Developing a CNP-Based Therapy to Strengthen Dysregulated Connective Tissue Signaling in Hypermobile Ehlers-Danlos Syndrome

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Hypermobile Ehlers-Danlos Syndrome (hEDS) is a connective tissue disorder characterized by collagen disorganization and impaired extracellular matrix signaling (ECM), resulting in chronic joint dislocations, severe tissue fragility, and progressive mobility loss that can confine patients to wheelchairs by their 40s. Affecting ~1 in 5,000 individuals, no therapy directly addresses the disrupted signaling pathways; treatment is limited to symptom management via pain control or physical therapy. This research engineered a recombinant fusion protein combining C-type natriuretic peptide (CNP), a short-lived signaling molecule essential for connective tissue regulation, with a Pentraxin scaffold (through a G4S linker) to extend circulatory half-life from 3 minutes to 30 hours – a ~600-fold improvement. The design enables sustained NPR-C receptor activation, restoring ECM signaling, supporting cellular communication, and promoting tissue stability. The CNP-Pentraxin construct was expressed in ExpiCHO cells, maintaining 93% viability across three transfections. Calcium-dependent PE affinity purification yielded 0.035-0.100 mg purified protein, and SDS-PAGE analysis confirmed a 26.07 kDa (CNP-PTX-2 molecular weight) band. Substantial protein remained in load and flow-through fractions, hinting at interference between the CNP domain and Pentraxin binding regions. Redesign with an HRV3C protease cleavage site between the fusion protein and CNP propeptide region is being incorporated to improve expression efficiency. Preliminary computational docking analysis showed favorable CNP-Pentraxin-NPR-C receptor engagement. Functional validation remains ongoing, but findings demonstrate molecular feasibility and support continued optimization toward a signaling-based therapeutic for hEDS.

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