New Flow Cytometry Software Enables High Throughput Determination of Apoptotic Mechanism

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INTRODUCTION

The study of the role of mitochondria in cell health and apoptosis has become increasingly important. Here, we study the inter-relationships between mitochondrial membrane potential, apoptosis, cell death and mitochondrial superoxide stress using the multiplexing power of the guava easyCyte 8HT benchtop flow cytometry platform and Incyte software when cells are treated with multiple inducers

Using the guava® easyCyte™ 8HT flow cytometer with InCyte™ software (Millipore) and the FlowCellect™ MitoDamage Kit (Millipore), we demonstrate that simultaneous dose-response data can be obtained on mitochondrial membrane potential change, apoptosis and cell death using inducers such as staurosporine. Further, the MitoStress Kit demonstrates that stauropsorine also induces an increase in mitochondrial superoxide stress and apoptosis almost simultaneously, which is in agreement with the current literature on mode of staurosporine action.

METHODS

The FlowCellect MitoDamage kit includes: MitoSense Red, which is responsive to mitochondrial potential changes; CF488A-conjugated Annexin V, which binds to phosphatidylserine (PS) on the surface of apoptotic cells; and 7AAD, a membrane-impermeant dead cell dye. Cellular mitochondrial potential changes cause a shift from high Red2 to low Red2 fluorescence. Increased phosphotidylserine exposure causes an increase in green fluorescence. Increased necrosis results in increased red fluorescence.

Jurkat cells were treated with multiple concentrations of inducers such as diamide and staurosporine; treated with kit reagents; and analysed on the guava easyCyte 8HT instrument. Heat maps and EC50 curves were easily generated *(Figure 1)* within the InCyte software enabling simultaneous comparison of multiple data sets.

RESULTS AND CONCLUSIONS

Our data demonstrate how the combination of InCyte software with MitoDamage kits gives flow cytometry users the power to quickly draw conclusions about the biological significance of multi-parametric apoptosis data. The combination also enables responses for the same sample to be compared across multiple reagents, which is highly valuable in time-based and dose-based tracking.

Increased concentrations of staurosporine resulted in dissipation of mitochondrial membrane potential and entry into apoptosis (as measured by Annexin V binding). The EC50 values for both mitochondrial membrane change and Annexin V binding were very similar with EC50 of 0.45 uM and 0.43 uM, respectively, demonstrating that simultaneous analysis of these parameters allows for greater precision in measurements. The coincidence of these two phenotypes provides support for the biological linkage of staurosporine-mediated apoptosis to changes in mitochondrial membrane potential. Further research is required to determine the precise pathway by which inducers such as staurosporine and diamide result in mitochondria-associated apoptosis.

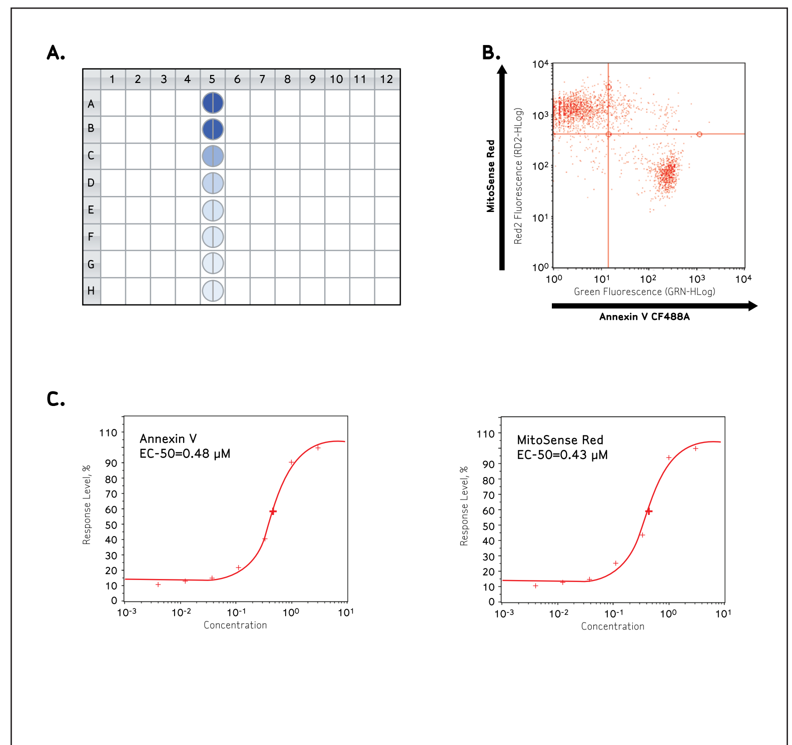

Figure 1. Correlation of mitochondrial potential with staurosporine-induced apoptosis. Treatment of Jurkat cells with staurosporine followed by staining with the FlowCellect MitoDamage Kit allowed for simple generation of a heat map (A), dot plots (B), and dose response curves with EC50 values for mitochondrial membrane potential change and Annexin V binding (C).

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