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Orthotopic Gastric Cancer Targetable Magnetic Nano Complex for T2 Turbo spin echo(TSE) MR Imaging

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Abstract - Novel diagnostic technique has been developed in many research area using targetable contrast agents with magnetic resonance imaging (MRI) for cancer diagnosis. It is efficient for cancer diagnosis to use MRI with biocompatible targeting moiety and magnetic nanoparticles (MNPs). Thus, we synthesized MNPs using thermal decomposition method which enable sensitive T2- or T2 Turbo spin echo (TSE) weighted magnetic resonance imaging. And it was coated with Hyaluronic acid (HA). Also we carried out that gastric cancer cell line (MKN45) which has cancer stem cell property was injected in heterotopic mouse model. And then magnetic resonance sequence (T2) for imaging effects and targeting ability were analyzed into MNPs conjugated HA. We noted that MDA-MB-231 cell which high-expressed CD44 ligand was showed contrast enhance efficiency through magnetic nanoparticles because of combining a lot of HA. As a result of these studies, we conclude that HA coated magnetic nanoparticles can be effectively used as a novel probe for visualizing of Gastric cancer stem cell.

Key word**:** *Gastric cancer, **C**ontrast agent, CD44, MRI(Magnetic Resonance Imaging), MNPs(Magnetic nano-particles)*

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1. Introduction

Molecular imaging provides as a tool to diagnose cancer at the cellular and molecular levels. It not only allows early and accurate tumor localization in diagnostic cancer imaging, but also has a potential to visualize the biological processes of tumor growth, metastasis and response to treatment[1-10]. Molecular MR imaging (Magnetic resonance imaging) has emerged as a key factor for the diagnosis of cancer[11-18]. Since it has advantages over noninvasive, good anatomical image due to high resolution, high contrast and 3-dimensional information in real time more than nuclear medicine (PET, SPECT), optical imaging compared to other imaging modality[19-23]. And also, molecular MR imaging is able to detect simultaneously metabolism of cells and tissues and

its physiological information and structural information, noninvasive and biological processes occurring in the deep tissues to provide the quantitative information[24-25]. Molecular MR imaging can observe a variety of imaging lesions as multi-modality in the diagnosis of gastric cancer. Many MR contrast agents have been used for good quality imaging[32-37]. However passive contrast agents are not enough to reach their target goals specifically. Thus, we are aiming to develop intelligent targetable contrast agent using Hyaluronic acid (HA)[38-44]. In particular, HA has become known to interact on CD44 receptor. Then gastric cancer is known to being overexpressed CD44 receptor which as marker of cancer stem cell[45-50]. It is so crucial for MR probe to early gastric cancer diagnostic point of view. Because CD44, important as cancer stem cell marker, is interacted with Hyaluronic acid. Hyaluronic acid which is a linear hydrogel with negative charge containing two alternating units of D-glucuronic acid (GLcUA) and N-acetyl-D-glucosamine (GLcNAc) with molecular weight of 105–107. HA has frequently been used for medical purposes such as a viscoelastic biomaterial in surgery. Especially, it is well known that various human tumor cells (gastric, ovarian, colon, lung, stomach, etc.) over-express HA-binding receptors, CD44[51-56]. In this study, molecular MR imaging were investigated to find biological processes which occur in gastric cancer. T2 weight sequence was simultaneously used to confirm for better diagnostic possibility and targeting effect was demonstrated through Hyaluronic acid conjugated magnetic nanoparticles in heterotopic xenograft gastric cancer model. And various experiments were conduct to evaluate specific binding affinity and diagnostic effectiveness through in vivo and in vitro.

2. EXPERIMENTAL METHOD

2.1. Materials

Polysorbate 80, ethylenediamine, 1,4-dioxane (99.8%), 4-dimethylaminopyridine, triethylamine, and succinic anhydride (SA) were purchased from Sigma Aldrich Chemical Co. Phosphate buffered saline (PBS: 10 mM, pH 7.4), Roswell Park Memorial Institute-1640 (RPMI-1640), fetal bovine serum and antibiotic-antimycotic solution were purchased from Gibco and dialysis membrane (molecular weight cut off: 1,000 Da) was obtained from Spectrum laboratory. Hyaluronic acid (HA, 1,000,000 Da) was purchased from Yuhan Phrmaceutical Corporation (Seoul, Korea). MDA-MB-231 cell lines (American Type Culture Collection) were grown in medium containing 5% fetal bovine serum and 1% Antibiotic-Antimycotic at 37°C, humidified 5% CO_2 atmosphere. gastric cancer stem cells (MDA-MB-231) were purchased from American Tissue Type Culture. Ultrapure deionized water was used for all of the syntheses.

2.2. Synthesis of magnetic nanoparticles

To synthesize monodispersed magnetic nanoparticles (MNPs), 2 mmol of iron (III) acetylacetonate, 1 mmol of manganese(II) acetylacetonate, 10 mmol of 1,2-hexadecanediol, 6 mmol of dodecanoic acid, and 6 mmol of dodecylamine were dissolved in 20 mL of benzyl ether under an ambient nitrogen atmosphere. The mixture was then pre-heated to 200°C for 2 hours and refluxed at 300°C for 30 minutes. After the reactants were cooled at room temperature, the products were purified with an excess of pure ethanol. Approximately 11 nm of MNPs were synthesized using the seed-mediated growth method.

2.3. Preparation of hyaluronan-modified magnetic nanoparticles (HA-MNPs)

HA-MNPs were prepared by the nano-emulsion method. 30 mg of magnetic nanoparticle were dissolved in 4 mL hexane (organic phase). This organic phase was poured into 20 mL deionized water (aqueous phase) containing 30 mg HA. The solution was ultra-sonicated in an ice-cooled bath for 20 min at 200 W and stirred overnight at room temperature to evaporate the organic solvent. The resulting suspension was centrifuged three times for 20 min each at 3000 rpm. After the supernatant was removed, the precipitated HA-MNPs were re-dispersed in 5 Ml deionized water. The size distribution and zeta potential of HA-MNPs were analyzed by laser scattering (ELS-Z, Otsuka Electronics); morphologies were confirmed using a transmittance electron microscope (TEM, JEM-2100, JEOL Ltd. Japan.). Finally, the relaxivity (R2) data of HA-MNPs solution were measured through magnetic resonance (MR) imaging analysis.

2.4. Biocompatibility tests for HA-MNPs

The cytotoxic effect of HA-MNPs against MDA-MB-231 cells (gastric cancer cell line) was evaluated by measuring the inhibition of cell growth using the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MDA-MB-231 cells were maintained in RPMI containing 5% fetal bovine serum (FBS) and 1% antibiotics at 37 ˚C in a humidified atmosphere with 5% CO^2. MDA-MB-231 cells (1.0 x 10^4 cells/well) were implanted in a 96-well plate at 37 ˚C overnight and the cells were treated with various concentrations of HA-MNPs for 4 hours. The MTT assay was then performed, in which yellow tetrazolium salt was reduced to purple formazan crystals in metabolically active cells. The relative percentage of cell viability was determined as the ratio of formazan intensity in viable cells treated with HA-MNPs to the intensity in non-treated (control) cells. Cell viability was normalized to non-treated cells (which were considered as having 100% cell viability).

2.5. Heterotopic animal model and experimental procedure

All animal experiments were conducted with the approval of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Female BALB/C-Slc nude mice at 6 weeks of age were anesthetized by intraperitoneal injection of a Zoletil/Rompun mixture and injected with 200 mL containing 1.0 x 10^7 MDA-MB-231 cells suspended in saline into the femoral region. After cancer cell implantation, MR imaging was performed between 4 and 5 weeks. After MR imaging organ MR imaging performed too.

2.6. MR imaging

Animal, solution and MR imaging experiments were performed with a 3 Tesla Siemens clinical MRI instrument using a wrist coil with T2 weight sequence. (TR: 14.85 ms, TE : 5.65 ms ,Slice thickness : 1.0mm, FOV read : 100mm)

3. RESULT AND DISCUSSION

3.1. Preparation of MNPs and HA-MNPs

Monodispersed magnetic nanoparticles were synthesized using thermal decomposition and solubilized in nonpolar organic solvent, as previously reported. And HA conjugated MNP was synthesized using EDC and sulfo-NHS method. As shown in Figure 1, the characteristic band of HA-MNPs conjugates were verified by FT-IR spectra, which

exhibits O-H stretching at 3200-3400 cm^{-1} , C=O stretching at 1100-1300 cm^{-1} , CO-NH(amide) bonds at 1630-1680 cm^{-1} and CH_2 bending in HA at 1430-1470 cm^{-1} .

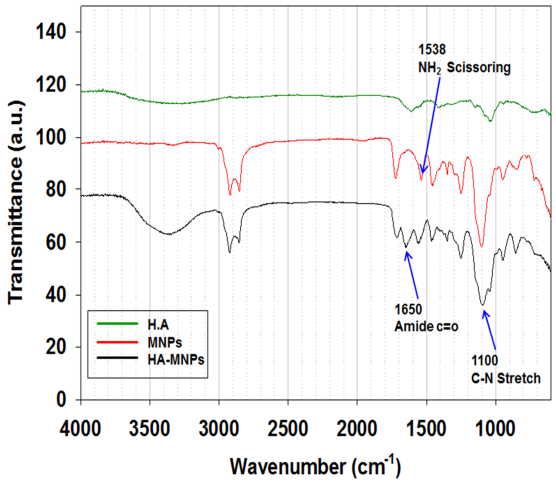

Figure 1. Fourier transform infrared spectra of HA (green line), MNP (red line) and HA-MNP(black line), i:CO-NH(amide) bonds

As MR agents, uniform MNPs (12nm) were synthesized at a high temperature via a thermal decomposition process. The size distribution and morphology of MNPs were confirmed by transmission electron microscopy (Figure 2) which showed no significant differences in size or morphology between HA-MNPs and MNP.

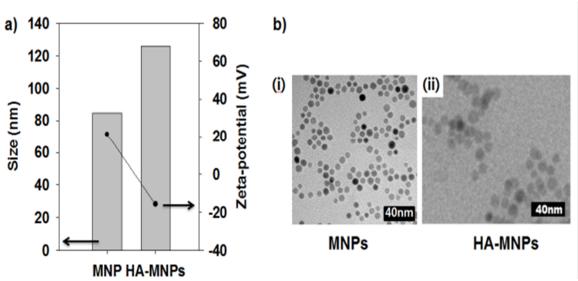

Figure 2. (a) is The average size (gray bar) and zeta potential (black circle) and (b) is TEM images of (i) magnetic nanoparticles (ii) hyaluronan-modified magnetic nanoparticles (HA-MNP)

The size of the water-soluble MNPs and HA-MNPs were determined to be 84.6 ± 32.4 nm and 137 ± 53.2 nm, respectively. After the conjugation of Hyaluronic acid MNPs, the size slightly increased due to the large molecular weight of Hyaluronic acid (1000kDa). In addition, the surface charge of aminated MNPs also changed from 20.62 ± 1.96 mV(aminated water soluble MNP) to -17.76 ± 1.64 mV (HA coated MNP) due to the presence of HA (Figure 1).

3.2. Solubility and magnetic sensitivity of HA-MNPs

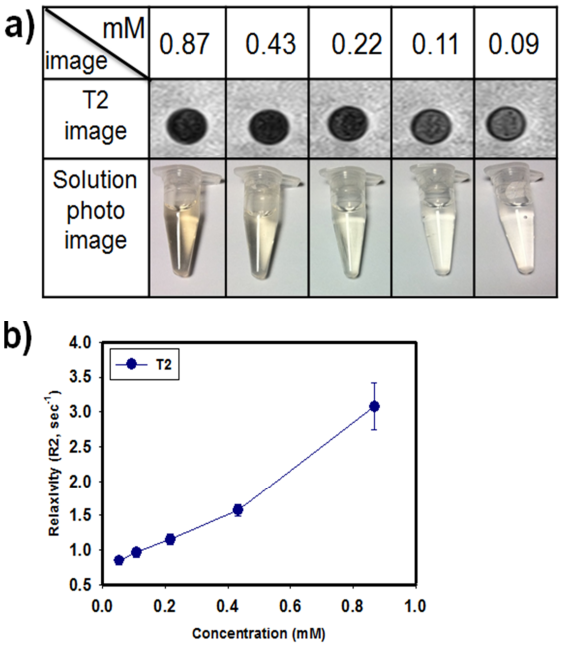

Figure 3. (a) Photographs and T2 solution MR images of HA-MNPs each conditions and (b) R2 relaxivity graph for the magnetic ion concentration.

To assess the potential use of HA-MNPs as MR imaging agents, we performed MR imaging experiments using HA-MNPs, exhibiting the highest magnetic properties with appropriate size to avoid RES detection and prolong retention in the circulation. In Figure 3 (a), the T2-weighted MR image exhibited a strong black color, which signified a decrease in signal intensity for the thicker HA-MNP solution. In Figure 3 (b) represent change of intensity between of T2-weighted MR solution image.

3.3. In vivo MR image

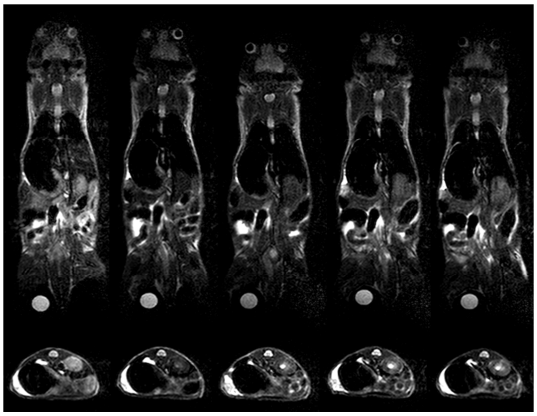

Figure 4. T2 TSE MR images of tumor-bearing mice after intravenous injection of HA-MNPs. FOV: 100mm, ST: 1.00mm, TR: 14.85ms, TE: 5.65ms, coil elements: Wrist coil, Imaging modality: Siemens MR scanner

In Figure 4, MR signal enhancement was identified after HA-MNPs injection. Initially, the center of tumor instantly darkened, and enhanced MR imaging signal intensity at surrounding vessels was simultaneously observed. In T2 weight MR images, clear anatomic details were observed, and there was no artifact due to a difference in susceptibility.

4. CONCLUSION

In summary, we synthesized HA-MNPs as MR imaging agents for effective diagnosis for CD44-overexpressing gastric cancer. HA-MNPs were prepared by the nano-emulsion method. 30 mg of magnetic nanoparticle were dissolved in 4 mL hexane (organic phase). The cytotoxic effect of HA-MNPs against MKN45 cells (gastric cancer cell line) was evaluated by measuring the inhibition of cell growth using the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Female BALB/C-Slc nude mice at 6 weeks of age were anesthetized by intraperitoneal injection of a Zoletil/Rompun mixture and injected with 200 mL containing 1.0 x 107 MDA-MB-231 cells suspended in saline into the femoral region. After cancer cell implantation, MR imaging was performed between 4 and 5 weeks. MR imaging experiments were performed with a 3 Tesla Siemens clinical MRI instrument using a wrist coil with T2 weight sequence. (TR: 14.85 ms, TE : 5.65 ms ,Slice thickness : 1.0mm, FOV read : 100mm)

We noted that MDA-MB-231 cell which high-expressed CD44 ligand was showed contrast enhance efficiency through magnetic nanoparticles because of combining a lot of HA. As a result of these studies, we conclude that HA coated magnetic nanoparticles can be effectively used as a novel probe for visualizing of gastric cancer stem cell.

Abbreviations

MRI: magnetic resonance imaging; MNPs: magnetic nanoparticles; TSE: turbo spin echo; HA: hyaluronic acid

Competing interests

The authors declare that there are no competing interests.

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