38. Han YF, Cao GW. Role of nuclear receptor NR4A2 in gastrointestinal inflammation and cancers. *World J Gastroenterol.* 2012; **18**(47): 6865–73.
39. McMorrow JP, Murphy EP. Inflammation: a role for NR4A orphan nuclear receptors? *Biochem Soc Trans.* 2011; **39** (2): 688–93.
40. Mix KS, Attur MG, Al-Mussawir H, Abramson SB, Brinckerhoff CE, Murphy EP. Transcriptional repression of matrix metalloproteinase gene expression by the orphan nuclear receptor NURR1 in cartilage. *J Biol Chem.* 2007; **282**(13): 9492–504.
41. Saijo K, Winner B, Carson CT, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell.* 2009; **137**(1): 47–59.
42. Wang Z, Benoit G, Liu J, et al. Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature.* 2003; **423**(6939): 555–60.
43. Mattes H. NR4A subfamily of receptors and their modulators. In: Ottow EWH, eds. *Nuclear Receptors as Drug Targets. Methods and Principles in Medicinal Chemistry* 39. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim: Wiley-VCH; 2008. 431–52.
44. Oki S, Raveney BJE, Doi Y, Yamamura T. Versatile orphan nuclear receptor NR4A2 as a promising molecular target for multiple sclerosis and other autoimmune diseases. In: Shibasaki MIM, Osada H, eds. *Chembiomolecular Science: At the Frontier of Chemistry and Biology.* Springer Japan, Tokyo: Springer; 2012. 193–200.
45. Castillo SO, Baffi JS, Palkovits M, et al. Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the Nurr1 gene. *Mol Cell Neurosci.* 1998; **11**(1–2): 36–46.
46. Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. *Science (New York, NY).* 1997; **276**(5310): 248–50.
47. Baffi JS, Palkovits M, Castillo SO, Mezey E, Nikodem VM. Differential expression of tyrosine hydroxylase in catecholaminergic neurons of neonatal wild-type and Nurr1-deficient mice. *Neuroscience.* 1999; **93**(2): 631–42.
48. Sakurada K, Ohshima-Sakurada M, Palmer TD, Gage FH. Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. *Development (Cambridge, England).* 1999; **126**(18): 4017–26.
49. Lammi J, Huppunen J, Aarnisalo P. Regulation of the osteopontin gene by the orphan nuclear receptor NURR1 in osteoblasts. *Mol Endocrinol.* 2004; **18**(6): 1546–57.
50. Pirih FQ, Tang A, Ozkurt IC, Nervina JM, Tetradis S. Nuclear orphan receptor Nurr1 directly transactivates the osteocalcin gene in osteoblasts. *J Biol Chem.* 2004; **279**(51): 53167–74.
51. Sekiya T, Kashiwagi I, Inoue N, et al. The nuclear orphan receptor Nr4a2 induces Foxp3 and regulates differentiation of CD4+ T cells. *Nat Commun.* 2011; **2**: 269.
52. Sekiya T, Kashiwagi I, Yoshida R, et al. Nr4a receptors are essential for thymic regulatory T cell development and immune homeostasis. *Nat Immunol.* 2013; **14**(3): 230–7.
53. Bartholomaus I, Kawakami N, Odoardi F, et al. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. *Nature.* 2009; **462** (7269): 94–8.
54. Kawakami N, Nagerl UV, Odoardi F, Bonhoeffer T, Wekerle H, Flugel A. Live imaging of effector cell trafficking and autoantigen recognition within the unfolding autoimmune encephalomyelitis lesion. *J Exp Med.* 2005; **201** (11): 1805–14.
55. Aranami T, Yamamura T. Th17 Cells and autoimmune encephalomyelitis (EAE/MS). *Allergol Int.* 2008; **57**(2): 115–20.
56. Kawakami N, Lassmann S, Li Z, et al. The activation status of neuroantigen-specific T cells in the target organ determines the clinical outcome of autoimmune encephalomyelitis. *J Exp Med.* 2004; **199**(2): 185–97.
57. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol.* 2008; **26**: 57–79.
58. Allard EL, Hardy MP, Leignadier J, et al. Overexpression of IL-21 promotes massive CD8+ memory T cell accumulation. *Eur J Immunol.* 2007; **37**(11): 3069–77.
59. King C, Ilic A, Koelsch K, Sarvetnick N. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell.* 2004; **117**(2): 265–77.
60. McGuire HM, Vogelzang A, Ma CS, et al. A subset of interleukin-21+ chemokine receptor CCR9+ T helper cells target accessory organs of the digestive system in autoimmunity. *Immunity.* 2011; **34**(4): 602–15.
61. Parrish-Novak J, Dillon SR, Nelson A, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature.* 2000; **408** (6808): 57–63.
62. Ettinger R, Kuchen S, Lipsky PE. Interleukin 21 as a target of intervention in autoimmune disease. *Ann Rheum Dis.* 2008; **67**(Suppl 3): iii83–6.
63. Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, Qi CF, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J Immunol.* 2004; **173**(9): 5361–71.
64. Bubier JA, Sproule TJ, Foreman O, et al. A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-Yaa mice. *Proc Natl Acad Sci USA.* 2009; **106**(5): 1518–23.
65. Monteleone G, Monteleone I, Fina D, et al. Interleukin-21 enhances T-helper cell type I signaling and interferon-gamma production in Crohn's disease. *Gastroenterology.* 2005; **128**(3): 687–94.

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66. Fina D, Sarra M, Fantini MC, et al. Regulation of gut inflammation and th17 cell response by interleukin-21. *Gastroenterology*. 2008; **134**(4): 1038–48.
67. Liu SM, Lee DH, Sullivan JM, et al. Differential IL-21 signaling in APCs leads to disparate Th17 differentiation in diabetes-susceptible NOD and diabetes-resistant NOD.Idd3 mice. *J Clin Investig*. 2011; **121**(11): 4303–10.
68. McGuire HM, Vogelzang A, Hill N, Flodstrom-Tullberg M, Sprent J, King C. Loss of parity between IL-2 and IL-21 in the NOD Idd3 locus. *Proc Natl Acad Sci USA*. 2009; **106**(46): 19438–43.
69. Herber D, Brown TP, Liang S, Young DA, Collins M, Dunussi-Joannopoulos K. IL-21 has a pathogenic role in a lupus-prone mouse model and its blockade with IL-21R.Fc reduces disease progression. *J Immunol*. 2007; **178**(6): 3822–30.
70. Spolski R, Kashyap M, Robinson C, Yu Z, Leonard WJ. IL-21 signaling is critical for the development of type I diabetes in the NOD mouse. *Proc Natl Acad Sci USA*. 2008; **105**(37): 14028–33.
71. Young DA, Hegen M, Ma HL, et al. Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis. *Arthritis Rheum*. 2007; **56**(4): 1152–63.
72. Liu SM, King C. IL-21-producing Th cells in immunity and autoimmunity. *J Immunol*. 2013; **191**(7): 3501–6.
73. Zhou L, Ivanov II, Spolski R, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol*. 2007; **8** (9): 967–74.
74. Sonderegger I, Kisielow J, Meier R, King C, Kopf M. IL-21 and IL-21R are not required for development of Th17 cells and autoimmunity in vivo. *Eur J Immunol*. 2008; **38** (7): 1833–8.
75. Coquet JM, Chakravarti S, Smyth MJ, Godfrey DI. Cutting edge: IL-21 is not essential for Th17 differentiation or experimental autoimmune encephalomyelitis. *J Immunol*. 2008; **180**(11): 7097–101.
76. Korn T, Bettelli E, Gao W, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature*. 2007; **448**(7152): 484–7.
77. Nurieva R, Yang XO, Martinez G, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*. 2007; **448**(7152): 480–3.
78. Li Y, Wang H, Long Y, Lu Z, Hu X. Increased memory Th17 cells in patients with neuromyelitis optica and multiple sclerosis. *J Neuroimmunol*. 2011; **234**(1–2): 155–60.
79. Jones JL, Phuah CL, Cox AL, et al. IL-21 drives secondary autoimmunity in patients with multiple sclerosis, following therapeutic lymphocyte depletion with alemtuzumab (Campath-1H). *J Clin Investig*. 2009; **119**(7): 2052–61.
80. Linhares UC, Schiavoni PB, Barros PO, et al. The ex vivo production of IL-6 and IL-21 by CD4+ T cells is directly associated with neurological disability in neuromyelitis optica patients. *J Clin Immunol*. 2013; **33**(1): 179–89.
81. Wang HH, Dai YQ, Qiu W, et al. Interleukin-17-secreting T cells in neuromyelitis optica and multiple sclerosis during relapse. *J Clin Neurosci*. 2011; **18**(10): 1313–7.
82. Wu A, Zhong X, Wang H, et al. Cerebrospinal fluid IL-21 levels in Neuromyelitis Optica and multiple sclerosis. *Can J Neurol Sci*. 2012; **39**(6): 813–20.
83. Ralph JA, McEvoy AN, Kane D, Bresnihan B, FitzGerald O, Murphy EP. Modulation of orphan nuclear receptor NURR1 expression by methotrexate in human inflammatory joint disease involves adenosine A2A receptor-mediated responses. *J Immunol*. 2005; **175**(1): 555–65.
84. Dubois C, Hengerer B, Mattes H. Identification of a potent agonist of the orphan nuclear receptor Nurr1. *Chem Med Chem*. 2006; **1**(9): 955–8.
85. Hintermann S, Chiesi M, von Krosigk U, Mathe D, Felber R, Hengerer B. Identification of a series of highly potent activators of the Nurr1 signaling pathway. *Bioorg Med Chem Lett*. 2007; **17**(1): 193–6.
86. Chintharlapalli S, Burghardt R, Papineni S, Ramaiah S, Yoon K, Safe S. Activation of Nur77 by selected 1,1-Bis (3′-indolyl)-1-(p-substituted phenyl)methanes induces apoptosis through nuclear pathways. *J Biol Chem*. 2005; **280**(26): 24903–14.
87. Morita K, Kawana K, Sodeyama M, Shimomura I, Kagechika H, Makishima M. Selective allosteric ligand activation of the retinoid X receptor heterodimers of NGFI-B and Nurr1. *Biochem Pharmacol*. 2005; **71**(1–2): 98–107.
88. Wansa KD, Muscat GE. TRAP220 is modulated by the antineoplastic agent 6-Mercaptopurine, and mediates the activation of the NR4A subgroup of nuclear receptors. *J Mol Endocrinol*. 2005; **34**(3): 835–48.
89. Beaudry G, Langlois MC, Weppe I, Rouillard C, Levesque D. Contrasting patterns and cellular specificity of transcriptional regulation of the nuclear receptor nerve growth factor-inducible B by haloperidol and clozapine in the rat forebrain. *J Neurochem*. 2000; **75**(4): 1694–702.
90. Maheux J, Ethier I, Rouillard C, Levesque D. Induction patterns of transcription factors of the nur family (nurr1, nur77, and nor-1) by typical and atypical antipsychotics in the mouse brain: implication for their mechanism of action. *J Pharmacol Exp Ther*. 2005; **313**(1): 460–73.
91. Karussis D. The diagnosis of multiple sclerosis and the various related demyelinating syndromes: a critical review. *J Autoimmun*. 2014; **49c**: 134–42.

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Austin Journal of Clinical Immunology

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Research Article

Molecular Network of NLRP3 Inflammasome Activation-Responsive Genes in a Human Monocyte Cell Line

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Received: May 05 2014; **Accepted:** June 11 2014; **Published:** June 13 2014

Abstract

Background: Inflammasome, activated by pathogen-derived and host-derived danger signals, constitutes a multimolecular signaling complex that serves as a platform for caspase-1 (CASP1) activation and interleukin-1β (IL-1β) maturation. The activation of NLRP3 inflammasome requires two-step signals. The first "priming" signal enhances gene expression of inflammasome components. The second "activation" signal promotes the assembly of inflammasome components. Deregulated activation of NLRP3 inflammasome contributes to the pathological processes of Alzheimer's disease (AD) and multiple sclerosis (MS). However, at present, the precise mechanism regulating NLRP3 inflammasome activation and deactivation remains largely unknown.

Methods: By genome-wide gene expression profiling, we studied the molecular network of NLRP3 inflammasome activation-responsive genes in a human monocyte cell line THP-1 sequentially given two-step signals.

Results: We identified the set of 83 NLRP3 inflammasome activation-responsive genes. Among them, we found the NR4A nuclear receptor family NR4A1, NR4A2, and NR4A3, the EGR family EGR1, EGR2, and EGR3, the IκB family NFKBIZ, NFKBID, and NFKBIA as a key group of the genes that possibly constitute a negative feedback loop for shutting down inflammation following NLRP3 inflammasome activation. By molecular network analysis, we identified a complex network of NLRP3 inflammasome activation-responsive genes involved in cellular development and death, and immune and inflammatory responses, where transcription factors AP-1, NR4A, and EGR serve as a hub.

Conclusion: NLRP3 inflammasome activation-responsive genes constitute the molecular network composed of a set of negative feedback regulators for prompt resolution of inflammation.

Keywords: Inflammasome; NLRP3; NR4A1; NR4A2; NR4A3

Introduction

Inflammasome serves as a multi molecular signaling complex involved in activation of caspase-1 (CASP1) and maturation of interleukin-1β (IL-1β) and IL-18 [1,2]. A wide variety of exogenous and endogenous stimuli, characterized by microbe-derived pathogen-associated molecular patterns (PAMPs) and host- or environment-derived danger-associated molecular patterns (DAMPs), are recognized by an intracellular sensor called the NOD-like receptors (NLRs), resulting in rapid induction of inflammasome formation by ordered assembly of self-oligomerizing components.

Among various classes of inflammasome, the nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3 (NLRP3) inflammasome has been most intensively studied. It is composed of NLRP3, the adaptor molecule named apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and the precursor form of the cysteine protease pro-CASP1 [1,2]. NLRP3 contains a central nucleotide-binding and oligomerization (NACHT) domain essential for activation of the signaling complex via ATP-dependent oligomerization, flanked by a C-terminal leucine-rich repeat (LRR) pivotal for ligand sensing and autoregulation and a N-terminal pyrin (PYD) domain involved in a homotypic protein-protein interaction between NLRP3 and ASC. The molecular interaction of NLRP3 with ASC recruits pro-CASP1 by a homotypic interaction of caspase activation and recruitment (CARD) domains between ASC and pro-CASP1. Subsequently, the proximity-induced pro-CASP1 oligomerization causes autocatalytic activation of CASP1, resulting in processing of pro-IL-1β or pro-IL-18 into biologically active IL-1β and IL-18. Both of them act as a central regulator for induction of cytokines and chemokines that amplify inflammation by recruiting immune effector cells.

The activation of NLRP3 inflammasome requires two-step signals (Figure 1) [3,4]. The first "priming" signal termed Signal 1, such as microbe-derived lipopolysaccharide (LPS), enhances gene expression of inflammasome components and target proteins via activation of transcription factor nuclear factor-kappa B (NF-κB). The second "activation" signal termed Signal 2 promotes the organized assembly of inflammasome components. The second signal involves three major mechanisms, such as generation of reactive oxygen species (ROS), lysosomal protease leakage, and the potassium efflux [1,2]. Mitochondria often serve as the principal source of ROS. Blockade of mitophagy induces accumulation of ROS-generating mitochondria that activates NLRP3 inflammasome [5]. Furthermore, oxidized

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Citation: Kawana N, Yamamoto Y, Kino Y and Jun-ichi Satoh. Molecular Network of NLRP3 Inflammasome Activation-Responsive Genes in a Human Monocyte Cell Line. Austin J Clin Immunol. 2014;1(4): 10.

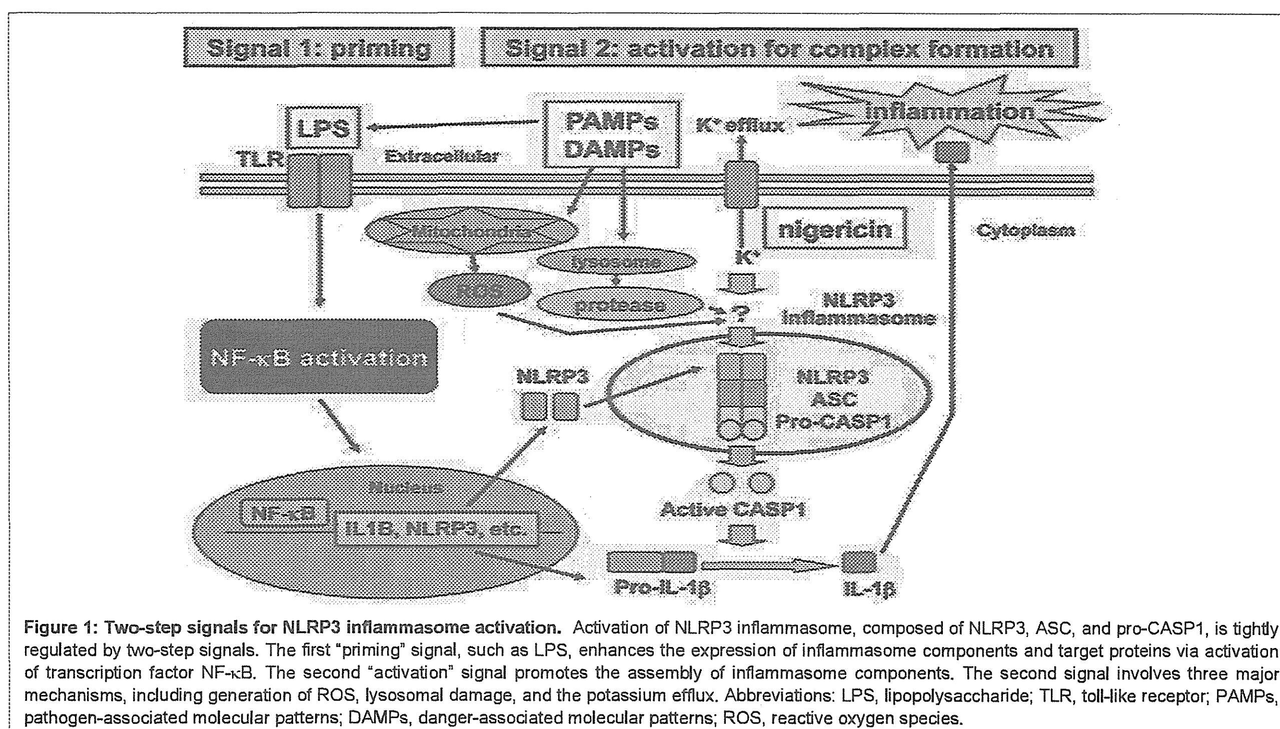

Figure 1: Two-step signals for NLRP3 inflammasome activation. Activation of NLRP3 inflammasome, composed of NLRP3, ASC, and pro-CASP1, is tightly regulated by two-step signals. The first "priming" signal, such as LPS, enhances the expression of inflammasome components and target proteins via activation of transcription factor NF-κB. The second "activation" signal promotes the assembly of inflammasome components. The second signal involves three major mechanisms, including generation of ROS, lysosomal damage, and the potassium efflux. Abbreviations: LPS, lipopolysaccharide; TLR, toll-like receptor; PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns; ROS, reactive oxygen species.

mitochondrial DNA directly activates NLRP3 inflammasome following induction of apoptosis [6]. By serving as an inducer of two-step signals, a diverse range of danger signals armed with PAMPs, such as *Listeria monocytogenes*, *Candida albicans*, and influenza A virus and those with DAMPs, such as amyloid-β (Aβ), uric acid and cholesterol crystals, asbestos, silica, alum, hyaluronan, and adenosine 5'-triphosphate (ATP), promptly activate the NLRP3 inflammasome [7,8].

Deregulated activation of NLRP3 inflammasome contributes to the pathological processes of various diseases, such as type 2 diabetes, Alzheimer's disease (AD), and multiple sclerosis (MS) [9-11]. Lack of NLRP3 inflammasome components skews microglial cells to an anti-inflammatory M2 phenotype with an enhanced capacity of amyloid-β (Aβ) clearance in a mouse model of AD [10]. *Nlrp3*-knockout mice showed reduced severity of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, characterized by substantial attenuation of inflammation, demyelination and astrogliosis [12]. In active inflammatory demyelinating lesions of MS, reactive astrocytes and perivascular macrophages expressed all three components of NLRP3 inflammasome, such as NLRP3, ASC, and CASP1, along with IL-1β, suggesting that biochemical agents and monoclonal antibodies designed to block specifically NLRP3 inflammasome activation might be highly effective in treatment of active MS [11]. However, at present, the precise mechanism regulating NLRP3 inflammasome activation and deactivation remains largely unknown. In the present study, by genome-wide gene expression profiling, we attempts to clarify the comprehensive molecular network of NLRP3 inflammasome activation-responsive genes in a human monocyte cell line given consecutively two-step signals.

Materials and Methods

NLRP3 inflammasome activation

A human monocyte cell line THP-1 was obtained from RIKEN Cell Bank (Saitama, Japan). The cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), 55 μM 2-mercaptoethanol, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (feeding medium). To load the Signal 1, the cells were incubated for 3 hours with or without 0.2 μg/ml lipopolysaccharide (LPS; Sigma, St. Louis, MO, USA). To load the Signal 2, they were washed twice by Phosphate-Buffered Saline (PBS) and incubated further for 0.5 or 2 hours with 10 μM nigericin sodium salt (Wako Pure Chemical, Osaka, Japan) dissolved in ethanol or the equal v/v% concentration of ethanol (vehicle). Then, protein extract of the cells was processed for western blot analysis with a rabbit antibody against the C-terminal peptide of the human CASP1 p10 protein (sc-515, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a rabbit antibody against the peptide mapping at amino acid residues of 117-269 of the human IL-1β protein (sc-7884, Santa Cruz Biotechnology).

Microarray analysis

Total cellular RNA was isolated by using the TRIZOL plus RNA Purification kit (Invitrogen). The quality of total RNA was evaluated on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Three hundred ng of total RNA was processed for cDNA synthesis, fragmentation, and terminal labeling with the GeneChip Whole Transcript Sense Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA, USA). Then, the labeled cRNA was processed for hybridization at 45°C for 17 hours with Human Gene 1.0 ST Array (28,869 genes; Affymetrix). The arrays were washed in the

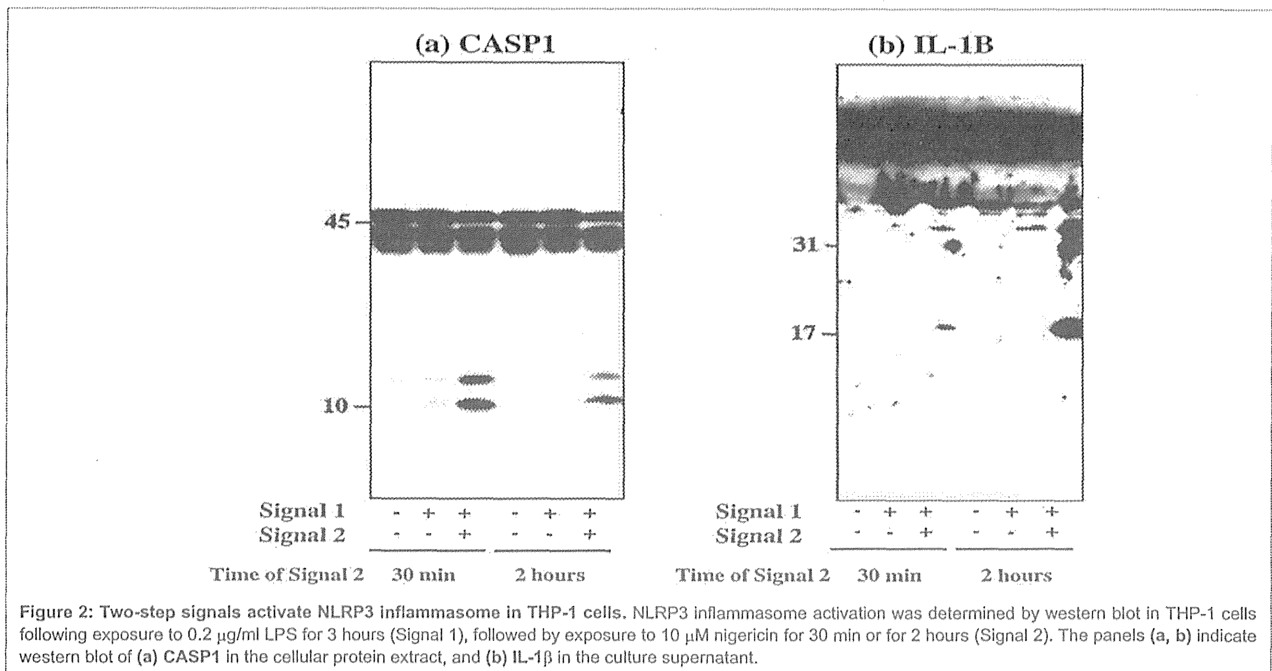

Figure 2: Two-step signals activate NLRP3 inflammasome in THP-1 cells. NLRP3 inflammasome activation was determined by western blot in THP-1 cells following exposure to 0.2 μg/ml LPS for 3 hours (Signal 1), followed by exposure to 10 μM nigericin for 30 min or for 2 hours (Signal 2). The panels **(a, b)** indicate western blot of **(a) CASP1** in the cellular protein extract, and **(b)** IL-1β in the culture supernatant.

Gene Chip Fluidic Station 450 (Affymetrix), and scanned by the Gene Chip Scanner 3000 7G (Affymetrix). The raw data were expressed as CEL files and normalized by the Robust Multi Array average (RMA) method with the Expression Console software (Affymetrix).

Quantitative reverse transcription (RT)-polymerase chain reaction (qPCR) analysis

DNase-treated total RNA isolated from THP-1 cells was processed for cDNA synthesis using oligo(dT)$_{12-18}$ primers and Super Script II reverse transcriptase (Invitrogen). Then, cDNA was amplified by PCR in Light Cycler ST300 (Roche Diagnostics, Tokyo, Japan) using SYBR Green I and a panel of sense and antisense primer sets following: 5'ccagcactgccaaactggactact3' and 5' acagctcagcaaagccagggatct3' for an 162 bp product of nuclear receptor subfamily 4, group A, member 1 (NR4A1); 5'ccaaagccgaccaagacctgcttt3' and 5'ctgtgcaagaccaccccattgcaa3' for an 124 bp product of nuclear receptor subfamily 4, group A, member 2 (NR4A2); 5'gagggctgcaagggctttttcaag3' and 5' gagggctgagaaggttcctgttgt3' for a 242 bp product of nuclear receptor subfamily 4, group A, member 3 (NR4A3); and 5'ccatgttcgtcatgggtgtgaacca3' and 5'gccagtagaggcagggatgatgttc3' for a 251 bp product of the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene that serves as an endogenous control. The expression levels of target genes were standardized against the levels of G3PDH detected in the corresponding cDNA samples. All the assays were performed in triplicate.

Molecular network analysis

To identify biologically relevant molecular networks, we imported corresponding Entrez Gene IDs into Ingenuity Pathways Analysis (IPA) (Ingenuity Systems, Redwood City, CA, USA), KeyMolnet (Institute of Medicinal Molecular Design, Tokyo, Japan), or Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) 9.1. STRING is an open-access database, while IPA and KeyMolnet are commercial resources.

STRING is a database that contains known and predicted, physiological and functional protein-protein interactions composed of 5,214,234 proteins from 1133 organisms [13]. STRING integrates the information from numerous resources, including experimental repositories, computational prediction methods, and public text collections. By uploading the list of UniProt IDs or Gene Symbols, STRING illustrates the union of all possible association networks.

IPA is a knowledgebase that contains approximately 3,000,000 biological and chemical interactions and functional annotations with definite scientific evidence. By uploading the list of Gene IDs and expression values, the network-generation algorithm identifies focused genes integrated in a global molecular network. IPA calculates the score p-value that reflects the statistical significance of association between the genes and the networks by the Fisher's exact test.

KeyMolnet contains knowledge-based contents on 164,000 relationships among human genes and proteins, small molecules, diseases, pathways and drugs [14]. They include the core contents collected from selected review articles with the highest reliability. By importing the list of Gene ID and expression values, KeyMolnet automatically provides corresponding molecules as nodes on the network. The neighboring network-search algorithm selected one or more molecules as starting points to generate the network of all kinds of molecular interactions around starting molecules, including direct activation/inactivation, transcriptional activation/repression, and the complex formation within one path from starting points. The generated network was compared side by side with 501 human canonical pathways of the KeyMolnet library. The algorithm counting the number of overlapping molecular relations between the extracted network and the canonical pathway makes it possible to identify the canonical pathway showing the most significant contribution to the

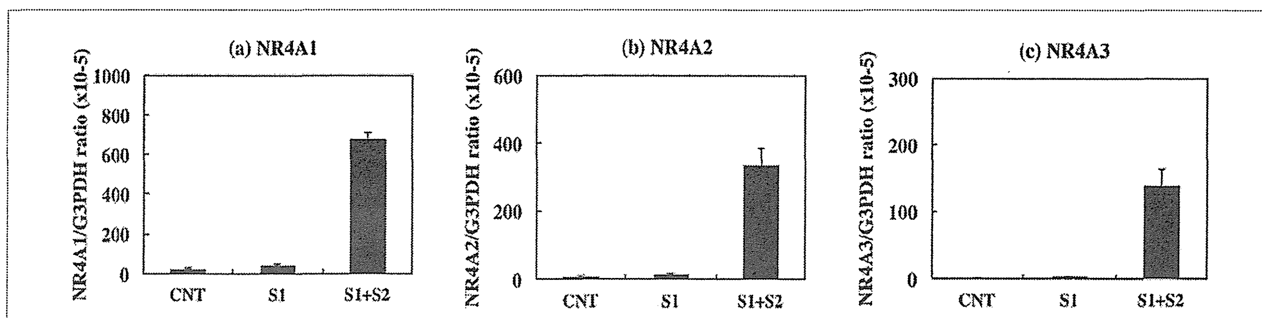

Figure 3: Upregulated expression of NR4A family members in THP-1 cells during NLRP3 inflammasome activation. The levels of expression of NR4A1, NR4A2, and NR4A3 transcripts in THP-1 cells following exposure to 0.2 μg/ml LPS for 3 hours (Signal 1; S1), followed by exposure to 10 μM nigericin for 2 hours (Signal 2; S2) were determined by qPCR. They were standardized against the levels of G3PDH detected in the corresponding cDNA samples. The panels **(a-c)** indicate qPCR of **(a) NR4A1**, **(b) NR4A2**, and **(c) NR4A3**. The bars represent **CNT** (LPS -, nigericin -), **S1** (LPS +, nigericin -), and **S1+S2** (LPS+, nigericin +).

extracted network.

Results

NLRP3 inflammasome activation in THP-1 cells following introduction of two-step signals

First, by western blot analysis, we studied NLRP3 inflammasome activation in THP-1 treated initially with exposure to 0.2 μg/ml LPS for 3 hours (Signal 1), followed by exposure to 10 μM nigericin for 30 min or 2 hours (Signal 2). The consecutive load of Signal 1 and Signal 2 markedly activated NLRP3 inflammasome in THP-1 cells, as indicated by production of cleaved products of CASP1 (Figure 2, panel a) and IL-1β (Figure 2, panel b). In contrast, the introduction of Signal 1 alone was not enough to activate NLRP3 inflammasome in THP-1 cells (Figure 2, panels a and b).

Gene expression profile during NLRP3 inflammasome activation

Next, we studied the genome-wide gene expression profile of THP-1 cells pretreated with 0.2 μg/ml LPS for 3 hours (Signal 1), washed by PBS, and exposed to 10 μM nigericin or vehicle for 2 hours (Signal 2). Then, total RNA was immediately processed for gene expression profiling on a Human Gene 1.0 ST Array. To identify NLRP3 inflammasome activation-responsive genes, we extracted the set of 83 annotated and protein-coding genes that satisfied fold change (FC) in Signal 1 (the presence of LPS versus the absence of LPS) smaller than 2-fold and FC in Signal 2 (the presence of nigericin versus the absence of nigericin) greater than 2-fold (Table 1). This gene enrichment procedure minimized the genes that were activated simply by exposure to LPS alone but not directly related to NLRP3 inflammasome activation.

Most notably, three members of NR4A nuclear receptor family, such as NR4A1 (NUR77), NR4A2 (NURR1), and NR4A3 (NOR1), were identified as those ranked within top 10 genes. Coordinated up regulation of NR4A1, NR4A2, and NR4A3 in NLRP3 inflammasome-activated THP-1 cells was validated by qPCR (Figure 3, panels a-c). Signal 1 alone mildly elevated expression of these mRNA levels, whereas introduction of Signal 2 after Signal 1 markedly elevated the levels of NR4A1, NR4A2, and NR4A3 transcripts with a 16-fold, 25-fold, or 51-fold increase, respectively. We also identified early growth response (EGR) family members, such as EGR1, EGR2, and EGR3, which belong to a family of zinc finger transcription factors involved in the regulation of cell growth, differentiation, and survival, NF-κB inhibitor (IκB) family members, such as NFKBIZ, NFKBID, and NFKBIA, along with a panel of pro inflammatory cytokines and chemokines, including CCL3, CCL3L3, IL8, CXCL2, CCL20, IL23A, and TNFSF9, as a subgroup of NLRP3 inflammasome activation-responsive genes.

Molecular network of NLRP3 inflammasome activation responsive genes

Next, by using three different bioinformatics tools for molecular network analysis based on knowledgebase, we studied biologically relevant molecular networks for the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells. The core analysis of IPA identified the networks defined as "Auditory and Vestibular System Development and Function, Embryonic Development, Organ Development" (p = 1.00E-32), "Cell Cycle, Cellular Development, Cell Death and Survival" (p = 1.00E-30) (Figure 4), and "Connective Tissue Disorders, Immunological Disease, Inflammatory Disease" (p = 1.00E-26) as top three most relevant functional networks. These results suggest that NLRP3 inflammasome activation-responsive genes play a pivotal role in cell development, death, and immune and inflammatory responses. KeyMolnet by the neighboring network-search algorithm operating on the core contents extracted the highly complex molecular network composed of 455 molecules and 529 molecular relations. The network showed the most statistically significant relationship with canonical pathways termed as "transcriptional regulation by AP-1" (p = 3.82E-184), "transcriptional regulation by NR4A" (p = 2.28E-105), and "transcriptional regulation by EGR" (p = 2.78E-99) (Figure 5). These results suggest a central role of transcription factors AP-1, NR4A, and EGR in regulation of expression of NLRP3 inflammasome activation-responsive genes, by acting as a hub of the molecular network.

Finally, STRING extracted a protein-protein interaction network, composed of 35 core molecules derived from the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells. In this network, both the set of NR4A family members NR4A1, NR4A2, and NR4A3 and EGR transcription factors EGR1, EGR2, and EGR3 constituted a close and intense protein interaction subnetwork (Figure 6).

Table 1: The set of 83 up-regulated genes in THP-1 monocytes following activation of NLRP3 inflammasome.

Rank	FC Related to Signal 1	FC Related to Signal 2	Entrez Gene ID	Gene Symbol	Gene Name
1	1.06819645	18.61247501	8013	NR4A3	nuclear receptor subfamily 4, group A, member 3
2	1.942378012	12.91651537	6348	CCL3	chemokine (C-C motif) ligand 3
3	1.63109973	11.69111	414062	CCL3L3	chemokine (C-C motif) ligand 3-like 3
4	1.100615838	11.24166642	9308	CD83	CD83 molecule
5	1.819566773	10.85127008	3576	IL8	interleukin 8
6	1.292541852	7.633454043	1960	EGR3	early growth response 3
7	0.948867136	6.576691539	4929	NR4A2	nuclear receptor subfamily 4, group A, member 2
8	1.116320272	5.51767318	3164	NR4A1	nuclear receptor subfamily 4, group A, member 1
9	1.842348508	5.271896351	64332	NFKBIZ	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
10	1.268131184	4.992502002	643616	MOP-1	MOP-1
11	1.222058201	4.99018398	1959	EGR2	early growth response 2
12	1.716614387	4.456895103	5734	PTGER4	prostaglandin E receptor 4 (subtype EP4)
13	1.067764134	4.401932449	10746	MAP3K2	mitogen-activated protein kinase kinase kinase 2
14	1.076240121	4.353030131	2920	CXCL2	chemokine (C-X-C motif) ligand 2
15	1.443866138	4.329651804	6364	CCL20	chemokine (C-C motif) ligand 20
16	1.506881527	4.037790353	5743	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
17	1.143021068	3.908082725	153020	RASGEF1B	RasGEF domain family, member 1B
18	1.00701348	3.793627448	1958	EGR1	early growth response 1
19	1.188818931	3.318906546	23645	PPP1R15A	protein phosphatase 1, regulatory (inhibitor) subunit 15A
20	0.978133301	3.154899408	65125	WNK1	WNK lysine deficient protein kinase 1
21	1.116953399	3.113268501	84807	NFKBID	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, delta
22	1.431860551	3.025219884	51561	IL23A	interleukin 23, alpha subunit p19
23	0.654486344	2.985745104	645188	LOC645188	hypothetical LOC645188
24	1.082721348	2.867304268	1843	DUSP1	dual specificity phosphatase 1
25	1.877501415	2.813972064	8870	IER3	immediate early response 3
26	1.458901009	2.788511085	9021	SOCS3	suppressor of cytokine signaling 3
27	0.930381294	2.730662487	728715	LOC728715	ovostatin homolog 2-like
28	1.251031395	2.703465614	2353	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog
29	1.994627015	2.654181457	27289	RND1	Rho family GTPase 1
30	0.877732964	2.64583117	23499	MACF1	microtubule-actin crosslinking factor 1
31	1.18363314	2.591793912	7538	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
32	0.768263434	2.584281103	79101	TAF1D	TATA box binding protein (TBP)-associated factor, RNA polymerase I, D, 41kDa
33	1.895682029	2.568793654	90668	LRRC16B	leucine rich repeat containing 16B
34	0.916615124	2.536018037	259296	TAS2R50	taste receptor, type 2, member 50
35	0.895110685	2.535538194	728741	LOC728741	hypothetical LOC728741
36	0.870604266	2.532650507	84319	CMSS1	cms1 ribosomal small subunit homolog (yeast)
37	0.474895831	2.525788794	4072	EPCAM	epithelial cell adhesion molecule
38	1.667878267	2.514873802	1326	MAP3K8	mitogen-activated protein kinase kinase kinase 8
39	1.107775084	2.496005315	8744	TNFSF9	tumor necrosis factor (ligand) superfamily, member 9
40	1.024389944	2.491488658	4616	GADD45B	growth arrest and DNA-damage-inducible, beta
41	0.97810347	2.470592388	2354	FOSB	FBJ murine osteosarcoma viral oncogene homolog B
42	1.017380957	2.461870724	643036	SLED1	RTFV9368
43	1.017380957	2.377675786	2152	F3	coagulation factor III (thromboplastin, tissue factor)

44	1.038770533	2.373054125	1973	EIF4A1	eukaryotic translation initiation factor 4A, isoform 1
45	1.596962012	2.3683134	4792	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
46	0.872659044	2.354224669	1736	DKC1	dyskeratosis congenita 1, dyskerin
47	1.254570022	2.347010028	50515	CHST11	carbohydrate (chondroitin 4) sulfotransferase 11
48	0.818985035	2.34454831	50840	TAS2R14	taste receptor, type 2, member 14
49	0.649089802	2.278082518	85028	SNHG12	small nucleolar RNA host gene 12 (non-protein coding)
50	0.978928228	2.273044623	2889	RAPGEF1	Rap guanine nucleotide exchange factor (GEF) 1
51	0.689249392	2.247537218	55795	PCID2	PCI domain containing 2
52	0.827575589	2.246739728	54765	TRIM44	tripartite motif-containing 44
53	1.067300921	2.243145194	1263	PLK3	polo-like kinase 3 (Drosophila)
54	0.767788042	2.229552244	337867	UBAC2	UBA domain containing 2
55	1.306111439	2.229215371	3759	KCNJ2	potassium inwardly-rectifying channel, subfamily J, member 2
56	1.925222241	2.191743556	80149	ZC3H12A	zinc finger CCCH-type containing 12A
57	0.882964289	2.185060168	58155	PTBP2	polypyrimidine tract binding protein 2
58	1.545906426	2.181251323	56895	AGPAT4	1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)
59	1.05509141	2.155321381	10896	OCLM	oculomedin
60	1.05361515	2.15489714	9659	PDE4DIP	phosphodiesterase 4D interacting protein
61	0.986553364	2.153150265	3047	HBG1	hemoglobin, gamma A
62	0.87493697	2.150450624	100507607	NPIPB9	nuclear pore complex interacting protein family, member B9
63	1.201327908	2.147514699	259292	TAS2R46	taste receptor, type 2, member 46
64	0.885483295	2.144478729	51574	LARP7	La ribonucleoprotein domain family, member 7
65	0.970156229	2.132807866	9839	ZEB2	zinc finger E-box binding homeobox 2
66	0.700126731	2.102345827	100133941	CD24	CD24 molecule
67	1.471640204	2.097753274	6303	SAT1	spermidine/spermine N1-acetyltransferase 1
68	0.796744464	2.080051151	9572	NR1D1	nuclear receptor subfamily 1, group D, member 1
69	1.754590053	2.069409283	10129	FRY	furry homolog (Drosophila)
70	1.117049405	2.06451372	5586	PKN2	protein kinase N2
71	1.084905208	2.058951728	339883	C3orf35	chromosome 3 open reading frame 35
72	1.007649566	2.047104863	1195	CLK1	CDC-like kinase 1
73	1.001286612	2.046307571	1185	CLCN6	chloride channel 6
74	1.005938423	2.043756057	338442	HCAR2	hydroxycarboxylic acid receptor 2
75	0.88066058	2.04297423	6144	RPL21	ribosomal protein L21
76	1.048011825	2.039547357	1844	DUSP2	dual specificity phosphatase 2
77	1.361895488	2.039480914	3092	HIP1	huntingtin interacting protein 1
78	0.951119813	2.038925421	388022	LOC388022	hypothetical gene supported by AK131040
79	0.888482949	2.018363478	144132	DNHD1	dynein heavy chain domain 1
80	0.972189862	2.012125102	23049	SMG1	SMG1 homolog, phosphatidylinositol 3-kinase-related kinase (C. elegans)
81	0.89112764	2.007348359	6181	RPLP2	ribosomal protein, large, P2
82	0.798221473	2.005195646	23329	TBC1D30	TBC1 domain family, member 30
83	1.206469961	2.003702064	3726	JUNB	jun B proto-oncogene

To activate NLRP3 inflammasome, THP-1 cells were initially exposed to 0.2 μg/ml LPS for 3 hours (Signal 1). They were then washed by PBS and exposed to 10 μM nigericin for 2 hours (Signal 2 after Signal 1). At 5 hours after initiation of the treatment, total RNA was isolated and processed for gene expression profilong on a Human Gene 1.0 ST Array. The set of 83 genes that satisfy fold change (FC) related to Signal 1 (LPS + versus LPS -) smaller than 2-fold and FC related to Signal 2 (nigericin + versus nigericin -) greater than 2-fold are shown with FC, Entrez Gene ID, Gene Symbol, and Gene Name.

Figure 4: IPA molecular network of NLRP3 inflammasome activation-responsive genes. Entrez Gene IDs corresponding to the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells (Table 1) were imported into the core analysis tool of IPA. The functional network defined as "Cell Cycle, Cellular Development, Cell Death and Survival" is shown. Red nodes indicate NLRP3 inflammasome activation-responsive genes.

Figure 5: KeyMolnet molecular network of NLRP3 inflammasome activation-responsive genes. Entrez Gene IDs corresponding to the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells (Table 1) were imported into KeyMolnet. The neighboring network-search algorithm operating on the core contents extracted the highly complex molecular network. Red nodes represent NLRP3 inflammasome activation-responsive genes, while white nodes exhibit additional nodes extracted automatically from the core contents of KeyMolnet to establish molecular connections. The molecular relation is indicated by solid line with arrow (direct binding or activation), solid line with arrow and stop (direct inactivation), solid line without arrow (complex formation), dash line with arrow (transcriptional activation), and dash line with arrow and stop (transcriptional repression). The cluster of NR4A1, NR4A2, and NR4A3 is highlighted by blue circle.

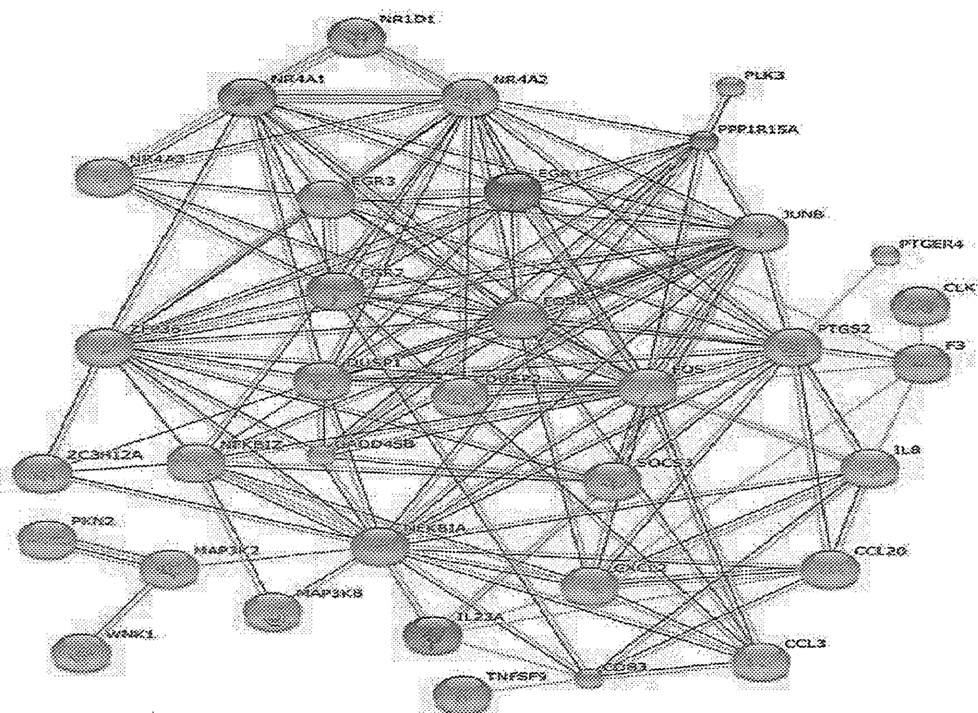

Fig. 6. STRING molecular network of NLRP3 inflammasome activation-responsive genes. Gene Symbols corresponding to the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells (Table 1) were imported into STRING. The set of 35 molecules constructing the protein-protein interaction network are shown on the evidence view of STRING.

Discussion

By genome-wide gene expression profiling, we identified the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells sequentially given two-step signals. Among them, we found three members of NR4A nuclear receptor family, such as NR4A1, NR4A2, and NR4A3, three members of EGR family, such as EGR1, EGR2, and EGR3, three members of IκB family, such as NFKBIZ, NFKBID, and NFKBIA as a noticeable subset of NLRP3 inflammasome activation-responsive genes. By molecular network analysis, we found that they play a central role in cellular development and death, and immune and inflammatory responses, where transcription factors AP-1, NR4A, and EGR serve as a hub in the molecular network. Because THP-1 is a spontaneously immortalized human monocytic cell line derived from an acute monocytic leukemia patient, the possibility could not be excluded that the molecular network we identified does not represent the physiological network of non-malignant human monocytes.

NR4A1, NR4A2, and NR4A3 are three closely related, highly homologous nuclear transcription factors of the steroid/thyroid hormone receptor superfamily, categorized as orphan nuclear receptors because of lack of their cognate ligands [15]. They are encoded by immediate early genes, rapidly induced by exposure of the cells to the serum, growth factors, cytokines, and peptide hormones. NR4A receptors act as a transcription factor for a battery of downstream genes involved in cell proliferation, apoptosis, DNA repair, inflammation, and angiogenesis [16]. Accumulating evidence indicates that NR4A family exerts not only proinflammatory but also anti-inflammatory effects on various cell types. NR4A receptors play a pivotal role in development of regulatory T (Treg) cells in the thymus [17]. Knockdown of either NR4A1 or NR4A3 elevates the levels of production of IL-1β, IL-8, and MCP-1 in THP-1 cells [18]. By binding directly to NF-κB p65, a central regulator of innate and adaptive immune response, NR4A1 recruits the CoREST corepressor complex on gene promoter and inhibits transcription of proinflammatory genes in mouse microglia and astrocytes [19]. Adenosine monophosphate released from apoptotic cells, when metabolized to adenosine, activates macrophages to express NR4A1, NR4A2, and NR4A3 that play a role in suppression of inflammation during engulfment of apoptotic cells [20]. Recently, we found that NR4A2 is one of vitamin D receptor-target genes with protective function against development of MS by analyzing a chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) dataset derived from immortalized B cells and THP-1 cells [21]. All of these observations suggest that NR4A proteins, whose expression is induced by proinflammatory mediators, serve as a safety valve for shutting down sustained inflammation that is amplified by NLRP3 inflammasome activation. Consistent with this view, IκB family members acting as a negative regulator of NF-κB activation, such as NFKBIZ, NFKBID, and NFKBIA [22-24], are coordinately induced along with enhanced expression of NR4A family, suggesting that these molecules constitute a negative feedback loop for NLRP3 inflammasome activation.

EGR family constitutes a family of zinc finger transcription factors very rapidly and transiently induced in various cell types without *de novo* protein synthesis following exposure to mitogenic signals [25,26]. EGR1 functions as a positive regulator for T and B cell functions, by regulating transcription of the genes encoding key cytokines and costimulatory molecules, while EGR2 and EGR3 act as a negative regulator essential for induction of anergy [27]. EGR1 downregulates the expression of itself by binding to an EGR1-binding site located on its own promoter [28]. Furthermore, EGR1 directly activates transcription of NR4A1 (nur77) in mouse IgM+ B cells [29]. Deletion of EGR2 and EGR3 in mouse T and B cells causes a lethal autoimmune syndrome characterized by excessive production of proinflammatory cytokines accompanied by overactivation of STAT1 and STAT3 [30]. Importantly, we identified SOCS3, a potent inhibitor of STAT3 activation [31], as one of NLRP3 inflammasome activation-responsive genes (Rank 26 in Table 1). These observations suggest the working hypothesis that the EGR family members are actively involved in resolution of sustained inflammation amplified by NLRP3 inflammasome activation.

Conclusion

By genome-wide gene expression profiling, we identified the set of 83 NLRP3 inflammasome activation-responsive genes in THP-1 cells. Among them, we found NR4A nuclear receptor family, EGR family, and IκB family as a group of the genes that possibly constitute a negative feedback loop for shutting down sustained inflammation following NLRP3 inflammasome activation. By molecular network analysis, we found that NLRP3 inflammasome activation-responsive genes play a pivotal role in cellular development and death, and immune and inflammatory responses, where transcription factors AP-1, NR4A, and EGR act as a hub in the molecular network.

Acknowledgement

This work was supported by the JSPS KAKENHI (C25430054), and the Intractable Disease Research Center (IDRC) project, the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, and the grant from the National Center for Geriatrics and Gerontology (NCGC 26-20). The authors would thank Ms. Aki Takaoka for her invaluable help in microarray analysis. The microarray data are available from the Gene Expression Omnibus (GEO) under the accession number GSE58959.

References

1. Schroder K, Tschopp J. The inflammasomes. Cell. 2010; 140: 821-832.
2. Menu P, Vince JE. The NLRP3 inflammasome in health and disease: the good, the bad and the ugly. Clin Exp Immunol. 2011; 166: 1-15.
3. Wang H, Mao L, Meng G. The NLRP3 inflammasome activation in human or mouse cells, sensitivity causes puzzle. Protein Cell. 2013; 4: 565-568.
4. Walsh JG, Muruve DA, Power C. Inflammasomes in the CNS. Nat Rev Neurosci. 2014; 15: 84-97.
5. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011; 469: 221-225.
6. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. Immunity. 2012; 36: 401-414.
7. Di Virgilio F. Liaisons dangereuses: P2X(7) and the inflammasome. Trends Pharmacol Sci. 2007; 28: 465-472.
8. Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol. 2008; 9: 857-865.
9. Jourdan T, Godlewski G, Cinar R, Bertola A, Szanda G, Liu J, et al. Activation of the Nlrp3 inflammasome in infiltrating macrophages by endocannabinoids mediates beta cell loss in type 2 diabetes. Nat Med. 2013; 19: 1132-1140.
10. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature. 2013; 493: 674-678.
11. Kawana N, Yamamoto Y, Ishida T, Saito Y, Konno H, Arima K, et al. Reactive astrocytes and perivascular macrophages express NLRP3 inflammasome in active demyelinating lesions of multiple sclerosis and necrotic lesions of neuromyelitis optica and cerebral infarction. Clin Exp Neuroimmunol. 2013; 4: 296-304.
12. Gris D, Ye Z, Iocca HA, Wen H, Craven RR, Gris P, Huang M. NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. J Immunol. 2010; 185: 974-981.
13. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013; 41: D808-815.
14. Satoh J. Bioinformatics approach to identifying molecular biomarkers and networks in multiple sclerosis. Clin Exp Neuroimmunol. 2010; 1: 127–140.
15. Zhao Y, Bruemmer D. NR4A orphan nuclear receptors: transcriptional regulators of gene expression in metabolism and vascular biology. Arterioscler Thromb Vasc Biol. 2010; 30: 1535-1541.
16. Mohan HM, Aherne CM, Rogers AC, Baird AW, Winter DC, Murphy EP. Molecular pathways: the role of NR4A orphan nuclear receptors in cancer. Clin Cancer Res. 2012; 18: 3223-3228.
17. Sekiya T, Kashiwagi I, Yoshida R, Fukaya T, Morita R, Kimura A, Ichinose H. Nr4a receptors are essential for thymic regulatory T cell development and immune homeostasis. Nat Immunol. 2013; 14: 230-237.
18. Bonta PI, van Tiel CM, Vos M, Pols TW, van Thienen JV, Ferreira V, et al. Nuclear receptors Nur77, Nurr, and NOR-1 expressed in atherosclerotic lesion macrophages reduce lipid loading and inflammatory responses. Arterioscler Thromb Vasc Biol. 2006; 26: 2288-2294.
19. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell. 2009; 137: 47-59.
20. Yamaguchi H, Maruyama T, Urade Y, Nagata S. Immunosuppression via adenosine receptor activation by adenosine monophosphate released from apoptotic cells. Elife. 2014; 3: e02172.
21. Satoh J, Tabunoki H. Molecular network of chromatin immunoprecipitation followed by deep sequencing-based vitamin D receptor target genes. Mult Scler. 2013; 19: 1035-1045.
22. Muta T, Yamazaki S, Eto A, Motoyama M, Takeshige K. IkappaB-zeta, a new anti-inflammatory nuclear protein induced by lipopolysaccharide, is a negative regulator for nuclear factor-kappaB. J Endotoxin Res. 2003; 9: 187-191.
23. Tergaonkar V, Correa RG, Ikawa M, Verma IM. Distinct roles of IkappaB proteins in regulating constitutive NF-kappaB activity. Nat Cell Biol. 2005; 7: 921-923.
24. Kuwata H, Matsumoto M, Atarashi K, Morishita H, Hirotani T, Koga R, et al. IkappaBNS inhibits induction of a subset of Toll-like receptor-dependent genes and limits inflammation. Immunity. 2006; 24: 41-51.
25. Christy B, Nathans D. DNA binding site of the growth factor-inducible protein Zif268. Proc Natl Acad Sci U S A. 1989; 86: 8737-8741.
26. Beckmann AM, Wilce PA. Egr transcription factors in the nervous system. Neurochem Int. 1997; 31: 477-510.
27. Gómez-Martín D, Díaz-Zamudio M, Galindo-Campos M, Alcocer-Varela J. Early growth response transcription factors and the modulation of immune

response: implications towards autoimmunity. Autoimmun Rev. 2010; 9: 454-458.

28. Cao X, Mahendran R, Guy GR, Tan YH. Detection and characterization of cellular EGR-1 binding to its recognition site. J Biol Chem. 1993; 268: 16949-16957.

29. Dinkel A, Warnatz K, Ledermann B, Rolink A, Zipfel PF, Bürki K, et al. The transcription factor early growth response 1 (Egr-1) advances differentiation of pre-B and immature B cells. J Exp Med. 1998; 188: 2215-2224.

30. Li S, Miao T, Sebastian M, Bhullar P, Ghaffari E, Liu M, et al. The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells. Immunity. 2012; 37: 685-696.

31. Carow B, Rottenberg ME. SOCS3, a Major Regulator of Infection and Inflammation. Front Immunol. 2014; 5: 58.

Austin J Clin Immunol - Volume 1 Issue 4 - 2014
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Citation: Kawana N, Yamamoto Y, Kino Y and Jun-ichi Satoh. Molecular Network of NLRP3 Inflammasome Activation-Responsive Genes in a Human Monocyte Cell Line. Austin J Clin Immunol. 2014;1(4): 10.

2 ヒト腸内細菌叢のMetagenomics

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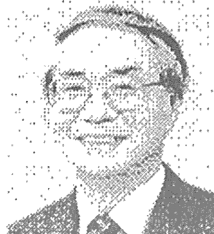

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2006年より現職
ヒトゲノム解読（21番染色体等）に貢献

Key words：メタゲノム，腸内細菌，次世代シークエンサー，16S遺伝子

Abstract

数百種類，数百兆個と見積もられる人体に生息する常在細菌群（ヒト常在菌叢）の研究は，近年における国際的な大型プロジェクトの推進，次世代シークエンサーの進歩，Metagenomics（メタゲノム解析）等を背景にしたゲノム科学的アプローチにより大きく前進した。とくに，ヒト腸内細菌叢の微生物生態と機能面での全体像が俯瞰され，さらに，病態と腸内細菌叢の異常（dysbiosis）との関係も明らかとなって来た。これらの知見は，ヒト常在菌叢が従来の想像を越えて，宿主の生体恒常性に深く関わることを示唆する。

はじめに

近代細菌学はパスツールやコッホの時代に体系化された。従って，人体やさまざまな環境中に生息する細菌群の研究では，その初期においてそこに生息する個々の細菌の分離培養とそれらの系統や細菌学的特性の解析が主に行われた（図1）。その後，1980年代にDNAを扱う分子生物学的手法が出現し，培養を介さず細菌叢を解析する方法が汎用されるようになった。その主な方法はPCR（ポリメラーゼ連鎖反応）を用いて，構成細菌種の16S rRNA（16S）遺伝子を細菌叢DNAから選択的に一括増幅し，それらの配列多様性から，細菌種の特定や系統関係を調べるものである。この培養を介さない方法は，多くの難培養性細菌（実験室で分離培養することが困難または不可能な細菌）の検出に有効であったが，それらの性質を知るにはそれらを分離培養する必要があった。この培養法とメタ16S解析法のジレンマを打破する手法としてメタゲノム（全構成細菌の集合ゲノム）解析が1998年に提唱された。開発当初では，メタゲノムは主に特定の遺伝子を狙い撃ち的に同定するためのリソースとして利用された。その後2004年に，細菌叢を網羅的（ランダム）にシークエンスして，そこに存在する遺伝子を情報学的に枚挙する方法が開発された。今日，前者を環境ゲノミクスまたは機能メタゲノミクス，後者をメタゲノミクスとよぶ（図1）。ヒト腸内細菌叢のメタゲノム解析は2006年と2007年に米国と日本のグループにより相次いで論文となった[1,2]。その後，次世代シークエンサー（NGS：Next Generation Sequencers）の実用化にともない，大量データに基づいたヒト腸内細菌叢のメタゲノム研究が大きく前進した（表）。本稿では，ヒト腸内細菌叢のメタゲノム解析の現状といくつかの知見を紹介する。

Metagenomics of the human gut microbiome：
Masahira Hattori, Center for Omics and Bioinformatics, Graduate School of Frontier Science, The University of Tokyo

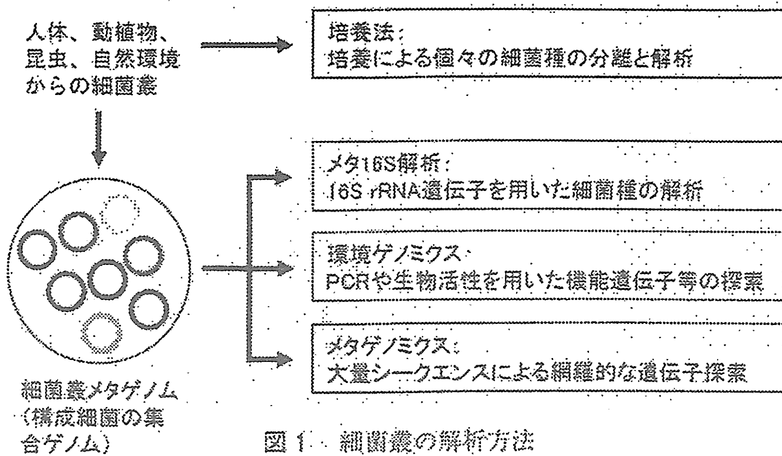

図1　細菌叢の解析方法

表　主なメタゲノム解析プロジェクトの現状

被験者国	被験者数	用いたシークエンサーまたはシークエンス法	同定されたユニーク遺伝子数（M：百万）	発表年	文献
米国	2	サンガー法	0.05M	2006	1
日本	13	サンガー法	0.8M	2007	2
国際コンソーシアムIHMCの発足				2008	—
スペイン	39	HiSeq	3.3M	2010	5
デンマーク(1)	85	HiSeq			
デンマーク(2)	207	HiSeq	?	2013	13
中国	368	HiSeq	4.3M	2012	7
米国	90	HiSeq	4.9M	2012	6
ベネズエラ（南米）・マラウィ（アフリカ）	15	454	?	2012	12
アイルランド	27	HiSeq	2.5M	2012	8
スウェーデン	145	HiSeq	6.0M	2013	9
ロシア	95	SOLiD	?	2013	10
日本	100	454/MiSeq/Ion PGM	4.6M	—	未発表

1．NGSを用いた腸内細菌叢解析技術

今日汎用されているNGSを用いたヒト腸内細菌叢の解析法の全体概略を図2に示す。大きく3つの解析が行われ，それらは①16S遺伝子データを元にしたメタ16S解析，②メタゲノム解析，③ヒトから分離された個々の細菌株のゲノム解析である。このほか，被験者の年齢やBMI，既往症，日頃の食事内容等のさまざまなメタデータも収集されている。メタ16Sデータからは細菌種の特定や菌種組成等の細菌データが得られる（詳細は後述）。メタゲノム解析からは上述した通り，遺伝子情報を元にした細菌叢の機能データが得られる（詳細は後述）。ヒト分離株のゲノムデータはメタゲノム及びメタ16Sデータの菌種帰属や菌種組成等の解析におけるリファレンスゲノムとして有用されている。これまでに6,000株以上が収集されており，データベースは日々アップデートされている（http://www.hmpdacc.org/）。

1-1．メタ16S解析

メタ16S解析の基本プロセスを図3に示す。16S遺伝子の可変領域（本稿ではV1-V2領域を示す）を共通プライマーでPCR増幅し，増えた16SアンプリコンをNGSに供して，約5000リード／サンプルの16S配列データを得る。ついで，配列類似度（本稿では96%類似度を閾値とした）を指標に16Sリードをクラスタリングし，配列が類似したリードをグループ化する。各グループは異なった16S配列，つまり異なった菌種ユニットを意味するのでOperational Taxonomic Unit（OTU）とよぶ。得られるOTU数は菌種数に近似され，細菌叢の多様性を調べる尺度になる。各OTUの16S配列データを既知菌種の16Sデータベース及びゲノムデータベースに相同検索することで，各

OTU は配列類似度に依存して既知菌種あるいはもっとも近縁の既知菌種に帰属される。また，各 OTU を構成する 16S リード数は細菌叢でのそれぞれの相対的な割合とみなせる。つまり，これら一連の解析から細菌叢を構成する菌種名とそれらの組成を知ることができる。このほか，各 OTU の 16S 配列から細菌叢を構成する細菌種の系統関係（系統樹）も知ることができる。この系統樹を構成する菌種及び存在量を比較することで異なった細菌叢間の類似性を数値化でき，この解析法を UniFrac- 距離及び主座標分析（PCoA）と言う[3]。UniFrac 解析は，例えば，疾患患者群の腸内細菌叢の異常（dysbiosis）を健常者群との比較から評価することに有用される（図４）。

メタ 16S 解析はメタゲノム解析よりもコストが安価であり，NGS を用いることで十分量の配列データの取得と 100 サンプルのような多サンプルの同時解析（数日内）も可能である。また，皮膚細菌叢のような微量の細菌叢 DNA にも対応できる。一方，メタ 16S 解析には PCR 増幅プロセスがあり，その増幅バイアスによる定量性の欠如を否めないが，それを軽減したヒト腸内細菌叢のメタ 16S 解析法も開発されている[4]。

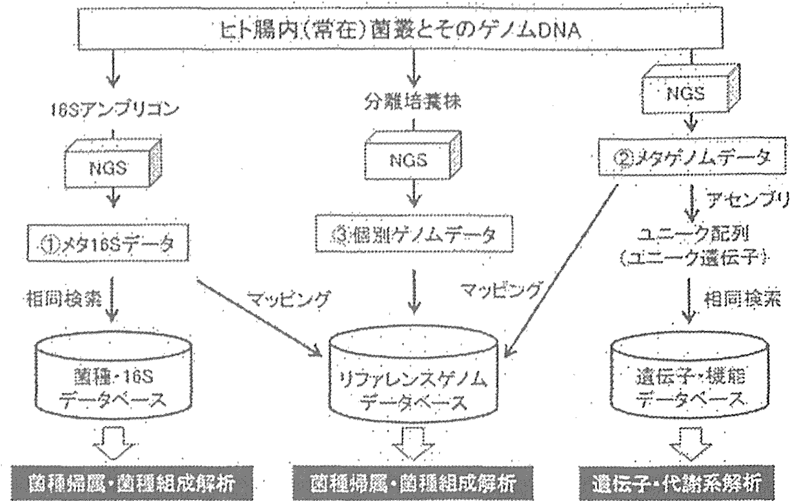

図２　NGS を用いた腸内（常在）細菌叢解析法の全体概略

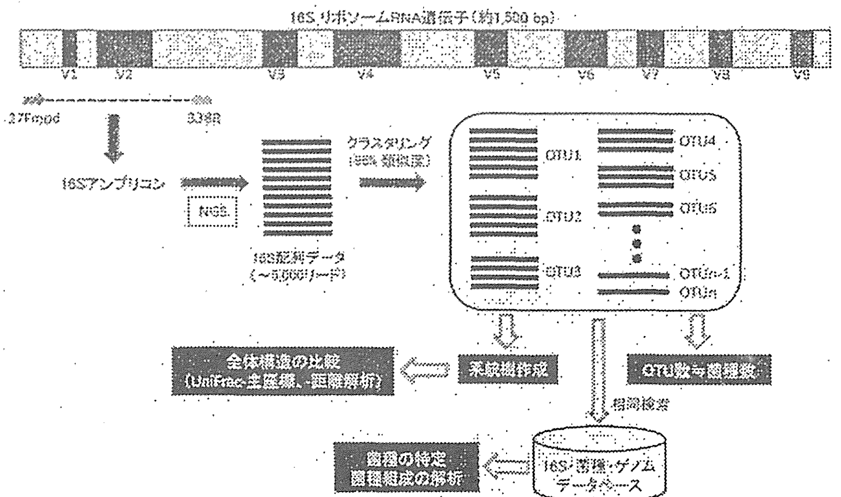

図３　16S リボソーム RNA 遺伝子配列をベースとした細菌叢解析

1-2. メタゲノム解析

メタゲノム解析は細菌叢メタゲノムの断片配列データを大量に収集し，その配列データから遺伝子等の機能情報を情報学的（バイオインフォマティクス）に解析する手法である。基本的な操作は，NGS から得られる大量のメタゲノムリードをアセンブリして非重複（ユニーク）ゲノム配列データ（コンティグとシングルトン）を取得する。ついで，その配列中に遺伝子予測プログラムを用いて遺伝子配列を同定する。得られた遺伝子配列をクラスタリングして高い

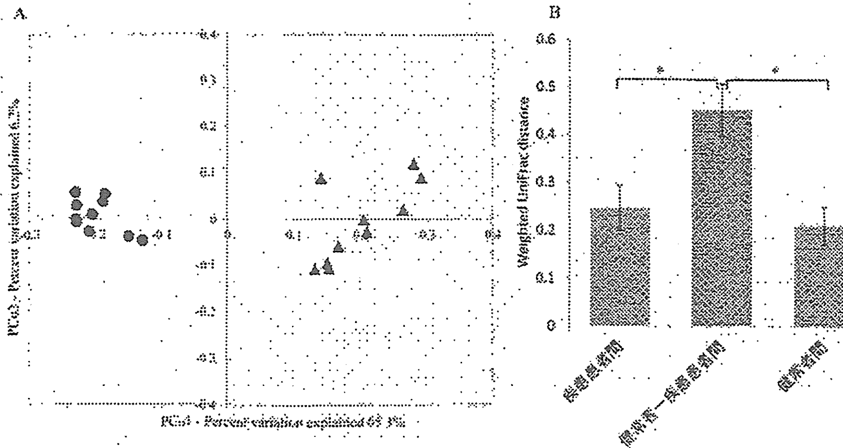

図 4　腸内細菌叢の UniFrac 解析例

(A) UniFrac 距離に基づく PCoA 解析。健常者群の各個人の腸内細菌叢（●）とある疾患患者群の各個人の腸内細菌叢（▲）がそれぞれ異なるクラスターを形成しており，両群は異なった構造の細菌叢を持つことを示す。

(B) 平均 UniFrac 距離値。健常者群－疾患患者群間の UniFrac 距離の値が健常者群内および疾患患者群内と比較して有意に高く，両群の細菌叢が有意に異なることを示す。エラーバーは標準誤差を示す。
* は t test における統計学的な有意さを示す($p<0.01$)。

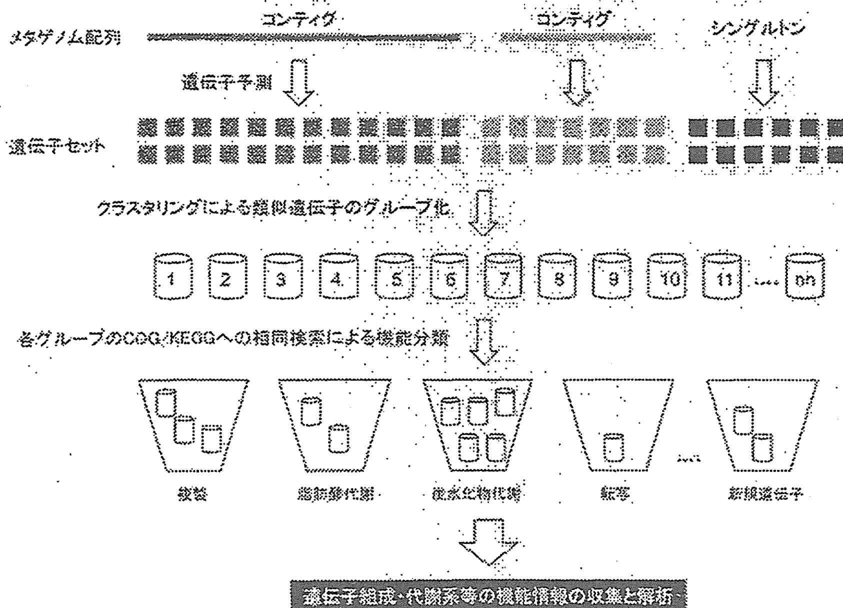

図 5　ヒト腸内（常在）細菌叢メタゲノムデータの情報学的解析プロセス

配列類似度をもつ遺伝子群をグループ化する。ついで，それらを COG (Clusters of Orthologous Groups) や KEGG (Kyoto Encyclopedia of Genes and Genomes) 等の機能既知遺伝子のデータベースに相同検索することで，各グループあるいはそれを構成する各遺伝子を機能分類し，細菌叢がもつ代謝系等の機能特性を明らかにする（図5）。

メタゲノム解析では，対象とする細菌叢の複雑さや多様性に依存して，取得する配列データ量を考慮する必要がある。少ないデータ量から検出される遺伝子の多くは優占菌種に由来することになり，細菌叢全体の機能特性を正確に評価することはできない。幸い NGS を用いることにより，今日では，検体あたり数百万リード以上（塩基数にして数億塩基以上）という大量のデータ取得も可能であり，1つのプロジェクトで 100 名程度のヒト腸内細菌叢から数百万のユニーク遺伝子が同定されている（表参照）。

メタゲノム解析では，メタゲノムリードを上述したリファレンスゲノムに直接マッピング（相同検索，配列類似度閾値：≥95%）することで，各リードの菌種帰属と各ゲノムにマップされるリード数から菌種組成を見積もることができる（図2）。本方法はそのプロセスに PCR 操作がなく，メタ 16S 解析（図3）よりもより定量性の高い細菌叢解析法となる。メタゲノムデータから得られた菌種組成や遺伝子組成データを主成分分析や階層式クラスタリング等の統計手法を駆使することで異なった細菌叢間の相違を調べることもできる。なお，今日のヒト腸内細菌叢のマッピングでは，メタゲノムリードの約 80% がリファレンスゲノムにマップされる。

しかし，このマッピング法はリファレンスゲノムが十分に収集されていないマウスや他の環境の細菌叢にはあまり有効ではない。

2．ヒト腸内細菌叢メタゲノム研究の現状

我が国では，2005年に筆者らが中心となったHuman MetaGenome Consortium Japan（HMGJ）が発足し，同年パリでヒトマイクロバイオーム研究の最初の国際会議が開催された。そして，2006年に米国グループが，2007年にHMGJがヒト腸内細菌叢のメタゲノム解析を世界に先駆けて論文発表した[1,2]。これらの先駆的論文では，腸内細菌叢がヒト代謝系を補完する多くの代謝系を有することや腸内細菌叢に特徴的な機能遺伝子の特定等が行われた。その後，2008年に日米欧中などからなるInternational Human Microbiome Consortium（IHMC）が設立され，それと同時に，米国NIHのHuman Microbiome Project（HMP），フランスを中心とした欧州連合（EU）＋中国BGI（Beijing Genomics Institute）のMetagenomics of the Human Intestinal Tract（MetaHIT）Projectが開始された。これらのプロジェクトではNGSを駆使し，100名規模でのメタゲノム解析が進められた。2010年にMetaHITが124名のスペイン人とデンマーク人の腸内細菌叢メタゲノムを発表した[5]。2012年にはHMPによるアメリカ人の腸内や皮膚，口腔等の18部位の常在菌叢[6]，MetaHIT/BGIによる345名の中国人[7]，178名のアイルランド人[8]の腸内細菌叢がそれぞれ論文となった。さらに，2013年には145名のスウェーデン人[9]，96名のロシア人[10]の腸内細菌叢が発表された（表）。筆者らも100名以上の日本人腸内細菌叢メタゲノムデータの解析を進めている（未発表）。これらの1,000名を越える被験者の腸内細菌叢からは1,000万以上のユニーク遺伝子が同定されており，この数はヒト遺伝子数（～2.5万）をはるかに凌駕する。これらの研究では，健常者腸内細菌叢の基本的な全体構造[5,6,11]，年齢や地域あるいは食習慣等による構造の違い[8,12]，くわえて，2型糖尿病や肥満等の疾患患者の腸内細菌叢の解析が行われた[5,7,9,13]。今日，腸内細菌叢の異常（dysbiosis）が消化器系だけでなく代謝系や神経系を含む全身的な疾患と関係することが明らかになって来ている[14]。

米国HMPの第2期（2013～）では，早産の経験者を含む妊婦の常在菌叢と宿主（母子）の特性，炎症性腸疾患（IBD）の腸内細菌叢と宿主の特性，前糖尿病状態から2型糖尿病への移行期にある患者の腸内細菌叢と宿主の特性について，ヒト多型，メタゲノム，メタトランスクリプトーム，メタボローム等の宿主データの収集も含めた研究が進められている[15]。

3．ヒト腸内細菌叢の全体像

メタゲノム解析及びメタ16S解析からヒト常在菌叢の全体像がきわめて正確に明らかになって来た。構成細菌種の大部分は4つの門（Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria）の菌種で占められるが，その相対的な組成は個人間や生息部位，年齢によって高い多様性を示す[6,11]。例えば，Firmicutes門は腸内の最優占菌種であり，Actinobacteria門やProteobacteria門は口腔や皮膚，鼻腔でその組成比が高くなる。マイナー菌種であるFusobacteria門は口腔細菌叢で相対的に多くなる。TM7門のような口腔内でしか検出できない菌種もいる。個人間の多様性はこれら菌種の有無や組成比の違いに起因する。例えば，124名の欧州人の腸内細菌叢メタゲノム解析では，124名全員に共通した菌種はわずか18菌種であった[5]。すなわち，常在菌叢にはヒトゲノムのような血縁同士の高い遺伝性はほとん

どなく，きわめて個人に特異的である。一方，ヒト腸内細菌叢は，Bacteroides 属，Prevotella 属，Ruminococcus 属の 3 属がそれぞれのタイプで優占菌種となる 3 つのエンテロタイプに分類できる[11]。エンテロタイプは人種や地域に関係なく，一様に分布していると考えられているが，最近の筆者らの解析から，日本人の多くは Ruminococcus タイプであり，欧米人の多くは Bacteroides タイプであることが分かった。なお，Prevotella タイプはベネズエラやアフリカの原住民に多い。エンテロタイプは地域や食習慣によって，その分布状態が偏っているらしい（筆者ら，未発表）。

ヒト腸内細菌叢の遺伝子数については，上述したように，1,000 万以上のユニーク遺伝子が検出されている。興味あることに，腸内細菌叢の菌種組成は各個人間で大きく異なるが（上述），遺伝子（機能）組成はほとんど個人間で差がない[16]。この事実は，各菌種がもつ遺伝子組成が腸内細菌叢の形成に大きく関係することを示唆している。上述した 4 門の優占菌種は，とくに人体での生息に適した遺伝子（機能）を獲得し，長い進化の中で選択されてきた菌種と考えられる。

ヒト腸内細菌叢を特徴づける遺伝子（機能）は，豊富な炭水化物代謝系の機能群である[1,2]。このことから，腸内細菌の主なエネルギー源は宿主が消化できない植物由来の多糖類であると考えられる。また，その代謝産物は酢酸や酪酸，ビタミンなどのヒト細胞に有用なものである。つまり，ヒトと腸内細菌は相互扶助的な関係にある。もうひとつの特徴は，腸内細菌叢には鞭毛や化学走性などの細胞運動に関わる遺伝子群がきわめて少ないことである[2]。腸内ではその蠕動運動のために自ら餌に向かって移動する必要がなく，宿主免疫のターゲットとなる鞭毛を持つ多くの病原菌との識別等，これらを持たない細菌種の選択と優占化は，常在菌叢が生体恒常性の維持に密接に関係することを示唆する。

おわりに

上述したように常在菌叢研究の国際化と NGS の実用化により，ヒト腸内細菌叢の構造実態と機能に関する多くの知見がこの 5 年間に蓄積された。また，疾患患者の腸内細菌叢解析から，消化管だけでなく，全身的な疾患にも腸内細菌叢の dysbiosis が密接に関係することが明らかになった[15]。今後は，腸内細菌叢の dysbiosis に関わる外的内的要因の解明及びそのヒトへの作用機構を多面的に解明することがきわめて重要な課題になると考えられる。

文　献

1) Gill SR, Pop M, Deboy RT, *et al* : Science 312: 1355-1359, 2006.
2) Kurokawa K, Itoh T, Kuwahara T, *et al* : DNA Res. 14: 169-181, 2007.
3) Hamady M, Lozupone C, Knight R: ISME J. 4: 17-27, 2010.
4) Kim SW, Suda W, Kim S, *et al* : DNA Res. 20: 241-253, 2013.
5) Qin J, Li R, Raes J, *et al* : Nature 464: 59-65, 2010.
6) Human Microbiome Project Consortium: Nature 486: 207-214, 2012.
7) Qin J , Li Y, Cai Z, *et al* : Nature 490: 55-60, 2012.
8) Claesson MJ, Jeffery IB, Conde S, *et al* : Nature 488: 178-184, 2012.
9) Karlsson FH, Tremaroli V, Nookaew I, *et al*: Nature 498: 99-103, 2013.
10) Tyakht AV, Kostryukova ES, Popenko AS, *et al*: Nat Commun. 4: 2469, 2013.
11) Arumugam M, Raes J, Pelletier E, *et al* : Nature 473: 174-180, 2011.
12) Yatsunenko T, Rey FE, Manary MJ, *et al* : Nature 486: 222-227, 2012.
13) Le Chatelier E, Nielsen T, Qin J, *et al* : Nature 500: 541-546, 2013.
14) Clemente JC, Ursell LK, Parfrey LW, Knight R.: Cell 148: 1258-1270 (2012).
15) Proctor LM: 米国 NIH HMP (Human Microbiome Project) の概要．大野博司，服部正平（編）「常在細菌叢が操るヒトの健康と疾患」実験医学増刊 32 (5) 38-40 (2014).
16) Turnbaugh PJ, Hamady M, Yatsunenko T, *et al* : Nature 457: 480-484, 2009.

ヒト腸内細菌マイクロバイオームの特徴

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Summary
近年におけるメタゲノム解析法の開発，国際プロジェクトの進展や次世代シークエンサー（NGS）の進歩などにより，ヒト腸内細菌叢研究は体系だったマイクロバイオーム研究としてこの5～6年間で世界的に大きく前進した．これらの研究から，ヒト腸内細菌叢の全体構造やさまざまな疾患との関連，また，宿主に作用する機能菌種の特定やその作用機構が明らかになってきた．これらの成果から，腸内細菌叢がこれまでの想像を超えて，宿主のさまざまな生理状態に密接に関与し，腸にとどまらず免疫系，代謝系，あるいは神経系などの全身にかかわることがわかってきた．

Key words
メタゲノム　腸内細菌　次世代シークエンサー（NGS）

はじめに

ヒトマイクロバイオーム（ヒト常在菌叢を構成する細菌種の集合ゲノム）を包括的に解析する計画は，わが国では，2005年にわれわれが中心となって設立されたHuman MetaGenome Consortium Japan（HMGJ）の発足にはじまる．同年パリでヒトマイクロバイオーム研究の最初の国際会議が日米欧などからの研究者が集まって開催された．そして，2006年に米国グループ[1]が，2007年にわが国のHMGJ[2]がヒト腸内細菌叢のメタゲノム解析を世界に先駆けて論文発表した．これらの研究によって腸内細菌叢が有する遺伝子や代謝系（＝機能）などが同定され，ヒト腸内マイクロバイオームの機能特性がはじめて明らかにされた．ついで，2008年には日米欧中などの研究者からなるInternational Human Microbiome Consortium（IHMC）が設立された[3]．それと同時に，米国のHuman Microbiome Project（HMP），欧州連合（European Union：EU）と中国Beijing Genomics Institute（BGI）のMetagenomics of the Human Intestinal Tract（MetaHIT）プロジェクトが開始された．HMPは口腔や皮膚，腸内などの全身の常在菌叢，MetaHITプロジェクトは腸内細菌叢に特化したプロジェクトである．本稿では，この5～6年間で世界規模に進められたヒト腸内細菌叢研究を解説する．

1 ヒト腸内細菌叢研究の全体概要

今日のIHMCが進めているヒト常在菌叢研究

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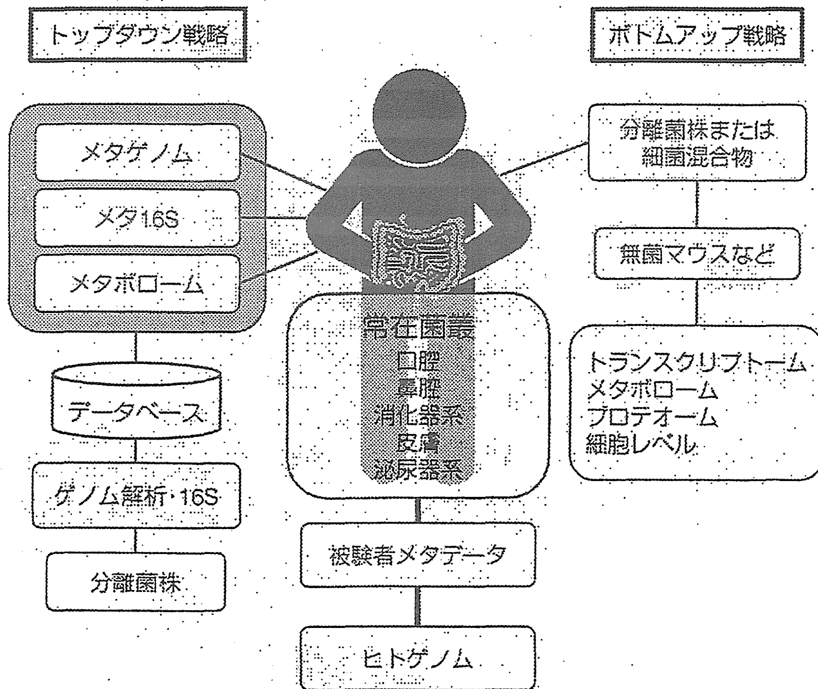

図 1．ヒト常在菌叢研究の全体概要

の全体概要を図1に示す．ヒトを対象とした研究では，健常および疾患患者からの常在菌叢（腸内細菌叢の多くは糞便から調製）のメタゲノム，16S ribosomal RNA（16S）遺伝子，代謝物（メタボローム）を収集・解析する．また，ヒトのさまざまな部位から分離培養できたヒト常在菌株のゲノム解析もおこなう．このほか，被験者の遺伝的背景（多型情報など）や食習慣，年齢や body mass index（BMI），既往症などのさまざまな宿主メタデータの収集も細菌叢データを解釈するうえできわめて大事である．

このような細菌叢のメタ 16S やメタゲノムデータの収集・体系化，疾患細菌叢の研究（後述）が進む一方で，宿主に作用する常在菌種の探索・特定や宿主-常在菌間相互作用のメカニズムの解明に関する研究も活発になってきた．たとえば，ヒト腸内細菌叢の優占菌種の一つである *Bifidobacterium* による大腸菌 O157 感染死の防御機構の解明[4]，大腸癌と関連する *Fusobacterium* の特定[5,6]，T 細胞の分化にかかわる *Bacteroides*[7]，マウスのセグメント細菌（segmented filamentous bacteria：SFB）[8]，ヒト *Clostridium*[9]，プロバイオティック *Clostridium* 株[10] などの同定，より最近では，T 細胞の分化にかかわる常在菌由来の酪酸の同定[11] などがある．これらの研究では，おもにマウスなどのモデル動物を利用し，宿主の細胞や遺伝子レベルなどのさまざまなデータを統合したオーミクスデータによるアプローチによるものが多い．このような生物学的実験から機能菌種を探索・特定する研究はボトムアップ戦略であり，上述した多数の細菌叢データを情報学や統計学で解析する研究はデータ駆動型のトップダウン戦略である．この二つの戦略が両輪となって常在菌叢研究は今後もさらに高度化され，想像を超えた常在菌（叢）の機能が明らかにされると見込まれる．

2 次世代シークエンサーを用いた腸内細菌叢解析

近年における次世代シークエンサー（Next Generation Sequencers：NGS）の進歩は，従来と