A11 Comparison of coacervation property and secondary structure of human tropoelastin in and its mutants

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Elastin is the core protein of the elastic fiber and confers elasticity to connective tissues such as arterial walls, ligaments, lungs and skin. Tropoelastin is the soluble precursor of insoluble elastin. The extracellular self-assembly of tropoelastin is called coacervation, which is a crucial step in elastogenesis. Recently, it is suggested that the mutation of tropoelastin is related to some congenital diseases. Supravalvular aortic stenosis (SVAS) is a kind of heart disorder which exhibit symptoms of congenital aortic valve stenosis. Cutis laxa (CL) shows skin laxity and pulmonary and cardiovascular compromise. SVAS and CL found to be linked to mutations within the tropoelastin gene that may alter the ability of the elastin precursor to undergo normal self-assembly.

Domains 16 and 17 of tropoelastin are deleted in tropoelastin of SVAS patients, whereas the frameshift mutation of domain 32 is observed in the tropoelastin in CL patients. To explore the contribution of domains 16 and 17 in SVAS and domain 32 in CL to coacervation, tropoelastin constructs derived from two diseases were designed: domain 16 and 17-deleted tropoelastin (Δ16&17) as the SVAS model and domain 32-mutated tropoelastin (CL) as the CL model. Other tropoelastin construct in which C-terminal domain 36 was deleted (Δ36) was also designed. Their coacervation properties and secondary structure were compared with normal human tropoelastin (HTE).

Coacervation property was analyzed by measuring the turbidity of each tropoelastin at 400 nm. Each tropoelastin solution was subjected to increasing temperature from 5°C to 60°C at a rate of 0.5°C/min. It was shown that the initiations of turbidity formation for coacervation of Δ16&17 and Δ36 occurred at higher temperatures than HTE did. Furthermore, the intensity of turbidity formation for coacervation of Δ16&17 was smaller than HTE. Coacervation property was further evaluated by initiation temperature (Tᵢ), midpoint temperature (Tₘ), and complete temperature (Tₐ). The Tᵢ, Tₘ, and Tₐ of Δ16&17 were higher than HTE, suggesting that these domains are important for the self-assembly of tropoelastin. On the contrary, the CL showed lower Tᵢ, Tₘ, but higher Tₐ than HTE.

Since it is said that the formation of β-structure as a secondary structure is important for coacervation, the secondary structure of these tropoelastin constructs were examined by CD and FT-IR measurements. The CD spectra of these tropoelastin constructs gave similar spectra showing large negative band at 200 nm in H₂O and phosphate buffer saline (PBS). It is suggested that all tropoelastin constructs have random structure in H₂O and PBS primarily and b-sheet structure in 50% trifluoroethanol primarily. In FT-IR measurement, HTE showed higher content of b-sheet structure than other tropoelastin constructs, suggesting that β-structure of HTE contributes to the formation of coacervation of tropoelastin.

A12 Involvement of Epipakin in HeLa Cell Clustering and Liver Reorganization after Partial Hepatectomy

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[Aim] Although epipakin, originally identified as an epiderma l autoantigen in a patient with subepidermal blistering disease, has been described to be widely expressed in human tissues, its precise roles remain unclear. In this study, we examined expression and localization of epipakin in cultured HeLa cells and regenerating liver tissue to explore the roles of epipakin in cells.

[Methods] HeLa cells cultured on Matrigel, as well as the liver tissue sections from regenerating liver after 70% hepatectomy, were immunostained for epipakin and other plakin family members, cytoskeletal proteins, or cell adhesion molecules including classical and desmosomal cadherins.

[Results and Discussion] Epipakin showed a similar distribution to those of vimentin and desmoplakin in well-spread HeLa cells cultured on polystyrene surface. In HeLa cell clusters formed after culture on Matrigel, however, epipakin displayed a peculiar localization to the outermost surface of HeLa cell clusters, but not to the adhering cell surface to the neighboring cells. Dual immunostaining for epipakin and N-cadherin suggested an elimination of epipakin localization from N-cadherin-mediated cell-cell attachment surface. In regenerating liver, epipakin expression was transiently elevated with a peak at 3 days post-hepatectomy and then returned to the basal level by 2 weeks post-hepatectomy. Although epipakin was distributed throughout cells in hepatocyte islands at 3 days post-hepatectomy, the epipakin localization was altered to the sinusoidal surface of parenchymal cells, as seen in HeLa cell clusters, namely, after formation of hepatocyte plates with cell-cell adhesions at 5 to 7 days post-hepatectomy.

From these results, it is suggested the possible involvement of epipakin in multicellular organization by cell-cell interaction mediated by classical or desmosomal cadherins as followed by intermediate filament reorganization, as seen in HeLa cell clusters and regenerating liver tissues.

HeLa細胞クラスター形成および部分肝切除後の肝再構築におけるエピプラキンの関与

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