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Heat-Inducible Gene Expression System by Applying Alternating Magnetic Field to Magnetic Nanoparticles

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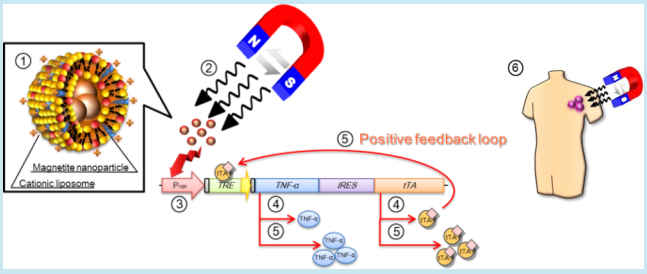
Supporting Information

ABSTRACT: By combining synthetic biology with nanotechnology, we demonstrate remote controlled gene expression using a magnetic field. Magnetite nanoparticles, which generate heat under an alternating magnetic field, have been developed to label cells. Magnetite nanoparticles and heat-induced therapeutic genes were introduced into tumor xenografts. The magnetically triggered gene expression resulted in tumor growth inhibition. This system shows great potential for controlling target gene expression in a space and time selective manner and may be used for remote control of cell functions via gene expression.



KEYWORDS: *inducible gene expression, transcriptional amplification, magnetite nanoparticles, heat shock protein promoter, tetracycline-responsive transactivator*

Gene therapy promises a wide range of applications in medicine, including cancer treatment. Similar to chemotherapy, the therapeutic efficacy depends on the dosage (gene expression level) and timing. Thus, controlled gene expression is essential for gene therapy. Synthetic biology focuses on designing artificial gene expression systems. Numerous gene circuits have been developed to control gene expression.[1,2] Among them, the tetracycline (Tet)-inducible system[3] and heat shock protein (HSP) promoter system[4] have been used as effective inducible gene expression systems. For Tet-inducible systems, a synthetic promoter comprised of a Tet-responsive element (TRE) sequence and minimal cytomegalovirus (CMV) promoter (P_{CMVmin}) is placed upstream of a target gene. The promoter induces target gene expression in response to the Tet-responsive transcriptional activator (tTA). For HSP promoter systems, the human HSP70B' promoter is often used because of its tight inducibility and negligible basal expression in most cell types.[5] Expression of genes under the control of the HSP70B' promoter is up-regulated by various cellular stresses, including heat stress. Upon exposure to stress, heat shock factor proteins activated in a cell bind to the heat shock element DNA sequence in the HSP70B′ promoter.[6] Such binding induces expression of the target gene. However, the activity of the HSP70B′ promoter is not as strong as that of virus-derived constitutive promoters such as the CMV promoter.[7] Using a synthetic biological approach, we have previously constructed a heat-inducible transgene expression system incorporating a transcriptional positive feedback loop that enhances the transcriptional activity of HSP70B′ promoter.[8] This system relies on the strict heat inducibility of the HSP70B′ promoter, which acts as a "switch", and the high-level gene expression mediated by the Tet-inducible system that acts as an "amplifier".

Nanotechnology is becoming increasingly important in medicine. Magnetite nanoparticles have been used for drug delivery systems (DDSs)[9] and cancer diagnosis as contrast-enhancement agents in magnetic resonance imaging.[10] The magnetic properties and functional addition by surface modification of nanoparticles have extended their medical usability.[11] For DDSs using magnetite (Fe_3O_4) nanoparticles, liposomal coatings provide a promising approach. Accumulation of magnetite nanoparticles in tumor cells can be enhanced by conferring a positive charge on the liposomal surface. Thus, magnetite cationic liposomes (MCLs) have been developed by encapsulating 10 nm magnetite nanoparticles in cationic liposomes.[12] In addition, magnetite particles generate heat under an alternating magnetic field (AMF).[13] Local hyperthermia can be induced by targeted delivery of magnetite nanoparticles and exposure to an AMF. AMF generators to induce hyperthermia have also been developed. AMF is mainly generated by a solenoid coil driven by a transistor inverter at a frequency of 100−500 kHz.[14,15] In preclinical studies using animal models with several types of tumors,[16] MCLs were directly injected into the tumors, and heat generation by AMF exposure induced strong therapeutic effects.

Remote activation of target cells to trigger specific gene expression *in vivo* can provide a useful research tool and a potential means to control gene expression in clinical settings.[17,18] Here, we combined synthetic biology with nanotechnology to convert a local heating signal using

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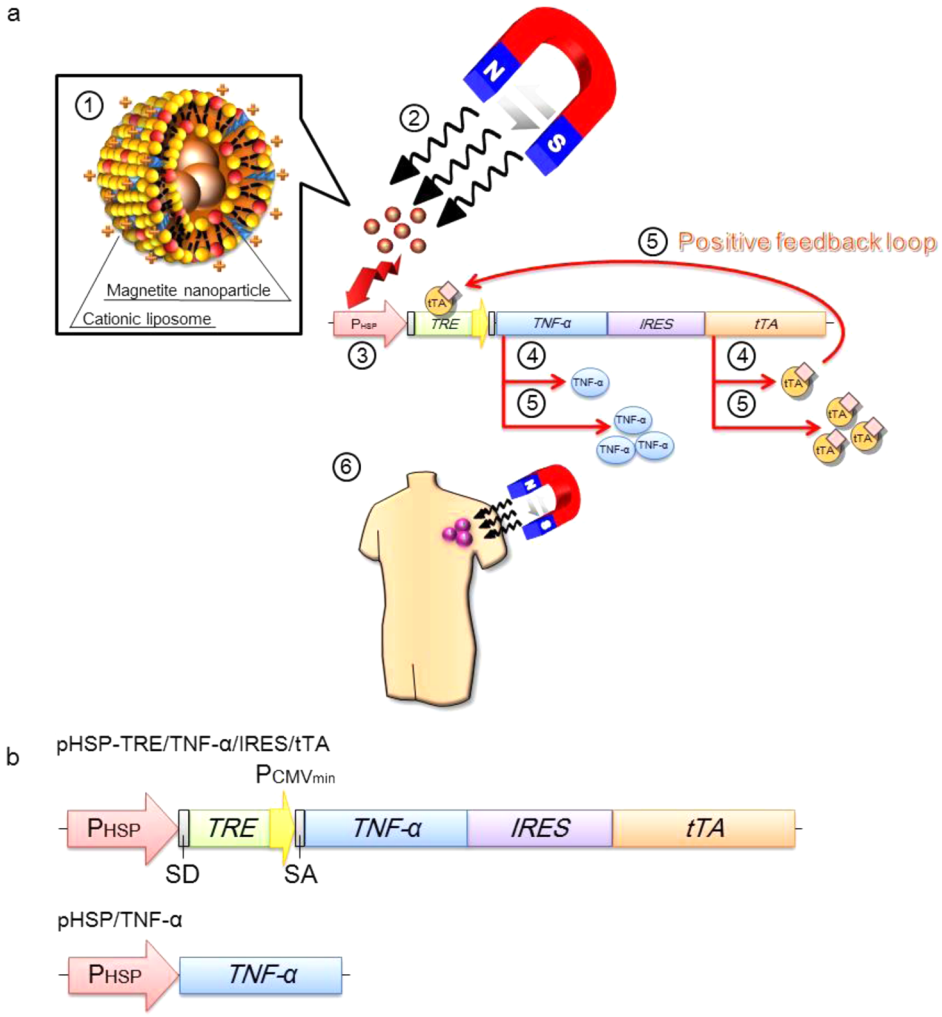

Figure 1. Overview of the magnetically triggered gene expression system. (a) Schematic diagram of the strategy for the magnetically triggered gene expression system. (1) Magnetite cationic liposomes (MCLs) bind to the membrane of target cells via electrostatic interaction. (2) Exposure to an alternating magnetic field (AMF) induces local magnetite nanoparticle heating (3) that activates the HSP70B' promoter. (4) Expression of both TNF-α and tTA genes are driven by the HSP70B' promoter and bicistronic IRES. (5) tTA activates the TRE/P_{CMVmin} promoter to induce further expression of TNF-α and tTA genes, providing a transcriptional positive feedback loop. (6) Therapeutic gene expression can be controlled by AMF exposure from outside the body. (b) The heat-inducible therapeutic gene expression plasmid with (pHSP-TRE/TNF-α/IRES/tTA) or without (pHSP/TNF-α) the Tet-transactivator system. P_{HSP}, human HSP70B' promoter; SD, splice donor sequence; *TRE*, Tet-responsive element; P_{CMVmin}, cytomegalovirus minimal promoter; SA, splice acceptor sequence; *TNF-α*, tumor necrosis factor-α; *IRES*, internal ribosomal entry site; *tTA*, Tet-responsive transactivator.

magnetite nanoparticles and AMF exposure into high-level gene expression at a specific site. We investigated the *in vivo* feasibility of this approach for cancer gene therapy using a tumor xenograft model.

As outlined in Figure 1a, our goal was to establish a magnetic switch to control gene expression. MCLs were used to magnetically label cells via the electrostatic interaction between the cationic liposomes and cell membrane. By applying an external AMF, the magnetite nanoparticles in MCLs generate heat. To enable strict control of gene expression from outside of the body, a heat shock-inducible system was selected (Figure 1b), since the human HSP70B' promoter exhibits heat inducibility with extremely low background activity.[8] As shown in Figure 1a, the heat-activated HSP70B' promoter drives bicistronic gene expression of tumor necrosis factor (TNF)-α and tTA genes, which is mediated by an internal ribosomal entry site (IRES). Then, the tTA binds and activates the TRE/PCMVmin placed upstream of the target gene, which, in turn, induces further expression of TNF-α and tTA genes, providing a transcriptional positive feedback loop. Consequently, therapeutic gene expression can be controlled by AMF exposure from outside of the body.

First of all, to investigate the toxicity of MCLs in medium, the human lung adenocarcinoma cell line A549 was incubated with various concentrations of MCLs. The amount of magnetite taken up by the cells increased when MCLs were added at 25−200 pg/cell to the culture medium (Supporting Information Figure S1a). Cell viability did not decrease at 100 pg MCLs/cell (Supporting Information Figure S1b), whereas a significant decrease in cell viability was observed at a higher concentration (200 pg MCLs/cell). Therefore, in the following *in vitro* experiments, MCLs were added at 100 pg/cell. MCL uptake started rapidly and almost reached a plateau at 6 h after MCL addition (Supporting Information Figure S1c). When MCLs were added to the culture medium at 100 pg/cell, the maximum uptake (13.5 pg MCLs/cell) was achieved after 24 h.

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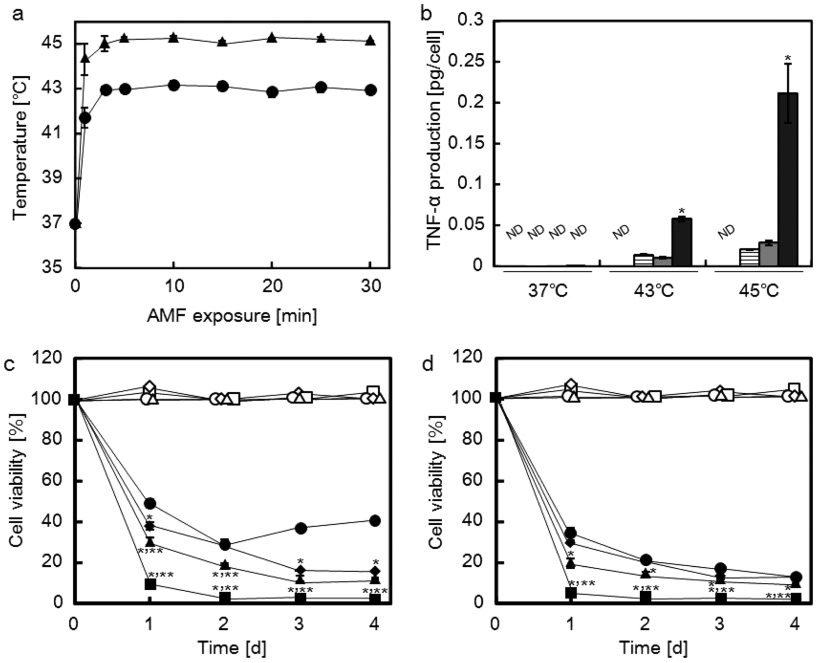

Figure 2. AMF-inducible gene expression *in vitro*. (a) MCL-induced heat generation *in vitro*. A549 cells magnetically labeled with MCLs (100 pg/cell) were harvested as cell pellets, and then exposed to an AMF for 30 min. The temperature of the cell pellets was measured using an optical fiber probe, and maintained at 43 °C (circles) or 45 °C (triangles) by adjustment of the electrical power of the AMF. (b) TNF-α production after AMF exposure. A549 cells were transfected with a mock plasmid (white columns), pHSP/TNF-α (stripe columns), pHSP-TRE/TNF-α/IRES/tTA plus Dox addition (gray columns) or pHSP-TRE/TNF-α/IRES/tTA (black columns), and then incubated at 37 °C or heated at 43 or 45 °C. At 24 h after AMF exposure, culture medium was collected to measure the TNF-α level by ELISA. *P < 0.05 versus pHSP/TNF-α at each temperature. ND indicates "no detection", where the minimum detectable range of TNF-α using the ELISA kit is <1.6 pg/mL, according to the manufacturer's instructions. (c, d) Cell viability after AMF exposure. A549 cells were transfected with the mock plasmid (circles), pHSP/TNF-α (diamonds), pHSP-TRE/TNF-α/IRES/tTA plus Dox addition (triangles) or pHSP-TRE/TNF-α/IRES/tTA (squares). The cells were incubated at 37 °C (open symbols) or exposed to the AMF for 30 min (closed symbols). The temperature was maintained at 43 °C (c) or 45 °C (d) by adjusting the electrical power of the transistor inverter during AMF exposure. *P < 0.05 versus the mock plasmid with AMF exposure (closed circles) and **P < 0.05 versus pHSP/TNF-α with AMF exposure (closed triangles) at each temperature, respectively. Data are expressed as the mean ± SD of triplicates.

To evaluate the performance of the AMF-inducible gene expression system, the system was first tested *in vitro*. After incubation with MCLs, the cells were harvested as cell pellets in 1.5 mL microtubes. A magnetic field was created using a horizontal coil (inner diameter, 7 cm; length, 7 cm) with a transistor inverter.[12] The cell pellets were subjected to AMF exposure for 30 min. In this study, the temperature was controlled at 43 or 45 °C by manually adjusting the electrical power of the transistor inverter during AMF exposure. We periodically sampled the data by monitoring the display of the optical fiber thermometer. The magnetic field frequency and intensity were 118 kHz and 30.6 kA/m (384 Oe), respectively. The volume of the cell pellets was approximately 50 μL, and the magnetite concentration in the cell pellets was 6.5 mg/cm^3. As shown in Figure 2a, the temperature at the center of the cell pellets increased immediately upon AMF exposure and reached 43 or 45 °C within 3 min. Then, the temperature was maintained for 30 min by adjusting the electrical power of the AMF.

To investigate whether TNF-α production in cells transfected with the therapeutic gene expression plasmid (pHSP-TRE/TNF-α/IRES/tTA or pHSP/TNF-α; Figure 1b) was triggered by AMF exposure, TNF-α production at 1 day after AMF exposure was measured by an enzyme-linked immunosorbent assay (ELISA) (Figure 2b). Without AMF exposure (37 °C), TNF-α production in cells transfected with the therapeutic gene expression plasmid was not detected. When cells were transfected with pHSP/TNF-α, substantial TNF-α production was observed by heating at 43 or 45 °C, indicating that the HSP70B' promoter is activated by magnetic heating of nanoparticles. Compared with pHSP/TNF-α, TNF-α production was significantly enhanced by the positive feedback loop mediated by the Tet-system in cells transfected with pHSP-TRE/TNF-α/IRES/tTA. Since the transfection efficiency in this experiment was 14% (Supporting Information Figure S2), the TNF-α concentration may not be a lethal level for A549 cells. Although the TNF-α gene expression mediated by the positive feedback loop may reach a plateau in the cells due to the limitation of protein synthesis or the cytotoxicity, further high level of TNF-α expression may be achieved by improving the transfection efficiency. The interaction between tTA and TRE is inhibited by doxycycline (Dox). Therefore, gene expression mediated by the positive feedback was switched off by addition of Dox. Following Dox addition, TNF-α production in cells transfected with pHSP-TRE/TNF-α/IRES/tTA decreased to a similar level as that in cells transfected with pHSP/TNF-α. In addition, the similar results were also obtained for the reporter gene assay (Supporting Information Figure S3), suggesting that the augmentation of TNF-α production in cells transfected with pHSP-TRE/TNF-α/IRES/tTA was attributed to the positive feedback loop mediated by the Tet-system. Next, *in vitro* therapeutic effects of heat-induced TNF-α gene therapy were investigated (Figure 2c and d). Without AMF exposure (37 °C), no cytotoxic effects were observed in the cells, even when the cells were transfected with pHSP-TRE/TNF-α/IRES/tTA. With AMF exposure,

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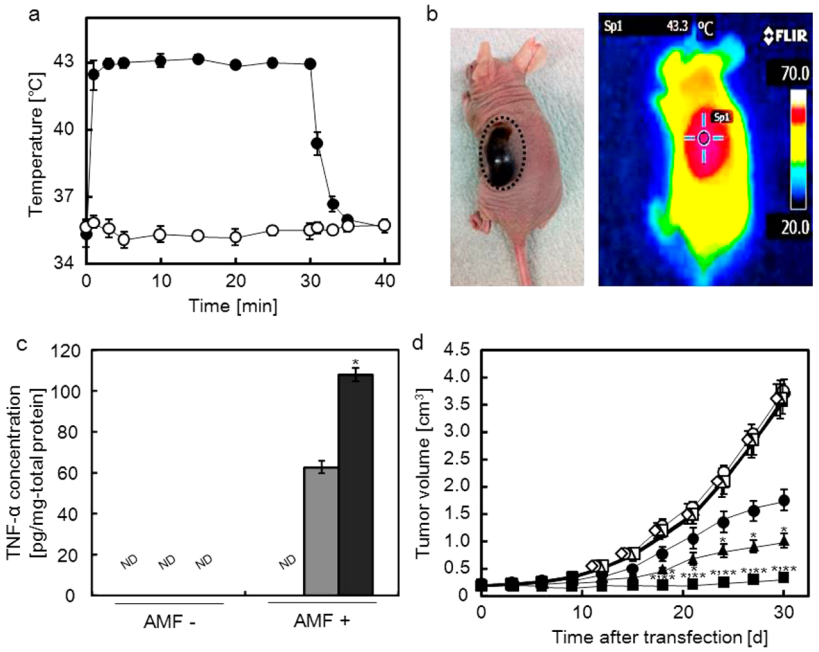

Figure 3. AMF-inducible gene expression *in vivo* and therapeutic effects of heat-induced TNF-α gene therapy. (a) Tumor temperature during AMF exposure. MCLs were injected into tumors, and then the mice were exposed to an AMF. Temperatures at the tumor surface (closed circles) and in the rectum (open circles) were measured by optical fiber probes. Data are expressed as the mean ± SD of five mice. (b) Infrared thermography of a mouse exposed to the AMF. (left) Bright field image. The tumor is encircled by a dotted line. (right) Infrared thermography. (c) TNF-α production in tumor tissues after AMF exposure. Tumors were transfected with the mock plasmid (white columns), pHSP/TNF-α (gray columns), or pHSP-TRE/TNF-α/IRES/tTA (black columns). The mice were then exposed to the AMF for 30 min. TNF-α concentration in tumor homogenates was determined by ELISA at 1 day after AMF exposure. The human TNF-α ELISA does not detect mouse TNF-α. TNF-α concentration (pg/mg total protein) represents pg of TNF-α/mg of total protein in tumor homogenates. Data are expressed as the mean ± SD of triplicates. Each group contained five mice. *P < 0.05 versus pHSP/TNF-α. ND means "no detection", where the minimum detectable range of TNF-α using the ELISA kit is <1.6 pg/mL, according to the manufacturer's instructions. (d) Therapeutic effects of heat-induced TNF-α gene therapy on tumor growth. Tumor tissues were transfected with the mock plasmid (circles), pHSP/TNF-α (triangles), or pHSP-TRE/TNF-α/IRES/tTA (squares). The mice were then treated with (closed symbols) or without (open symbols) AMF exposure for 30 min. As a control group, tumors were not transfected or exposed to the AMF (diamonds). Each group contained five mice. *P < 0.05 versus the mock plasmid with AMF exposure (closed circles) and **P < 0.05 versus pHSP/TNF-α with AMF exposure (closed triangle). Data are expressed as the mean ± SD of five mice.

substantial cell death was caused by heating at 43 or 45 °C for 30 min. In the absence of the therapeutic gene plasmid, cell viability decreased to 50% at 1 day after AMF exposure (day 1). When heated at 43 °C, cell viability was maintained at 40% until day 4 (Figure 2c), whereas cell viability gradually decreased until day 4 after heating at 45 °C (Figure 2d). For cells transfected with pHSP/TNF-α and heated at 43 °C (Figure 2c), a significant decrease of cell viability was observed compared with that in cells without the plasmid (heating alone). For cells transfected with pHSP/TNF-α and heated at 45 °C (Figure 2d), there was no further decrease in cell viability compared with heating alone. On the other hand, cell viability decreased drastically in cells transfected with pHSP-TRE/TNF-α/IRES/tTA and exposed to the AMF. In addition, cell viability showed a significant decrease compared with that in cells transfected with pHSP/TNF-α and heated at 43 °C (Figure 2c) or 45 °C (Figure 2d). The decrease in cell viability was moderated by Dox addition (Figure 2c and d), suggesting that the augmented cytotoxic effect using pHSP-TRE/TNF-α/IRES/tTA after AMF exposure was owing to enhanced therapeutic gene expression mediated by the positive feedback loop. To further evaluate the efficacy of the gene circuit, we measured the AMF exposure time required for sufficient cell death with or without Dox addition (Supporting Information Figure S4). The AMF exposure time required for 50% cell death with or without Dox addition was 12.0 or 29.3 min, respectively (for 90% cell death, 20.3 or 40.0 min, respectively). These results indicate that the transcriptional positive feedback loop significantly enhanced the *in vitro* cell-killing effects of hyperthermia.

To evaluate the *in vivo* therapeutic effects of heat-induced TNF-α gene therapy, tumor growth and therapeutic gene expression in mice with tumor xenografts were investigated. At 1 day after MCL injection into tumor tissue, the AMF was applied to the whole mouse. Figure 3a shows the temperature at the tumor surface and in the rectum as the body temperature during AMF exposure. The tumor temperature reached 43 °C within 3 min, which was maintained for 30 min by tuning the electrical power of the AMF. When AMF exposure was stopped, the tumor temperature decreased to the normal body temperature within 10 min. In contrast, the temperature in the rectum hardly increased above 36 °C throughout AMF exposure. Moreover, infrared thermography revealed that the heat was generated only in the tumor tissue containing magnetite nanoparticles (Figure 3b). TNF-α concentrations in tumor homogenates were measured following AMF exposure. As shown in Figure 3c, without AMF exposure, no TNF-α was detected in tumors transfected with the mock plasmid (pETBlue II) or the therapeutic gene expression plasmids (pHSP/TNF-α or pHSP-TRE/TNF-α/IRES/tTA). Similarly, TNF-α gene expression was not detected in tumors transfected with the mock plasmid and treated with MCL-

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mediated hyperthermia. In contrast, TNF-α was detected in tumors transfected with the therapeutic gene expression plasmids and treated with MCL-mediated hyperthermia. A significantly higher concentration of TNF-α was observed in tumors transfected with pHSP-TRE/TNF-α/IRES/tTA compared with that in tumors transfected with pHSP/TNF-α (Figure 3c). Exogenous TNF-α expression was not detected in the serum of mice under all conditions. Tumor growth after pHSP-TRE/TNF-α/IRES/tTA transfection is shown in Figure 3d. A control group of five mice was subjected to MCL injection, but neither gene transfer nor AMF exposure was carried out. In the control group, tumors grew progressively in the mice, and the average tumor volume at day 30 after transfection was 3.6 $\pm$ 0.3 cm^3. When tumor tissues were treated with plasmid transfection and MCL injection, but without AMF exposure, the tumor volume increased to the same level as that in the control group. For the hyperthermia group with mock vector transfection, tumor growth was significantly suppressed compared with that in the control group, and the average tumor volume at day 30 was 1.8 $\pm$ 0.2 cm^3. When tumor tissues underwent pHSP/TNF-α transfection and MCL-mediated hyperthermia (heat-induced TNF-α gene therapy group using pHSP/TNF-α), tumor growth was significantly suppressed compared with that in the hyperthermia group with mock vector transfection, and the tumor volume at day 30 was 1.0 $\pm$ 0.1 cm^3. When tumor tissues underwent pHSP-TRE/TNF-α/IRES/tTA transfection and MCL-mediated hyperthermia (heat-induced TNF-α gene therapy group using pHSP-TRE/TNF-α/IRES/tTA), the tumor volume was strongly arrested at day 30, and the average tumor volume at day 30 was 0.3 $\pm$ 0.1 cm^3. These results demonstrate that a magnetic switch to control gene expression by heating magnetite nanoparticles with AMF exposure is operable *in vivo* and heat-induced TNF-α gene therapy is effective for cancer treatment.

Spatiotemporal control of target gene expression is a promising approach for gene therapy. Furthermore, remote activation of specific cells and tissues to express genes *in vivo* can provide a potent tool for biological experiments and clinical applications. For this purpose, physical stimuli using optical,[19] electrical,[20] and magnetic[21] methods have been developed. Among these methods, magnetic techniques are advantageous for *in vivo* use because magnetic fields can penetrate deeply into tissues, whereas light waves have difficulties in penetrating tissue, and electrical stimulation requires implants. As a preclinical study, we have used a solenoid-type AMF generator and athymic mice. However, it is difficult to scale up the coil size in the solenoid-type applicator for clinical application, because a very large coil would be required to accommodate a human body, which may be accompanied by a serious risk associated with the high voltage between the two solenoid ends. Therefore, a new device called a "ferrite core-inserted solenoid type" has been developed. In this device, a ferrite core inside of a solenoid coil is equipped to concentrate the magnetic field generated by the solenoid coil, resulting in emission of the magnetic field from the surface of the device. Thus, the ferrite core-inserted solenoid-type applicator enables heating of a target region outside of the coil. Clinical trials using a ferrite core-inserted solenoid-type applicator have already begun.[22] These AMF applicators may be useful for a magnetic switch to control gene expression by heating magnetite nanoparticles in humans.

Magnetite nanoparticles absorb energy and generate heat in response to an AMF. Targeting of magnetite nanoparticles can be achieved by coating with biomaterials. For cell specificity *in vitro*, Huang et al. used streptavidin-conjugated magnetite nanoparticles to target cells expressing genetically engineered membrane protein markers containing a biotinylated acceptor peptide.[17] In the present study, MCLs were used to target the cell membrane, which are applicable *in vivo* by direct injection into tissues.[23] MCLs showed a high cell-binding capacity because of the exterior cationic liposomes.[24] The amount of MCL uptake differs among cell types. Generally, tumor cell lines exhibit higher uptake of MCLs compared with that of normal cells. Some tumor cells have been shown to possess high endocytotic activities, and MCL uptake may depend on such activities of target cells. Unexpectedly, A549 cells exhibited low MCL uptake (13.5 pg/cell; Supporting Information Figure S1c) compared with that of other tumor cell lines. For example, human U251-SP glioma,[25] and human hepatoma HepG2[26] exhibit MCL uptake at 58.9 and 48.9 pg/cell, respectively. The mechanism of MCL uptake has not been fully elucidated. Nevertheless, A549 cells were successfully heated to 43 and 45 °C by AMF exposure *in vitro* (Figure 2a), suggesting that MCLs are a superior tool for universal magnetic labeling of cells. Moreover, we showed that the induced temperature increase was highly localized at the tumor sites where MCLs were injected *in vivo* (Figure 3). Although MCLs were directly injected into tumors in this study, antibody-conjugated magnetoliposomes (AML) may be applied for specific tumor targeting *in vivo*.[27] By applying this concept to our system, plasmid-loaded AMLs could be engineered where antibodies target magnetite nanoparticles and DNA vectors to cancer cells, and then magnetic fields are applied. Cho et al. developed magnetic nanoparticles conjugated to a targeting antibody for death receptor 4 on cancer cells.[28] By functionalizing magnetic nanoparticles, various magnetic switches that stimulate cell functions would be designable.

In the present study, AMF exposure was used as a switch to induce gene expression. Heat generation by AMF exposure resulted in TNF-α expression driven by the HSP70B' promoter, whereas no TNF-α production was observed without AMF exposure (Figures 2b and 3c), indicating the stringent heat-inducibility of the HSP70B′ promoter. This strict regulation enabled us to combine the transcriptional positive feedback loop mediated by the Tet-transactivator system, although leakage of tTA gene expression under the HSP70B′ promoter without AMF exposure could potentially stimulate the positive feedback loop and amplify TNF-α gene expression. Thus, we successfully constructed a heat inducible gene expression system incorporating a transcriptional positive feedback loop for amplification of target gene expression.

The TNF-α gene was chosen as a model therapeutic gene because of its cytotoxic effect by inducing apoptosis in a wide range of cancer cell types. However, the clinical use of TNF-α in humans has been limited by systemic toxicity.[29] In our study, the heat-induced TNF-α expression resulted in strong anticancer effects by the combination of hyperthermia with TNF-α gene therapy (Figures 2c, d and 3d). Hyperthermic killing of A549 cells *in vitro* increased as the heating temperature increased (Figure 2c and d). The tumor temperature was raised to 43 °C for the *in vivo* study to assess the effect of TNF-α gene expression in the xenograft model. As a result, we observed a strong therapeutic effect of the heat-induced TNF-α gene therapy (Figure 3d). High temperatures

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are required for cancer therapy by hyperthermia combined with gene therapy. However, hyperthermic cell death might be undesirable in other cell-based therapies such as regulation of plasma glucose by controlling insulin gene expression through magnetic heating of nanoparticles.[18] Interestingly, a recent report indicated that dispersed single magnetite nanoparticles are able to increase the temperature of their immediate surroundings without a perceptible temperature rise.[30] Thus, local heating within cells using magnetite nanoparticles and AMF exposure may enable the regulation of exogenous gene expression without thermal damage of target cells.

In conclusion, we combined synthetic biology with nanotechnology to control gene expression *in vivo* by external stimulus. As a result, the magnetically triggered high-level therapeutic gene expression caused strong therapeutic effects in tumor xenografts. These results indicate that this strategy would be applicable for the development of novel therapies based on remote manipulation of cell functions via gene expression.

■ ASSOCIATED CONTENT

Supporting Information

Description of materials and methods used and a supporting figure showing cellular uptake of magnetite nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lu, T. K., Khalil, A. S., and Collins, J. J. (2009) Next-generation synthetic gene networks. *Nat. Biotechnol. 27*, 1139−1150.

(2) Prindle, A., Selimkhanov, J., Danino, T., Samayoa, P., Goldberg, A., Bhatia, S. N., and Hasty, J. (2012) Genetic circuits in *Salmonella typhimurium*. *ACS Synth. Biol. 1*, 458−464.

(3) Stieger, K., Belbellaa, B., Le, Guiner, C., Moullier, P., and Rolling, F. (2009) *In vivo* gene regulation using tetracycline-regulatable systems. *Adv. Drug Delivery Rev. 61*, 527−541.

(4) Huang, Q., Hu, J. K., Lohr, F., Zhang, L., Braun, R., Lanzen, J., Little, J. B., Dewhirst, M. W., and Li, C. Y. (2000) Heat-induced gene expression as a novel targeted cancer gene therapy strategy. *Cancer Res. 60*, 3435−3439.

(5) Leung, T. K., Rajendran, M. Y., Monfries, C., Hall, C., and Lim, L. (1990) The human heat-shock protein family. Expression of a novel heat-inducible HSP70 (HSP70B′) and isolation of its cDNA and genomic DNA. *Biochem. J. 267*, 125−132.

(6) Cotto, J. J., Kline, M., and Morimoto, R. I. (1996) Activation of heat shock factor 1 DNA binding precedes stress-induced serine phosphorylation. Evidence for a multistep pathway of regulation. *J. Biol. Chem. 271*, 3355−3358.

(7) Wada, K., Taniguchi, A., and Okano, T. (2007) Highly sensitive detection of cytotoxicity using a modified HSP70B' promoter. *Biotechnol. Bioeng. 97*, 871−876.

(8) Yamaguchi, M., Ito, A., Okamoto, N., Kawabe, Y., and Kamihira, M. (2012) Heat-inducible transgene expression system incorporating a positive feedback loop of transcriptional amplification for hyperthermia-induced gene therapy. *J. Biosci. Bioeng. 114*, 460−465.

(9) Hoare, T., Santamaria, J., Goya, G. F., Irusta, S., Lin, D., Lau, S., Padera, R., Langer, R., and Kohane, D. S. (2009) A magnetically triggered composite membrane for on-demand drug delivery. *Nano Lett. 9*, 3651−3657.

(10) Gao, J., Chen, K., Luong, R., Bouley, D. M., Mao, H., Qiao, T., Gambhir, S. S., and Cheng, Z. (2012) A novel clinically translatable fluorescent nanoparticle for targeted molecular imaging of tumors in living subjects. *Nano Lett. 12*, 281−286.

(11) Gupta, A. K., Naregalkar, R. R., Vaidya, V. D., and Gupta, M. (2007) Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications. *Nanomedicine (Lond). 2*, 23−39.

(12) Shinkai, M., Yanase, M., Honda, H., Wakabayashi, T., Yoshida, J., and Kobayashi, T. (1996) Intracellular hyperthermia for cancer using magnetite cationic liposomes: *In vitro* study. *Jpn. J. Cancer Res. 87*, 1179−1183.

(13) Gazeau, F., Lévy, M., and Wilhelm, C. (2008) Optimizing magnetic nanoparticle design for nanothermotherapy. *Nanomedicine (Lond). 3*, 831−844.

(14) Kawai, N., Ito, A., Nakahara, Y., Honda, H., Kobayashi, T., Futakuchi, M., Shirai, T., Tozawa, K., and Kohri, K. (2006) Complete regression of experimental prostate cancer in nude mice by repeated hyperthermia using magnetite cationic liposomes and a newly developed solenoid containing a ferrite core. *Prostate 66*, 718−727.

(15) Gneveckow, U., Jordan, A., Scholz, R., Brüss, V., Waldöfner, N., Ricke, J., Feussner, A., Hildebrandt, B., Rau, B., and Wust, P. (2004) Description and characterization of the novel hyperthermia- and thermoablation-system MFH 300F for clinical magnetic fluid hyperthermia. *Med. Phys. 31*, 1444−1451.

(16) Ito, A., Shinkai, M., Honda, H., and Kobayashi, T. (2005) Medical application of functionalized magnetic nanoparticles. *J. Biosci. Bioeng. 100*, 1−11.

(17) Huang, H., Delikanli, S., Zeng, H., Ferkey, D. M., and Pralle, A. (2010) Remote control of ion channels and neurons through magnetic-field heating of nanoparticles. *Nat. Nanotechnol. 5*, 602−606.

(18) Stanley, S. A., Gagner, J. E., Damanpour, S., Yoshida, M., Dordick, J. S., and Friedman, J. M. (2012) Radio-wave heating of iron oxide nanoparticles can regulate plasma glucose in mice. *Science 336*, 604−608.

(19) Wang, X., Chen, X., and Yang, Y. (2012) Spatiotemporal control of gene expression by a light-switchable transgene system. *Nat. Methods. 9*, 266−269.

(20) Buvinic, S., Almarza, G., Bustamante, M., Casas, M., López, J., Riquelme, M., Sáez, J. C., Huidobro-Toro, J. P., and Jaimovich, E. (2009) ATP released by electrical stimuli elicits calcium transients and gene expression in skeletal muscle. *J. Biol. Chem. 284*, 34490−34505.

(21) Deckers, R., Quesson, B., Arsaut, J., Eimer, S., Couillaud, F., and Moonen, C. T. (2009) Image-guided, noninvasive, spatiotemporal control of gene expression. *Proc. Natl. Acad. Sci. U.S.A. 106*, 1175−1180.

(22) Ito, A., and Kobayashi, T. (2008) Intracellular hyperthermia using magnetic nanoparticles: A novel method for hyperthermia clinical applications. *Thermal Medicine 24*, 113−129.

(23) Ito, A., Shinkai, M., Honda, H., and Kobayashi, T. (2001) Heat-inducible TNF-α gene therapy combined with hyperthermia using magnetic nanoparticles as a novel tumor-targeted therapy. *Cancer Gene Ther. 8*, 649−654.

(24) Ito, A., and Kamihira, M. (2011) Tissue engineering using magnetite nanoparticles. *Prog. Mol. Biol. Transl. Sci. 104*, 355−395.

(25) Le, B., Shinkai, M., Kitade, T., Honda, H., Yoshida, J., Wakabayashi, T., and Kobayashi, T. (2001) Preparation of tumor-specific magnetoliposomes and their application for hyperthermia. *J. Chem. Eng. Jpn. 34*, 66−72.

(26) Ito, A., Jitsunobu, H., Kawabe, Y., and Kamihira, M. (2007) Construction of heterotypic cell sheets by magnetic force-based 3-D coculture of HepG2 and NIH3T3 cells. *J. Biosci. Bioeng. 104*, 371−378.
(27) Ito, A., Kuga, Y., Honda, H., Kikkawa, H., Horiuchi, A., Watanabe, Y., and Kobayashi, T. (2004) Magnetite nanoparticle-loaded anti-HER2 immunoliposomes for combination of antibody therapy with hyperthermia. *Cancer Lett. 212*, 167−175.
(28) Cho, M. H., Lee, E. J., Son, M., Lee, J. H., Yoo, D., Kim, J. W., Park, S. W., Shin, J. S., and Cheon, J. (2012) A magnetic switch for the control of cell death signalling in *in vitro* and *in vivo* systems. *Nat. Mater. 11*, 1038−1043.
(29) Spriggs, D. R., and Yates, S. W. (1992) Cancer chemotherapy. Experience with TNF administration in humans. In *Tumor Necrosis Factors: The Molecules and their Emerging Role in Medicine* (Beutler, B., Ed.), pp 383−406, Raven Press, New York.
(30) Creixell, M., Bohórquez, A. C., Torres-Lugo, M., and Rinaldi, C. (2011) EGFR-targeted magnetic nanoparticle heaters kill cancer cells without a perceptible temperature rise. *ACS Nano 5*, 7124−7129.