Differential Expression of NUP98-ERBB4-PSEN1-NRG1 (NEPN) Signaling Axis as Potential Blood-Based Biomarker for Viral Myocarditis

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Background: Myocarditis, inflammation of the myocardium, is a leading cause of unexpected heart failure in young adults, commonly attributable to viral infections. Myocarditis manifests in a wide range of clinical presentations, from asymptomatic to acute heart failure. The current gold standard for diagnosis relies upon invasive endomyocardial biopsy, requiring histopathological demonstration of inflammation with/without associated myocyte damage. Under these guidelines, diagnostic sensitivity in independent published studies is

Design: HeLa cells and human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) were CVB3- or sham-infected. Western blot analysis and confocal microscopy were performed to determine the expression and subcellular localization of NEPN signaling axis proteins in tissue culture. In addition, tissue and blood from murine models of viral myocarditis (A/J and C57BL/6 mice) were collected at 4 dpi (viremic phase) and5 to 8 dpi (acute phase), and were analyzed by Western blot analysis, IHC and IF to detect protein expression.

Results: NEPN proteins all show changes in expression and subcellular localization throughout the infection time course. NUP98 is upregulated and cleaved during infection. NRG1 is upregulated in both lysates and supernatants. ERBB4 co-localizes at the cell membrane with the protease PSEN1. ERBB4 and NRG1 cleavage fragments are secreted and detected in both tissue culture supernatant and mouse plasma. In the plasma of the less susceptible C57BL/6 mice, ERBB4 and NRG1 cleavage fragments increase throughout the viremic and acute phases. However, in the highly susceptible A/J mice, ERBB4 and NRG1 cleavage fragments were not detected in the plasma until 6 dpi (acute phase), when they are highly upregulated as compared to C57BL/6 mice ($p=0.001$).

Conclusion: Differential expression, subcellular localization and cleavage fragments of NEPN signaling axis proteins were detected during the pathogenesis of viral myocarditis. These composite changes may be useful in the development of a blood-based diagnostics.