Emergence of the Wallerian degeneration pathway as a mechanism of secondary brain injury

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Augustus Volney Waller was a renowned British neurophysiologist who birthed the axon degeneration field in 1850 by describing curling and fragmentation of the glosopharyngeal and hypoglossal cranial nerves of a frog following a transection injury. The degeneration of axons after a transection injury is now known as Wallerian degeneration (WD). Waller’s work was expanded by Santiago Ramón y Cajal who described in detail the morphological stages of WD from monitory fragmentation of the axon and the granular disintegration of the neurofibris to the final resorption of the axon. Interest in this field grew rapidly in the early 1990’s with the novel discovery of axonal degeneration genes. A forward genetic screen was conducted in Drosophila melanogaster and applied it to the axon degeneration field. In a seminal experiment, Sarm1 possession of enzymatic NAD cleavage activity which is key for its pro-degenerative function (Essuman et al., 2017), making it an attractive target for drug development programs. SARM1 NAD cleavage activity is further induced following axotomy and NMNAT2 depletion. Several studies suggest that a toxic rise in NMNAT2 substrate NMN as a consequence of NMNAT2 loss is important to regulate and increase SARM1 activity. Downstream of SARM1 are final common execution events such as raised intracellular calcium and calpain activation. These terminally dismantle the axon before it is phagocytosed by immune cells. The fine details of the WD process are still being elucidated and it is likely that beyond the core pathway there will be various parallel streams of input and modulating processes. At present known modulatory factors include MAPK, SKP1A, FBX045, SCG10, Pobbled and the ZNF13-AKT-GSK3B-CRM2 pathways. Loss-of-function mutations in the highwire gene in Drosophila and the mammalian ortholog MYCBP2(PHR-1) is another such factor. The highwire gene encodes a large protein with E3 ubiquitin ligase activity that modulates levels of dNMNAT and it is associated with a strong delay in axon degeneration both in vitro and in vivo. Although the vista of WD has broadened significantly in the past 30 years, the story is by no means complete, and further discoveries will be required to fully describe this evolutionarily conserved death pathway. A simplified linear WD pathway is shown in Figure 1.

Our understanding of WD continues to advance at pace. The striking protection achieved with Sarm1 deletion (lifesaving rescue in certain circumstances) has encouraged pharmaceutical companies to target the WD pathway, due to its possible role in neurological disorders affecting both the peripheral and central nervous system (Coleman and Höke, 2020). Despite some differences between WD in the peripheral nervous system and central nervous system, namely in the inflammatory response to injury and the time required to clear myelin debris by different glial cells, the axon death pathway discussed above continues degeneration of axons and myelin in both the peripheral nervous system and central nervous system axons. One of these diseases is traumatic brain injury (TBI) (Hill et al., 2016). An estimated 10 million people per year suffer a TBI; it is one of the leading causes of death in many parts of the world and can have profound individual and socioeconomic consequences. TBI differs from many traditional diseases in so far as it is not primarily driven by genetic mutation or an aberration of normal physiology. Instead it is an acquired insult caused by an external force at a single moment in time. What follows can be understood in terms of subsequent biological consequences of that initial act – known as secondary brain injury. While the cause of the primary neurological injury is usually obvious, the multitude of processes driving secondary injury are concealed at the cellular and subcellular levels (Hill et al., 2016).

In 1956, Sabina Strich described a dementia-like phenotype with histological evidence of white matter degeneration in 5 patients following severe head injury. Experimental evidence of diffuse axonal pathology in large-animals subjected to trauma was reported in the 1980s and is supported by recent evidence of progressive white matter degeneration, SARM1, an axonal isoform, is now known as a major player in traumatic brain injury in humans. The relationship of these findings to WD has not been validated with certainty. A timeline showing the relative discoveries in WD and TBI science is presented in Additional Figure 1.

Not all axonal injury and death is via the WD pathway and acknowledgement of this fact is important if we are to avoid inadvertently attributing effects to WD when it is actually due to modulation of distinct secondary injury mechanism. Primary traumatic axotomy occurs when an injury leads to direct axon transection. Even in severe TBI this is thought to affect relatively small numbers of axons and so this is unlikely to be a major player in patient outcomes following injury. A common criticism of WD research is that if axon-soma disconnection has occurred (as in transection) then what would be the functional benefit of delaying, or even permanently stopping, as the axon is no longer an intact functional neurological unit. This is a valid statement and it seems logical that those transected nerves are beyond salvage. However, whether temporarily delaying the physical fragmentation of axons, and the associated signaling and glial events that may accompany it, has any beneficial or harmful effect on the long-term health of neighbouring axons is not known. Perhaps more important than transection injury is the case of sub-transection injuries. There is evidence that these partial injuries, including axonal stretch due to rapid centripetal deceleration forces, result in microtubular fractures that impair axonal transport and induce WD (Tang-Schomer et al., 2012). However, unlike a transection injury, this might be recoverable if initiation of the WD pathway were delayed by inducing a proposed mechanistic link between high impact trauma and WD, and a therapeutic rational for blocking it. Additional direct evidence for WD as a secondary brain injury mechanism remains limited. Radiological descriptions of WD in diffuse axonal injury exist across various imaging modalities but a robust correlation of advanced technologies such as diffusion-weighted injury and tractography with postmortem tissue are lacking. Therefore,
Perhaps the most convincing evidence for WD in TBI comes from several in vivo animal studies. The first concerns a WD expressing mouse that was exposed to a single weight-drop cortical-contusion injury. It was found to have less motor and cognitive deficit than uninjured control animals (Fox and Faden, 1998). WD conferred protection also against blast mediated TBI. Other examples include a closed cortical-injury in a Sarm1−/− mouse that demonstrated reduced neuronal loss and cognitive impairment following injury, and an impact acceleration model of Thy1-eYFP-H/Sarm1−/− mice that found a reduction in the number of axonal lesions early after injury (Henninger et al., 2016; Ziogas and Koliatsos, 2018). Sarm1 deletion also reduces axon damage and improves functional outcomes in in models of mild TBI (Marion et al., 2019; Maynard et al., 2020). The modelling of TBI in Drosophila is a recent development. We used a Drosophila model of trauma to investigate if a loss-of-function model of the highwire gene would affect outcomes from TBI and found that it protected the fly against trauma-induced premature death and behavioral deficits. We also demonstrated injury induced loss of a subset of dopaminergic neurons related to locomotion that was robustly rescued by highwire deficiency (Hill et al., 2020). These neurons degenerate in genetic PINK1/Parkin loss of function models of Parkinson’s disease but can be directly rescued by highwire deletion (Loreto et al., 2020). This is intriguing given that Parkinsonism is a well-recognized sequela of TBI and WD is emerging as a potential therapeutic target in this disease.

The ultimate proof of WD’s involvement in human TBI necessitates examination in the human condition. Modelling disease using human organotypic explants, organoids, or human induced pluripotent stem cell lines are an option for in vitro experimentation. The expression of NMNAT2 and SARM1 levels varies between individuals – sometimes pathologically so – but the effect of this on outcomes following TBI and other neurodegenerative diseases remains unexplored in humans. Targeted pharmacological blocking of WD offers an opportunity to directly limit the impact of neurodegeneration and interest in developing safe, brain-penetrant drugs is high. While the role of WD as a significant secondary brain injury mechanism in trauma has yet to be established beyond doubt, preclinical modelling in animal models may represent an exciting modifiable target that has the potential to improve outcomes from TBI.

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