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*Original Article*

# Angiopoietin-1 facilitates recovery of hematopoiesis in radiated mice

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**Abstract:** Angiopoietin-1 (Ang-1) plays a critical role in the regulation of endothelial cell survival and vascular maturation and stability. However, its role in hematopoiesis is not clear. Here, we determined effect of Ang-1 on the recovery of hematopoiesis in radiated mice. By injecting an Ang-1 plasmid, we found that Ang-1 was preferentially expressed in bone marrow (BM) of femur from radiated mice. This injection resulted in elevated blood counts and serum VEGF level. The blockade in S phase of cell cycle in mouse BM stromal cells following radiation was attenuated by injection of Ang-1 plasmid. In addition, injection of Ang-1 plasmid attenuated the radiation-mediated inhibition of Tie2 expression. Furthermore, through analyzing Notch1 expression, we found that injection of Ang-1 plasmid increased Notch mRNA expression in radiated mice. In conclusion, these findings suggest that Ang-1 facilitates the recovery of hematopoiesis in radiated mice with the involvement of Notch signaling pathway.

**Keywords:** Angiopoietin-1, hematopoiesis, Notch, signaling pathway

## Introduction

Radiation, chemotherapy or conditioning of bone marrow transplantation (BMT) damages blood vessels, sinus and stroma of bone marrow [1, 2]. Rebuilding the BM microenvironment plays a crucial role in hematopoietic recovery after BMT. At present, little is known about self-repair of BM stroma after BM damage by radiation or chemotherapy. The injury of BM stroma makes it difficult for donor cells to rebuild BM hematopoietic microenvironment. Until now, there is no effective way to prevent the damage or facilitate the recovery of BM stroma. Recently, it has been shown that the recovery of hematopoiesis can be accelerated by BM niche vascular reconstruction [3]. However, the underlying mechanism by which angiogenesis is formed in BM is unclear.

Angiopoietin-1 (Ang-1) is a member of angiopoietin family (Ang-1, Ang-2, Ang-3 and Ang-4) and the ligand of the vascular endothelium-specific receptor tyrosine kinase Tie2 [4]. Ang-1, along with family member Ang-2, is a key regulator for angiogenesis events including vascular stabilization and remodeling [4]. Ang-1 is critically important in vessel maturation and is also involved in migration, adhesion and survival of endothelial cells [5]. Ang-1/Tie2 signalling inhibits vascular endothelial growth factor (VEGF)-mediated vascular permeability via several downstream signaling cascades [6]. It has been shown that hematopoietic stem and progenitor cells regulate the regeneration of their niche by secreting Ang-1 [7].

There are two forms of angiogenesis: vasculogenesis and angiogenesis [8]. Vasculogenesis is the process that gives rise to primitive blood vessels during embryonic development. A number of growth factors such as VEGF and fibroblast growth factor (FGF) are involved in vasculogenesis. By binding to its specific receptors (VEGFR1 and VEGFR2), VEGF promotes vasculogenesis by stimulating proliferation and migration of endothelial cells [9]. It has been shown that VEGF recruits BM hematopoietic stem cells and endothelial progenitor cells and promotes proliferation of microvessels and blood niche in BM, thereby inducing hematopoiesis [10]. Angiogenesis is the process of new

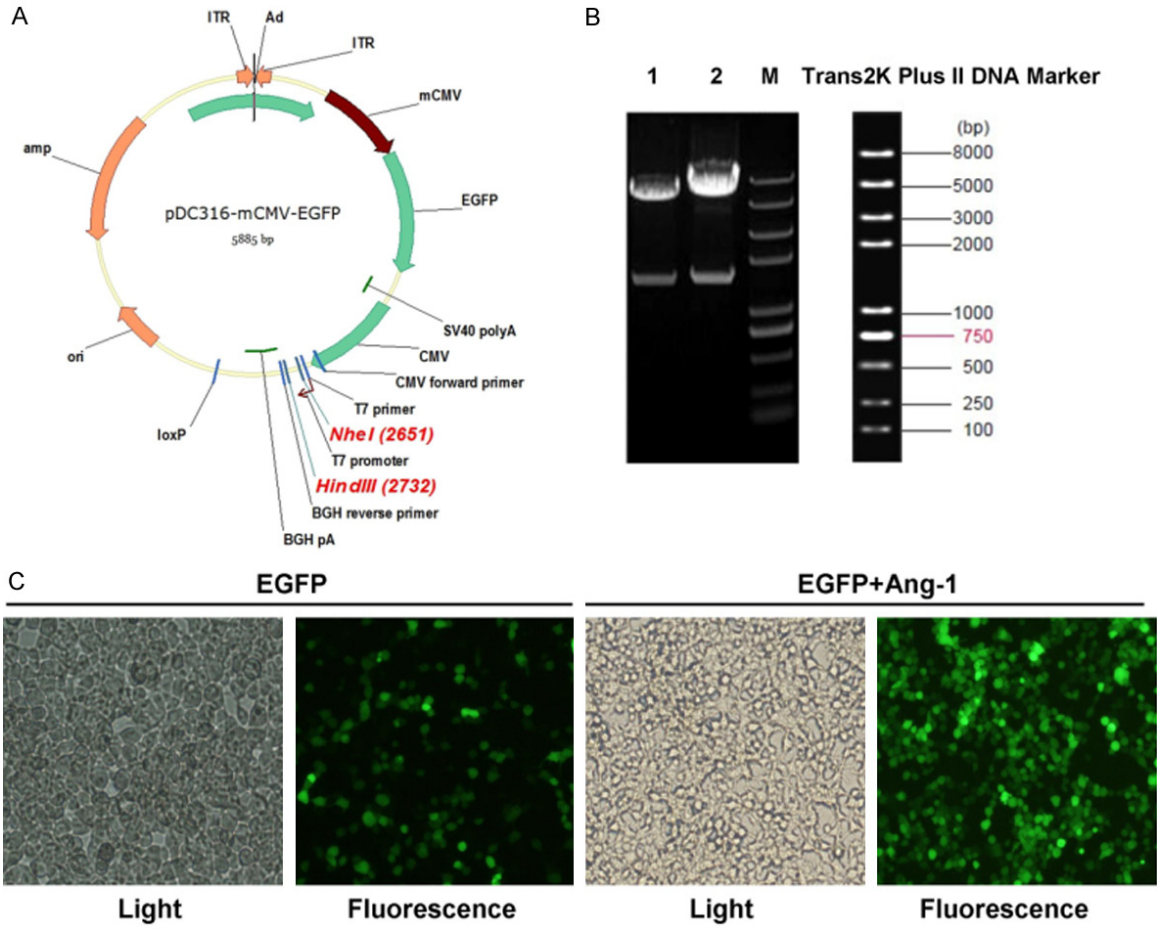


**Figure 1.** Transfection efficiency of Ang-1 plasmid in vitro. A. The scheme of Plasmid pDC316-mCMV-EGFP-ANGPT1. B. The confirmation of the product after dual enzyme digestion by agarose gel. The PCR size of target gene is 1497 bp. C. Infection efficiency of Ang-1 plasmid in vitro. The 293T cells were transfected with either Ad-EGFP or Ad-EGFP/hAng-1 plasmid and the fluorescence intensity was measured by light or fluorescent microscope.

blood vessel formation from primitive vascular plexus or existing vessels, involving the remodeling and expansion of vascular network (8). Ang-1 is important in angiogenesis by facilitating transformation of endothelial cell layer to multi-cellular vascular structures [11].

Given the critical role of Ang-1 in angiogenesis, whether it affect hematopoiesis remains unclear. In the present study, we constructed and injected an adenoviral Ang-1 plasmid into Kunming mice to express exogenous Ang-1. We measured Ang-1 expression in peripheral blood, liver and femur on different days. To determine whether expression of exogenous Ang-1 has effect on parameters of hematopoiesis, we measured blood counts, cell cycles, serum VEGF level, CD34 and Tie2 expression and Notch mRNA expression in radiated mice injected with Ang-1 plasmid.

## Materials and methods

### *Plasmid construction*

Plasmid pDC316-mCMV-EGFP-ANGPT1 was constructed using vector pDC316-mCMV-EGFP (**Figure 1A**) as follows.

*A.* Polymerase chain reaction (PCR) assay. PCR was performed in a 50 μl final volume, containing 10 μl 5× Fast fu buffer, 4 μl dNTP Mix (2.5 mM), 1 μl Fast pfu polymerase, 1 μl cDNA, 2 μl primers (each) and 30 μl $ddH_2O$. The primers for ANGPT1 included NdeI and HindIII restriction sites. The pDC316-ANGPT1-NheI-Forward: 5'-CTAGCTAGCCACCATGACAGTTTTCC TTTCCTTTGCTTTCC-3'; pDC316-ANGPT1-HindIII-Reverse: 5'-CCC AAGC TTTCAAAAATCTAAAGGTCGAATCATC-3'. The PCR reaction was performed using a program as following: initial denaturation step at 95°C for 3 min, then 30 cycles of

amplification reaction with denaturation at 95°C for 20 sec, annealing at 55°C for 30sec and extension at 72°C for 2 min, and followed by a final extension step at 72°C for 5 min. The PCR product was separated by electrophoresis in 1.5% agarose gel with a molecular size of 1497 bp.

*B.* Enzyme digestion of plasmid DC316-mCMV-EGFP and ANGPT1 by Nhe I and Hind III. Enzyme digestion of plasmid and PCR product was performed in a 20 μl final volume. For the plasmid, the mixture contains 1 μl DC316-mCMV-EGFP, 2 μl 10× Buffer, 2 μl 10× BSA, 1 μl Nhe, 1 μl HindIII and dd$H_2O$ up to 20 μl. For the PCR product, the mixture is 14 μl PCR product, 2 μl 10× Buffer, 2 μl 10× BSA, 1 μl Nhe and 1 μl Hind III. The mixtures were incubated in water bath at 37°C for 2 h. The fragments from enzyme digestion were confirmed by PCR (**Figure 1B**).

*C.* DNA ligation and bacteria transformation. Ligation of fragments of plasmid DNA and ANGPT1 PCR product was conducted in a 10 μl final volume. The mixture contains 6 μl fragment of ANGPT1 PCR product, 2 μl fragment of pDC316-mCMV-EGFP plasmid DNA, 1 μl 10× DNA Ligase Buffer and 1 μl T4 DNA Ligase. The mixtures were incubated in water bath at 22°C for 2 h.

After ligation, 10 μl ligation mixture was suspended and incubated with 100 μl competent bacteria on ice for 30 min. After at 42°C for 45 sec and on ice for 2 min, the suspension was mixed with 400 μl LB medium and incubated in a thermostatic shaker for 1 h. After centrifuge, 400 μl volume was removed and the leftover was resuspended and evenly coated on a LB plate containing 100 μg/ml Ampicillin. The plate was incubated at 37°C overnight. The single colony was amplified by inoculating two single colonies on a LB plate containing 100 μg/ml Ampicillin and shaking at 250 r/min overnight. The clone was ready for confirmation and experiments.

*Establishment of radiated mouse model*

Kunming mice were randomly divided into four groups with 10 mice in each group, including control group, radiation group, Ang-1 group and EGFP group. Radiation was performed by using a 60Co-γ ray therapy machine to radiate whole body of mice. The total dose was 6.0 Gy with an absorbed dose rate of 0.56 Gy/min. For Ang-1 or EGFP treatment group, 0.1 ml ($3\times10^8$ pfu/ml) Ad-EGFP/hAng-1 plasmid or Ad-EGFP plasmid was injected into each mouse via the tail vein 24 hours after radiation, respectively.

*Cell cycle assay*

Cell cycle of stromal cells was determined by flow cytometry following the manufacturer instruction (Lianke Bio Ltd, Hangzhou, China). The DNA content was assessed by staining the ethanol-fixed cells with PI. BM was harvested from tibia and femur of normal and radiated mice. Stromal cells were generated by culturing BM cells with 10% MEM/F12 medium for 10 days. The cells were harvested and fixed in ice-cold 70% ethanol overnight at 4°C. After centrifugation, cells were incubated with 1 ml Reagent A for 30 min at 37°C in dark. Cells were analyzed using a Accuri C6 flow cytometer (Becton Dickinson, San Jose, CA, USA).

*ELISA assay*

ELISA assay was performed according to the manufacturer's instruction (mouse Ang-1 ELISA Kit, Uscn Life, Wuhan, China). Briefly, serum was collected from radiated mice injected with or without Ang-1 plasmid. 50 μl samples were added into each well and incubated with 50 μl Buffer A at 37°C for 1 h. After wash, 100 μl Buffer B were added and incubated at 37°C for 30 min. After wash, 90 μl substrate solution were added and incubated in dark at 37°C for 15 min. The reaction was stopped with 50 μl stop solution. The optical density of each well was determined using a micro-plate reader (Spectra Plus 384, MD) set to 450 nm.

*Immunohistochemistry assay*

The mouse femur was fixed by immersing it with 10% formaldehyde for 48 h and then decalcificated by treating the fixed femur tissue with 10% EDTA solution for 25 days with replacement of decalcification solution once every 7 d. The slides were made using Rotary Slicer (RM2135, LEICA) following the manufacturer instruction. After Xylene dewaxing and gradient alcohol rehydration, the slides were incubated with 3% $H_2O_2$ 37°C for 20 min to block endogenous peroxidase. The slides then were incubated with a primary antibody at 4°C overnight. After rinse with PBS 3 times, the slides were incubated with a secondary antibody at 37°C for 30 min. DAB staining was then

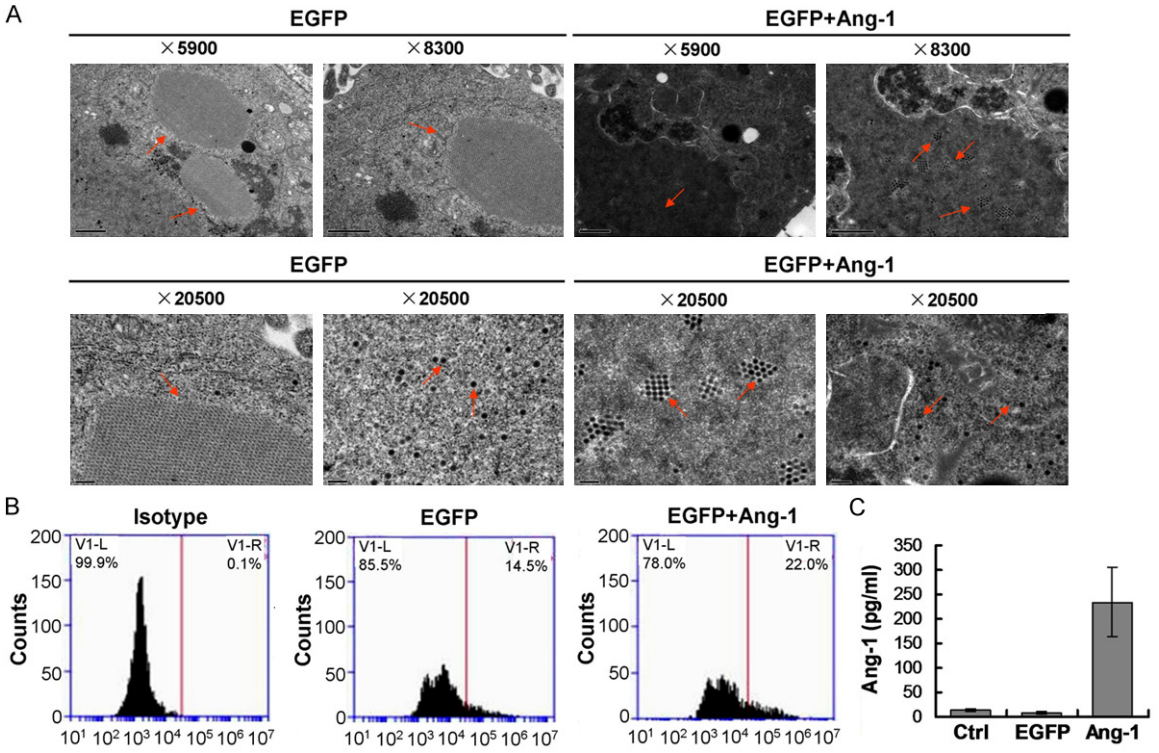


**Figure 2.** Electron microscope and the flow cytometry assay. A. The images by electron microscope showing the virus within transfected 293T cells indicated by red arrows. B. Histograms of flow cytometry showing fluorescence in mouse PBMCs transfected with either Ad-EGFP or Ad-EGFP/hAng-1 plasmid. Expression level of fluorescence was gated and calculated based on isotype control. C. Graph showing Ang-1 concentration by ELISA in the culture supernatant of cells transfected with or without Ad-EGFP/hAng-1 plasmid.

performed and the slides were restained with Hematoxylin and dried with air for microscopy. Primary antibodies were Ang-1 (abcam, Cat#: ab94684, 1:100), CD34 (abnova, Cat#: PAB-18289, 1:200), Tie2 (Novus Biologicals, Cat#: NBP 1-69753, 1:200). Secondary antibody was goat anti-rabbit HRP Antibody (abcam, cat.#: ab136817, 1:500).

*Immunofluorescence assay*

The liver was harvested from normal or radiated mice injected with or without Ang-1 plasmid. The tissue was embedded and sliced using a freezing microtome. The slice was then incubated with 100 μL DAPI staining solution at room temperature in dark for 15 min. After wash, the slice was ready for microscopic analysis.

*Notch mRNA expression by quantitative PCR assay*

Total RNA was isolated using TRIzol (Invitrogen). The cDNA was generated using RevertAid First Strand cDNA synthesis Kit (Fermentas). The primers were: Notch1-Forward GGGTTTCAAAGTGTCAGAGGC; Notch1-Reverse: TCGTCCATCAGAGCACCATC. Actin-Forward: GAGACCTTCAACACCCCAGC; Actin-Reverse: ATGTCACGCACGATTTCCC. The quantitative PCR was performed on CFX connect Real-Time PCR System and analyzed using software BIO-RAD CFX Manager.

*Statistical analysis*

The values were expressed as mean ± standard deviation. Statistical analysis was performed using the Student t test. The difference was considered to be statistically significant at $P<0.05$.

## Results

*Transfection efficiency of Ang-1 plasmid in vitro*

After completion of constructing Ad-EGFP/hAng-1 plasmid, we tested whether the adenoviral plasmid efficiently infected cells in vitro. We first transfected 293T cells with either

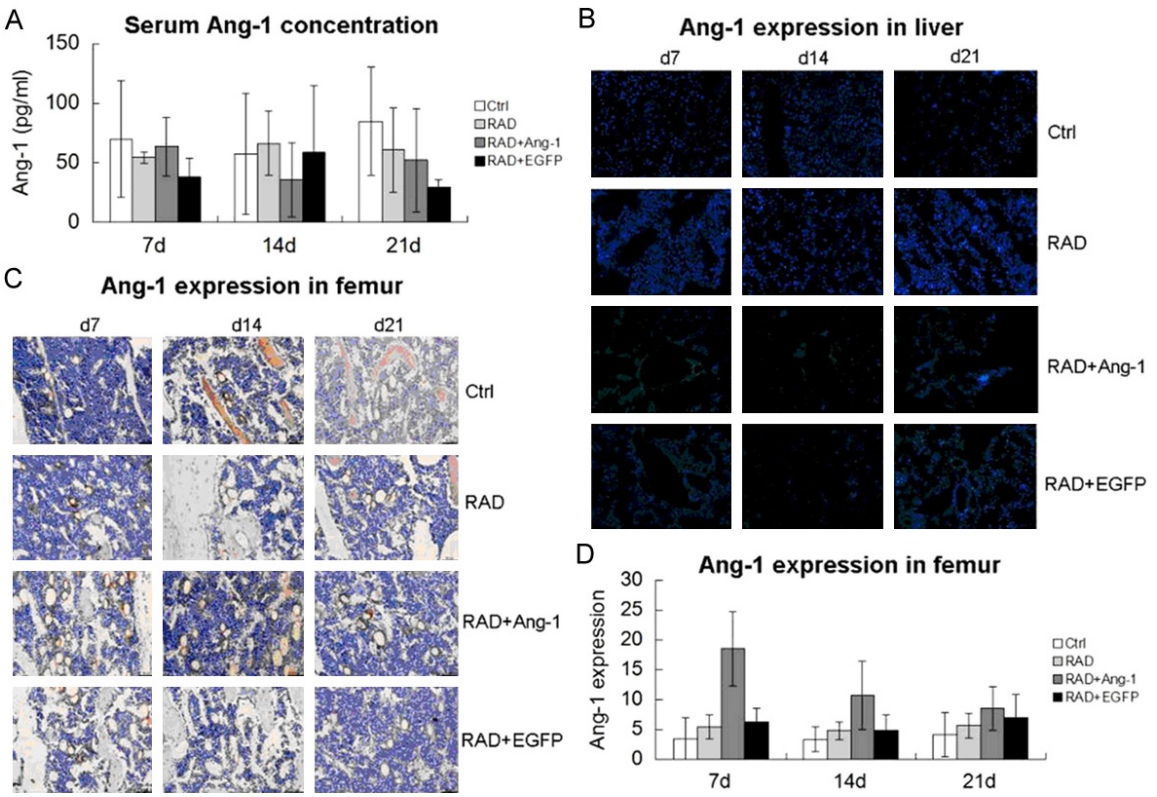


**Figure 3.** Expression of exogenous Ang-1 in radiated mice. (A) Graph showing serum Ang-1 concentration by ELISA in Kuming mice radiated alone or injected with Ad-EGFP or Ad-EGFP/hAng-1 plasmid. Serum was collected on day 7, 14, and 21 after plasmid injection. The normal Kuming mice were used as a control. (B-D) Fluorescence (B) or immuno-histochemistry (C) images showing Ang-1 expression in liver (B) or femur (C) of radiated mice injected with Ad-EGFP or Ad-EGFP/hAng-1 plasmid. The green fluorescence and brown or tan staining represents Ang-1 expression in (B) and (C), respectively. A quantitative analysis counting average numbers of positive Ang-1-stained cells from 3 fields of each femur slide was graphed in (D).

Ad-EGFP or Ad-EGFP/hAng-1 plasmid and observed expression of GFP by fluorescent microscope. As shown in **Figure 1C**, the fluorescence intensity was bright in both transfections and the majority of cells were fluorescent 72 h after transfection, suggesting a high transfection efficiency. Next, we utilized electron microscope to visualize the virus inside transfected cells. Under electron microscope, considerable quantities of viral particles can be seen in the cytoplasma and nuclei of 293T cells transfected with either Ad-EGFP or Ad-EGFP/hAng-1 plasmid (**Figure 2A**). The viruses scattered over or lined up in the cytoplasma and nuclei. By flow cytometry analysis, we were able to quantify the transfection efficiency. Compared to isotype control, approximately 22% of cells were transfected with Ad-EGFP/hAng-1 plasmid (**Figure 2B**). Strongly supporting this finding, Ang-1 concentration was dramatically elevated in the culture supernatant of cells transfected with Ad-EGFP/hAng-1 plasmid while cells transfected with Ad-EGFP plasmid showed negligible level of Ang-1 in culture supernatant. Taken together, these in vitro results indicated that this adenoviral plasmid was able to efficiently infected cells.

*Expression of exogenous Ang-1 in radiated mice*

Next, we wanted to test whether injection of Ang-1 plasmid resulted in exogenous expression of Ang-1 in radiated mice. To do this, we collected peripheral blood, liver and femur from normal or radiated mice injected Ad-EGFP or Ad-EGFP/hAng-1 plasmid. By ELISA, we found that there was no significant difference of serum Ang-1 concentration in normal or radiated mice injected with or without Ang-1 plasmid (**Figure 3A**). However, we found that Ang-1 expression was increased in both tissues of liver and femur from radiated mice injected with Ang-1 plasmid. Using immuno-fluorescence assay, we observed that green fluorescence, the marker of exogenous Ang-1 expres-

sion, was stained in interior vascular wall of liver in mice injected with Ang-1 plasmid. As controls, the green fluorescence was absent from the liver tissue in mice injection without Ang-1 plasmid (**Figure 3B**). Similar finding was seen in femur from mice injected with Ang-1 plasmid. By immuno-histochemistry, we observed that Ang-1 staining (brown or tan staining) was enhanced in marrow cavity of femur from mice injected with Ang-1 plasmid (**Figure 3C**). A quantitative analysis was performed by counting average numbers of positive Ang-1-stained cells from 3 fields of ea

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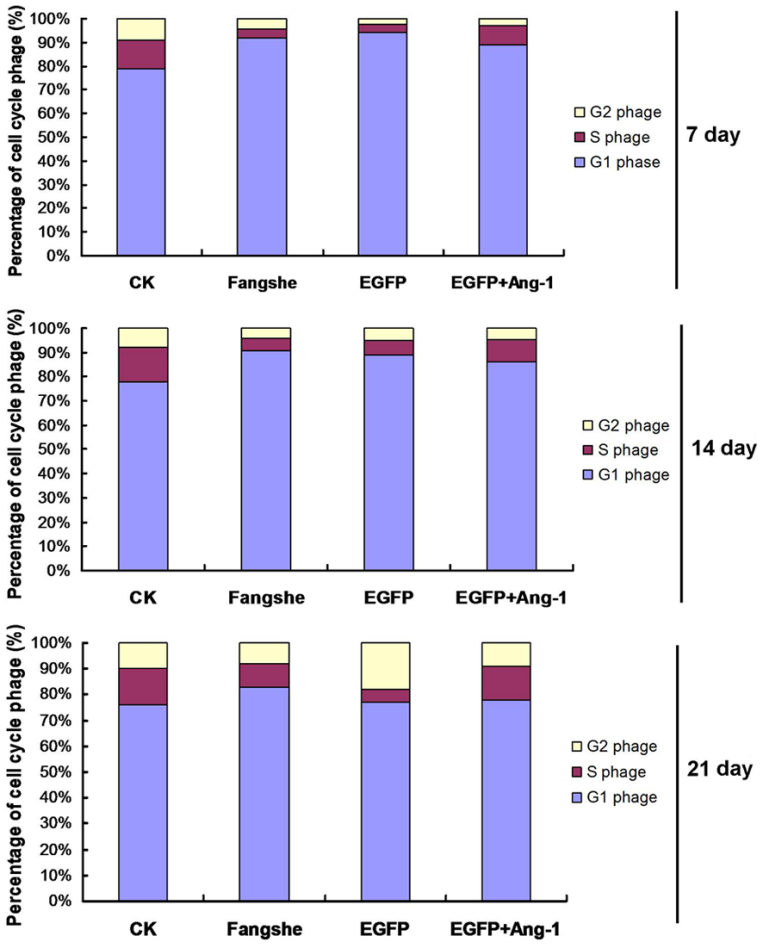


**Figure 5.** Effect of Ang-1 on cell cycle of BM stromal cells in radiated mice. Left panel: histograms of flow cytometry showing cell cycles of BM stromal cells from radiated mice injected with Ad-EGFP or Ad-EGFP/hAng-1 on different days. A quantitative analysis calculating G1, S or G2 phase was graphed in right panel.

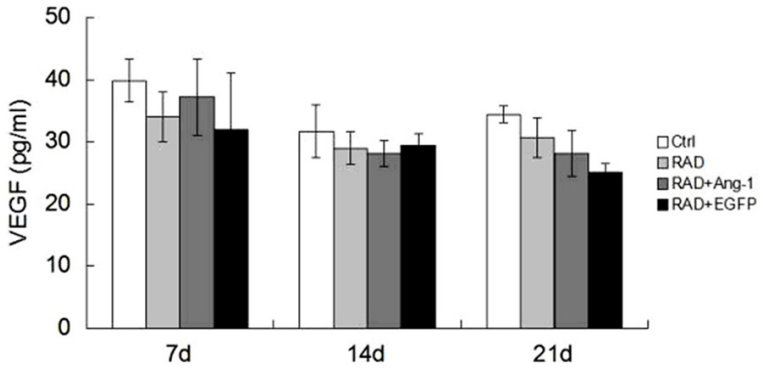


**Figure 6.** Effect of Ang-1 on serum VEGF level in radiated mice. A graph showing serum level of VEGF by ELISA in radiated mice injected with Ad-EGFP or Ad-EGFP/hAng-1 on different days.

ELISA in radiated mice injected with or without Ang-1 plasmid. As shown in **Figure 6**, the radiation caused a modest decrease in VEGF concentration compared to normal mice. When injected with Ang-1 plasmid, serum VEGF level elevated in radiated mice compared to radiated mice injected with EGFP plasmid on day 7. We did not see a difference of serum VEGF level in radiated mice injected with Ang-1 or EGFP plasmid on day 14 and 21 (**Figure 6**).

*Effect of Ang-1 on expression of CD34 and Tie2 in radiated mice*

We next determined the effects of Ang-1 on CD34 expression in femur of radiated mice using immuno-**histo**chemistry. As shown in **Figure 7A**, we did not see a difference of CD34 staining (brown or tan staining) in femur of radiated mice injected with Ang-1 or EGFP plasmid on all time points (7 d, 14 d and 21 d), suggesting that CD34 may not be involved in Ang-1-mediated protection of BM injury.

Tie2 is expressed on hematopoietic stem cells and plays an important role in hematopoiesis. We also determined effect of Ang-1 on Tie2 expression in femur of radiated mice using immuno-histochemistry. As shown in **Figure 7B**, radiation resulted in a decrease of Tie2 expression (brown or tan staining) in femur of mice. Injection of Ang-1 plasmid attenuated the inhibition of Tie2 expression by radiation, mostly on day 7. Compared to mice injected with EGFP plasmid, mice injected with Ang-1 plasmid had more Tie2 staining cells (brown or tan staining) on all time points.

*Effect of Ang-1 on Notch expression in radiated mice*

To explore the underlying mechanism by which Ang-1 promotes the recovery of radiated-induced BM injury, we determined effect of Ang-1 on Notch expression in radiated mice by

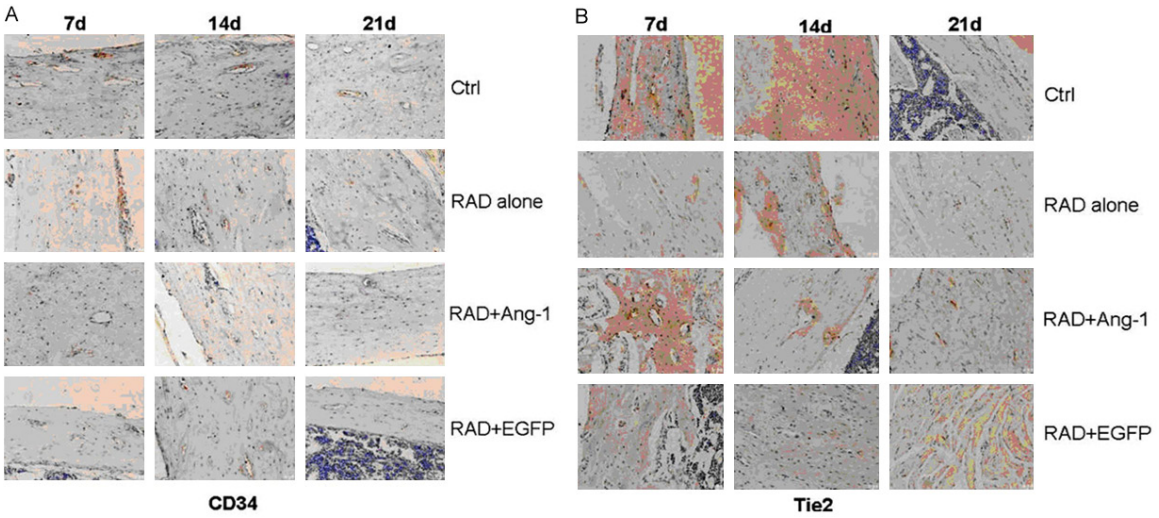


**Figure 7.** Effect of Ang-1 on expression of CD34 and Tie2 in radiated mice. (A, B) Images of immuno-histochemistry showing CD34 (A) and Tie2 (B) expression in femur of radiated mice injected with Ad-EGFP or Ad-EGFP/hAng-1 on different days. The brown or tan staining represents positive CD34 or Tie2 expression.

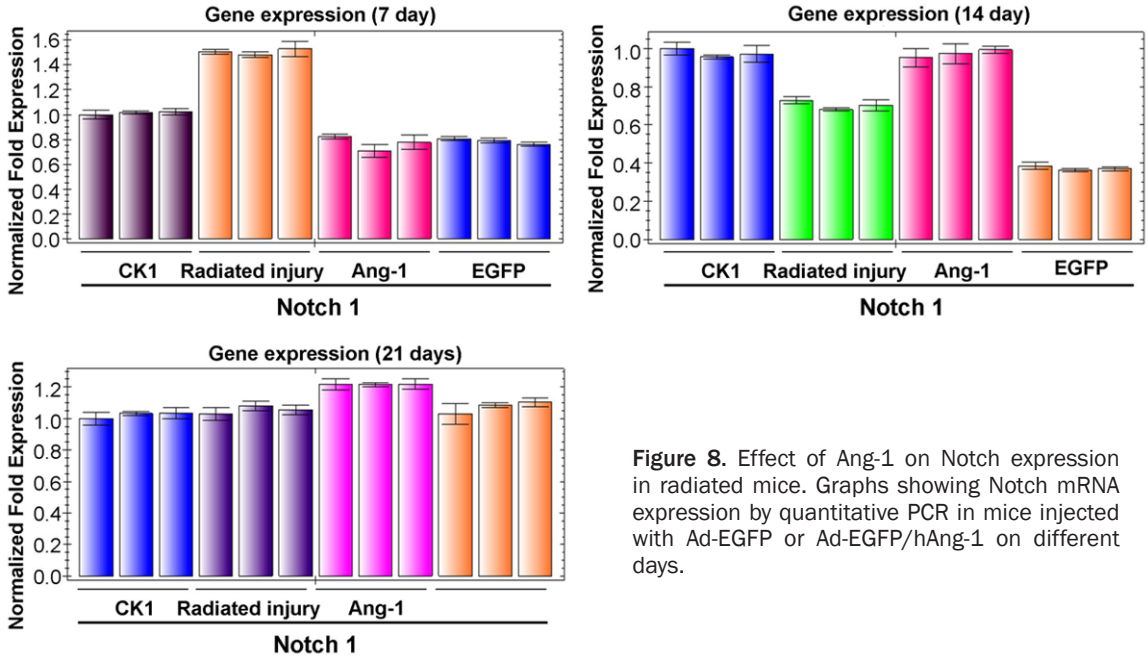


**Figure 8.** Effect of Ang-1 on Notch expression in radiated mice. Graphs showing Notch mRNA expression by quantitative PCR in mice injected with Ad-EGFP or Ad-EGFP/hAng-1 on different days.

quantitative PCR. As shown in **Figure 8**, Notch mRNA expression level was enhanced by radiation on day 7. However, on day 14, mice injected with Ang-1 plasmid showed increased Notch mRNA expression compared to mice radiated alone or mice injected with EGFP plasmid. Although diminished on day 21, Notch mRNA expression level remained higher in mice injected with Ang-1 plasmid than mice injected with EGFP plasmid. These results indicated that Notch signaling pathway may be involved in Ang-1-mediated recovery of radiated-induced BM injury.

## Discussion

Hematopoietic reconstitution post-BMT usually begins after the recovery of hematopoietic microenvironment. Improving the hematopoietic microenvironment promotes the recovery of hematopoiesis. A variety of factors were involved in the recovery of hematopoiesis. In

the present study, by using an in vivo system, we showed that Ang-1 facilitates the recovery of hematopoiesis in mice following radiation.

BM is the organ to generate RBCs, WBCs and platelets. BM suppression or injury leads to lower counts of one or more types of blood cells. Therefore, measuring blood counts can serve as an indicator of whether BM is suppressed [12]. Supporting this, we found that radiation decreased blood counts of WBCs and platelets in mice. With injection of Ang-1 plasmid, blood counts of WBCs and platelets recovered in radiated mice, suggesting a role of Ang-1 in the recovery of hematopoiesis.

It has been shown that Ang-1 and VEGF play an important role in the recovery of hematopoiesis after chemotherapy-induced BM suppression [13]. By binding to its specific receptor, VEGF stimulates proliferation and migration of endothelial cells and increases vascular permeability and promotes angiogenesis [14]. In addition, VEGF signaling pathway is able to recruit hematopoietic stem cells and endothelial progenitor cells, promote growth of BM capillary and blood niche, thereby inducing hematopoiesis [15]. We found that serum VEGF level elevated in radiated mice injected with Ang-1 plasmid, suggesting VEGF plays a role in Ang-1-mediated recovery of hematopoiesis.

Tie2 receptor is mainly expressed on endothelial cells as well as hematopoietic stem/progenitor cells. Ang-1 binding to Tie2 on endothelial cells activates Tie2 phosphorylation, induces and maintains migration, survival of endothelial cells [16, 17]. This interaction also attracts vascular smooth muscle cells to surround and support endothelial cells and form the wall of blood vessels, which promotes remodeling, maturation and function of blood vessels. Regarding the role in hematopoiesis, studies have shown that Ang-1/Tie2 pathway promotes bone marrow mesenchymal stem cell survival by maintaining hematopoietic stem cell quiescence in the bone marrow niche [5, 18]. In contrast, other studies found that Tie2 activation contributes to hemangiogenic regeneration [19]. Under physiological condition, Tie2 expression is weak in blood vessels of mouse bone marrow [20]. The BM suppression by 5-FU treatment increases plasma level of VEGF and vascular Tie2 expression . The inhibition of Tie2 results in a reduced angiogenesis and delayed recovery of hematopoiesis [19]. In the present study, we found that mice injected Ang-1 plasmid had increased Tie2 expression in femur. Given the role of Tie2 in hematopoiesis, our results further confirmed that Ang-1 is involved in the recovery of radiation-mediated BM injury.

We showed that Ang-1 plays an important role in the recovery of hematopoiesis suppressed by radiation. However, the underlying mechanism is unclear. Here, we found that Notch signaling is involved in Ang-1-mediated recovery of hematopoiesis. The Notch pathway is an evolutionarily conserved signalling pathway fundamentally important in regulating development, including blood cell development in invertebrates and vertebrates [21]. During hematopoiesis, blood cells at different stages express Notch proteins and interact with stromal cells expressing Notch ligands in bone marrow to activate Notch signaling [22]. Through the interaction, Notch signaling pathway regulates survival, proliferation and differentiation of hematopoietic stem/progenitor cells. It has been shown that expression of Notch proteins (Notch1 and Notch4) increased when endothelial cells were stimulated to enhance vascular formation [23]. Down-regulation of Notch1 expression on endothelial cells by siRNA technology reduce the formation of blood vessels [23], suggesting an important role of Notch signaling in angiogenesis. In the present study, we found that mice injected with Ang-1 plasmid showed increased Notch mRNA expression, indicating Notch signaling is involved in Ang-1-mediated recovery of hematopoiesis.

In summary, we provide a line of evidence showing the role of Ang-1 in the recovery of radiation-induced BM suppression, including elevated blood counts and VEGF secretion, increased G1 phase of cell cycle, up-regulated expression of Tie2 and Notch. These findings not only accelerate our better understanding in the recovery of hematopoiesis after radiation, but may have therapeutic potential value for patients with BM suppression.

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**Disclosure of conflict of interest**

None.

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## References

[1] Yu Q, Li B, Li P, Shi Z, Vaughn A, Zhu L, Fu S. Plasma microRNAs to predict the response of radiotherapy in esophageal squamous cell carcinoma patients. Am J Transl Res 2015; 7: 2060-2071.

[2] Galotto M, Berisso G, Delfino L, Podesta M, Ottaggio L, Dallorso S, Dufour C, Ferrara GB, Abbondandolo A, Dini G, Bacigalupo A, Cancedda R, Quarto R. Stromal damage as consequence of high-dose chemo/radiotherapy in bone marrow transplant recipients. Exp Hematol 1999; 27: 1460-1466.

[3] He N, Zhang L, Cui J, Li Z. Bone marrow vascular niche: home for hematopoietic stem cells. Bone Marrow Res 2014; 2014: 128436.

[4] Fukuhara S, Sako K, Noda K, Nagao K, Miura K, Mochizuki N. Tie2 is tied at the cell-cell contacts and to extracellular matrix by angiopoietin-1. Exp Mol Med 2009; 41: 133-139.

[5] Fukuhara S, Sako K, Noda K, Zhang J, Minami M, Mochizuki N. Angiopoietin-1/Tie2 receptor signaling in vascular quiescence and angiogenesis. Histol Histopathol 2010; 25: 387-396.

[6] Mammoto T, Parikh SM, Mammoto A, Gallagher D, Chan B, Mostoslavsky G, Ingber DF, Sukhatme VP. Angiopoietin-1 requires p190 RhoGAP to protect against vascular leakage in vivo. J Biol Chem 2007; 282: 23910–23918.

[7] Zhou BO, Ding L, Morrison SJ. Hematopoietic stem and progenitor cells regulate the regeneration of their niche by secreting Angiopoietin-1. Elife 2015; 4: e05521.

[8] Patan S. Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodeling. J Neurooncol 2000; 50: 1-15.

[9] Koch S, Claesson-Welsh L. Signal transduction by vascular endothelial growth factor receptors. Cold Spring Harb Perspect Med 2012; 2: a006502.

[10] Gerber HP, Malik AK, Solar GP, Sherman D, Liang XH, Meng G, Hong K, Marsters JC, Ferrara N. VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature 2002; 417: 954-958.

[11] Witzenbichler B, Maisonpierre PC, Jones P, Yancopoulos GD, Isner JM. Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. J Biol Chem 1998; 273: 18514-1821.

[12] Nieboer P, de Vries EG, Mulder NH, Sleijfer DT, Willemse PH, Hospers GA, Gietema JA, Sluiter WJ, van Der Graaf WT. Long-term haematological recovery following high-dose chemotherapy with autologous bone marrow transplantation or peripheral stem cell transplantation in patients with solid tumours. Bone Marrow Transplant 2001; 27: 959-966.

[13] Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tateno M, Hicklin DJ, Zhu Z, Witte L, Crystal RG, Moore MA, Rafii S. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med 2001; 193: 1005-1014.

[14] Lamalice L, Le Boeuf F, Huot J. Endothelial cell migration during angiogenesis. Circ Res 2007; 100: 782-794.

[15] Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. Recruitment of Stem and Progenitor Cells from the Bone Marrow Niche Requires MMP-9 Mediated Release of Kit-Ligand. Cell 2002; 109: 625–637.

[16] Kim I, Kim HG, So JN, Kim JH, Kwak HJ, Koh GY. Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3'-Kinase/Akt signal transduction pathway. Circ Res 2000; 86: 24-29.

[17] Daly C, Wong V, Burova E, Wei Y, Zabski S, Griffiths J, Lai KM, Lin HC, Ioffe E, Yancopoulos GD, Rudge JS. Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). Genes Dev 2004; 18: 1060-1071.

[18] Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 2004; 118: 149-161.

[19] Kopp HG, Avecilla ST, Hooper AT, Shmelkov SV, Ramos CA, Zhang F, Rafii S. Tie2 activation contributes to hemangiogenic regeneration after myelosuppression. Blood 2005; 106: 505-512.

[20] De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS, Sampaolesi M, Naldini L. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 2005; 8: 211-226.

[21] Gazave E, Lapébie P, Richards GS, Brunet F, Ereskovsky AV, Degnan BM, Borchiellini C, Vervoort M, Renard E. Origin and evolution of

the Notch signalling pathway: an overview from eukaryotic genomes. BMC Evol Biol 2009; 9: 249.

[22] Bigas A, Robert-Moreno A, Espinosa L. The Notch pathway in the developing hematopoietic system. Int J Dev Biol 2010; 54: 1175-1188.

[23] Morrow D, Cullen JP, Cahill PA, Redmond EM. Cyclic strain regulates the Notch/CBF-1 signaling pathway in endothelial cells: role in angiogenic activity. Arterioscler Thromb Vasc Biol 2007; 27: 1289-1296.