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# A Novel Strategy of Dual Inhibition of Distinct Metabolic Features in Osteosarcoma

*Shingo Kishi, Kanya Honoki, Yasuhito Tanaka  
and Hiroki Kuniyasu*

## Abstract

Mitochondria are the places for the energy production of the cells, while reactive oxygen species (ROS) are also produced alongside. In recent years, it has been reported that cancer stem cells metabolize predominantly through oxidative phosphorylation (OXPHOS) rather than glycolysis. Targeting OXPHOS achieved by suppression of ATP synthesis through mitochondrial ATP synthase could be a potential therapeutic option against cancer stem cells. Since c-Myc inhibition is considered to lead a metabolic flux to OXPHOS from glycolysis, the combinatory inhibition of both OXPHOS and glycolysis could be a strong candidate for the treatment of malignant tumors. In this chapter, we will discuss about the mitochondria metabolism as the potential therapeutic target in osteosarcoma stem cells, and the synergistic effects of combination of OXPHOS inhibitor with c-Myc inhibitor, which target both OXPHOS-dominant cancer stem cells and glycolysis-dominant non-cancer stem cells, will be discussed.

**Keywords:** osteosarcoma, mitochondria, metabolism, OXPHOS, c-Myc

## 1. Introduction

Intratumor heterogeneity, which is the basis of tumor evolution, is the fundamental challenge in cancer medicine. Intratumor heterogeneity is considered to be involved in several important aspects in cancer biology such as disease relapse and metastatic behaviors as well as drug resistance. Over the past decades, a small subset of tumor cells, so-called cancer stem cells (CSCs), have been proposed to be a hierarchical organizer of the tumor heterogeneity [1] and play a critical role in tumor relapse, metastasis, drug resistance, and tumor propagation in many cancer types including osteosarcoma (OS) [2–6]. At the apex of the heterogeneity in the tumor, CSCs possess the capacity of self-renew and tumorigenicity which generate the bulk of tumor with more differentiated progenies [7]. Conventional anticancer therapies target the bulk of heterogeneous tumor mass resulting in tumor shrinkage, but CSCs could trigger the relapse by differentiation into non-stem tumor cells. Thus, targeting CSCs could represent an integral component for developing more effective treatment strategies against cancer.

Now, we have evidences that cancer heterogeneity not only is generated by genetically distinct subclones but is also driven by phenotypic and functional

heterogeneity within each subclone [8, 9]. One of the distinct phenotypes of CSCs is their cellular metabolism mechanisms for energy production. Tumor cells reprogram their metabolic machinery to meet their needs during tumor growth known as Warburg effect which shifts their ATP production from via oxidative phosphorylation (OXPHOS) to glycolysis even in the microenvironment with abundant oxygen concentration [10]. Over the past years, the metabolic phenotype of CSCs has been intensely investigated, and it was originally hypothesized that CSCs would reflect the normal tissue hierarchy where multipotent stem cells are fundamentally glycolytic, while differentiated somatic cells rely on OXPHOS [11]. Although the differentiated cells increase their dependency upon the glycolysis during the acquisition of the transformed phenotype, these changes might be cell specific, and some cells might adapt to neoplastic transformation by increasing their dependency on OXPHOS [12]. As a matter of fact, the metabolic phenotype of CSCs are controversial suggesting that it could be a tumor-type- or cell line-dependent manner such as breast and nasopharyngeal CSCs relying on glycolysis [13, 14], while lung, glioblastoma and pancreatic ductal adenocarcinoma CSCs relying on OXSPHOS [15–17]. In osteosarcoma (OS), a CSC-like cell line, 3AB-OS, exhibited its metabolic dependency on glycolysis compared to the parental MG-63 cells [18]. However, this came from only one cell line chemically treated long time from MG-63 which bore the *ras* gene mutation unusually found in OS. Controversially, there is the evidence that the transformed mesenchymal stem cells (MSCs), which are supposed to be the sarcoma-initiating cells or sarcoma stem cells, showed the increased OXPHOS and had a capability to switch to glycolysis to adapt to their microenvironments [19]. The discrepancy in these metabolic profiles of CSCs would be due to multifactorial causes. The first possible explanation is the plasticity of these cells in response to the microenvironment and the stages of harvesting in terms of differentiation/dedifferentiation [20]. Another possible cause would be the lack of precise definition of CSCs and their heterogeneity due to the various techniques for CSC isolation [21]. Furthermore, the metabolic status of CSCs can be affected by cross talk between CSCs and cancer-associated stroma in the microenvironment. For instance, OS cells directly increase their mitochondrial biogenesis using this energy-rich metabolite such as lactate that is abundantly provided by MSC as an effect of the altered microenvironmental conditions induced by OS cells, and the lactate produced by MSC promotes the migratory ability of OS cell [22].

Actually, there are several reports indicating that CSCs have augmented utilization of extracellular catabolites such as pyruvate, lactate to support OXPHOS, [23] and mitochondrial metabolism could be a potential target for an effective elimination of CSCs [17]. Previous studies suggested that inhibition of the OXPHOS pathway reduced sphere formation and tumor formation capacity, which demonstrated the vulnerability of CSCs to mitochondria-targeted therapies [24], and several agents such as salinomycin targeting CSCs through inhibition of mitochondrial biogenesis and OXPHOS are currently under investigation for cancer treatment [25].

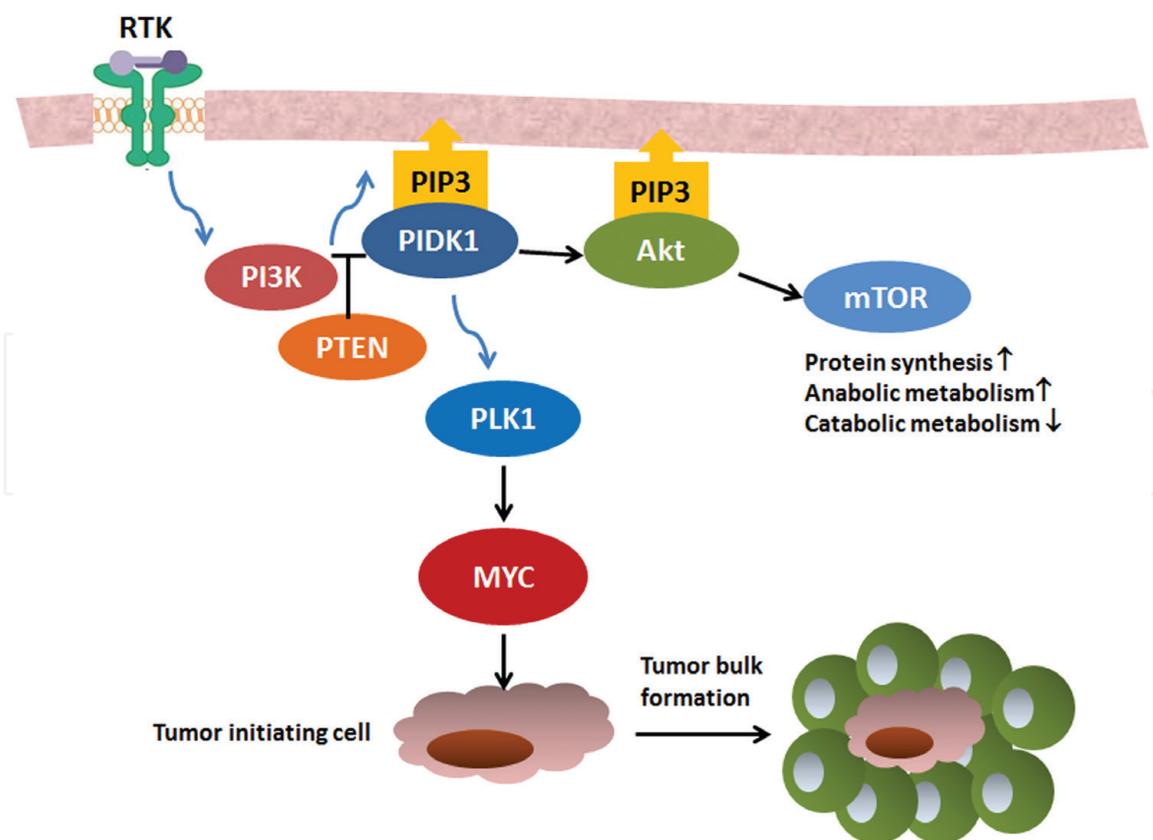
Here, we will discuss about the mitochondrial metabolism as a potential therapeutic target in osteosarcoma, especially its stem-like cell populations.

## **2. Myc is a key regulator of cancer cell metabolism**

Glucose is one of the major nutrients that mammalian cells utilize to synthesize new organelles as well as to generate high-energy molecules, such as ATP, NADH/NADPH, and FADH. c-Myc played an important role in regulation of glycolysis through its target glucose metabolism genes [26], and those genes directly regulated by c-Myc include glucose transporter GLUT1, hexokinase 2

(HK2), phosphofructokinase (PFKM), and enolase 1 (ENO1) [27, 28]. Myc was also observed to upregulate lactate dehydrogenase A (LDHA) to generate NAD<sup>+</sup>, which is a cofactor required for the glycolysis particularly by GAPDH [29]. Through the upregulation of these genes, c-Myc contributes directly to the Warburg effect (aerobic glycolysis) and the ability of transformed cells to convert glucose to pyruvate even under adequate oxygen tension. Myc also regulates protein synthesis, ribosome biogenesis, nucleotide biosynthesis, as well as fatty acid and cholesterol metabolism. Cancer cells take advantage of these Myc's broad reaches to reprogram and augment the most critical processes for survival, particularly metabolism.

In addition to the metabolic reprogramming, c-Myc also contributes to the cell cycle regulation in corporation with PI3K (phosphatidylinositol-3,4,5-triphosphate)/Akt/mTOR (mammalian target of rapamycin) and Wnt pathway. The role of PI3K-Akt-mTOR and Wnt pathways in regulation of the cell cycle progression in cancer cell has been proposed (**Figure 1**). The activation of insulin/insulin-like growth factor (IGF) receptor by nutrients/growth factors activates PI3K-Akt pathway. The phosphorylated Akt activates mammalian target of rapamycin complex 1 (mTORC1), and the activated mTORC1 upregulates the protein synthesis but inhibits autophagy. Inhibition of autophagy rescues Dvl (Dish homolog in mammalian), and this leads to activation of Wnt pathway. Wnt pathway is the key pathway in activation of cell cycle, and the activated Wnt pathway upregulates the CyclinD; the c-Myc; the matrix metalloproteinases, COX-2, peroxisome proliferator-activated receptors (PPARs); and the growth factors and their receptors and downregulates E-cadherin, the cell cycle inhibitor p16INK4a (ARF), and p53 [30]. Wnt pathway thus regulates the cancer cell entry into the cell cycle through the production of



**Figure 1.** *c-Myc* contributes to the cell cycle regulation in corporation with PI<sub>3</sub>K/Akt/mTOR and Wnt pathway. The activation of insulin/IGF receptor by nutrients/growth factors activates PI<sub>3</sub>K-Akt pathway. The phosphorylated Akt activates mTORC1, which eventually leads to activation of Wnt pathway. Wnt pathway is the key pathway in activation of cell cycle, and the activated Wnt pathway upregulates the CyclinD, the *c-Myc* which would be involved in tumor initiation as well as tumor progression.

CyclinD. CyclinD complexes with cyclin-dependent kinase 4/6 (Cdk4/6), inactivates the tumor suppressor protein retinoblastoma (Rb), and promotes the entry of the cell from G0 to G1 phase of cell cycle as well as metabolic reprogramming through the c-Myc activation as described above. In this context, c-Myc is acknowledged to play an important role to activate genes involved in predominantly cell cycle regulation, cellular metabolism, and protein synthesis, especially specific to G0-G-S transition as well as glycolysis and Krebs cycle, chromatin structure, and its transcriptional networks in cancer cells and embryonic stem cells as well [31, 32]. Given its crucial role in cancer progression and maintenance, c-Myc has been the ideal target for cancer therapy, and c-Myc targeting strategy in cancer therapy has been investigated in various means such as direct inhibition by antisense oligonucleotide [33] and siRNA [34], indirect inhibition by blocking transcription with BET bromodomain inhibitors such as JQ-1 [35], and blocking mRNA translation with mTOR inhibitor [36]. After a long time struggling on targeting c-Myc in cancer therapy, we are now witnessing a renewed interest in making Myc inhibition for the future cancer therapy. In the aspect of c-Myc contribution to the metabolism, c-Myc promotes a Warburg-like glycolytic phenotype through dual mechanism of upregulation of key glycolytic enzymes as described above, and suppression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 $\alpha$ ) by direct inhibition through binding to its promoter [17]. PGC-1 $\alpha$  is crucial for the anti-oxidative capacity and mitochondrial metabolism in cancer [37, 38] and contributes to the maintenance of CSC's phenotype of self-renewal through controlling intracellular ROS levels. Myc/PGC-1 $\alpha$  balance controls the metabolic phenotype of cancer cells as c-Myc dominance shifts to Warburg phenotype of differentiated cancer cells and PGC-1 $\alpha$  dominance shifts to OXPHOS-dependent phenotype of CSCs [17]. Therefore, combining OXPHOS inhibition with c-Myc inhibition could provide a new multimodal approach for targeting the distinct metabolic features in cancer therapy.

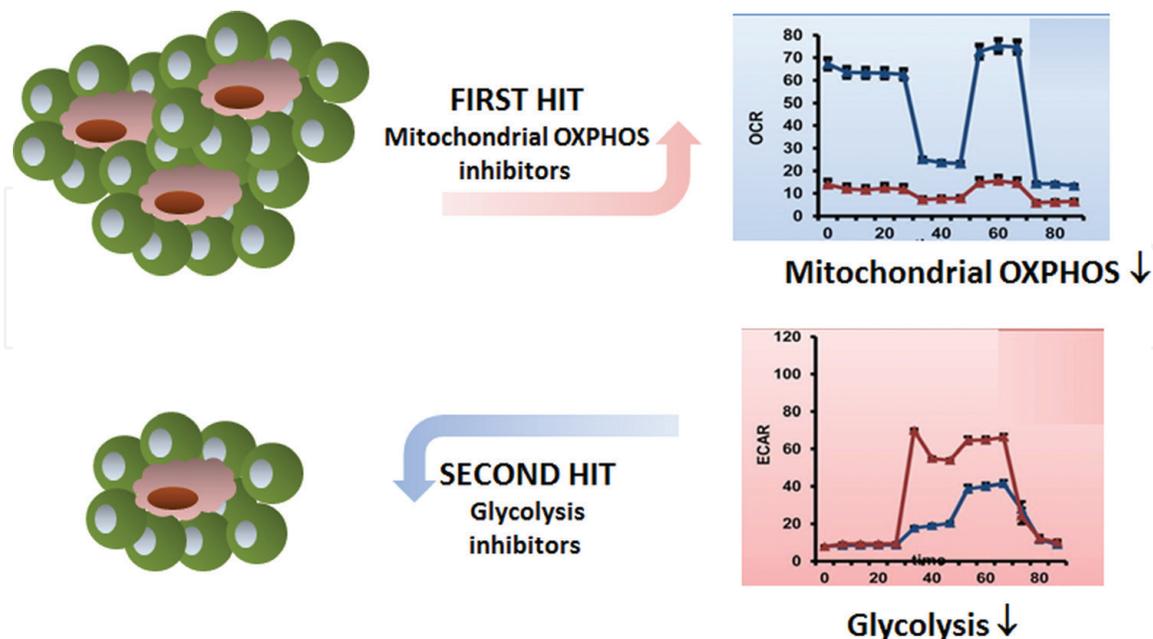
### **3. Cancer stem cell metabolism: a potential therapeutic target**

CSCs also known as tumor-initiating cells (TICs) are a rare population of tumor cells with stem cell properties, which are thought to generate the tumor bulk and considered to drive the malignant growth, treatment resistance, minimal residual disease, and metastases. Along with the role of cancer drivers, CSCs also exhibit stem cell properties such as self-renewal and multilineage differentiation capacity [39]. CSCs are considered to be resistant to chemotherapy or radiations, both of which successfully destroy differentiated non stem-like cancer cells. As a matter of fact, even though current conventional anticancer therapies could achieve a transient control over the disease, a large number of patients experience tumor relapse or metastatic dissemination after an initial treatment with apparent disease-free period. Since CSCs, resisting to conventional therapies, attributes these biological behaviors, eradication of CSCs could be a promising target to totally exterminate the disease of cancer [40].

Although much is known regarding metabolic pathways important for cancer cell survival, the potential for therapeutic metabolic alteration of CSCs has not been fully uncovered [41], but recent studies indicate that metabolism and stemness are highly intertwined processes in tumor tissues, and CSCs possibly have different metabolic properties compared to non-CSCs.

Glucose homeostasis is reciprocally controlled by the catabolic glycolysis/OXPHOS and by the anabolic gluconeogenesis pathway. In the catabolic reaction, when oxygen is absent, glycolysis predominantly controls the metabolism of ATP

production, while in the presence of oxygen, OXPHOS predominantly regulates the maximal ATP production in the mitochondria. Cancer cells preferentially metabolize glucose rather than OXPHOS even in the presence of oxygen defined as Warburg effect/aerobic glycolysis by the activation of some key genes such as c-Myc. In contrast to the somatic cells which primarily utilize OXPHOS, pluripotent stem cells including embryonal stem cells and induced pluripotent stem cells rely on glycolysis [42]. Therefore, it has to be pointed out that the biological functions of CSCs are different from those of differentiated cancer cells, making their phenotype more similar to normal stem cell, and metabolism is not the exception. Since aerobic glycolysis/Warburg effect has been widely accepted and recognized as a peculiar hallmark of cancer cells, it may be reasonable to expect that, conversely, CSC metabolism is mostly oriented toward mitochondrial OXPHOS. Actually, it is broadly accepted that ATP production in CSCs depends on glycolysis or OXPHOS in a tumor-type-dependent manner. The heterogeneity and plasticity of CSCs probably determine their primary source of energy to survive. The recent investigation has suggested that CSCs may display a broader repertoire of biochemical behavior in response to different environmental conditions, and accumulating evidence has indicated that CSCs utilize OXPHOS not only relying on the glycolysis [41, 43]. CSC metabolism show a highly plastic profile which allows to fulfill the energy requirements, according to the most suitable environmental condition. This metabolic flexibility of CSCs has been shown in diverse tumor types demonstrating that CSCs efficiently gain energy production from glycolysis when OXPHOS is blocked [44]. Recently, De Francesco et al. proposed the “two metabolic hit strategies” for eradication of CSCs (Figure 2) [45]. They demonstrated that the prolonged treatment with a mitochondria-interfering agent like doxycycline drastically impairs oxygen consumption rate (OCR) and mitochondrial respiration in MCF7 breast cancer cells. Such impairment in mitochondrial activity represents a first metabolic hit that constrains cellular metabolism of the surviving cancer cell subpopulation toward a



**Figure 2.** The treatment with a mitochondria-interfering agent impairs oxygen consumption rate (OCR) and mitochondrial respiration in cancer cells as a metabolic first hit that constrains cellular metabolism of the surviving cancer cell subpopulation toward a predominantly glycolytic phenotype, resulting in metabolic inflexibility, as evidenced by the increased extracellular acidification rate (ECAR). The use of a glycolysis inhibitor may therefore act as the second metabolic hit that efficiently targets CSCs by halting their biochemical machinery (X axis, time (min); Y axis, pmoles/min/mg protein; red line, mitochondria inhibitor treatment; blue line, control).

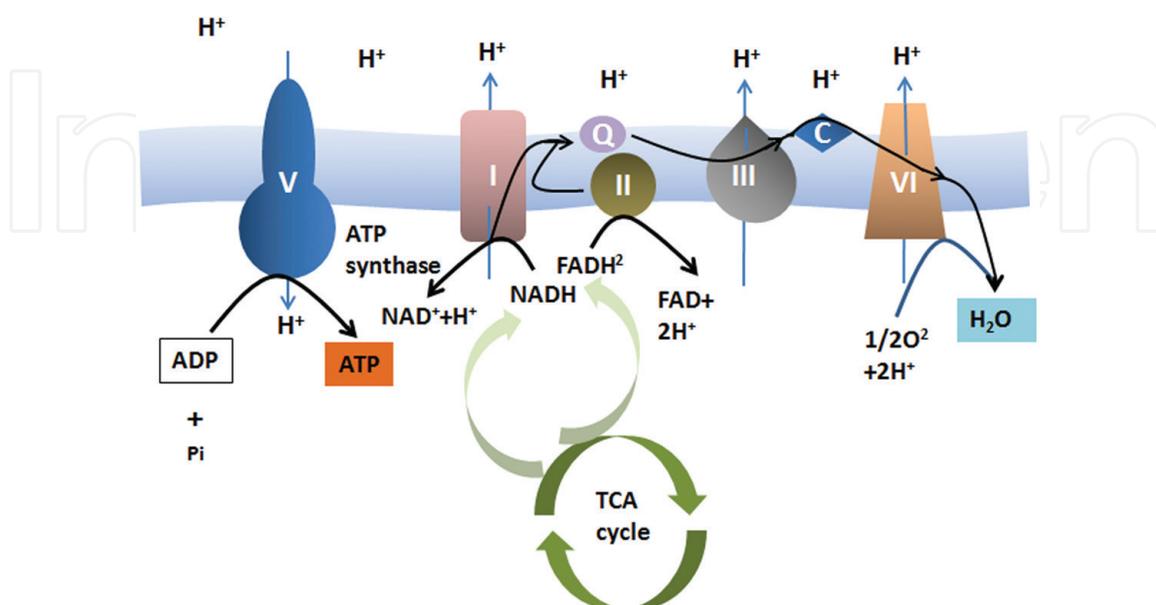
predominantly glycolytic phenotype, resulting in metabolic inflexibility, as evidenced by the increased extracellular acidification rate (ECAR). In this sequence, the glycolysis inhibitor potentially acts a second metabolic hit which effectively targets CSCs. Thus, specific metabolic-oriented pharmacological intervention could reverse CSC metabolic plasticity toward an inflexible biochemical phenotype, representing a new synthetic-lethal metabolic strategy for eradicating CSCs.

#### 4. Targeting mitochondrial physiology in cancer stem cells

Mitochondria are key organelles involved in several processes related to cell proliferation and survival, and their most important function is the generation of ATP which holds cell metabolism. As the main energy producers, mitochondria produce ATP using tricarboxylic acid (TCA) cycle and OXPHOS. They also generate reactive oxygen species (ROS) during this process, which are sometimes harmful to the cells when produced excessively.

Because mitochondria play a key role in the alteration of oxidative stress and energy status, their functional characteristics have been considered to verify stemness like stem cell stability and pluripotency [46, 47]. Mitochondrial metabolic activity and antioxidant enzyme expression have shown to be closely related to the cell differentiation [48, 49]. Thus, we could assume that stem cell mitochondria play important roles in maintaining stemness and differentiation. However, whether the roles of CSC mitochondria are similar to stem cell mitochondria or so-called differentiated cancer cells in general is not clear. Based on the previous reports, the CSCs might be more differentiated than normal stem cells, and the mitochondrial properties of CSCs are possibly different from those of stem cells or general cancer cells [39, 50].

Mitochondria have a multi-level network of antioxidant and OXPHOS systems (**Figure 3**). Mitochondrial redox balances are regulated by the mitochondrial inner membrane electrochemical gradient. As shown in **Figure 3**, nicotinamide adenine dinucleotide (NADH) from TCA cycle is oxidized by Complex I in the electron transport chain (ETC) of OXPHOS. Electrons from Complex I and II are



**Figure 3.**

Mitochondrial redox balances are regulated by the mitochondrial inner membrane electrochemical gradient. NADH from TCA cycle is oxidized by Complex I in the ETC. Electrons from Complex I and II are transferred to coenzyme Q<sub>10</sub> and then passed on to Complex III, cytochrome c, Complex IV, and finally O<sub>2</sub> to generate H<sub>2</sub>O. Complex V (FoF<sub>1</sub>-ATP synthase) generates ATP from ADP for the cellular energy source.

transferred to coenzyme Q10 and then passed on to Complex III, cytochrome c, Complex IV, and finally O<sub>2</sub> to generate H<sub>2</sub>O. Complex V (ATP synthase; F<sub>0</sub>-F<sub>1</sub>) generates ATP from ADP as well as inorganic phosphate Pi.

A powerful strategy focused on mitochondrial biogenetics as a target to eradicate CSCs involves inhibition of the ETC complex, with consequent ROS overproduction. Dong et al. demonstrated that the suppression of mitochondrial Complex I activity inhibited oxygen consumption and induced glycolysis in breast CSCs as a result of loss of fructose-1,6-biphosphatase implying that overproduction of ROS and reduction in glucose metabolism might be effective against breast CSCs [11]. Hirsch et al. also showed that metformin, the first-line antidiabetic drug, selectively killed the CSCs in breast cancer cell line through the inhibition of Complex I [51]. Furthermore, atovaquone, an FDA-approved antimalarial drug, inhibits the propagation of breast cancer cell line MCF7-derived CSCs through the selective OXPHOS inhibition by targeting the CoQ10 dependence of mitochondrial Complex III [52]. This has been explored in the context of therapy as indicated by Alvero et al. using the novel isoflavone derivative NV-128 which significantly decreased mitochondrial function, as shown by a decrease in ATP, Complex I and Complex IV levels, and induced cell death in ovarian CSCs [53]. Finally, mitochondria-targeted vitamin E succinate (MitoVES) has been well characterized as an agent, which potentiates the ability to induce apoptosis in breast CSCs [54]. Reduction of mitochondrial membrane potential, overproduction of mitochondrial ROS, and inhibition of mitochondrial biogenesis have been demonstrated to affect CSC proliferation and survival [55]. These inputs indicate that the maintenance of CSC proliferation may not only be dependent on glycolysis, but is also based on mitochondrial activity. Therefore, the specific mitochondrial-targeted compounds which induce cell death in chemoresistant CSCs are promising novel therapeutic venue to treat cancer patients with relapsed or metastatic diseases. The most important point is that mitochondria from CSCs are not indistinguishable to those from differentiated non stem-like tumor cells in divergent aspects. Since CSCs are considered to be heterogeneous and adaptive metabolic profiles, the future therapy targeting cellular metabolism should be designed in a form of simultaneous or selective blockade of both glycolysis and mitochondrial respiration to completely eradicate CSCs [20]. Consequently, dual inhibition of glycolytic and mitochondrial energy pathways has proven to be effective against tumor growth in a number of preclinical cancer models. For instance, dual inhibition of glycolysis by 2-deoxyglucose (2-DG) and OXPHOS by metformin is effective in vivo against breast cancer cell xenograft model [56]. However, hexokinase inhibitors such as 2-DG and 3-bromopyruvate have been unfortunately discontinued in clinical trials. There has been an elegant study demonstrating sarcoma cells to be more sensitive than normal cells to dual inhibition of glycolysis with 2-deoxyglucose and OXPHOS with oligomycin or metformin [57]. Recently, Kang et al. have demonstrated that ALDH inhibitor gossypol combined with mitochondrial Complex I inhibitor phenformin resulted in up to 80% ATP depletion in non-small cell lung cancer, which induced significant tumor regression in the cancer xenograft model [58]. These warrant that a key molecule regulating cancer energy metabolism can be a therapeutic target.

Meanwhile, other mitochondria-related processes like activation of developmental signaling pathways including Hedgehog, Notch, and Wnt are also the druggable targets; the drug targeting Notch and Hedgehog pathway has been developed [59], and numerous molecules acting on mitochondria has been used or being tested in clinical trials [60]. Adding these attempts targeting mitochondria, we will emphasize that the dual inhibition of metabolic pathways could be an approach with greater potential to eradicate heterogeneous CSCs rather than singularly targeting glycolysis or OXPHOS pathway.

## 5. Novel approach to target cellular metabolism in osteosarcoma

Osteosarcoma and mitochondria have been investigated since 1970; however, those are mostly limited to the morphological characteristics by microscopic or electron microscopic observation [61, 62].

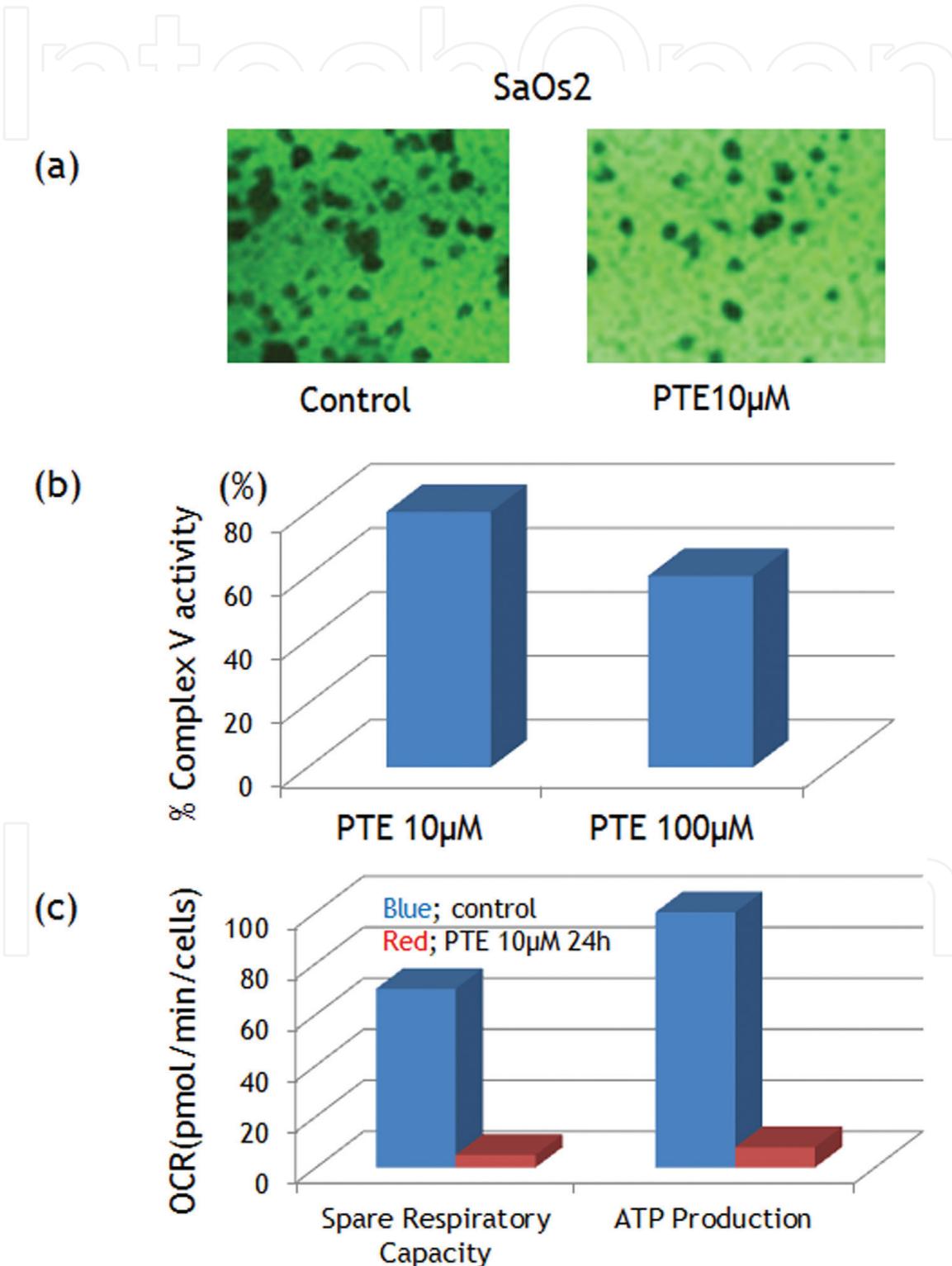
Giang et al. demonstrated that highly invasive and metastatic cell lines were more relied on Warburg effect-like glycolysis than their parental cell lines which showed similar mitochondrial oxygen consumption rate to fetal osteoblasts, suggesting that highly metastatic and invasive cell lines were in the state of suppression of mitochondrial function and upregulation of glycolysis. They suggested that the mechanism of mitochondrial dysfunction was the results of mitochondrial permeability transition such as mitochondrial swelling, depolarization, and membrane permeabilization, and they also demonstrated that this mitochondrial dysfunction and the Warburg effect are reversed by the treatment with mitochondrial permeability transition inhibitor sanglifehrin A [63]. These results indicated that osteosarcoma cells might possess the metabolic plasticity in response to their microenvironment especially hypoxia-reoxygenation caused by an irregular blood supply within tumors, an immature and leaky vasculature, and an abnormal and constantly changing vessel network architecture.

Another biochemical mechanism which contributes to the glycolytic rate of tumor cells is the inhibition of mitochondrial ATP synthase (F<sub>1</sub>F<sub>0</sub>-ATPase) by the natural inhibitor protein IF<sub>1</sub> [64]. Barbato et al. reported that IF<sub>1</sub> modulates the mitochondrial membrane potential and oxidative phosphorylation rate in osteosarcoma cells suggesting that interaction between IF<sub>1</sub> and F<sub>0</sub>F<sub>1</sub>-ATPase might regulate the OXPHOS and glycolysis [65]. However, the detailed mechanisms regulating the cellular bioenergetics by IF<sub>1</sub> have been still controversial in cancer cells under the complex microenvironment.

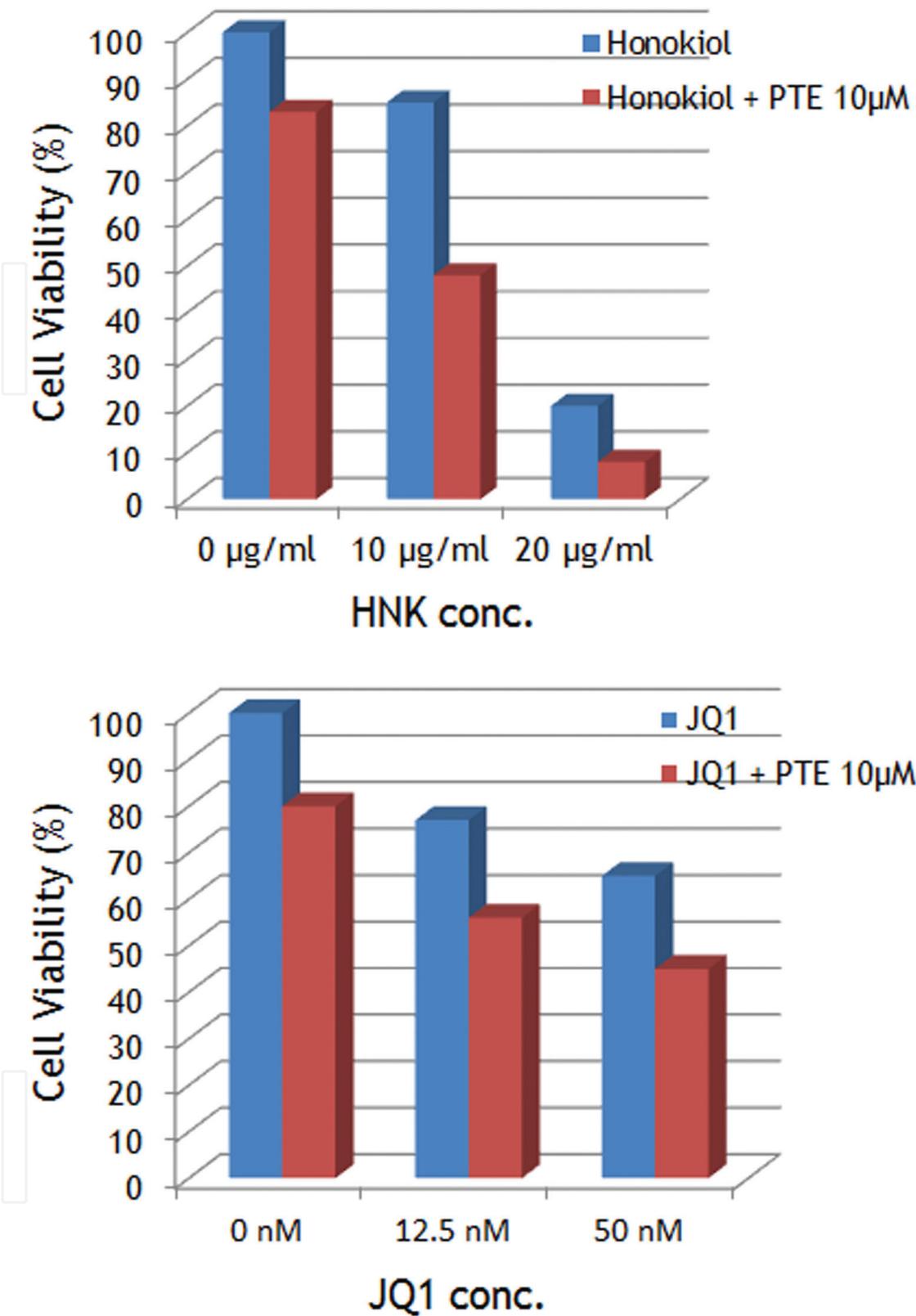
Recently, novel strategy targeting CSCs through phytochemicals and their analogs has been proposed, and mitochondria are one of their potential targets [66]. Among the various compounds, pterostilbene (PTE), which is a methylated resveratrol derived from plants, has been shown to inhibit CSC properties in breast cancer [67, 68], glioma [69], hepatocellular carcinoma [70], and lung cancer [71] through the inhibition of multiple pathways which are possibly related to the CSC propagation such as Wnt, Hedgehog, Notch, and PI3K/Akt. Honokiol (HNK), which is the extract from *Magnolia obovata*, has shown its various antitumor effects through the inhibition of several pathways such as PI3K/Akt/mTOR, Wnt, and c-Myc [72–74]. Besides these effects of PTE and HNK, we have identified that PTE in combination with HNK could be the possible metabolism-targeted therapy against osteosarcoma as a “two hit” or “dual inhibition” of metabolic pathways, OXPHOS and glycolysis. PTE treatment on human osteosarcoma cell lines SaOS2, U2OS, and MG63 reduced viabilities of all cell lines in dose-dependent manner, and expression of stem cell marker such as Oct3, NS, and CD44 and the ability of sphere formation were also decreased in terms of sphere number and size (**Figure 4a**). PTE reduced the activity of F<sub>0</sub>F<sub>1</sub>-ATP synthase, Complex V predominantly (**Figure 4b**), and the mitochondrial oxygen consumption rates and synthetic amount of ATP were also decreased in spheroid condition (**Figure 4c**). These results suggest that PTE possibly targets stem cell population which preferably relies on OXPHOS, suppressing ATP synthesis via F<sub>0</sub>F<sub>1</sub>-ATP synthase inhibition as well as increased ROS production in osteosarcoma cells, and changes metabolic flux to glycolysis-dependent feature.

As aforementioned before, c-Myc promotes a Warburg-like glycolytic phenotype through the upregulation of key glycolytic enzymes along with the

suppression of PGC-1 $\alpha$ . There are several reports regarding the anticancer activity of HNK which is possibly associated with c-Myc as well as JQ1, a BET bromodomain inhibitor [74]. Thus, we conducted dual inhibition of OXPHOS by PTE and c-Myc inhibition by HNK or JQ1. The results showed that both of these agents synergistically inhibited osteosarcoma cell growth in a dose-dependent manner (**Figure 5**). Now, we are conducting an investigation of dual metabolic inhibition via in vivo experiments using our own established rat osteosarcoma model.



**Figure 4.** (a) Pterostilbene (PTE) treatment on human osteosarcoma cells reduced the ability of sphere formation in terms of sphere number and size. (b) PTE reduced the activity of FoF<sub>1</sub>-ATP synthase, Complex V. (c) The mitochondrial OCR rates and synthetic amount of ATP were also decreased in spheroid condition (Kishi et al. unpublished data).



**Figure 5.** Dual inhibition of OXPHOS by PTE and c-Myc inhibition by Honokiol (HNK) or JQ1 showed the synergistic effect on inhibition of osteosarcoma cell growth in a dose-dependent manner (Kishi et al. unpublished data).

The above results suggested that c-Myc inhibitor could lead to metabolic flux to OXPHOS and PTE could lead to metabolic flux to glycolysis. Thus, these exerted a great synergistic effect with “two metabolic hits” or “dual metabolic inhibition” of distinct metabolic features, OXPHOS and glycolysis, and it could be a novel therapeutic strategy against osteosarcoma, possibly targeting both stemlike cell population and general tumor cell population.

## 6. Conclusions

Prognosis of the patients with osteosarcoma has been improved; actually, we could say “dramatically,” over the last quarter-century. However, it is also true that it has reached to plateau without any breakthroughs, and nearly 30% of patients still have to face very severe poor prognosis, especially with metastatic disease. We need to develop a novel treatment to combat such a poor prognostic situation. Targeting the distinct metabolic features of OXPHOS and glycolysis with the concept of “two metabolic hits”/“dual metabolic inhibition” strategy could bring a new insight into the field of osteosarcoma treatment, and some natural compounds such as pterostilbene and honokiol could be the possible candidates to achieve this aim.

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## Conflict of interest

All authors have no “conflict of interest” to be declared.

## Author details

Shingo Kishi<sup>1,2</sup>, Kanya Honoki<sup>2\*</sup>, Yasuhito Tanaka<sup>2</sup> and Hiroki Kuniyasu<sup>1</sup>

<sup>1</sup> Department of Molecular Pathology, Nara Medical University, Kashihara, Japan

<sup>2</sup> Department of Orthopedic Surgery, Nara Medical University, Kashihara, Japan

\*Address all correspondence to: [kahonoki@naramed-u.ac.jp](mailto:kahonoki@naramed-u.ac.jp)

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