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No influence of tumor growth by intramuscular injection of hepatocyte growth factor plasmid DNA: safety evaluation of therapeutic angiogenesis gene therapy in mice

Atsushi Matsuki,[a,b,c] Seiji Yamamoto,[b] Hironori Nakagami,[a] Motokuni Aoki,[d] Katsuto Tamai,[b] Kunio Matsumoto,[e] Toshikazu Nakamura,[e] Toshio Ogihara,[d] Yasufumi Kaneda,[b] and Ryuichi Morishita[a,*]

[a] *Division of Clinical Gene Therapy, Biomedical Research Center, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan*
[b] *Division of Gene therapy Science, Biomedical Research Center, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan*
[c] *Digestive and General Surgery, Graduate School of Medical and Dental Science, Niigata University, Asahimachi, Niigata 951-8122, Japan*
[d] *Department of Geriatric Medicine, Biomedical Research Center, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan*
[e] *Division of Biochemistry, Department of Oncology, Biomedical Research Center, Graduate School of Medicine, Osaka University, Suita, Osaka 565-0871, Japan*

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Abstract

Recently, a novel therapeutic treatment for ischemic diseases using angiogenic growth factors to augment collateral artery development has been proposed. As intramuscular injection of naked human hepatocyte growth factor (HGF) plasmid DNA induced therapeutic angiogenesis in several animal test subjects, we have started a clinical trial to treat peripheral arterial disease. However, one might assume that over-expression of angiogenic growth factors could enhance tumor growth. To resolve this issue, we examined the over-expression of HGF in tumor bearing mice. Tumors on their backs were prepared with an intradermal inoculation of A431, human epidermoid cancer cells expressing c-Met. These mice were intramuscularly injected with human HGF plasmid or control plasmid into the femoral muscle. Human HGF concentration was increased only in the femoral muscle, but not in blood. Although recombinant HGF stimulated the growth of A431 cells in vitro, temporally and locally HGF elevation in hindlimb had no effect on tumor growth in mice.

Keywords: Angiogenesis; Safety; Cancer; Peripheral vascular disease; Gene therapy

Critical limb ischemia is estimated to develop in 500–1000 individuals per million per year [1]. In a large proportion of these patients, the anatomical extent and the distribution of arterial occlusive disease make the patients unsuitable for operative or percutaneous revascularization. Most importantly, there is no optimal medical therapy for critical limb ischemia. Therefore, novel therapeutic modalities are needed to treat these patients. Recently, the efficacy of therapeutic angiogenesis using gene transfer of angiogenic growth factors such as VEGF (vascular endothelial growth factor) has been tested in human patients with critical limb ischemia and myocardial ischemia [2–4]. Thus, the strategy for therapeutic angiogenesis using angiogenic growth factors should be considered for the treatment of patients with critical limb ischemia or myocardial infarction. In addition to VEGF, hepatocyte growth factor (HGF) originally cloned as hepatocyte mitogen [5] is also of particular interest, as HGF exclusively stimulated the growth of endothelial cells without replication of vascular smooth muscle cells (VSMC) [6–8]. Indeed, others and we have previously reported that HGF is a potent angiogenic growth factor that is useful for effective therapeutic angiogenesis in several animal tests [9,10]. Based upon the preclinical study, we have started human clinical trials of gene

* Corresponding author. Fax: +81-6-6879-3409.
E-mail address: morishit@gts.med.osaka-u.ac.jp (R. Morishita).

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therapy to treat peripheral arterial disease using naked plasmid DNA encoding the HGF gene by intramuscular injection into ischemic legs. Although human clinical trials using angiogenic growth factors including HGF do not exhibit severe adverse effects at the present time, one might assume that the potential risks such as the promotion of tumor growth might exist. As HGF is well known as a pleiotropic effector of cells expressing the c-Met tyrosine kinase receptor [11–13], HGF produced by mesenchymal cells may act predominantly on cells of epithelial origin in an endocrine and/or paracrine fashion [14,15]. HGF especially regulates cell growth, cell motility, and morphogenesis of various types of cells [5]. HGF-Met signaling clearly plays a role in tumor development and progression, as c-Met was originally isolated as the product of human oncogene, trp-met, encodes an altered Met protein possessing constitutive, ligand-independent tyrosine kinase activity, and transforming ability [16,17]. The oncogenic capability of HGF-Met signaling is likely due to the inappropriate use of mitogenic and angiogenic signals. HGF-Met signaling also induces the invasiveness and metastatic potential [18–20]. To answer this safety question, we examined the effects of local over-expression of HGF by intramuscularly injection of plasmid DNA on tumor growth. The present study documented no influence of HGF plasmid DNA gene therapy on tumor growth despite the increase in local HGF concentration in mice.

Materials and methods

Construction of plasmids. To produce a HGF expression vector, human HGF cDNA (2.2 kb) was inserted into pVAX1 (3.0 kb) (Invitrogen, Carlsbad, CA, USA) at the *Bam*HI and *Not*I sites. pVAX1 not containing HGF cDNA was used as a control. pCMV-luciferase-GL3 (7.4 kb) was constructed by cloning the luciferase gene from the pGL3-Promoter Vector (Promega, Madison, WI, USA) into pcDNA3 (5.4 kb) (Invitrogen, USA) at the *Hin*dIII and *Bam*HI sites. Plasmids were purified with the Qiagen plasmid isolation kit (Hilden, Germany).

Human recombinant HGF. Human recombinant HGF was purified from a culture medium of Chinese hamster ovary cells or C-127 cells transfected with an expression plasmid containing human HGF cDNA [9,21].

Cell scattering and proliferation assay. A431 cells were cultured in DMEM supplemented with 10% FBS. Then the cells were plated in 6-well tissue culture dishes at 1×10^5 cells/well in 3.0 ml DMEM with 1% FBS and cultured for 48 h in the absence or presence of various concentrations of HGF [21]. For proliferation assay, A431 cells were cultured in DMEM supplemented with 10% FBS. Then the cells were plated in 96-well tissue culture dishes at 3×10^3 cells/well in 200 μl DMEM with 1% FBS and cultured for 24 h. After washing with DMEM, the cells were cultured for 48 h in DMEM with 1% FBS in the absence or presence of various concentrations of HGF [21]. The number of cells was counted with MTS colorimetric assay system (Promega, USA).

In vivo gene transfer using direct intramuscular injection approach. All procedures were approved by the Osaka University Committee on Animal Research. Six-week-old male BALB/cA nu/nu mice bearing tumor on their back were prepared by an intradermal inoculation of 5×10^6 A431 cells in 0.1 ml PBS [21,22]. A431 human epidermoid carcinoma cells express c-Met/HGF receptor but scarcely produce HGF. A431 cells produce IL-1, one of the HGF-inducing soluble factors [21,23]. One week later, the mice received an intramuscular injection of 200 μg human HGF plasmid DNA in 0.1 ml saline on hindlimb [24,25]. After 4 weeks observation, mice were killed under anesthesia and examined for body weight, tumor weight, tumor invasion, central necrosis, and metastasis. HGF concentrations in blood and tumor tissues were measured by enzyme-immunoassay (EIA).

In vivo gene transfer into the tumor tissues. A431 cells were intradermally implanted into nude mice same as above and one week later, the direct injection of 200 μg human HGF plasmid DNA or empty vector was injected into tumor tissues. After 4 weeks observation, mice were killed under anesthesia and examined for tumor properties.

Measurement of HGF concentration in hindlimb and other tissues. To document the successful transfection of HGF vector into the hind limb, we examined the production of human immunoreactive HGF [8,26]. Before and at 1, 4, 7, and 14 days after transfection, the hindlimb of the mouse transfected with HGF vector, opposite leg, back skin, liver, and blood serum were removed and stored at −70 °C until use. On the day of extraction, the tissue was thawed at 4 °C, weighed, and homogenized by polytron in assay solution. Each specimen was centrifuged at 20,000*g* for 30 min at 4 °C, to remove the lysate. The concentration of HGF in the hindlimb was determined by EIA using anti-human HGF antibody [8,26]. In brief, rabbit anti-rat or anti-human HGF IgG was coated on 96-well plates (Corning, NY) at 4 °C for 15 h. After blocking with 3% bovine serum albumin in phosphate-buffered saline (PBS), the conditioned medium was added to each well and the preparation was incubated for 2 h at 25 °C. The wells were washed three times with PBS containing 0.025% Tween 20 (PBS–Tween), biotinylated rabbit anti-human HGF IgG was added, and the preparation was incubated for 2 h at 25 °C. After washing with PBS–Tween, the wells were incubated with horseradish peroxidase-conjugated streptavidin–biotin complex in PBS–Tween. The enzyme reaction was initiated by adding substrate solution composed of 2.5 mg/ml *o*-phenylenediamine, 100 mM sodium phosphate, 50 mM citric acid, and 0.015% H_2O_2. The enzyme reaction was stopped by adding 1 M H_2SO_4 and absorbance at 490 nm was measured. The antibody against human HGF reacts with only human HGF and not with rat HGF [27]. Mouse endogenous immunoreactive HGF was also measured by EIA using anti-mouse HGF antibody, as the antibody against mouse HGF reacts with only mouse HGF, and not with human HGF [26]. The lower detection limit of this method was 0.1 ng/ml.

Assay for luciferase activity. The mice transfected with luciferase gene were killed under anesthesia at 24 h post-injection. The tissues (tumor, left hindlimb, and right hindlimb) were harvested and placed individually in flat-bottomed 1.5 ml Eppendorf microfuge tubes. Luciferase activity assay was performed as described previously [28]. Luciferase levels were normalized by determining the protein concentrations of the tissue extracts [28]. Luciferase units were expressed as relative light units (RLU) per microgram of tissue protein.

Statistical analysis. All statistical analyses were done with the Stat View, release 4.11 (Abacus Concepts, CA, USA). The results were compared with Student's *t* test. All data are expressed as means ± SD. The differences were considered statistically significant at a P value of <0.05.

Results

As co-expression of c-Met/HGF receptor and HGF molecules in the same cell, which generates an autocrine stimulatory loop, is reported to be oncogenic [18,29], we initially identified the in vitro effects of HGF on the growth cells expressing c-Met. We chose A431 human epidermoid carcinoma cells, since A431 cells express

c-Met, but rarely produce HGF. Initially, we examined the effects of HGF on tumor scattering. As shown in Fig. 1, addition of rHGF induced the scattering of A431 cells in a dose-dependent manner. In addition to scattering, a significant increase in the number of A431 cells was observed under rHGF stimulation in a dose-dependent manner (Fig. 2, $P < 0.01$).

Using these A431 cells, we next examined the effects of local over-expression of HGF on tumor growth in mice harboring A431 tumors. Initially, we measured tissue HGF concentration in tumor induced by A431 cells implantation. As reported previously, A431 cells themselves rarely produced HGF, but secreted the HGF inducers for fibroblasts such as interleukin 1 [21,23]. Indeed, as shown in Fig. 3A, human HGF concentration both in A431 cells and the medium of A431 cells in vitro could not be detected. Similarly, neither could mouse HGF concentration be detected in either the A431 cells or the medium of A431 cells in vitro. In contrast, human HGF concentration was readily detected in tumor tissues in vivo. Tumors induced by A431 cells most likely secrete human HGF locally. In addition, mouse endogenous HGF concentration was also readily detectable by tumor harboring. As A431 cells secreted inducers for HGF such as interleukin 1 [21,23], endogenous HGF levels would be induced. In this

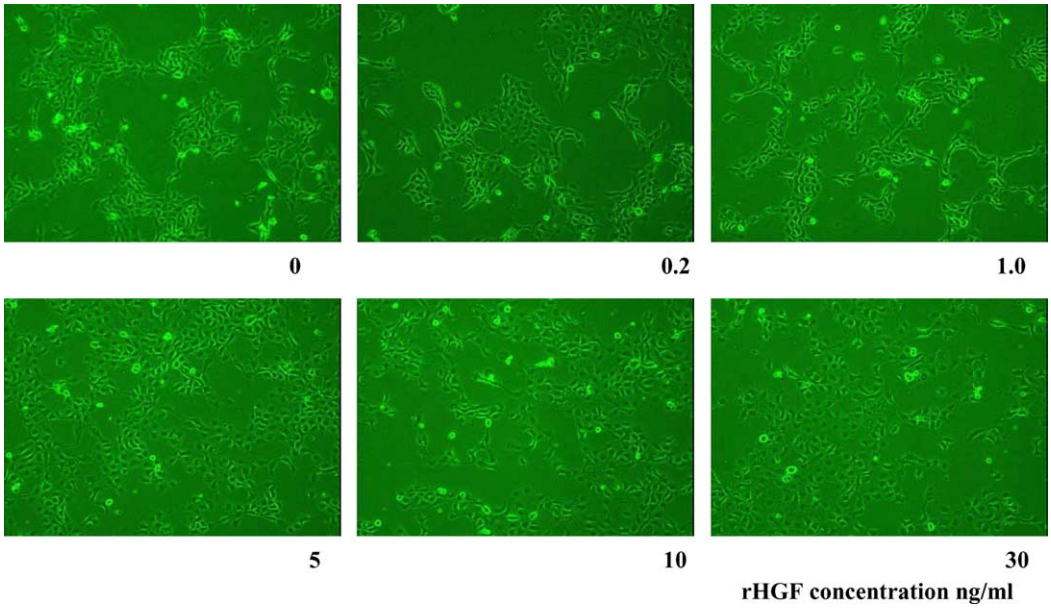

Fig. 1. Effects of human recombinant HGF protein (rHGF) on cell scattering. A431 cells were plated at a density of 1×10^5 cells/well on 6-well plates and cultured for 48 h in various concentrations of rHGF.

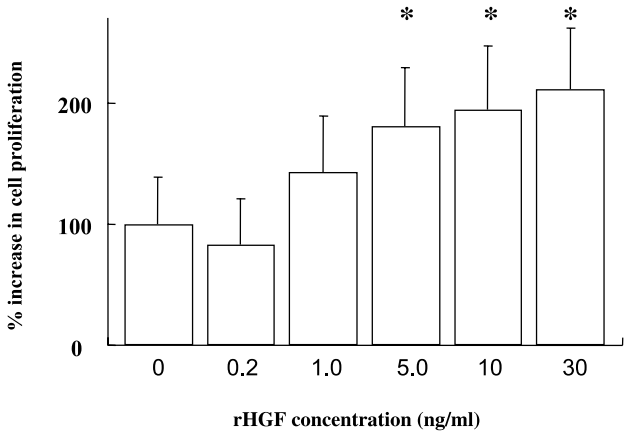

Fig. 2. Effects of rHGF on cell proliferation. A431 cells were plated at a density of 3×10^3 cells/well on 96-well plates and cultured for 48 h in various concentrations of rHGF for every 16 wells. Cell numbers of each well were measured using MTS assay.

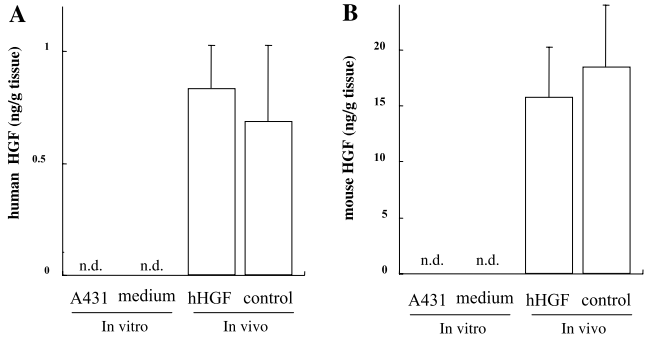

Fig. 3. HGF concentration in vitro and in vivo. (A) Human HGF concentration in A431 cells and medium in vitro and tumor tissues in vivo. The mice received intradermal injections of A431 cells (5×10^6 cells) on their back on day 0, intramuscularly injections of human HGF plasmid (hHGF, $n = 3$) or empty vector (pVAX1, $n = 3$) on hindlimb on day 7. Their tumors were resected on day 28 and HGF concentration was measured by EIA. (B) Mouse HGF concentration in same objects.

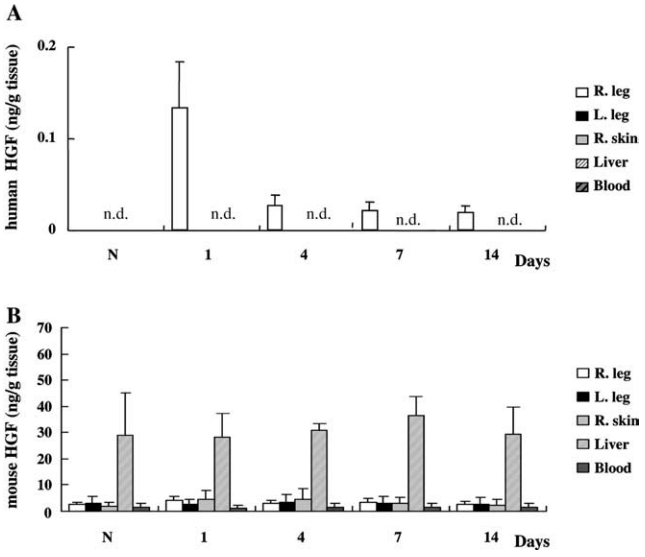

Fig. 4. HGF concentration in various tissues. (A) Change of human HGF concentration in right femoral muscle (R. leg), left femoral muscle (L. leg), right back skin (R. skin), liver, and blood of BALB/cA nu/nu mice before (N), and 1, 4, 7, and 14 days after intramuscularly injection of 200 μg naked human HGF plasmid on right hindlimb $n = 4$. (B) Change of mouse HGF concentration in the same tissues of BALB/cA nu/nu mice before and after intramuscular injection of 200 μg naked human HGF plasmid on right hindlimb $n = 4$.

model, naked plasmid DNA was intramuscularly injected into hindlimb. As shown in Fig. 4A, human HGF concentrations were only detected in the right hindlimb directly transfected with human HGF plasmid. Other organs such as contra-lateral left hindlimb, skin, and liver did not exhibit a detectable HGF level. In addition, neither could serum HGF concentration be detected. In contrast, mouse endogenous HGF concentration could be detected in all tissues. However, HGF level in the liver was extremely high as compared to other organs. Local transfection of HGF plasmid DNA did not affect mouse endogenous HGF level (Fig. 4B). Interestingly, the human and mouse HGF levels in the tumors were also not affected by intramuscular injection of HGF plasmid DNA (Figs. 3A and B).

Based upon the increase in local HGF concentration, we examined the effects of over-expression of HGF in hindlimb on tumor growth. Transfection of human HGF plasmid DNA into peripheral arterial disease mouse model at same dosage resulted in a significant increase in blood flow and the capillary density (data not shown). However, as expected, there was no significant difference in tumor size, weight, invasion, and central necrosis between the mice intramuscularly transfected with human HGF plasmid and control vector (Fig. 5). In addition, no significant difference in tumor size, weight, invasion, and central necrosis could be detected between the mice intramuscularly transfected with control vector and the untreated mice (Fig. 5). Metastasis was not detected in all mice. None of the mice transfected with HGF gene died during the observation period.

Since mice were bearing tumors on their right back, we examined the site effects of direct injection of HGF plasmid DNA. There was no significant difference in tumor properties between the mice intramuscularly injected with HGF plasmid on the right hindlimb and the left hindlimb (data not shown). We next examined whether intratumor injection of HGF plasmid DNA accumulates tumor invasion and growth. There was no significant difference in tumor size between the mice injected with empty vector into tumor tissues and the untransfected mice. In addition, no significant difference in tumor size could be detected between the mice injected with HGF plasmid DNA and empty vector

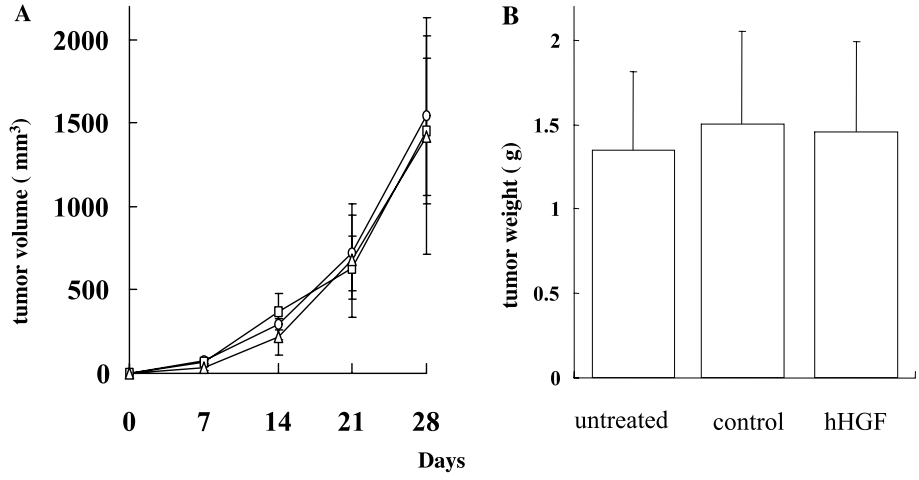

Fig. 5. Growth of tumor cells in vivo. (A) Effect of intramuscular injection of naked human HGF plasmid on the growth of tumor cells in vivo. Mice received intradermal injections of A431 cells (5×10^6 cells) on their right back on day 0 and intramuscular injections of human HGF plasmid (hHGF; ○, $n = 9$) or empty vector (pVAX1; △, $n = 9$) on left hindlimb on day 7 or not (N; □, $n = 9$). Tumor volume on their back was calculated as $1/2 \times \text{length} \times \text{width}^2$ (length > width). (B) Tumor weight on day 28. Tumors were dissected on day 28 and measured by weight.

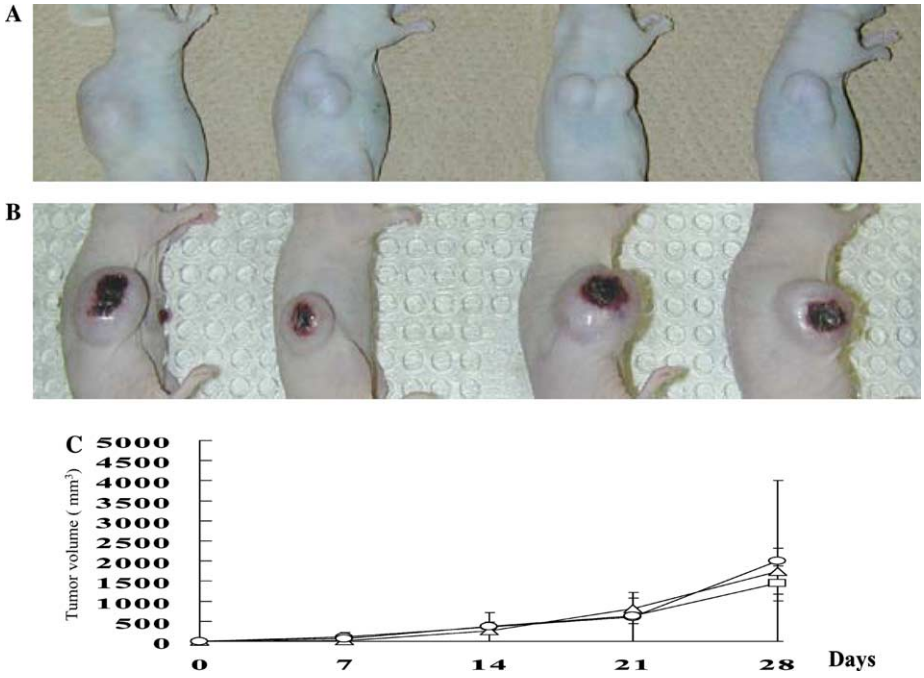

Fig. 6. Effect of direct injection of naked human HGF into the tumor on the growth of tumor. Mice were intradermally injected with A431 cells (5×10^6 cells) on their right back on day 0. Direct injection of human HGF plasmid (○, $n = 4$) or empty vector (△, $n = 4$) into tumor tissues was performed on day 7, whereas control mice were not transfected (□, $n = 9$). (A) Mice transfected with human HGF plasmid DNA (○, $n = 4$). (B) Mice transfected with empty control vector (△, $n = 4$). (C) Effect of direct injection of naked human HGF into the tumor on the growth of tumor.

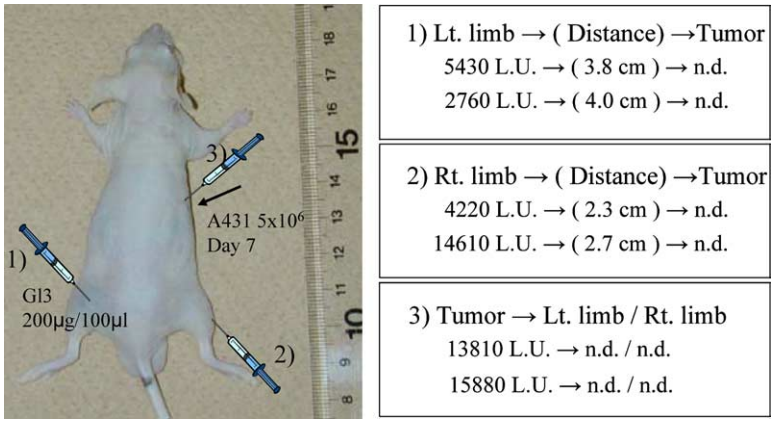

Fig. 7. Measurement of luciferase activity after injection of plasmid DNA: (1) Intramuscular injection into left hindlimb, (2) intramuscular injection into right hindlimb, and (3) injection into tumor. n.d., not detected.

(Fig. 6). Distant metastasis could not be detected in all mice. Finally, we tested the possibility that plasmid DNA should occur within the tumor by leakage of plasmid DNA from the hindlimb to the circulating system. As shown in Fig. 7, our present study denied this possibility. Intramuscular injection of pCMV-luciferase-GL3 plasmid DNA into the hindlimb resulted in the detection of luciferase activity only in hindlimb, while no luciferase activity could be detected in the tumor. In contrast, even with an injection into the tumor, luciferase activity was only detected in the tumor and not the hindlimb. These results demonstrate that the transfection of the injection of plasmid DNA results in the limited expression within the injection site.

Discussion

As the feasibility of therapeutic angiogenesis using angiogenic growth factors such as VEGF, FGF, and HGF has been reported in experimental models [9], in vivo transfer of these genes into the muscle is currently being tested as gene therapy for untreatable peripheral arterial disease. In this study, we evaluated the most

important safety issue regarding the use of plasmid vector encoding angiogenic growth factors in the patients who have tumors. The two important questions are: (1) the promotion of tumor by the intramuscular injection of naked plasmid DNA and (2) the expression of transgene in tumors by the leakage of plasmid DNA from the injected sites. In this study, we chose HGF as a model of safety evaluation, since the previous reports demonstrated the oncogenic capability of HGF-Met signaling, the invasiveness, and metastatic potential [18–22]. Indeed, we confirmed the stimulatory effects of human rHGF on scattering and proliferation using A431 cells in vitro. Although HGF transgenic mice develop a remarkably broad array of histologically distinct tumors of both mesenchymal and epithelial origin [30], in this study, no change was detected in tumor growth in mice intramuscularly transfected with HGF plasmid DNA into the hindlimb of tumor bearing mice. In addition, no evidence of metastasis and death could be detected throughout the observation period. Although human and mouse HGF concentrations were significantly increased in tumor tissues in vivo than in A431cells in vitro, there was no significant difference in local HGF concentration in tumor tissue between the mice intramuscularly injected with human HGF plasmid and control vector, while local human HGF was overexpressed in the hindlimbs. In fact, human HGF concentration was significantly elevated only in femoral muscle where human HGF plasmid was injected, but not elevated in other tissues and blood. In addition, despite an extremely high concentration of HGF in tumor tissues on mice, there was no significant difference in circulating HGF concentration between the tumor bearing mice and no treatment mice. How about the comparability of the present study with human clinical trial? In this study, we employed 200 μg human HGF plasmid DNA by the intramuscular injection into the hindlimb, while the body weights of the mice were pretty small about 30 g/body. Even at this dose, we confirmed that transfection of HGF plasmid DNA is enough to induce blood flow and the number of capillary density. That amount per body weight is equal to about 400–600 mg in a human case. In a human clinical trial, a total of 4 or 8 mg of human HGF plasmid DNA was used in trial. Even in extremely large amounts of human HGF plasmid, no promotion of the tumor was detected. Regarding the expression of plasmid DNA within the tumor from the leakage of plasmid DNA from the hindlimb to the circulating system, our present study denies this possibility. Intramuscular injection of plasmid DNA resulted in the detection of luciferase activity only in the hindlimb, while no luciferase activity could be detected in the tumor. These results denied the potential side effects of therapeutic angiogenesis using plasmid DNA in cancer patients with peripheral arterial disease.

However, there are still some elements of this series of safety evaluations to limit the conclusions. In this study, we used the mice bearing the tumors on their backs, but in clinical situation there are many different types of tumors that arise from variable tissues. In addition, other angiogenic growth factors such as VEGF may not be same as HGF, since serum concentration of VEGF, but not HGF, was increased after gene transfer into the human legs using naked plasmid DNA [31]. Nevertheless, the present study clearly demonstrated that local over-expression of HGF did not induce tumor promotion. HGF gene therapy is safe for mice tumor and further studies have to be done in the case of human diseases. The present information is important to consider the stimulation of new vessel formation by HGF as a new therapeutic option in angiogenesis-dependent conditions such as wound healing, inflammatory diseases, ischemic heart disease, myocardial infarction, and peripheral arterial disease.

Acknowledgments

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References

[1] Second European Consensus Document on Chronic Critical Leg Ischemia, Circulation 84 (1991) IV 1–26.
[2] J.M. Isner, K. Walsh, J. Symes, A. Pieczek, S. Takeshita, J. Lowry, K. Rosenfield, L. Weir, E. Brogi, D. Jurayj, Arterial gene transfer for therapeutic angiogenesis in patients with peripheral artery disease, Hum. Gene Ther. 7 (1996) 959–988.
[3] I. Baumgartner, A. Pieczek, O. Manor, R. Blair, M. Kearney, K. Walsh, J.M. Isner, Constitutive expression of phVEGF165 after intramuscularly gene transfer promotes collateral vessel development in patients with critical limb ischemia, Circulation 97 (1998) 1114–1123.
[4] D.W. Losordo, P.R. Vale, J.F. Symes, C.H. Dunnington, D.D. Esakof, M. Maysky, A.B. Ashare, K. Lathi, J.M. Isner, Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia, Circulation 98 (1998) 2800–2804.
[5] T. Nakamura, T. Nishizawa, M. Hagiya, T. Seki, M. Shimonishi, A. Sugimura, K. Tashiro, S. Shimizu, Molecular cloning and expression of human hepatocyte growth factor, Nature 342 (1989) 440–443.
[6] Y. Nakamura, R. Morishita, S. Nakamura, M. Aoki, A. Moriguchi, K. Matsumoto, T. Nakamura, J. Higaki, T. Ogihara, A vascular modulator, hepatocyte growth factor, is associated with systolic pressure, Hypertension 28 (1996) 409–413.
[7] Y. Nakamura, R. Morishita, J. Higaki, I. Kida, M. Aoki, A. Moriguchi, K. Yamada, S. Hayashi, Y. Yo, H. Nakano, K. Matsumoto, T. Nakamura, T. Ogihara, Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic

fibroblast growth factor but not with vascular endothelial growth factor, J. Hypertens. 14 (1996) 1067–1072.
[8] S. Hayashi, R. Morishita, J. Higaki, M. Aoki, A. Moriguchi, I. Kida, S. Yoshiki, K. Matsumoto, T. Nakamura, Y. Kaneda, T. Ogihara, Autocrine–paracrine effects of overexpression of hepatocyte growth factor gene on growth of endothelial cells, Biochem. Biophys. Res. Commun. 220 (1996) 539–545.
[9] R. Morishita, S. Nakamura, S. Hayashi, Y. Taniyama, A. Moriguchi, T. Nagano, M. Taiji, H. Noguchi, S. Takeshita, K. Matsumoto, T. Nakamura, J. Higaki, T. Ogihara, Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy, Hypertension 33 (1999) 1379–1384.
[10] E. Van Belle, B. Witzenbichler, D. Chen, M. Silver, L. Chang, R. Schwall, J.M. Isner, Potentiated angiogenic effect of scatter factor/hepatocyte growth factor via induction of vascular endothelial growth factor: the case for paracrine amplification of angiogenesis, Circulation 97 (1998) 381–390.
[11] E. Gherardi, M. Sharpe, K. Lane, A. Sirulnik, M. Stoker, Hepatocyte growth factor/scatter factor (HGF/SF), the c-met receptor and the behaviour of epithelial cells, Symp. Soc. Exp. Biol. 47 (1993) 163–181.
[12] K. Matsumoto, T. Nakamura, Hepatocyte growth factor: molecular structure, roles in liver regeneration, and other biological functions, Crit. Rev. Oncogenesis 3 (1992) 27–54.
[13] J.S. Rubin, D.P. Bottaro, S.A. Aaronson, Hepatocyte growth factor/scatter factor and its receptor, the c-met proto-oncogene product, Biochim. Biophys. Acta 1155 (1993) 357–371.
[14] E. Sonnenberg, D. Meyer, K.M. Weidner, C. Birchmeier, Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development, J. Cell Biol. 123 (1993) 223–235.
[15] M. Stoker, E. Gherardi, M. Perryman, J. Gray, Scatter factor is a fibroblast-derived modulator of epithelial cell mobility, Nature 327 (1987) 239–242.
[16] C.S. Cooper, M. Park, D.G. Blair, M.A. Tainsky, K. Huebner, C.M. Croce, G.F. Vande Woude, Molecular cloning of a new transforming gene from a chemically transformed human cell line, Nature 311 (1984) 29–33.
[17] M. Park, M. Dean, K. Kaul, M.J. Braun, M.A. Gonda, G. Vande Woude, Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors, Proc. Natl. Acad. Sci. USA 84 (1987) 6379–6383.
[18] S. Bellusci, G. Moens, G. Gaudino, P. Comoglio, T. Nakamura, J.P. Thiery, J. Jouanneau, Creation of an hepatocyte growth factor/scatter factor autocrine loop in carcinoma cells induces invasive properties associated with increased tumorigenicity, Oncogene 9 (1994) 1091–1099.
[19] E.M. Rosen, J. Knesel, I.D. Goldberg, L. Jin, M. Bhargava, A. Joseph, R. Zitnik, J. Wines, M. Kelley, S. Rockwell, Scatter factor modulates the metastatic phenotype of the EMT6 mouse mammary tumor, Int. J. Cancer 57 (1994) 706–714.
[20] K.M. Weidner, J. Behrens, J. Vandekerckhove, W. Birchmeier, Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells, J. Cell Biol. 111 (1990) 2097–2108.
[21] T. Nakamura, K. Matsumoto, A. Kiritoshi, Y. Tano, Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: in vitro analysis of tumor-stromal interactions, Cancer Res. 57 (1997) 3305–3313.
[22] K. Date, K. Matsumoto, K. Kuba, H. Shimura, M. Tanaka, T. Nakamura, Inhibition of tumor growth and invasion by a four-kringle antagonist (HGF/NK4) for hepatocyte growth factor, Oncogene 17 (1998) 3045–3054.
[23] W. Jiang, S. Hiscox, K. Matsumoto, T. Nakamura, Hepatocyte growth factor/scatter factor, its molecular, cellular and clinical implications in cancer, Crit. Rev. Oncol. Hematol. 29 (1999) 209–248.
[24] Y. Taniyama, R. Morishita, M. Aoki, H. Nakagami, K. Yamamoto, K. Yamazaki, K. Matsumoto, T. Nakamura, Y. Kaneda, T. Ogihara, Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat and rabbit hindlimb ischemia models: preclinical study for treatment of peripheral arterial disease, Gene Ther. 8 (2001) 181–189.
[25] T. Couffinhal, M. Silver, L.P. Zheng, M. Kearney, B. Witzenbichler, J.M. Isner, Mouse model of angiogenesis, Am. J. Pathol. 152 (1998) 1667–1679.
[26] A. Yamada, K. Matsumoto, H. Iwanari, K. Sekiguchi, S. Kawata, Y. Matsuzawa, T. Nakamura, Rapid and sensitive enzyme-linked immunosorbent assay for measurement of HGF in rat and human tissues, Biomed. Res. 16 (1995) 105–114.
[27] Y. Tsurumi, M. Kearney, D. Chen, M. Silver, S. Takeshita, J. Yang, J.F. Symes, J.M. Isner, Treatment of acute limb ischemia by intramuscularly injection of vascular endothelial growth factor gene, Circulation 96 (1997) II-382–II-388.
[28] M. Tsujie, Y. Isaka, H. Nakamura, E. Imai, M. Hori, Electroporation-mediated gene transfer that targets glomeruli, J. Am. Soc. Nephrol. 12 (2001) 949–954.
[29] S. Rong, M. Bodescot, D. Blair, J. Dunn, T. Nakamura, K. Mizuno, M. Park, A. Chan, S. Aaronson, G.F. Vande Woude, Tumorigenicity of the met proto-oncogene and the gene for hepatocyte growth factor, Mol. Cell. Biol. 12 (1992) 5152–5158.
[30] H. Takayama, W.J. LaRochelle, R. Sharp, T. Otsuka, P. Kriebel, M. Anver, S.A. Aaronson, G. Merlino, Diverse tumorigenesis associated with aberrant development in mice overexpressing hepatocyte growth factor/scatter factor, Proc. Natl. Acad. Sci. USA 94 (1997) 701–706.
[31] J.M. Isner, I. Baumgartner, G. Rauh, R. Schainfeld, R. Blair, O. Manor, S. Razvi, J.F. Symes, Treatment of thromboangiitis obliterans (Buerger's disease) by intramuscularly gene transfer of vascular endothelial growth factor: preliminary clinical results, J. Vasc. Surg. 28 (1998) 964–973.