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Structural Characterization of TIR Domains Interaction

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Abstract : Toll-like receptors (TLRs) play central role in the innate immune response and inflammation by recognizing pathogen-associated molecular patterns (PAMPs). A fundamental basis of TLR signalling is dependent upon the recruitment and association of adaptor molecules that contain the structurally conserved Toll/interleukin-1 receptor (TIR) domain. MyD88 (myeloid differentiation primary response gene 88) is the universal adaptor for TLRs and cooperates with Mal (MyD88 adapter-like protein, also known as TIRAP) in TLR4 response which is predominantly used in inflammation, host defence and carcinogenesis. Up to date two possible models of MyD88, Mal and TLR4 interactions have been proposed. The aim of our studies is to confirm or abolish presented models and accomplish the full structural characterisation of TIR domains interaction. Using molecular cloning methods we obtained several construct of MyD88 and Mal TIR domain with GST or 6xHis tag. Gel filtration method as well as pull-down analysis confirmed that recombinant TIR domains from MyD88 and Mal are binding in complexes. To examine whether obtained complexes are homo- or heterodimers we carried out cross-linking reaction of TIR domains with BS3 compound combined with mass spectrometry. To investigate which amino acid residues are involved in this interaction the NMR titration experiments were performed. 15N MyD88-TIR solution was complemented with non-labelled Mal-TIR. The results undoubtedly indicate that MyD88-TIR interact with Mal-TIR. Moreover 2D spectra demonstrated that simultaneously Mal-TIR self-dimerization occurs which is necessary to create proper scaffold for Mal-TIR and MyD88-TIR interaction. Final step of this study will be crystallization of MyD88 and Mal TIR domains complex. This crystal structure and characterisation of its interface will have an impact in understanding the TLR signalling pathway and possibly will be used in development of new anti-cancer treatment.

Keywords : cancer, MyD88, TIR domains, Toll-like receptors
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