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Improved anticancer efficacy of doxorubicin mediated by human-derived cell-penetrating peptide dNP2

Yucheng Xiang, Wei Shan, Yuan Huang*

Key Laboratory of Drug Targeting and Drug Delivery System (Ministry of Education), West China School of Pharmacy, Sichuan University, No. 17, Block 3, South Renmin Road, Chengdu 610041, PR China

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ABSTRACT

Although cell penetrating peptides (CPPs) have been extensively studied as an approach to deliver anti-cancer drugs into the tumor cells for the last 30 years, no FDA-approved CPP-based drugs are available, implying that the existing CPPs may have less efficiency in human or have side effects such as toxicity. Herein, we established a tumor targeting drug delivery system by attaching a human-derived cell-penetrating peptide dNP2 (CKIKKVKKKGRKKIKKVKKKGRK) to N-(2-hydroxypropyl)-methacrylamide (HPMA) copolymer doxorubicin conjugates. Firstly, in vitro cytotoxicity of free dNP2 peptide and dNP2-modified blank HPMA copolymer were examined. A classic CPP-R8 (CRRRRRRRR) was chosen for comparison and the results showed that 200 μM free R8 reduced cell viability to 68.4% but dNP2 did not induce any toxicity at the same concentration. After conjugation to HPMA copolymer, a similar trend was also observed which indicated the excellent biocompatibility of dNP2. Next, effect of dNP2 modification on cellular uptake, DNA damage, apoptosis and anticancer activity of HPMA copolymer doxorubicin conjugates were evaluated. It was excited that dNP2 modified HPMA copolymer (P-(dNP2)-DOX) not only had a higher uptake by HeLa cell compared with non-modified copolymer (P-DOX) but resulted in an enhanced drug distribution in nuclei. Furthermore, P-(dNP2)-DOX exhibited greater DNA damage ability (10.5 folds higher than P-DOX) in comet assay and induced more apoptosis cells (46.0%). P-(dNP2)-DOX also showed a stronger cell cytotoxicity (3-fold to P-DOX) as well as in 3D tumor spheroid assay (inhibition rate 78%). All these results suggested that the human-derived cell-penetrating peptide dNP2 could facilitate tumor nuclear-accumulation of anti-cancer drugs and improve anticancer efficacy. More importantly, dNP2 has less toxicity compared with classic CPP-R8 thus shows the potential for the clinic cancer therapy.

1. Introduction

Despite great effort has been made, the incidence of cancer is predicted to rise a further 75% over the next two decades (Stewart, 2017) and the clinical outcomes of conventional cancer therapies need further improvement (Gotwals et al., 2017). Many chemotherapeutic drugs used in clinic have severe adverse effects and suffer from poor pharmacokinetic (Maeda et al., 2013; Spencer et al., 2015). The targeted delivery of drugs to tumor tissues is fundamental to the realization of effective therapies. Although the number of studies on tumor-targeted nanomedicine has grown vastly, the unsatisfied performance of such agents has not met the high expectations in preclinical or clinical trials (Wilhelm et al., 2016). Combination of targeting therapeutics to cancer tissue and crossing cellular and subcellular membrane barrier is the primary challenge (Li et al., 2015; Shan et al., 2016; Yang et al., 2016).

Cell-penetrating peptides (CPP) have been shown to be one promising avenue for the efficient intracellular delivery of various drug delivery systems (Koren and Torchilin, 2012; Raucher and Ryu, 2015). Since the transactivator of transcription (TAT) protein of HIV was reported to mediate efficient intracellular delivery in 1988, cell-penetrating peptides have been widely used in nanocarrier modification for the last 30 years. However, no FDA-approved CPP-based drugs are available, yet implying that the existing CPPs may have less efficiency in human or have side effects such as toxicity (Katayama et al., 2011) or adaptive immune response (Fang et al., 2013).

To address this, several human-derived cell penetrating peptides were developed and considered to be promising alternatives for efficient intracellular delivery (Monte et al., 2016; Sudo et al., 2017; Zhao et al., 2011). dNP2 (KIKKVKKKGRKKIKKVKKKGRK) is a cell-permeable peptide derived from human, recently been reported by Sangho Lim and his colleagues (Lim et al., 2015). The dNP2 peptide was identified and optimized from the human novel leucine zipper-containing

* Corresponding author.
E-mail address: huangyuan0@163.com (Y. Huang).

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