

Immunohistochemical and serological evaluation of axonal degeneration after acute through sub-acute cervical spinal cord injury (SCI)

Gerry Shaw^{1,8}, Anna F Fusco^{2, 3, 4, 5, 6, 7}, Sabhya Rana^{3, 5, 6, 7}, Sruti Rayaprolu⁷, Maya M MacIntyre^{4,6,7}, Marda Jorgensen⁸, David D Fuller^{3,4,5,6}

¹Neuroscience PhD Program, University of Florida; ²Neuroscience Department, University of Florida; ³Physical Therapy Department, University of Florida; ⁴College of Medicine, University of Florida; ⁵McKnight Brain Institute, University of Florida; ⁶Breathing Research and Therapeutics Center, University of Florida; ⁷Center for Translational Research In Neurodegenerative Disease, University of Florida; ⁸EnCor Biotechnology Inc, Gainesville, FL

We developed novel antibodies to epitopes on the neurofilament light chain (NF-L) which are either exposed on degeneration induced proteolysis (Degenotag™ reagents) or alternately bind only forms of NF-L seen in healthy axons (NF-L-ct reagents). We collected blood and tissue sections spanning the caudal medulla to C8 at 1-hr, 6-hr, 1-day, 3-days and 10-days post injury in rats given unilateral 150 kydne C4 contusion spinal cord injuries (SCI). We stained sections with Degenotag™ and NF-L-ct reagents and compared with antibodies to two other neurodegeneration markers, amyloid precursor protein (APP) and neurofilament medium chain (NF-M) antibodies RMO-44 and RMO-14. We also quantified and compared the Degenotag™ staining with the NF-L blood signal as measured with the Quanterix SIMOA™ NF-L assay. APP staining paralleled that of NF-L-ct reagents for the most part, showing little overlap with the Degenotag™ staining pattern. In contrast both the RMO-44 and RMO-14 staining pattern was very similar to that seen with Degenotag™ reagents and distinct from that seen with APP. We conclude that injury induced proteolysis rapidly destroys both the NF-L-ct signal and the APP signal, while revealing the Degenotag™, RMO-14 and RMO-O44 staining. The blood NF-L levels determined with the SIMOA™ assay were increased at 6-hr and peaked at 1-3 days after SCI, correlating well with the quantification of Degenotag™ positive material in spinal fiber tracts. These findings throw light on the significance of multiple immunocytochemical markers of neurodegeneration and reveal the morphological correlate of the SIMOA™ NF-L assay.

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