Fluorescent indicator could help scientists identify useful drugs that modulate process of cell death

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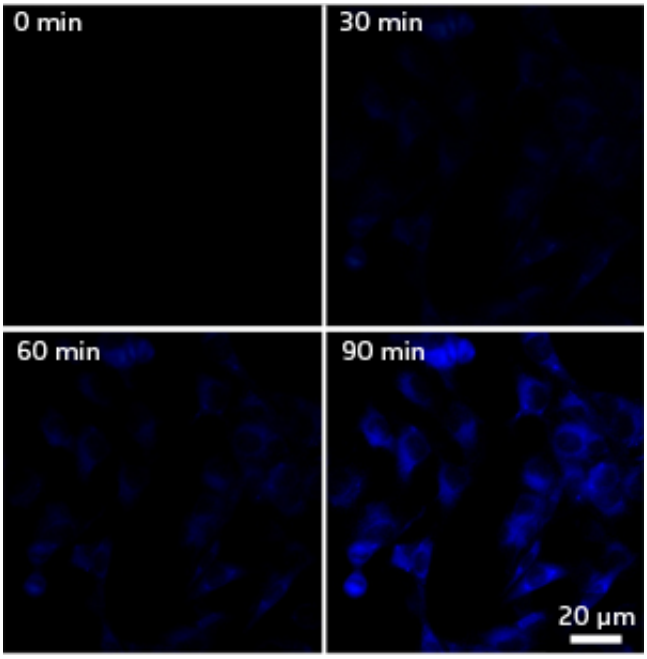

Treatment with the drug staurosporine triggers onset of apoptosis in cultured cells, resulting in increased blue fluorescent signal over time. Credit: 2012 American Chemical Society

Apoptosis is a programmed death mechanism that eliminates unwanted or injured cells from the body. Defects in apoptotic regulation can lead to serious physiological problems such as tissue damage or uncontrolled cancerous growth. Apoptosis is therefore a prominent target for drug

development.

A molecular probe devised by Bin Liu and Ben Tang of the A*STAR Institute of Materials Research and Engineering, Singapore, could accelerate drug discovery by giving scientists a clear view of apoptotic onset in living cells. Recent years have seen an explosion in the use of fluorescent molecules to track biological processes, but existing probes have proven inadequate for monitoring cell death. "Commercial apoptosis probes do not have good cell permeability, and their background signal is also quite high," explains Liu.

As an alternative, the researchers employed a mechanism called 'aggregation-induced emission', initially developed in Tang's laboratory. Their probe contains a molecule called tetraphenylethene (TPE), which normally gathers into insoluble fluorescent clumps in aqueous media. This accumulation can be prevented by attaching a short polypeptide sequence that renders the probe water-soluble.

In the presence of an enzyme known as caspase-3, however, this polypeptide is clipped off. "Caspase-3 exists in all cells in an inactive form, but the enzyme becomes activated upon induction of apoptosis," says Liu. Dying cells therefore produce a fluorescent signal as TPE is released and begins to aggregate. The researchers initially demonstrated the effectiveness of their probe in solutions containing different concentrations of caspase-3, and showed that their molecule is a highly responsive sensor. Compounds that inhibit caspase-3-blocked fluorescent signaling confirm that the probe is exclusively activated by this enzyme.

Liu and Tang achieved similar success in cultured cells, and pretreatment with their probe allowed them to directly monitor the onset of apoptosis over the course of 90 minutes (see image). "We observed very low background and strong, time-dependent signaling," says Liu, "which

opens new opportunities to continuously monitor biological processes." To test the molecule's usefulness for drug screening, the researchers observed cultured cells after treatment with a selection of apoptosis-inducing drugs. Based on the rate of increase in fluorescence, they were able to compare the relative efficacy of each drug, demonstrating the probe's promise as a valuable clinical tool.

The researchers have now begun optimizing the probe for use in vivo, which should allow scientists to conduct more meaningful drug testing in live animals. The team will determine whether the probe can be used for continuous monitoring of drugs taken either orally or injected.

More information: Shi, H. et al. Real-time monitoring of cell apoptosis and drug screening using fluorescent light-up probe with aggregation-induced emission characteristics. *Journal of the American Chemical Society* 134, 17972–17981 (2012). pubs.acs.org/doi/abs/10.1021/ja3064588

Luo, J. et al. Aggregation-induced emission of 1-methyl-1,2,3,4,5-pentaphenylsilole. *Chemical Communications*, 1740–1741 (2001). pubs.rsc.org/en/content/articl … ing/2001/cc/b105159h

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