Development of a Dual-Enzyme-Responsive Turn-on Fluorescent Probe for Cancer Stem Cell Imaging

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Cancer stem cell (CSC) is known as a subpopulation of tumor that is responsible for cancer proliferation, metastasis, and therapeutic resistance.[1] The development of fluorescent probes that can visualize and detect CSC is important to evaluate cancer malignancy. Aldehyde dehydrogenase 1A1 (ALDH1A1) is known as a reliable biomarker of CSC. Recently, we have developed an ALDH1A1-responsive fluorescent probe **C5S-A** based on a near-infrared (NIR) cyanine dye bearing a formyl group.[2] **C5S-A** can work as a turn-on fluorescent probe for CSC imaging; however, **C5S-A** cannot identify CSC among stem cells because ALDH1A1 is also overexpressed in normal stem cell (NSC).[3] We envisioned that a turn-on fluorescent probe which responds to not only ALDH1A1 but another enzyme overexpressed in tumor cells can distinguish CSCs from NSC s.

Here, we report a hemicyanine-based dye **CHO_βgal** that emits fluorescence only after reacting with both ALDH1A1 and β-galactosidase, which are overexpressed in cancers[4] (Figure 1a). The fluorescence intensity greatly increased after treating two enzymes with **CHO_βgal**, whereas those remained almost unchanged after incubating with one of enzymes or without enzymes (Figure 1b).

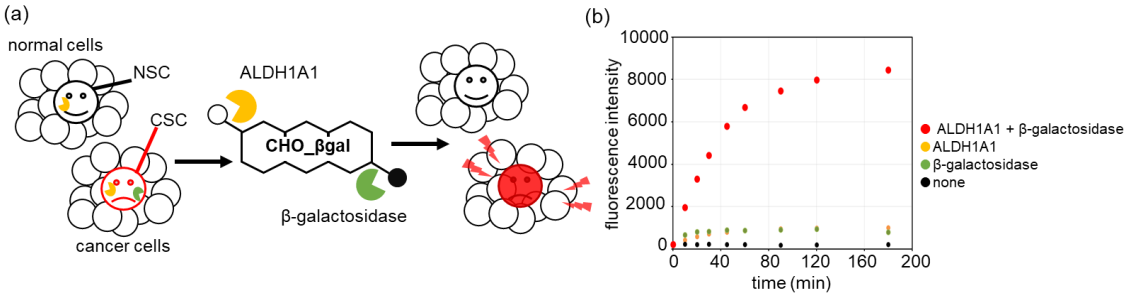

Figure 1. (a) Conceptual scheme of CSC-selective visualization utilizing **CHO_βgal**. (b) Time-dependent fluorescence intensity change of **CHO_βgal** at 660 nm after treatment of ALDH1A1 (200 nM) and/or β-galactosidase (10U).

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