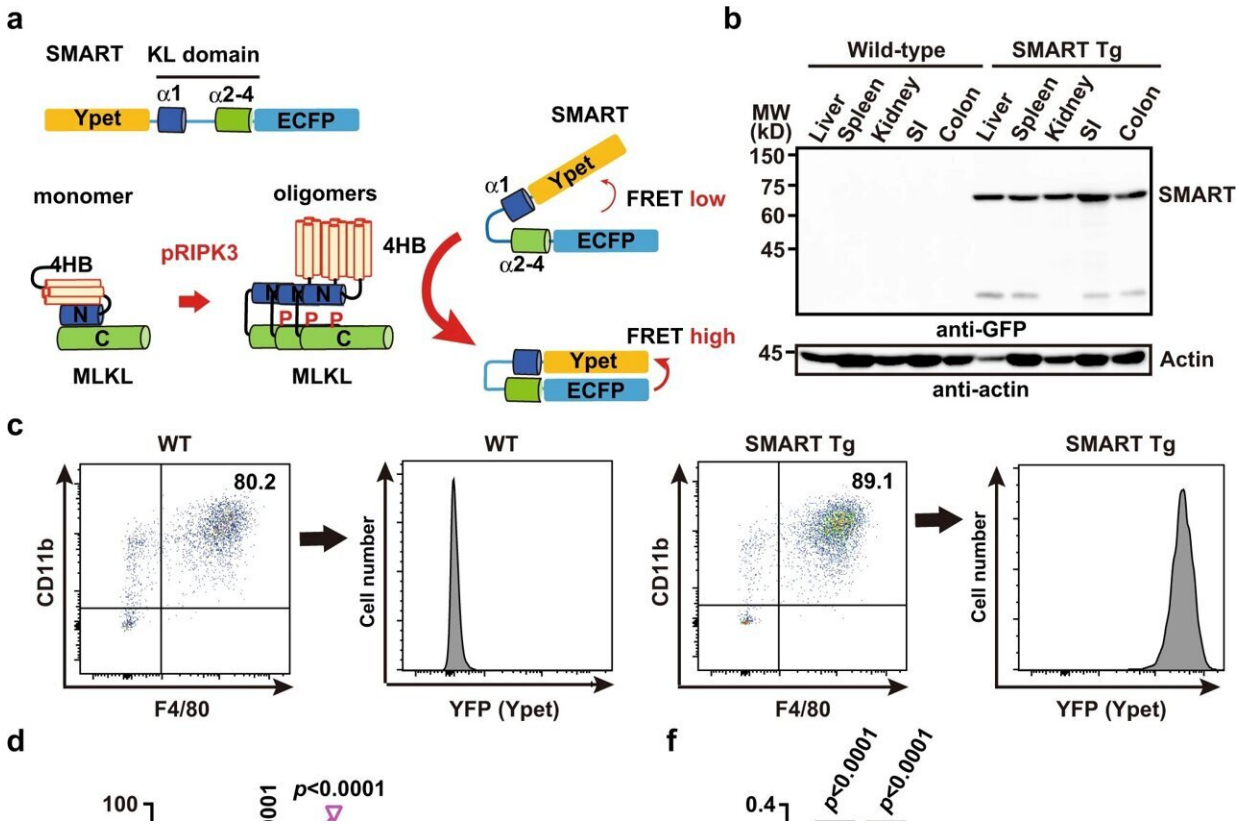
FRET-based biosensor visualizes execution of necroptosis in vivo

December 9 2022



Generation of SMART Tg mice. **a** Depiction of the SMART biosensor structure (*top*) and its activation mechanism (bottom). SMART is composed of N-terminal Ypet, a modified α1 to α4 helices of the KL domain of MLKL, and C-terminal ECFP. Upon necroptosis induction, activated and phosphorylated RIPK3 (pRIPK3) phosphorylates MLKL, resulting in oligomer formation of MLKL. Then, oligomers of MLKL induces conformational changes of SMART, possibly through the interaction, thereby increasing FRET efficiency. KL pseudokinase domain, Ypet modified yellow fluorescent protein, ECFP enhanced cyan

fluorescent protein, pRIPK3 phosphorylated RIPK3. **b** Western blots probed with anti-GFP antibody show expression of the SMART biosensor in various murine tissues. Tissue extracts were prepared from the indicated organs of 8-week-old wild-type or SMART Tg mice. Results are representative of two independent experiments. **c** Mice were intraperitoneally injected with thioglycollate, then peritoneal cells were recovered by washing the peritoneal cavity with ice-cold PBS on day 4 after injection. Isolated cells were stained with the indicated antibodies and analyzed with flow cytometry. The percentages of $CD11b^{+}F4/80^{+}$ cells indicate the fraction of macrophages; the levels of YFP detected in these cell populations indicate the expression of SMART. Results are representative of three independent experiments. WT wild-type. **d** Peritoneal macrophages from SMART Tg mice were untreated or stimulated with BV6 (1 μM) + zVAD (20 μM) or BV6 (1 μM) + zVAD (20 μM) + GSK'872 (5 μM) for the indicated times. Cell death was assessed with the LDH release assay. Results are mean ± SD of triplicate samples, and they are representative of five independent experiments. **e**, **f** Peritoneal macrophages derived from SMART Tg mice were stimulated as described in **d**, and FRET/CFP ratios were calculated. Pseudocolored images show cellular changes in FRET/CFP ratio values in response to the indicated stimulations (**e**). FRET/CFP responses are color-coded according to the color scales (right). White arrowheads indicate cells undergoing necroptosis. Scale bars, 20 μm. Maximum changes detected in the FRET/CFP ratios (**f**). Results are mean ± SE (n = 11 cells per condition). Each dot indicates an individual cell. Results are representative of four independent experiments. Statistical significance was determined with two-way ANOVA with Dunnett's multiple comparison test (**d**) or one-way ANOVA with Turkey's multiple comparison test (**f**). Credit: *Communications Biology* (2022). DOI: 10.1038/s42003-022-04300-0

Necroptosis is a form of regulated cell death (RCD) similar to apoptosis, the most commonly studied type of RCD. In contrast to apoptosis, plasma membrane rupture in necroptotic cells occurs at early time points. For this reason, scientists think that necroptosis elicits strong inflammation in surrounding tissues and plays a role in inflammation-associated diseases. However, it is not well understood where and when

necroptosis occurs in physiological and pathological conditions in vivo.

To address this issue, Prof. Nakano's group previously developed a biosensor for necroptosis called SMART (Sensor for MLKL activation by RIPK3 based on FRET). This biosensor is designed based on fluorescence resonance energy transfer (FRET). In a *Nature Communications* paper published in 2018, they successfully characterized necroptosis in vitro using this FRET biosensor.

Now, they went one step further; they developed transgenic mice with the FRET biosensor SMART for in vivo observation of necroptosis. "The goal of our project is to see when and where necroptosis occurs in vivo, and to understand its role in pathological contexts," said Dr. Murai, the lead author of the study.

In their experiments, they first confirmed that necroptosis can be monitored in primary macrophages or murine embryonic fibroblasts that were derived from SMART Tg mice. Then, they applied a cisplatin-induced acute kidney injury model to SMART Tg mice.

"After many trials and errors, we were finally able to monitor the execution of necroptosis in proximal tubular epithelial cells in cisplatin-injected SMART Tg mice. In contrast to apoptosis, it was rather difficult to induce massive necroptosis in particular tissues at relatively short periods, which is essential for efficiently monitoring cell death using two-photon microscopy in vivo," said Prof. Nakano, the senior author of the study.

"We believe that SMART Tg mice serve as a promising tool to visualize necroptosis in vivo and help us to better understand the role of this relatively newly discovered form of cell death, necroptosis, in pathophysiology of diseases."

These results were published in *Communications Biology*.

More information: Shin Murai et al, Generation of transgenic mice expressing a FRET biosensor, SMART, that responds to necroptosis, *Communications Biology* (2022). DOI: 10.1038/s42003-022-04300-0

Provided by Toho University