NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

Reg. No. 200604393R

Research Theme: Cell Adhesion and Signaling

Research Project Title: Mechanisms of Integrins Mediated Cell-Cell Adhesion & Therapeutics Development

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Co-supervisor/ Collaborator(s) (if any):

Project Description

a) Background:

Integrins are a large family of type I transmembrane cell adhesion molecules that are involved in many biological processes, including immunity, wound healing, and the development of metazoans. Integrins are unique signaling receptors that carry out bi-directional signaling inside-out and outside-in. Integrins mediated signaling are directly correlated with several diseases like cancer, autoimmune diseases, inflammations etc. In humans there are 24 specific integrins that can be categorized based on ligand-binding specificities or tissue expressions. Each integrin is composed of an α and a β subunit that are non-covalently associated, and each subunit has a large extracellular domain that binds ligand, a transmembrane domain and a short cytoplasmic tail (CTs). The ligand-binding properties of integrins are tightly regulated by cytoplasmic proteins that interact with the integrin cytoplasmic tails. These interactions regulate the conformation of integrin by allostery that modulates its ligand-binding affinity. A large number of cytoplasmic proteins have been identified to interact directly with the integrin CTs, potentially forming multi-protein complexes. Characterization of the multi-protein complexes is deemed essential not only to understand sequence of events of complex formation but also protein/protein interface would be a viable target for therapeutic development. There is still very limited information on how multiprotein-complex involving different integrin CT-interacting partners function temporally and spatially to regulate integrin activation, and it remains to be discovered how integrin CT-interacting partners modulate the functions of one another. This is compounded by the fact that there are subtle but important differences in the sequence composition of the integrin CTs. Hence, a ubiquitous model of integrin regulation is insufficient to explain the varied signaling properties of different integrins reported in different cell types such as β3 integrins in fibroblast compared with β2 integrins in leukocytes.

b) Proposed work:

We are investigating β2 integrins that are only expressed in leukocytes and they are critical for a functional immune system. There are four members of the β2 integrins: αLβ2, αMβ2, αXβ2 and αDβ2. Our current and future research aims to obtain a comprehensive understanding of the network of interactions at atomic resolution. We investigate interactions between the β2 cytosolic tail of integrins of leukocytes with its negative and positive protein regulators using NMR spectroscopy and *in vivo* functional analyses. Works from my laboratory have determined 3-D structures of β2-CT and α-CTs of αL (J. Biol. Chem. 2009), αM (J. Biol. Chem. 2011), αX (PLOS-One, 2012) and α4/paxillin (PLOS-One, 2013) and mapped interactions between α and β CTs of leukocytes. These results have provided important molecular insights for activation and regulation of β2 integrins and also showed critical structural and interface, α/β CTs variations with other integrins.

We are currently working on protein regulators e.g. talin, docking protein 1 (Dok1), 14-3-3, filamin and kindlin3 that interact with phosphorylated and non-phosphorylated CTs. Phosphorylation at certain sequence motifs in β-CTs recruit specific regulatory proteins. Dok1 and filamin are negative regulator of integrins, whereas as talin and 14-3-3 are known to be positive regulators. Dok1 is known to bind to phosphorylated tyrosine in NPxpY motif in β3 integrin and also in other proteins. However, in β2 CT the motif, NPXF, contains a nonphosphorytable Phe. It remained unclear how Dok1 could bind to β2 CT. On the other hand, phosphorylation of Thr in TTT motif of β2 CT enables binding of 14-3-3 protein. Our work demonstrated an alternate phosphor switch for β2 CT recognized by Dok1 in NPLFKpS (Scientific Reports | 5:11630 | DOI: 10.1038/srep11630). Recently, we discovered a novel ternary complex between talin/pTβ2CT/14-3-3ζ (J. Mol. Biol. 2016). Talin and 14-3-3ζ synergistically bind to pTβ2CT activating β2 integrin. Our more recent works demonstrated interactions among positive and regulator proteins e.g. 14-3-3ζ and Dok1 and filamin (unpublished). Collectively, these works open a new paradigm in integrins activation and regulations.

Future researches aim to examine following questions. 1. How the multi-protein complexes form in CTs and regulate activation and repression of integrins? 2. How do the trans-membrane domains (TMs) of integrins influence intracellular multi-protein complexes? Recent works demonstrated that TMs are not a passive linker between cytosolic domain and extra-cellular region of integrins. 3. Designing drugble peptide based inhibitors that would disrupt multi-protein complexes and modulate cellular function of integrins. We believe that our multipronged approaches, use of NMR, x-ray sensitive biophysical methods (ITC, SPR etc) and in combination with cell based functional analyses will provide an in depth understating of integrin signaling.

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