Ultrasound in Med. & Biol., Vol. 34, No. 11, pp. 1857–1867, 2008

0301-5629/08/$–see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2008.03.019

● *Original Contribution*

TRANSFECTION EFFICIENCY OF TDL COMPOUND IN HUVEC ENHANCED BY ULTRASOUND-TARGETED MICROBUBBLE DESTRUCTION

JIAN-LI REN, ZHI-GANG WANG, YONG ZHANG, YUAN-YI ZHENG, XING-SHENG LI, QUN-XIA ZHANG, ZHAO-XIA WANG, and CHUAN-SHAN XU

Institute of Ultrasound Imaging, The Second Affiliated Hospital of Chongqing Medical University, Chongqing City, China

(*Received* 30 *October* 2007; *revised* 26 *January* 2008; *in final form* 6 *March* 2008)

Abstract—The aim of the present study was to explore the gene transfection efficiency of Tat peptide/plasmid DNA/ liposome (TDL) compound combined with ultrasound-targeted microbubble destruction (UTMD) in human umbilical vein endothelial cell (HUVEC). Tat peptide, plasmid DNA (pIRES2-EGFP-HGF) and Lipofectamine™ 2000 were used to prepare the TDL compound. Microbubbles were prepared using mechanic vibration. The expression of the report gene enhanced green fluorescent protein (EGFP) was observed using fluorescent microscopy and flow cytometry. The viability of HUVEC was measured by MTT assay. mRNA and protein of HGF was analyzed by reverse transcription–polymerase chain reaction and Western Blot. The intensity of green fluorescence and the gene transfection efficiency of TDL compound + microbubbles + ultrasound group were higher than those of other groups, and no significantly different viability was found between TDL compound + microbubbles + ultrasound group and the other groups. The HGF mRNA and HGF protein of TDL compound + microbubbles + ultrasound group were higher than those of other groups. Our finding demonstrated that UTMD could enhance the transfection efficiency of TDL compound without obvious effects on the cell viability of HUVEC, suggesting that the combination of UTMD and TDL compound might be a useful tool for the gene therapy of ischemic heart disease. (E-mail: xcshan@163.com)

Key Words: **Ultrasound-targeted microbubble destruction, Cell-permeable peptides, TDL compound, Gene transfection, Ischemic heart disease.**

INTRODUCTION

Ischemic heart disease is a major health problem worldwide. Although great efforts have been made in its treatment, no ideal treatment is available. Exploring novel therapeutic strategies can improve the clinical outcomes. Gene therapy shows considerable promise as a new modality for treating ischemic heart disease. Many genes have been applied for gene therapy in ischemic heart disease, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), hypoxia-inducible factor-1alpha (HIF-1α) and angiopoietin-1 (Ang-1) (Bougioukas et al. 2007; Hao et al. 2007; Iwakura et al. 2003; Kido et al. 2005; Losordo and Dimmeler 2004; Shim et al. 2006; Yamaguchi et al. 2005). Hepatocyte growth factor, a recently characterized growth factor with a disulfide-linked heterodimer structure, is originally identified in the plasma of rats after partial hepatectomy and its receptor, c-Met, is a transmembrane tyrosine kinase protooncogene. Hepatocyte growth factor participates in mitogenesis (Rubin et al. 1991), motogenesis (Stoker et al. 1987), morphogenesis (Montesano et al. 1991) and angiogenesis (Bussolino et al. 1992). Recent studies have shown that HGF is a cardioprotective factor through angiogenesis, anti-apoptosis, antioxidative stress and antifibrosis (Ahmet et al. 2002; Kitta et al. 2001; Taniyama et al. 2002), suggesting HGF could be a promising therapeutic gene for treating ischemic heart disease (Jayasankar et al. 2005; Yamaguchi et al. 2005). Gene therapy requires an efficient and safe carrier for delivering therapeutic genes to targeted cells. There are two kinds of gene delivery vector, *viz.*, viral vector and nonviral vector (Miyazaki et al. 2006; Saras-

Address correspondence to: Dr. Chuan-Shan Xu, Institute of Ultrasound Imaging, Second Affiliated Hospital, Chongqing Medical University, No.76, Lin Jiang Road, YuZhong District, Chongqing City, 400010, China. E-mail: xcshan@163.com

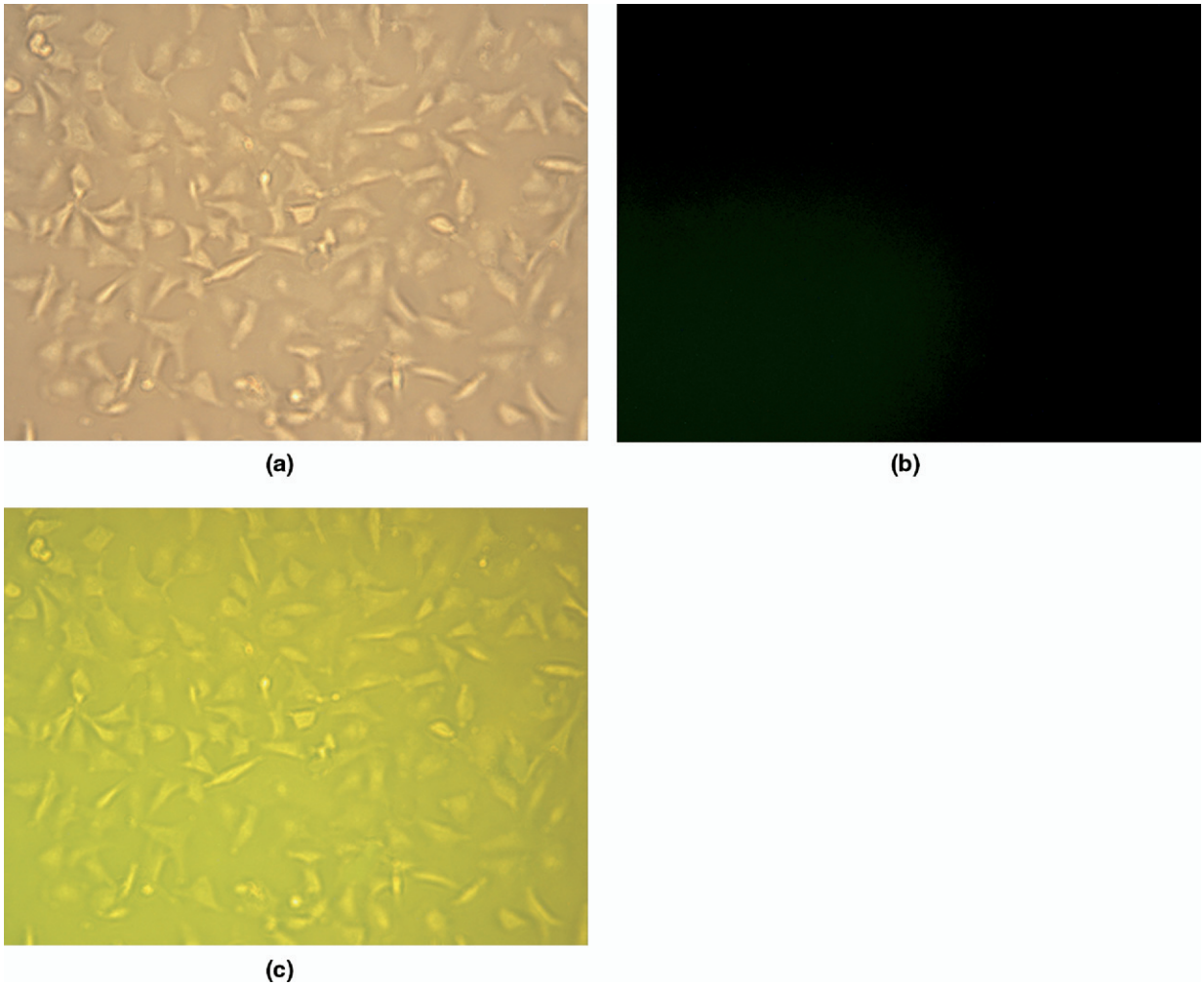

Fig. 1. Fluorescent microscopic images of pIRES2-EGFP-HGF expression in HUVEC (×200). Cells were transfected with all treated factors. Cells were cultured for 24 h and the reporter gene expression was observed using fluorescent microscopy. C indicates blanked control group; D indicates plasmid DNA; T indicates TDL compound; M indicates microbubbles; U indicates ultrasound. (a-c) C group; (d-f) T group; (g-i) T+M group; (j-l) T+U group; (m-o) T+M+U group; (p-r) D+M+U group. Bright field images (a,d,g,j,m,p); fluorescence images (b,e,h,k,n,q); overlay image (c,f,i,l,o,r).

wathi et al. 2007; Xia et al. 2004). Viral vector is the current favorites for intracellular gene delivery, but it has disadvantages such as immunoreactions, toxicity, nonspecificity, high cost, size limits of exogenous DNA and the possibility of random integration of the vector DNA into the host genome (Haider et al. 2008). Nonviral vector has more advantages such as little immunoreactions, lower toxicity, ease and safety in preparation, high gene encapsulation capability and simplicity of use (El-Aneed et al. 2004; Zhang et al. 2007). However, the lower efficiency of nonviral vector needs to be overcome.

Cationic liposome has a better membrane affinity and DNA condensation and can enhance the *in vitro* transfection efficiency. But the toxicity and *in vivo* low transfection efficiency remain the limitations for cationic liposome applied to gene therapy (Alton et al. 1999; Madry et al. 2001). In recent years, cell-permeable peptides (CPPs) have been used widely as cellular delivery vectors for their remarkable functions carrying macromolecular substance directly and actively through cellular membrane into plasma or nucleus without cytotoxicity (Astriab-Fisher et al. 2002; Ignatovich et al. 2003; Nakanishi et al. 2003; Rudolph et al. 2003; Tung et al. 2002). Cell-permeable peptides have many advantageous properties such as high affinity of cellular membrane, fast speed of permeating membrane, fast degradation and no destructiveness to the cellular membrane. Transactivating transcriptional activator protein (Tat peptide) from human immunodeficiency virus type 1(HIV-1 Tat), one of most popular CPPs, has membrane translocation