

Supplementary information, Data S1

Materials and Methods

Time lapse imaging for caspase activity

Cell suspensions were transferred to a thermostated chamber with a glass cover-slip bottom, allowed adhering for 24 h, cells were transfected with MitoDsRed for 24 h before treatment with staurosporine. Cells were then incubated with 10 mM DEVDase (green) in RPMI 1640 plus 10% FCS, 10 mM HEPES for half an hour before washing with PBS [1]. Samples were excited using λ 488/543 nm, and images were acquired every 3 min and brightness/contrast settings were adjusted so that the fluorescent signal of cells without fluorescent substrate was at the background level. The simultaneous recording of mitochondrial morphology (red) and fluorescent images of activated caspases (green) by DEVDase (PhiPhiLux™) was carried out by a ZEISS LSM510 laser scanning confocal microscope system.

Time lapse of GFP-cyto.c release

Cell suspensions were transferred to a thermostated chamber with a glass cover-slip bottom, allowed adhering for 24 h, then cells were co-transfected with pEGFP-C3-cyto.c and MitoDsRed (DNA, 10:1) for 36 h before treatment with staurosporine. Cells with GFP-labeled cyto.c correctly located at mitochondria were chosen to perform the time lapse imaging. Samples were excited using λ 488/543 nm, and images were acquired every 2 min. The simultaneous recording of mitochondrial morphology (red) and fluorescent images of GFP-cyto.c was carried out by a ZEISS LSM510 laser scanning confocal microscope system.

Reference

- 1 Komoriya A, Packard BZ, Brown MJ, Wu ML, Henkart PA. Assessment of caspase activities in intact apoptotic thymocytes using cell-permeable fluorogenic caspase substrates. (Translated from eng) *J Exp Med* 2000; **191**:1819-1828 (in eng).