Abstract

To the PhD thesis titled

"**Molecular recognition between cadherins and nectins that drive cell-cell adhesion**"

by

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The ability of a cell to interact with another cell is the fundamental property of a multicellular organism. This interaction and their subsequent organization, orchestrate the formation of higher order multicellular structures to perform various biological functions. Cell to cell adhesion is elemental to facilitate these intercellular interactions and it is achieved through the formation of multiprotein complexes that organize and assemble to form intercellular junctions at the contact sites of interacting cells. Cell adhesion molecules are the cell surface proteins that function in structuring these cell-to-cell junctions. Of the many junctions, adherens junction primarily operates to mechanically link cells through their cytoskeletal actin belt. Interactions mediated by the membrane glycoproteins, cadherins and nectins, form these adherens junctions. The study undertaken aims to understand the interactions mediated by these cadherins and nectins in details, and the various crosstalk involving them, to comprehend the understanding of their function as cell adhesion molecules in assembly of adherens junction.

Although extensive research suggests that classical cadherins engage in homophilic interaction to facilitate cell adhesion, reports of heterophilic recognition between them have recently surfaced. **In the first objective of the work, the molecular and structural bases of the heterophilic interacting cadherin pair, the E- and N-cadherin, was thoroughly researched.** Surface plasmon resonance-based and fluorosphere based binding studies show the interaction between the membrane distal ectodomain of E- and N-cadherin. Site direct mutagenesis and complementary interaction studies further identify some critical residues that are involved in this heterophilic association.

Cadherins and nectins colocalize and cooperate to build the architecture of adherens junction. **Accounting to previous references of the direct crosstalk between N-cadherin and nectin-2, other such direct crosstalk was hypothesized and investigated in the second objective of the work.** Through surface plasmon resonance-based protein-protein interaction studies, a direct recognition between E-cadherin and nectin-4 is reported that is validated through cell-based binding studies. Furthermore, single molecule force spectroscopy studies show the interaction between E-cadherin and nectin-4 to be strong and stable with k_{off} value of $0.062 \pm 0.03\ s^{-1}$.

Cell adhesion function of the heterophilic interactions under study was demonstrated in the third objective of the work. By performing *in vitro* cell aggregation assays, the adhesion behavior of the heterophilic interactions under study, was elucidated. The results show that E- and N-cadherin and E-cadherin and nectin-4 interactions can drive cell adhesion by forming heterophilic aggregates.

In spite of cadherins and nectins being in research since longtime, there are increasing studies that demonstrate the numerous interactions mediated by them and the functional significance of it. They thus continue to be the important regulators of various physiological processes in our body. With regard to this, the current findings can provide insight to the complex interactions that they participate in while performing their function.

Keywords: cell adhesion, cadherin, nectin, protein-protein interaction, surface plasmon resonance, site directed mutagenesis, single molecule force spectroscopy, flow cytometry, cell aggregation assay