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Inhibition of hepatocellular carcinoma growth using immunoliposomes for co-delivery of adriamycin and ribonucleotide reductase M2 siRNA

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ABSTRACT

The chemotherapy combined with gene therapy has received great attention. We developed targeted LPD (liposome-polycation-DNA complex) conjugated with anti-EGFR (epidermal growth factor receptor) Fab' co-delivering adriamycin (ADR) and ribonucleotide reductase M2 (RRM2) siRNA (ADR-RRM2-TLPD), to achieve combined therapeutic effects in human hepatocellular carcinoma (HCC) overexpressing EGFR. The antitumor activity and mechanisms of ADR-RRM2-TLPD were investigated. The results showed that RRM2 expression was higher in HCC than in non-HCC tissue, and RRM2 siRNA inhibited HCC cell proliferation, suggesting that RRM2 is a candidate target for HCC therapy. ADR-RRM2-TLPD delivered ADR and RRM2 siRNA to EGFR overexpressing HCC cells specifically and efficiently both *in vitro* and *in vivo*, resulting in enhanced therapeutic effects (cytotoxicity, apoptosis and senescence-inducing activity) compared with single-drug loaded or non-targeted controls, including ADR-NC-TLPD (targeted LPD co-delivering ADR and negative control siRNA), RRM2-TLPD (targeted LPD delivering RRM2 siRNA) and ADR-RRM2-NTLPD (non-targeted LPD co-delivering ADR and RRM2 siRNA). Mechanism studies showed that p21 is involved in the combined therapeutic effect of ADR-RRM2-TLPD. The average weight of the orthotopic HCC in mice treated with ADR-RRM2-TLPD was significantly lighter than that of mice treated with other controls. Thus, ADR-RRM2-TLPD represents a potential strategy for combined therapy of HCC overexpressing EGFR.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide [1]. Gene therapy using small interfering RNA (siRNA) represents a potent therapeutic approach in tumor therapy and offers an elegant alternative to HCC chemotherapy [2]. Ribonucleotide reductase M2 (RRM2), which plays an important role in DNA synthesis and repair, is an important target for tumor gene therapy, with several RRM2 inhibitors such as GTI-2040 currently in the clinical trial phase [3]. To date, there have been very limited data regarding the therapeutic efficacy of RRM2 suppression in HCC [4]. Furthermore, the effectiveness of targeting a single gene in HCC tends to be largely impaired by activation of other signal pathways [5,6]. To enhance the therapeutic efficacy of gene therapy in HCC, one rational strategy is to combine chemotherapy with RRM2 siRNA-based gene therapy.

The chemotherapy combined with gene therapy has recently received great attention [7–9]. Combined process is far more effective than standard chemotherapy or gene therapy by itself and has no additional side effects. Lin et al. performed a combined therapy of TRAIL (TNF-related apoptosis-inducing ligand) gene therapy and chemotherapy, resulting in an additive effect with minimal toxicity [7]. In one such effort, we investigated the combined effect of adriamycin-based (ADR, one of the most active agents in HCC) chemotherapy and RRM2 siRNA in HCC therapy. This

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