Therapeutic arteriogenesis and vessel branching study of DLL4/VEGF delivery system

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Statement of Purpose: The importance of therapeutic angiogenesis has been learned in terms of many vascular diseases. However, the arteriogenesis and understanding of on how vessel branching affect therapeutic efficacy remains incomplete. One important state of the art study results has shown that many different kinds of endothelial cells are involved in this process, offering various functions in building the vascular networks. Tip endothelial cell is one of the most important cells in vessel branching with their filopodial structure, similar to that of axon in the nervous system for sensing and responding to the environmental cues. The filopodia plays an important role in building the original vascular network. The expression of VEGF, platelet derived growth factor (PDGF)-BB and DLL4 are upregulated specifically in the tip cells as verified previously [1]. In this study, a delivery system of both DLL4 and VEGF encapsulated in reactive oxygen species (ROS) sensitive hydrogel was developed. The function of DLL4 in vessel branching was studied and the synergetic effect of DLL4/ VEGF in terms of vessel formation was explored *in vitro* before delivered *in vivo* on CLI model. Then, the therapeutic effects of our delivery system on the model of murine hind limb ischemia was evaluated using different parameters

Methods: The ROS sensitive hydrogel was synthesized by N-Isopropylacrylamide (NIPAAm), 2-hydroxyethyl methacrylate (HEMA) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl acrylate (AAcPB), using benzoyl peroxide (BPO) as initiator. Von Willebrand Factor (vWF) antibody, alpha smooth muscle actin (α-SMA) and EphrinB2 were used as received from Abcam. Transforming growth factor beta 1, recombinant human delta-like 4 (DLL4) and human VEGF 165 (VEGF) were purchased from Peprotech. The primary human arterial endothelial cells (HAECs) were purchased from Cell application Inc. For in vitro studies, 3D collagen gel model was created for lumen formation, branching of endothelial cells and expression of ephrinB2 and ephB4. The groups included TGFβ, DLL4 with TGFβ, and DLL4+VEGF with TGFβ. HAECs were first grown to around 95% confluence in complete growth medium in T-75 flask and then switched to the 35 mm petri dish. The procedure was subjected to a scratch assay with 200 µL pipette tips. Additionally, cell proliferation was performed by Picogreen dsDNA assay and verified by live cell imaging. For animal experiments, 9-week-old female mice (C57BL/6 type) were provided by Jackson Laboratories and were randomly divided into 4 groups, which were surgery only; gel; gel+DLL4 and gel+DLL4+VEGF. The hindlimb ischemia was introduced by ligation of the commonly used femoral artery, followed by vein ligation of the iliac artery according to a previously reported [2]. Blood perfusion and motor performance were measured post-surgery at day0, 3, 7 and 14. After 2 weeks, tissue samples were collected and processed for IHC and histology study.

Results: To evaluate whether DLL4 leads to increased branching density of HAECs, we compared the branching points in terms of a hypoxic and TGFβ rich environment as a mimic microenvironment of ischemia. In DLL4/TGFβ group, an increased number of branching points was displayed without severe defects. In terms of cell function, we found that the DLL4/TGFβ increased the cell growth and migration of ECs, and these functions were further improved by the addition of VEGF. Besides, DLL4/VEGF group showed improved expression of both EphB4 and ephrinB2 under hypoxic and upregulated TGFβ. The vessel formation was also significantly increased by the addition of DLL4/VEGF. In vivo animal test showed that the delivered DLL4/VEGF improved outcomes in the critical limb ischemia model including enhanced blood flow, motor performance and increased ischemic score. Furthermore, the IHC staining study showed that the mature vessel density and arterial density were both upregulated by DLL4/VEGF added group. Moreover, H&E images in DLL4/VEGF group showed significantly higher central nuclei density than that of control groups as well as improved diameter of muscle fiber diameter and muscle area, indicating muscle tissue regeneration and function recovery.

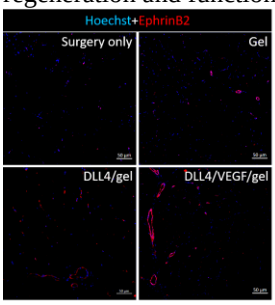

Figure 1. a.

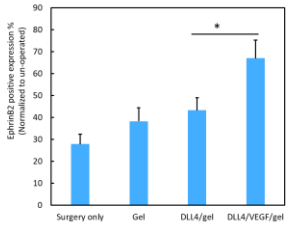

Figure 1. b.

Figure.1 a). Representative IHC images of animal tissue samples stained with Hoechst for nucleus and ephrinB2 for arteries; b). and quantification of arterial expression

Conclusions: Our study showed that DLL4 improved vessel branching by stimulating the growth of filopodial structure of ECs. Similarly, the vessel density was largely increased. Besides, the combination of VEGF/DLL4 further improved the vessel formation and ECs function under hypoxic condition with the presence of TGFβ. In animal model, the injection of DLL4/VEGF with hydrogel showed fastest recovery of ischemia tissue as well as muscle repair based on the significant improved muscle fiber diameter, central nuclei, expression for specific markers in therapeutic angiogenesis at a protein level.

References:

[1] T. Tammela et al, Nature. 454.7204 (2008): 656.
[2] Xu Y et al. Acta biomaterialia, 2015, 26: 23-33.