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Death is not the end

In the time it takes you to read this sentence, millions of cells within your body would have scarified themselves for the greater good of your heath. Apoptosis, Greek for '*leaves falling from a tree'*, has been vastly studied for decades leading to the intricate mapping of its stringent molecular pathways. Perhaps, this is why apoptosis is now frequently overlooked in the rapidly evolving field of cell death consisting of everything 'tosis', such as necroptosis, ferroptosis, pryoptosis, NETosis and PANtosis (*1*). However, as the field moves on from the excitement of classical apoptosis, an intriguing question has remained – what happens during the final moments of apoptotic cell death?

In 1972, John Foxton Ross Kerr, an Australian pathologist, was the first to describe the morphological changes that occur during apoptosis (*2*). Since then, plasma membrane blebbing (the formation of balloon-like structures on the cell surface) has been highly regarded as a morphological hallmark of apoptotic cell death. Kerr *et al.* also noted the formation of small fragments generated from the apoptotic cell, "…*we shall call them apoptotic bodies*" (*2*). However, apoptotic bodies have been largely dismissed of any functional significance and are assumed to form through a mere stochastic event. Nevertheless, perhaps aspiring to follow in the footprints of Australia's renowned apoptosis researchers, in 2015 we began to discover that apoptotic bodies are indeed, more than just debris.

Discovery of a novel 'beads-on-a-string' membrane structure

Monocytes are the warriors of our immune system, constantly at the heart of infection and inflammation where they fight to maintain our health. As a consequence, there are countless disease settings in addition to physiological homeostasis, where monocytes die through apoptosis. But, what happens in the final moments of a monocytes life? To answer this question, we turned to time-lapse confocal microscopy which provides an unparalleled approach to visualize the entire process

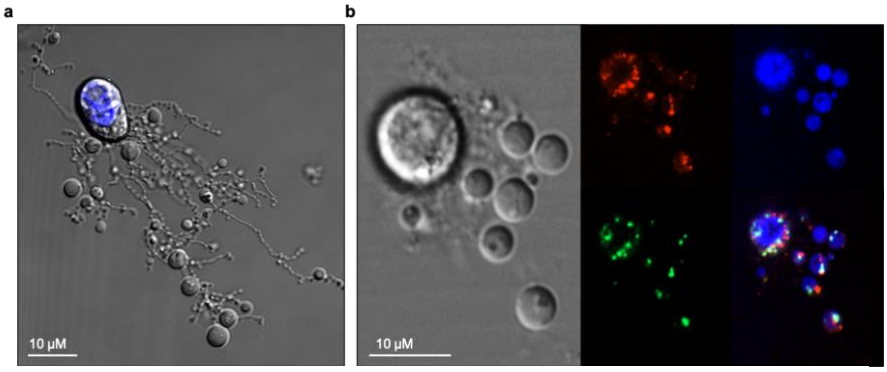

Figure 1. *Confocal microscopy images of apoptotic monocytes generating beaded apoptopodia, approximately 3 hours post UV irradiation.* ***a)*** *Blue = Hoechst.* ***b)*** *Red = LysoTracker, Green = MitoTracker, Blue = TO-PRO-3.*

of cell death from conception to completion. By capturing images every minute, we were able to witness the surprisingly dynamic nature of monocyte apoptosis, a process we colloquially refer to as *the 'dance of death'*. In contrast to a stochastic event as once believed, apoptotic monocytes follow a coordinated three-step process including plasma membrane blebbing, the formation of long beaded necklace-like protrusions we coined beaded apoptopodia, and the fragmentation into apoptotic bodies (*3*, *4*). Beaded apoptopodia, generated exclusively during apoptosis, can extend up to 10 times the length of the apoptotic cell and undergo a segmentation-like event to form their characteristic 'beads-on-a-string' morphology (see figure) (*4*). In their final moments, beaded apoptopodia fragment to release abundant apoptotic bodies which are generally ~1 μm in diameter and depleted of all nuclear content, representing a unique mechanism of apoptotic body formation (*4*).

Identifying the regulators of beaded apoptopodia

Surprisingly, beaded apoptopodia shared very few mechanistic similarities to other well-characterized membrane protrusions such as filopodia and lamellipodia, which are exclusively actin-rich and dependent (*5*). Thus, this drove us to further explore the mechanistic force responsible for beaded apoptopodia formation. Morphologically, apoptotic monocytes share a striking similarity with neurons which both exhibit long membrane structures (either beaded apoptopodia or an axon/dendrites, respectively) that extend from an individual cell body (*6*, *7*). In line with this, one of the key proteins involved in axonal guidance and dendrite growth was identified in an unbiased proteomic screen on beaded apoptopodia-derived apoptotic bodies – a protein called Plexin B2 (*8*, *9*). Through a CRISPR/Cas9-based approach, we discovered a novel role of Plexin B2 in mediating the formation of beaded apoptopodia and the subsequent fragmentation into apoptotic bodies (*9*). Moreover, like many of the key apoptotic regulators, we found Plexin B2 to be targeted by caspases 3 and 7 however, the cleavage product was enriched in monocytes apoptotic bodies and now represents a novel marker for extracellular vesicle research (*9*).

Interestingly, another protein with well characterized roles in neuronal function, the membrane channel Pannexin 1, was also found to coordinate the disassembly of apoptotic monocytes (*4*, *10*). In contrast to Plexin B2, Pannexin 1 was identified as a negative regulator whereby pharmacological inhibition led to an increase in monocyte apoptopodia and apoptotic bodies (*4*). The extension of the neuron axon and their dendrites ultimately facilitates effective communication between neighbouring

cells, a process driven by dynamic membrane changes and trafficking (*11*). Similarly, we also identified a key role of vesicular trafficking in the biogenesis of beaded apoptopodia and together, highlights a surprising parallel between two distinct cellular processes of opposing nature (*4*).

The double edge-sword of apoptotic body formation

The clear mechanistic control of beaded apoptopodia formation leaves one asking, why? Akin to you and I who cut up a steak with a knife and fork during dinner, perhaps the organized fragmentation of apoptotic cells could also provide small, 'bite-sized' pieces to aid their engulfment – with Plexin B2 the knife, and Pannexin 1 the fork. Through impairing cell disassembly (using a Plexin $B2^{-/-}$ system) and monitoring phagocytosis, we demonstrated that dying cell disassembly via beaded apoptopodia was indeed required for efficient phagocytic clearance *in vitro* and *in vivo* (*9*).

Although beneficial under homeostatic conditions, disease settings may provide a contrasting scenario where the vast number of monocyte apoptotic bodies could outcompete rapid clearance and play a pathogenic role. Given the prevalence of monocytes and cell death during infectious disease (*12*), we assessed the functional consequence of monocyte apoptotic body formation during influenza A virus (IAV) infection. IAV-infected monocytes disassemble into abundant apoptotic bodies which harbour influenza biomolecules and virions (*13*). Like a Trojan horse, such apoptotic bodies could propagate infection to surrounding cells and elicit a robust anti-viral immune response. Thankful to the unexpected and still unclear mechanistic similarities to neurons, we identified that a commonly prescribed antipsychotic (Haloperidol) that normal targets neuronal dopamine receptors, could impair the disassembly of apoptotic monocytes and thus, limit the propagation of IAV and reduce disease burden (*13*). Altogether, the findings from this thesis challenge a long-standing paradigm and demonstrate that apoptotic body formation is not simply a stochastic event, but a tightly controlled molecular process with important functional consequences.

References

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