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# A recombinant trans-membrane protein hMnSOD–R9 inhibits the proliferation of cervical cancer cells in vitro

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**Abstract** Human manganese superoxide dismutase (hMnSOD) is a new type of cancer suppressor. Nonamer of arginine (R9) is an efficient protein transduction domain (PTD). The aim of the study was to improve the transduction efficiency of hMnSOD and investigate its activity in vitro. In this study, we designed, constructed, expressed, and purified a novel fusion protein containing the hMnSOD domain and R9 PTD (hMnSOD–R9). The DNA damaged by Fenton's reagent was found to be significantly reduced when treated with hMnSOD–R9. hMnSOD–R9 fusion protein was successfully delivered into HeLa cells. The MTT assay showed that proliferation of various cancer cell lines were inhibited by hMnSOD–R9 in a dose-dependent manner. In addition, the cell cycle of HeLa cells was arrested at the sub-G0 phase by hMnSOD–R9. hMnSOD–R9 induced apoptosis of HeLa cells in a dose-dependent manner. With hMnSOD–R9 treatment, *Bax*, *JNK*, *TBK1* gene expression was increased and *STAT3* gene expression was gradually down-regulated in HeLa cells. We also found that apoptosis was induced by hMnSOD–R9 in HeLa cells via up-regulation of cleaved caspase-3 and down-regulation phospho-STAT3 pathway. These results indicated that hMnSOD–R9 may provide benefits to cervical cancer treatment.

**Keywords** hMnSOD–R9 · Expression · DNA damage protection · Trans-membrane · Apoptosis

## Introduction

In human tissues, there are three known isoforms of SOD: the mitochondrial manganese SOD (MnSOD), the cytosolic copper–zinc SOD (Cu/ZnSOD), and the extracellular SOD (EcSOD) [1]. Mainly located in mitochondrial matrix, MnSOD is well known as one of the major antioxidant enzymes against superoxide free radicals and catalyzes dismutation of superoxide radical anion into hydrogen peroxide [2]. Furthermore, MnSOD has been proposed to be a new type of tumor suppressor gene [3]. Most types of cancer cells have reduced the expression of MnSOD compared with their normal cell counterparts. The region of chromosome 6q25.3, where MnSOD is located, is deleted in many cancers cells. Numerous studies have demonstrated that transfection of MnSOD cDNA into various cancer cells resulted in decreasing their tumorigenicity [4–6].

Although MnSOD offers great potential as a therapeutic molecule in many cancers, therapeutic use of MnSOD is limited by poor penetration in tissues and inability to cross cellular membrane. Protein transduction domains (PTDs)

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