Apoptosis-targeted low molecular weight heparin derivatives for cancer therapy

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ABSTRACT SUMMARY

Various angiogenesis inhibitors and apoptosis-targeting agents have been therapeutically applied in preclinical cancer models, some of which have been tested in clinical trials. In a previous study, we demonstrated that LHT7, a low molecular weight heparin (LMWH)-taurocholate conjugate, has strong antiangiogenic and tumor-suppressive activity and diminished anticoagulant properties. In this study, we developed LHT7-ApoPep-1, an apoptosis-homing peptide-conjugated variant of LHT7. LHT7-ApoPep-1 exhibited antiangiogenic activity in endothelial cell tube-formation assays and apoptotic cell-targeting ability in tumor cell binding assays; it also showed little toxicity toward healthy cells. Administration of LHT7-ApoPep-1 in mouse xenograft models of breast carcinoma delayed tumor growth compared to LHT7-only, and histological evaluations revealed decreased vessel formation and increased apoptotic area in tumor tissues. Moreover, an examination of LHT7-ApoPep-1-Cy7.5 localization within the body using in vivo live imaging showed accumulation at the tumor site of tumor-bearing mice, with a more prolonged circulation time and enhanced intensity compared to LHT7-Cy7.5. Inspection of the tumor microenvironment revealed that Cy5.5-labeled LHT7-ApoPep-1 was located on and near CD31-positive vessels in tumor tissue. We conclude that LHT7-ApoPep-1 has antiangiogenic and apoptosis-targeting properties and exerts antitumor effects by suppressing tumor vessel growth and homing to apoptotic cells within the tumor.

INTRODUCTION

Angiogenesis and apoptosis are prominent features of the environment of growing tumors [1]. Angiogenesis is a highly exquisite process that is critical to reproduction, wound healing, and progression of malignant tumors [2]. In the process of tumor angiogenesis, as well as during growth and/or tumor metastasis, tumors secrete multiple growth factors, some of which interact with heparan sulfate; these factors are potential targets for the development of angiogenesis inhibitors. The anticoagulant properties of heparin, a plentiful sulfated polysaccharide, are well known, but heparin also has an important role as a growth factor-inhibitory agent. In the past decade, it has been reported that heparin binds angiogenic growth factors and affects tumor progression through modulation of tumor vascularization and the spread of tumor cells.
mg of ethylene amine derivative of taurocholic acid (Et-TCA) was added in water and allowed to react overnight. The product, LHT7-ApoPep-1, was dialyzed with membrane (2000 Da MWCO) to remove EDC, HOSu and unreacted Et-TCA, and was lyophilized by vacuum drying (yield=60%).

In vivo studies used the mouse models of human tumor xenografts (5-wk old female athymic nude mice) and treatment strategies as follows. Briefly, MDA-MB-231 human breast cancer cells (1.0×10⁶) were injected subcutaneously into the back of a mouse. After the tumors had grown to approximately 50e80 mm³, ApoPep-1, LHT7-ApoPep-1 or LHT7 were injected via the tail vein. Mice were divided into six groups (n=5/group) receiving different intravenous (i.v.) injections, as follows: (1) saline (control group); (2) ApoPep-1; (3) LHT7, 5 mg/kg; (4) LHT7-ApoPep-1, 0.2 mg/kg; (5) LHT7-ApoPep-1, 1 mg/kg; and (6) LHT7-ApoPep-1, 5 mg/kg.

RESULTS AND DISCUSSION

In xenograft experiments, LHT7-ApoPep-1 dramatically suppressed tumor growth compared with the control group and was significantly more effective than LHT7. The progression of tumor size is closely associated with the formation of new vasculature in tumor tissues. A histological analysis of MDA-MB-231 tumor tissues showed that LHT7-ApoPep-1 inhibited blood vessel formation and effectively increased apoptosis in tumors. Since LHT7-ApoPep-1 retarded the formation of new blood vessels in tumor tissues, tumor growth was effectively slowed.

In tumor-bearing mice treated with fluorescently labeled drugs, fluorescence intensity in mice injected with LHT7-ApoPep-1 was greater and more prolonged than in mice injected with LHT7-Cy7.5. This elevated fluorescence signal of LHT7-ApoPep-1 in tumors is likely attributable to an extended residence time in the tumor resulting from targeting both apoptotic and proliferative vasculature. In addition, this ability of labeled LHT7-ApoPep-1 to home to tumor tissue reveals its potential for the non-invasive monitoring of tumors following therapy, as has been demonstrated for ApoPep-1.

CONCLUSION

We conclude that LHT7-ApoPep-1 has apoptosis-targeting properties and exerts antitumor effects by homing to apoptotic cells within the tumor.

REFERENCES

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