Barts and The London
School of Medicine and Dentistry

The Blizard Institute

Real Time Flow Cytometry for the Kinetic Analysis of Oncosis

G. Warnes, Flow Cytometry Core Facility

INTRODUCTION

Flow cytometry has been used to investigate apoptosis using various assays including Sub G_1 analysis of DNA content of cells, annexin V assay, caspase activity amongst but a few of a wide range of assays. However none of these very different approaches has been able to determine the difference between cells undergoing apoptosis and oncosis. Oncosis is a better term for the commonly misused term necrosis or accidental cell death. Here for the first time flow cytometric analysis of mitochondrial function in real-time shows that cells undergoing oncosis lose mitochondrial activity within the first hour of the oncotic process whilst cells undergoing apoptosis only lose mitochondrial activity after several hours.

METHODS

Time Course Experiments

Jurkat cells were treated for 1-30h with 1µM staurosporine (STS), Heat shock 42^{0}C & 56^{0}C, 0.25% & 1% sodium azide, 0.01% & 0.1% Triton X-100. Cells were labelled with annexin V-FITC, MitoTracker dye DiIC$_1$(5) (40nM), and DAPI (200ng/ml) to determine live, apoptotic and dead cells for each time point (n=3). The percentage of live cells with functioning mitochondria was also determined over 1-30h by use of the MitoTracker dye DiIC$_1$(5) (n=3). 100,000 events were acquired on a BD FACSCanto II flow cytometer.

Real Time Kinetic Analysis of Oncosis

Untreated Jurkat cells were labelled with annexin V-FITC, MitoTracker dye DiIC$_1$(5), plasma membrane potential dye, *bis*-oxonal (100nM) and DAPI to determine live, apoptotic and dead cells. Decrease in DiIC$_1$(5) fluorescence indicates mitochondrial inner membrane depolarization; whilst increase in *bis*-oxonal fluorescence indicates plasma membrane depolarization. Baseline levels of live cell DiIC$_1$(5) and *bis*-oxonal fluorescence's were made for 30 seconds then 1µM staurosporine (STS) was added, or cells were heat shocked at 42^{0}C or 56^{0}C, or treated with 0.25% or 1% sodium azide or 0.01% Triton X-100. Changes in mitochondrial function and plasma membrane potential over 25 minutes were then measured by real-time flow cytometry. 2,00,0000 events were acquired on a BD LSR II flow cytometer collected over 25 minutes.

Image Analysis of Oncosis

Untreated Jurkat cells were labelled with annexin V-FITC, MitoTracker dye DiIC$_1$(5), plasma membrane potential dye, *bis*-oxonal and DAPI to enable imaging of live cells. Cells were then treated with 1µM staurosporine (STS), or cells were heat shocked at 42^{0}C and 56^{0}C, or treated with 1% sodium azide or 0.01% Triton X-100 for 30 min. Live cells were then imaged on a Zeiss 510 confocal to show changes in mitochondrial function and plasma membrane potentials.

RESULTS

Annexin V Assay

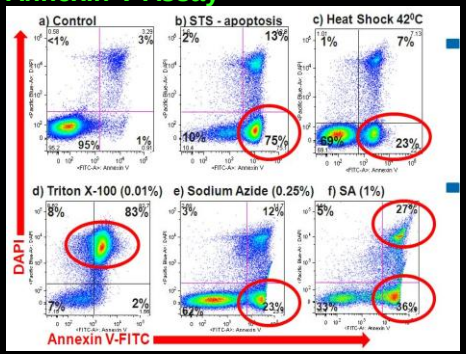

Figure 1. Jurkat cells were treated with for 5h a) control, b) 1µM staurosporine (STS), c) Heat shock 42^{0}C, d) 0.01% Triton X-100, e) 0.25% sodium azide (SA) f) 1% SA. Cells were labelled with annexin V-FITC and DAPI to determine live, apoptotic and dead cell populations (n=3).

Annexin V Time Course

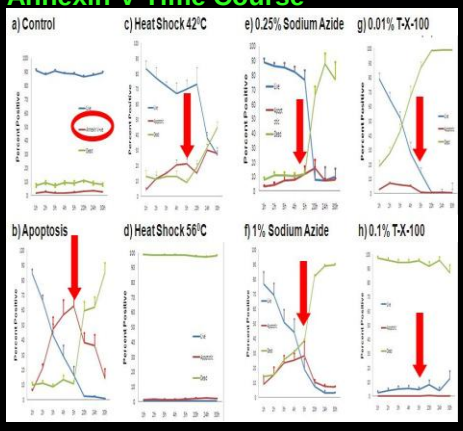

Figure 2. Jurkat cells were treated with for 1-30h a) control, b) 1µM staurosporine (STS), c) Heat shock 42^{0}C d) HS 56^{0}C, e) 0.25% sodium azide f) 1% sodium azide g) 0.01% Triton X-100, h) 0.1% Triton X-100. Cells were labelled with annexin V-FITC and DAPI to determine live, apoptotic and dead cells for each time point (n=3). Error bars indicate SEM.

Real-Time Kinetic Analysis of Oncosis

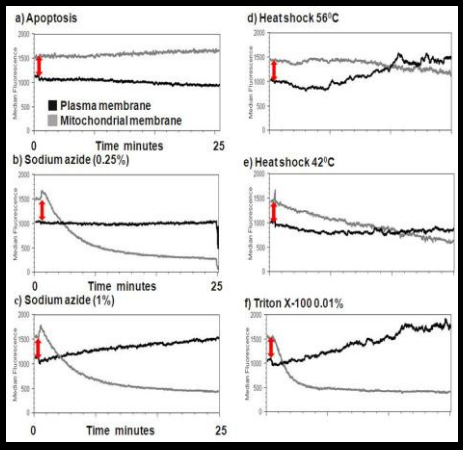

Figure 3. Untreated Jurkat cells were labelled with annexin V-FITC, MitoTracker dye DiIC$_1$(5), plasma membrane potential dye, *bis*-oxonal and DAPI to determine live, apoptotic and dead cell populations. Baseline levels of live cell mitochondrial function and plasma membrane potential were made by monitoring changes in DiIC$_1$(5) fluorescence and *bis*-oxonal intensity for 30 seconds. Then as indicated by the arrow different reagents were then added including, a) 1µM staurosporine (STS), b) 0.25% sodium azide c) 1% sodium azide, d) cells were heat shocked at 56^{0}C, e) cells were heat shocked at 42^{0}C, f) 0.01% Triton X-100. Real-time changes in mitochondrial function and plasma membrane potential were then measured over 25 minutes. 2,00,0000 events were acquired on a BD LSR II flow cytometer over 25 minutes.

Mitochondrial Dysfunction

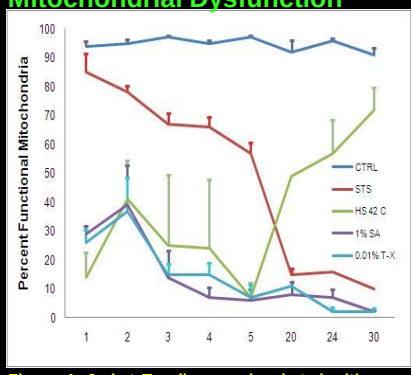

Figure 4. Jurkat T cells were incubated with no treatment, 1µM STS, HS 42^{0}C, 1% sodium azide (SA) or 0.01% Triton X-100 for 1-30h (n=3). Cells were labelled with annexin V-FITC, MitoTracker DiIC$_1$(5) and DAPI to determine the level of mitochondrial dysfunction in the live cell population for each time point. Error bars indicate SEM.

Imaging Oncosis

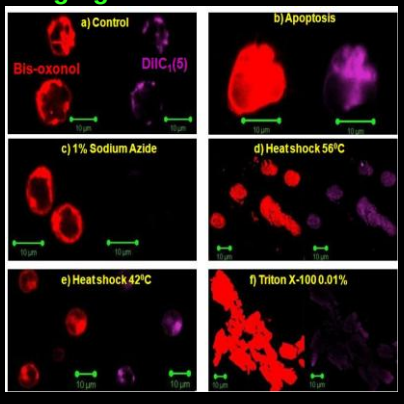

Figure 5. Untreated Jurkat cells were labelled with annexin V-FITC, MitoTracker dye DiIC$_1$(5), plasma membrane potential dye, *bis*-oxonal and DAPI to determine live, apoptotic and dead cells. Cells were a) untreated, b) treated with 1µM staurosporine (STS), c) treated with 1% sodium azide, d) cells were heat shocked at 56^{0}C, e) or at 42^{0}C, f) treated with 0.01% Triton X-100 for 30 min. Live cells were imaged on a Zeiss 510 confocal microscope. Decrease in DiIC$_1$(5) fluorescence indicates mitochondrial dysfunction. Whilst increases in *bis*-oxonal intensity indicates plasma membrane depolarization.

CONCLUSIONS

- Annexin V was not significantly up-regulated during oncosis
- Real-Time flow cytometric analysis showed that oncosis reduced mitochondrial function and depolarized the plasma membrane whilst apoptosis did not significantly induce any such changes
- The decrease in mitochondrial function & plasma membrane depolarization was confirmed by image analysis
- Please see publication in *Cytometry 79A*, p181-191, 2011

Please contact:- Dr Gary Warnes
g.warnes@qmul.ac.uk
tel. no. 020-7882-2402