Synopsis of Original Research Paper

A search for low-molecular weight ligands that regulate a dynamic equillibrium of tight-junction

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The cellular maintenance of tight junctions (TJs) is considered as dynamic remodeling processes of equilibrium between internalization / degradation and generation of claudin-based TJ strands. While the mechanism of biogenesis of TJs driven by ZO-1 and its paralogs were well understood, the molecular mechanism behind TJs' turnover remains unknown. Recently, the E3 ubiquitin ligase ligand of Numb-protein X1 (LNX1p80) was identified as a responsible factor that binds to claudin-1 and promotes its endocytosis. Since the first PDZ domain of ZO-1 is indispensable for claudin interaction in the TJ biogenesis, a competition between ZO-1 and LNX1p80 against claudin is assumed. We analyzed in vitro binding activity of the several claudin-derived peptides and the other peptides derived from the TJ-related proteins. We found that some of the claudin-derived peptide could bind LNX1-PDZ2, whereas none of claudins bind LNX1-PDZ3. Notably, all of these claudin-derived peptides bound ZO1-PDZ1. CAST and JAM-4 are the strong binders to LNX1-PDZ2 domain, which also did not bind ZO1-PDZ1. For further clarifying the molecular recognition mechanisms underlying the claudin competition among LNX1 and ZO-1 PDZ domains, we started the structurl studies. We also succeeded in determining the NMR structure of mouse ZO1-PDZ1 domain, which was further subjected to a virtual screening study for identifying ZO1-PDZ1 inhibitors. We succeeded in crystallizing the LNX1-PDZ2 in a certain condition, and the structural determination at 1.5Å resolution was mostly completed. This high-resolution structure of LNX1-PDZ1 is also planned to be subjected to a virtual screening study. These structural informations of TJ-related PDZ domains will provide the molecular basis towards discovery and development of TJ-regulating (promoting and inhibiting) small molecular weight compounds.