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Effects of 3.7 T–24.5 T high magnetic fields on tumor-bearing mice*

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Since high magnetic field (MF) intensity can improve the image quality and reduce magnetic resonance imaging (MRI) acquisition time, the field intensity of MRIs has continued to increase over the past few decades. Although MRIs in most current hospitals are 0.5 T–3 T, there are preclinical studies have been carried out using 9.4 T MRI, and engineers are also putting efforts on building MRIs with even higher MFs. However, the accompanied safety issue of high-field MRIs is an emergent question to address before their clinical applications. In the meantime, the static magnetic field (SMF) has been shown to inhibit tumor growth in previous studies. Here, we investigated both the safety issue and the anti-tumor potentials of 3.7 T–24.5 T SMFs on GIST-T1 gastrointestinal stromal tumor-bearing nude mice. We followed up the mice three weeks after their exposure to high SMF and found that none of the mice died or had severe organ damage, except for slightly decreased food intake, weight gain, and liver function. Moreover, the tumor growth was inhibited by 3.7 T–24.5 T SMFs (up to $\sim$ 54%). It is interesting that the effects are more dependent on MF gradient than intensities, and for the same gradient and intensity, mice responded differently to hypogravity and hypergravity conditions. Therefore, our study not only demonstrated the safeness of high SMFs up to 24.5 T on mice but also revealed their anti-tumor potentials in the future.

Keywords: static magnetic field (SMF), high magnetic field (MF), tumor, magnetic field gradient

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1. Introduction

High static magnetic field (SMF) is essential for MRI (magnetic resonance imaging) and MRS (magnetic resonance spectroscopy) to provide high-quality images and adequate information.[1] In recent few years, 21.1 T MRI has been used to image mice and rat brain, which got much higher resolution[2–4] than 9.4 T or 3 T MRIs. MRS of 21.1 T has also been used to reveal metabolic properties in stroked rats.[5]

Over the past decades, there have been multiple cellular experiments to study the effects of high SMFs, and the exact effects depended on various cellular and MF parameters, including but not limited to cell and MF types, MF intensity and homogeneousness.[6–18] For example, Schiffer *et al.* found that there was no influence on cell cycle progression using conditions relevant to patients during MRI examinations of 1.5 T and 7 T;[7] no obvious effects were observed for CHO-K1 cells after exposure to a 10 T SMF;[8] for immortalized hamster cells and human primary fibroblasts cells, 13 T SMF had very little effects on their cell cycle distribution and cell viability.[9] Meanwhile, Qian *et al.* tested high MFs of up to 16 T on osteoblast-like cells (MG63 and MC3T3E1) and found that the osteoblast ultrastructure and function, as well as the association of MACF1 with actin and microtubule cytoskeleton, were affected.[10,19] Apparent biological effect of 7 T–16.7 T high SMFs was also observed on mosquito and Heliothis virescens egg hatching[11,12] and cleavage planes in frog eggs.[15] Moreover, cellular ATP content was found to decrease by 8.5 T and 9 T SMFs in multiple cell types.[13,20] There are also multiple studies reporting that high SMFs could change cell orientations, such as rat Schwann cells,[14] red blood cells,[21,22] bull sperm,[23,24] as well as mitotic spindles in human cells.[16] However, so far, only a very few studies have been carried out using high SMFs above 20 T,[16,25,26] which is mainly limited by the availability of high field magnets that are compatible to biological samples.

In the comprehensive and inspiring review by Budinger and Bird, the authors summarized the safety issue, magnet

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technologies and specific neuroscience applications of high-field MRIs.[27,28] They concluded that there are no foreseen barriers either in the technical or human safety aspects of brain MRI and MRS at MFs up to 20 T. They suggested that people should put their efforts to develop high field MRI and MRS up to 20 T, admitting that this is conditioned on results of recommended experiments to verify the predicted level of physiological effects beyond 9.4 T. However, for animal levels, there are very limited studies about the safety issues of high SMFs between 9.4 T–20 T using animals. In 2016, Pais-Roldan *et al.* investigated the effects of a 14 Tesla MRI scanner on zebrafish larvae and showed that 14 T SMF treatment for 2 hours induced otolith fusion and aberrant swimming behavior.[29] More importantly, except for the unicellular organism Paramecium,[25,26] there are no reported experimental studies about whole animals in high SMFs above 20 T. It should be noted that although Budinger and Bird mentioned in their review that no deleterious observations have been reported from extensive studies on rodents at 21.1 T by NHMFL investigators other than temporary effects on the vestibular apparatus,[28] there are no experimental data or information about the biological effects on these mice.

Besides these safety issues, another major goal of our work was to investigate the anti-tumor potentials of high SMFs because multiple studies have reported the anti-tumor effects of moderate intensity SMFs[30–33] and higher MF intensity seem to have more obvious effects.[31,32] Here we used a water-cooled magnet that provides a 24.5 T SMF in the center, and descending MF intensities with various gradient off-the-center, to investigate the effects of 3.7 T–24.5 T SMFs on tumor-bearing mice. No deleterious effects were observed in 3.7 T–24.5 T SMF-treated mice and the tumor growth was reduced in multiple SMF conditions, which revealed the anti-cancer potential of high SMFs.

2. Methods

2.1. Construction of the mice exposure system for 24.5 T ultra-high magnet

A water-cooled magnet (WM2) in Chinese High Magnetic Field Laboratory (CHMFL, Hefei, China) was used to generate 3.7 T–24.5 T SMFs. The cylinder bore of the magnet is 50 mm in diameter and 1300 mm long. To investigate the biological effect of the magnetic field provided by the magnet, we designed and constructed a set of mice exposure system with accurate temperature and gas control. We designed two sets of devices to specifically fit in the WM2 magnet, each of which consists of two coaxial nonmagnetic stainless-steel tubes. The outer diameter of the outer tube (OT) is 49 mm and the inner diameter of the inner tube (IT) is 43 mm. Each mouse was housed in a nonmagnetic stainless-steel tube (38.5 mm diameter, 80 mm long) and eight of them were stacked together into the longer tube (41 mm diameter, 700 mm long) before each experiment. To provide proper air supply, we used a nonmagnetic stainless-steel air tube with 10 mm outer diameter held by a height-adjusting nut, which can be adjusted according to the position of the mice in the magnet. Meanwhile, a pump was used to circulate the air inside the cylinder bore, which blew the air into the cylinder bore at 450 L per minute. Furthermore, the air conduit was placed into ice water to avoid overheating caused by the pump. A platinum resistance temperature sensor (PT100) connected to a temperature display was used to monitor the temperature inside the mice tube, which was controlled at 22–24 °C by thermal conduction from temperature-controlled water that flowed through the space between the IT and OT. By adjusting the temperature of the water, the temperature inside the mice tube can be controlled.

2.2. Static magnetic field exposure

After the GIST-T1 tumor volume reached 100–200 mm^3, we placed the tumor-bearing mice into individual mice tubes, stacked them into the long tube before they were inserted into the magnet (as SMF-treated group) or left outside of the magnet (as "Sham control" group). Both groups had eight mice. The tube was precisely measured, marked and adjusted to ensure the fifth mouse is precisely located at the center position of the magnet. The MF exposure time was 3 hours in total (increasing field for 15 min, constant 24.49 T field for 2.5 hr and reducing field for 15 min) for each time, three times in total, on day 1, 4, and 7, respectively. It should be noted that although the maximal MF intensity that WM2 can reach is 25 T at the center of the magnet, we maintained it steadily at $\sim$ 24.5 T for better stability. The Sham control group and MF group were handled identically during the whole experiment, except for MF exposure. After the last SMF exposure, the mice were fed normally by sterilized food and autoclaved water for another two weeks.

The food and water consumption, body weight and tumor growth were all measured daily during the whole experiment. Tumor volumes were calculated as tumor volume $(mm^3) = [(W^2 \times L)/2]$, where width ($W$) is defined as the smaller diameter of the tumor and length (L) is the larger diameter. Blood samples were collected by removing eyeball on day 22 (endpoint) of the study. 200 μL blood samples with 0.08% (M/V) ethylenediamine tetraacetic acid disodium salt (EDTA) were used for blood routine examination by an automatic hematology analyzer (Sysmex, Japan). In addition, the

heart, liver, spleen, lungs, and kidneys of all mice were collected, weighed and fixed with 4% formaldehyde (#10010018, Sinopharm Chemical Reagent) after blood sampling.

For blood biochemistry analysis, the blood samples were placed at room temperature for 4 hrs, protected from light and collected the serum by centrifugation. The serum was stored at −80 °C or dry ice until it was analyzed by an automatic analyzer (HITACHI 7020, Japan) in the Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China), including alanine aminotransferase (ALT), aspartic transaminase (AST), alkaline phosphatase (AKP), total protein (TP), albumin (ALB), total bilirubin (TBIL), blood urea nitrogen (BUN), serum creatinine (CREA), calcium (Ca), phosphorus (P), iron ion (Fe), total cholesterol (CHOL), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and blood glucose (GLU).

2.3. GIST-T1 tumor mice model

Twenty 4-week-old female specific pathogen-free (SPF) BALB/c (nu/nu) mice were from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). Each five mice were housed in one polycarbonate cage and all mice were kept in an animal room under SPF condition. The mice were fed with sterilized food and autoclaved tap water freely. The protocol involving animals was approved by the ethical and humane committee of Hefei Institutes of Physical Science, Chinese Academy of Sciences and carried out strictly in accordance with the related regulations. After one week, five million human gastrointestinal stromal tumor (GIST-T1) cells were suspended in 150 μL PBS and injected into the subcutaneous space on the right flank of nu/nu mice. For SMF exposure, we chose 16 mice whose GIST-T1 tumors had reached 100–200 mm^3 after seven days of inoculation. The mice were randomly divided into two groups, Sham control group and SMF exposure group. After MF exposure, we maintained the mice in another room since they were no longer qualified to return back to the SPF animal room, fed them with sterilized food and autoclaved water for another two weeks to monitor their behaviors.

2.4. Hematoxylin-eosin (HE) staining

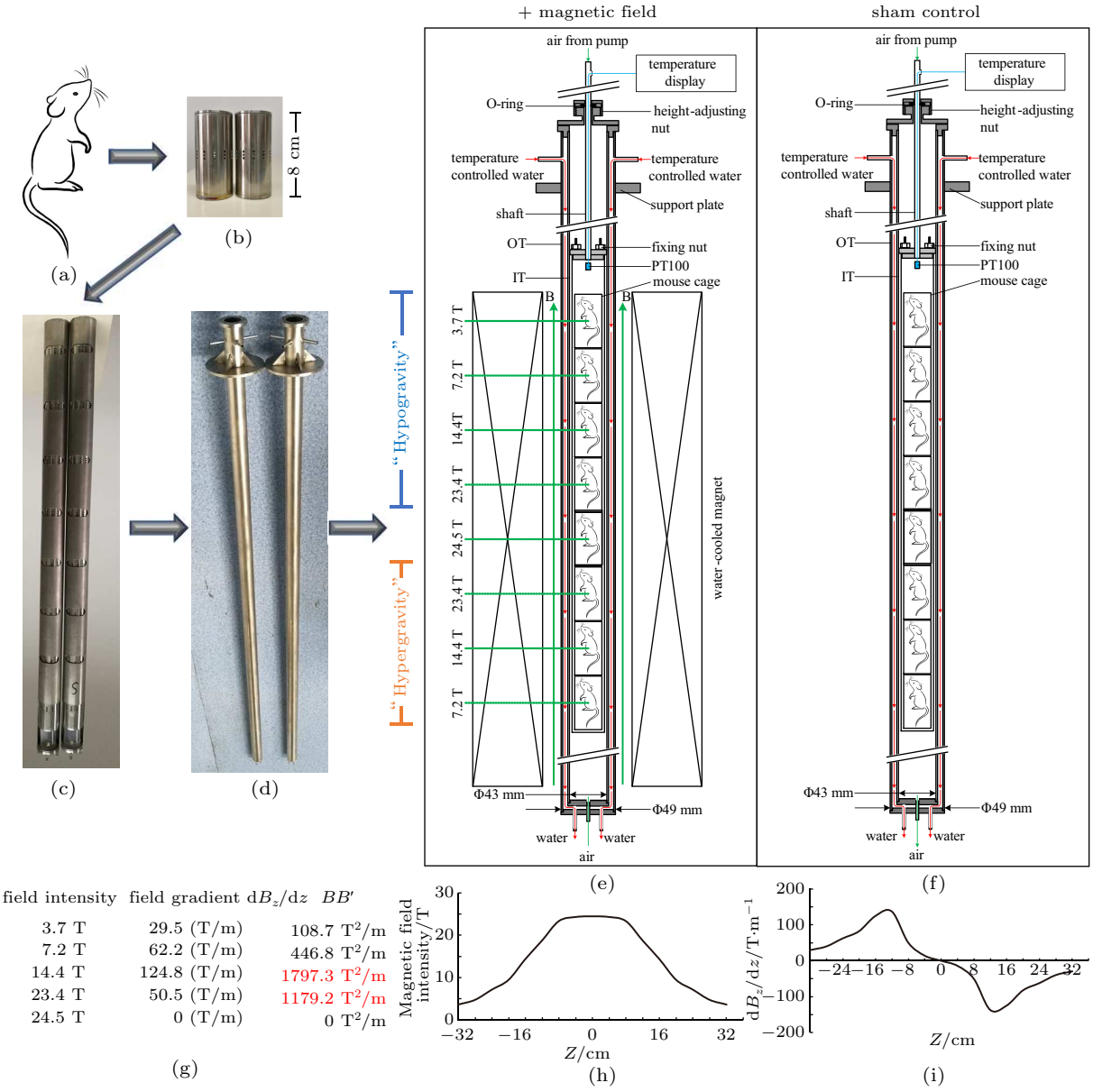
After being fixed in 4% formaldehyde for 24 hr, the heart, liver, spleen, lung, kidney and tumor tissues were placed in the running water overnight to remove formaldehyde. Then the tissues were dehydrated, cleared and immersed in wax before they were embedded in paraffin. The paraffin-embedded specimens were sectioned at 5 μm thickness and then immersed in xylene twice, 15 min each time. Next, the sections were hydrated in decreasing concentrations of ethanol (100, 95, 85 and 75%) for 3 minutes followed by a 5-min immersion in running water before they were dipped into the hematoxylin (#ZLI-9610, Beijing Zhongshan) for 5 min. After washing the slides in running water for 5 min, they were stained with 1% (V/V) hydrochloric acid solution in absolute ethyl alcohol for 1 s before they were placed into 1% eosin (#ZLI-9610, Beijing Zhongshan) for 3 s. Subsequently, after washing for 5 min in running water, the sections were dehydrated through 85%, 95% alcohol and two changes of 100% alcohol for 3 min each. The alcohol was extracted with two changes of xylene. Finally, one or two drops of mounting medium were added and covered with a coverslip.

3. Results

3.1. 3.7 T–24.5 T high static magnetic fields setup

Each mouse was placed in a mice container, which was then inserted individually into a long tube (Figs. 1 and S1). For the experimental group, the mice tube was inserted into the water-cooled magnet #2 (WM2) in the Chinese High Magnetic Field Laboratory, Chinese Academy of Sciences, Hefei, China. The Sham control group was kept in the same room, but not inserted in the WM2 magnet. Since we have eight mice in the Sham control group that had essentially the same condition, we took the mean value as control (CT) for all figures in this paper. In the meantime, we used the data of these mice as a "Sham control range" in the figures as references to help justify the influences of the high MF exposure.

The experimental and the Sham control sets have identical gas, humidity, and temperature control systems and they were all operated side-by-side. The water-cooled magnet that provides 24.5 T ultra-high SMF in the center, and 3.7 T–23.4 T off the center (Figs. 1(g)–1(i)). The upper part of the magnet mimics the "hypogravity" condition because the MF gradient provides an upward direction force on the mice. The lower part of the magnet mimics the "hypergravity" condition because the MF gradient provides a downward direction force on the mice. The mouse in the center was exposed to a 24.5 T SMF with negligible gradient and magnetic force. The MF intensity, MF gradient as well as the BB' (MF intensity × MF gradient) information for the center position of each mice container are included (Fig. 1(g)). Mice at 14.4 T had the largest BB', which means that they experienced the strongest magnetic force. For mice at the positions of both 14.4 T and 23.4 T, the BB' values are larger than 1370 T^2/m, which is required to lift water and make water "weightless".



field intensity	field gradient dB_z/dz	BB'
3.7 T	29.5 (T/m)	108.7 T^2/m
7.2 T	62.2 (T/m)	446.8 T^2/m
14.4 T	124.8 (T/m)	1797.3 T^2/m
23.4 T	50.5 (T/m)	1179.2 T^2/m
24.5 T	0 (T/m)	0 T^2/m

Fig. 1. (color online) The mice exposure system for 3.7 T–24.5 T ultra-high magnetic fields. (a) The BALB/c (nu/nu) mouse bearing GIST-T1 tumor, (b) the mice containers, (c) the inner mice tube, (d) the outer mice tube, (e, f) the design of the mice exposure system. Two identical sets were made, one used in the magnet as the experimental set (e) while the other was placed outside of the magnet to serve as the 'Sham control' (f). (g) Magnetic field intensity, field gradient, and the magnetic field intensity × field gradient for the center position of each mice container. (h) Magnetic field intensity distribution within the mice tube inside the magnet (water-cooled magnet #2 of Chinese High Magnetic Field Laboratory). (i) Magnetic field gradient distribution within the mice tube inside the magnet.

3.2. 3.7 T–24.5 T SMF slightly influenced the food consumption and body weight of tumor-bearing mice.

The mice were exposed to high SMFs for 3 hours each time, three times in total, 3 days apart. To check for possible long-term effects, the mice were maintained and observed for 22 days since the first SMF exposure before they were sacrificed. We recorded their food and water consumptions each day during the whole experiment period and found that the SMF-treated mice had 6.4%–14.8% decreased water and food intake (Figs. 2(a) and 2(b)), although the water intake decrease was not statistically significant (Fig. 2(b)). In fact, some of the mice appeared to be irritated after the first exposure, especially the mouse in the center of the magnet, who was exposed to the highest field SMF (24.5 T). However, this effect was transient because the mice rapidly recovered to their normal alert state a few hours after exposure. In addition, it was interesting that the mice did not show any discomfort after the second or third exposure, which shows that they were adaptive to these exposure conditions.

We also recorded their body weight each day and found that the gain of body weight in most mice was not obviously affected, except for the 14.4 T-treated mice in the hypograv-

ity condition (Fig. 2(c)). It should be noted that the 14.4 T position has the largest BB' (MF intensity × MF gradient = 1797.3 T^2/m), which indicates that mice at this position were exerted the strongest magnetic force. The relative organ weight was also measured for each mouse at the end of the experiment. For heart, spleen, lung, and kidney, there were no noticeable organ changes for all eight SMF exposure conditions (Fig. 2(d)). In contrast, the liver weight was affected in some SMF exposure conditions, although none of them are substantial (Fig. 2(d)).

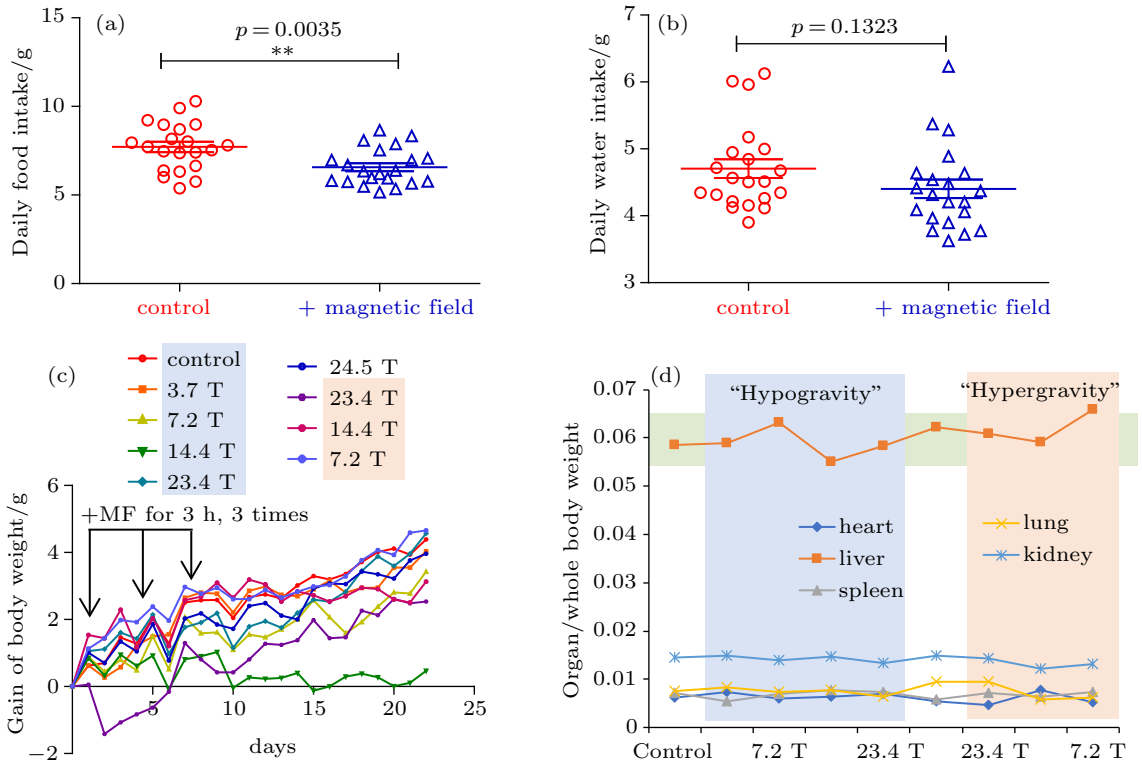


Fig. 2. (color online) Food and water consumption, gain of body weight and organ weight changes of GIST-T1 tumor-bearing mice caused by high magnetic fields. (a) and (b) Food and water consumption of each mouse per day. The data were collected for each cage of mice and then divided by the number of mice in each cage. Data represent mean ±SD. p values were calculated using unpaired Student's t test: $^{**}p < 0.01$. (c) Gain of body weight was measured each day for each mouse. (d) The relative weight of organ to body weight was calculated. For control (CT), the mean value from eight mice was used. The blue shaded area means the mice were under the circumstances of "hypogravity". The pink shaded area shows the mice were under the circumstances of "hypergravity". The green shaded area shows the liver reference range defined by eight mice in the Sham control group (0.054–0.065).

3.3. 3.7 T–24.5 T high magnetic fields have negligible effects on the blood routine of GIST-T1 tumor-bearing mice

To get a better assessment of the effects of these ultra-high SMFs on the tumor-bearing mice, we collected their blood and did blood routine test and blood biochemistry examination. We tested 13 different indicators in the blood routine test, including the concentration of red blood cell (RBC), platelet (PLT) (Fig. 3(a)), white blood cell (WBC) (Fig. 3(b)), the mean corpuscular volume (MCV) and mean platelet volume (MPV) (Fig. 3(c)), hemoglobin concentration (HGB) and mean corpuscular hemoglobin concentration (MCHC) (Fig. 3(d)), hematocrit (HCT) and thrombocytocrit (PCT) (Fig. 3(e)), lymphocyte (LYMPH), and monocyte (MONO) (Fig. 3(f)), neutrophil (NEUT) and eosinophil (EO) (Fig. 3(g)). It is interesting that although there are fluctuations in the SMF-treated mice, most of them are within the control range set by the eight mice in the Sham control group, which means that they belong to normal variations among individual mice. The only exceptions are 7.2 T and 14.4 T in the hypogravity condition, which induced decreased white blood cells.

We noticed that for the same MF intensity, the "hypogravity" and "hypergravity" conditions had differential effects. For example, 7.2 T in the hypogravity condition and hypergravity condition sometimes produced opposite effects (Fig. 3(g)). In addition, the highest MF intensity 24.5 T, which is in the center of the magnet and has no MF gradient, did not produce the strongest effects as we predicted (Fig. 3(h)).

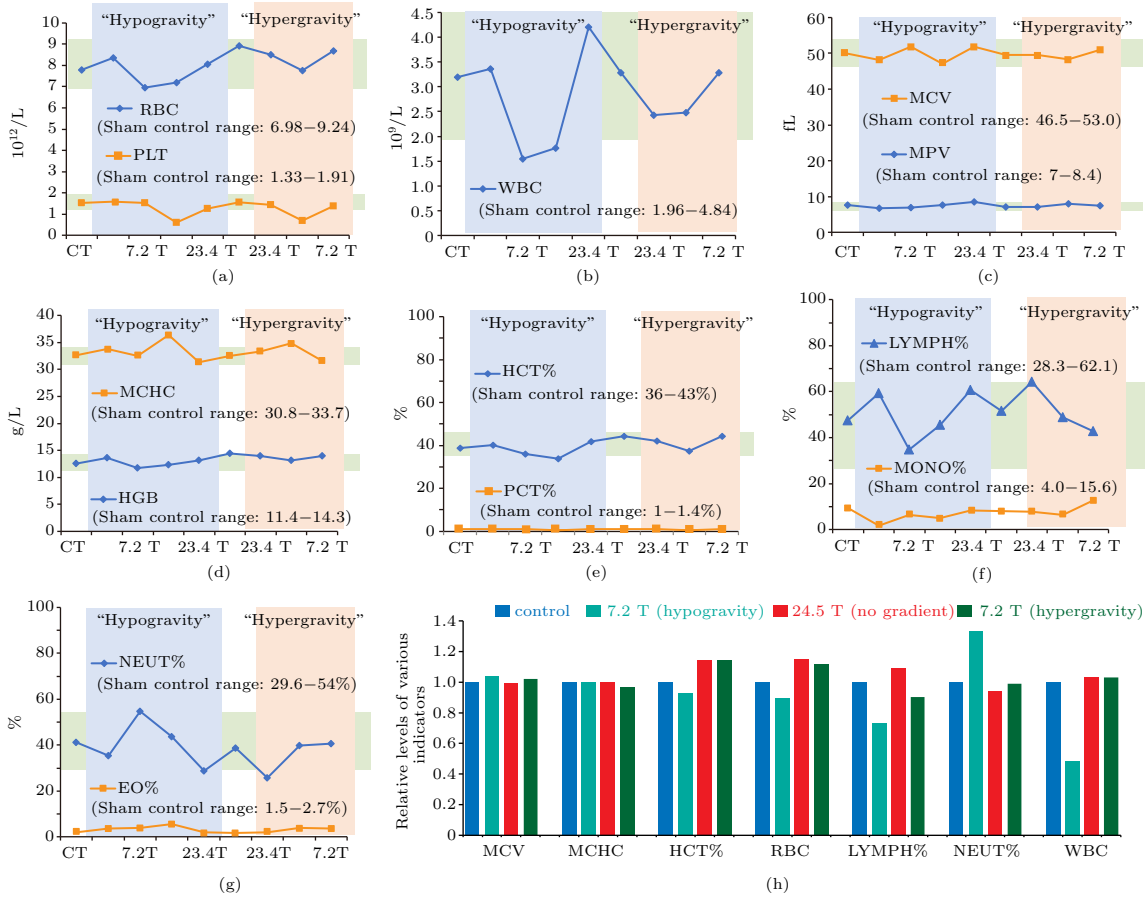


Fig. 3. (color online) Blood routine test results for 3.7 T–24.5 T high magnetic field-treated GIST-T1 tumor-bearing mice. (a), (b) The concentration of red blood cell (RBC), platelet (PLT), white blood cell (WBC); (c) mean corpuscular volume (MCV) and mean platelet volume (MPV); (d) hemoglobin concentration (HGB) and mean corpuscular hemoglobin concentration (MCHC); (e), (f), (g) percentage of hematocrit (HCT), thrombocytocrit (PCT), lymphocyte (LYMPH), monocyte (MONO), neutrophil (NEUT), and eosinophil (EO) were measured for each mouse. For control (CT), the mean value from eight mice was used. The blue shaded area means the mice were under the circumstances of "hypogravity". The pink shaded area shows the mice were under the circumstances of "hypergravity". The green shaded area shows the reference range defined by eight mice in the Sham control group. (h) The relative levels of MCV, MCHC, HCT%, RBC, LYMPH%, NEUT%, and WBC were compared between Sham control, 24.5 T (no gradient), and 7.2 T (in hypogravity and hypergravity conditions).

3.4. Gradient high magnetic fields have moderate effects on liver and kidney functions of GIST-T1 tumor-bearing mice

We also did blood biochemical test for liver and kidney functions for 3.7 T–24.5 T high MF-treated GIST-T1 tumor-bearing mice. Alanine transaminase (ALT) activity (Fig. 4(a)), aspartate transaminase (AST) (Fig. 4(b)), alkaline phosphatase (AKP) (Fig. 4(c)), total protein (TP), albumin (ALB) (Fig. 4(d)), total bilirubin (TBIL) (Fig. 4(e)), blood urea nitrogen (BUN) (Fig. 4(f)), serum creatinine (CREA) (Fig. 4(g)) were measured for each mouse. For most indicators of liver and kidney functions, high MFs did not severely affect them. However, we noticed that in some specific MF exposure conditions, the liver and kidney functions were affected (Fig. 4). It is interesting that 24.5 T SMF, which is the highest MF intensity, did not affect these indicators. In contrast, some SMFs with gradient produced noticeable effects on the liver and kidney functions. For example, 23.4 T SMF in the hypogravity condition moderately increased the ALT level (Fig. 4(a)), indicating a liver functional abnormality. Moreover, similar to blood routine test above, we found that for the same MF intensity, the "hypogravity" and "hypergravity" conditions had differential effects on these blood biochemistry results. In addition, the highest MF intensity 24.5 T, which has no MF gradient, did not cause obvious changes (Fig. 4(h)).

3.5. Gradient high magnetic fields have moderate effects on metabolisms of tumor-bearing mice

We also did the blood biochemical test of metabolism-related indicators for 3.7 T–24.5 T high MF-treated GIST-T1 tumor-bearing mice. Blood glucose (GLU) (Fig. 5(a)), calcium (Ca) (Fig. 5(b)), phosphorus (P) (Fig. 5(c)), iron (Fe) (Fig. 5(d)), cholesterol (CHOL) (Fig. 5(e)), triglyceride (TG) (Fig. 5(f)), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) (Fig. 5(g)) were measured for each mouse. Multiple indicators were affected by some high MF conditions, such as blood glucose (Fig. 5(a)), phosphorus (P) (Fig. 5(c)) and iron (Fe) (Fig. 5(d)). Similar to above, the highest MF intensity 24.5 T, which has no MF gradient, did not cause obvious changes while some gradient MFs had effects on metabolisms (Fig. 5(h)).

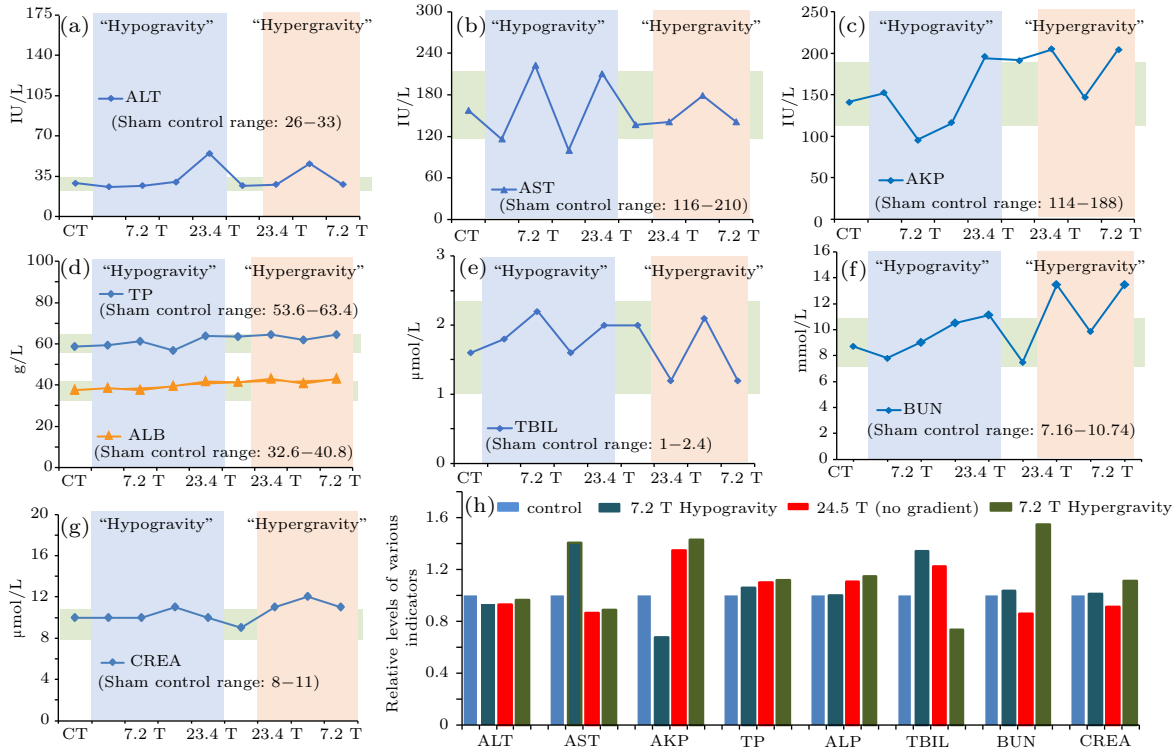


Fig. 4. (color online) The biochemical test results of liver and kidney functions for 3.7 T–24.5 T high magnetic field-treated GIST-T1 tumor-bearing mice. (a) Alanine transaminase (ALT) activity, (b) aspartate transaminase (AST), (c) alkline phosphatase (AKP), (d) total protein (TP), albumin (ALB), (e) total bilirubin (TBIL), (f) blood urea nitrogen (BUN), (g) serum creatinine (CREA) were measured for each mouse. For control (CT), the mean value from eight mice was used. The blue shaded area means the mice were under the circumstances of "hypogravity". The pink shaded area shows the mice were under the circumstances of "hypergravity". The green shaded area shows the reference range defined by eight mice in the Sham control group. (h) The relative levels of ALT, AST, AKP, TP, ALB, TBIL, BUN, and CREA were compared between Sham control, 24.5 T (no gradient), and 7.2 T (in hypogravity and hypergravity conditions).

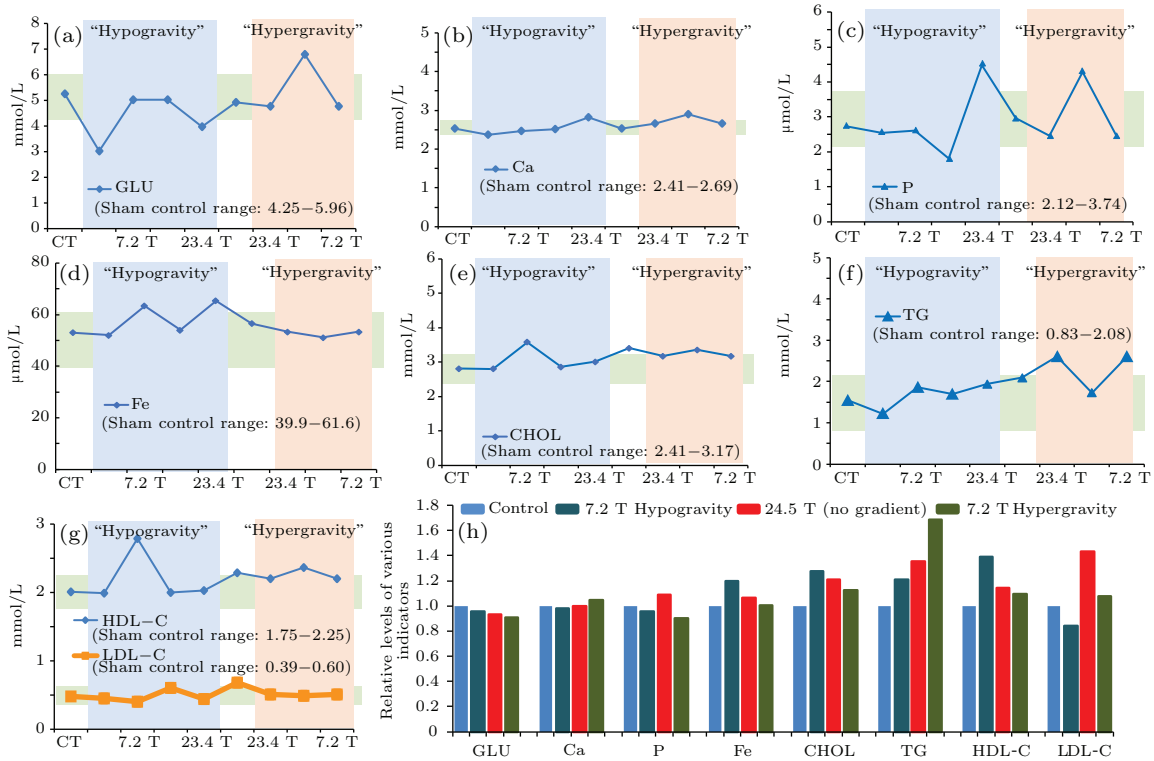


Fig. 5. (color online) The blood biochemical test results of metabolism-related indicators for 3.7 T–24.5 T high magnetic field-treated GIST-T1 tumor-bearing mice. (a) Blood glucose (GLU), (b) calcium (Ca), (c) phosphorus (P), (d) iron (Fe), (e) cholesterol (CHOL), (f) triglyceride (TG), (g) high density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured for each mouse. For control (CT), the mean value from eight mice was used. The blue shaded area means the mice were under the circumstances of "hypogravity". The pink shaded area shows the mice were under the circumstances of "hypergravity". The green shaded area shows the reference range defined by eight mice in the Sham control group. (h) The relative levels of GLU, Ca, P, Fe, CHOL, TG, HDL-C, and LDL-C were compared between Sham control, 24.5 T (no gradient), and 7.2 T (in hypogravity and hypergravity conditions).

Next, we did Hematoxylin-eosin(HE) staining for the liver, kidney and tumor tissues of 3.7 T–24.5 T high MF-treated GIST-T1 tumor-bearing mice. Representative HE stain images of liver, kidney, and tumor in control and MF-treated mice are shown (Figs. 6, S2, and S3). We observed some liver abnormalities in high MF-treated mice, which is consistent with the above-mentioned blood biochemistry results. The kidney and tumor tissue appear normal.

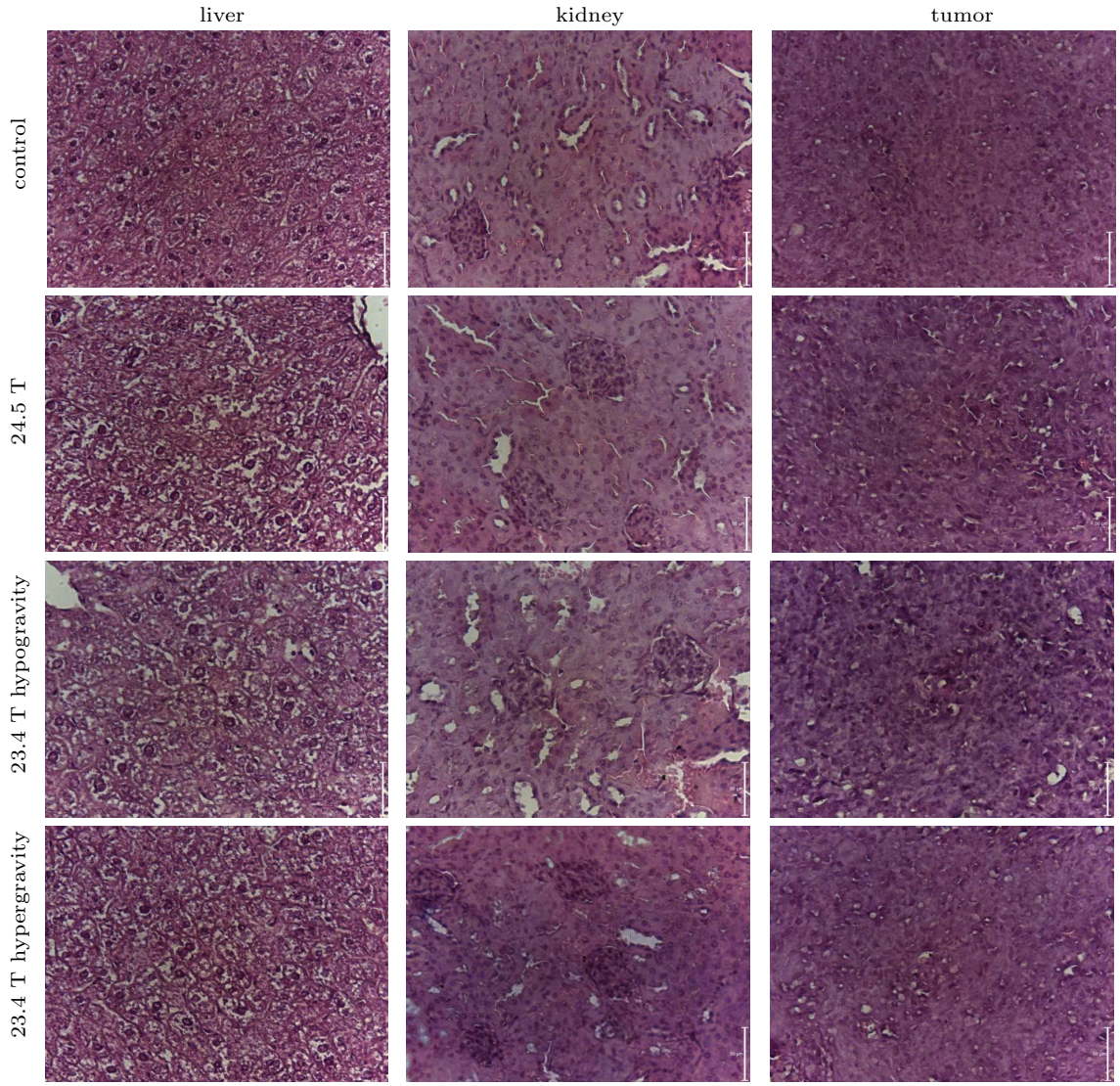


Fig. 6. (color online) Hematoxylin-eosin (HE) stains of liver, kidney, and tumor tissue of GIST-T1 tumor-bearing mice in Sham control, 23.4 T and 24.5 T high SMF treatment groups. Representative images are shown. Scale bar: 50 μm.

3.6. GIST-T1 tumor growth in mice was inhibited by high static magnetic field

Besides safety issues, we also investigated the impacts of high MFs on tumor growth (Fig. 7). It is obvious that different MF conditions have differential effects on GIST-T1 tumor growth in mice (Fig. 7(a)). For example, for the same MF intensity of 23.4 T, the GIST-T1 tumor growth in mice was obvious reduced by 23.4 T in hypergravity condition, but not in hypogravity condition (Fig. 7(b)); for 14.4 T, both hypergravity and hypogravity conditions reduced tumor growth, while the hypogravity affected more obviously (Fig. 7(c)); for 7.2 T, the GIST-T1 tumor growth in mice was obviously reduced by 7.2 T in hypergravity condition, but not in hypogravity condition (Fig. 7(d)), which is similar to 23.4 T. For the 24.5 T high SMF with no gradient, the tumor growth was also inhibited (Fig. 7(e)). We took all the data from eight different SMF exposure conditions together and found that they generally inhibited the tumor growth rate in GIST-T1 tumor-bearing mice (Fig. 7(f)). Although the final tumor weight shows that the tumor inhibition efficacy of different SMF exposure conditions are different, in which the tumor growth inhibition (TGI) could reach 42% in 14.4 T in hypogravity condition and 54% in 7.2 T in hypergravity condition (Fig. 7(g)), the overall TGI for all 8 different SMF exposure conditions are about 17% (Fig. 7(h)).

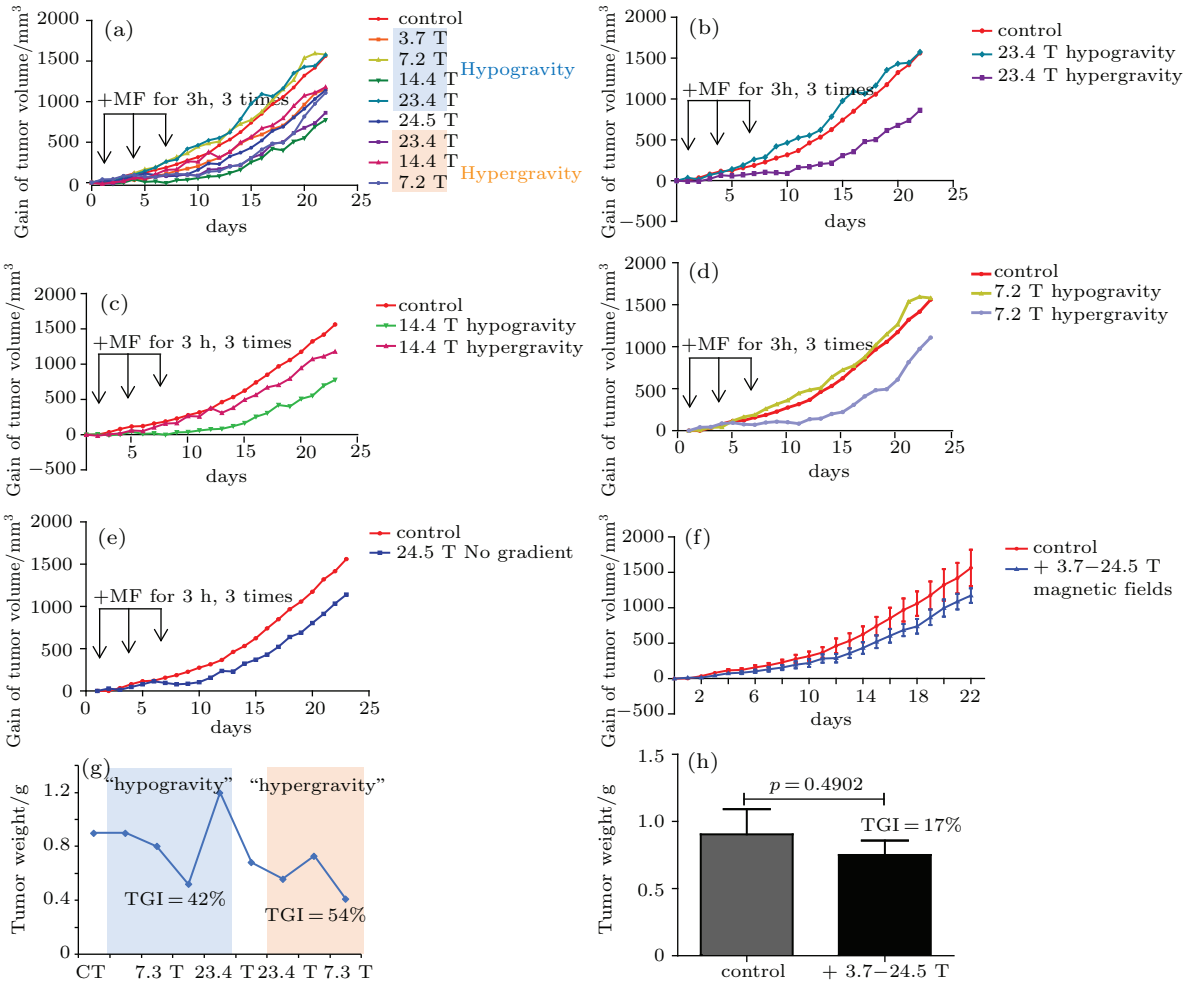


Fig. 7. (color online) GIST-T1 tumor growth in mice was inhibited by static magnetic field. Black arrows show that mice were exposed to static magnetic field for 3 h each time. (a) Gain of tumor volume was measured each day for each mouse under different magnetic field intensity. (b) Gain of tumor volume in mice under the condition of 23.4 T hypogravity and hypergravity was compared. (c) Gain of tumor volume was measured under the condition of 14.4 T hypogravity and hypergravity for each mouse. (d) Gain of tumor volume was measured under the condition of 7.2 T hypogravity and hypergravity for each mouse. (e) Gain of tumor volume was compared between control group and 24.5 T group. (f) Gain of tumor volume was measured each day for each mouse. (g) Each mouse tumor weight in control and different magnetic field exposed groups. (h) Tumor weight in control and exposed groups. Data represent mean ±SD. p value was calculated using unpaired Student's t test.

4. Discussion

Our results show that not only MF intensity but also MF gradient and change of gradient direction all contribute to the biological effects of high MFs on mice. In fact, the MF gradient and change of gradient direction seem to have more impacts than MF intensity per se. MF gradient has been frequently applied in diamagnetic levitation studies, which could be used as a novel ground-based microgravity simulator to facilitate researches of weightlessness condition.[34–36] The MF gradient and change of gradient direction determine whether a diamagnetic sample is in a "hypogravity condition" or a "hypergravity condition", which could produce differential effects on cells.[10,19] Our results report that the GIST-T1 tumor-bearing mice responded differently in different MF exposure conditions. Mice in the upper half tube of the magnet responded differently than the lower half tube of the magnet. More research is needed to further validate these results and unravel the underlying mechanisms.

It has been reported previously that 5 T SMF could suppress food and water consumption, as well as weight gain in mice,[37] which are similar to some of our observed phenomenon. Here we found the weight gain of tumor-bearing mice was only affected by some SMF conditions; for example, the 14.4 T in hypogravity condition that has the highest BB' value, which indicates the largest magnetic force on mice. In addition, we further analyzed their blood and organs, which indicate that the liver function, kidney function, and metabolism are moderately affected by some MF exposure conditions, mostly with a magnetic gradient. However, it should be mentioned that none of the changes are grievous enough to affect apparent organ weight or cause severe organ damage, except for some moderate liver abnormalities.

We have to admit that the big drawback of our study is the limited sample size. Since limited magnet availability and repeated exposure in our experimental design, we could only test 16 mice in this study. However, we found that for most

of these high SMF exposure conditions, tumor growth was inhibited. More interestingly, the tumor growth was inhibited to different extent by different MF exposure conditions, depending on MF intensity, gradient, as well as in hypogravity or hypergravity conditions. Based on our experimental results about moderate intensity magnetic fields, the tumor suppressing effects are directly correlated with the magnetic field intensity and treatment time. Generally speaking, longer magnetic field exposure usually generates stronger effects. However, due to the limitation of magnet availability and the extremely high cost of machine operating, it is not realistic for now to test more magnetic field exposure conditions or to test a large number of mice. We believe with the development of magnet technology, especially superconducting technology, we will be able to systematically investigate the biological effects of high magnetic fields and to explore their medical potentials in the future.

5. Conclusion

In this study, we investigated the effects of a water-cooled magnet, which has its maximum intensity of 24.5 T in the center, and 3.7 T–23.4 T off the center, on nude mice bearing GIST-T1 tumors. Different positions inside the magnet have different MF intensities and gradient, as well as different gradient direction, which generates hypogravity-like and hypergravity-like conditions. The mice were exposed to 3.7 T–24.5 T for 3 times, 9 hours in total, and we observed them for three weeks and examined their health conditions. None of them died during or after exposure and there was no severe organ damage either. Further detailed blood biochemistry analysis and tissue examination revealed that the liver function was mildly disturbed. More importantly, the tumor growth was inhibited by these 3.7 T–24.5 T SMFs and it is interesting that the exact biological effects varied not only between MF intensities, field gradient, but also hypogravity or hypergravity conditions. More research is needed to further validate these conclusions and more exposure conditions should be explored in the future for their clinical applications.

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