INTRODUCTION

Flow cytometry has been used to investigate apoptosis using various assays including Sub G, 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 necrosis. 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°C & 56°C, 0.25% & 1% sodium azide, 0.01% & 0.1% Triton X-100. Cells were labelled with annexin V-FITC, MitoTracker dye DiIC(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 measuring V potentials (n=100,000 events were acquired on a BD FACScan). Alive, apoptotic and dead cells for each time point (n=3). Error bars indicate SEM.

Real Time Kinetic Analysis of Oncosis

Untreated Jurkat cells were labelled with annexin V-FITC, MitoTracker dye DiIC(5), plasma membrane potential dye, bis-oxonol (100nM) and DAPI to determine live, apoptotic and dead cells. Decrease in DiIC(5) fluorescence indicates mitochondrial inner membrane depolarization; whilst increase in bis-oxonol fluorescence indicates plasma membrane depolarization. Baseline levels of live cell DiIC(5) and bis-oxonol fluorescence were made for 30 seconds then 1μM staurosporine (STS) was added or cells were heat shocked at 42°C or 56°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, 20,000,000 events were acquired on a BD LSR II flow cytometer collected over 25 minutes.

RESULTS

Annexin V Assay

Figure 1. Jurkat cells were treated with for 6h a) control, b) 1μM staurosporine (STS), c) Heat shock 42°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).

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.

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