Mitochondrion-mediated iron accumulation promotes carcinogenesis and Warburg effect through reactive oxygen species in osteosarcoma

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Abstract

Background

Iron metabolism disorder is associated with cancer, however its underlying mechanisms of the carcinogenesis and energy metabolism in osteosarcoma is yet unknown.

Methods

In this study, depletion of mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) were generated by shRNA. The carcinogenesis, migration and invasion were detected by soft agar formation, transwell and wound healing assay. The xenograft tumor mouse model was used to evaluate the role of iron in vivo. Seahorse metabolic analyser was used to assess the metabolism of SAOS-2 and U2OS cells. Iron assay kit was used to detect the level of iron in vitro. Expression of Warburg key genes were measured by Western blot. Reactive oxygen species (ROS) were detected by Dichlorodihydrofluorescein diacetate (DCFH-DA).

Results

We demonstrated that mitochondrion-mediated iron accumulation promoted the carcinogenesis and Warburg effect through reactive oxygen species (ROS) in osteosarcoma cell lines. Knock-out of mitoferrin rescued the iron-mediated ROS production. Iron-mediated ROS affected osteosarcoma cells metabolism via down-regulating AMPK signaling pathway, which increased mTORC1 activity. Furthermore, we found that iron chelator could reduce the tumorigenicity in vivo and in vitro.

Conclusions

Mitochondrion iron accumulation contributes to the development of cancer, and deprivation of iron might be a novel effective strategy in osteosarcoma.

Background

Iron metabolism is of central importance to numerous biological processes, including DNA replication, cell-cycle progression, electron transport chain and microsomal electron transport proteins, as well as the production of iron-sulfur proteins (1, 2). Iron is absorbed from the duodenum by DMT1 and then transported by transferrin, which is regulated by hepcidin and cellular iron exporter ferroportin (FPN1) (3, 4). Notably, mitochondrion is the main consumer of iron in cytoplasm for its function of synthesis or assembly many important proteins such as heme and iron sulfur clusters (5, 6). Mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) are the main iron importers in mitochondrion, which controlled the iron homeostasis by transferring the ferrous iron to the mitochondrial matrix (7, 8). Accumulating evidence indicates that mitochondrial iron accumulation is associated with human disease such as pulmonary and cardiovascular disorders (9-11). However, little is known about the role of mitoferrin in the development of cancer.

Several large epidemiological studies have demonstrated that iron is correlated directly with esophageal cancer, colorectal cancer, liver cancer, and lung cancer (12-15). Dietary intake of high-iron is associated with increased colorectal cancer risks (16). Moreover, iron overload promotes carcinogenesis and tumorigenesis in colorectal and lung cancer (17-19). Some studies have demonstrated that iron could induce reactive oxygen species (ROS) and promote carcinogenesis (20). In addition, mitoferrin-mediated iron-dependent ROS accumulation promotes pancreatic tumorigenesis and Warburg effect in mice (21).

Recently, several studies have demonstrated that reactive oxygen species (ROS) regulate Warburg effect directly or indirectly (21-24). Here, we hypothesized that iron-induced reactive oxygen species were capable of regulating of Warburg effect via mitoferrin in osteosarcoma cell lines SAOS-2 and U2OS.
Methods

Cell lines and reagents

Human osteosarcoma cell line SAOS-2 and U2OS were obtained from American Type Culture Collection (ATCC, USA). Both cell lines were cultured in RPMI 1640 medium (Gibco, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS) in a concentration of 5% CO2 and 37°C incubator. Ferric ammonium citrate (FAC) and Deferoxamine (DFO) were purchased from Sigma (St. Louis, MO, USA).

Cell viability assay

Cell Counting Kit-8 (CCK-8; Beyotime, Shanghai, China) was obtained from Beyotime and be used to detect the viability of two cell line according to the manufactories’ protocol. FAC (100 μM), DFO ((100 μM)) and PBS were added to different groups respectively. Then the 96-well plate was assessed at 450 nm.

Plate colony formation

Cells were collected and resuspended in RPMI 1640 medium containing 100 μM FAC and 100 μM DFO, respectively. Then, the cells were transferred to 6-well plates with the density of 500 cells per well and incubated for 14 days. At last, colonies were stained and counted.

EdU cell proliferation assay

Edu Cell Proliferation Kit (Beyotime, Shanghai, China) was used to detect the proliferation of two cell lines visually according to the manufactories’ protocol. After a co-culture of 100 μM FAC and DFO for 24 hours, cells were stained and photoed by Olympus FSX100 microscope (Olympus, Tokyo, Japan).

Soft-agar colony formation assay

Two percent agar solution was made at a proper temperature combined with culture medium containing 100 μM FAC or 100 μM DFO, respectively. Then the mixed medium was added into 24-well plate, and moved to 4 °C refrigerator immediately until it turns into a solid state. Then cell suspension containing each type of cell was added on to the top of solidified agar (500 / 50μL). Thereafter, 2 % agar solution containing each conditional medium (1:6 v/v) along with Matrigel (1:30 v/v). After 2 weeks of culture, colony numbers were counted.

Trans-well assay

Trans-well chambers (Corning Costar, Cambridge, MA, USA) were used to detect the migration and invasion ability of cells. In brief, cells were cultured in FBS-free medium overnight. Then it was transferred to the upper chambers (10^5 cells / well). Diluted (1:6) Matrigel (BD Bioscience, San Diego, CA, USA) were used to detect the invasion ability of the cells. After 24 hours incubation, the cells on the upper surface (non-migrated or non-invasion cells) were gently removed. The cells on the opposite surface (migrated or invasion cells) were counted and imaged.

Wound healing assay

SAOS-2 and U2OS cells were cultured in 12-well plates. After cells were confluence, wound area was made in cell monolayer by pipette tips. FBS-free RPMI 1640 medium containing 100 μM FAC or 100 μM DFO were added and incubated for 24 hours. The wound closure was captured and the percentage of arear was evaluated by ImageJ software (USA).

Seahorse XF24 Respirometry assay

Seahorse Bioscience Extracellular Flux Analyzer (XF24, Seahorse Bioscience Inc., North Billerica, MA, USA) was used to detect the oxygen consuming rate (OCR), and extracellular acid rate (ECAR). Mito Stress Test Kit from
Agilent was used according to the manufacturer’s protocol. Briefly, $1 \times 10^6$ cells were seeded in the 24-well plate in conditional culture medium and incubated overnight. Then the cells were washed with XF media (1% FBS) then cultured in a CO2-free incubator at 37°C for 2 h. ECAR and OCR measurements were performed. OCR and ECAR were measured in a typical 8 minutes cycle of mix (2 to 4 min), dwell (2 min), and measure (2 to 4 min).

**Mitochondrial extraction**

Mitochondrial isolation and extraction were performed according to the manufacturers’ protocol. Mitochondria/Cytosol Fractionation Kits (ab65320, Abcam). Briefly, cells were harvested after culturing in different conditional medium for 24 hours. A number of $5 \times 10^7$ cells was centrifuged at 600 x g for 5 minutes at 4 °C. Cells were resuspended with cytosol extraction buffer mix after washing with cold PBS. Then the cells were incubated 10 minutes and performed the task with grinder on ice.

**Iron assay**

Iron assay was performed according to the manufacturers’ protocol of Iron Assay Kit (ab83366, Abcam) as previously described (21).

**Western blot analysis**

Cells were collected after stimulated with 100 μM FAC or 100 μM DFO for 24 hours. Cellular proteins were extracted by RIPA lysis buffer containing protease and phosphatase inhibitors. SDS-PAGE was used to separate the proteins. After running process, gels were transferred to PVDF membranes and immersed in primary antibodies. The next day, membranes were incubated with secondary antibodies and be visualized by chemiluminescence detection kit (Beyotime). Slc25a28 antibody (ab90170 , 1:100) was from Abcam. antibodies specific for SLC25A37/ Mitoferrin1 (26469-1-AP, 1:100) and Glut1 (66290-1-Ig , 1:100) were purchased from Proteintech. Anti-phospho-AMPK (Thr172) antibody (#2535S, 1:100), Anti-AMPKα Antibody (#2532, 1:100), anti-p70-S6K (9202S, 1:100), anti-phospho-p70-S6K (Thr389) (9234S, 1:100), anti-Hexokinase 2 (2867S, 1:100), anti-phospho-4EBP1 (Thr70) (13396, 1:100) and anti-4EBP1 (9644s, 1:100) were from Cell Signaling Technology. The anti-GAPDH antibody (BM1623, 1:1000), anti-β-actin antibody (BM0627, 1:1000), anti-α-tubulin antibody (BM1452, 1:1000), anti-rabbit IgG-HRP antibody (BA1054, 1:5000), and anti-mouse IgG-HRP antibody (BA1050, 1:5000) were purchased from Boster Biological Technology (Wuhan, China).

**TCGA database and analysis**

The correlation of mitochondrial-related genes and Warburg genes was analyzed by GEPIA web tools (http://gepia.cancer-pku.cn/) based on the TCGA database.

**ROS production detection**

Dichlorodihydrofluorescein diacetate (DCFH-DA; Beyotime, Shanghai, People's Republic of China) was used to detect ROS production according to the manufacturers’ protocol. Briefly, $5 \times 10^5$ cells were planted in the 6-well plate in different conditional culture medium (100 μM FAC or 100 μM DFO) for 24 h. at the day of measurement, then the culture medium was removed. Next, FBS-free medium with DCFH-DA was added to the dish and then incubated for 20 minutes. The fluorescence intensity of cells was detected by microplate reader.

**RNA extraction and qRT-PCR**

Cells treated with 100 μM FAC or 100 μM DFO for 24 hours were collected for RNA extraction. RNeasy Mini Kit (Qiagen, Valencia, USA) was used to extract the RNA. Then it was reverse-transcribed into cDNA. The mRNA expression levels were assessed by qRT-PCR system (Applied Biosystems, Foster, CA, USA). The primers we used are listed in supplementary Table 1.

**Cell transfection**

SAOS-2 and U2OS cells were transfected with shRNA. Human SLC25A37 shRNA and human SLC25A28 shRNA sequences were designed by Biomics Biotech (Shanghai, China). We selected the most effectively one according
to the results of qPCR and Western blot. The most effective sequence of SLC25A37 shRNA and human SLC25A28 shRNA are listed in supplementary Table 2. Lentivirus medium of shRNA was generated by HEK293T cells. Then it was used to infect the two cell lines. The transfection procedure was performed according to the manufacturers’ protocol of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Puromycin (2μg/ml) was used to screen stable cell lines.

**Mouse xenograft tumor model**

The animal experiments in this study was approved by the Animal Ethics Committee of Shanghai Pudong Hospital, in accordance with the National Institutes of Health (NIH) Guide for the animal treatments of Laboratory Animals. A number of 2 * 10^6 SAOS-2 cells resuspended in 200 µl FBS-free RPMI 1640 medium was injected subcutaneously into the arms of nude mice (6-week-old, female). Tumor volumes were measured every two days. Deferoxamine (DFO) 16mg / kg or normal saline (NS) was injected intraperitoneally for 2 weeks. Deferoxamine (DFO) was purchased from Sigma (St. Louis, MO, USA). After 2 weeks, all animals were sacrificed.

**Statistical analysis**

All experimental data was presented as the mean ±SD (n ≥ 3). GraphPad Prism (version 7, GraphPad Software, La Jolla, CA, USA) was used to analyse the data. Student’s t-test was used between treated and control group. One-way ANOVA was used for multiple groups. LSD-t test was applied when data needed to be compared with control in multiple groups. P<0.05 was considered to be significant.

### Results

**Iron chelator DFO decrease tumorigenicity in vivo.**

Based on the epidemiological evidences that cancer patient links increased body iron storage (25), so we do not set a iron-treated group in animal experiment. We used Deferoxamine (DFO), a chelator used in clinic which could chelate iron efficiently, to explore whether iron was involved in the tumorigenesis of osteosarcoma in vivo. DFO group showed a smaller tumor volume and lower weight compared with control group (Fig. 1b, c). These results indicated that DFO could inhibit the development of osteosarcoma, which implied that iron maybe was involved in the tumorigenesis of osteosarcoma.

**Iron promotes osteosarcoma cell lines proliferation.**

Two osteosarcoma cell lines SAOS-2 and U2OS were treated with exogenous iron (ferric ammonium citrate, FAC), DFO or PBS at indicated times. We found FAC could increase the viability of both cell lines (Fig. 2a).

The colony formation assay showed an increase of colony number in FAC group, whereas DFO group showed a decrease of colony number compared to control group (Fig. 2b). The results of EdU cell proliferation assay showed a increase of cell percentage in cells treated with FAC (Fig. 2c, d). Together, these results demonstrated that the cell proliferative ability could be promoted by FAC treatment while DFO could rescue this process, indicating that iron could be a promoter in osteosarcoma cell lines proliferation.

**Iron promotes osteosarcoma carcinogenesis and increases migration and invasion ability of both cells.**

The soft-agar colony formation assay was used to detect the carcinogenesis of osteosarcoma. Colony number of FAC-treated showed a significant increase compared to other groups (Fig. 3a). In the trans-well assay, FAC group showed more SAOS-2 and U2OS cells than other groups (Fig. 3b). The wound healing assay, FAC group showed a smaller wound area (Fig. 3c). Collectively, these results indicated iron promotes carcinogenesis, invasion and migration ability of both osteosarcoma cells carcinogenesis, invasion and migration.

**Mitochondrion-mediated iron accumulation promotes Warburg effect.**
Warburg effect, as one of the hallmarks of cancer, presented as a metabolic disorder. Even in oxygen condition cancer cells acquire energy by aerobic glycolysis. Seahorse metabolic analyzer is used to detect the extracellular acidification (ECAR) and oxygen consumption rates (OCR). FAC-treated group showed a higher ECAR compared to that of control group, whereas DFO-treated group showed a reduced ECAR. OCR results in FAC group were significantly lower than other groups in both cell lines (Fig. 4a-d). We further wanted to investigate the places where iron accumulated in cells. Iron assay was used to detect the iron content in cytoplasm and mitochondrion according to the instruction of Iron Assay Kit. Mitochondrion to cytoplasm ratio indicated that iron was accumulated more in mitochondrion than in cytoplasm (Fig. 4e). Next, mitochondrial iron importer proteins mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) were detected by western blot. We found FAC could increase the expression of mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) (Fig. 4f). Taken together, these results showed that iron promoted Warburg effect of osteosarcoma cells through the mitochondrion-mediated iron accumulation.

Mitochondrion-mediated iron accumulation increases ROS production by mitoferrin.

Studies have shown that iron could increase ROS production and promote carcinogenesis (20, 21). We then explored ROS production in each group by DCFH-DA, and then found FAC-treated group with a significant increase of ROS (Fig. 4g). Next, we wanted to explore the underlying mechanisms of iron-mediated ROS production. Depletion of SLC25A37 and SLC25A28 by RNAi could significantly reduce ROS production when cells treated by FAC (Fig. 4h). The expression of shRNA was confirmed by western blot and qPCR(Supplementary Fig. 1). Collectively, our results demonstrated that iron could increase ROS production by mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28).

SLC25A37 had a positive correlation with key genes of Warburg effect.

Based on the results described above, GEPIA 2 online tools which based on TCGA database, was used to investigate the correlation of SLC25A37 and Warburg key genes including HK2, GLUT1, GAPDH, PGK1, ENO1, PKM and LDHA (http://gepia2.cancer-pku.cn/#correlation). P < 0.05 was considered as statistically significant. HK2, GLUT1, PGK1, ENO1 and PKM were found positive correlation with SLC25A37 in sarcoma patients (Fig. 5a).

Iron increased Warburg key protein HK2 and GLUT1 expression, which were associated with poor prognosis in osteosarcoma patients.

According to the results we found by GEPIA 2 online tools and several studies reported, the expression of Warburg key proteins HK2 and Glut1 were significantly increased compared to that of control group, while DFO rescued the expression of HK2 and Glut1 (Fig. 5b). Further, we evaluated the correlations between those protein expressions and the survival of osteosarcoma patients. We found that HK2 and Glut1 high expression associated with poor prognosis in osteosarcoma patients, P = 0.023 and p = 0.013 respectively (Fig. 5c).

Iron-induced AMPK inhibition increased mTORC1 activity

AMP-activated protein kinase (AMPK) worked as a regulator of cell metabolism and a sensor of energy, when inhibited, it could activate the mTOR complex 1 (mTORC1) signaling pathway. We next investigated whether this pathway is involved in iron-induced metabolism abnormal. The inhibition of p-AMPK and subsequently the activation of mTORC1 was observed (Fig. 6a, b). Moreover, iron-induced activation of p-AMPK was observed in a dose-dependent manner (FAC 10 µM and 100 µM). Taken together, iron could inhibit AMPK and subsequently activate mTORC1.

Discussion

Iron metabolism is of central importance to numerous biological processes, including DNA replication, cell-cycle progression, electron transport chain and microsomal electron transport proteins, as well as the production of iron-sulfur protein (1, 2). Iron overload promotes carcinogenesis and tumorigenesis in colorectal and lung cancer (17–19). Several studies have demonstrated the therapeutic effects of iron chelator in lung cancer, pancreatic
cancer, multiple myeloma and colorectal cancer(15, 17–19, 21, 25). However, no study has yet focused on the correlation between iron and osteosarcoma, as well as the underlying mechanisms of how iron affected the energy metabolism in osteosarcoma. In the present study, we generated a tumor-load model by injecting SAOS-2 cell line in nude mice. After 21 days of DFO treatment, tumor volume and weight were significantly reduced. These results implied that iron maybe a promoter in osteosarcoma. Based on the epidemiological evidences that cancer patient links increased body iron storage (25), we do not set a iron-treated group in animal experiment though. To our surprise, iron chelator DFO could significantly reduce the tumor volume and weight, which implied that iron chelator maybe a therapeutic alternative in osteosarcoma.

The underlying mechanisms of iron-mediated carcinogenesis is yet unknown. Studies have reported that intracellular iron excess could result in the upregulation and activation of ROS, P53 nuclear and cytosolic degradation, gp130 / STAT3 signaling pathway and iron-sulfur cluster, all of which could promote carcinogenesis and tumorigenesis(17). Iron-mediated ROS promotes Kras-driven pancreatic tumorigenesis (21). Ferroportin-mediated ROS up-regulation promotes the proliferation of multiple myeloma(26). However, ferric iron entered cells via transferrin receptor (TFR1) and be transformed into ferrous iron, which was released by divalent metal transporter 1 (DMT1) (17). Mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) are the main iron importers in mitochondrion, which controlled the iron homeostasis by transferring the ferrous iron to the mitochondrial matrix (7, 8). Hence, we speculated that iron in mitochondrion (ferrous iron) could indicate the part of activated iron intracellular, based on the Fenton reaction. Accordingly, we speculated that mitochondrion-mediated iron accumulation may play an important role in the development of cancer through ROS. Interestingly, our findings suggested that iron not only promoted the proliferation, migration and invasion of osteosarcoma, but also the Warburg effect by iron-mediated ROS.

Warburg effect, as one of the hallmarks of cancer, presented as a metabolic disorder. Warburg effect is associated closely with proliferation, metastasis and drug resistance of cancer cell. In our study, seahorse metabolic analysis showed a higher aerobic glycolysis and a decrease of oxygen consumption rate, a classical Warburg phenotype, which implied that iron could be a factor in this process. Several studies have demonstrated that reactive oxygen species (ROS) regulate Warburg effect directly or indirectly (21–24). In normal cells, ROS production is mainly from mitochondrion intracellular (27). Interestingly, mitochondrion-mediated iron accumulation increased ROS production in both osteosarcoma cell lines, whereas knock out of mitoferrin rescued the iron-mediated ROS. The present work proved that mitochondrion-mediated iron accumulation played a critical role in Warburg effect in osteosarcoma. We further explored the correlation of mitoferrin genes and Warburg key genes. Surprisingly, mitoferrin 1 (SLC25A37) had a positive correlation with HK2, GLUT1, PGK1, ENO1 and PKM, whereas mitoferrin 2 (SLC25A28) had a negative correlation with those genes. Moreover, expression of HK2 and GLUT1 was increased in iron-treated group in our work. Based on the results previously described, high expression of HK2 and GLUT1 were associated with poor prognosis in osteosarcoma patients according to TCGA database. These results suggested that iron maybe a risk factor, through which increases the expression of Warburg key genes of osteosarcoma patients in clinic.

AMP-activated protein kinase (AMPK) worked as a regulator of cell metabolism and a sensor of energy, it could activate the mTOR complex 1 (mTORC1) signaling pathway when AMPK is inhibited. PI3k-AKT-mTOTC axis is an effective alternative proved by several clinical trials in the treatment of cancer (28). Recently, study has found the inhibition of HK2 activated AMPK and thus suppressed its down-stream mTORC1 in lung cancer (29). We further investigated the AMPK-mTORC1 signaling axis at the end of our work. The inhibition of p-AMPK and activation of its down-stream mTORC1, as indicated by the level of S6K1 (Thr389) and 4EBP1 (Thr70) phosphorylation, was observed when osteosarcoma cells treated with iron. Taken together, we demonstrated iron could affect osteosarcoma cells metabolism by ROS and AMPK signaling pathway. These findings, in turn, suggested that iron chelator could be an effective therapeutic alternative in osteosarcoma.

**Conclusions**

Our results highlighted the iron-induced carcinogenesis of osteosarcoma, and proved that mitochondrion-mediated iron accumulation promotes carcinogenesis and Warburg effect of osteosarcoma through reactive oxygen species (ROS) and AMPK signaling pathway.
oxygen species. These findings may also provide some clues to elucidating the underlying mechanism of iron-dependent disease, especially in osteosarcoma. These findings in turn suggested that iron chelator maybe a therapeutic alternative in osteosarcoma.

**Abbreviations**

DMT1, divalent metal transporter 1; FPN1, ferroportin 1; ROS, reactive oxygen species; FAC, Ferric ammonium citrate; DFO, Deferoxamine; DCFH-DA, Dichlorodihydrofluorescein diacetate; OCR, oxygen consumption rate; ECAR, extracellular acid rate; AMPK, AMP-activated protein kinase; mTORC1, mTOR complex 1.

**Declarations**

**Ethics approval and consent to participate**

The animal experiments in this study was approved by the Animal Ethics Committee of Shanghai Pudong Hospital, in accordance with the National Institutes of Health (NIH) Guide for the animal treatments of Laboratory Animals.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ Contributions**

Shuo Ni, Yanbin Kuang and Yin Yuan contributed equally to this work.
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Supplementary Materials

Supplementary Figure S1: Depletion of mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) were tested by Western blot and qPCR. (a) Western blot showed the expression of mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) on protein level. (b) qPCR results of depletion of mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) by shRNA. (* p < 0.05, ** p < 0.01, ns: no significant, mean ± SD, n = 3).

Supplementary Table S1: Specific primers for the qRT-PCR assay.

Supplementary Table S2: RNAi sequence of mitoferrin.

Figures

![Figure a](image1.png)

![Figure b](image2.png)
Figure 1
Deferoxamine (DFO) inhibited osteosarcoma development. (a) Representative images of tumors injected with 2*10^6 SAOS-2 cells subcutaneously (n=6). (b) Tumor volumes were measured by the indicated time (n=6). (c) Tumor weight (n=6). (* p < 0.05, ** p < 0.01, mean ± SD, n=6).
Deferoxamine (DFO) inhibited proliferation of SAOS-2 and U2OS cells in vitro, whereas ferric ammonium citrate
(FAC) promoted this process. (a) Cell viability assay of SAOS-2 and U2OS cells treated with 100 µM FAC, 100 µM DFO or PBS for 0-72 hours. (b) Plate colony formation assay of different groups. (c-e) Images and percentages of EdU-positive cells. (* p < 0.05, ** p < 0.01, mean ± SD, n=3).
Iron promoted carcinogenesis, migration and invasion of osteosarcoma cells SAOS-2 and U2OS. (a) Soft-agar colony formation assay showing ferric ammonium citrate (FAC) promoted the growth of osteosarcoma cells. (b) Ferric ammonium citrate (FAC) increased the migration and invasion of SAOS-2 and U2OS cells. (c) Ferric ammonium citrate (FAC) showed a smaller wound area after 24 hours incubation. (* p < 0.05, ** p < 0.01, mean ± SD, n=3).
Mitochondrion-mediated iron accumulation promotes Warburg effect through reactive oxygen species. (a-d) Seahorse metabolic analysis of extracellular acidification (ECAR) and oxygen consumption rates (OCR) in both cell lines. (e) Mitochondrion iron accumulation was higher than cytoplasm. (f) Ferric ammonium citrate (FAC) increased mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) expression. (g) Ferric ammonium citrate (FAC) increased reactive oxygen species (ROS) production. (h) Depletion of mitoferrin 1 (SLC25A37) and mitoferrin 2 (SLC25A28) by shRNA rescued the ROS production by FAC. (* p < 0.05, ** p < 0.01, ns: no significant, mean ± SD, n=3).
Mitoferrin genes co-expressed with Warburg key genes in sarcoma patients, high expression of HK2 and Glut1 were associated with poor prognosis in sarcoma patients. (a) Mitoferrin 1 (SLC25A37) had a positive correlation of Warburg key genes. (b) Ferric ammonium citrate (FAC) increased HK2 and Glut1 expression. (c) High expression of HK2 and Glut1 were associated with poor prognosis in sarcoma patients.
Iron-induced AMPK inhibition increased mTORC1 activity. (a) Ferric ammonium citrate (FAC) inhibited expression of phospho-AMPK. (b) Ferric ammonium citrate (FAC) increased phosphor-4EBP1 and phosphor-S6k1 activity.
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