Supporting Information

Hyaluronate – Flt1 peptide conjugate / epirubicin micelles for theranostic applications to liver cancers

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Experimental

**The formation of HA-Flt1 peptide conjugates/EPI micelles.** To encapsulate EPI in the HA-Flt1 peptide conjugate micelles, we used the oil in water (O/W) emulsion method. The synthesis and characterization of HA-Flt1 peptide conjugate was described in detail in our previous report. The HA-Flt1 peptide conjugate was dissolved in PBS at a concentration of 2 mg/ml. Then, EPI·HCl (Wako chemical, Japan) was substituted with tetraethylamine to dissolve in chloroform. The concentration of EPI solution was 4 mg/ml. The solutions of HA-Flt1 peptide conjugate and EPI were mixed at a weight ratio of 1 : 2 and sonicated with a tip sonicator for 15 to 20 min in an ice-bath. After finishing sonication, the complex solution stirred overnight to evaporate chloroform. Then, HA-Flt1 peptide conjugate / EPI micelles were filter with a PTFE syringe filter. The final solution was diluted and characterized by DLS (Zetasizer Nano ZS, Malvern Instruments, UK), TEM (Hitachi, Japan), and UV-Vis spectroscopy.

**Intravital microscopy and biodistribution of HA-Flt1 peptide conjugates/EPI micelles.** To investigate the dynamics of micelles in the liver, the intravital real time laser scanning confocal microscopy was carried out after intravenous injection of HA-Flt1 peptide conjugate / EPI micelles to anesthetized Balb/c mice at an age of 7 weeks. After cutting the abdomens, we imaged liver region at systole and diastole in heart using a home built microscope and a water immersion objective lens (×60, Olympus, Japan). The fluorescence of injected HA-pep/micelles was captured with the intravital confocal microscope within 30 min. In addition, the biodistribution analysis of HA-Flt1 peptide conjugate / EPI micelles was carried out in tumor mice model. B16F10 melanoma cells were proliferated and injected subcutaneously in the flank of C57BL/6 mice at an age of 7 weeks. The mice were anesthetized via intraperitoneal injection of a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). During in vivo experiments, the mice were kept under anesthesia, and the body temperature was maintained at 37°C. HA-Flt1 peptide conjugate / EPI micelles were administered to mice at an average age of 7 weeks by tail-vein injection. As a control, the normal mice were also injected with the same amount of HA-Flt1 peptide conjugate / EPI micelles intravenously. After 3 days, both mice were sacrificed for the bio-imaging of their livers, spleens, and kidneys with the image analyser. A halogen lamp with a 625 nm filter was used for the excitation and the fluorescence images were obtained through a 790 nm emission filter. We have complied with the POSTECH institutional ethical use protocols for animals.

**Treatment of hepatocellular carcinoma.** For cancer treatment, The SV40-transgenic mouse was used as a spontaneous hepatocellular carcinoma (HCC) model. The HA-Flt1 peptide conjugate / EPI micelles were intravenously injected to the HCC mice twice a week at a dose of 3 mg/kg of EPI. As a control, we administered the same volume of PBS via intravenous injection. After 4 weeks post-injection, the liver tumor was imaged by MRI. The tumor volume was calculated using the formula \((\text{length} \times \text{width}^2) / 2\).
Figure S1. The original intravital microscopic images of HA – Flt1 peptide conjugate / EPI micelles in the liver tissue at diastole and systole state of heart (scale bar = 25 μm).

References