RECOMBINANT NANOPARTICLES DECORATED WITH A NEAR-INFRARED FLUORESCENT PROTEIN FOR IN VIVO IMAGING

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Key words: nanoparticles, recombinant, infrared fluorescent protein, IVIS spectrum

Introduction: Viral capsids are naturally occurring nanoparticles which have prospects of utilization in an *in vivo* imaging and targeted delivery. Hepatitis B virus capsid protein (HBcAg) self-assembles into spherical particles with a diameter of 32 nm. Each particle is composed of 240 protein subunits. We developed HBcAg particles decorated with the near-infrared fluorescent protein (NIRFP). Each particle (HBcAg/NIRFP) carries multiple moieties of the NIRFP. The HBcAg/NIRFP particles were injected into mice central bloodstream to study biodistribution.

Methods: Genes for the HBcAg and NIRFP were constructed *de novo* and the HBcAg was fused to the NIRFP using a molecular design (termed SplitCore) which allows inserting of the NIRFP into surface-exposed loops of the HBcAg so that multiple moieties (240 molecules) of the fluorescent protein are presented on a surface of the HBcAg/NIRFP particle. The gene for the HBcAg/NIRFP fusion protein was placed into an *E.coli* expression plasmid. Upon bacterial expression the HBcAg/NIRFP particles were purified using gradient ultracentrifugation. The integrity of the particles was confirmed by measurement of sizes using a dynamic light scattering and electron microscopy. Mice were injected into tale veins with 50-100 micrograms of the HBcAg/NIRFP particles to study the biodistributions using the IVIS Spectrum.

Results: Particles with floating density 1.12 g/ml were obtained by lysing the biomass of the *E.coli* expression strain and subjecting the lysate to ultracentrifugation in sucrose gradient. Dynamic light scattering (DLS) showed presence in the preparation of nanoparticles with uniform size distribution (hydrodynamic radius 21 nm). Electron microscopy revealed abundance in the preparation of nanoparticles with sizes completely compatible with the results of the DLS. Upon injection of mice with a solution of the HBcAg/NIRFP particles the nanopartilces' biodistribution was studied using IVIS Spectrum in vivo Imaging System (PerkinElmer). The biodistributions shown varying time patterns with increasing accumulation in organs of reticuloendothelial system.

Conclusion: Viral nanoparticles decorated with the infrared fluorescent protein are efficient tools to visualize the biodistributions.