

Astrocytic role of Thy-1 induced inhibition of axonal sprouting

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Thy-1 as an “anti-sprouting” factor: Since the identification of Thy-1 (CD-90) in 1964 by Reif and Allen, the precise functions of this glycosyl phosphatidylinositol-anchored surface protein within the central nervous system have been difficult to characterize. Thy-1 is a 110-amino acid cellular adhesion molecule that plays a role in cell-cell communication by interacting with other cellular adhesion molecules, including integrins (Leyton et al., 2001). While found on a diverse array of cells within the body, Thy-1 is present on the surface of almost every neuron in the brain (Morris and Grosfeld, 1989). In Reif and Allen’s 1964 study, they found that Thy-1 levels increased in the brain nearly 100-fold during post-natal development (Reif and Allen, 1964). Later, Xue et al. (1990) elaborated on this discovery and found that Thy-1 protein levels increased following the cessation of axonal and dendritic growth in the olfactory system. It was subsequently shown that neuronal Thy-1 inhibited neurite outgrowth on astrocytes *in vitro*, but not Schwann cells or embryonic glia, and anti-Thy-1 antibody is able to counteract the inhibition (Tiveron et al., 1992). Moreover, Thy-1 is localized to the ends of axon terminals, which may account for the ability of Thy-1 to block axon growth (Herrera-Molina et al., 2012).

These findings, and others, led many to describe Thy-1 as an “anti-sprouting” factor, suggesting that Thy-1 protein specifically prevents axonal outgrowth. However, we believe this description of Thy-1 may be missing the context of the interplay between Thy-1 and axonal sprouting. We would like to shift the conversation back to the question of whether elevated Thy-1 prevents axonal growth or if cessation of axonal outgrowth increases Thy-1 levels. Adding to the complexity in this paradigm is that even if the termination of axon growth stimulates Thy-1 levels to increase, the increased Thy-1 levels would be changing the biochemical makeup of the axonal plasma membrane and still would prevent future axonal outgrowth. Thus, while referring to Thy-1 as an “anti-sprouting” factor is not completely inaccurate, it does not tell the whole story. This debate began as early

as 1990 when Xue et al. (1990) found a spatial difference in increased Thy-1 mRNA and protein levels and hypothesized that additional regulation is needed to stimulate Thy-1 protein expression, regulation possibly due to the termination of axonal outgrowth. While the cessation of axonal outgrowth in the developing olfactory system is difficult to determine, because the axons grow in multiple stages, the authors did suggest that increased Thy-1 protein levels are either linked to or slightly follow the termination of axonal outgrowth. Thus, we believe it is important to definitively answer the question if increased Thy-1 inhibits axonal outgrowth, or if cessation of axonal outgrowth leads to increased Thy-1. Experiments utilizing organotypic or co-culture injury models with temporal knockdown and/or overexpression of Thy-1 may help clarify the role and timing of Thy-1 mediated inhibition of axonal sprouting.

Signaling through Thy-1: More recently, research has focused on finding an endogenous ligand for neuronal Thy-1 and the downstream signaling pathways that are activated. In 2001, it was discovered that astrocytic β_3 integrin binds neuronal Thy-1 and is sufficient to inhibit neurite outgrowth (Leyton et al., 2001). Integrin proteins function as heterodimers of alpha (α) and beta (β) subunits. Reports have demonstrated the binding of neuronal Thy-1 to astrocytic $\alpha_v\beta_3$ initiates neurite retraction through the clustering of Thy-1 (Herrera-Molina et al., 2012). Additionally, neuronal Thy-1 is able to bind astrocytic $\alpha_v\beta_5$ (Keasey et al., 2013), indicating that $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are astrocytic ligands for neuronal Thy-1. These results suggest that the inhibition that Thy-1 has on neurite outgrowth can act indirectly through astrocytes, which is consistent with the results from Tiveron et al. (1992). However, Thy-1 interactions and intracellular signaling appear to be complicated. The increased Thy-1 levels appear to stabilize neuronal processes with the underlying cytoskeletal (Herrera-Molina et al., 2012), but the need to interact with integrin dimers on astrocytes suggests that astrocytes may play a role in inhibition. Thus, understanding

the signaling pathways activated in neurons and astrocytes may be key to unlocking the specific mechanisms that Thy-1 utilizes to prevent axonal sprouting.

Studies have demonstrated signaling pathways activated by Thy-1 (Leyton et al., 2019), and specifically within astrocytes, the interaction between neuronal Thy-1 and astrocytic $\alpha_v\beta_5$ activated focal adhesion kinase and c-Jun N-terminal kinase signaling proteins (Keasey et al., 2013). Interestingly, Keasey et al. (2013) also demonstrated that the interaction between neuronal Thy-1 and astrocytic $\alpha_v\beta_5$ suppressed levels of ciliary neurotrophic factor (CNTF), a neurotrophic factor that is found exclusively within astrocytes in the central nervous system. We have previously demonstrated that CNTF promotes neurite outgrowth (Askvig and Watt, 2019), suggesting that modulating CNTF may be important in the astrocytic role of the neurite inhibition via Thy-1.

The astrocytic role: The supraoptic nucleus (SON) of the hypothalamus has been extensively studied due to the ability to elicit a neuroregenerative response following injury. Our lab has shown that axotomy of the hypothalamo-neurohypophysial tract of the SON in 35-day-old rats results in axonal sprouting from contralateral, uninjured axons, but the same injury in 125-day-old rats does not elicit a post-injury sprouting response (Askvig and Watt, 2019). We have evidence suggesting that CNTF promotes the post-injury axonal sprouting response in the 35-day-old rat via the astrocytes in the SON (Askvig and Watt, 2019). Consequently, we hypothesized that cellular adhesion molecules may be modulating the post-injury response in the SON and changing with age to inhibit the ability of new axons to grow following injury, and we were especially interested in Thy-1 due to the induced suppression of CNTF reported by Keasey et al. (2013). To this point, we recently demonstrated that Thy-1 levels are significantly elevated in the 125-day SON when the absence of axonal sprouting occurred following injury, compared to significantly lower Thy-1 protein levels in the 35-day SON rat when a collateral sprouting response repopulates the axons following axotomy (Askvig et al., 2020). While this may not be surprising considering previously published reports, our results still do not clarify if Thy-1 levels increase before or after the termination of the post-injury axonal sprouting response in the SON. However, it

does provide us with a working hypothesis that increased Thy-1 levels in the 125-day-old rat SON prevents CNTF from promoting axonal sprouting following injury.

Surprisingly, while previous reports have demonstrated that Thy-1 is exclusively localized to neurons, we found Thy-1 immunoreactivity presents on astrocytes in the SON (Askvig et al., 2020). Others have reported the presence of Thy-1 on astrocytes *in vitro* and suggested that the astrocytic Thy-1 is most abundant on the astrocytes in direct contact with neurons (Brown et al., 1984). However, it should be noted that with the low-resolution microscopy utilized in these studies and the proximity of neurons to astrocytes in the SON, future experiments utilizing higher resolution microscopy paired with proximity ligation assay would be needed to determine if astrocytes in the SON do contain Thy-1. The astrocytes within the SON do retain several traits that normally are only present in astrocytes during development, including the extensive ensheathment of the neurons, thus providing a possible explanation for the expression of Thy-1 on astrocytes in the SON.

These observations are the first reported demonstrating the presence of astrocytic Thy-1 *in vivo*, and if confirmed, it creates an interesting paradigm in the SON. Tiveron et al. (1992) hypothesized that neuronal Thy-1 directly initiated an inhibitory signal for axon growth across the membrane of the neuron. While they did pose the question of whether the action of Thy-1 may be indirect, with neuronal Thy-1 inducing the astrocyte to modify its surface so as to inhibit neurite extension; they had evidence suggesting this is not the case (Tiveron et al., 1992). However, with the evidence from Keasey et al. (2013), the role of astrocytes in inhibiting axon growth may be due to repressing CNTF levels in the astrocyte, a protein that can stimulate axonal sprouting in the brain. A new wrinkle in Thy-1 mediated inhibition of axonal sprouting exclusively related to the SON is to understand the possible role of astrocytic Thy-1. Specifically, it will be important to confirm the localization of astrocytic Thy-1, to determine the ligands for astrocytic Thy-1, and to determine if astrocytic Thy-1 affects axonal outgrowth in the SON. We also observed localization of the integrin ligands for Thy-1 on astrocytes and neurons in the SON (Askvig et al., 2020) and binding studies between Thy-1 and integrin heterodimers in the SON are ongoing. Complicating the process is that the binding

of integrin proteins to Thy-1 can occur either between two molecules of different cells (trans) (Leyton et al., 2001) or within the plasma membrane of a singular cell (cis) (Fiore et al., 2015). Thus, if Thy-1 is found on astrocytes in the SON, the possibility of cis/trans signaling between Thy-1 and integrin heterodimers on astrocytes and/or the neurons could be quite complicated. The elucidation of the signaling pathways activated by Thy-1-integrin binding would require pharmacological or genetic inhibition studies utilizing organotypic cultures that maintain cell to cell interactions and *in vivo* cytoarchitecture, before ultimately utilizing *in vivo* approaches to confirm the *in vitro* organotypic results.

Conclusions: Understanding the role of Thy-1 in axonal sprouting and as an age-related factor will require integration of knowledge about neuron-astrocyte signaling with an *in vivo* approach. To deduce the many factors of Thy-1 communication, we need to better understand Thy-1 interactions, intracellular signaling, and the cells involved. Key to figuring out the role *in vivo* will be to look for subsets of neurons and astrocytes that signal through Thy-1. To answer the question of whether Thy-1 inhibits axonal sprouting directly or if cessation of axonal outgrowth increases Thy-1, perhaps we need to start looking in new directions. The possibility of astrocytic Thy-1 opens the door for new functions and interactions that have not been previously described, which may add to the elusive roles of this cell adhesion molecule in the nervous system.

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