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Tumor-induced disorder of iron metabolism in major organs: a new insight from chemical speciation of iron

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Abstract

Objective: To investigate the evolution of iron speciation in major organs of tumor-bearing mice and its role in cancer formation and cancer-associated complications.

Methods: The concentration and chemical speciation of iron in the spleen, liver, lung, kidney, heart, blood, muscle, and tumor tissue of healthy mice and tumor-bearing mice were studied by synchrotron radiation-based total reflection X-ray fluorescence spectrometry (SR-TXRF) coupled with X-ray absorption spectroscopy (XAS).

Results: The TXRF and XAS results showed that the iron content, especially the ferritin content, significantly decreased in the blood and spleen but significantly increased in the liver, lung, and muscle of mice after tumor implantation. The chemical speciation of iron in the tumor mainly comprised ferrous-sulfide-like iron and ferritin.

Conclusion: The tumors disturbed the iron metabolism in major organs, and the evolution of iron may be involved in iron deficiency anemia, cancer growth, and immunity. Additionally, iron speciation-based markers may be further developed as clinical indicators for cancer and cancer-associated complications.

Keywords

Chemical speciation, iron metabolism, cancer, synchrotron radiation, total reflection X-ray fluorescence spectrometry, X-ray absorption spectroscopy, mouse model

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Introduction

Iron is an essential element that regulates multiple physical functions in the human body, including energy metabolism, oxygen metabolism, hematopoiesis, muscle function, and others.[1] However, iron is also crucially involved in the formation of cancer and cancer-associated complications.[2,3] Previous

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studies have demonstrated that iron is a powerful promoter of cancer growth, invasion, and metastasis.[3] Additionally, iron homeostasis is often distorted in patients with cancer; thus, iron deficiency anemia is a common complication in these patients.[2] However, cancer-associated iron distortion is not fully understood in terms of iron speciation.

Different chemical species of iron perform different physical functions.[1,2] For example, iron exists in homeostasis between the ferrous state and ferric state and mediates electron transfer in the human body. Iron–sulfur (FeS) complexes serve as parts of cofactors for enzymes of mitochondrial energy metabolism and DNA synthesis. Ferrous iron plays a key role in oxygen transportation of red cells. Ferritin participates in iron storage in cells and tissues. However, iron is also potentially toxic; free iron ion can induce the formation of free oxygen radicals and damage cells and tissues.[4] Thus, elucidating the homeostasis of iron in terms of iron speciation is vital to understanding how iron homeostasis is distorted in patients with cancer. However, precise quantification of the chemical speciation of iron in cells and tissues remains challenging because of the highly complex chemical speciation and low concentration of iron in cells and tissues.

Synchrotron radiation-based total reflection X-ray fluorescence spectrometry (SR-TXRF) provides highly sensitive quantifying concentrations of elements at a parts-per-billion level, and synchrotron radiation-based X-ray absorption spectroscopy (SR-XAS) can precisely quantify the chemical speciation of elements in cells.[5,6] Thus, TXRF has been widely used to determine the copper, iron, and zinc content in numerous types of cells, such as the HT-29 and HCA-7 colorectal adenocarcinoma cell lines.[7] Meanwhile, XAS is the primary method used to analyze the chemical speciation of elements in cells and tissues and has thus been applied to studying the stability of zinc oxide quantum dots in KB cells,[8] the fate of superparamagnetic iron oxide nanoparticles in human mesenchymal stem cells,[9] and similar phenomena. In this study, the concentration and chemical speciation of iron in the spleen, liver, lung, kidney, heart, blood, muscle, and tumor tissue of healthy mice and tumor-bearing mice were fully studied by SR-TXRF coupled with SR-XAS. This study provides a comprehensive understanding of how cancer distorts iron homeostasis in terms of the concentration and chemical speciation of iron and will help in the future development of iron-based cancer treatment.

Materials and methods

Sample preparation

Human malignant glioma U87-MG cells were cultured in low-glucose Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin. All cells were cultured at 37°C in a humidified 5% carbon dioxide environment.

Six-week-old female BALB/c nude mice were purchased from Shanghai SLRC Laboratory Animal Co. (Shanghai, China) and raised in an animal facility under filtered air. All experiments were performed in accordance with the Guidelines for the Care and Use of Research Animals of Wenzhou Medical University. To establish the tumor-bearing mouse models, U87-MG cells (1×10^6 cells in 100 μL phosphate-buffered saline) were subcutaneously injected into the legs of the mice. After cell injection, the mice were raised for an additional 21 days with a standard pellet diet and pure water to allow the tumor to reach approximately 500 mm^3. The spleen, liver, lung, kidney, heart, blood, and muscle of both tumor-bearing mice ($n = 3$) and healthy mice ($n = 3$) and the tumors of tumor-bearing mice ($n = 3$) were collected,

freeze-dried, ground, and stored at −80°C until analysis.

SR-TXRF and SR-XAS analysis

Both the SR-TXRF analysis and Fe K-edge SR-XAS studies were performed on the 1W1B beamline at Beijing Synchrotron Radiation Facility. The energy of the electron beam in the storage ring during data collection was 2.5 GeV with a current intensity of 200 mA.

For the SR-TXRF analysis, the organ samples were irradiated with a 10-keV beam of energy. The fluorescence spectra of the samples were then recorded for 5-s dwell times with a 19-element germanium array solid detector in TXRF mode. All SR-TXRF spectra were fitted and analyzed with the PyMca version 5.1.1 program.[10] The peak areas of the iron fluorescence signals were calculated to represent the relative iron contents in different organ samples.

For Fe K-edge SR-XAS analysis, an Si(111) double crystal monochromator was employed. The iron reference samples of ferrous sulfide (FeS), iron–cysteine (Fe-cysteine), iron–citrate (Fe-citrate), ferritin, and ferrous sulfate ($FeSO_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and analyzed in the transmission mode. The iron reference sample of Fe-cysteine was prepared by mixing 5 ml of 50 mM ferric nitrate and 5 ml of 500 mM cysteine. The Fe-cysteine pellets were collected and analyzed in the fluorescence mode with a Lytle detector. To collect the Fe K-edge X-ray absorption near-edge structure (XANES) spectra of organ samples, the fluorescence mode with a 19-element germanium array solid detector was used. The XANES spectra of organ samples and reference compounds were analyzed with the WinXAS version 3.1 program.[11] The spectra were normalized by fitting first- and second-order polynomial functions to the pre- and post-edge regions, respectively. Quantitative edge fitting analysis of the XANES spectra (7090–7180 eV) was then performed using the program LSFitXAFS.[12] After component screening, FeS, Fe-cysteine, Fe-citrate, ferritin, and $FeSO_4$ were chosen to fit the XANES spectra of the organs by linear combination fitting using a least-squares algorithm. For the linear combination fitting analysis, individual fitted fractions were constrained to lie between 0 and 1, and the sum of all fractions was forced to 1.

Results

Effect of cancer on iron concentrations of major organs

In this study, SR-TXRF was used in situ to determine the variation in the iron content of major organs of tumor-bearing mice (Figure 1). Further analysis by fitting and quantifying the peak areas of the iron fluorescence signals in the SR-TXRF spectra revealed that the blood contained the largest amount of iron among all tested organs (Figure 1(b) and 1(c)). In the tumor-bearing mice, the iron contents of blood, spleen, and heart were significantly lower than those in the control mice. In contrast, SR-TXRF analysis indicated higher iron contents in the liver, kidney, and muscle in tumor-bearing mice than control mice (Figure 1(c)). Moreover, the iron content of tumor tissue was significantly higher than that of normal muscle.

Cancer-induced iron speciation evolution in major organs of mice

Fe K-edge SR-XAS analysis was performed to further analyze the chemical speciation of iron in major organs. First, the Fe K-edge XANES spectra of a series of iron-containing references resembling the iron speciation in tissues, including FeS, Fe-cysteine, Fe-citrate, ferritin, and $FeSO_4$, were obtained (Figure 2). Next, Fe K-edge XANES spectra

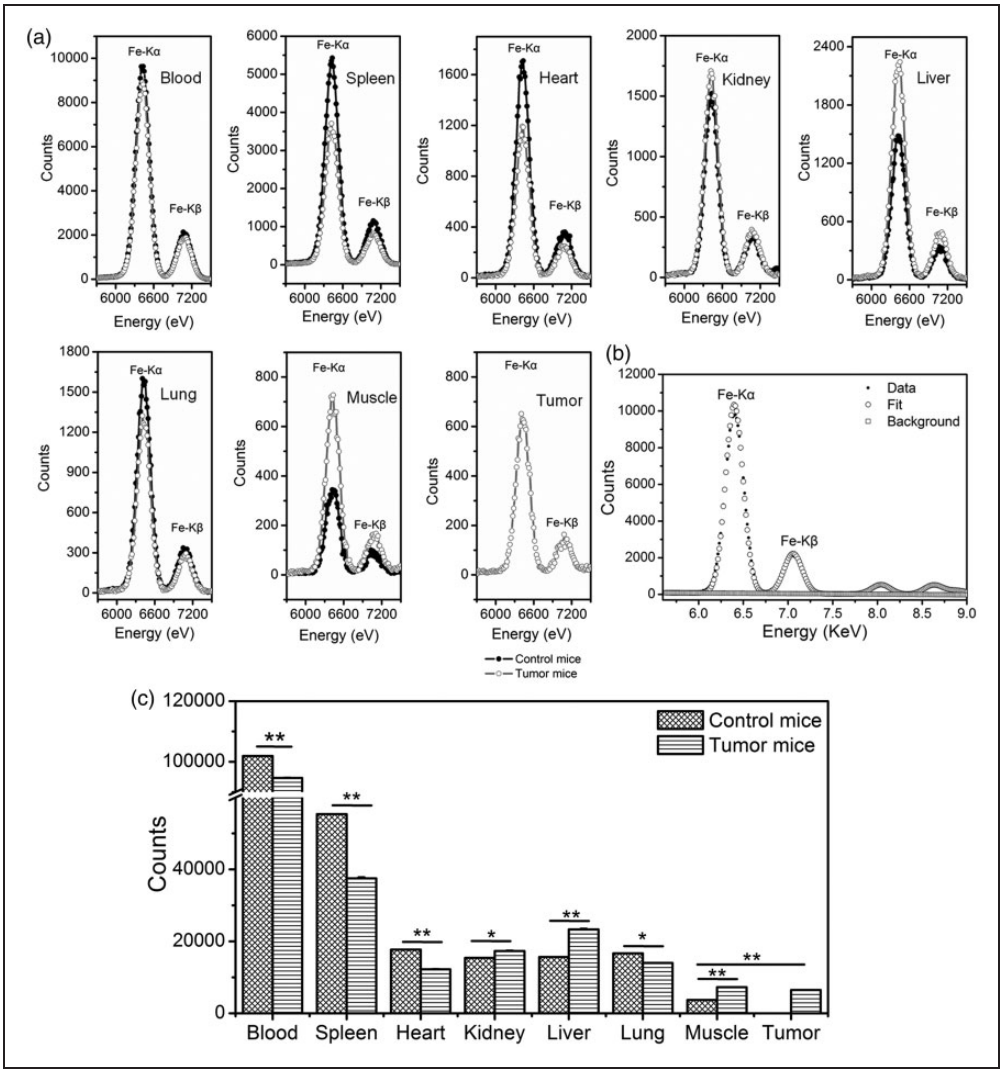

Figure 1. Total reflection X-ray fluorescence assay of iron content in major organs of control mice and tumor-bearing mice. $^{**}P < 0.01$, $^{*}P < 0.05$.

of spleen, liver, lung, kidney, heart, blood, muscle, and tumor tissue of healthy mice and tumor-bearing mice were measured (Figure 3). The Fe K-edge XANES spectra differed among tissues, indicating highly diverse iron speciation in different tissues. Moreover, the Fe K-edge XANES spectra of organs in tumor-bearing mice differed from the corresponding organs in healthy mice, indicating that the cancer altered the iron speciation in most organs. To quantitatively identify the chemical speciation of iron in the tested organs, the Fe K-edge XANES spectra of the tested organs were fitted with the Fe K-edge XANES spectra of the references using a linear combination fitting

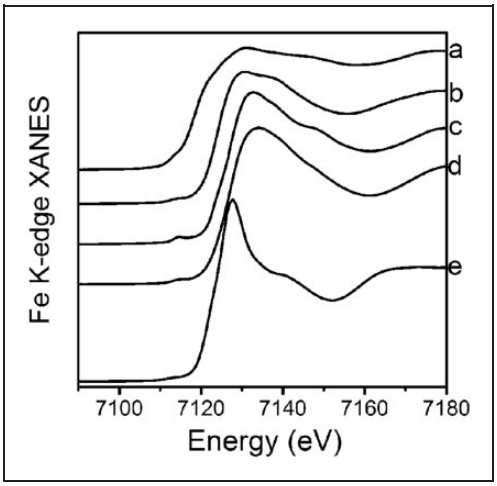

Figure 2. Fe K-edge X-ray absorption near-edge structure (XANES) spectra of iron reference samples of (a) ferrous sulfide, (b) iron–cysteine, (c) ferritin, (d) iron–citrate, and (e) and ferrous sulfate.

method (Table 1). Fe-cysteine-like species and Fe-citrate-like species were the predominant iron species in the blood of healthy mice, representing 42.7% and 35.5% of the total iron species, respectively. In the blood of tumor-bearing mice, Ferritin-like species represented 4.4% and Fe-citrate-like species represented 41.5% of the total iron species in the blood of tumor-bearing mice; these percentages were lower and higher, respectively, than those in healthy mice. Similar to the percentages in the blood, the percentages of ferritin-like species and Fe-citrate-like species in the spleen were lower and higher, respectively, in the tumor-bearing mice than control mice. Unlike the percentages in the blood and spleen, the percentage of ferritin-like iron in the liver, lung, and kidney was higher in the tumor-bearing mice than control mice. The iron speciation was quite similar between tumor tissue and muscle; in tumor tissue, it mainly comprised FeS-like iron (50.1%) and ferritin (49.9%). However, more ferritin and less FeS-like iron were found in tumor tissue than in normal muscle.

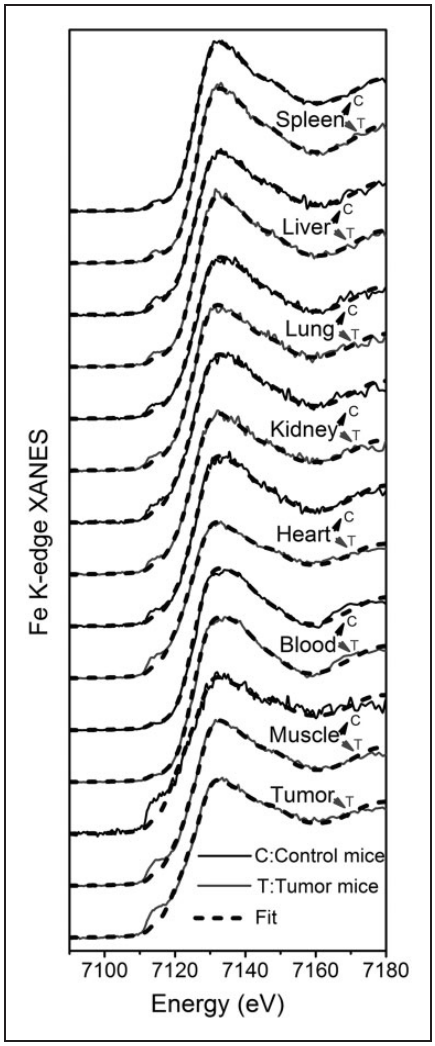

Figure 3. Chemical speciation of iron in the major organs of control mice (C) and tumor-bearing mice (T) as determined by Fe K-edge X-ray absorption near-edge structure (XANES) spectra.

Discussion

Cancer induces distortion of iron homeostasis in patients with cancer.[2,3] Clinical research has shown that the iron content is the predominant indicator of iron distortion. Thus, the iron content in the major organs of tumor-bearing mice was assessed

Table 1. Fitting results of Fe K-edge X-ray absorption near-edge structure (XANES) spectra of the major organs in control mice and tumor-bearing mice.

Sample	FeS (%)	Fe-cysteine (%)	Ferritin (%)	Fe-citrate (%)	$FeSO_4$ (%)	RSS
C-Spleen	6.5	0.0	86.2	0.2	7.1	0.05
T-Spleen	9.0	0.0	65.0	19.2	6.8	0.10
C-Liver	25.2	4.8	66.4	3.1	0.5	0.18
T-Liver	15.8	0.0	72.8	7.2	4.2	0.13
C-Lung	23.2	13.9	36.5	26.3	0.0	0.22
T-Lung	25.9	3.1	62.5	0.6	7.9	0.25
C-Kidney	35.5	11.6	29.9	22.7	4.6	0.33
T-Kidney	31.8	2.3	58.5	0.0	7.4	0.25
C-Heart	27.8	9.4	41.4	19.9	1.5	0.23
T-Heart	46.3	0.0	47.1	0.0	6.6	0.22
C-Blood	8.6	42.7	13.1	35.5	0.0	0.21
T-Blood	10.3	43.8	4.4	41.5	0.0	0.16
C-Muscle	61.7	0.0	38.3	0.0	0.0	1.11
T-Muscle	38.9	0.0	60.0	0.0	0.0	0.31
T-Tumor	50.1	0.0	49.9	0.0	0.1	0.46

[a]Fitting was performed within the XANES spectra from 7090 to 7180 eV using the LSFitXAFS program.
FeS, ferrous sulfide; Fe-cysteine, iron–cysteine; Fe-citrate, iron–citrate; FeSO4, ferrous sulfate; RSS, residual sum of squares; C, control mice; T, tumor-bearing mice.

by an SR-TXRF method for the first time in the present study. In the blood, iron is mainly stored in the red blood cells and serves as a central component of hemoglobin, which enables the red blood cells to carry oxygen and deliver it to the body's tissues. The iron content in blood was significantly lower in the tumor-bearing mice than control mice (Figure 1). This lower iron content may be attributed to the lower number of circulating red blood cells. In addition, the iron content in the spleen was significantly lower in the tumor-bearing mice. The lower iron content in the spleen may decrease erythropoiesis, inducing iron deficiency anemia in mice with cancer. The significantly lower iron content in the hearts of tumor-bearing mice also indicated cancer-induced functional iron deficiency because oxygen transportation in the heart and oxygen-based cardiac muscle activity are iron-dependent.[1] In contrast to the blood, spleen, and heart, the iron content in the liver, kidney, and muscle was higher in the tumor-bearing mice than control mice. Moreover, the iron content in tumor tissue was higher than that in normal muscle (Figure 1). Previous studies have shown that iron is crucial for cancer growth, invasion, and metastasis.[3,13] Cancer cells are rapidly dividing cells that require a great amount of iron for their DNA replication.[13,14] Thus, tumor tissues may steal iron from normal tissues and cause iron deficiency anemia.

In addition to the iron content, the chemical status of iron is another key factor that determines the function and toxicity of iron. For example, FeS complexes serve as central parts of numerous enzymes, while free iron ion is a powerful generator of oxygen free radicals.[4,15] Thus, elucidating the evolution of the chemical speciation of iron in the major organs is crucial for understanding cancer-induced complications and will help in the future development of iron-based cancer treatment.

Ferritin in the blood and spleen serves as the iron reserve in the body.[16] Decreased ferritin in combination with decreased iron content in the spleen further indicates that cancer may result in iron deficiency anemia.[2] Iron deficiency anemia may be caused by the consumption of a large amount of the body's iron by a tumor, preventing iron from producing enough hemoglobin-containing red blood cells.[2,13] In contrast to the ferritin-like species, the Fe-citrate-like species was increased in both the blood and spleen in tumor-bearing mice in the present study. The concentration of Fe-citrate-like species in blood is a crucial factor involved in systemic bacterial infection. The immune system of the human body initiates a process known as iron withholding to keep iron unavailable to bacteria, thus resisting bacterial infection.[17,18] Fe-citrate-like species are relatively easily accessed by bacteria. The cancer-induced increase of Fe-citrate-like iron in the blood may increase the risk of systemic bacterial infection.

We found that ferritin-like iron in the liver, lung, and kidney was higher in tumor-bearing mice than control mice. The cancer-induced high iron content (Figure 1) and high ferritin-like iron (Figure 3 and Table 1) in liver may induce liver dysfunction. Previous studies have shown that excess iron may result in the development of liver dysfunction, and the sustained high iron content in the liver will lead to cirrhosis and liver cancer.[19,20] These findings indicate that cancer may induce iron-associated complications such as anemia, immune deficiency, and liver dysfunction.

In the present study, the iron speciation in the tumor tissue was quite similar to that in the muscle and mainly comprised FeS-like iron and ferritin. The tumor was implanted in the leg muscle of the mice and grew in a microenvironment similar to that of muscle. Our findings indicated that the physiological characteristics of a tumor may be affected by its microenvironment, leading it to exhibit an iron speciation similar to that of muscle. However, more ferritin and less FeS-like iron were found in tumor than those in normal muscle. Numerous studies have shown that iron is a crucial element for cancer cell proliferation.[3,21] Ferritin is the source of reserve iron in most cells and is crucial for cell metabolism and proliferation. The high percent of ferritin in tumors suggests that cancer cells can plunder iron from surrounding cells and tissues and store it as ferritin to sustain their rapid proliferation. With respect to FeS-like iron, FeS complexes have been found as central parts of numerous enzymes involved in mitochondrial energy metabolism. The FeS complexes of electron transfer proteins are capable of generating a proton gradient to allow adenosine triphosphate (ATP) synthase to synthesize ATP, thus playing a central role in the electron transfer chain of energy metabolism.[15,22] ATP is required for the contraction of muscle cells. Thus, FeS complex-containing proteins are abundant in muscle. Interestingly, FeS-like iron was also abundant in the tumors in this study. The tumors grew quickly at the time of sample harvesting, indicating that a large proportion of the tumor cells were highly active. This suggests that ATP was crucial to the highly active cancer cells, and FeS complex-containing proteins were therefore also needed to sustain the energy metabolism of the cancer cells.

In summary, SR-TXRF and SR-XAS were successfully used to explore the concentration and chemical speciation of iron in the major organs of tumor-bearing mice. The SR-TXRF and SR-XAS results showed that the iron content, especially the ferritin content, significantly decreased in the blood and spleen but significantly increased in the liver, lung, and muscle of mice after tumor implantation. Moreover, the chemical speciation of iron in the tumor tissue was quite similar to that in muscle and mainly comprised FeS-like iron and ferritin. These results suggest that the cancer-induced iron

evolution may be involved in iron deficiency anemia, cancer growth, metastasis, immunity, and other phenomena. These findings are not only crucial for improving our understanding of cancer formation and cancer-induced complications but are also vital for the further development of new iron speciation-based clinical indicators of cancer and cancer-associated complications.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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