A multiplex assay to simultaneously monitor apoptosis and necrosis using the Cellaca® PLX Image Cytometer

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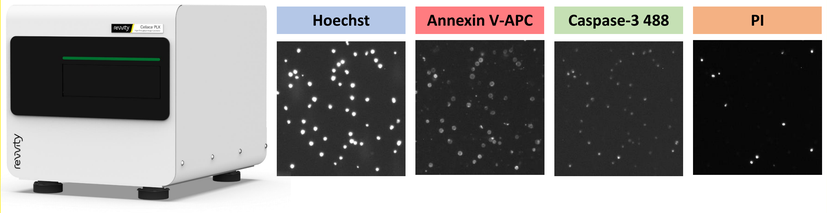
1. ABSTRACT

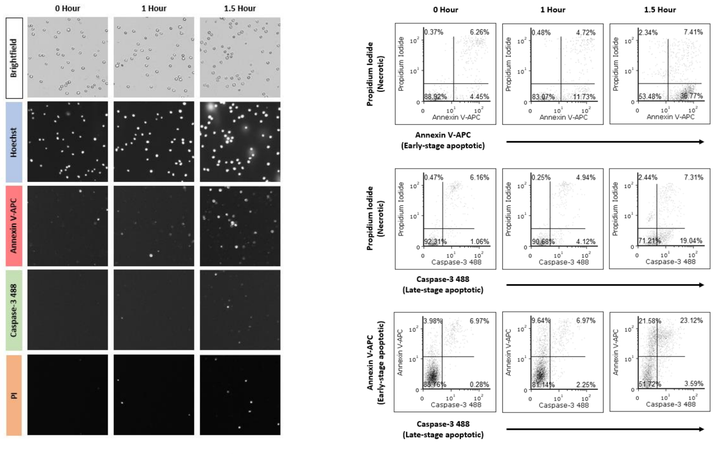
Apoptosis is a critical pathway for programmed cell death that cancer cells can evade to ensure survival. For pharmaceutical drug discovery, it is important to characterize and compare cancer therapeutics (i.e., small molecules, antibody drugs, cell therapies) that can initiate the process of apoptosis, allowing for the identification of potential therapeutic candidates. In this work, we developed and demonstrated a multiplex detection method for monitoring apoptosis and necrosis with Annexin V, Caspase-3, and Propidium Iodide (PI) using the Cellaca® PLX Image Cytometer. First, apoptosis was induced in Jurkat and K562 cell lines with staurosporine over the course of 24 h, and were monitored at 0, 1, 1.5, 2, 4, 20, and 24 h timepoints. Samples were stained with Hoechst 33342 (total dye), Annexin V-APC (early-stage apoptosis), Caspase-3 488 (late-stage apoptosis), and PI (necrosis) at each timepoint and evaluated using the Cellaca® PLX. Results showed that apoptotic factors and cascades were successfully detected along the pathway from early to late-stage apoptosis, and ultimately necrosis. A clear trend was observed during the first 1.5 h showing differences of up to 15% between the single Annexin V+ and Caspase-3+ populations in treated Jurkat cells, however, a significant increase in double positive apoptotic/necrotic cells for Annexin V+PI+ and Capase-3+PI+ was not observed until 20 h. Upon further analysis amongst apoptotic populations, Annexin V+ only populations were higher than Caspase-3+ only populations by up to 20% between 0-1.5 hours. Conversely, K562 cells did not exhibit a notable change in apoptotic and necrotic populations due to low sensitivity to staurosporine. The proposed image-based detection method using the Cellaca® PLX may provide an effective and efficient tool for rapid and reliable simultaneous detection of early, late-stage apoptosis, and necrosis. This may allow researchers to better characterize and screen potential cancer therapeutic drug candidates in a high-throughput manner to determine their treatment efficacy.

2. CELLACA® PLX IMAGE CYTOMETRY

- The Cellaca® PLX is a high-throughput image cytometer capable of brightfield and fluorescence based cytometric analysis
- Applications on the Cellaca® PLX include direct cell counting, high-throughput viability, apoptosis detection, fluorescent protein expression, and immune cell phenotyping
- A 4-color detection panel was used to stain staurosporine treated Jurkat and K562 cell lines for early- and late-stage apoptosis, and necrosis
- Samples were stained with Hoechst (total), Annexin V-APC (early-stage apoptosis), Caspase-3 488 (late-stage apoptosis), and PI (necrosis) at 0, 1, 1.5, 2, 4, 20, and 24 h timepoints



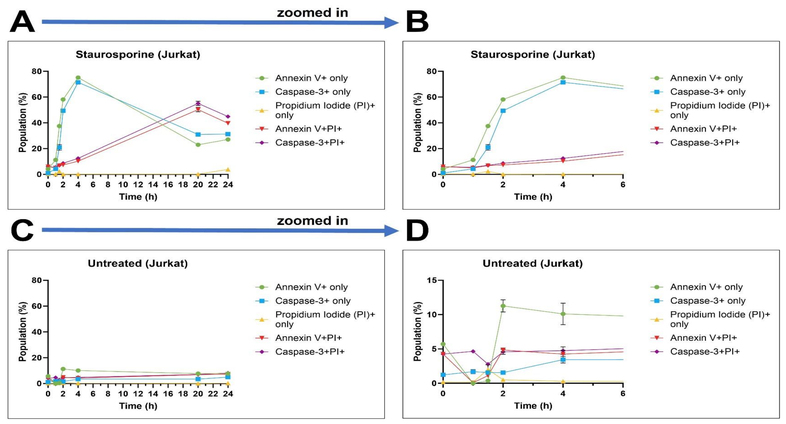
3. JURKAT PANEL



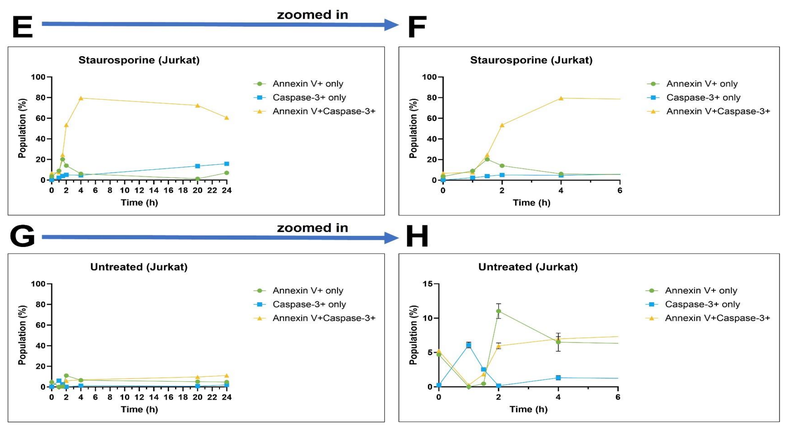
- Representative images of staurosporine treated Jurkat cells stained with Hoechst, Annexin V-APC, Caspase-3 488, and PI acquired using the Cellaca® PLX
- Scatter plots identifying an increase in early- and late-stage apoptotic, and necrotic populations in staurosporine treated Jurkat cells

4. JURKAT POPULATION ANALYSIS

Annexin V, Caspase-3, and PI Jurkat population analysis

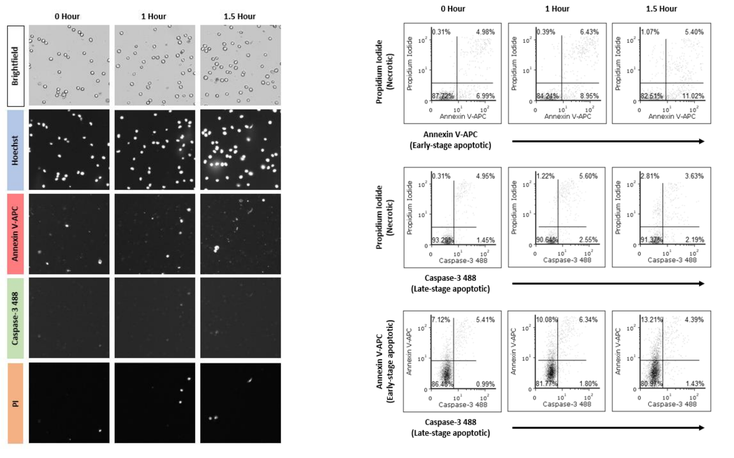


Annexin V and Caspase-3 Jurkat population analysis



- Time-dependent early- and late-stage apoptotic, and necrotic identification comparing Annexin V-APC, Caspase-3 488, and PI populations in staurosporine treated and untreated Jurkat cells
- Higher Annexin V+ only populations were detected in comparison to Caspase-3 488+ only populations between 0-2 h, indicating more cells in the early apoptotic phase. Populations were almost identical from 4 h and onward, suggesting that cells transitioned from early- to late-stage apoptosis
- Annexin V+PI+ and Caspase-3 488+PI+ populations increased drastically between 4-20 h, while single Annexin V+ and Caspase-3 488+ populations decreased, indicating that membrane integrity was compromised
- Extremely low levels of PI+ only populations were detected throughout, suggesting that all necrotic cells were labeled for apoptotic markers simultaneously

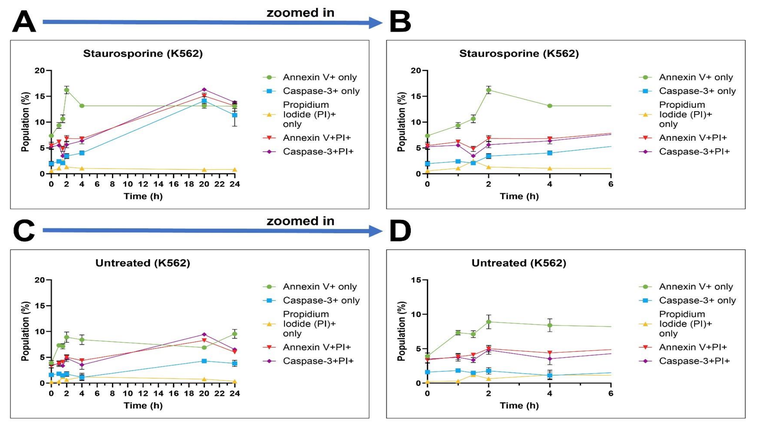
5. K562 PANEL



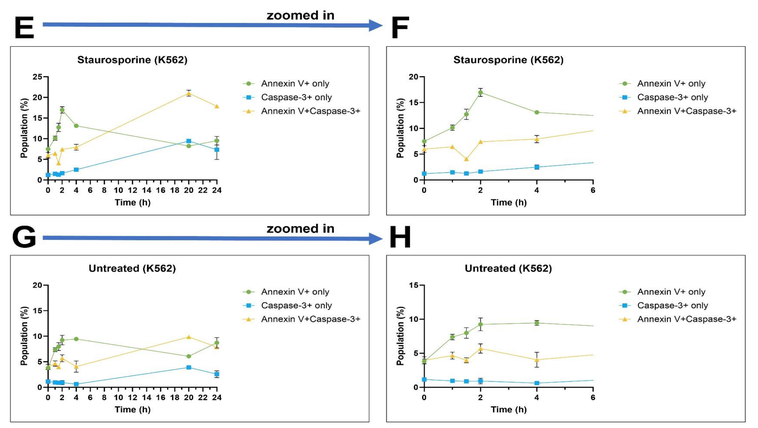
- Representative images of staurosporine treated K562 cells stained with Hoechst, Annexin V-APC, Caspase-3 488, and PI acquired using the Cellaca® PLX
- Scatter plots identifying a minimal increase in early- and late-stage apoptotic, and necrotic populations in staurosporine treated K562 cells

6. K562 POPULATION ANALYSIS

Annexin V, Caspase-3, and PI K562 population analysis



Annexin V and Caspase-3 K562 population analysis



- Time-dependent early- and late-stage apoptotic, and necrotic identification comparing Annexin V-APC, Caspase-3 488, and PI populations in staurosporine treated and untreated K562 cells
- Interestingly, K562 cells showed more resistance to staurosporine drug treatment than Jurkat cells, as Annexin V+ only and Caspase-3 488+ only populations upheld low levels of apoptosis during the 24 h period
- Subsequently, Annexin V+PI+ and Caspase-3 488+PI+ populations also remained low for 24 h, suggesting minimal apoptosis and necrosis occurred
- Very low levels of PI+ only populations were detected throughout, suggesting that few cells had compromised membrane integrity

7. CONCLUSION

- We demonstrated a 4-color panel simultaneously identifying early- and late-stage apoptosis, and necrosis on the Cellaca® PLX
- Using the Cellaca® PLX we showed results for the following in staurosporine treated Jurkat cells:
  - Higher Annexin V+ only than Caspase-3 488+ only populations between 0-2 h
  - Increase in Annexin V+PI+ and Caspase-3 488+PI+ populations between 4-20 h, while a decrease in Annexin V+ only and Caspase-3 488+ only populations were observed concurrently
  - Minimal levels of PI+ only populations detected over the 24 h period
- Using the Cellaca® PLX, results for staurosporine treated K562 cells displayed low levels of apoptosis and necrosis during the 24 h study
- Employing this image cytometer and 4-color panel may prove useful for researchers screening therapeutic drug candidates