Technology watch

IMPROVING CANCER CELL IMAGING

Visualization of cancer cells *in vivo* using injectable molecular imaging probes has clear clinical implications for detecting cancers and monitoring therapy. However, the currently available probes are limited in part by high levels of background signal, leading to a low tumour-to-normal-tissue detection ratio. Yasuteru Urano, Hisataka Kobayashi and colleagues have developed an activatable fluorescent probe that has minimal background signal and a high tumour-to-normal-tissue detection ratio, and is targeted to cancer cells expressing a particular antigen.

The authors developed a series of fluorescent probes using 2,6-dicarboxyethyl-1,3,5,7-tetramethyl boron-dipyrromethene (BODIPY); this fluorophore was selected because it is unaffected by pH and can be coupled to proteins. Addition of various *N*,*N*-dialkylated anilines allowed them to create probes with different pK_a values whose fluorescence was activatable by acidic pH. The probes were then coupled to the anti-ERBB2 monoclonal antibody trastuzumab. Trastuzumab was used because it is internalized after binding to ERBB2 on the cell's surface, and the complex is then trafficked to the lysosome, which has an acidic pH.

In vitro analysis of three different probes containing different aniline moieties in NIH3T3 cells expressing ERBB2 showed that all became fluorescent after internalization and that there was minimal background signal. One probe, *N*,*N*-diethylaminophenyl BODIPY (DiEtN-BDP)–trastuzumab, had a stronger signal, so the authors selected this for *in vivo* studies. Metastatic lung tumours were created in nude mice by intravenous injection of the ERBB2-expressing NIH3T3 cells. *In situ* and *ex vivo* imaging of tumour-bearing lungs and non-tumour-bearing hearts with the DiEtN-BDP–trastuzumab pH-activatable probe or an 'always-on' control probe showed a 22-fold higher fluorescent tumour-to-heart ratio for the pH-activatable probe. Furthermore, the probe was not activated in tumours created by ERBB2-negative NIH3T3 cells. Treatment with ethanol significantly reduced the signal from the pH-activatable probe in tumours, indicating that only viable cells were detected by the probe.

The authors also created a second probe, DiEtN-BDP–galactosamine-conjugated serum albumin (GSA), which targets ovarian cancer cells, and tested it in a mouse model of peritoneal ovarian cancer metastasis. DiEtN-BDP–GSA specifically identified peritoneal micrometastases with minimal background signal, and facilitated microendoscopic surgery and tumour resection in live mice by allowing visualization of micrometastases that were invisible in white light endoscopic images.

Urano, Kobayashi and colleagues emphasize the versatility of their pH-activatable probe, as it contains two moieties: one that can be any cancer-specific macromolecule that is internalized following receptor binding on the surface of a cancer cell, and one that can be modified to change the fluorescence colour or pH threshold. Such probes have potential as *in vitro* imaging tools and for clinical imaging of tumours.

ORIGINAL RESEARCH PAPER Urano, Y. *et al.* Selective molecular imaging of viable cancer cells with pH-activatable fluorescence probes. *Nature Med.* 7 Dec 2008 (doi:10.1038/nm.1854)