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Protein transduction domain-hA20 fusion protein protects endothelial cells against high glucose-induced injury

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ABSTRACT. We constructed a plasmid containing a protein transduction domain (PTD) and a human A20 (hA20) gene fragment; the fusion protein was obtained by highly expressing this plasmid in the yeast *Pichia pastoris* GS115. The plasmid was obtained by adding 9xArg and *Eco*RI recognition sites to the end of the primer, and 6xHis-Tag and *Not*I recognition sites to its end. After sequencing, the hA20 gene fragment was inserted into plasmid pPIC9k to construct expression vector pPIC9k-PTD-hA20; then, we transfected GS115 with the vector and induced PTD-hA20 protein expression. We purified protein from the yeast fermentation supernatant using a nickel column. Human umbilical vein

endothelial cells (HUVECs) were cultured in high glucose medium (30 mM glucose) and in high glucose medium containing different concentrations of protein. Apoptosis of HUVECs was assayed by TUNEL 72 h later. The biological activity tests indicated that the fusion protein not only passed through the cell membrane freely, but also inhibited apoptosis of HUVECs induced by high glucose levels. We conclude that the fusion protein PTD-hA20 has potential for clinical use.

Key words: Protein transduction domain; hA20; Fusion protein; HUVECs; High glucose; Apoptosis

