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Fluorescence-conjugated CD166 anti-peptide for detecting colorectal cancer stem-like tumor *in vivo*

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Cancer stem cells (CSCs) possess characteristics associated with normal stem cells and may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. They involved in drug resistance, metastasis and relapse of cancers can significantly affect tumor therapy. Therefore, it is important to develop specific therapies targeted probe at CSCs for improvement of survival and quality of life of cancer patients. Studies have indicated that the CD166 protein has been considered as a specific marker for colorectal CSCs detection. In addition to monoclonal antibodies, small molecules such as anti-peptides could provide more advantages for CSCs detection *in vivo*. Here, we attended to design the CD166 anti-peptide (CD166ap) to detecting the CSCs *in vitro* and *in vivo*. To obtain CSCs, the human colorectal cancer cells (HCT-15) were seeded into selection media (DMEM/F12, 0.4% bovine serum albumin, 2% B27, 5 μg/mL bovine insulin, 4 μg/mL heparin, 20 ng/mL fibroblast growth factor 2, and 20 ng/mL epidermal growth factor) at a density of 1000 cells per mL. Under these conditions, only CSCs and early progenitor cells survive and proliferate, whereas differentiated cells die. Next, we designed a CD166ap (amino acid: KDSEGYESYNGNLGSQC {It is known that human CD6 proteins have the ability to bind the human CD166 proteins specifically (Chappell PE et al., Structure. 2015, 23:1426-1436). Depending on the two proteins binding sites, we designed the amino acid sequence of CD166 anti-peptide. However, the N- and C-terminal amino acid (lysine and cysteine) were added for conjugating with fluorescence, nuclear medicine chelator and Polyethylene glycol (PEG).} and conjugated with fluorescence for CSCs binding assay by flow cytometry and immunofluorescence stain. For *in vivo* imaging detection, the media-induced CSCs (2×106) were subcutaneous inoculated into the right flank of nude mice (n=5 per each group) and grew for one week. Then, the fluorescence conjugated CD166ap was IV injected into animal model for *in vivo* imaging system and biodistribution assay. The primary spheres that derived from HCT-15 cells under serum-free conditions and which are highly enriched for CSCs at 48 hours. These induced CSCs overexpressed the reprogramming factors such as OCT4, c-myc, Nanog and anti-apoptosis factor (Survivin). Moreover, they also showed the characteristic of drug resistance compared with cancer cells. In CSCs targeted probe binding assay, the CD166ap and antibody revealed the quite binding capability in CSCs. The *in vivo* imaging assay, we found that CD166ap specifically targeted to CSCs-induced xenograft model and accumulated in tumor area. In conclusion, we designed a specific probe for CSCs detection *in vivo* successfully. In addition, the CD166ap may label radioisotope for nuclear medicine imaging and conjugate drug for CSCs therapy in clinical.

Biography

Siao Syun Guan received his PhD Degree from the National Taiwan University, College of Medicine Graduate Institute of Toxicology in 2015. His research interests include biomarker discovery and drug development for tumor diagnosis by nuclear medical imaging. He is a Deputy Engineer in Division of Isotope Application, Institute of Nuclear Energy Research in Taiwan. The programs he has participated in includes: Nuclear Medicine in Diagnosis of Central Nervous Diseases (2008-2009), Development of Gastric Cancer Detection Kit (2010-2013), Colorectal Cancer Capsule Endoscopy (2012), Peptide-Based Tumor Target Probe (2014) and Radioactive Protein Labeling Technology (2015-2016). He is currently the Co-Program Manager for Tumortheranostics Drug Development (2017).

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