Enhancement of anti-tumor activity of hybrid peptide by conjugation with thiolated carboxymethyl dextran via disulfide linkers

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ABSTRACT SUMMARY
In this study, to improve the anti-tumor activity of EGFR2R-lytic hybrid peptide, we prepared a thiolated nanoparticles form through the disulfide bonds between thiolated carboxymethyl dextran (CMD) and cysteine-conjugated peptide. In vitro release studies confirmed that the peptide was released from the CMD-s-s-peptide in the presence of glutathione (GSH). In vivo release studies indicated that the anti-tumor activity of the CMD-s-s-peptide was more effective than that of free peptide treatment, and showed high tumor accumulation in xenograft mice models. These results demonstrate that the thiolated nanoparticles would be potentially useful carriers for sustained release of peptide, and that can enhance the anti-tumor activity of peptide in vivo.

INTRODUCTION
We previously reported that the EGFR2R-lytic hybrid peptide had cytotoxic and anti-tumor activities both in vitro and in vivo 1. Furthermore, to improve the peptide pharmacokinetics and its anti-tumor activity after intravenous (i.v.) injection, we prepared a gelatin hydrogel nanoparticles based on the ionic interaction between anionic gelatin and cationic peptide. The gelatin hydrogel nanoparticles exhibited a long circulation time in the blood and higher anti-tumor activity than that of the free peptide 2. CMD is a dextran derivative and one of the frequently used macromolecular carriers for the delivery of drugs because of its relatively low immunogenicity 3. Moreover, it is reported that the thiolated CMD had potential for peptide drug delivery 4. In the present study, we investigated the capacity of a prepared thiolated nanoparticles based on thiolated CMD for controlled release of peptide and its influence on the anti-tumor activity in vivo.

EXPERIMENTAL METHODS
To synthesize the thiolated CMD-peptide conjugates, the thiolated CMD (10 mg) was hydrated in 1 ml 0.1M phosphate buffer solution (PBS) pH 3.5. Cysteine-conjugated peptide was dissolved in water and buffered to pH 7.4. Both solutions were mixed at ratio of 1:2 (peptide: polymer) and hydrogen peroxide was added to this mixture drop by drop to achieve a final concentration of 0.06% (v/v). The reaction was allowed to proceed at room temperature under an argon atmosphere for 3 h. The unreacted peptide was removed by ultrafiltration with Amicon Ultra centrifugal filter devices. The peptide content in CMD-s-s-peptide was determined by a NanoDrop spectrophotometer. Then, we carried out the in vitro release studies of peptide from the conjugates. Thirty-microliters of CMD-s-s-peptide (10 mg/ml) were incubated in same volumes of PBS solution containing 2 µM GSH or 2 mM GSH or 1 mM DTT (positive control) at 37 °C with moderate shaking. After different incubation periods, the released peptide samples were recovered and analyzed by a NanoDrop spectrophotometer. Furthermore, we performed the in vivo release studies of peptide from the conjugates and its influence on the anti-tumor activity in human tumor xenograft mice models. BxPC-3 pancreatic cancer cells were implanted subcutaneously into athymic female nude mice (day 0). On day 5, the mice were randomized and i.v. injection of saline (control), peptide (1 mg/kg) and equivalent peptide-loading CMD-s-s-peptide (1 mg/kg) and thiolated CMD (5 mg/kg) three times a week for a total of nine doses. Tumors were measured with a caliper, and the tumor volumes were calculated as follows: width 2 x length x 0.5. In addition, we also investigated the biodistribution of the fluorescently-labeled CMD-s-s-peptide after i.v. administration.

RESULTS AND DISCUSSION
In vitro release rate of peptide from the CMD-s-s-peptide conjugates in the presence of GSH is shown in Figure 1. Peptide could release from the CMD-s-s-peptide conjugates in extracellular plasma GSH level (2 µM), and it was shown that the release was sustained over a time period of 120 h. Figure 2 shows the results of in vivo release studies. The mice
that were treated with the saline showed a progressive increase in tumor volume, reaching 1136 mm$^3$ by day 72. In contrast, the mice that were treated with the peptide or the CMD-s-s-peptide showed significant tumor growth inhibition, the mean tumor sizes were 846 mm$^3$ and 535 mm$^3$, respectively. The mice were treated with the CMD-s-s-peptide had significantly smaller tumors than that of the mice treated with peptide alone. These findings suggest that the anti-tumor activity of the CMD-s-s-peptide was more effective than that of peptide alone.

In addition, biodistribution of the fluorescently-labeled peptide or CMD-s-s-peptide data showed the fluorescence intensity in tumors in mice that were treated with the CMD-s-s-peptide was more than 2-fold higher than that in tumors in mice that were treated with peptide alone. It was clearly demonstrating that the thiolated nanoparticles could improve the accumulation of the peptide into tumors. Therefore, the accumulated peptide in tumor tissues could selectively kill the tumor cells and enhance their anti-tumor activity.

CONCLUSION
The present study demonstrated that the thiolated nanoparticles were one of the useful carriers for our synthesized EGFR2R-lytic hybrid peptide delivery and contributed to enhance its anti-tumor activity in vivo.

REFERENCES

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