available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Del-1 gene transfer induces cerebral angiogenesis in mice

Yongfeng Fan[a], *Wei Zhu*[a,d], *Michael Yang*[a], *Yiqian Zhu*[a,d], *Fanxia Shen*[a], *Qi Hao*[a], *William L. Young*[a,b,c], *Guo-Yuan Yang*[a,b], *Yongmei Chen*[a,*]

[a]*Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California San Francisco, 1001 Potrero Avenue, Room 3C-38, San Francisco, CA 94110, USA*
[b]*Department of Neurological Surgery, University of California San Francisco, San Francisco, CA, USA*
[c]*Department of Neurology, University of California San Francisco, San Francisco, CA, USA*
[d]*Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai, China*

ARTICLE INFO

Article history:
Accepted 1 May 2008
Available online 10 May 2008

Keywords:
Angiogenesis
Brain
Del-1
Gene transfer
Ischemia

ABSTRACT

Developmental endothelial locus-1 (Del-1) is a novel angiomatrix protein that has been shown to stimulate a potent angiogenic response and promote functional recovery in hindlimb and cardiac ischemia in animal models; however, its impact on cerebral angiogenesis is unknown. In this study, we investigated whether Del-1 overexpression via gene transfer induces cerebral angiogenesis in a murine model, and examined Del-1 expression after ischemic stroke. Cerebral Del-1 overexpression was achieved with AAV (adeno-associated virus) transduction system via stereotactic injection. Control mice were injected with AAV-lacZ. Del-1 gene transduction led to a significant induction of cerebral angiogenesis compared to AAV-lacZ treatment at 4 weeks after gene transfer (Del-1: 97±7 *vs* lacZ: 64±28, vessels/field, $p<0.05$). Mice transduced with AAV-Del-1 showed significantly elevated vascular densities for up to 6 weeks after gene delivery. In addition, double immunofluorescent staining showed co-localization of endothelial cell marker CD31 with BrdU (specific marker for proliferating cells), indicating that Del-1 promoted endogenous endothelial cell proliferation and angiogenesis. Our immunohistochemical staining also showed that Del-1 expression was markedly up-regulated in the peri-infarct area at 3 days after permanent focal cerebral ischemia compared to the sham-operated non-ischemic control. Our data suggest that Del-1 may participate in modulating cerebral angiogenesis, and that gene transfer of Del-1 may provide a novel and potent means for stimulating cerebral angiogenesis.

Published by Elsevier B.V.

1. Introduction

Angiogenesis, the sprouting of new capillaries from pre-existing vessels, is important in both health and disease (Carmeliet and Jain, 2000; Risau, 1997). Developmental endothelial locus-1 (Del-1) was a recently cloned and characterized unique matrix protein that is expressed by endothelial cells during embryological vascular development (Hidai et al., 1998). Del-1 is a 52-kD protein that contains 3 epidermal growth factor (EGF) repeats, and an arginine-glycine-aspartic acid (RGD) motif. The RGD motif of Del-1 binds $\alpha v\beta 5$ integrin, which, in turn, leads to increased angiogenic transcription

* *Corresponding author*. Fax: +1 415 206 8907.
E-mail address: meichen@anesthesia.ucsf.edu (Y. Chen).

Abbreviations: AAV, adeno-associated virus; AAV-Del-1, adeno-associated viral Del-1 vector; Del-1, developmental endothelial locus-1; MCAO, middle cerebral artery occlusion; sCBF, surface cerebral blood flow; VEGF, vascular endothelial growth factor

0006-8993/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.brainres.2008.05.003

factor HoxD3 expression. HoxD3 activates αvβ3 and uPA, resulting in the transformation of resting endothelial cells to an angiogenic state (Penta et al., 1999; Rezaee et al., 2002). We have previously reported that HoxD3 overexpression can induce brain angiogenesis (Chen et al., 2004).

Del-1 becomes quiescent at the time of birth, and is no longer expressed in normal adult tissues. It has been found re-expressed in a number of human tumors as well as in ischemic muscles, which may play an important role in adult angiogenesis (Aoka et al., 2002; Ho et al., 2004). In addition to promoting adherence and migration of endothelial cells, Del-1 also acts as an endothelial cell survival agent through up-regulating Bcl-2 expression (Pollman et al., 1999). Exogenous application of Del-1 has been demonstrated to augment angiogenesis and improve blood flow and tissue function in murine models of hind-limb ischemia (Ho et al., 2004; Zhong et al., 2003) and cardiac ischemia (Kown et al., 2003). The function of Del-1 in the normal and the ischemic brain has not been documented previously. In this study, we examined whether exogenous application of Del-1 can induce cerebral angiogenesis, and whether Del-1 is expressed after cerebral ischemia.

2. Results

2.1. *Construction of AAV expression vectors*

Gene transfer has the potential to maintain a sufficient concentration of transduced protein over a long period of time from a single administration (Burger et al., 2005; Monahan and Samulski, 2000). To investigate whether Del-1 might play a role in cerebral angiogenesis, we first developed adeno-associated viral (AAV) vectors with Del-1 or lacZ. AAV vector is a single-stranded DNA virus, and belongs to the nonpathogenic, helper-dependent member of the parvovirus family. It has several advantages over other viral vectors, including low immunogenicity, the ability to mediate long-term transgene, and infect both dividing and non-dividing cells (Zaiss et al., 2002). To construct AAV-Del-1, we inserted the human Del-1 cDNA between two ITRs of pAAV-MC plasmid to generate the pAAV-Del-1. CMV promoter was used to control gene expression in this vector. Fig. 1A shows the structure of the two AAV vectors with Del-1 or lacZ. Fig. 1B shows confirmation by gel electrophoresis of correct Del-1 cDNA inserted into pAAV-CMV after double-enzyme digest. After purification and concentration using CsCl gradient centrifuge, we obtained AAV-Del-1 as high as 10^{13} gene copy determined by dot blot hybridization (Figs. 2A and B). AAV-lacZ was generated as a control vector in the same fashion and at the same titer.

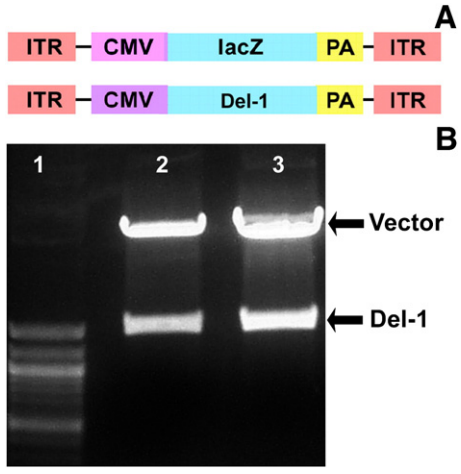

Fig. 1 – Construction and production of AAV-Del-1. (A) Structure of the 2 AAV vectors with Del-1 or lacZ (as a control). CMV promoter was used to control gene expression in this vector. (B) The cDNA encoding human Del-1 was PCR-amplified by using the primers 5′ CG GAA TTC ATG AAG CGC TCG GTA GCC GT 3′ and 5′ CCC AAG CTT tc att cct cct ctg tgc agc 3′. The fragment was cloned into the plasmid pAAV-MC with EcoR I/Hind III, and tested by double-enzyme cutting and sequencing. Gel image shows electrophoresis of recombinant pAAV-Del-1 after EcoR I/Hind III digestion. Lane 1: molecular size marker. Lanes 2 and 3: Del-1 fragment at 1.2 k base pairs.

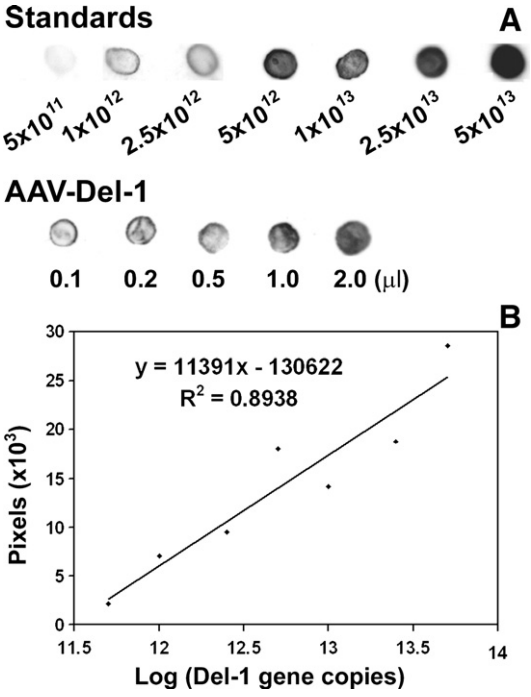

Fig. 2 – Dot blot analysis of AAV-Del-1 gene copies. (A) Photomicrograph shows AAV-Del-1 titers using dot blot hybridization. Del-1 gene fragment was amplified by PCR and used as standards. Upper panel in A shows the intensities of dot blot increases with the loading doses of the standards. Low panel in A is a representative blot image of AAV-Del-1 with different loading doses. After hybridization and exposure, the real pixels of dots were measured using software Image J. (B) Standard curve, X-axis is log scale of gene copy, and Y-axis is linear scale of pixels. We obtained AAV-Del-1 of 1.4×10^{13}/ml.

2.2. *Del-1 gene transfer induced cerebral angiogenesis*

To determine whether Del-1 increases vascular density in the brain, we performed intraparenchymal AAV injection. AAV-