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LETTER

Comment on: PH20 is not expressed in murine CNS and oligodendrocyte precursor cells

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A recent paper by Marella and coworkers[1] in the *Annals of Clinical and Translational Neurology* reports that a hyaluronidase, called PH20, does not influence the maturation of oligodendrocyte progenitor cells (OPCs). This finding is in contrast to work in our laboratory showing that OPCs digest hyaluronan (HA), and that PH20, but not other hyaluronidases, as well as PH20-generated digestion products of HA potently block OPC maturation and remyelination.[2] The basis for the claim that PH20 does not influence OPC maturation is that in the Marella et al. paper, the authors report that the effects of a preparation of hyaluronidase, bovine testicular hyaluronidase (whose hyaluronidase activity is mostly PH20), are due entirely to contaminating fibroblast growth factor-2 (FGF-2; also called basic fibroblast growth factor). These authors also failed to find any effect of a recombinant form of PH20 from their company on OPC maturation (the study was conducted by Halozyme Therapeutics, who are developing PH20 for clinical applications).

While it remains unclear if our preparation of bovine testicular hyaluronidase was contaminated with sufficient FGF-2 to impact OPC maturation, Marella et al. failed to acknowledge that we also reported that transducing the PH20 cDNA into OPCs potently inhibited OPC maturation to a far greater degree than other hyaluronidases.[2] This experiment demonstrates that elevated PH20 directly blocks OPC maturation, given that contaminating factors did not exist in these experimental conditions. In addition, we recently reported[3] that both bovine testicular hyaluronidase and a preparation of recombinant PH20 both had nearly identical effects on hippocampal neural stem cell proliferation and differentiation both in vitro and in vivo, and that these effects were dependent on the CD44 transmembrane hyaluronan receptor. These data similarly indicate that PH20 activity and not contaminating factors are playing a role in the biological effects of adding PH20 to cells in the nervous system. To further confirm this finding, we have recently obtained new data showing that the preparation of recombinant bovine PH20 used by Su and coworkers also potently blocks OPC maturation.

We also reported, using size exclusion chromatography with multi-angle laser light scattering, that distinct hyaluronidases generated HA digestion products that varied in their size ranges.[2] We found that PH20-digested HA but not HA digested by another hyaluronidase blocked OPC maturation. Marella et al. reported that such digestion products have no effect on OPC maturation, and claim that HA digestion products would not be varied in size as we reported.[1] This seems highly unlikely, since we have recently repeated our findings using highly enriched preparations of different sizes of HA digestion products and find that only specific sizes have this effect (manuscript in revision), and because at any given time in tissues, there are likely to be a variety of HA sizes depending on the activities of HA synthases, hyaluronidases, and other agents that promote HA catabolism.

We[2,4] and others[5] also previously reported that the PH20 hyaluronidase is elevated in demyelinating white matter lesions as well as oligodendrocyte progenitor cells. PH20 was also reported to be present, albeit at low levels, in the rat brain in a study on traumatic brain injury.[6] These studies found PH20 in the brain, under certain conditions, using both antibody-based techniques and PCR. However, Marella and coworkers[1] report that PH20 is not expressed in the murine central nervous system or in rodent or human demyelinating lesions at the protein or RNA levels. They report that most available PH20 antibodies are not reliable, but that their antibody is reliable. While we agree that PH20 antibodies can react with other proteins, and that PH20 transcripts are not detectable in the normal adult central nervous system, we consistently find transiently increased PH20 transcripts under certain

culture conditions in OPCs and astrocytes, and in tissues following insults to the brain. While we cannot rule out that other hyaluronidases may also contribute to remyelination failure, we believe that PH20 and/or hyaluronidases with activities that are similar to PH20 play a role in regulating OPC behaviors following insults to the brain and spinal cord.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

1. Marella M, Ouyang J, Zombeck J, et al. PH20 is not expressed in murine CNS and oligodendrocyte precursor cells. Ann Clin Transl Neurol 2017;4:191–211.
2. Preston M, Gong X, Su W, et al. Digestion products of the PH20 hyaluronidase inhibit remyelination. Ann Neurol 2013;73:266–280.
3. Su W, Foster SC, Xing R, et al. CD44 transmembrane receptor and hyaluronan regulate adult hippocampal neural stem cell quiescence and differentiation. J Biol Chem 2017;292:4434–4445.
4. Hagen MW, Riddle A, McClendon E, et al. Role of recurrent hypoxia-ischemia in preterm white matter injury severity. PLoS ONE 2014;9:e112800.
5. Sloane JA, Batt C, Ma Y, et al. Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci USA 2010;107:11555–11560.
6. Xing G, Ren M, Verma A. Divergent temporal expression of hyaluronan metabolizing enzymes and receptors with craniotomy vs. controlled-cortical impact injury in rat brain: a pilot study. Front Neurol 2014;5:173.

