Methods 112 (2017) 105–123

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Multi-parametric imaging of cell heterogeneity in apoptosis analysis

Ivan A. Vorobjev [a,b,c,*], Natasha S. Barteneva [d,e]

[a] A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Russian Federation
[b] Department of Cell Biology and Histology, M.V. Lomonosov Moscow State University, Russian Federation
[c] School of Science and Technology, Nazarbayev University, Kazakhstan
[d] Program in Cellular and Molecular Medicine, Boston Childrens Hospital, Harvard Medical School, MA, USA
[e] Department of Pediatrics, Harvard Medical School, MA, USA

ARTICLE INFO

Article history:
Received 23 April 2016
Received in revised form 14 June 2016
Accepted 5 July 2016
Available online 5 July 2016

Keywords:
Apoptosis
Fluorescent microscopy
Imaging flow cytometry
Flow cytometry
Programmed cell death
Multicolor imaging

ABSTRACT

Apoptosis is a multistep process of programmed cell death where different morphological and molecular events occur simultaneously and/or consequently. Recent progress in programmed cell death analysis uncovered large heterogeneity in response of individual cells to the apoptotic stimuli. Analysis of the complex and dynamic process of apoptosis requires a capacity to quantitate multiparametric data obtained from multicolor labeling and/or fluorescent reporters of live cells in conjunction with morphological analysis. Modern methods of multiparametric apoptosis study include but are not limited to fluorescent microscopy, flow cytometry and imaging flow cytometry.

In the current review we discuss the image-based evaluation of apoptosis on the single-cell and population level by imaging flow cytometry in parallel with other techniques. The advantage of imaging flow cytometry is its ability to interrogate multiparametric morphometric and fluorescence quantitative data in statistically robust manner. Here we describe the current status and future perspectives of this emerging field, as well as some challenges and limitations. We also highlight a number of assays and multicolor labeling probes, utilizing both microscopy and different variants of imaging cytometry, including commonly based assays and novel developments in the field.

Contents

Abbreviations: 7-AAD, 7-aminoactinomycin D; AB, antibody; AcrDIMs, 3,6-bis((1-alkyl-5-oxo-imidazolidin-2-yliden)imino)acridine hydrochlorides; AIF, apoptosis-inducing factor; AnV, annexin V; AO, acridin orange; BRDU, bromodeoxyuridine; AVD, apoptotic volume decrease; Bax, Bcl-2 associated X-protein; BID, BH_3-interacting-domain death agonist; BODIPY, boron-dipyrromethene-based fluorescent lipid dyes; bp, base pair; CAD, caspase-activated DNAse; CCD, charging coupling devices; C-FLIP, FLICE-like inhibitory protein; CMOS, Complimentary Metal Oxide Semiconductor; DAPI, (4′,6-diamidino-2-phenylindole); DIC, differential interference contrast; DISC, death-inducing signaling complex; EGFP, enhanced green fluorescent protein; γH2AX, histone H2AX; FADD, Fas-associated death domain protein; FCCP, *p*-trifluoromethoxy carbonyl cyanide phenyl hydrazine; FITC, fluorescein isothiocyanate; FLICA, fluorescently labeled inhibitors of caspases; FMK, fluoromethylketone; FP, fluorescent protein; FRET, Förster resonance energy transfer; FSC, forward scatter; FSC-H, forward scatter height; FSC-W, forward scatter width; HCS, high-content screening; HIV-gp120, HIV envelope glycoprotein GP120; HLA, human leukocyte antigen; IAPs, inhibitory apoptotic proteins; IFC, imaging flow cytometry; IS, Imagestream; Lamp 1, lysosomal-associated protein 1 or CD107a; LC3, microtubule-associated protein 1 light chain 3; Lyso-ID, lysosomal selective dye, suitable for live cell imaging (Enzo Life Sciences, USA); LSC, laser scanning cytometry; LSCM, laser scanning confocal microscopy; MOMP, mitochondria outer membrane permeabilization; PARP, poly ADP ribose polymerase; PE, phycoerythrin; PI, propidium iodide; PS, phosphatidylserine; ROCK1, rho-associated, coiled-coil-containing protein kinase 1; SB, Sytox Blue; SSC, side scatter; STS, staurosporine; TMRE, teramethylrhodamine ethyl ester; TMRM, Tetramethylrhodamine methyl ester; TNF, tumor-necrotic factor; TRAIL, TNF-related apoptosis inducing ligand; TRITC, tetramethylrhodamine; UV, ultraviolet; XIAP, X-linked inhibitor of apoptosis protein.

* Corresponding author at: Laboratory of Cell Motility, A.N. Belozersky Institute for Physical and Chemical Biology, M.V. Lomonosov Moscow State University, Leninskie Gory 1, bldg. 40, Moscow 119991, Russian Federation.
E-mail address: vorob@libro.genebee.msu.ru (I.A. Vorobjev).

http://dx.doi.org/10.1016/j.ymeth.2016.07.003
1046-2023/

1. Introduction

Apoptosis is one of the processes of programmed cell suicide triggered by external or internal signals. Animal cells have different suicide programs [1,2] and many excellent reports describe several alternative cell death modes (caspase-independent apoptosis-like programmed cell death, autophagy, programmed necrosis or necroptosis) [3–8]. Major characteristic of apoptosis making it different from the other pathways of cell death is prolonged integrity of the plasma membrane when cell structure and metabolism are devastated by specific enzymes [9–11] and ATP production [12]. Another hallmark of apoptosis program is the activation of specific proteases, namely caspases and specific DNAses. Activation of caspases happens in at least two steps with first activation of regulatory caspase 8 and/or caspase 9 and consequent activation of executive caspase 3 (and occasionally some others – caspases 6 and 7). DNAse activation happens after activation of regulatory caspases, but might proceed independently of them. At the end of apoptosis execution cells in vivo are usually engulfed by other cells and gradually digested, while in vitro cells loose plasma membrane integrity and eventually die. Morphological features characteristic for apoptosis start with prolonged blebbing, continue with shrinkage of the cytoplasm with organelles [13,14]. Finally, shrinkage and fragmentation of cell nucleus and in some cases fragmentation of cells with formation of apoptotic bodies happens [15,16]. However, the aforementioned data represent the average estimation for cell population. At the single cell level not all events have been described so far and thus some of apoptotic events hardly could be put in line with others. Taking into account asynchrony in the apoptosis execution more detailed analysis becomes essential.

Modern cell death research actively uses microscopy and flow cytometry, and particularly exciting recent development – imaging flow cytometry (IFC). Over the past ten years IFC has undergone rapid development and has now spatial resolution sufficient for structure analysis at subcellular level and spectral resolution up to 10 colors for the quantitation of multiple fluorescent parameters in addition to bright field and side scatter.

Cytometric methods for the detection of apoptosis by single probes were excellently reviewed by Wlodkowic and others [17], thus we will focus mainly on the multiparameter evaluation of this process by imaging instrumentation such as Imagestream technology platform and multicolor fluorescent microscopy. Here we review the IFC field, discuss the new and elegant approaches to analyze apoptosis sequence and apoptosis regulators, and provide evidence that multiparametric IFC in combination with more traditional microscopy and flow cytometry can serve for further understanding of mammalian apoptosis.

2. Apoptosis sequence

Initially apoptosis had been defined as “shrinkage necrosis” [18] and two phases of this process were described at this time: cell