論文の内容の要旨

Title: Innate Immunity Interactome Dynamics

(自然免疫のインタラクトーム・ダイナミクス)

Name: アスマ　モハメド　モハメド　ラベ　エルザワハリ

Introduction:

The Immune system of host defends against invading pathogens and it has two main subsystems which are Innate immunity and acquired immunity[1].To initiate immune responses , it is crucial to recognize pathogens.

Recognition of pathogens is mediated by pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs), which are molecular structures that are common in pathogens[2, 3].
Once PAMP have recognized, PRRs initiate signaling cascades to achieve first line of defense toward invading pathogens.[3]

A group of major players in innate immunity response are Toll-like receptors (TLRs) which are transmembrane proteins [3] that have specificity in recognizing pathogens[4, 5]. TLRs specificity is based on PRRs, which recognize different PAMPs[6] such as nucleic acids, lipids, lipoproteins and proteins from different pathogens as viruses, fungi, bacteria and parasites[6, 7].

One of TLRs member proteins is (Toll like receptor 4) TLR4, which can be stimulated by many PAMPs including Lipopolysaccharide (LPS)[8] which is an essential part for the structure of outer membrane of gram negative bacteria.

Once TLR4 recognize LPS, it initiates signaling cascades of immune response as a result of LPS stimulation[8, 9].

TLRs signaling pathways can be divided into MyD88-dependent pathways, which leads to the induction of cytokines, and TRIF-dependent pathways, which leads to induction of type I interferon and inflammatory cytokines.[7]

In TLRs members, TLR4 is unique in that it is the only TLR member, which uses both MyD88 and TRIF dependent pathways.[7]

LPS stimulates TLR4, which triggers immune response through both MyD88 and TRIF dependent pathways, activation of MyD88 and TRIF pathways induces inflammatory cytokines and type I interferon.[7]

In addition, innate immunity signaling cascades involve protein-protein interactions (PPIs) as well as protein–DNA interactions as Transcription factor binding.

Thousands of PPIs that are related to innate immunity has been curated as static interactions, however, PPIs involved in innate immunity are dynamic rather than static.

In this study, we did interactome analysis by combining PPI with dynamic gene expression data to infer interactome dynamics, the stage at which highest number of interactions perturbed, as well as interactome differences between different stages during immune response.

Interactome Dynamics: *Global overview*

We constructed a network of protein-protein interactions (PPIs) of 4822 proteins with 10545 interactions.

To acquire a global view of interactome dynamics during immune response we identified the time point that has the largest number of interactions resulted from a smallest number of up/down regulated proteins after stimulation with LPS. We found that 1hr is the point at which largest number of PPIs might affect by up/down regulation of smallest number of proteins. This indicates that up/down regulated proteins at 1hr after LPS stimulation may have largest effect on interactome during immune response.

In order to identify interactome dynamics, we considered interactome dynamics from two perspectives: First we identified protein complexes from network of proteins that are encoded by genes that show differential expression during the whole time course of immune response, these proteins with their interactions constitute differentially expressed network.

Second: we identified differences in up/down regulation in interactome between each two time points during immune response (Differential networks) as well as core proteins/interactions that were conserved in interactome during immune responses.

Differentially expressed network

We constructed a network of protein-protein interactions for differentially expressed proteins and their interactors. Differentially expressed proteins are proteins whose genes showed statistically significant differential expression throughout the whole time course of immune response. The network of differentially expressed proteins consists of 3379 proteins with 5472 interactions, we identified protein modules from this network based on density of a protein complex using MCODE [11].

For functionally significant modules Gene Ontology (GO) analysis and enriched KEGG pathways were explored, many modules have functions related to immunity, and there is enrichment for many identified modules with pathways in cancer such as leukemia. A link between innate immunity and cancer has been previously reported [12].

Time specific networks and differential networks

Protein-protein interactions exist as a static interactome map, while time-course gene expression data during immune response is dynamic and it shows dynamics of gene expression during immune response, combining PPI data with gene expression data allowed us to identify interactome dynamics during immune response.

We constructed different interactome maps for different time points during immune response based on gene expression values. We constructed network for proteins that are encoded by genes that showed significant up/down regulation after stimulation for each time point separately.

From interactome maps that are time specific during immune response, we constructed differential PPI networks between each successive time points such that differential PPI network between half hr and one hour has only proteins and interactions that are unique to half hour PPI map but not to one hour PPI map. We identified differences in PPI networks between each two successive time points' networks throughout the immune response time course from 0.5 hr to 24hrs after LPS stimulation by construction of differential maps and identification of protein complexes (modules) for each differential map.

For instance, half hr network was characterized by down regulation of protein complex which consists of [Syt1-clstn1-Syp-Vamp2-Atp6v0a1] subunits, this module is responsible for transmission of nerve impulse and cell-cell signaling, in addition half hr after stimulation interactome was characterized by up regulation [ticam1/tbk1/traf6] subunits of a protein complex which consists of [Ticam1-Tbk1-Traf6-Tlr3]. This protein module has an extremely important role in immune response. Moreover, Ticam1-Tbk1-Traf6-Tlr3 complex exist in 16hr-24hr differential network with all its subunits up regulated.

In 6hr-8hr differential network, we identified a protein complex that is related to circadian rhythm and consists of Cry2-Per3-Per2 subunits, this complex is down regulated during immune response and showed up regulation starting from 16hrs after stimulation.

From functionally identified modules during immune response we could summarize interactome dynamics during immune response.

Toll like receptors (TLRs) interactome dynamics

Toll like receptors play crucial role in innate immunity. Mice have 12 TLRs which are TLR1-TLR9,TLR11-TLR13 where TLR10 is not functional. However, human have 10 TLRs (TLR1-TLR10) [7].we constructed TLRs network, the network includes eight TLRs(Tlr2,Tlr3,Tlr4,Tlr6,Tlr7,Tlr8,Tlr9,Tlr13) with their interactors, we constructed a network of 49proteins and 66 interactions.
TLRs interactome network at half hour and 3 hours after LPS stimulation are shown in Fig.1.
For instance, at half hour after stimulation Tlr3 was significantly down regulated while Ticam1 was significantly up regulated, at one hr after stimulation Tlr2, Src and Birc3 were significantly up regulated, however 2hrs after stimulation Tlr2, 3,6,7,Src, Cav1 and Birc3 showed significant up regulation while S100a8 showed significant down regulation.
At 3 hours after stimulation there is significant up regulation for Tlr3,Tlr6,Tlr7,Tlr9,Cav1 and Src while there is significant down regulation for S100a8.

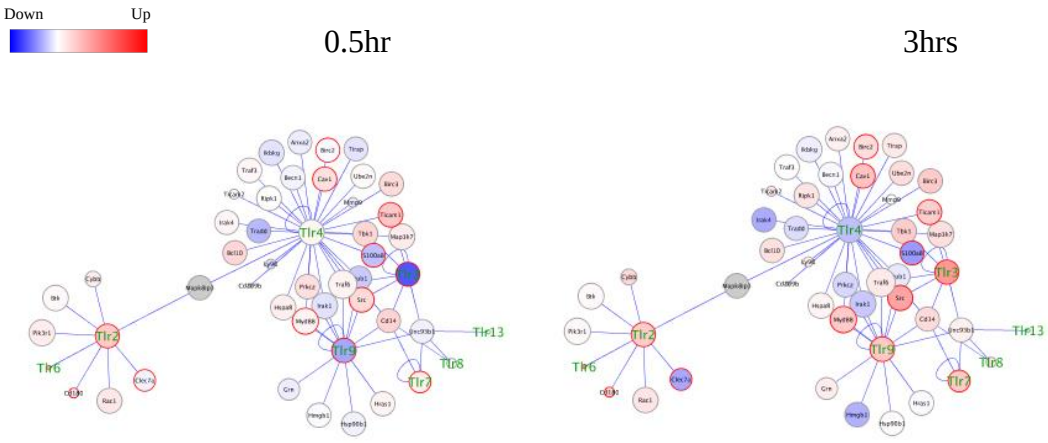

Fig.1: TLRs interactome network at half hr and 3 hours after stimulation.

Node colour represents gene expression level ,blue colour indicates downr egulation while red colour indicates up regulation.

Discussion

In order to identify interactome dynamics during immune response we considered dynamics from two perspectives: First we identified protein modules in a differentially expressed network. We found that some identified modules have role in innate immunity, cell cycle, signaling, phosphorylation, transcription, DNA repair, chromosome organization, transport, development and differentiation. In

addition some modules showed enrichment to P53 pathway, pathways in cancer colorectal cancer and acute myeloid leukemia. Previous review reported a link between innate immunity and cancer [12]. In addition, some identified modules have a role in circadian rhythm. Previous review had discussed a link between Circadian rhythm and immunity[13].

Second we identified protein modules specific to a certain time point. Half hour after stimulation was characterized by up regulation of genes encoding proteins responsible for immune response, cytokine production, Interferon-beta production, IL-6 production, positive regulation of NF-kappaB transcription factor activity and down regulation of Cell-cell signaling and transmission of nerve impulse.

Moreover, in this study we introduced PWDM, which is a novel way to identify differences between different networks either in terms of either proteins or interactions.

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