Supplement

Assay protocols.

After SM treatments for the preset times, multiparametric assays were performed according to the assay panel shown in Table1.This panel consisted of six assays containing almost all common cytotoxic response assays. Each assay was devised according to the principle of fluorescence tagging and followed the criterion of lowest interference between different fluorescence probes or markers. The detail procedures described as below.

The basal procedures for assay 1 were suitable for all other assays; for example, the cells in 96-well assay plates were fixed in phosphate-buffered saline (PBS) containing 4% formaldehyde for 20min and then permeabilized using 0.1% Triton X-100 for 20min at room temperature (RT). For cell immunofluorescence examination, blocking was performed with PBS containing 5% BSA for 30min at RT. After all labeling and washing procedures, the sample plates were kept in PBS and prepared for image acquisition. In order to ensure the reproducibility of all assays, cell number was measured across all six assays. The procedures for each assay were as follows.

For assay 1, after the cells were fixed and permeabilized, 50μl PBS containing 1μM Hoechst33342 and 1mU/mlAlexa Fluor 488–phalloidin was added to the cells and incubated at RT for 1h to allow fluorescent labeling of the nucleus and microfilaments. Next, the cells were washed with PBS and exposed to the blocking solution (100μl/well).Subsequently, primary antibody to α-tubulin (dilution,1:500)and the secondary antibody (Alexa Fluor 546 donkey anti-mouse antibody; dilution, 1:500) were successively added to the system, and incubation was continued at 4°C overnight and for 1h at RT in the dark, respectively. Finally, the cells were washed three times, and incubated with PBS containing 100ng/ml Cell Mask DeepRed at RT for 1h to label the cytoplasm in order to display the whole-cell shape and texture.

For assay 2, 50μl cell culture medium containing1μM MitoTracker Red, 4μM Hoechst33342, and 0.4μM TOTO-3 iodide (for labeling mitochondrial membrane potential [MMP], nuclear and nuclear membrane permeability [NMP], respectively) was added 30min before the end of SM treatment (treated time was 8, 24 and 48h) respectively). Then the cells were fixed and a standard immunofluorescence procedure, as described above, was followed to allow the antibody-dependent labeling of MnSOD. MnSOD primary antibody and Alexa Fluor 488 donkey anti-mouse secondary antibody were used at a dilution of 1:500.

Assay 3 was devised for detecting reactive oxygen species (ROS) release and GSH level in the cells. SM treatment was performed for 0.25, 0.5, 2, 4, or 6h. A mixed stain solution containing CM-H2DCFDA, mBCI, and Hoechst33342 was prepared shortly before use. The three stains mentioned were dissolved separately and sequentially blended in 1×Hank's balanced salt solution (HBSS), at final concentrations of 1μM, 100μM, and 1μM, respectively. After SM treatment, the culture solution was discarded, and 100μl stain solution was added to each well; then, the incubation was continued for 45min. After 45 min, the stain solution was carefully replaced with pre-warmed (37°C) primary culture medium for 5min. Finally, the cells were washed once and balanced in 1×red-free HBSS at a concentration of 200μl/well at RT.

Assay 4 detected changes in the DNA-damage marker pH2AX, the autophagy flux marker LC3B, and Lyso Tracker Red in lysosomes after SM treatments for 0.5, 2, 4, 6, 8, and 24h. A 50-μl aliquot of cell culture medium containing 200nM LysoTracker Red and 4μM Hoechst33342 was added to each well 30min before the end of SM treatment. After that, the cells were fixed and

permeabilized, and a standard immunofluorescence procedure was conducted. Mouse anti-pH2AX primary antibody and rabbit anti-LC3B primary antibody were used at a dilution of 1:1000, and their corresponding secondary antibodies, namely, Alexa Fluor 647 donkey anti-mouse antibody and Alexa Fluor 488 donkey anti-rabbit antibody, were used at a dilution of 1:500.

Assay 5 detected cell apoptosis and necrosis after 24, 36 and 48h of SM treatment. The cell culture medium was replaced with 40µl/well annexinV dyeing buffer (Alexa Fluor 488–annexin V)at a dilution of 1:20 in binding buffer (140mM NaCl, 10mM Hepes, 2.5mM CaCl2at pH7.4) supplemented with 1µM Hoechst33342 and 3µM PI. The plates were then incubated at RT for 30min to allow fluorescent labeling to phosphatidyl serine (PS), a marker for cell membrane eversion, nuclear, and NMP. Next, 160µl/well binding buffer was added, and 100µl of 12% formaldehyde diluted in binding buffer without the stains was added for cell fixation for 20min at RT. Then, the binding buffer was replaced with 200µl/well PBS, and image acquisition was performed.

Assay 6 was devised to assess cell cycle. The protocol of SM treatment and nuclear staining were the same as that for assay1.