MULTI-MODALITY IMAGING OF ENDOTHELIAL PROGENITOR CELLS MEDIATES NEOVASCULARIZATION AND MUSCLE REGENERATION IN ISCHEMIA MUSCLE

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TARGET AUDIENCE This paper was focus on the cell tracking by optical imaging and MRI *in vivo* and the therapeutic effect assessment of endothelial progenitor cells by MRI and histology. The researchers of studying molecular imaging should be interested in this topic.

PURPOSE As obstructive vascular disease is the major cause of mortality in the world (1), cell-based strategies aimed at developing novel therapies or improving current therapies are currently under study. Endothelial progenitor cells (EPCs) play an important role in tissue repairing and regeneration in ischemic muscle (2). However, the EPCs survival and efficacy after transplantation is unclear in cardiovascular cell therapy. In this study, we demonstrated the labeled EPC migration by multi-modality imaging and the effects of EPCs on vascular and skeletal muscle regeneration in a mouse model of hind limb ischemia.

METHODS Bone marrow-derived EPCs were characterized with immunofluorescent staining and flow cytometry. The multi-modality probe was devised that the conjugation with the bacterial cytosine deaminase (bCD) and the poly-L-lysine (PLL) moiety was functionalized with Gd^{3+}-DOTA chelators and flurorophore probe including rhodamine and Cy5.5 (3). After intracardiac injection of labeled or unlabeled EPCs (1×10^6, 150μl each) and saline (150μl each) into nude mice after 24 hours of hind limb ischemia, MR imaging, optical imaging, inductively coupled plasma-mass spectrometry (ICP-MS) and immunohischemistry detected *in vivo* and *in vitro* the presence of EPCs in ischemia muscle. We also used MR imaging, near-infrared spectroscopy (NIRS) system and histopathology to evaluate muscle fiber regeneration and the blood oxygen saturation.

RESULTS The characteristic markers of EPCs, CD34 (52.73%), CD133 (14.28%) and VEGFR2 (61.63%), were examined by flow cytometry. Results presented in figure 1 show that these cells were positive for hematopotetic stem cell and progenitor cell markers, including CD34, CD133 and CXCR4. We also observed CD31, VEGFR2, and vWF positive cells (the markers of endothelial cell). MR imaging, optical imaging, ICP-MS and immunohischemistry revealed the migration and formation of labeled EPCs to capillary vessel at days 3 to 5 after injection (Figure 2 and 3). A significant recovery of hemoglobin oxygen saturation and significant increased survival muscular fibers in the ischemic hind limb were observed at 14 and 21 days after EPCs treated. In addition, the majority of muscular fibers were intact. SDF-1 and VEGF expression were upregulated by Western-Blot in ischemic muscle compared with control group. In addition, EPCs transplantation increased the ratio of complete salvage of ischemic hind limb.

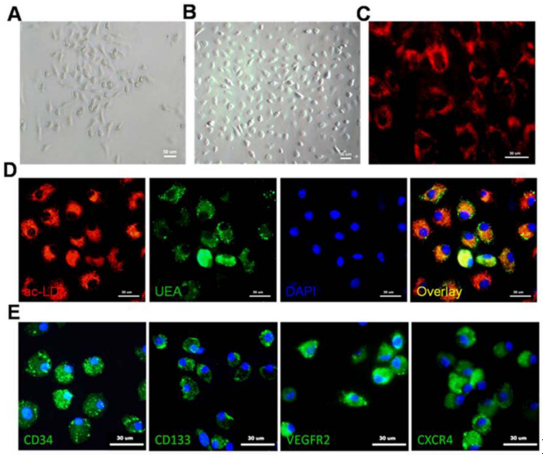

Fig.1

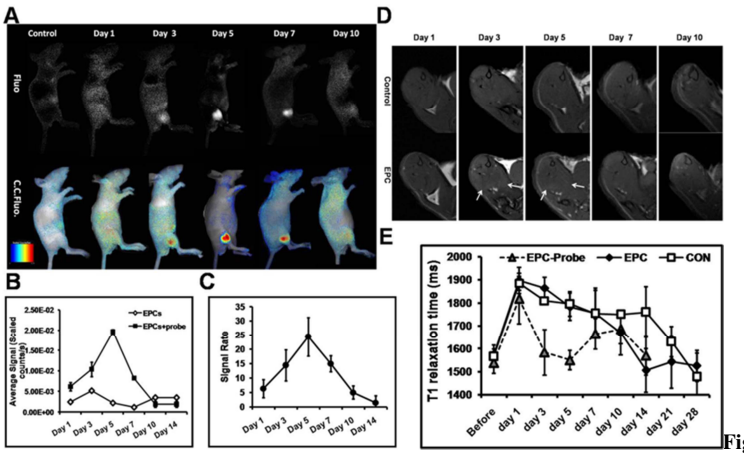

Fig.2

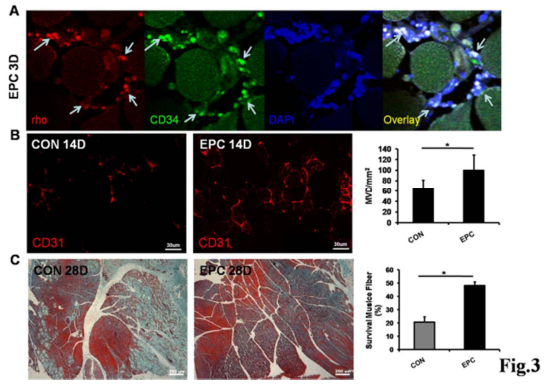

Fig.3

Figure 1. Morphological changes and immunocytochemical analysis in mouse bone marrow-derived EPCs. **A**) MNCs changed from globe-like shape to being thin and flat, and then round and fusiform at day 7. **B**) At days 14, the cells exhibited a typical fusiform or "cobblestone" morphology. **C**) Flurorescence image of multi-modality probe labeled cells. **D**) The EPCs were able to take up Dil-labeled acetylated low-density lipoproteinand bind the endothelial specific lectin FITC-labeled lectin after 14 days in culture, which were colocalized in >95% cell. **E**) Positive for CD34, CD133, VEGFR2, CXCR4 marker. (bar = 30 μm).

Figure 2. *In vivo* tracking of labeled-EPCs by optical imaging and MRI in hindlimb muscle. *In vivo* (left-lateral position, **A**) representative optical imaging showed that the signal of ischemic hindlimb at 1, 3, 5, 7 and 10 days after probe labeled EPCs transplantation was significantly stronger than that of control group. The average signals (**B**) of ischemia muscle and signal ratio (probe-EPCs/EPCs, **C**) were calculated from ROI analyses in two groups. Representative T1 weighted images **D**) and the T1 relaxation time **E**) at different times after probe labeled-EPCs

Figure 3. Rhodamine-labeled and specific markers positive (Alex488-CD34) EPCs migrated into microvessel walls after 3 days of transplantation **(A)**. At day 14 after ischemia, the number of microvessels by staining with CD31 of the ischemic hindlimb in EPCs transplantation group was significantly increased compared with that in control group **(B)**. At day 28 after ischemia, the survival muscle fibers of mice with EPCs treated were more than with the control group using Masson's staining **(C)**. (bar = 30 μm).

Discussion EPCs were not only direct participation into the forming neovasculature of ischemic tissues and organs via vasculogenesis, but also indirect contribution to neovascularization though paracrine aspect (2). After transplanted EPCs at 3 to 7 days, the labeled EPCs homed to ischemia muscle tissue detected *in vivo* by MRI and optical imaging and *in vitro* by ICP-MS. In addition, rhodamine-labeled and $CD34^+$/$VEGFR2^+$/$CXCR4^+$/$CD133^+$ co-labeling cells were found in the vessel wall, suggesting that the cultured EPCs from bone marrow could contribute to vessel formation by incorporating into foci of new vessel formation. The mechanism of how the intracardiac transplanted EPCs homing to sites of ischemic is still uncertain. However, SDF-1, E-selectin and soluble CD146 could increase late EPCs chemotaxis, angiogenic capacity, and vascularisation in ischemic hind limb model (4-6).Various cytokines and other secreted pro-angiogenic factors were demonstrated the presence, such as VEGF, SDF-1, HGF, IGF-1, etc. VEGF can not only promote EC proliferation including angiogenesis, but also promote the growth of myogenic fibers and protect the myogenic cells from apoptosis (7-8). We also demonstrated that EPCs transplantation promoted angiogenesis and increased blood oxygen saturation in ischemia muscle measured by NIRS and improved muscle fiber regeneration of ischemic hind limb using MR imaging *in vivo* and histology *in vitro*.

Conclusion Our results showed that the transplanted bone marrow-derived EPCs were tracked by the multi-modality molecular probe *in vivo* and *in vitro* in a mouse model of hind limb ischemia. Later growth EPCs from bone marrow did not incorporate into vessel formation, but the paracrine secretion of angiogenic factors promoted the neovascularization and muscle fiber regeneration in ischemia tissue.

Reference (1) Yusuf S. et al, Circulation. 2001; 104: 2746-2753. (2) Alev C., et al, *Antioxid Redox Signal.* 2011;15:949-65. (3) Ju, S., et al, *Mol Imaging*. 2011; **10**: 359-369. (4) Oh IY., et al, *Blood.* 2007; 110: 3891-3899. (5) Harhouri K., et al, *Blood*. 2010; 115: 3843. (6) Frederick JR., et al. *Circulation*. 2010; 122: S107-S117. (7) Arsic N., et al. *Mol Ther*. 2004; 10: 844-854. (8) Rissanen TT., et al, *Am J Pathol*. 2002; 160: 1393-1403.