy generation process in cells [27]. Carbon compounds produced from the catabolism of some nutrients can be used in the TCA cycle to then form ATP via ETC. Most of the metabolites resulting from nutrient catabolism are converted to acetyl-CoA through different pathways, before being used in the TCA cycle. Glucose is oxidized to form pyruvate at the cytosol in glycolysis. Pyruvate is then transported into the mitochondrial matrix, where it is oxidized and reacts with coenzyme-A, forming acetyl-CoA. Pyruvate can also be carboxylated to directly form oxaloacetate, an intermediate of the TCA cycle [7,28]. Meanwhile, in the beta-oxidation reaction of fatty acids, long-chain fatty acids are catabolized to produce two carbon molecules which ultimately form acetyl-CoA. In addition, glutamine, which is catabolized through glutaminolysis, will form glutamate, which is then converted in the mitochondria to alfa-ketoglutarate, an intermediate of the TCA cycle [28].

The TCA cycle begins with the incorporation of acetyl-CoA with oxaloacetate (OAA) to form citric acid [7]. Citrate is then converted to isocitrate. Next, an oxidative decarboxylation process occurs, where isocitrate is converted to α-ketoglutarate (α-KG) followed by the formation of NADH and the release of CO2. The α-KG molecule formed then undergoes oxidative decarboxylation to also become succinyl-CoA. The reaction produces NADH and releases CO2. The resulting NADH is then used in complex I of ETC [7,27]. Succinyl-CoA is then converted to succinate followed by the release of GTP. Furthermore, succinate is oxidized to produce fumarate. The reaction is followed by the transfer of two hydrogen atoms to FAD to produce FADH2, which acts as an electron carrier in complex II of ETC. In addition, the reaction for the formation of succinate in the TCA cycle is catalyzed by the enzyme succinate dehydrogenase which also plays a role in complex II ETC. Fumarate is then converted to malate and then malate is converted to oxaloacetate. The oxaloacetate formed can react again with acetyl-CoA to form citrate [27].

Overall, a full cycle of TCA will produce 1 mole of ATP and the by-products are 3 moles of NADH and 1 mole of FADH2. NADH and FADH2 will be used in complex I (NADH dehydrogenase) and complex II (Succinate dehydrogenase) of ETC, respectively. Complexes I and II will transfer their

electrons through the ETC and generate ATP via OXPHOS. NADH in complex I will donate electrons to coenzyme Q and start the ETC process [27,29]. The electron transfer is mediated by enzymes containing flavin. These electrons can then enter into complex II or III [29].

Complex III and cytochrome c then pass electrons to complex IV. A proton gradient is formed by complexes I, II, and IV as electrons move through the inner mitochondrial membrane. The protons then enter the mitochondrial matrix with the help of complex V, ATP synthase, and result in the production of ATP. Complexes I–V allow oxidative phosphorylation to occur and generate up to 38 moles of ATP per mole of glucose [7,29]. The TCA and OXPHOS cycles are closely related because the oxidation of NADH and FADH2 in complexes I and II is required for the TCA cycle to continue [27].

The TCA and OXPHOS cycles that occur in mitochondria play an important role in producing far more energy than the glycolysis process [7]. Adequate energy supply through aerobic metabolic processes is known to support the process of cell survival and proliferation. Aerobic energy metabolism through the TCA and OXPHOS cycles is known to be maintained in some cancer cells, one of which is lung cancer cells [6,29]. The relationship between mitochondria and cell proliferation in cancer is shown in Figure 2.

Mitochondria and cancer cell proliferation

In addition to providing the main energy source (ATP) for cell survival and proliferation, mitochondria are indispensable for cancer cells because of their ability to process metabolic intermediates from several metabolic pathways through the TCA cycle and provide building blocks for anabolic processes. TCA and OXPHOS cycle regulation is important in cancer cell survival. The TCA cycle is central to cell metabolism because many substrates can enter the cycle. Several enzymes of the TCA cycle are frequently mutated or deregulated in cancer, including aconitase (also known as aconitate hydratase, AH), isocitrate dehydrogenase (IDH), fumarate hydratase (FH), succinate dehydrogenase (SDH), and alfa-ketoglutarate dehydrogenase complex (KGDHC) [23,30]. In addition, a metabolite in the TCA cycle, namely acetyl-CoA is also involved in chromatin modification, DNA methylation, and post-translational modification of proteins that alter their function [27].

Todisco et al (2019) demonstrated the role of the TCA cycle in hepatocellular carcinoma. Todisco suggested decreased gene expression and enzyme activity related to the TCA cycle, glutamine, malate/aspartate metabolism, and citrate/pyruvate transport [31]. Excessive conversion of glucose to lactate, as well as the export of citrate from the mitochondria to the cytosol by the dicarboxylate antiporter solute carrier family 25 member 1 (citrate transporter) (SLC25A1), induces tumor cells to use anaplerotic reactions to replenish the TCA cycle. This is then achieved through increased glutaminolysis [23].

Mitochondrial glutamate is converted to alfa-KG catalyzed by glutamate dehydrogenase 2 (GLUD2), or glutamate-oxaloacetate transaminase 2 (GOT2). The alfa-KG compound undergoes oxidative carboxylation in the TCA cycle to produce citrate. Citrate is then exported to the cytosol via SLC25A1 and converted to oxaloacetate and acetyl-CoA by ATP citrate lyase (ATP citrate lyase, ACLY), which is ultimately used in the synthesis of fatty acids and steroids. Therefore, developing tumors exhibit the ability to oxidatively process glutamine in generating energy via the TCA and ETC cycle [26,27,32,33].

Increased glutamine transport and glutaminolysis allow various types of cancer, including myeloma and glioma, to obtain most of the energy and macromolecules [34]. Enzymes such as ACLY and acetyl-CoA carboxylase (acetyl coenzyme A carboxylase, ACC), which control lipid synthesis, are frequently overexpressed in certain cancer. ACLY is overexpressed in several cancers such as lung, cervical, and breast cancer cells [35,36], whereas ACC is overexpressed in lung and hepatocellular carcinoma cells [37].

The association of heme with mitochondrial function, cell proliferation, and malignancy

Heme is known to have a key role in supporting mitochondrial function in the process of energy formation through oxidative phosphorylation. Heme compounds play a role in compiling protein complexes involved in oxidative phosphorylation so as to enable the process of electron transfer and ATP synthesis to work properly. The process of energy formation through OXPHOS can run well when the oxygen supply in the cells is in sufficient quantities. On the other hand, heme synthesis is also needed to maintain the expression of several hemoproteins essential for survival and proliferation [38]. Dysregulated heme metabolism known to promotes oxidative stress, which causes DNA, lipid, and protein damage [38].

Many studies have been carried out about the biosynthesis of heme in cancer. Several studies on cancer cells showed an increase in heme levels [39,40], activity of proteins containing heme [6,39,41], and the expression of heme transport proteins [39]. Based on those studies, not only succinyl co-A but also heme production and the entire heme biosynthetic pathway is promoted in tumors. Several studies have shown that the expression and activity of ALAS1, porphobilinogen deaminase, and uroporphyrinogen decarboxylase tend to increase in several types of cancer. Meanwhile, inhibition of heme biosynthesis by succinyl acetone showed a decrease in tumor cell survival and proliferation [6,39,42].

Several studies have shown that most tumor cells produce ATP via the oxidative phosphorylation pathway [43]. Study using the human lung carcinoma cell line A549, showed that the induction of heme biosynthesis by ALA increased OXPHOS in that cell line [44]. Similarly, the increase of heme synthesis that associated with high oxygen consumption was observed in another study of lung cancer and in myeloid leukemia, [6,39]. High oxygen consumption in the cells can be an indicator of OXPHOS running. Administration of heme synthesis inhibitors causes a decrease in oxygen consumption and the expression of several hemoproteins such as cytoglobin and cytochromes [6]. Another study on colorectal cell lines (Caco2) showed that inhibition of heme synthesis-export system by ALAS1 & FLVCR1 silencing reduced cell survival and proliferation [45,46].

Increased heme synthesis in tumors known to contribute to the activity of certain hemoproteins. There are several hemoproteins that play an essentials role in cancer, such as myoglobin; tryptophan 2,3-dioxygenase (TDO) [47]; indolamine-2,3-dioxygenase 1 and 2 (IDO1/2) [48]; mitochondrial cytochromes; cytochrome P450; and cyclooxygenase [43,49]. Study on lung cancer have shown that the expression of hemoproteins, such as cytoglobin, cytochrome c, cytochrome P450 family-1-subfamily member B 1 (CYP1B1), and prostaglandin endoperoxide synthase 2 (COX2) is higher in tumor cells compared to normal cells. [6] In this regard, the levels of cytoglobin and cytochrome c are known to depend on the rate of heme synthesis and the intracellular heme concentration.

The increase in heme biosynthesis in cancer cells may also be related to the regulation of p53, a protein that regulates various biological processes [50]. Shen et al (2014) showed that, heme regulates p53 stability directly. Hem interacts with the C-terminal heme-responsive motif (HRM) of the p53 protein [51]. The interaction between hem with p53 inhibit p53 ability to binds with DNA, which indicates that heme can control transcription of p53 target genes. It also showed that p53 degradation via the ubiquitin-proteasome system, is triggered by binding of heme to p53. This suggests that tumorigenesis is associated with iron ion overload.

In vivo studies have shown that excess iron ion levels in cancer cells can maintain the rate of heme synthesis, and have a direct effect on the stability and function of p53 [52]. Changes in heme metabolism which occur over tumor development progression are known to influence the dysregulation of p53 [51]. Furthermore, it is known that heme production is increased in cancer to induce cataplerosis of the TCA cycle.

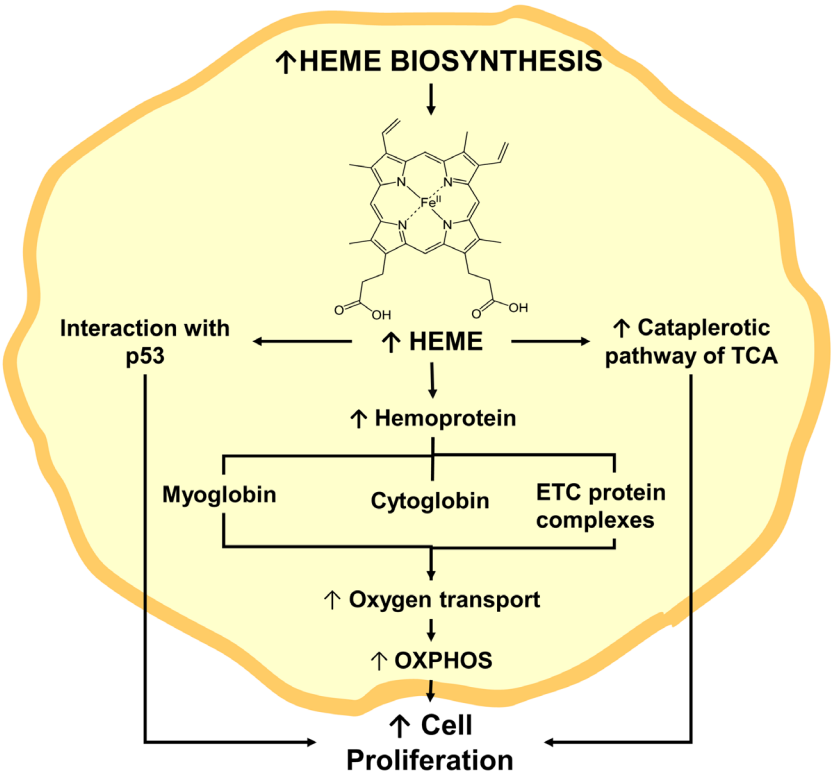

Figure 3. The association of heme biosynthesis with mitochondrial function and cell proliferation in cancer. Heme biosynthesis play an important role in cell proliferation through various ways such as supporting the function of OXPHOS in mitochondria through the mechanism of oxygen transport into cells, interaction with tumor suppressor protein (p53), and increase cataplerotic pathway of TCA cycle to avoid the accumulation of intermediates from this cycle that can be toxic to cancer cells

The increased of heme production is mainly aimed at using succinyl-CoA. Studies in hereditary leiomyomatosis and renal cell cancer (HLRCC) with alteration in the enzyme fumarate hydratase found that the enhanced heme production is essential to avert a build-up of hazardous intermediates of the TCA cycle. Heme oxygenase 1 (HMOX1) degrades the heme generated in system. This suggests that heme synthesis does not design to boost intracellular heme sources [53].

Based on these explanations, heme is recognized to have a role in various ways of cancer cells proliferation. The increase in heme biosynthesis is known to be related to its role in supporting the function of OXPHOS in mitochondria through the mechanism of oxygen transport into cells and as a prosthetic group of protein complexes that make up ETC [6,39,41]. In addition, heme can contribute to cancer cells proliferation through its interaction with the tumor suppressor protein, namely p53 [50,51]. Furthermore, the increase in heme biosynthesis in some cancer cells aims to trigger the cataplerotic pathway of the TCA cycle to avoid the accumulation of intermediates from this cycle that can be toxic to cancer cells [45,53]. Although the increase in heme biosynthesis in tumors has different goals, multiple studies have found that it is linked to an increase in tumor cell proliferation. The association of heme with mitochondrial function and cell proliferation in cancer is showed in Figure 3.

Conclusion

Heme and mitochondria are recognized to play a critical role in cell energy production, which may subsequently be used to trigger cell proliferation. Heme has been proven in several studies to have an essential function in triggering cell proliferation in tumors. Based on the fact that heme is an important cofactor in electron transport chain complexes, and that there is an increase in heme biosynthesis in tumors, it is possible to investigate

the role of heme, in tumor therapeutic approaches. Inhibition of heme biosynthesis can be used as an approach to inhibit cancer cell proliferation and survival.

Author contributions

RN collects literature and writes manuscript under SWAJ's guidance. SWAJ contributed in funding, ideas, collect literture, directing and guiding the writing and finalization of the manuscript.

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Declaration of competing interest

None.

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