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Effects of Astroglia on Motor Neurons in Spinal Muscular Atrophy

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Review of Martin et al.

Hereditary proximal spinal muscular atrophy (SMA), a severe neuromuscular disorder and a leading genetic cause of infant death, is characterized by loss of motor neurons in the ventral horn of the spinal cord, resulting in progressive muscle atrophy and weakness (Lunn and Wang, 2008). SMA is an autosomal recessive disease that is caused by homozygous disruptions of the survival motor neuron 1 (*SMN1*) gene, resulting in reduced levels of SMN protein (Lefebvre et al., 1997). Although humans have a second, highly homologous copy of the SMN gene, named *SMN2*, it undergoes alternative splicing that truncates the transcript and therefore only produces small amounts of full-length SMN protein. Nonetheless, *SMN2* copy number variation in patients is a major modifier of disease severity, because the number of copies directly influences SMN protein levels.

Although the genetic underpinnings of SMA have been known for several decades, it is not clear how loss of SMN gives rise to SMA (Monani, 2005; Burghes and Beattie, 2009). Previous work has indicated that SMN is involved in several cellular processes, however, including pre-mRNA splicing, transcription termination, RNA trafficking, local translation regulation and stress granule formation (Monani, 2005; Burghes and Beattie, 2009; Singh et al., 2017). Many studies on SMA focus exclusively on the perturbation of these processes in motor neurons as a result of SMN deficiency. However, accumulating evidence suggests that other cell types, including non-neuronal cells like neuroglia, also play important roles in SMA (Hamilton and Gillingwater, 2013). A convincing argument for the involvement of non-motor neuron cells in SMA is the finding that only systemic restoration of SMN levels in SMA mouse models provides long-term rescue of the phenotype: increasing SMN in neurons alone is less effective (Hua et al., 2011; Martinez et al., 2012). Moreover, Schwann cells, the predominant glial cells of the peripheral nervous system that produce myelin and regulate neuromuscular junction formation and maintenance, show intrinsic defects in transgenic mouse models for SMA (Hunter et al., 2014, 2016). Therefore, insight into the contribution of non-neuronal cells is crucial for a better understanding of SMA pathology, and it may lead to the identification of novel therapeutic targets.

Astrocytes have also been found to contribute to SMA. These cells carry out numerous vital functions in the nervous system. For example, they influence synaptic communication and they support neuronal development and survival by secreting multiple beneficial factors (e.g., growth factors). Astrocytes are increasingly recognized as key players in several neurodegenerative diseases, including motor neuron disease (Pekny et al., 2016). SMN-deficient astrocytes show marked alterations and impairments, such as increased expression of glial fibrillary acidic protein and shorter process length (which are indicative of reactive astrocytosis), abnormal calcium homeostasis, reduced neurotrophin production, and diminished support of motor neuron synapse formation *in vitro* (McGivern et al., 2013; Zhou et al., 2016). Notably, a selective increase of SMN in astrocytes increases life span in SMA mice, although it does not prevent motor neuron death (Rindt et al., 2015). How astrocytes mediate these effects in SMA remains unknown.

In a recent study published in *The Journal of Neuroscience*, Martin et al. (2017) revealed a novel aspect of astrocyte dysfunction in SMA. Through the use of astrocyte-conditioned medium (ACM), i.e., medium that had been incubated with SMN-deficient murine astrocytes, they observed motor neuron defects in culture and identified a potential role for astrocyte-secreted monocyte chemoattractant protein 1 (CCL2/MCP1) in causing these defects.

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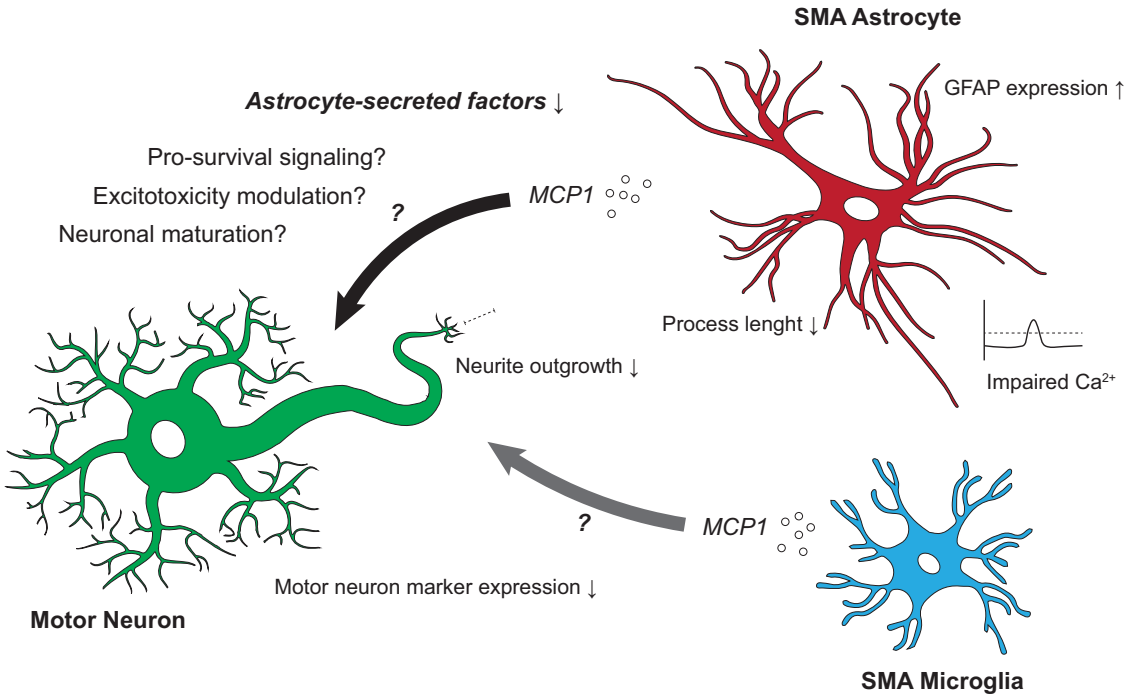

Figure 1. Abnormal motor neuron– glia interactions in SMA. Astrocytes secrete factors to support neuron development and function. Reduced levels of secreted factors derived from cultures of murine astrocytes, specifically MCP1, induced motor neuron defects in culture. Glia and glia-secreted molecules could act as important drivers of SMA pathology. Astrocyte image based on original image from *Cancer Research UK*.

The authors isolated primary astrocytes from spinal cord of both wild-type (WT) and SMA neonatal mice. These astrocytes were cultured for 1 week, and medium that had been on these cultures was collected every 48 h. Collected ACM was then added to cultures of isolated primary mouse motor neurons or motor neurons derived from human induced pluripotent stem cells (hiPSCs). Compared with neurons cultured in WT ACM, those grown in medium from SMA astrocytes had significantly shorter neurites and lower expression of motor neuron-specific expression markers, indicating motor neuron loss (Martin et al., 2017, their Figs. 1, 2*A*,*B*). hiPSC-motor neurons derived from SMA patient fibroblasts showed similar neurite aberrations when cultured in SMA ACM (Martin et al., 2017, their Fig. 2*C*,*D*). This indicated that SMA ACM negatively affects both WT and SMA motor neuron health *in vitro*.

To identify the factors present or lacking in the SMA ACM that are responsible for these detrimental effects on motor neurons, Martin et al. (2017) compared the expression of 32 chemokines in WT and SMA ACM. ELISAs were subsequently performed to measure protein concentration of select chemokines. Of course, the astrocyte secretome contains many more factors than these 32 chemokines, pointing out a drawback to this experimental approach. Nevertheless, ELISA showed a substantial decrease of MCP1 protein levels in SMA ACM compared with WT ACM (Martin et al., 2017, their Fig. 3).

MCP1 is a well-studied chemotactic protein that has been previously found to improve neuron survival and promote neuronal activity and neurite outgrowth *in vitro*. Both motor neurons and astrocytes have been shown to express CCR2, a receptor for MCP1 (see references in Martin et al., 2017). Martin et al. (2017) measured MCP1 mRNA levels in WT and SMA mouse spinal cords of different ages (Martin et al., 2017, their Fig. 4), and found reduced MCP1 levels in spinal cords of young mice with SMA. To determine whether MCP1 affects neuronal survival and neurite outgrowth in culture, primary mouse motor neurons were supplemented with recombinant MCP1. The addition of exogenous MCP1 stimulated neurite outgrowth and increased the expression of a motor neuron marker (Martin et al., 2017, their Fig. 5). Similarly, MCP1 supplementation increased neurite outgrowth in WT and SMA hiPSC-derived motor neurons (Martin et al., 2017, their Fig. 6). Importantly, the addition of exogenous MCP1 to astrocytes alone did not influence the effects of ACM on neurons, demonstrating that the effect on motor neurons is direct and is not due to autocrine signaling on astrocytes. Conversely, addition of an anti-MCP1 neutralizing antibody to WT ACM impaired neurite outgrowth and reduced the expression of motor neuron markers in primary mouse motor neurons as well as in WT and SMA hiPSC motor neurons (Martin et al., 2017, their Figs. 7, 8).

Overall, these results suggest that abnormal levels of glia-secreted factors, including the chemokine MCP1, in SMA contribute to defects in cultured motor neurons (Fig. 1).

Martin et al. (2017) propose that the restoration of deficient astrocyte-secreted factors be explored as a therapeutic approach. Perhaps such an approach could complement therapies that aim to increase SMN protein levels (e.g., via gene therapy and *SMN2*-targeting strategies; Foust et al., 2010; Corey, 2017; d'Ydewalle et al., 2017). However, several questions should be addressed before taking this step.

First, no data on the purity of the astrocyte cultures is provided. Therefore, it is possible that other cells, including immunoregulatory cells like microglia, were present in the astrocyte cultures that were used. Reduced numbers or secretion of MCP1 by such cells in the SMA condition may have contributed to the observed defects. Based on previous expression data, it seems likely that microglia are an important source of MCP1 (Orre et al., 2014; Zhang et al., 2014, 2016). Future work

could address this possibility by measuring the expression of microglia marker Iba-1 in cultures or by purifying the astrocytes via fluorescence-activated cell sorting. If future therapies target particular cell types, it will be important to accurately identify the cells and mechanisms that mediate specific SMA-related defects. Yet, supplementing motor neurons with MCP1 remains a potential therapeutic option, regardless of the cellular source.

Another unresolved question is how MCP1 achieves its advantageous downstream effects on motor neurons. It has been previously shown that MCP1 can be protective against HIV-1 transactivator protein (Tat)-induced neurotoxicity, in a CCR2-dependent manner (Yao et al., 2009). Removal or blocking of CCR2 on motor neurons would reveal whether the effect of MCP1 is mediated by CCR2 signaling or via other pathways. Through such pathways, MCP1 might, for example, inhibit apoptosis or prevent excitotoxicity in neurons (Madrigal et al., 2009). Dissecting the mechanisms in more detail could result in novel therapeutic targets and enhanced therapeutic efficacy.

Finally, as pointed out by Martin et al. (2017), modulating chemokine levels might not be a viable approach in patients because of the proinflammatory properties of these molecules. In fact, there is evidence that elevation of MCP1 levels and gliosis occur as SMA progresses (Rindt et al., 2015). Astrocytic loss-of-function during early development might shift toward a toxic gain-of-function in later stages of the disease, narrowing the therapeutic window of opportunity and the range of efficacy in patients. It is worth noting that the role of neuroinflammation and microglia, major immune effector cells in the nervous system, has been largely unexplored in SMA (Rindt et al., 2015). It is possible, for example, that abnormal synaptic pruning by microglia contributes to the motor neuron deafferentation observed in SMA. Interactions of astrocyte-secreted factors with other cells, including Schwann cells, microglia, and other astrocytes, should be investigated before seriously considering the use of such factors as a therapy.

In summary, glia-secreted factors appear to be an important contributor to motor neuron defects in SMA. Future studies should aim to investigate the variety of secreted factors and the roles of these molecules in more detail. These insights might also be relevant to other neurological disorders. Upcoming studies will benefit from advances in the generation of different types of hiPSC-derived glial cells and gene-editing techniques to accurately mimic patient cells in a nonxenogeneic setup. Nonetheless, any results generated *in vitro* must ultimately be validated in *in vivo* experimental model systems for SMA. It would be important to confirm whether the restoration of glia-secreted factors, such as MCP1, ameliorates disease progression in SMA mice before moving on to develop therapies.

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