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Autophagy Inhibition in Pain: Role of a microRNA

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Abstract. Neuropathic pain caused by peripheral nerve injury (PNI) leads to the activation and infiltration of microglial cells and to a neuroinflammatory-induced pain state. miRNAs and autophagy are two main factors and/or mechanisms which have the ability to alter the pain state. In this study, miR-195 was shown to be markedly increased after PNI and associated with the pain phenotype. In addition, inhibition of autophagy in vivo led to p62 accumulation, decreased production of LC₃, and inhibition of ATG14.

Chronic Pain and Autophagy. Chronic pain affects nearly one hundred million Americans and refers to pain that persists in the absence of ongoing stimuli (IOM, 2011; IASP, 2014). When chronic pain results from pain arising in response to a lesion or disease affecting the nervous system, it is considered neuropathic (IASP, 2014). It can be characterized by mechanical allodynia, hyperalgesia, and/or spontaneous pain (Bennett and Xie, 1988). It is understood that neuropathic pain results from altered neuronal activity and it is associated with a long-lasting peripheral- and central-sensitization and possible permanent changes of the associated neurons, neuroglia, and immune cells at or near the site of injury. The interactions of these cells drive neuroinflammation – the inflammation of tissue within the peripheral and central nervous systems – and are the most likely the culprit for generating and sustaining the sensitization of nociceptive neurons that leads to chronic pain (Gao, 2010; Ji, 2014).

Recently, advances in autophagy (self-eating) have been in the scientific spotlight with Ohsumi and colleagues being awarded the 2016 Nobel Prize in Physiology and Medicine. Autophagy is a lysosomemediated intracellular process in which proteins are targeted and degraded by a double-membraned organelle, known as the autophagosome. It has recently been shown that autophagy is involved in the regulation of inflammatory response by suppressing neuroinflammation after ischemic brain injury in the rat cortex, as well as in other nervous tissue (Zhou, 2011; Luo, 2016).

Involvement of miRNAs. The paper by Yuan and colleagues in the journal *Glia* is the only scientific evidence describing a role for non-coding RNA (ncRNA), specifically a microRNA, on autophagy in neuropathic pain symptomology and etiology (Yuan, 2011).

For a long time, it was thought that protein-coding RNA (mRNA) was the main player in influencing the central dogma, and of course, protein regulation and degradation. miRNAs are typically 22 nucleotides in length and along with other types of ncRNAs account for greater than 80% of the mammalian transcriptome (Riddihough, 2005; Claverie, 2005; Gu, 2016). These endogenous ncRNAs can change gene regulation; they are key post-transcriptional regulators with the ability to modify the expression of mRNA targets and corresponding proteins (ie., inhibition of protein degradation) and can effect ribosomal trafficking (Bali, 2014; Hengst, 2006; Johnston 2005; Chen, 2004). miRNAs are highly abundant in the nervous system, uniquely expressed in specific cell types (including brain and neuronal tissue) and can be indicative of particular disease states (Bali, 2014; Zhao, 2013). The study by Yuan and colleagues sought to explore whether miR-195, a potent regulator of neuroinflammation and neuropathic pain (the target of which is unclear), plays a role in autophagy in a chronic pain model.

Expression of miR-195 is known to be upregulated after sciatic nerve injury (Yu, 2011), however it was not known whether miR-195 is increased after spinal nerve injury and whether it contributes to neuroinflammation and autophagic flux in cells. Yuan and colleagues utilized the spinal nerve ligation model of neuropathic pain, where the L5 spinal nerve was isolated and tightly ligated distal to the dorsal root ganglia with a silk suture (Kim and Chung, 1992), to monitor the impact of miR-195 on neuropathic pain and autophagy.

Remarkably, Yuan et al found that miR-195 was dramatically increased in rats after spinal nerve ligation of L5 in microglial cells, coinciding with pain-like behavior and inhibition of microglial autophagy activation, as well as, suppressed neuroinflammation in vivo and in vitro. miR-195 expression levels increased 2-days post-injury and were elevated for 14 days compared to sham. This result was confirmed with northern blotting. In addition, miR-195 levels were increased in cultured spinal microglial cells from day 2 till day 14 post-injury.

Wistar rats were subjected to behavioral testing (tactile allodynia and cold hypersensitivity) and intrathecal administration of an miR-195 agonist mimic or an miR-195 inhibitor. miR-195 agonists increased expression of miR-195 in spinal microglia and these animals showed increased pain behavior compared to miR-195 inhibitor injections. Thus increased miR-195 is correlated with increased neuroinflammation and neuropathic pain symptoms.

miRNA-195 and Autophagy Inhibition. To unravel the molecular mechanism underlying miR-195 function the authors assessed the autophagy pathway in a series of elegant experiments. Consistent with inhibition of autophagy activation in vivo LC3 expression was assayed in miR-195 animals and microglial cells. LC3, when lipidated, is

attached to both faces of the phagophore during degradation (Tanida, 2004). LC3 changes were seen in the dorsal horn (DH) at days 0, 2, 5 and 14 post-spinal nerve ligation (SNL): specifically, SNL resulted in a decreased ratio of LC3-II to LC3-I conversion as well as an accumulation of p62, a marker for autophagy impairment. p62 is a ubiquitin-binding protein that acts as an autophagy substrate that directly binds to LC3 and is then incorporated into autophagosomes and is degraded (Berliocchi, 2011). miR-195 inhibitor administration leads to a significant increase in autophagy activation as evidence by increased LC3 production via the conversion of LC3-I to LC3-II proteins in microglial cells. Similar results were found in vitro when miR-195 was given to microglial cells directly.

The next significant finding from Yuan and colleagues is that ATG14 is targeted by (regulated by) miR-195. ATG14 is essential for the localization of Vsp34 to the autophagosome in mammalian cells for the formation of the class III PtdIns3K complex (Ohsumi, 2011). miR-195 markedly inhibited ATG14 protein expression in microglial cells, and suppressed autophagy. When the gene was overexpressed, it rescued the activation of autophagy in miR-195-treated microglial cells.

The Future of miRNAs and Autophagy in Pain. Future implications for these pivotal findings is their possible application in the clinic. miR-195 may serve as a useful clinical biomarker of neuropathic pain. Treating chronic pain symptoms is difficult to do given the many players involved in the underlying neuroinflammatory processes. More research would need to be done to see if miR-195 is present in circulating macrophages and after nerve injury in the peripheral nervous system. This may shed insight into the implications of non-coding RNAs in treatment. Some other future studies could look at differential expression studies in other peripheral cells (in monocytes, Schwann cells, DRG sensory neurons), which could contribute to finding better treatment options as well. High expression of miR-195 in circulating macrophages may lead to the infiltration into the DRG and sciatic nerve contributing to peripheral sensitization via the release of pro-inflammatory mediators. It is possible that this may alter neuron-glia cross-talk and neuronal functions leading to symptoms of neuropathic pain or it may influence neuronal functions directly by suppressing autophagic genes within neurons or indirectly by influencing the functioning of immune or glial cells. As stated earlier, more studies are needed on this subject, given that there are so few.

Figure 1

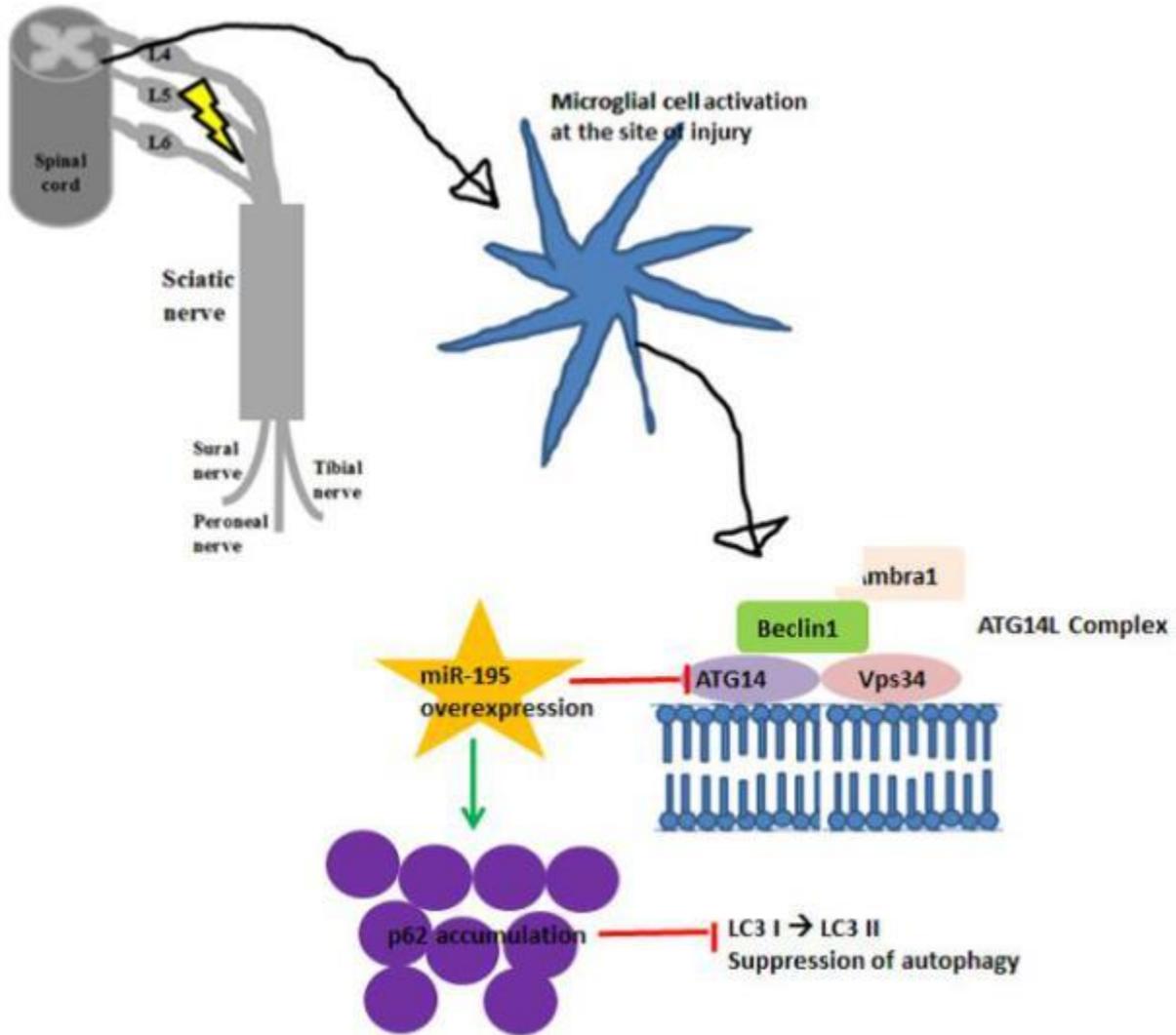


Figure 1. Model of miR-195 inhibition of autophagy in SNL injury. When miR-195 is overexpressed, autophagy induction does not occur, leading to p62 accumulation and a failure to convert LC₃ I to LC₃ II, as well as hindrance of the formation of the ATG14L complex, which is important in autophagosome formation.

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