The Mechanism and Function of Dynamic Blebbing in Human Embryonic Stem Cells

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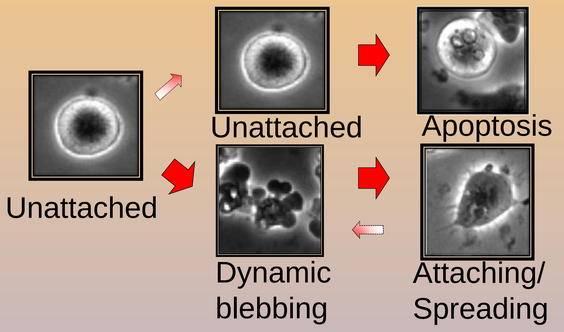
Introduction

Dynamic blebs are membrane protrusions that appear and disappear from cell surfaces. They are not equivalent to apoptotic blebs associated with cell death. In animal cells, dynamic blebbing is observed during cytokinesis and some types of cell migration.

Dynamic vs Apoptotic Blebbing in in hESC



To study blebbing in human embryonic stem cells (hESC), we used a new video technology, the Nikon BioStation IM which combines an incubator, microscope, and cooled CCD camera in a compact body, allowing time-lapse imaging of cells for minutes, days, or even weeks.

Comparison of Blebbing in 4 Cell Types

We compared dynamic blebbing in DU145 human prostate cancer cells, mouse embryonic fibroblasts, mouse embryonic stem cells, and hESC using a BioStation IM. Each cell type underwent dynamic blebbing before attachment to its substrate. However, hESC produced more blebs, blebbed more rapidly, and blebbed longer (50 minutes vs 10-25 minutes) than the other cell types.

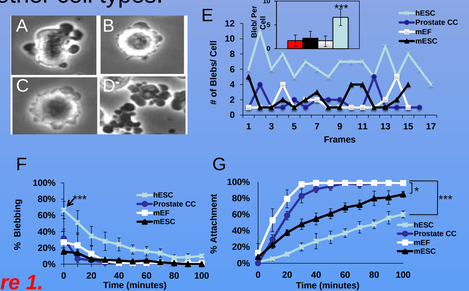


Figure 1.

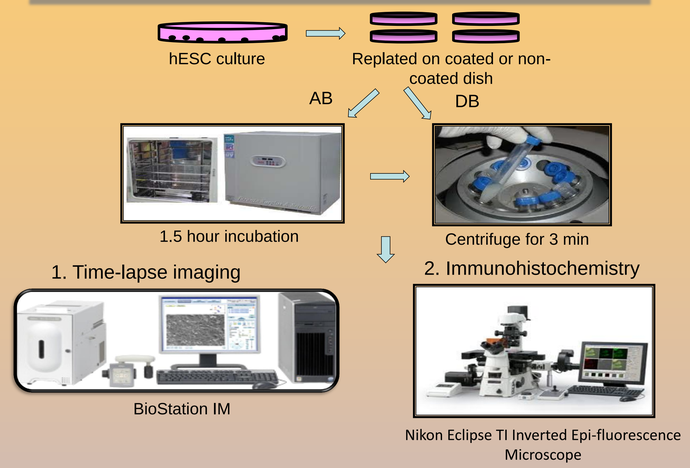
Purpose

•To better characterize apoptosis in hESC and develop better methods to inhibit apoptosis in cultured hESC.
•To understand the mechanism and function of dynamic blebbing in hESC.

Acknowledgements

We are grateful for support from CIRM, TRDRP, IGERT, Randy Myers and the entire Talbot Lab.

Methods & Materials



Results

Time Line of Dynamic Blebbing, Attachment, Rounding, and Apoptotic Blebbing in hESCs.

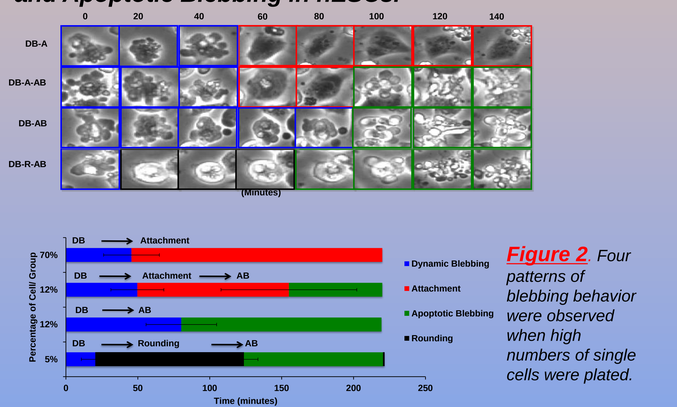


Figure 2. *Four patterns of blebbing behavior were observed when high numbers of single cells were plated.*

The mitochondrial outer membrane potential and activation of caspases 3&7 in dynamic and apoptotic cells.

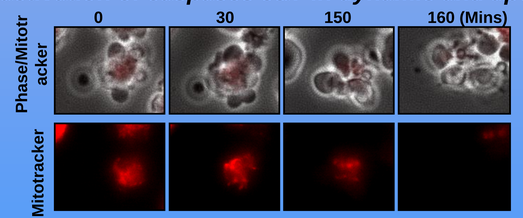


Figure 3. *Apoptotic blebbing cells showed diminished fluorescence indicating a loss of mitochondrial membrane potential.*

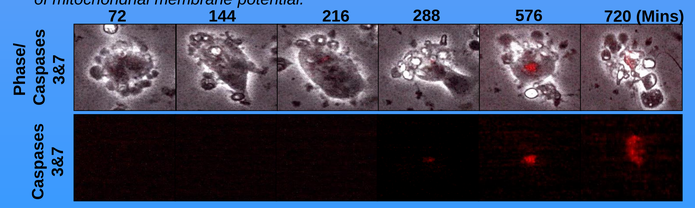


Figure 4. *After cells had incubated on non-coated dishes, activated caspases 3&7 were detected, and shortly thereafter apoptotic blebs appeared.*

Results

The duration of bleb formation and retraction in dynamically and apoptotically blebbing cells

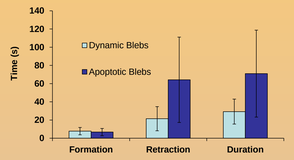


Figure 5. *Most dynamic and apoptotic blebs formed within 10 seconds of bleb initiation. However, apoptotic blebs took much longer to retract (average = 64 seconds for 69 cells) than dynamic blebs (average = 22 seconds for 80 cells) after expansion.*

Distribution of the cytoskeleton in dynamically and apoptotically blebbing cells.

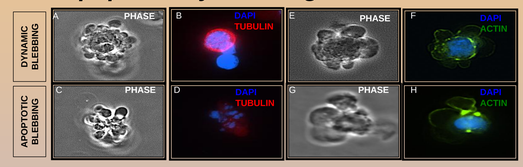


Figure 6. *During formation of dynamic blebs, microtubules formed a thick band around the nucleus, but did not extend into blebs. There were no microtubules in apoptotically blebbing cells with fragmented nuclei. In dynamic and apoptotic cells, some blebs had actin beneath their membranes while others did not. In apoptotic cells, much of the actin was concentrated in hot spots in the cortex.*

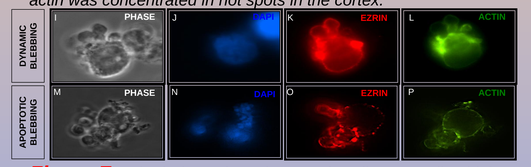


Figure 7. *In both dynamic and apoptotic cells, expanding bleb membranes had ezrin and either lacked associated actin or had a continuous band of actin subjacent to the membrane.*

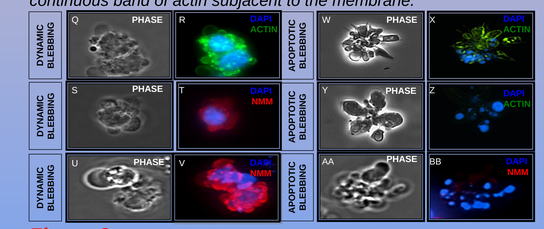


Figure 8. *In dynamically blebbing cells, small retracting blebs had intense actin and myosin labeling adjacent to the bleb membrane. In early stages of apoptosis, actin and myosin were adjacent to bleb membranes, and actin was sometimes fragmented and formed hot spots in blebs. In apoptotic cells with highly fragmented nuclei, actin and myosin were not observed in the blebs or cell center.*

Cytochalasin D, ROCKi, and Blebbistatin but not Nocodazole inhibited dynamic blebbing in hESC.

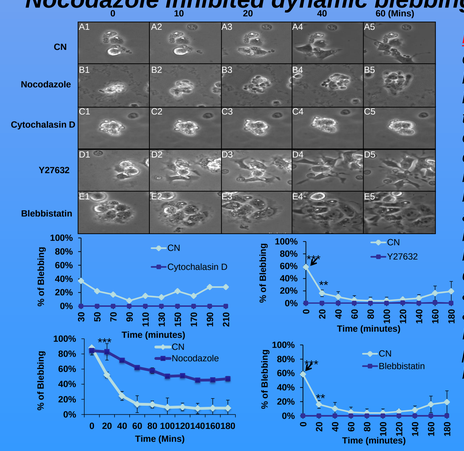


Figure 9. *In living cells, dynamic blebbing was inhibited during treatment with cytochalasin D; cells remained round with few or no blebs and did not attach. ROCK inhibitor and blebbistatin stopped dynamic blebbing and accelerated cell attachment. Nocodazole prolonged dynamic blebbing.*

The role of the cytoskeleton in apoptotic blebbing.

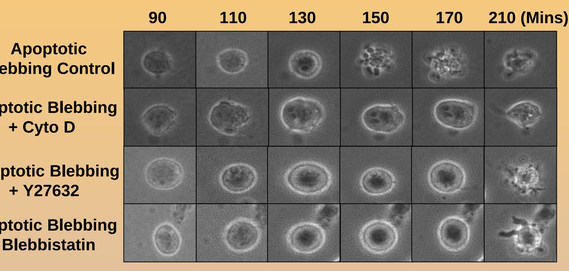


Figure 10. *In apoptotic cells, cytochalasin D stopped apoptotic blebbing. ROCK inhibitor and blebbistatin delayed apoptotic blebbing.*

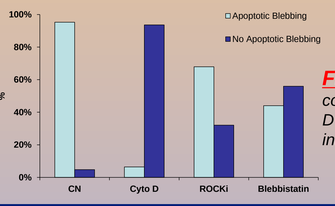


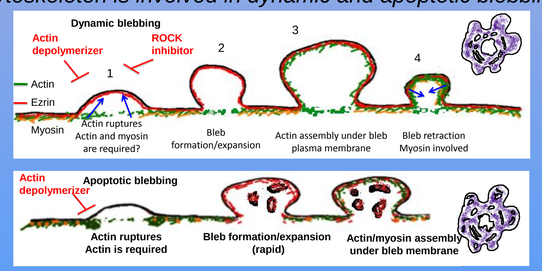
Figure 11. *Apoptotic blebbing was completely inhibited by cytochalasin D but only partially inhibited by ROCK inhibitor and blebbistatin.*

Conclusion

•Activated caspases 3&7 and loss of mitochondrial membrane potential were only observed in apoptotically blebbing cells, indicating that type II apoptosis, which is mitochondrial-dependent, occurred in cultured hESC.

•Real time imaging of live cells showed that formation of dynamic and apoptotic blebs occurs in 10 seconds, while retraction takes 22 seconds for dynamic bleb and 64 seconds for apoptotic blebs on average.

•Based on the fluorescent labeling and depolymerizer experiments, we propose a model to explain how the cytoskeleton is involved in dynamic and apoptotic blebbing.



•ROCKi inhibited dynamic blebbing and accelerated cell attachment. Unexpectedly the ROCKi did not completely block apoptotic blebbing.

Future Directions

To develop video bioinformatics tools to quantify dynamic and apoptotic blebbing in single hESC.
•To follow dynamic blebbing cells in time-lapse videos.
•To distinguish dynamically blebbing cells from apoptotically blebbing cells.