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Soluble Fms-like Tyrosine Kinase-1 Expression Inhibits the Growth of Multiple Myeloma in Nude Mice

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Abstract Angiogenesis is an essential factor in the growth and progression of hematological malignancies including multiple myeloma (MM). Vascular endothelial growth factor and its receptors have been shown to be targets for treating tumors. This study explores the effect of adenovirus-mediated delivery of soluble vascular endothelial growth factor receptor Fms-like tyrosine kinase-1 (sFLT-1) on the growth of MM cell line KM3 in nude mice. sFLT-1 cDNA was amplified by reverse transcription-polymerase chain reaction from human umbilical vein endothelial cells and was used as a transgene to construct an adenoviral vector carrying sFLT-1 (ADV-sFLT). Cell proliferation and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assays were carried out to evaluate the effect of ADV-sFLT on human umbilical vein endothelial cells and KM3 cells *in vitro*. Eighteen female BALB/c nude mice were inoculated subcutaneously with KM3 cells, and they were randomly divided into three groups and injected intravenously with ADV-sFLT, ADV-LacZ, or phosphate-buffered saline (PBS). The volume of KM3 xenografts was measured twice a week. Three weeks after the initial treatment, the volume of MM xenografts in the mice treated with ADV-sFLT, ADV-LacZ, or PBS was 770.32±28.73 mm^3, 1983.36±43.72 mm^3, and 2042.05±82.31 mm^3, respectively (P<0.01, ADV-sFLT versus ADV-LacZ or PBS). The value of microvessel density was 29.17±6.85, 79.17±7.35, and 78.83±8.54 in the tumors treated with ADV-sFLT, ADV-LacZ, and PBS, respectively (P<0.01, ADV-sFLT versus ADV-LacZ or PBS). This study suggested that the adenovirus-mediated sFLT-1 gene greatly inhibits MM-derived tumor growth and angiogenesis in mouse xenograft, and might serve as a new therapy for MM.

Keywords multiple myeloma; gene therapy; adenovirus; vascular endothelial growth factor; receptor

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Multiple myeloma (MM) accounts for 1% of all malignancies and 10% of malignant hematological neoplasms. Despite the use of conventional and high-dose chemotherapy, it remains incurable [1]. Novel approaches need to be found to improve the treatment efficiency of MM. Drugs such as thalidomide achieve responses in patients with refractory and relapsed MM based on its antiangiogenic activity [2–4]. The bone marrow microenvironment has become a target to treat MM.

Angiogenesis is associated with the growth, progression and metastasis of most solid tumors [5]. As a result, decreased angiogenesis with novel antiangiogenic agents appears to be a promising and exciting therapeutic approach for cancer. Further studies have also suggested that angiogenesis plays a very important role in hematological malignancies including MM and there is a strong correlation between microvessel density (MVD) and disease progression and poor prognosis of MM [6–10]. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and exerts its biological functions by interacting with its receptors such as Fms-like tyrosine kinase-1 (FLT-1) and fetal liver kinase-1 (Flk-1)/kinase insert domain-containing receptor (KDR) [11]. It has been reported that the patients of MM overexpress VEGF and have high levels of serum VEGF correlated with poor prognosis [12]. Importantly, FLT-1 protein but not Flk-1 is expressed in MM cell lines and MM patient cells [13]. It has been found

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that VEGF binds to FLT-1 with an affinity 7–10 times higher than to KDR. These data provided the framework for targeting VEGF and FLT-1 in the novel therapeutics of MM. Several anti-VEGF drugs such as GW654652 and PTK787 have been shown to have anti-MM activity *in vitro* [14,15].

One novel method of inhibiting the angiogenic action of VEGF is to use soluble FLT-1 (sFLT-1), a potent and selective inhibitor of VEGF. sFLT-1 is a known, endogenously expressed, alternatively spliced form of the FLT-1 receptor [16]. It is devoid of the FLT-1 transmembrane domain and the entire intracellular tyrosine kinase-containing region. sFLT-1 binds to VEGF with the same affinity and specificity as that of the full-length receptor, but this binding does not initiate signal transduction [17, 18]. In addition, the mutant consisting of the first three ectodomains of FLT-1 is reported to be able to bind to VEGF with affinity similar to that of sFLT-1, indicating the sFLT-1(1–3) could be sufficient as a potent inhibitor of VEGF mediating angiogenesis [17]. sFLT-1 inhibits the angiogenic action of VEGF in two ways. First, by sequestering VEGF, thus making it unavailable for angiogenic action; and second, by heterodimerizing with the extracellular ligand binding region of the membrane spanning FLT-1 and Flk-1/KDR receptors, thereby blocking the phosphorylation and activation of downstream signal transduction pathways for endothelial cell proliferation [16,18].

Several studies have shown that adenovirus-mediated or plasmid-mediated gene transfer of sFLT-1 inhibited tumor angiogenesis and growth both *in vitro* and *in vivo* [19–22]. Almost all of these studies focused on solid tumor therapy.

In this study, we investigated the effects of sFLT-1 gene therapy on the hematological tumor MM in nude mice. We constructed the sFLT-1 gene that codes the 1–3 immunoglobulin (Ig)-like domains and established an adenoviral vector expressing sFLT-1. We used an adenoviral vector for the intravenous delivery of sFLT-1 and showed its therapeutic effect in a murine model system of human MM.

Materials and Methods

Cell culture

KM3 human MM cell line was kindly presented by Dr. Jian HOU (Second Military Medical University, Shanghai, China). KM3 and human embryonic kidney cell line 293 from Amercian Type Culture Collection (ATCC, Manassas, USA) were maintained in complete Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco BRL, Garlsbad, USA), 1% penicillin and streptomycin. Human umbilical vein endothelial cells (HUVECs) were obtained from ATCC and grown in DMEM containing 10% fetal bovine serum supplemented with 75 μg/ml endothelial cell growth supplement (ECGS) (Sigma-Aldrich, St. Louis, USA). All of the cells were grown at 37 ºC in a humidified atmosphere of 5% CO_2.

Cloning of the 1–3 Ig-like domains of FLT-1

Total cellular RNA was isolated from HUVECs with Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The isolated RNA was applied as a template for the first strand cDNA synthesis by reverse transcription (RT) and cDNA used as a template for polymerase chain reaction (PCR). The upstream sense primer (5′-TTGGTACCCATGGTCAGCTACTGGGACA-3′, nt 250–268, *Kpn*I), and the downstream antisense primer (5′-GGAAGCTTCCTCAATGCACTGAGGTGTT-3′ nt 1230–1213, *Hin*dIII) were designed to amplify the coding sequence for the 1–3 Ig-like domains of FLT-1 according to the sequence information from GenBank (accession No. X51602) [17,19]. The amplified product was subcloned directly into the PMD-18T easy cloning vector (TaKaRa, Dalian, China) following the manufacturer's instructions. The resultant clone, T-sFLT, was confirmed by DNA sequencing and restriction digestion analysis (*Kpn*I and *Hin*dIII).

Construction of recombinant adenoviral vector expressing sFLT-1

T-sFLT was digested with *Kpn*I and *Hin*dIII restriction enzymes and ligated to the pShuttle-CMV vector (Stratagene, Glen Burnie, USA). After sequence verification, the resultant plasmid was named pShuttle-sFLT. This shuttle vector was then linearized with *Pme*I and used for homologous DNA recombination with AdEasy adenoviral vector (Stratagene) containing the adenovirus genome in the *Escherichia coli* strain BJ5183. The plasmid containing the genome of Ad-sFLT was obtained as a result of this recombination and designated pAd-sFLT. Insertion of the pShuttle-sFLT in the plasmid was confirmed by PCR analysis and restriction mapping with *Pac*I digestion.

Recombinant adenovirus ADV-sFLT expressing sFLT-1 was generated by transfection of 293 cells with *Pac*I-digested pAd-sFLT and confirmed by PCR analysis. The virus titer expressed in p.f.u./ml was determined by plaque formation assay using 293 cells. The control virus ADV-LacZ was constructed similarly.

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Western blot analysis for sFLT-1

Conditioned media and cell lysates were generated by infecting KM3 cells at a multiplicity of infection (MOI) of 100 of either ADV-sFLT or ADV-LacZ for 72 h. Cell lysates and conditioned media were electrophoretically separated on a 12% sodium dodecyl sulfate-polyacrylamide gel under reducing conditions. The gel was then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, USA), probed by rabbit polyclonal antibody FLT (Santa Cruz Biotechnology, Santa Cruz, USA), then reacted with horseradish peroxidase-labeled anti-rabbit mouse antibody. Detection of the bands was carried out using an enhanced chemiluminescent system (Cell Signaling Technology, Danvers, USA).

Verification of expression of sFLT-1 by enzyme-linked immunosorbent assay (ELISA)

Expression of sFLT-1 was verified with the ELISA method using a commercial kit (VEGFR1, referred to in this study as sFLT-1; Boster, Wuhan, China) according to the instructions provided by the manufacturer. KM3 cells were planted in a 6-well plate at a density of 3×10^5 cells/well and infected with ADV-sFLT or ADV-LacZ at a MOI of 100. The supernatants were taken at 12, 24 and 48 h and subjected to ELISA detection. Briefly, the 96-microwell plate provided in the kit was coated with mouse monoclonal anti-sFLT-1 antibody and washed with wash buffer. Cell supernatant samples were diluted 1:50 by sample dilutant, and 100 μl of diluted sample was added to the microwell plate in duplicate. Similarly, sFLT-1 standards ranging from 0.03 to 2 ng/ml were added to the microwell plates in duplicate. After washing four times with wash buffer, 200 μl of biotin-conjugated polyclonal antibody against sFLT-1 was added and incubated at room temperature for 2 h. After washing the wells again three times, tetramethylbenzidine substrate solution was added and incubated at room temperature for 30 min. The enzymatic reaction was stopped by adding 100 μl of stop solution to the wells, and absorbance was determined by spectrophotometric readings at 450 nm. The sFLT-1 concentration in the cell supernatant samples was calculated based on the standard curve.

HUVEC proliferation inhibition assay

Conditioned media was obtained from KM3 cells infected with ADV-sFLT or ADV-LacZ. HUVECs were grown in 12-well plates at 37 ºC in DMEM. At 80% confluence, DMEM was removed, the cell layers were washed with PBS, and 1.0 ml of 5×conditioned medium was added. After 15 min, purified recombinant human VEGF165 (R&D Systems, Minneapolis, USA) at 10 ng/ml was added to each well and incubated at 37 ºC. After 72 h, the cells were trypsinized, and the number of viable cells was counted using a Trypan blue assay.

KM3 proliferation inhibition assay

The inhibitory effect of ADV-sFLT on KM3 cell growth was assessed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), which was cleaved by viable cells to yield a dark blue formazan product. KM3 cells were infected with ADV-sFLT or ADV-LacZ at an MOI of 100 for 2 h, then the infection media was removed. Serum-free DMEM medium with or without VEGF165 (10 ng/ml) was added. The cells were incubated for 1, 2, 3 and 4 d and a cell count was carried out daily by MTT assay.

In vivo animal studies

To test the effect of ADV-sFLT on the growth of MM *in vivo*, 4-week-old BALB/c female nude mice were divided into three groups with six mice in each group. All experimental procedures conformed with institutional guidelines for the care and use of laboratory animals in Sun Yat-sen University (Guangzhou, China), and to the NIH Guide for Care and Use of Laboratory Animals (revised 1985). All mice were inoculated with 3×10^7 KM3 cells subcutaneously on day 0. The mice were then given 1×10^9 p.f.u. of ADV-sFLT, ADV-LacZ or PBS, injected into the tail vein on day 1. Animal weight and tumor measurements were obtained twice a week. Tumor volume was calculated using the formula $V=L\times W^2/2$ (L, length; W, width). Animals were killed at the end of day 21 or when the one-dimensional tumor diameter exceeded 2.0 cm. To evaluate the expression of sFLT-1 in the plasma of treated mice, plasmas were obtained when the mice were killed, then subjected to ELISA detection as described above.

Immunohistochemical analysis for microvessel formation

Angiogenesis was measured by estimating the MVD. The tumor specimens of mice were harvested under anesthesia and fixed immediately in formalin. To analyze the microvessel formation in tumors, antigen retrieval was carried out by boiling the sections for 10 min in 0.01 M citrate buffer in a microwave oven. Tumor sections of five micrometers were then treated with 3% goat serum for 1 h at 25 ºC to reduce nonspecific staining followed by 1 h incubation at 37 ºC with goat anti-mouse CD31 polyclonal antibody (Boster) at a concentration of 1:100.

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The regions of highest MVD (“hot spot” regions) were scanned at low magnification (100×) and counted at higher magnification (200×). MVD was expressed as the average number of vessels in three hot spots.

Statistical analysis

Data were reported as the mean±SE of measurement. $P<0.05$ was considered statistically significant in all of the analyses. Analysis of significance of difference among multiple groups was carried out by ANOVA. The statistical analyses were carried out with SPSS software, version 12.0 for Windows (SPSS, Chicago, USA).

Results

Cloning and validation of sFLT-1

The sFLT-1 was obtained by PCR amplification from HUVECs and was subcloned into PMD-18T easy vector [**Fig. 1(A)**]. Restriction digestion mapping and DNA sequencing of this vector confirmed the presence of sFLT-1 [**Fig. 1(B)**].

Construction of recombinant adenovirus expressing sFLT-1

As a first step of constructing an adenovirus, the sFLT-1 was subcloned into the pShuttle-CMV vector, generating pShuttle-sFLT. Recombinant adenovirus pAd-sFLT was then generated through co-transfecting the pShuttle-sFLT with an E1A/B-deleted adenoviral backbone vector. Presence of sFLT-1 in the recombinant adenovirus was confirmed by PCR (980 bp). Restriction digestion of pAd-sFLT with *Pac*I resulted in 3 kb and 30 kb fragments. The 3 kb fragment indicated the presence of pShuttle-sFLT (**Fig. 2**).

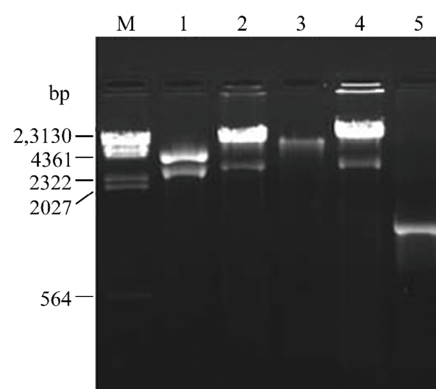

Fig. 2 Recombinant adenovirus expressing soluble Fms-like tyrosine kinase-1 (sFLT-1) identified by *Pac*I enzyme digestion and polymerase chain reaction (PCR)
M, marker λ *Hin*dIII digest; 1, pShuttle-sFLT digested with *Pac*I; 2 and 4, pAd-sFLT digested with *Pac*I; 3, pAd-easy plasmid digested with *Pac*I; 5, PCR product of pAd-sFLT plasmid.

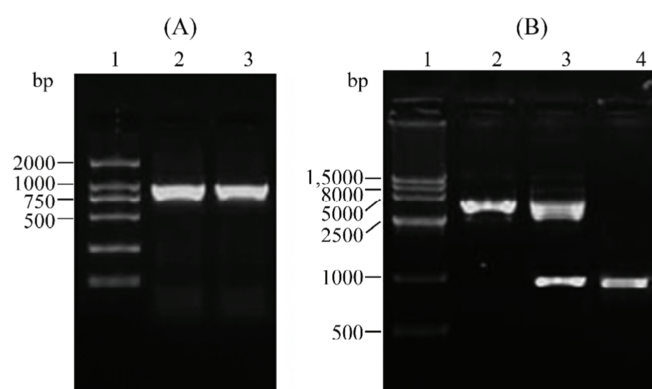

Fig. 1 Reverse transcription-polymerase chain reaction (RT-PCR) and cloning of 1–3 immunoglobulin-like domains of soluble Fms-like tyrosine kinase-1 (sFLT-1)
(A) RT-PCR product of sFLT-1. 1, marker DL 2000; 2 and 3, 980 bp PCR products. (B) Cloning plasmid T-sFLT was identified by double enzyme digestion and PCR. 1, marker; 2, cloning plasmid was digested with *Hin*dIII; 3, cloning plasmid was digested with *Kpn*I and *Hin*dIII; 4, PCR product of cloning plasmid.

Expression of sFLT-1

To determine the expression/secretion of sFLT-1 *in vitro*, KM3 cells were infected with 100 MOI of ADV-sFLT. This conditioned media and cell lysate were subjected to Western blot analysis. The results showed that sFLT-1 was present in conditioned media and cell lysate from ADV-sFLT but not ADV-LacZ, confirming the adenovirus-mediated secretion of sFLT-1. The sFLT-1 identified by Western blot was approximately 37 kDa [**Fig. 3(A)**]. The expression of sFLT-1 was further subjected to ELISA verification. The results showed that the KM3 cells infected with ADV-sFLT efficiently secreted sFLT-1 [**Fig. 3(B)**].

Inhibition of HUVEC proliferation by ADV-sFLT

The concentrated conditioned media obtained from ADV-sFLT, ADV-LacZ infected cells and uninfected cells was applied to the HUVECs grown in 12-well plates, followed by stimulation with VEGF of 10 ng/ml. The cell count after 72 h incubation was $(9.62\pm1.32)\times10^4$, $(32.37\pm2.64)\times10^4$ and $(33.00\pm2.55)\times10^4$ in ADV-sFLT, ADV-LacZ and PBS, respectively ($P<0.01$). Conditioned media from

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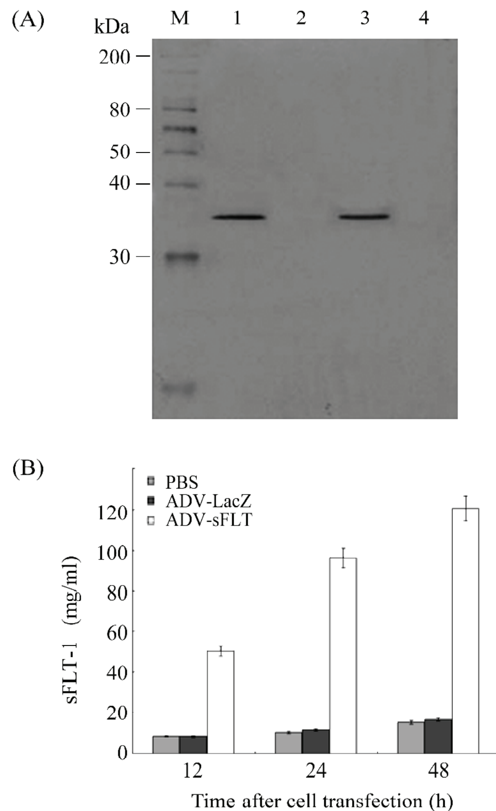

Fig. 3 Identification of the expression and secretion of soluble Fms-like tyrosine kinase-1 (sFLT-1) in multiple myeloma KM3 cells with Western blot and enzyme-linked immunosorbent assay (ELISA)

(A) Expression of sFLT-1 detected by Western blot. 1, culture supernatant of KM3 transfected by ADV-sFLT; 2, culture supernatant of KM3 transfected by ADV-LacZ; 3, cell lysates of KM3 transfected by ADV-sFLT; 4, cell lysates of KM3 transfected by ADV-LacZ. (B) Secretion of sFLT-1 in KM3 cells detected by ELISA at 12, 24 and 48 h after transfection.

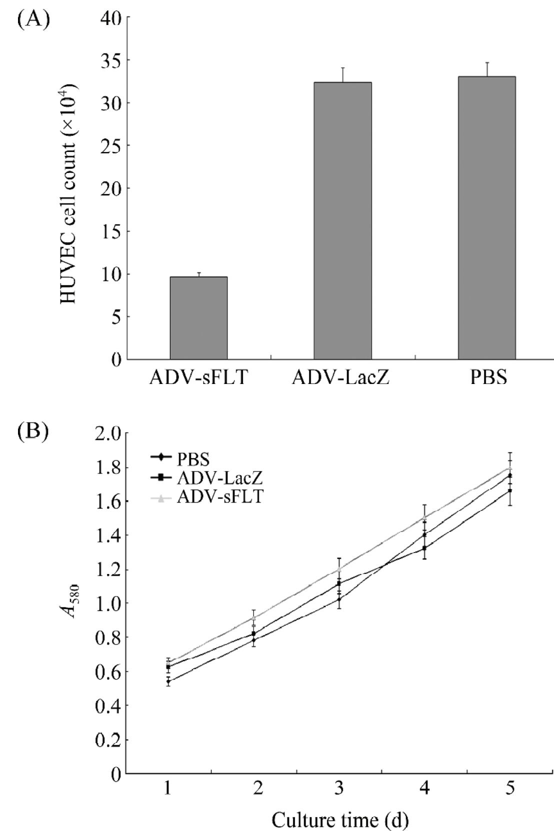

Fig. 4 Effect of adenoviral vector carrying soluble Fms-like tyrosine kinase (ADV-sFLT) on proliferation of human umbilical vein endothelial cells (HUVECs) and multiple myeloma KM3 cells *in vitro*

(A) Effect of ADV-sFLT on proliferation of HUVECs and KM3 cells *in vitro*, $P<0.01$, ADV-sFLT versus ADV-LacZ or PBS. (B) Effect of ADV-sFLT on proliferation of KM3 cells *in vitro* by MTT assay, $P>0.05$, ADV-sFLT versus ADV-LacZ or PBS.

ADV-sFLT cells inhibited HUVEC proliferation by 70% and 71% compared with conditioned media from ADV-LacZ and PBS, respectively [**Fig. 4(A)**].

Inhibition of KM3 proliferation by ADV-sFLT *in vitro*

MTT proliferation test showed that there was no significant difference among the KM3 cells treated with ADV-sFLT, ADV-LacZ or PBS ($P>0.05$) in the presence or absence of VEGF. The results showed that ADV-sFLT can not inhibit the proliferation of KM3 cells directly *in vitro* [**Fig. 4(B)**].

Effect of ADV-sFLT on MM growth *in vivo*

At day 21, the mean size of tumors in the ADV-sFLT, ADV-LacZ and PBS groups reached approximately 770.32±28.73 mm^3, 1983.36±43.72 mm^3, and 2042.05±82.31 mm^3, respectively. The growth of tumors treated with ADV-sFLT therapeutic viruses was inhibited by 157% and 165% compared with the ADV-LacZ and PBS groups, respectively ($P<0.01$). These results suggested that sFLT-1 gene therapy was effective in suppressing the growth of xenografted MM (**Fig. 5**). Plasma levels of sFLT-1 in ADV-sFLT, ADV-LacZ and PBS were determined by ELISA. The average sFLT-1 concentration level in the ADV-sFLT, ADV-LacZ and PBS groups was 9.29±1.19 ng/ml, 0.94±0.17 ng/ml and 0.76±0.16 ng/ml, respectively. The expression level of sFLT-1 in ADV-sFLT was almost 10-fold higher than the ADV-LacZ and PBS groups, which were almost negligible (**Fig. 6**).

Effect of ADV-sFLT on tumor microvessel formation

To determine whether sFLT-1 gene therapy inhibited

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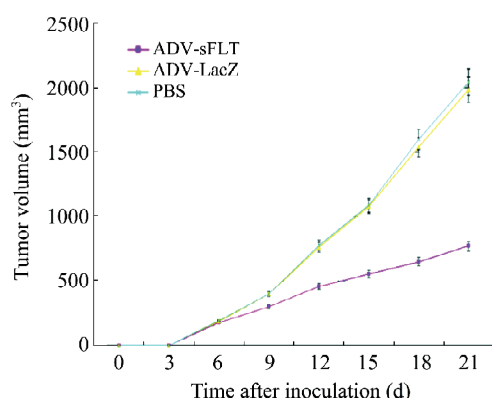

Fig. 5 Effect of adenoviral vector carrying soluble Fms-like tyrosine kinase (ADV-sFLT), ADV-LacZ, and phosphate-buffered saline (PBS) on tumor growth of multiple myeloma KM3 cells in xenograft mice

$P<0.01$, ADV-sFLT versus ADV-LacZ or PBS.

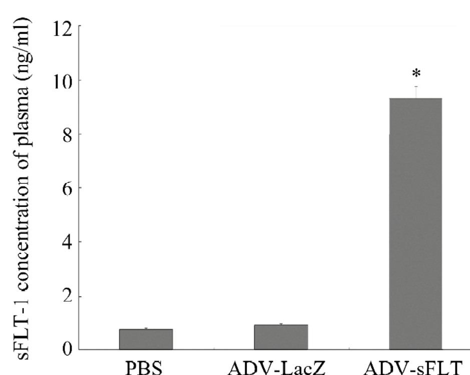

Fig. 6 Plasma levels of soluble Fms-like tyrosine kinase-1 (sFLT-1) as determined by enzyme-linked immunosorbent assay

The concentration of sFLT-1 was very high after intravenous injection of adenoviral vector ADV-sFLT compared with ADV-LacZ and phosphate-buffered saline (PBS) (*$P<0.01$).

intratumoral vessel formation, we assessed the MVD in the tumor nodules resected from ADV-sFLT and control mice using endothelial cell-specific CD31 immunostaining. The mean number of microvessels in the ADV-sFLT, ADV-LacZ and PBS groups was 29.17±6.85, 79.17±7.35 and 78.83±8.54, respectively ($P<0.01$). As shown in **Fig. 7**, the density of CD31$^+$ vascular endothelial cells was dramatically reduced in tumor nodules resected from mice treated with ADV-sFLT, suggesting a significant antiangiogenic effect of sFLT-1 gene therapy against

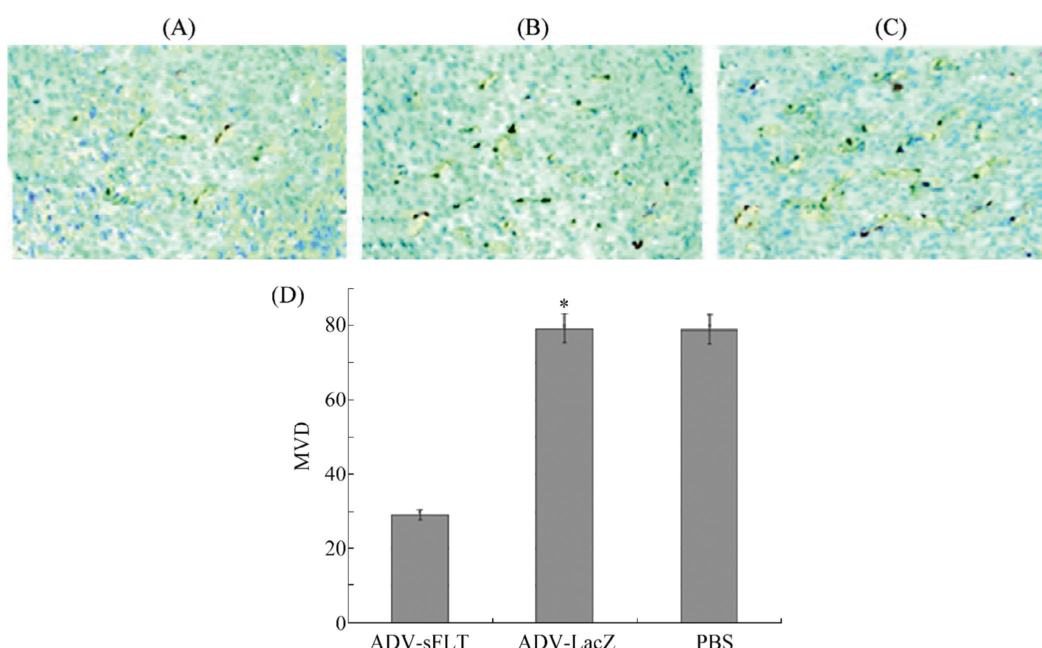

Fig. 7 Effect of adenoviral vector carrying soluble Fms-like tyrosine kinase (ADV-sFLT), ADV-LacZ, and phosphate-buffered saline (PBS) on intratumoral vessel formation in multiple myeloma KM3 xenograft mice

Immunohistochemical analysis for microvessel formation with CD31 antibody in KM3 tumors treated by ADV-sFLT (A), ADV-LacZ (B) or PBS (C). (D) Comparative analysis of microvessel density (MVD) in different groups. Data are presented as mean±SE (n=6). The ANOVA test was used to analyze the results (*$P<0.01$, ADV-sFLT versus ADV-LacZ or PBS). Magnification, 200×.

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experimental MM.

Discussion

Antiangiogenic therapy shows promise as a strategy for cancer treatment because most tumor growth is dependent on the formation of new blood vessels. Several approaches have been tested recently for their antitumor effectiveness in delivering sFLT-1 gene therapy *in vivo* [19–22]. Mahasreshti *et al*. reported that adenovirus-mediated sFLT-1 significantly inhibited the proliferation of ovarian tumor [20]. In addition, sFLT-1 resulted in a significant increase in the survival times of mice compared with control mice. In another study, Ye *et al*. observed that systemic delivery of the sFLT-1 gene mediated by mammalian cells could be effective in inhibiting tumor angiogenesis and growth [19]. These data showed that sFLT-1 gene therapy was practical and effective. Nearly all of these studies focused on solid tumors. Preclinical studies show that VEGF directly triggers MM cell growth, migration and survival, increases osteoclast activity and chemotaxis, and inhibits dendritic cell maturation [23]. Thus, VEGF and its receptors could be important targets for the development of new drugs and present an alternative method for the control of MM.

In this study, we applied adenovirus-mediated sFLT-1 gene therapy to a MM mouse model. We successfully constructed ADV-sFLT expressing the 1–3 Ig-like domains of sFLT-1, as verified by Western blot and ELISA assay. Results presented in this report showed that the transfer of the sFLT-1 gene effectively inhibited the growth of human MM tumors in immunodeficient mice. We observed the direct effect of ADV-sFLT on proliferation of KM3 cells in the presence/absence of VEGF. Unfortunately, we could not find any relationship between the proliferation of KM3 cells and ADV-sFLT. This result showed that ADV-sFLT exerted its anti-MM effect without affecting the tumor cells proliferation directly. To determine whether the inhibitory effect of ADV-sFLT on tumor growth was associated with the suppression of tumor angiogenesis, we examined the distribution of the endothelial cell-specific antigen CD31 in tumor section. As expected, the MVD of tumor was decreased in the ADV-sFLT group compared with the control. This result showed that ADV-sFLT exerted its biological effect mainly through inhibiting the angiogenesis of tumor without affecting the growth of tumor itself. According to our study, sFLT-1 can also inhibit the growth of hematological tumor. Systemic delivery of ADV-sFLT could be effective in inhibiting MM growth through decreasing the microvessel density of tumors.

Recombinant adenoviral vectors are effective tools for gene delivery because of their superiority of gene transfer efficacy *in vivo* in a wide spectrum of both dividing and non-dividing cell types. Potential side-effects of adenoviruses have been reported, such as liver toxicity and animal death [24,25]. However, no visible toxicity such as weight loss, change in animal behavior, decreased food or water intake, or premature mortality, was found in animals injected with ADV-sFLT in our study. Perhaps the time we observed was not long enough to find its side-effects.

Recently, several anti-VEGF targeted therapies have been used to inhibit proliferation of MM. Some artificially synthesized drugs such as GW654652 and PTK787/ZK222584, which are small-molecular tyrosine kinase inhibitors inhibiting VEGF receptors, can inhibit the proliferation of MM cells line and patients MM cell *in vitro* [14,15]. Another VEGF receptor tyrosine kinase inhibitor, pazopanib, can also inhibit growth and migration of MM cells *in vitro* and *in vivo* [26]. All of these drugs belong to non-native inhibitors and have certain toxicities to the human body. However, sFLT-1 is a known potent and selective endogenous inhibitor of VEGF-mediated angiogenesis. Theoretically it has no side-effects. Comparison of sFLT-1 with other synthesized drugs will be carried out in further research.

In conclusion, ADV-sFLT gene therapy targeted to bone marrow could effectively suppress the development of MM without any side-effects in a mouse xenograft model. The mechanism of its anti-MM is based on its antiangiogenic effect. This method might be more practical for clinical applications. It is further confirmation that sFLT-1 could serve as a new approach for the treatment of MM.

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