ORIGINAL ARTICLE

Targeted MR Imaging Adopting T1-Weighted Ultra-Small Iron Oxide Nanoparticles for Early Hepatocellular Carcinoma: An *in vitro* and *in vivo* Study

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Objective The purpose of this study was to produce an arginylglycylaspartic acid (RGD) peptide-modified ultra-small superparamagnetic iron oxide (Fe_3O_4) nanoparticles (NPs) for targeted magnetic resonance (MR) imaging of hepatocellular carcinoma (HCC) cells and verify its utility as a T1 positive MRI imaging contrast agent *in vitro* and *in vivo*.

Methods The carboxylated Fe_3O_4 NPs stabilized with sodium citrate were conjugated with polyethylene glycol (PEG)-linked RGD nanoparticles to form a novel target contrast agent Fe_3O_4 -PEG-RGD NPs. The specificity of Fe_3O_4 -PEG-RGD to bind RGD receptor was investigated *in vitro* by HepG2 cellular uptake and cell MR imaging, and *in vivo* by MR imaging of subcutaneous HepG2 tumors of nude mice.

Results The formed Fe_3O_4 -PEG-RGD NPs displayed good biocompatibility, and the ultrahigh r_1 relaxivity was $1.37 \text{ mM}^{-1}\text{S}^{-1}$. The synthesized Fe_3O_4 -PEG-RGD NPs were demonstrated spherical-like with an approximate diameter of 2.7 nm in similar size. The targeting effect to HepG2 cells was confirmed by *in vitro* cellular uptake and cell MR imaging. The *in vivo* MR imaging of nude mice demonstrated that the MR signal intensity enhancement of HepG2 tumor in Fe_3O_4 -PEG-RGD NPs treated mice was significantly higher than in mice treated with non-targeted Fe_3O_4 -mPEG NPs at the same post-administration time point.

Conclusion The results indicate that the Fe_3O_4 -PEG-RGD particles have potential utility as T1 positive contrast agent in targeted MR imaging.

HEPATOCELLULAR carcinoma (HCC) is the most frequent malignant tumor of liver worldwide, especially in China.^[1-4] It is the 5th most common carcinoma and causes one million deaths in the world annually.^[5] Early diagnoses of HCCs and surgical resection can enhance the survival rate and ensure the life quality of survivals.^[6] Desperately, it is very important to develop a sensitive method at an early date to detect hepatic nodules that possess HCCs. The common manifestation of HCCs in contrast enhanced CT and magnetic resonance imaging (MRI) is high density/signal intensity in hepatic arterial phase and low density/signal intensity in portal venous phase, namely washout.^[7-10] However, early staged HCCs less than 2 cm in size are often demonstrated atypically.^[11-14] About 65% of early staged HCCs, especially the well-differentiated nodules, are hypovascular and do not wash out in portal phase because of the retention of dual blood supply of both hepatic artery and portal vein.^[15-18] These uncharacteristic features cause difficulties in detection and differential diagnosis of early staged HCC.

The current imaging modalities for early HCCs diagnoses, *e.g.* CT, ultrasound (US), and MR imaging, are generally based on the typical blood kinetic features of enhancement. New diagnostic methods that do not depend on typical vascularity are needed. The emerging molecular imaging focus on tumor micro-environment, where MR imaging is commonly applied because of its significant advantages, such as superior soft tissue contrast, high spatial resolution, and free of radiation exposition.^[19-22] Molecular MR imaging has brought novel insights into diagnosis of multiple diseases.^[23-28]

Superparamagnetic iron oxide (Fe_3O_4) nanoparticles (NPs) can be applied as negative contrast agents for T2-weighted MR imaging.^[29-32] While small size Fe_3O_4 NPs (<5 nm) have been adopted as T1 contrast agents for MR imaging applications.^[33-35] In our previous study, we developed a method to synthesis arginyl-glycine-aspartate (RGD) peptide-functionalized ultra-small Fe_3O_4 NPs for targeted T1-weighted positive MR imaging.^[36] The objective of this work was to investigate its T1 positive MR intensity and the targeting effect of RGD-conjugated Fe_3O_4 NPs to HepG2 cells (a human hepatoma cell line) overexpressing $\alpha\text{v}\beta_3$ integrin (**Figure 1**), and to explore the possibility of its clinical application in tumor targeted MR imaging.

Figure 1. Schematic illustration of synthesis of Fe_3O_4 -PEG-RGD as a platform for targeted magnetic resonance imaging of HepG₂ cells.

MATERIALS AND METHODS

Materials

All of the chemicals in this study were purchased commercially and used without further purification. 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and diethylene glycol were from Aldrich (St Louis, MO, USA); RGD was from GL Biochem (Shanghai, China); Polyethylene glycol (PEG) with one end of amine group and the other end of maleimide group (NH_2 -PEG-Mal, $M_w=2000$) and PEG monomethyl ether with the other end of amine group ($m\text{PEG-NH}_2$, $M_w=2000$) were both obtained from Shanghai Yanyi Biotechnology Co. (Shanghai, China); Dulbecco's modified eagle medium (DMEM), phosphate buffer saline (PBS), fetal bovine serum (FBS), streptomycin, and penicillin were from Gibco (Carlsbad, CA, USA); sodium citrate, anhydrous ferric chloride ($\text{FeCl}_3>99\%$) were from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and applied as received. The water used in the experiments was purified by a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA, USA) with a resistivity higher than 18 $\text{M}\Omega\cdot\text{cm}$. HepG2 cells and 293T cells (a human embryonic kidney cell line) were purchased from Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China).

Synthesis of Fe_3O_4 -PEG-RGD NPs

Fe_3O_4 -PEG-RGD NPs were synthesized using a mild reduction route according to our previous study.^[29] In brief, sodium citrate was added to $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ (1081.00 mg), then the obtained black product was dispersed into water and lyophilized to get Fe_3O_4 NPs powder for further use. Next, we added NH_2 -PEG-Mal (20.00 mg) into 5 mL DMSO with vigorous magnetic

stirring at room temperature, followed by adding RGD (6.92 mg, in 2 mL DMSO) and stirring for 72 hours to obtain the product of NH₂-PEG-RGD in white powder. At last, the citric acid carboxyl of Fe₃O₄ NPs (56.00 mg, in 10 mL DMSO) was dissolved dropwise into the DMSO solution of NH₂-PEG-RGD (11.08 mg, 5 mL) under vigorous stirring at room temperature for 72 hours, followed by lyophilization to produce powder of Fe₃O₄-PEG-RGD NPs. We used Fe₃O₄ NPs powder and mPEG-NH₂ under the similar experimental conditions to produce powder of Fe₃O₄-mPEG NPs.

Characterization techniques

Hydrodynamic sizes and zeta potential were measured by a Malvern Zetasizer Nano (ZS model ZEN3600, Malvern, Worcestershire, UK) with a standard 633-nm laser. The morphologies and sizes of nanoparticles were evaluated on transmission electron microscopy (TEM) (2010F, JEOL, Tokyo, Japan) at a voltage of 200 kV. The Fe concentration was evaluated by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Leeman Prodigy, Hudson, NH, USA). The nanoparticles were dissolved with water to obtain Fe solution of different concentrations. The T1 relaxometry of the Fe₃O₄-PEG-RGD NPs was examined by positive MR imaging via a 0.5 T NMR Analyzing and Imaging system (NMI20-Analyst, Niumag Corporation Ltd, Shanghai, China).

Cytotoxicity assay and cell morphology observation

The cytotoxicity of the Fe₃O₄-PEG-RGD NPs was evaluated by MTT viability assay of HepG2 and 293T cells. The cells were seeded into a 96-well plate with a density of 1×10^4 per well and incubated in regular DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin for 24 h. Afterwards, the medium was replaced with a fresh medium containing Fe₃O₄-PEG-RGD NPs at different Fe concentrations. After 24 h incubation at 37°C and 5% CO₂, MTT (20 µL, 5 mg/mL) in PBS was added into each well and the cells were incubated for another 4 h under regular culture conditions. The medium was then carefully removed, and DMSO (200 µL) was added into each well to dissolve the formed formazan crystals. The absorbance at 570 nm of each well was measured using a microplate photometer (Multiskan™ MK3, Thermo Fisher Scientific, Hudson, NH). Mean and standard deviation for the triplicate wells were calculated. After treatment with Fe₃O₄-PEG-RGD NPs at different Fe concentrations (0–100 µg/mL)

for 24 h, the cell morphology was observed using a Leica DM IL LED inverted phase contrast microscope with magnification of 200× for each sample.

In vitro cellular uptake of the Fe₃O₄-PEG-RGD NPs

HepG2 cells and 293T cells were seeded in MEM medium and DMEM medium, respectively, supplemented with 1% penicillin-streptomycin and 10% FBS at 37°C with 5% CO₂. The specific uptake of the Fe₃O₄-PEG-RGD NPs by HepG2 cells was demonstrated using ICP-AES analysis of the cells handled with the nanoparticles for 4 hours. HepG2 cells were cultured in 12-well plates at a density of 2×10^5 cells per well in 1 mL DMEM and incubated at 37°C with 5% CO₂. Next day the medium was replaced by 1 mL fresh PBS (control), and the Fe₃O₄-PEG-RGD NPs at Fe concentration of 25, 50, 75, and 100 µg/mL respectively. After 4 hours, washed the cells three times with PBS, then trypsinized, centrifuged, and resuspended in 1 mL PBS to get the number of cells per well prior to being digested by aqua regia solution. Finally, ICP-AES analysis was performed to value the uptake of Fe₃O₄-PEG-RGD NPs by HepG2 cell. For comparison, the uptake by 293T cells was managed and valued under the same experimental conditions.

In vitro MR imaging of targeted cancer cells

HepG2 cells were cultured in a 6-well plate at a density of 3×10^6 cells per well in 2 mL DMEM, and incubated at 37°C with 5% CO₂. After a night culture to get the cells confluence, replaced with 2 mL fresh medium with Fe₃O₄-mPEG or Fe₃O₄-PEG-RGD NPs at Fe concentrations of 5, 10, 25, 50 and 100 µg/mL, respectively. Next, seeded the cells at 37°C with 5% CO₂ for 6 hours. Then, washed the HepG2 cells 5 times with PBS, trypsinized, centrifuged, and resuspended in 1 mL PBS in 2 mL Eppendorf tubes for MR performance. T1 positive MR imaging of the cell suspension was performed via a wrist receiver coil with inversion recovery sequence: TR=400 ms, TE=20 ms, point resolution=128 mm×128 mm, section thickness=0.5 mm, and number of excitation=1.

Animal model and *in vivo* MR imaging

Animal model of tumor

The animal studies were approved by the Committee on Use and Care of Animals Shanghai Jiaotong Uni-

versity affiliated General Hospital, and conducted in accordance with local humane animal care standard. Nude mice in about 4-5 weeks old were purchased from Shanghai Laboratory Animal Centre. We injected HepG2 cells suspension (5×10^6 cells in 0.2 ml folate-free RPMI1640 medium) into the right lateral thigh of nude mice, then maintained them under specific pathogen-free (SPF) conditions for at least 4 weeks. When the tumors reached 1.0 cm in diameter, the mice were adopted for *in vivo* MR imaging.

In vivo MR imaging

Nine tumor bearing mice were randomly divided into three groups: RGD-targeted Fe_3O_4 NPs injection group, Fe_3O_4 -mPEG injection group and PBS injection group (as control). Pentobarbital sodium of 40 mg/kg was injected to the tumor-bearing mice, then Fe_3O_4 -mPEG or Fe_3O_4 -PEG-RGD NPs dispersed in 0.3 mL PBS were administrated via tail veins at a dose of 450 μg Fe per mouse. MR imaging was performed on 3.0 T MR scanner (Discovery 750, GE Healthcare, USA) with a small animal coil (Magtron Inc., Shanghai, China) and FSE sequence: TR=600 ms, TE=11.7 ms, slice thickness=1.8 mm, FOV=3 cm $\times$ 3 cm, and point resolution=128 $\times$ 256. For each animal, T1-weighted MR images were obtained before and at 15, 30, 60, and 90 min after injection of the nanoparticles. The tumor MR signal intensities of three mice in each group were collected under each condition and described as mean and standard deviation (SD).

In vivo biodistribution and tumor uptake

After MR scanning, the tumor-bearing mice were euthanized at 12 hours after injection and then organs (heart, liver, spleen, lung, kidney) and tumors were resected and weighed. The organs were then cut into small pieces about 1–2 mm³ in size and digested by aqua regia solution (nitric acid/hydrochloric acid, v/v=1:3) for 24 hours. Then, Fe content in organs

were quantified by ICP-AES. For comparison, the mice without injection and the mice injected with the non-targeted Fe_3O_4 -mPEG NPs received the same procedures as controls. The tumor uptake of Fe_3O_4 -PEG-RGD NPs was further evaluated by TEM according to the methods described in previous paper.^[37, 38]

Statistical analysis

All statistical analyses were performed with SPSS (version 21.0). Data of tumor MR signal intensity were described as mean $\pm$ SD. One-way ANOVA statistical analysis was performed to compare the MR signal intensity of cells and animals. A *P* value < 0.05 was considered statistically significant.

RESULTES

Characterization of the Fe_3O_4 -PEG-RGD NPs

The synthesized Fe_3O_4 -PEG-RGD NPs were demonstrated spherical-like with an approximate diameter of 2.7 nm in similar size. Fe_3O_4 -PEG-RGD NPs can be dissolved in PBS, water, or cell culture fluid with quite good stability. The pristine citrate-stabilized Fe_3O_4 NPs have a fairly negative surface potential of -39.7 mV *via* the citrate stabilization. The surface potentials of the Fe_3O_4 -mPEG and Fe_3O_4 -PEG-RGD measured were -8.8 mV and -10.1 mV, respectively. The hydrodynamic sizes of the Fe_3O_4 -mPEG and Fe_3O_4 -PEG-RGD were 168.7 nm and 212.5 nm, respectively.

T1 relaxometry

The synthesized Fe_3O_4 -PEG-RGD NPs displayed good biocompatibility and ultrahigh r_1 relaxivity which was 1.37 mM⁻¹S⁻¹. Both the Fe_3O_4 -mPEG and Fe_3O_4 -PEG-RGD NPs showed increased MR signal intensity; with increasing of Fe concentration, the enhancement of T1 signal intensity by Fe_3O_4 -PEG-RGD increased, which was more distinct than that of Fe_3O_4 -mPEG (Figure 2).

Figure 2. The *in vitro* T1-weighted MR images of the Fe_3O_4 -mPEG (non-targeted) and Fe_3O_4 -PEG-RGD (targeted) NPs at Fe concentration of 0.05, 0.1, 0.2, 0.4, 0.8 mM, respectively.

In vitro cytotoxicity assay

The cell viabilities of HepG2 cells treated with Fe_3O_4 -PEG-RGD NPs at Fe concentrations of 10-100 $\mu\text{g/mL}$ all exceeded 80%, while no obvious cytotoxicity in 293T cells treated by Fe_3O_4 -PEG-RGD was observed in the same range of Fe concentration (Figure 3).

Targeted T1-weighted positive MR imaging

HepG2 cells in vitro

We explored the possibility of the Fe_3O_4 -PEG-RGD NPs as a nanoprobes for T1-weighted positive MR imaging of HepG2 cells *in vitro* (Figure 4). The MR signal intensity of the HepG2 cells treated with Fe_3O_4 -PEG-RGD NPs was significantly higher than that treated with Fe_3O_4 -

mPEG NPs at the same concentration of Fe ($F=10.2$, $P=0.009$). With the increase of Fe concentration, the enhancement of T1 signal intensity by Fe_3O_4 -PEG-RGD was more distinct than that by Fe_3O_4 -mPEG.

Xenografted HepG2 tumor model in vivo

Both targeted and non-targeted NPs injections produced MR contrast enhancement of HepG2 tumors in mice (Figure 5). The quantitative tumor MR signal intensity data show that, at the same time point (post-administrated 15 min, 30 min, 60 min, and 90 min), the tumor MR signal intensity enhancement of Fe_3O_4 -PEG-RGD NPs treated mice was significantly higher than that treated with Fe_3O_4 -mPEG NPs ($F=8.6$, $P=0.01$).

Figure 3. MTT assay of cell viability 24 hours after the treatment of Fe_3O_4 -PEG-RGD NPs. PBS were used as control ($n=3$). A. HepG2 cells; B. 293T cells.

Figure 4. *In vivo* T1-weighted MR image of human hepatoma cell HepG2 acquired 2 hours after treated with the Fe_3O_4 -mPEG (non-targeted) and Fe_3O_4 -PEG-RGD (targeted) NPs at Fe concentrations of 10, 25, 50, 75, and 100 $\mu\text{g/mL}$, respectively.

Figure 5. *In vivo* T1-weighted MR imaging of HepG₂ tumor model mice before and after injection of the Fe_3O_4 -mPEG (non-targeted) and Fe_3O_4 -PEG-RGD (targeted) NPs solution through tail vein. The arrows indicate the tumorous area.

***In vivo* bio-distribution**

The ICP-AES result data (Figure 6) showed there was a significant uptake of Fe in liver, while only a relatively small amount of Fe was absorbed by other organs (*e.g.*, heart and kidney) and the tumor tissue. Importantly, the Fe content in tumorous tissues after injection of Fe₃O₄-mPEG and Fe₃O₄-PEG-RGD NPs were much higher than the control mice without injection. The results fully illustrated the important RGD targeting role in effectively delivering the NPs to tumors, which consequently render effective MR imaging targeting on tumors.

***In vivo* tumor uptake of Fe₃O₄-PEG-RGD NPs**

On TEM images, we found a small number of elec-

Figure 6. The *in vivo* biodistribution of Fe in tumor and different organs of nude mice received injection of Fe₃O₄-mPEG (non-targeted) or Fe₃O₄-PEG-RGD (targeted) NPs (450 µg Fe, in 0.3 mL PBS).

tron-dense particles in the vacuoles of cytoplasm of HepG2 tumor cell in Fe₃O₄-PEG-RGD NPs treated model mice (Figure 7).

DISCUSSION

In this study, Fe₃O₄-PEG-RGD NPs were synthesized with a mild reduction route on the basis of our former study.^[33] Fe₃O₄ is usually regarded as T2 negative MR contrast agent because it decreases MR signal intensity,^[39,40] while small sized Fe₃O₄ NPs with diameter <5 nm can be adopted as T1 contrast agents. We found that most Fe₃O₄-PEG-RGD particles were demonstrated spherical in similar size, with an approximate diameter of 2.7 nm, which is very small comparing to Fe₃O₄ nanoparticles. The T1-weighted MR images clearly showed that both Fe₃O₄-mPEG and Fe₃O₄-PEG-RGD nanoparticles demonstrated increased MR signal intensity with the increase of Fe concentration.

It is important to know the potential cytotoxicity of Fe₃O₄-PEG-RGD NPs *in vitro* before being adopted *in vivo*. This study showed that after Fe₃O₄-PEG-RGD NPs treatment with Fe concentration in range of 10-100 µg/mL, the viability of HepG2 cells can remain higher than 80% of its origin. The result indicates that the prepared Fe₃O₄-PEG-RGD NPs have good cytocompatibility in the studied range of Fe concentration. We explored the possibility of Fe₃O₄-PEG-RGD NPs as a nanoprobe for T1-weighted positive MR imaging of HepG2 cancer cells *in vitro*, and found that both the Fe₃O₄-mPEG and Fe₃O₄-PEG-RGD NPs can produce

Figure 7. Transmission electron microscopy images of hepatocellular carcinoma in Fe₃O₄-PEG-RGD (targeted) NPs treated mice. A. Image of a hepatocellular carcinoma cell (10000×); B. a higher-magnified image of the highlight area on image (A) showing the distribution of Fe₃O₄-PEG-RGD NPs in the lysosomes (arrow) (30000×).

T1-positive MR contrast enhancement in HepG2 cells and the enhancing effect was correlated to Fe concentration. Additionally, at the same Fe concentration, the enhanced MR signal intensity of HepG2 cells by Fe₃O₄-PEG-RGD NPs was higher than that by Fe₃O₄-mPEG NPs without RGD. In particular, the MR signal intensity of the HepG2 cells handled with the Fe₃O₄-PEG-RGD NPs was significantly higher than that handled with the Fe₃O₄-mPEG NPs at the same Fe concentration. These results suggest that it is possible to adopt Fe₃O₄-PEG-RGD NPs as a nanoprobe of specific T1 positive MR imaging for α v β 3 overexpressing cancer cells *in vitro*.

We investigated the potential of using Fe₃O₄-PEG-RGD NPs as a nanoprobe for targeted T1-weighted MR imaging of tumors *in vivo*. It is clearly shown that intravenous injections of targeted and non-targeted NPs can both induce MR contrast enhancement of the HepG2 tumors. At 60 minutes post-injection, both nanoparticles are able to aggregate in the tumor region, reaching the maximum MR contrast enhancement which was likely due to the maximum accumulation of the nanoparticles in the tumor. The signal intensity gradually decreased subsequently, which was probably due to the metabolism process of the particles decreased its accumulation in the tumors. The quantitative data of tumor MR signal intensity showed that the post-administrated tumor MR signal intensity treated with Fe₃O₄-PEG-RGD NPs was significantly higher than that treated with non-targeted Fe₃O₄-mPEG NPs at the same time point. These results suggest that the Fe₃O₄-PEG-RGD NPs can be used as a nanoprobe for targeted T1-weighted positive MR imaging of tumors *in vivo* through the RGD-mediated active targeting pathway.

It is important to investigate the bio-distribution of the Fe₃O₄-PEG-RGD NPs *in vivo* for its applications as a contrast medium of MR imaging. ICP-AES data showed that the Fe contents in major organs and tumor tissue after injection of Fe₃O₄-mPEG and Fe₃O₄-PEG-RGD NPs were much higher than that of the control mice without injection. A significant uptake in liver was observed. Data of Fe bio-distribution suggested that the PEGylation modification of the Fe₃O₄ NPs allowed a portion of particles to escape from reticuloendothelial system and transfer to tumor tissue for effective MR imaging. The Fe content in the tumors of mice treated with targeted Fe₃O₄-PEG-RGD NPs was much higher than that treated with non-targeted Fe₃O₄-mPEG NPs, indicating the important role of RGD in targeting on and delivering to the tumor, and conse-

quently, effectively delineating tumors in MR imaging.

TEM imaging, a modality that identifies the intracellular distribution of nanoparticles, revealed the uptake of Fe₃O₄-PEG-RGD NPs in HepG2 cell. The detection of a small number of electron-dense particles in vacuoles of HepG2 cells of Fe₃O₄-PEG-RGD NPs treated mice indicated that the cellular uptake of Fe₃O₄-PEG-RGD NPs was probably through the RGD receptor-mediated pathway, instead of internalization by phagocytosis, or diffusion through cytoderm.

To conclude, as a specific nanometre targeted MR imaging probe, Fe₃O₄-PEG-RGD NPs targeting on α v β 3 can be used to study early staged HCC in animal models *in vivo* with high field MR imaging and provide additional information to improve early diagnosis of HCC.

Conflict of interests

None.

REFERENCES

1. Kavanaugh G, Williams J, Morris AS, et al. Utility of [18F] FSPG PET to image hepatocellular carcinoma: first clinical evaluation in a US population. *Mol Imaging Biol* 2016; 18(6):924–34. doi: 10.1007/s11307-016-1007-0.
2. Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2014; 10(5):761–80. doi: 10.1016/j.jgassur.2005.10.006.
3. Chalasani N, Said A, Ness R, et al. Screening for hepatocellular carcinoma in patient with cirrhosis in the United States: results of a national survey. *Am J Gastroenterol* 1999; 94(8):2224–9. doi: 10.1016/s0002-9270(99)00353-6.
4. Yamamoto M, Takasaki K, Otsubo T, et al. Favorable surgical outcomes in patients with early hepatocellular carcinoma. *Ann Surg* 2004; 239(3):395–9. doi: 10.1097/01.sla.0000114215.03112.e0.
5. Liu H, Xu Y, Wen S, et al. Targeted tumor computed tomography imaging using low-generation dendrimer-stabilized gold nanoparticles. *Chemistry* 2013; 19(20):6409–16. doi: 10.1002/chem.201204612.
6. Lim JH, Kim CK, Lee WJ, et al. Detection of hepatocellular carcinomas and dysplastic nodules in cirrhotic livers: accuracy of helical CT in transplant patients. *AJR Am J Roentgenol* 2000; 175(3):693–8. doi: 10.2214/ajr.175.3.1750693.
7. Semelka RC, Martin DR, Balci C, et al. Focal liver lesions: comparison of dual-phase CT and multisequence

- multiplanar MR imaging including dynamic gadolinium enhancement. *J Magn Reson Imaging* 2001; 13(3):397–401. doi: 10.1002/jmri.1057.
8. Tsekouras, Magkos YE, Kellas F, et al. High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men. *Am J Physiol Endocrinol Metab* 2008; 295(4):E851. doi: 10.1152/ajpendo.90545.2008.
9. Nobuhiro F, Akihiro N, Yoshiki A, et al. Significance of the signal intensity of gadoxetic acid-enhanced MR imaging for predicting the efficacy of hepatic arterial infusion chemotherapy in hepatocellular carcinoma. *Magnet Reson Med Sci* 2016; 15(1):111–20. doi: 10.2463/mrms.2015-0012.
10. Gan LU, Chang R, Jin H, et al. Typical CT and MRI signs of hepatic epithelioid hemangioendothelioma. *Oncology Letters* 2016; 11(3):1699–706. doi: 10.3892/ol.2016.4149.
11. Kim SA, Lee JM, Lee KB, et al. Intrahepatic mass-forming cholangiocarcinomas: enhancement patterns at multiphasic CT, with special emphasis on arterial enhancement pattern-correlation with clinicopathologic findings. *Radiology* 2011; 260(1):148–57. doi: 10.1148/radiol.11101777.
12. Forner A, Vilana R, Ayuso C, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008; 47(6):97–104. doi: 10.1002/hep.22356.
13. Kim I, Kim MJ. Histologic characteristics of hepatocellular carcinomas showing atypical enhancement patterns on 4-phase MDCT examination. *Korean J Radiol* 2012; 13(5):586–93. doi: 10.3348/kjr.2012.13.5.586.
14. Xu Y, Xiao A, Yang J, et al. Assessment of lipiodol deposition and residual cancer for hepatocellular carcinoma after transcatheter arterial chemoembolization via iodine-based material decomposition images with spectral computed tomography imaging: a preliminary study. *Iran J Radiol* 2015; 12(4):e26009. doi: 10.5812/iranjradiol.26009.
15. Khan AS, Hussain HK, Johnson TD, et al. Value of delayed hypointensity and delayed enhancing rim in magnetic resonance imaging diagnosis of small hepatocellular carcinoma in the cirrhotic liver. *J Magn Reson Imaging* 2010; 32(2):360–6. doi: 10.1002/jmri.22271.
16. Jia C, Dai C, Zhao X, et al. Use of hepatic blood inflow occlusion and hemihepatic artery retention in liver resection for hepatocellular carcinoma. *Translation Cancer Res* 2016; 5(5):598–606. doi: 10.21037/tcr.2016.10.50.
17. Wayne LW. Regulatory processes interacting to maintain hepatic blood flow constancy: Vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. *Hepatology Res* 2010; 37(11):891–903. doi: 10.1111/j.1872-034X.2007.00148.x.
18. Vanagas T, Gulbinas A, Pundzius J, et al. Radiofrequency ablation of liver tumors (II): clinical application and outcomes. *Medicina* 2010; 46(1):81–8. doi: 10.1159/000320314.
19. Pysz MA, Gambhir SS, Willmann JK. Molecular imaging: current status and emerging strategies. *Clin Radiol* 2010; 65(7):500–16. doi: 10.1016/j.crad.2010.03.011.
20. Mundt AP, Winter C, Mueller S, et al. Targeting activated microglia in Alzheimer's pathology by intraventricular delivery of a phagocytosable MR imaging contrast agent in APP23 transgenic mice. *Neuroimage* 2009; 46(2):367–72. doi: 10.1016/j.neuroimage.2009.01.067.
21. Oude Engberink RD, van der Pol SM, Dopp EA, et al. Comparison of SPIO and USPIO for in vitro labeling of human monocytes: MR detection and cell function. *Radiology* 2007; 243(2):467–74. doi: 10.1124/jpet.105.100149.
22. Xu Y, Yang J, Zhang Z, et al. MRI for discriminating metastatic ovarian tumors from primary epithelial ovarian cancers. *J Ovarian Res* 2015; 8:61. doi: 10.1186/s13048-015-0188-5.
23. Stoll G, Bendszus M, Perez J, et al. Magnetic resonance imaging of the peripheral nervous system. *J Neurol* 2009; 256(7):1043–51. doi: 10.1007/s00415-009-5064-z.
24. Anderson SA, Shukaliak-Quandt J, Jordan EK, et al. Magnetic resonance imaging of labeled T-cells in a mouse model of multiple sclerosis. *Ann Neurol* 2004; 55(5):654–9. doi: 10.1002/ana.20066.
25. Patterson AJ, Tang TY, Graves MJ, et al. In vivo carotid plaque MRI using quantitative T2* measurements with ultrasmall superparamagnetic iron oxide particles: a dose-response study to statin therapy. *NMR Biomed* 2011; 24(1):89–95. doi: 10.1002/nbm.1560.
26. Yang J, Wadghiri YZ, Hoang DM, et al. Detection of amyloid plaques targeted by USPIO-Abeta1-42 in Alzheimer's disease transgenic mice using magnetic resonance microimaging. *Neuroimage* 2011; 55(4):1600–9. doi: 10.1016/j.neuroimage.2011.01.023.

27. Liu H, Wang H, Xu Y, et al. Lactobionic acid-modified dendrimer-entrapped gold nanoparticles for targeted computed tomography imaging of human hepatocellular carcinoma. *ACS Appl Mater Interfaces* 2014; 6(9):6944–53. doi: 10.1021/am500761x.
28. Zhai C, Franssen GM, Petrik M, et al. Comparison of Ga-68-Labeled Fusarinine C-Based multivalent RGD conjugates and [(68)Ga]NODAGA-RGD-in vivo imaging studies in human xenograft tumors. *Mol Imaging Biol* 2016; 18(5):758–67. doi: 10.1007/s11307-016-0931-3.
29. Chen PJ, Hu SH, Hsiao CS, et al. Multifunctional magnetically removable nanogated lids of Fe₃O₄-capped mesoporous silica nanoparticles for intracellular controlled release and MR imaging. *J Mater Chem* 2011; 21(8):2535–43. doi: 10.1039/c0jm02590a.
30. Xie J, Chen K, Lee HY, et al. Ultrasmall c (RG-DyK)-coated Fe₃O₄ nanoparticles and their specific targeting to integrin $\alpha v \beta_3$ -rich tumor cells. *J Am Chem Soc* 2011; 130(24):7542–3. doi: 10.1021/ja802003h.
31. Li J, Zheng L, Cai H, et al. Facile One-Pot synthesis of Fe₃O₄@Au composite nanoparticles for Dual-Mode MR/CT imaging applications. *ACS Appl Mater Interfaces* 2013; 5(20):10357–66. doi: 10.1021/am4034526.
32. Chen J, Sun Y, Chen Q, et al. Multifunctional gold nanocomposites designed for targeted CT/MR/optical trimodal imaging of human non-small cell lung cancer cells. *Nanoscale* 2016; 8(28):13568–73. doi: 10.1039/c6nr03143a.
33. Wang G, Zhang X, Skallberg A, et al. One-step synthesis of water-dispersible ultra-small Fe₃O₄ nanoparticles as contrast agents for T1 and T2 magnetic resonance imaging. *Nanoscale* 2014; 6(5):2953–63. doi: 10.1039/c3nr05550g.
34. Zeng L, Ren W, Zheng J, et al. Ultrasmall water-soluble metal-iron oxide nanoparticles as T1-weighted contrast agents for magnetic resonance imaging. *Phys Chem Chem Phys* 2012; 14(8):2631–6. doi: 10.1039/c2cp23196d.
35. Yang J, Luo Y, Xu Y, et al. Conjugation of iron oxide nanoparticles with RGD-modified dendrimers for targeted tumor MR imaging. *ACS Appl Mater Interfaces* 2015; 7(9):5420–8. doi: 10.1021/am508983n.
36. Luo Y, Yang J, Yan Y, et al. RGD-functionalized ultrasmall iron oxide nanoparticles for targeted T1-weighted MR imaging of gliomas. *Nanoscale* 2015; 7(34):14538–46. doi: 10.1039/C5NR04003E.
37. Zhang Z, Hu Y, Yang J, et al. Facile synthesis of folic acid-modified iron oxide nanoparticles for targeted MR imaging in pulmonary tumor xenografts. *Mol Imaging Biol* 2016; 18(4):569–78. doi: 10.1007/s11307-015-0918-5.
38. Li K, Shen M, Zheng L, et al. Magnetic resonance imaging of glioma with novel APTS-coated superparamagnetic iron oxide nanoparticles. *Nanoscale Res Lett* 2014; 9(1):304–14. doi: 10.1186/1556-276X-9-304.
39. Jin J, Krishnamachary B, Barnett JD, et al. Human Cancer Cell Membrane-Coated Biomimetic Nanoparticles Reduce Fibroblast-Mediated Invasion and Metastasis and Induce T-Cells. *ACS Appl Mater Interfaces* 2019; 11(8):7850–61. doi: 10.1021/acsami.8b22309.
40. Clements TW, Sarsons C, Platnich CM, et al. Maltol-functionalized Fe₃O₄ nanoparticles as T2 magnetic resonance imaging contrast agents. *Chemistry Select* 2016; 1(8):1602–6. doi: 10.1002/slct.201600240.