ABSTRACT

Kim, Seong-Eun. Ph.D., Purdue University, May 2017. Development of Biopolymer-Based Nanoparticles toward Cancer Theranosis/Theragnosis. Major Professor: You-Yeon Won

Cancer is the leading cause of death worldwide, and is a huge economic burden to the society. Detecting and treating cancer at an early stage significantly increases the likelihood of treatment success. Recently, DNA methylation has been recognized as a promising cancer biomarker, because it is involved in tumorigenesis. Unfortunately, current methods of detecting DNA methylation (e.g., the bisulfite conversion method) are complicated and expensive. A simpler method with a higher sensitivity is desirable. To address this need, we developed a methyl binding domain 1 (MBD1) protein-based probe for detecting the methylation of CG dinucleotide sites on DNA by fluorescence correlation spectroscopy. In DNA samples with added MBD1 probes, the methylation level could be reliably detected at methylated nucleotide concentrations as low as 20 nM, regardless of DNA sequence. The range of detectable methylated nucleotide concentrations was 20 – 1900 nM, and the range of detectable methylation levels was 5% – 100%. Therefore, this system is a feasible platform for clinical use.

Real-time image-guided cancer surgery uses intra-operative imaging techniques to guide the surgical procedure. Fluorescence-guided surgery requires the use of fluorescence contrast agents for visualizing specific tissues. Most conventional near-infrared (NIR) fluorescent probes fall short for this application because of their insufficient chemical stability. So there is a need for better contrast agents. We demonstrate a new method of fluorophore encapsulation that may address this limitation. Our method utilizes genetically-engineered hepatitis B virus (HBV) capsid proteins as the encapsulating material. Fluorescent protein moieties, mCardinal (mC), and a cancer targeting peptide moiety (“affibody”) were

inserted into the HBV capsid protein by recombinant protein synthesis. The self-assembly of these genetically modified HBV capsid subunits results in virus-like nanoparticles encapsulating mC fluorophores and having their outer surfaces decorated with tumor-interacting affibodies. These affibody-conjugated fluorescent virus-like nanoparticles produce significantly stronger fluorescence signals, exhibit far greater stability against photobleaching, are readily internalized by cancer cells, are significantly less susceptible to degradation under intracellular conditions than unencapsulated fluorophores, and are effective at generating fluorescence contrast in tumor with less accumulation in the liver in vivo. These results suggest that these tumor-targeting virus-like fluorescent protein nanoparticles are promising for use as contrast agents for image-guided cancer surgery.

For potential cancer theranostic applications, we developed MBD1/DNA-templated gold nanoparticle (AuNP) arrays; because of their unique surface plasmon resonance (SPR) properties under NIR radiation, these AuNP arrays can be used for simultaneous photoacoustic (PA) imaging and photothermal (PT) therapy. MBD1 proteins bind to methylated cytosines (mC) on CG sequences (on linear DNA). The histidine-rich peptide on MBD1 can be used as a nucleation site for growing a AuNP. Therefore, MBD1/DNA complexes can be used as a template for synthesizing linear AuNP arrays (called hereafter “AMD”). AMD showed a very strong NIR absorption because of coupled SPRs among linearly arranged AuNPs. AMD exhibited a high PT conversion efficiency of 14.1%, a PA intensity that is about 4-fold higher than gold nanorods, and a cancer cell-targeting efficiency that is about 8-times as high as pristine MBD1. AMD is expected to be safe for clinical use, because MBD1 and DNA are biodegradable, and AuNPs are clearable from the body. AMD is a promising agent for cancer theranosis/theragnosis.