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研究課題名（和文）病原体特異的に活性化される自然免疫遺伝子ネットワークのシステム生物学的同定と比較

研究課題名（英文）Identification and comparison of pathogen-specific gene regulatory networks activated during the innate immune response using a systems biology approach

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交付決定額（研究期間全体）：（直接経費）　　2,000,000円

研究成果の概要（和文）：Gene response networks for five Toll-like receptors, activated by a distinct pathogenic component, were computationally predicted. Proteins showing a large change in their interactions when activated by distinct pathogens were identified. Upstream regulators of selected cytokines were identified.

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研究分野：総合生物

キーワード：遺伝子ネットワーク　免疫シグナル伝達　自然免疫

１．研究開始当初の背景

The innate immune system is the primary host response to invading pathogens. Immune cells produce distinct effects when exposed to components from different pathogens like viruses, bacteria, fungi and parasites. These diverse effects are mediated by pattern recognition receptors (PRRs) which recognize specific pathogen-associated molecular patterns (PAMPs) and trigger downstream signaling cascades leading to the expression of response genes. The Toll-like receptors (TLRs) are a family of highly conserved PRRs. Though the pathways triggered when TLRs bind to microbial components are well-studied, the response is not yet completely understood. Novel regulators have been identified in these pathways using perturbation studies. However, such studies fail to show the associations between perturbed genes and those with changed expression levels.

In order to address these issues, a novel computational method (TimeXNet) was developed that uses time-course gene expression profiles with a large molecular interaction network to identify the regulatory networks of response genes. TimeXNet was successfully used to study the response of mouse bone-marrow derived dendritic cells (BMDCs) to lipopolysaccharide (LPS).

２．研究の目的

The goal of this project was to use TimeXNet for the identification of the response of the innate immune system to various pathogens (other bacteria, viruses, fungi and parasites) and further, analyze their differences. Individual pathogen responses have been previously studied and a large amount of transcriptional data is available. However, a comprehensive analysis and comparison of the regulatory networks responsible for the distinct immune outcomes produced for different pathogens had not been done so far. The purpose of this project was to identify response gene networks from mouse BMDCs activated by diverse microbial components in order to characterize the differences between pathogen-specific responses of the innate immune system.

３．研究の方法

1) Dataset: After surveying Gene Expression Omnibus, Sequence Read Archive and Immgen, time-course gene expression patterns of dendritic cells activated with 5 pathogenic components (LPS, CpG, Poly I:C, PAM3CSK4, Gardiquimod), each activating a different Toll-like receptor (TLR), were selected (GEO accession: GSE17721). This dataset was selected because experimental conditions were uniform for all pathogenic components and the responses of the cellular and endosomal TLRs were available (Figure 1).

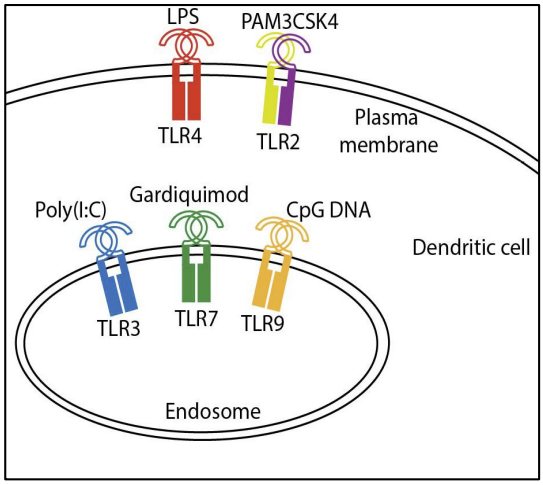

Figure 1.Cellular location of Toll-like Receptors studied

Protein-protein interactions were obtained from the database, HitPredict. HitPredict is a consolidated database of protein-protein interactions with reliability scores assigned to each interaction. HitPredict was updated for use in this project and now contains approximately 500,000 interactions from more than 100 species. Protein-DNA interactions where obtained from KEGG and TRANSFAC. Post-translational modifications were obtained from KEGG. Together, these three data formed the molecular interaction network used to predict the response network for each pathogenic component.

2) Analysis: a) Genes with more than 1.7 fold up-regulation at a p-value less than 0.05 were identified using the R software limma. b) TimeXNet was used to predict response gene networks for the 5 pathogenic components using the

up-regulated genes. TimeXNet uses minimum cost flow optimization to identify the most probable paths connecting highly expressed genes across different time points within a large molecular interaction network containing protein-protein, protein-DNA interactions and post-translational modifications. c) Differential interaction hubs, or genes with the largest change in the number of interactions between the 5 TLR response networks, were identified using the 5 response gene networks with differential interaction scoring. These genes potentially result in differential immune response triggered by various pathogens. d) Cytokines showing differential expression across pathogenic responses were selected for the purpose of identifying their upstream regulators that result in their differential expression patterns. Upstream regulators were identified as genes directly upstream, or separated by one or two edges from the cytokine within the response network predicted by TimeXNet. The predicted upstream regulators were tested for statistical significance by calculating 1000 random response networks using the selected gene expression profiles.

4．研究成果

Response gene networks of approximately 1000-1500 genes, most of which were up-regulated in response to each pathogen, were obtained for each of the 5 TLRs. Figure 2 shows the partial response networks of LPS (TLR4) and CpG (TLR9) along with the critical role played by the protein AKT3 in connecting the TLR and VEGF pathways.

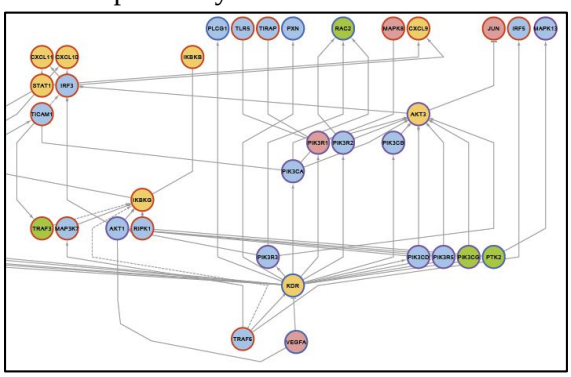

Figure 2. AKT3 network in TLR4 and TLR9 responses. Nodes are genes, edges are interactions. Node color denotes time of upregulation. Node colors indicate time of expression. Red: 0-1 hour, yellow: 2-4 hours, green: 6-8 hours, blue: unknown.

Interaction hubs, many of which are kinases, and their networks show various differences between the 5 responses (Figure 3). For instance, the protein SRC associates with different proteins in cell surface receptor activation versus endosomal receptor activation. Its activity also differs between the cell surface receptors, TLR2 and TLR4 responses, where it associates with transcription factors or hydrolases, and cell adhesion molecules respectively.

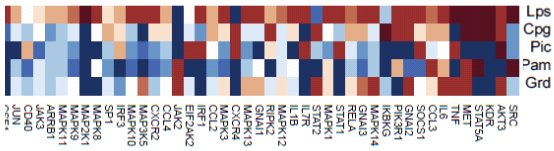

Figure 3. Differential interaction hubs identified for pathogenic response networks. Red: Large change in number of interactions compared to average. Blue: Small change in number of interactions compared to average.

Upstream regulators were identified for several cytokines like Il6, Il10, Il12a, Il12b, Ifna2, Il15 among others, all of which were differentially expressed across the 5 responses. Well-known regulators like Rela, Mapk10, Nlrp3, Bcl2l1, Prdm1 (Figure 4) were identified. Novel regulators were also identified and are being evaluated for potential experimental verification by collaborators.

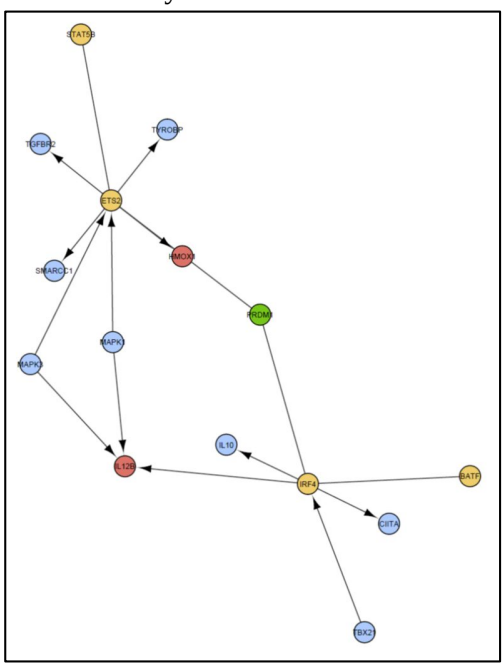

Figure 4. IL12b regulation by Prdm1 and Irf4 in TLR2 response in BMDCs.

Future prospects include performing a similar analyses in macrophages and comparing the differences in their response with those of BMDCs.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　6 件）
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3. Srihari S, Yong CH, Patil A, Wong L. Methods for protein complex prediction and their contributions towards understanding the organisation, function and dynamics of complexes. FEBS Lett. 589(19 Pt A):2590-602, 2015.
4. Patil A and Nakai K. TimeXNet: Identifying active gene sub-networks using time-course gene expression profiles. BMC Systems Biology Suppl 4:S2, 2014.
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6. Elzawahry A, Patil A, Kumagai Y, Suzuki Y and Nakai K. Innate immunity interactome dynamics. Gene Regulation and Systems Biology, 2014:8 1-15 (2014).

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2. Patil A. BioITWorld Expo 2015, Boston USA. 2015/4/21-2015/4/23.
3. Patil A and Nakai K. Identification of pathogen-specific response pathways in activated immune cells using a systems biology approach. 10th International Symposium of the Institute Network, The Alumni Hall “Furate”, Hokkaido University (Sapporo, Hokkaido). 2015/7/23-2015/7/24. (Poster)
4. Patil A and Nakai K. Identifying active gene sub-networks using time-course gene expression profiles using TimeXNet. GIW/ISCB-Asia 2014, Tokyo Japan. 2014/12/15-2014/12/17. (Selected talk)
5. Patil A and Nakai K. TimeXNet: Identifying active gene sub-networks using time-course gene expression profiles. InCoB 2014, Sydney, Australia. 2014/7/30-2014/8/2. (Selected talk)
6. Patil A and Nakai K. TimeXNet: Identifying active gene sub-networks using time-course gene expression profiles. ISMB, NetBIO SIG, 2014, Boston, USA. 2014/7/11-2014/7/15. (Selected talk)

〔その他〕
ホームページ等
1. HitPredict Database: http://hintdb.hgc.jp/htp/
2. TimeXNet Application: http://timexnet.hgc.jp/

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