Molecular Magnetic Resonance Imaging Approaches Used to Aid in the Understanding of the Tissue Regeneration Marker Met *In Vivo*: Implications for Tissue Engineering

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The levels of Met, a tyrosine kinase receptor for the hepatocyte growth factor or scatter factor, are elevated during tissue regeneration, and can be used to assess tissue regeneration associated with engineered tissue grafts. This study involved the development and assessment of a novel magnetic resonance imaging (MRI) molecular probe for the *in vivo* detection of Met in an experimental rodent (rat) model of disease (C6 glioma). The implication of using these probes in tissue engineering is discussed. The molecular targeting agent we used in our study incorporated a magnetite-based dextran-coated nanoparticle backbone covalently bound to an anti-Met antibody. We used molecular MRI with an anti-Met probe to detect *in vivo* Met levels as a molecular marker for gliomas. Tumor regions were compared to normal tissue, and found to significantly ($p < 0.05$) decrease MR signal intensity and T_2 relaxation in tumors. Nonimmune nonspecific normal rat IgG coupled to the dextran-coated nanoparticles was used as a control. Met levels in tumor tissues were confirmed in Western blots. Based on our results, *in vivo* evaluation of tissue regeneration using molecular MRI is possible in tissue engineering applications.

Introduction

IN TISSUE ENGINEERING it is often necessary to assess tissue regeneration associated with engineered tissue grafts to determine if successful tissue and/or cell growth is occurring. The levels of Met, a tyrosine kinase receptor for the hepatocyte growth factor or scatter factor (HGF/SF), are elevated during tissue regeneration. Met may also be an important marker to assess during cell growth in tissue constructs. The HGF/SF is a polypeptide growth factor with associated major roles in development and tissue regeneration in vertebrate organisms.¹ HGF/SF and its receptor Met, the tyrosine kinase encoded by the c-Met proto-oncogene, provide signals essential for the development of the placenta, liver, tongue, diaphragm, limb muscles, and certain groups of neurons.^{2–4} HGF/SF and Met are required in postnatal life for liver regeneration and the survival and activity of the endocrine pancreas.^{5–7} The functional expression of HGF and the HGF receptor/c-met in adult mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing.⁸

Molecular magnetic resonance imaging (mMRI) allows noninvasive observation of *in vivo* biological processes at the cellular and molecular level. mMRI utilizes the signaling component of a targeting probe to monitor alterations in

contrast-agent-mediated tissue relaxation times that occur as a result of the binding of the affinity component to molecular markers. The advantage of using mMRI is that the spatial location of *in vivo* molecular marker overexpression can be readily observed in heterogeneous deeply located tissues with good spatial resolution. Superparamagnetic iron oxide (SPIO)-based particles generate a negative signal contrast (T_2 relaxation contrast) that can be monitored with MRI.

The molecular targeting agents we used in our study incorporated a dextran-coated magnetite-based nanoparticle backbone covalently bound to an anti-c-Met antibody (Ab). The objective of this project was to develop and assess a novel MRI molecular probe for the *in vivo* detection of Met in an experimental rodent model for gliomas, which may potentially be used for *in vivo* diagnostic biomarkers for malignant human gliomas. Met is a potential marker that can also be used to evaluate tissue-engineered grafts or transplants to monitor tissue regeneration.

Materials and Methods

Rat glioma models

C6 gliomas. Three-month-old male Fischer 344 rats (250–350 g; $n = 4$) were anesthetized (3% isoflurane at 2.5 L/min

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oxygen) and placed on a stereotaxic device (Stoelting, Wood Dale, IL). The heads of anesthetized rats were immobilized, and using aseptic techniques, a 1 mm burr hole was drilled in the skull 2 mm anterior and 2 mm lateral to the bregma on the right-hand side of the skull. C6 cells (10^6 of a 10^6 /mL cell suspension) were injected in the cortex at a 3 mm depth from the dura.^{9,10} Rats were maintained on a choline-deficient diet throughout the studies, as the tumor cells have been shown to be tumorigenic in choline-deficient Fisher rats. C6 implanted cells (glial cell strain cloned from a rat glial tumor induced by N-nitrosomethylurea) yield intracerebral growth with minimal distant metastasis.^{11,12}

Synthesis of the anti-c-Met MRI contrast agent

Synthesis of nanoparticles involves coprecipitation of ferrous and ferric salts in an alkaline medium, with a surface complexing agents, such as dextran to provide colloid stability and biocompatibility.^{13–15} For this study, a dextran-based cross-linked iron oxide (CLIO) molecular-targeted IO nanoparticle-based anti-c-Met probe was synthesized. To recognize c-Met, a mouse monoclonal anti-c-Met Ab to the β -chain of c-Met, which has an extracellular domain¹⁶ (B-2: sc-8057), was used.

A monodispersed superparamagnetic iron oxide colloid (MION) was synthesized and cross linked with epichlorohydrin (Sigma-Aldrich, St. Louis, MO) to prepare an amine-terminated CLIO (CLIO-NH₂).^{17,18} Briefly, amination is achieved by the addition of concentrated ammonia, followed by heating at 37°C overnight.¹⁷ Low molecular weight materials are removed by dialysis against water using dialysis tubing (12–14K cutoff; Spectra/Por, Laguna Hills, CA).^{17,18} Air is then bubbled through the colloid for 24 h at 37°C.¹⁷ The colloid is subjected to pressure dialysis with the addition of 10 volumes of 5 mM sodium citrate, pH 8.^{17,18} These steps fully oxidize any ferrous iron and remove traces of low molecular weight materials.¹⁷ The nanoparticles were characterized by transmission electron microscopy and light scattering (Nanotrak particle size analysis).

The Ab-SPIO conjugates were prepared via disulfide exchange reactions in the following steps: (1) synthesis of amine-terminated CLIO: CLIO-NH₂, (2) N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) activation of CLIO-NH₂, (3) N-succinimidyl-S-acetylthioacetate (SATA) activation of IgG, and (4) synthesis of CLIO-IgG.¹⁹ Hetero-bifunctional cross-linking agents containing an amine-reactive group at one end and a disulfide bond with a good leaving group on the other end are used for making conjugates.¹⁹ The leaving group on the disulfide portion of the cross-linker permits efficient interchange with a free sulfhydryl on the antibody.¹⁹ SPDP is used for conjugation.¹⁹ The activated NHS ester end of the SPDP reacts with amine groups in SPIO to form an amide linkage.¹⁹ The 2-pyridyldithiol group at the other end reacts with sulfhydryl groups in the Ab to form a disulfide linkage.¹⁹ Ab IgG is activated with SATA, a thiolation reagent.¹⁹ The compound reacts with primary amines via its NHS ester end to form a stable amide linkage.¹⁹ The acetylated sulfhydryl group is stable until deacetylated with hydroxylamine.¹⁹ Thus, Ab molecules may be thiolated with SATA to create the sulfhydryl target functional groups necessary to couple the SPDP activated CLIO. The molar ratio of Fe to Ab is 1:26.

MRI experiments

In vitro mMRI was used to assess the change in T₂ relaxation with varying concentrations of CLIO nanoparticles, and the efficiency of the binding of the molecular targeting nanoprobe in rat glioma C6 cells and *in vivo* in a rat C6 glioma model. For determination of T₂ values of the IO nanoprobe in C6 cells, C6 cells with the IO nanoprobe, IO nanoprobe alone, or media alone, a rapid acquisition with relaxation enhancement (RARE) and variable repetition time (TR) method was used with the following parameters: TR = 3000 ms, 8 echoes (echo time [TE] = 15, 30, 45, 60, 75, 90, 105, 120 ms), number of averages = 2, matrix = 256 × 256, slice thickness = 1 mm, and estimated total scan time = 25 min. T₂ relaxations were calculated from a series of T₂-weighted images using a nonlinear two-parameter fitting procedure (Bruker Paravision software). The *in vitro* imaging of C6 cells (with or without IO nanoprobe) was used to establish if the IO nanoprobe binds specifically to the C6 cells.

In vivo MR experiments were carried out under general anesthesia (1–2% Isoflurane, 0.8–1.0 L/min O₂). MR equipment used was a Bruker Biospec 7.0 Tesla/30 cm horizontal-bore imaging spectrometer (Bruker BioSpin MRI GmbH, Ettlingen, Germany). Animals were imaged at 7–10 days after the cells were injected and then every 2–3 days until the desired volume (100–150 mm³) of the tumor. Anesthetized (2% Isoflurane) restrained rats were placed in an MR probe, and their brains localized by MRI. Images were obtained using a Bruker S116 gradient coil (maximum 2.0 mT/m/A), a 72 mm quadrature multi-rung RF coil for RF transmission, and a rat head coil for RF signal receiving. MRI was performed for the purpose of determining the volumes (mm³) and growth rates (tumor doubling times, with an average of 2.6 days²⁰) of each tumor for the C6 gliomas. Multiple ¹H-MR image slices were taken in the transverse (axial) or coronal (horizontal) planes using a RARE sequence with the following parameters: TR 3.0 ms, TE 60 ms to obtain T₂-weighted images, 128 × 128 matrix, four steps per acquisition (averages), 4 × 5 cm² field of view (FOV) (axial) or 6 × 5 cm² FOV (horizontal), 1 mm slice thickness, and 15 slices.²⁰ MR angiography images were obtained as previously described.²⁰

Rat brains were imaged at 0 (precontrast), 20, 40, 60, 120, and 180 min intervals postcontrast agent injection. Rats were injected intravenously with anti-c-Met, anti-VEGF-R2 antibodies (both rabbit anti-rat), or normal rat IgG, tagged with an IO-based contrast agent (CLIO-based) (200 μ L/200 g rat; 1 mg antibody/kg; 0.05 mmol Fe⁺³/kg). Multiple ¹H-MR image slices were taken in the transverse plane using a spin echo multislice (SEMS; TR 0.8 s, TE 23 ms, 128 × 128 matrix, four steps per acquisition, 4 × 5 cm² FOV, 1 mm slice thickness). For determination of T₂ values of the IO nanoprobe in rat bearing C6 gliomas, a RARE and variable TR method was used with the following parameters: TR = 3000 ms, 8 echoes (TE = 15, 30, 45, 60, 75, 90, 105, 120 ms), number of averages = 2, matrix = 256 × 256, slice thickness = 1 mm, and estimated total scan time = 25 min. T₂ maps were generated from the multi-echo data sets (Bruker Paravision software).

For both T₂-weighted images and T₂ maps, the same thresholding method was used. Intensities were measured in regions of interest (ROIs) taken in the background and in the normal tissue to ensure that the gray levels were identical

FIG. 1. (A) T_2 -weighted MR images (RARE, TR = 3.0 ms, TE = 60 ms) of a C6 glioma-bearing rat at 19 days after cell implantation. (B) Western blot levels of c-Met (140 kDa) indicate that there are higher levels in C6 glioma tumor (T) tissue compared with normal (N) tissue. β -Actin (43 kDa) levels are shown as a sample loading reference. MR, magnetic resonance; RARE, rapid acquisition with relaxation enhancement and variable repetition time; TR, repetition time; TE, echo time.

between pre- and postcontrast images. Difference images were then obtained by straightforward intensity difference between pre- and postcontrast images, on a pixel basis. The intensity range of each difference image was determined, and the intensity threshold taken as 65% of the maximum intensity observed for each image. Pixels above this threshold and within the brain were selected and represented as red pixels.

Brain extraction

Cardiac perfusion with phosphate-buffered saline (PBS) was performed while the rats were under anesthesia (Isoflurane). For brain tissue the heads were cut off using a guillotine. The skin and muscle were removed off the head. The bones (top of head) were removed, from the cerebellum to the olfactory bulb through the bregma. The ear bones were extracted from the brain; the optic chiasm and the olfactory bulb were excised to extract the brain. Transverse cuts were then performed to get samples for histology and Western blot analyses in the glioma and contralateral brain regions. Samples for Western blots were frozen in liquid nitrogen. For histology, brain tissue samples were fixed in Z-fixative (Zinc Formalin: formaldehyde 3.7%, zinc sulfate). The tissues were then washed with PBS and incubated with 15% sucrose before embedding in optimal cutting temperature compound and frozen in liquid nitrogen.

Western blot analysis of c-Met

The contralateral side of the brain was used as a control. Frozen tissue was weighed, sliced into thin pieces, and then thawed in lysis buffer containing proteases and phosphatase inhibitors. Tissues were disrupted and homogenized at 4°C, incubated on ice for 30 min, and centrifuged (10,000 g, 10 min, 4°C), and the supernatant containing the total cell lysate was collected. After determining protein concentrations, lysates were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Bio-Rad, Emeryville, CA), and transferred to nitrocellulose membranes. Western analysis was performed using an Ab against c-Met. Secondary Abs were labeled with horseradish peroxidase. The ECL Advance Western Blotting Detection Kit (Amersham Bioscience, Piscataway, NJ) was used to detect immunoreactive proteins. NIH Image J (NIH, Bethesda, MD) was used to calculate density regions in Western blot bands.

Prussian blue staining for IO nanoparticles

The IO-based nanoprobe in tissue cryosections were detected using Prussian blue staining, which involves the

treatment of sections with acid solutions of ferrocyanides. The ferric ion (+3) present in the IO-based nanoprobe from tissue sections combines with the ferrocyanide and results in the formation of ferric ferrocyanide, visible as a blue pigment in bright field imaging.

Statistical analyses

A significant decrease in T_2 relaxation indicated specific binding of the nanoprobe in glioma tissue. Statistical differences between the anti-c-Met probe and IgG contrast

FIG. 2. (A) T_2 -weighted MR images (RARE, TR = 3.0 ms, TE = 60 ms) and T_2 values of vials containing water, cell culture media and agarose, C6 cells in media, cross-linked iron oxide (CLIO) nanoparticles (no cells and no antibody), or C6 cells with the CLIO-anti-c-Met (antibody) nanoprobe. (B) T_2 -weighted MR images (RARE, TR = 3.0 ms, TE = 60 ms) and T_2 values of vials containing CLIO nanoparticles in PBS (phosphorylated saline, pH 7.4) at varying concentrations (vials 1–4: 3.3–0.4 μ g/ μ L, respectively), compared to PBS containing no CLIO nanoparticles (vial 5). For vial 1, a light outline has been added to show the vial boundary. PBS, phosphate-buffered saline.

FIG. 3. T₂-weighted MR images (RARE, TR = 3.0 ms, TE = 60 ms) at (a) preadministration and (b) 3 h (180 min) postadministration of the anti-c-Met probe in a cross-sectional image orientation and corresponding T₂ maps (d and e, respectively). Note a decreased T₂ in (e) (yellow circled region). (c) A signal intensity (SI) difference image [preadministration minus 3 h postadministration; (b) and (a)] depicts high SI areas (in red) resulting from the uptake of the anti-c-Met probe in the tumor (white oval region), compared to the contralateral brain tissue (showing no high SI regions). (f) A T₂ map difference image [preadministration minus 3 h postadministration; (e) and (d)] depicts decreased T₂ values (in red) within the tumor (white oval region).

T₂-weighted images at (g) preadministration and (h) 3 h postadministration of the anti-c-Met probe in a horizontal image orientation with a corresponding difference image (i) and changes in signal intensity (in red) within the tumor (white oval region). (j) Percentage changes in T₂ and SI decreases in tumor and contralateral brain tissue regions depicted in the difference images in (f) and (c), respectively, 3 h after administration of the anti-c-Met probe. Comparatively, percentage changes in T₂ and SI decreases in tumor and contralateral brain tissue regions from rats administered with an IgG-SPIO contrast agent are also shown. Both T₂ and SI are found to decrease significantly (*, $p < 0.05$) in tumor regions of the anti-c-Met-administered rats when compared to contralateral tissue values of the same animals or within tumor or contralateral brain tissues of IgG-SPIO controls (mean $\pm$ standard deviation; $n = 2$ with 3 ROIs taken per animal in tumor and normal brain tissues).

(k) Difference MR image (3 h postadministration minus preadministration) of a C6 glioma-bearing rat administered with an IgG-SPIO contrast agent. Note no selective uptake of the nonspecific IgG-SPIO contrast agent in either tumor (white oval region) or contralateral brain tissues. (l) Relative changes in T₂ relaxation (ms) over the course of 3 h in tumor and normal brain regions of rats administered with either the anti-c-Met probe or the IgG-SPIO contrast agent. The relative change in T₂ relaxation (mean $\pm$ standard deviation) is significantly altered (*, $p < 0.05$) in tumor regions of the anti-c-Met probe-administered rats compared to contralateral brain regions of the same animals or in either tumor or contralateral brain tissues of the IgG-SPIO controls. (m) Difference SI MR angiography image obtained between preadministration and 3 h postadministration of the anti-c-Met probe, depicting high SI regions of the vasculature associated with the tumor (white oval region) due to the presence of the anti-c-Met probe. The MRA is obtained in the rectangular outlined region of the rat brain in the image shown in (h). Color images available online at www.liebertonline.com/ten.

agent control group, and between tumor and nontumor regions, was analyzed with an unpaired, two-tailed Student's *t*-test by using commercially available software (InStat; GraphPad Software, San Diego, CA). A p -value < 0.05 was considered to indicate a statistically significant difference.

Results

Figure 1A is a representative MR image of a C6 rat glioma at 19 days postcell implantation. Western blot levels of c-Met in glioma tissue (T = tumor) compared to normal (N) brain

FIG. 4. Prussian blue staining of iron oxide nanoparticles in the contralateral or tumor regions of C6 glioma-bearing rats administered with either the anti-c-Met probe or the IgG-SPIO contrast agent (2 h postadministration). (a) Prussian-blue-stained histological slice obtained from the contralateral brain region of an anti-c-Met probe-administered rat [region depicted in T_2 -weighted MRI image (b)]. (c) Prussian-blue-stained histological slice from tumor region of an anti-c-Met probe-administered rat. (d) Prussian-blue-stained histological slice from the contralateral region of an IgG-SPIO contrast agent-administered rat [region depicted in T_2 -weighted MRI image (e)]. (f) Prussian-blue-stained histological slice from tumor region of an IgG-SPIO-administered rat. SPIO, superparamagnetic iron oxide. Color images available online at www.liebertonline.com/ten.

tissue are shown in Figure 1B, with higher levels of c-Met in glioma tissue compared to normal tissue. Immunofluorescence levels for c-Met in a C6 glioma were previously found to be elevated in tumor tissue compared to normal brain tissue.²¹

T_2 relaxation values of an anti-c-Met IO nanoprobe (CLIO-anti-c-Met) with C6 rat glioma cells is shown in Figure 2A. T_2 relaxation dramatically decreases in vials (compared with controls: water alone, culture media alone, or C6 cells in media) with the CLIO nanoparticles alone or in the presence of the C6 cells with an anti-c-Met Ab coupled to the nanoparticles. This indicates that changes in T_2 values can be used to detect the presence of the CLIO nano-probes. T_2 relaxation values as a function of CLIO nanoparticle concentration ($\mu\text{g}/\mu\text{L}$) are shown in Figure 2B, resulting in a decrease in T_2 relaxation as concentrations increase.

Figure 3 depicts pre- and post-anti-c-Met probe administration (3 h), and difference images with high signal intensities (in red), in cross-sectional (c) and horizontal (i) MR image orientations. Figure panels 3d and e are corresponding cross-sectional T_2 maps at preadministration and 3 h postadministration of the anti-c-Met probe, respectively. The highlighted regions in the MR SI (c) and T_2 map (f) difference images depict a common area of decreases in SI and T_2 , respectively. Regions in red in the difference images (Fig. 3c, i) highlight regions where the anti-c-Met targeting agent remained after 3 h. The T_2 map difference image also depicts regions (in red), some of which correlate with the signal intensity changes shown in Figure 3c. Figure 3j illustrates percentage changes in MR signal intensity and T_2 relaxation times within tumor and contralateral tissues in rat brains with a C6 glioma, after administration of the anti-c-Met probe or an IgG-SPIO contrast agent. Both signal intensity and T_2 relaxation decreases in the glioma as the anti-c-Met probe stays within the tumor of rats administered with the anti-c-Met probe, but not the IgG-SPIO contrast agent. In comparison the anti-c-Met probe does not get taken up very much in contralateral tissue. The most highlighted regions in the horizontal difference

image (i) seem to be in the vicinity of vascular structures or blood vessels. Figure 3l shows the relative change in T_2 relaxation in tumor and contralateral brain regions over the course of 3 h, with only a significant change ($p < 0.05$) in T_2 occurring in the tumors of anti-c-Met probe-administered rats, compared to little change in contralateral brain tissue, or the tumor or contralateral brain tissues of the IgG-SPIO controls. Figure 3m is a difference MR angiogram obtained between preadministration and 3 h postadministration of the anti-c-Met probe indicating highlighted bright regions in the vasculature associated with the tumor.

With the use of Prussian blue stain (Fig. 4), which is specific for IO particles, there was mainly staining only in the tumor region of the C6 glioma-bearing rat (Fig. 4b) administered with the anti-c-Met probe (Fig. 4c), and very little staining either in the contralateral side of the same rat (Fig. 4a) or within tumor (Fig. 4f) or contralateral (Fig. 4d) brain tissues in a glioma-bearing rat administered with the non-specific IgG-SPIO contrast agent.

Discussion

The study of tissue regeneration with high-resolution imaging is somewhat constrained by the lack of suitable probes coupled to a noninvasive imaging modality that can detect markers for these processes in living animals.

Our laboratory has experience in using mMRI to assess molecular markers associated with primary brain tumor (e.g., glioma) growth.²¹ Experimental rodent glioma models, such as the C6 model, have been widely used.^{11,22,23} We have used molecular-targeted MRI to observe the *in vivo* expression of c-Met that is overexpressed in hepatic tumors²⁴ and gliomas²¹ in experimental rodent models.

Magnetic IO-based nanocrystals are ideal as magnetic probes for MRI as a result of their signal-enhancing capabilities.²⁵ SPIO typically consists of two components, an IO core and a hydrophilic coating. We used a dextran hydrophilic coating in our

study. Studies have shown that polymer-coated nanoparticles, such as the dextran-coated nanoparticles, have minimal impact on cell viability and function, and low toxicity.²⁶ Other researchers have used SPIO for detecting inflammatory diseases via the accumulation of nontargeted SPIO in infiltrating macrophages²⁶ to specifically identify cell surface markers of cancer (e.g., transferrin,^{27,28} folate receptor,²⁹ underglycosylated MUC-1,³⁰ matrix metalloproteinase-2,³¹ hepsin,³² and Her-2/neu^{33,34})^{26,35} and cardiovascular disease (e.g., vascular adhesion molecule-1³⁶ and E-selectin³⁷).^{26,35,38}

c-Met, a tyrosine kinase receptor, and its ligand, the HGF (SF), are critical in cellular proliferation, motility, and invasion and are known to be overexpressed in gliomas.^{39–42} c-Met is crucially involved in invasive cell growth and motility during embryogenesis, and has been detected in invasive tumors, including gliomas, meningiomas, colorectal cancer, breast carcinomas, gastric carcinomas, and hepatocellular carcinomas (HCCs).^{39–48} In this study *in vivo* c-Met levels were found to significantly increase ($p < 0.05$, compared to contralateral brain tissue) in glioma tumors, with the use of the anti-c-Met probe and mMRI (Fig. 3), compared to a nonspecific IgG-SPIO control. Prussian blue staining was used to confirm the presence of the anti-c-Met IO-based probe only in the tumor region.

Of importance to tissue engineering, Met has been found to be associated with tissue regeneration, and high levels indicate increased cell growth¹ in the liver,^{5,6} pancreas,⁷ and mesenchymal stem cells.⁸ Therefore, mMRI in conjunction with an anti-Met nanoprobe can be used to monitor Met levels in tissue grafts or in stem cell transplantations to follow successful tissue growth.

This is the first attempt at detecting *in vivo* expression of Met using IO-based nanoprobe in conjunction with mMRI. Our mMRI data provide compelling evidence that this technique can be used to detect Met levels *in vivo* in gliomas, and that this method can be extended to tissue engineering applications.

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Disclosure Statement

No competing financial interests exist.

References

- Holmes, O., Pillozzi, S., Deakin, J.A., Carafoli, F., Kemp, L., Butler, P.J., Lyon, M., and Gherardi, E. Insights into the structure/function of hepatocyte growth factor/scatter factor from studies with individual domains. *J Mol Biol* **367**, 395, 2007.
- Bladt, F., Riethmacher, D., Isenmann, S., Aguzzi, A., and Birchmeier, C. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* **376**, 768, 1995.
- Caton, A., Hacker, A., Naeem, A., Livet, J., Maina, F., Bladt, F., Klein, R., Birchmeier, C., and Guthrie, S. The branchial arches and HGF are growth-promoting and chemoattractant for cranial motor axons. *Development* **127**, 1751, 2000.

- Schmidt, C., Bladt, F., Goedecke, S., Brinkmann, V., Zschiesche, W., Sharpe, M., Gherardi, E., and Birchmeier, C. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* **373**, 699, 1995.
- Borowiak, M., Garratt, A.N., Wüstefeld, T., Strehle, M., Trautwein, C., and Birchmeier, C. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci USA* **101**, 10608, 2004.
- Huh, C.G., Factor, V.M., Sánchez, A., Uchida, K., Conner, E.A., and Thorgeirsson, S.S. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci USA* **101**, 4477, 2004.
- Dai, C., Huh, C.G., Thorgeirsson, S.S., and Liu, Y. Beta-cell-specific ablation of the hepatocyte growth factor receptor results in reduced islet size, impaired insulin secretion, and glucose intolerance. *Am J Pathol* **167**, 429, 2005.
- Neuss, S., Becher, E., Wöltje, M., Tietze, L., and Jähnen-Dechent, W. Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing. *Stem Cells* **22**, 405, 2004.
- Schmidt, K.F., Ziu, M., Schmidt, N.O., Vaghiasa, P., Cargioloi, T.G., Doshi, S., Albert, M.S., Black, P.M., Carroll, R.S., and Sun, Y. Volume reconstruction techniques improve the correlation between histological and *in vivo* tumor volume measurements in mouse models of human gliomas. *J Neurooncol* **68**, 207, 2004.
- Badruddoja, M.A., Krouwer, H.G., Rand, S.D., Rebro, K.J., Pathak, A.P., and Schmainda, K.M. Antiangiogenic effects of dexamethasone in 9L gliosarcoma assessed by MRI cerebral blood volume maps. *Neurooncology* **5**, 235, 2003.
- Zhang, X., Wu, J., Gao, D., Fei, Z., Qu, Y., and Jing, J. Development of a rat C6 brain tumor model. *Chin Med J* **115**, 455, 2002.
- Zhou, R., Mazurchuk, R., and Straubinger, R.M. Antivascular effects of doxorubicin-containing liposomes in an intracranial rat brain tumor model. *Cancer Res* **62**, 2561, 2002.
- Shen, T., Weissleder, R., Papisov, M., Bogdanov, A., Jr., and Brady, T.J. Monocrystalline iron oxide nanocompounds (MION): physicochemical properties. *Magn Reson Med* **29**, 599, 1993.
- McCarthy, J.R., Kelly, K.A., Sun, E.Y., and Weissleder, R. Targeted delivery of multifunctional magnetic nanoparticles. *Nanomedicine* **2**, 153, 2007.
- Bulte, J.W., Douglas, T., Witwer, B., Zhang, S.C., Strable, E., Lewis, B.K., Zywicke, H., Miller, B., van Gelderen, P., Moskowitz, B.M., Duncan, I.D., and Frank, J.A. Magnetodendrimers allow endosomal magnetic labeling and *in vivo* tracking of stem cells. *Nat Biotechnol* **19**, 1141, 2001.
- Trusolino, L., and Comoglio, P.M. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. *Nat Rev* **2**, 289, 2002.
- Josephson, L., Tung, C.-H., Moore, A., and Weissleder, R. High-efficiency intracellular magnetic labeling with novel superparamagnetic-Tat peptide conjugates. *Bioconjug Chem* **10**, 186, 1999.
- Palmacc, S., and Josephson, L. Synthesis of polysaccharide covered superparamagnetic oxide colloids. U.S. Patent 5,262,176, 1993.
- Hermanson, G. *Bioconjugate Techniques*. New York: Academic Press, 1996, pp. 456–493, 494–527.
- Doblas, S., Saunders, D., Tesiram, Y.A., Kshirsagar, P., Pye, Q., Oblander, J., Gordon, B., Kosanke, S., Floyd, R.A., and Towner, R.A. Phenyl-tert-butyl nitron induces tumor

- regression and decreases angiogenesis in a C6 rat glioma model. *Free Radic Biol Med* **44**, 63, 2008.
21. Towner, R.A., Smith, N., Doblas, S., Tesiram, Y., Saunders, D., Cranford, R., Silasi-Mansat, R., Herlea, O., and Lupu, F. *In vivo* detection of c-Met expression in a rat C6 glioma model. *J Cell Mol Med* **12**, 174, 2008.
 22. Barth, R.F. Rat brain tumor models in experimental neuro-oncology: the 9L, C6, T9, F98, RG2 (D74), RT-2 and CNS-1 gliomas. *J Neurooncol* **36**, 91, 1998.
 23. Grobben, B., De Deyn, P.P., and Slegers, H. Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion. *Cell Tissue Res* **310**, 257, 2002.
 24. Towner, R.A., Smith, N., Tesiram, Y.A., Abbott, A., Saunders, D., Blindauer, R., Herlea, O., Silasi-Mansat, R., and Lupu, F. *In vivo* detection of c-Met expression in a rat hepatocarcinogenesis model using molecularly targeted magnetic resonance imaging. *Mol Imaging* **6**, 18, 2007.
 25. Huh, Y.-M., Jun, Y.-W., Song, H.-T., Kim, S., Choi, J.-S., Lee, J.-H., Yoon, S., Kim, K.-S., Shin, J.-S., Suh, J.-S., and Cheon, J. *In vivo* magnetic resonance detection of cancer by using multifunctional magnetic nanocrystals. *J Am Chem Soc* **127**, 12387, 2005.
 26. Thorek, D.L.J., Chen, A.K., Czupryna, J., and Tsourkas, A. Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Ann Biomed Eng* **34**, 23, 2006.
 27. Kresse, M., Wagner, S., Pfeifferer, D., Lawaczek, R., Elste, V., and Semmler, W. Targeting of ultrasmall superparamagnetic iron oxide (USPIO) particles to tumor cells *in vivo* by using transferring receptor pathways. *Magn Reson Med* **40**, 236, 1998.
 28. Ichikawa, T., Hogemann, D., Saeki, Y., Tyminski, E., Terada, K., Weissleder, R., Chiocca, E.A., and Basilion, J.P. MRI of transgene expression: correlation to therapeutic gene expression. *Neoplasia* **4**, 523, 2002.
 29. Choi, H., Choi, S.R., Zhou, R., Kung, H.F., and Chen, I.W. Iron oxide nanoparticles as magnetic resonance contrast agent for tumor imaging via folate receptor-targeted delivery. *Acad Radiol* **11**, 996, 2004.
 30. Moore, A., Medarova, Z., Potthast, A., and Dai, G. *In vivo* targeting of underglycosylated MUC-1 tumor antigen using a multimodal imaging probe. *Cancer Res* **64**, 1821, 2004.
 31. Kachra, Z., Beaulieu, E., Delbecchi, L., Mousseau, N., Berthelet, F., Moudmjan, R., Del Maestr, R., and Beliveau, R. Expression of matrix metalloproteinases and their inhibitors in human brain tumors. *Clin Exp Metastasis* **17**, 555, 1999.
 32. Kelly, K.A., Setlur, S.R., Ross, R., Anbazhagan, R., Waterman, P., Rubin, M.A., and Weissleder, R. Detection of early prostate cancer using a hepsin targeted imaging agent. *Cancer Res* **68**, 2286, 2008.
 33. Artemov, D., Mori, N., Okollie, B., and Bhujwala, Z.M. MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. *Magn Reson Med* **49**, 403, 2003.
 34. Funovics, M.A., Kapeller, B., Hoeller, C., Su, H.S., Kunstfeld, R., Puig, S., and Macfelda, K. MR imaging of the her2/neu and 9.2.27 tumor antigens using immunospecific contrast agents. *Magn Reson Imaging* **22**, 843, 2004.
 35. McCarthy, J.R., and Weissleder, R. Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Adv Drug Deliv Rev* **60**, 1241, 2009.
 36. Tsourkas, A., Shinde-Patil, V.R., Kelly, K.A., Patel, P., Wolley, A., Allport, J.R., and Weissleder, R. *In vivo* imaging of activated endothelium using an anti-VCAM-1 magneto-optical probe. *Bioconjug Chem* **16**, 576, 2005.
 37. Kang, H.W., Josephson, L., Petrovsky, A., Weissleder, R., and Bogdanov, A., Jr. Magnetic resonance imaging of inducible E-selectin expression in human endothelial cell culture. *Bioconjug Chem* **13**, 122, 2002.
 38. Sosnovik, D.E., Nahrendorf, M., and Weissleder, R. Magnetic nanoparticles for MR imaging: agents, techniques and cardiovascular applications. *Basic Res Cardiol* **103**, 122, 2008.
 39. Arrieta, O., Garcia, E., Guevara, P., Garcia-Navarrete, R., Ondarza, R., Rembao, D., and Sotelo, J. Hepatocyte growth factor is associated with poor prognosis of malignant gliomas and is a predictor for recurrence of meningiomas. *Cancer* **94**, 3210, 2002.
 40. Koochekpour, S., Jeffers, M., Rulong, S., Taylor, G., Klineberg, E., Hudson, E.A., Resau, J.H., and Vande Woude, G.F. Met and hepatocyte growth factor/scatter factor expression in human gliomas. *Cancer Res* **57**, 5391, 1997.
 41. Lamszus, K., Liang, J., Larterra, J., Zagzag, D., Way, D., Witte, M., Goldberg, I.D., and Rosen, E.M. Scatter factor promotes motility of human glioma and neuromicrovascular endothelial cells. *Int J Cancer* **75**, 19, 1998.
 42. Moriyama, T., Kataoka, H., Kawano, Y., Yokogami, K., Nakano, S., Goya, T., Uchino, H., Kono, M., and Wakasaka, S. Comparative analysis of expression of hepatocyte growth factor and its receptor, c-met, in gliomas, meningiomas and schwannomas. *Cancer Lett* **124**, 149, 1998.
 43. Amicone, L., Terradillos, O., Calvo, L., Costabile, B., Cichini, C., Della Rocca, C., Lozupone, F., Piacentini, M., Buendia, M.A., and Tripodi, M. Synergy between truncated c-Met (cyto-Met) and c-Myc in liver oncogenesis: importance of TGF-beta signaling in the control of liver homeostasis and transformation. *Oncogene* **21**, 1335, 2002.
 44. DiRenzo, M.F., Narshimhan, R.P., Olivero, M., Bretti, S., Giordano, S., Medico, E., Gaglia, P., Zara, P., and Comoglio, P.M. Expression of the Met/HGF receptor in normal and neoplastic tissues. *Oncogene* **6**, 1997, 1991.
 45. Jiang, Y., Xu, W., Lu, J., He, F., and Yang, X. Invasiveness of hepatocellular carcinoma cell lines: contribution of hepatocyte growth factor, c-met, and transcription factor Ets-1. *Biochem Biophys Res Commun* **286**, 1123, 2001.
 46. Yamashita, J., Ogawa, M., Yamashita, S., Nomura, K., Kuramoto, M., Saishoji, T., and Shin, S. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. *Cancer Res* **54**, 1630, 1994.
 47. Yu, Y., and Merlino, G. Constitutive c-MET signaling through a nonautocrine mechanism promotes metastasis in a transgenic transplantation model. *Cancer Res* **62**, 2951, 2002.
 48. Zeng, Z., Weiser, M.R., D'Alessio, M., Grace, A., Shia, J., and Paty, P.B. Immunoblot analysis of c-Met expression in human colorectal cancer: overexpression is associated with advanced stage cancer. *Clin Exp Metastasis* **21**, 409, 2004.

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