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Comprehensive analysis of human microRNA target networks

Jun-ichi Satoh* and Hiroko Tabunoki

* Correspondence: satoj@my-pharm.ac.jp
Department of Bioinformatics and Molecular Neuropathology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan

Abstract

Background: MicroRNAs (miRNAs) mediate posttranscriptional regulation of protein-coding genes by binding to the 3' untranslated region of target mRNAs, leading to translational inhibition, mRNA destabilization or degradation, depending on the degree of sequence complementarity. In general, a single miRNA concurrently downregulates hundreds of target mRNAs. Thus, miRNAs play a key role in fine-tuning of diverse cellular functions, such as development, differentiation, proliferation, apoptosis and metabolism. However, it remains to be fully elucidated whether a set of miRNA target genes regulated by an individual miRNA in the whole human microRNAome generally constitute the biological network of functionally-associated molecules or simply reflect a random set of functionally-independent genes.

Methods: The complete set of human miRNAs was downloaded from miRBase Release 16. We explored target genes of individual miRNA by using the Diana-microT 3.0 target prediction program, and selected the genes with the miTG score ≧ 20 as the set of highly reliable targets. Then, Entrez Gene IDs of miRNA target genes were uploaded onto KeyMolnet, a tool for analyzing molecular interactions on the comprehensive knowledgebase by the neighboring network-search algorithm. The generated network, compared side by side with human canonical networks of the KeyMolnet library, composed of 430 pathways, 885 diseases, and 208 pathological events, enabled us to identify the canonical network with the most significant relevance to the extracted network.

Results: Among 1,223 human miRNAs examined, Diana-microT 3.0 predicted reliable targets from 273 miRNAs. Among them, KeyMolnet successfully extracted molecular networks from 232 miRNAs. The most relevant pathway is transcriptional regulation by transcription factors RB/E2F, the disease is adult T cell lymphoma/leukemia, and the pathological event is cancer.

Conclusion: The predicted targets derived from approximately 20% of all human miRNAs constructed biologically meaningful molecular networks, supporting the view that a set of miRNA targets regulated by a single miRNA generally constitute the biological network of functionally-associated molecules in human cells.

Introduction

MicroRNAs (miRNAs) are a class of endogenous small noncoding RNAs conserved through the evolution. They mediate posttranscriptional regulation of protein-coding genes by binding to the 3' untranslated region (3'UTR) of target mRNAs, leading to translational inhibition, mRNA destabilization or degradation, depending on the degree of sequence complementarity [1]. During the biogenesis of miRNAs, the primary miRNAs (pri-miRNAs) are transcribed from the intra- and inter-genetic regions of the genome by RNA polymerase II, followed by processing by the RNase III enzyme Drosha into pre-miRNAs. After nuclear export, they are cleaved by the RNase III enzyme Dicer into mature miRNAs consisting of approximately 22 nucleotides. Finally, a single-stranded miRNA is loaded onto the RNA-induced silencing complex (RISC), where the seed sequence located at positions 2 to 8 from the 5' end of the miRNA plays a pivotal role in recognition of the target mRNA [2]. At present, more than one thousand of human miRNAs are registered in miRBase Release 16 http://www.mirbase.org. The 3'UTR of a single mRNA is often targeted by several different miRNAs, while a single miRNA concurrently reduces the production of hundreds of target proteins [3]. Consequently, the whole miRNA system (microRNAome) regulate greater than 60% of all protein-coding genes in a human cell [4]. By targeting multiple transcripts and affecting expression of numerous proteins, miRNAs play a key role in fine-tuning of diverse cellular functions, such as development, differentiation, proliferation, apoptosis and metabolism. Therefore, aberrant regulation of miRNA expression is deeply involved in pathological events that mediate cancers [5] and neurodegenerative disorders [6].

Recent advances in systems biology have made major breakthroughs by illustrating the cell-wide map of complex molecular interactions with the aid of the literature-based knowledgebase of molecular pathways [7]. The logically arranged molecular networks construct the whole system characterized by robustness, which maintains the proper function of the system in the face of genetic and environmental perturbations [8]. In the scale-free molecular network, targeted disruption of limited numbers of critical components designated hubs, on which the biologically important molecular interactions concentrate, efficiently disturbs the whole cellular function by destabilizing the network [9]. Therefore, the identification of the hub in the molecular network constructed by target genes of a particular miRNA helps us to understand biological and pathological roles of individual miRNAs. Recently, Hsu et al. studied the human microRNA-regulated protein-protein interaction (PPI) network by utilizing the Human Protein Reference Database (HPRD) and the miRNA target prediction program TargetScan [10]. They found that an individual miRNA often targets the hub gene of the PPI network, although they did not attempt to characterize relevant pathways, diseases, and pathological events regulated by miRNA target genes.

At present, the question remains to be fully elucidated whether a set of miRNA target genes regulated by an individual miRNA in the whole human microRNAome generally constitute the biological network of functionally-associated molecules or simply reflect a random set of functionally-independent genes. To address this question, we attempted to characterize molecular networks of target genes of all human miRNAs by using KeyMolnet, a bioinformatics tool for analyzing molecular interactions on the comprehensive knowledgebase.

Materials and methods

MicroRNA Target Prediction

The complete list of 1,223 human miRNAs was downloaded from miRBase Release 16 http://www.mirbase.org. We searched the target genes of individual miRNA on the Diana-microT 3.0 target prediction program (diana.cslab.ece.ntua.gr/microT), which was selected because of the highest ratio of correctly predicted targets over other prediction tools [11]. Diana-microT 3.0 calculates the miRNA-targeted gene (miTG) score that reflects the weighted sum of the scores of all conserved and non-conserved miRNA recognition elements (MRE) on the 3'UTR of the target mRNA. The miTG score correlates well with fold changes in suppression of protein expression [11]. To optimize the parameter of miRNA-target interaction, we considered the target genes with a cutoff of the miTG score equal to or larger than 20 as the highly reliable targets, because we found that the targets with the miTG score < 20 exhibited the significantly lower precision score, an indicator of correctness in predicted interactions [11], compared with those having the score ≧ 20 ($p = 2.78E-08$ by Mann-Whitney's U-test).

Molecular Network Analysis

Ensembl Gene IDs of target genes retrieved by Diana-microT 3.0 were converted into the corresponding Entrez Gene IDs by using the DAVID Bioinformatics Resources 6.7 program http://david.abcc.ncifcrf.gov[12], where non-annotated IDs were deleted. Then, Entrez Gene IDs of miRNA target genes were uploaded onto KeyMolnet.

KeyMolnet is a tool for analyzing molecular interactions on the literature-based knowledgebase that contains the contents on 123,000 molecular relationships among human genes and proteins, small molecules, diseases, pathways and drugs, established by the Institute of Medicinal Molecular Design (IMMD) (Tokyo, Japan) [13-15]. The core contents are collected from selected review articles and textbooks with the highest reliability, regularly updated and carefully curated by a team of expert biologists. KeyMolnet contains a panel of human canonical networks constructed by core contents in the KeyMolnet library. They represent the gold standard of the networks, composed of 430 pathways, 885 diseases, and 208 pathological events. Detailed information on all the contents is available from IMMD http://www.immd.co.jp/en/keymolnet/index.html upon request.

We utilized the neighboring network-search algorithm that selects the set of miRNA target genes as starting points to generate the network around starting points within one path, composed of all kinds of molecular interactions, including direct activation/inactivation, transcriptional activation/repression, and the complex formation. By uploading the list of Entrez Gene IDs onto KeyMolnet, it automatically provides corresponding molecules and a minimum set of intervening molecules as a node on networks. The generated network was compared side by side with human canonical networks described above. The algorithm that counts the number of overlapping molecules and/or molecular relations between the extracted network and the canonical network identifies the canonical network showing the most statistically significant contribution to the extracted network. This algorithm is essentially based on that of the GO::TermFinder [16]. The significance in the similarity between the extracted network and the canonical network is scored following the formula, where O = the number of overlapping molecules and molecular relations for the pathway or overlapping molecules alone for the disease and the pathological event between the extracted network and the canonical network, V = the number of molecules

and/or molecular relations located in the extracted network, C = the number of molecules and/or molecular relations located in the canonical network, T = the number of total molecules and/or molecular relations of KeyMolnet, currently composed of approximately 15,700 molecules and 123,000 molecular relations, and the × = the sigma variable that defines coincidence.

$$\text{Score} = -\log_2(\text{Score (p)}) \qquad \text{Score (p)} = \sum_{x=O}^{Min(C,V)} f(x) \qquad f(x) = {}_{C}C_{x} \cdot {}_{T-C}C_{V-x}/{}_{T}C_{V} \quad (1)$$

Results

Molecular Network of MicroRNA Target Genes

Among 1,223 human miRNAs examined, Diana-microT 3.0 predicted the targets from 532 miRNAs (43.5%). Among the 532 miRNAs, 273 miRNAs contained a set of highly reliable targets showing the miTG score ≧ 20. Among 273 miRNAs having reliable targets, KeyMolnet successfully extracted molecular networks from 232 miRNAs. They are comprised of 19% of total human miRNAs (microRNAome). Then, the generated network was compared side by side with human canonical networks of the KeyMolnet library, composed of 430 pathways, 885 diseases, and 208 pathological events. We found that not all 232 miRNAs contained entire categories of canonical networks because several miRNAs comprised relatively small numbers of targets. See Additional file 1 for all the information on 232 miRNAs and their target networks. When top three pathways, diseases, and pathological events were individually totalized, the most relevant pathway is 'transcriptional regulation by RB/E2F' (n = 39; 6.8% of total), followed by 'TGF-beta family signaling pathway' (n = 32; 5.6%) and 'transcriptional regulation by POU domain factor' (n = 24; 4.2%), the most relevant disease is 'adult T cell lymphoma/leukemia' (n = 68; 12.1%), followed by 'chronic myelogenous leukemia' (n = 65; 11.5%) and 'hepatocellular carcinoma' (n = 51; 9.1%), and the most relevant pathological event is 'cancer' (n = 97; 24.7%), followed by 'adipogenesis' (n = 46; 11.7%) and 'metastasis' (n = 36; 9.2%) (Figure 1 and Additional file 1).

Next, we identified the large-scale miRNA target networks by uploading targets greater than 100 per individual miRNA onto KeyMolnet (Table 1). Fifty-two miRNAs that construct such a large-scale miRNA target network include let-7, miR-9, 17, 19, 20, 26, 27, 29, 30, 32, 92, 93, 96, 98, 101, 106b, 124, 137, 147, 153, 218, 372, 429, 495, 506, 519, 520, 603, and their closely-related family members. The targets of these miRNAs established highly complex molecular networks, in which the pathways of 'transcriptional regulation by RB/E2F', 'transcriptional regulation by Ets-domain family', and 'transcriptional regulation by p53', the diseases of 'chronic myelogenous leukemia' and 'viral myocarditis', and the pathological event of 'cancer' were notably accumulated (Table 1). Importantly, distinct members belonging to the same miRNA family, for example, five miR-30 family members ranging from miR-30a to miR-30e constructed a virtually identical molecular network (Table 1).

Biological Implications of MicroRNA Target Networks

As described above, the present observations indicated that a set of miRNA target genes regulated by an individual miRNA generally constitute the biological network of

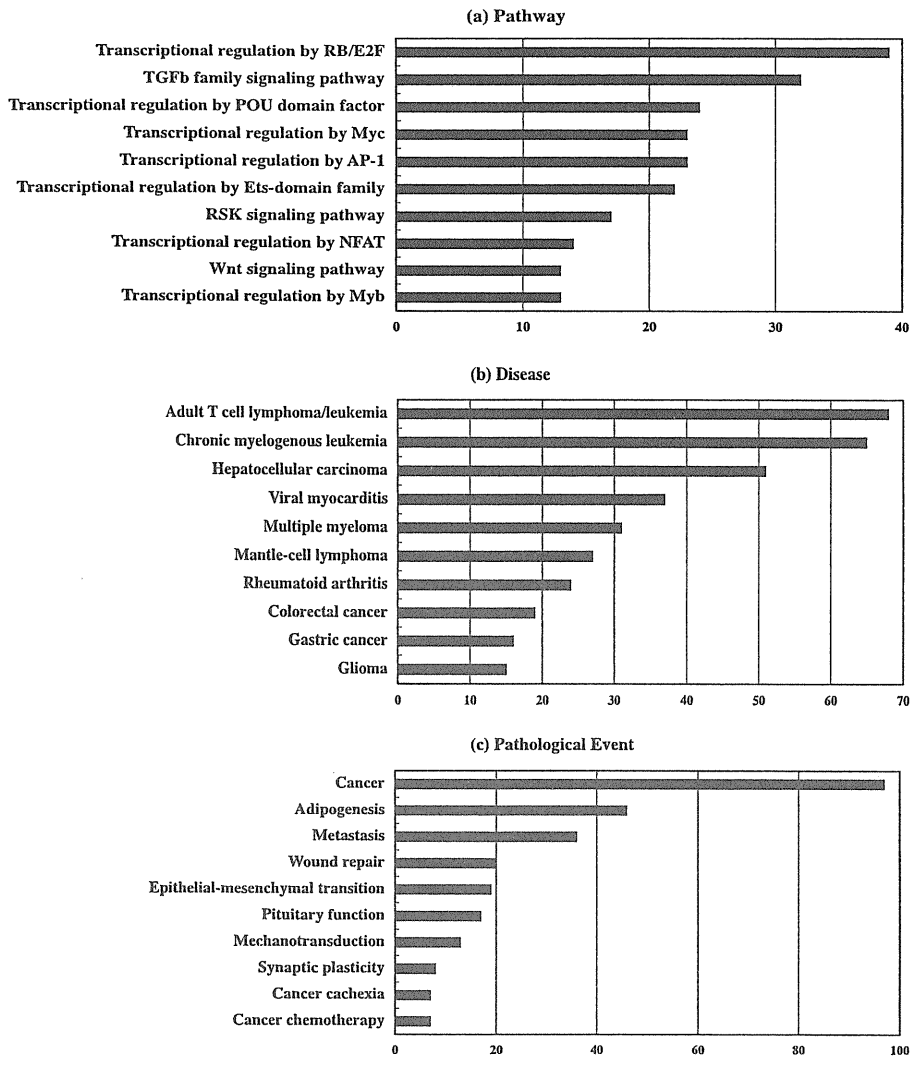

Figure 1 The pathways, diseases, and pathological events relevant to 232 miRNA target networks. Among 1,223 human miRNAs examined, Diana-microT 3.0 identified the set of reliable targets from 273 miRNAs. Among them, KeyMolnet extracted molecular networks from 232 miRNAs. The generated network was compared side by side with human canonical networks of the KeyMolnet library, composed of 430 pathways, 885 diseases, and 208 pathological events to identify the canonical network showing the most statistically significant contribution to the extracted network (see Table S1 for all the information). After top three pathways, diseases, and pathological events were individually totalized, the cumulated numbers of top 10 of **(a)** pathway, **(b)** disease, and **(c)** pathological event categories are expressed as a bar graph.

functionally-associated molecules in human cells. Therefore, it is highly important to obtain deeper insights into biological implications of miRNA target networks.

The protooncogene c-myb is a key transcription factor for normal development of hematopoietic cells. A recent study showed that miR-15a targets c-myb, while c-myb binds to the promoter of miR-15a, providing an autoregulatory feedback loop in human hematopoietic cells [17]. Consistent with this study, we found 'transcriptional regulation by myb' as the most relevant pathway to the miR-15a target network (the score = 602; the score p-value = 7.39E-182) (Figure 2 and Additional file 1). These observations propose a scenario that miR-15a synchronously downregulates both c-myb itself and downstream genes transcriptionally regulated by c-myb, resulting in

Table 1 The large-scale human microRNA target networks

MicroRNA	Number of Targets	Molecules in KeyMolnet Networks	Top Pathway	Score	p-Value	Top Disease	Score	p-Value	Top Pathological Event	Score	p-Value
hsa-let-7a	244	1022	Transcriptional regulation by p53	593	2.69E-179	Viral myocarditis	113	1.21E-34	Cancer	206	1.31E-62
hsa-let-7b	242	1016	Transcriptional regulation by p53	594	1.83E-179	Viral myocarditis	113	9.32E-35	Cancer	206	7.66E-63
hsa-let-7c	243	1020	Transcriptional regulation by p53	593	2.49E-179	Viral myocarditis	113	1.11E-34	Cancer	206	1.10E-62
hsa-let-7d	145	885	Transcriptional regulation by RB/E2F	836	2.18E-252	Chronic myelogenous leukemia	72	1.95E-22	Cancer	130	9.68E-40
hsa-let-7e	236	1111	Transcriptional regulation by p53	575	8.90E-174	Viral myocarditis	116	1.20E-35	Cancer	175	1.86E-53
hsa-let-7f	244	1022	Transcriptional regulation by p53	593	2.69E-179	Viral myocarditis	113	1.21E-34	Cancer	206	1.31E-62
hsa-let-7g	245	1022	Transcriptional regulation by p53	593	2.69E-179	Viral myocarditis	113	1.21E-34	Cancer	206	1.31E-62
hsa-let-7i	245	1022	Transcriptional regulation by p53	593	2.69E-179	Viral myocarditis	113	1.21E-34	Cancer	206	1.31E-62
hsa-miR-9	352	1115	Transcriptional regulation by PPARa	340	5.28E-103	Hepatocellular carcinoma	72	1.69E-22	Cancer	171	3.50E-52
hsa-miR-17	195	961	Transcriptional regulation by RB/E2F	971	3.27E-293	Chronic myelogenous leukemia	92	2.83E-28	Cancer	181	3.58E-55
hsa-miR-19a	226	1094	Transcriptional regulation by RB/E2F	760	2.10E-229	Chronic myelogenous leukemia	113	1.26E-34	Cancer	253	7.04E-77
hsa-miR-19b	225	1094	Transcriptional regulation by RB/E2F	760	2.10E-229	Chronic myelogenous leukemia	113	1.26E-34	Cancer	253	7.04E-77
hsa-miR-20a	165	1038	Transcriptional regulation by RB/E2F	856	1.64E-258	Chronic myelogenous leukemia	87	6.09E-27	Cancer	85	3.33E-26
hsa-miR-20b	198	981	Transcriptional regulation by RB/E2F	962	2.35E-290	Chronic myelogenous leukemia	98	3.39E-30	Cancer	183	6.98E-56
hsa-miR-26a	148	672	Transcriptional regulation by RB/E2F	919	1.76E-277	Chronic myelogenous leukemia	107	6.15E-33	Cancer	181	3.20E-55
hsa-miR-26b	148	672	Transcriptional regulation by RB/E2F	919	1.76E-277	Chronic myelogenous leukemia	107	6.15E-33	Cancer	181	3.20E-55
hsa-miR-27a	229	1192	Transcriptional regulation by CREB	1022	2.23E-308	Chronic myelogenous leukemia	95	1.96E-29	Cancer	194	3.05E-59
hsa-miR-27b	261	1337	Transcriptional regulation by CREB	1022	2.23E-308	Chronic myelogenous leukemia	94	4.51E-29	Cancer	211	4.11E-64
hsa-miR-29a	119	543	Transcriptional regulation by Ets-domain family	430	4.36E-130	Glioma	85	3.46E-26	Cancer	139	1.41E-42

Table 1 The large-scale human microRNA target networks *(Continued)*

hsa-miR-29b	118	578	Transcriptional regulation by Ets-domain family	422	1.15E-127	Glioma	82	1.55E-25	Cancer	146	1.44E-44
hsa-miR-29c	118	543	Transcriptional regulation by Ets-domain family	430	4.36E-130	Glioma	85	3.46E-26	Cancer	139	1.41E-42
hsa-miR-30a	455	1494	Transcriptional regulation by RB/E2F	777	9.43E-235	Chronic myelogenous leukemia	86	1.11E-26	Cancer	195	2.39E-59
hsa-miR-30b	455	1480	Transcriptional regulation by RB/E2F	781	1.08E-235	Chronic myelogenous leukemia	87	7.01E-27	Cancer	188	1.92E-57
hsa-miR-30c	454	1495	Transcriptional regulation by RB/E2F	778	6.13E-235	Chronic myelogenous leukemia	86	1.15E-26	Cancer	191	3.63E-58
hsa-miR-30d	452	1491	Transcriptional regulation by RB/E2F	778	7.28E-235	Chronic myelogenous leukemia	86	1.01E-26	Cancer	195	1.96E-59
hsa-miR-30e	455	1481	Transcriptional regulation by RB/E2F	780	1.29E-235	Chronic myelogenous leukemia	87	7.25E-27	Cancer	188	2.05E-57
hsa-miR-32	261	905	Transcriptional regulation by RB/E2F	842	2.74E-254	Gastric cancer	80	8.85E-25	Cancer	157	4.19E-48
hsa-miR-92a	219	642	Transcriptional regulation by MEF2	335	1.51E-101	Viral myocarditis	59	1.62E-18	Epithelial-mesenchymal transition	83	7.76E-26
hsa-miR-92b	258	701	Transcriptional regulation by MEF2	328	1.59E-99	Viral myocarditis	60	1.23E-18	Cancer	94	3.97E-29
hsa-miR-93	195	958	Transcriptional regulation by RB/E2F	972	2.37E-293	Chronic myelogenous leukemia	92	2.47E-28	Cancer	181	2.77E-55
hsa-miR-96	142	688	Transcriptional regulation by Ets-domain family	407	3.42E-123	Viral myocarditis	36	1.06E-11	Cancer	106	1.37E-32
hsa-miR-98	162	671	Transcriptional regulation by Myb	549	4.73E-166	Viral myocarditis	85	2.66E-26	Cancer	126	1.42E-38
hsa-miR-101	188	806	Transcriptional regulation by AP-1	492	1.10E-148	Hepatocellular carcinoma	70	6.40E-22	Cancer	127	4.26E-39
hsa-miR-106b	164	1028	Transcriptional regulation by RB/E2F	854	7.21E-258	Chronic myelogenous leukemia	87	5.48E-27	Cancer	85	2.93E-26
hsa-miR-124	285	1346	Transcriptional regulation by RB/E2F	756	3.57E-228	Chronic myelogenous leukemia	83	9.34E-26	Cancer	185	1.90E-56
hsa-miR-137	288	941	Transcriptional regulation by MITF family	339	1.19E-102	Adult T cell lymphoma/ leukemia	66	1.30E-20	Cancer	179	1.00E-54
hsa-miR-147	199	867	Transcriptional regulation by RB/E2F	805	4.06E-243	Chronic myelogenous leukemia	113	6.60E-35	Cancer	132	2.57E-40
hsa-miR-153	154	1019	Transcriptional regulation by Myb	507	2.35E-153	Multiple myeloma	60	6.44E-19	Cancer	174	4.31E-53

Table 1 The large-scale human microRNA target networks *(Continued)*

hsa-miR-218	155	830	Transcriptional regulation by AP-1	344	2.28E-104	Hepatocellular carcinoma	69	1.63E-21	Cancer	136	1.52E-41
hsa-miR-372	101	562	Transcriptional regulation by RB/E2F	1022	2.23E-308	Chronic myelogenous leukemia	85	1.90E-26	Cancer	144	2.75E-44
hsa-miR-429	123	634	Transcriptional regulation by RB/E2F	918	2.45E-277	Chronic myelogenous leukemia	76	1.71E-23	Cancer	130	5.28E-40
hsa-miR-495	156	601	Transcriptional regulation by Ets-domain family	431	2.14E-130	Rheumatoid arthritis	77	5.90E-24	Adipogenesis	79	1.32E-24
hsa-miR-506	394	1536	Transcriptional regulation by Ets-domain family	317	4.69E-96	Viral myocarditis	99	1.73E-30	Cancer	172	1.43E-52
hsa-miR-519a	281	1256	Transcriptional regulation by RB/E2F	811	5.32E-245	Chronic myelogenous leukemia	106	1.34E-32	Cancer	220	8.03E-67
hsa-miR-519b-3p	281	1256	Transcriptional regulation by RB/E2F	811	5.32E-245	Chronic myelogenous leukemia	106	1.34E-32	Cancer	220	8.03E-67
hsa-miR-519c-3p	281	1256	Transcriptional regulation by RB/E2F	811	5.32E-245	Chronic myelogenous leukemia	106	1.34E-32	Cancer	220	8.03E-67
hsa-miR-520a-3p	184	690	Transcriptional regulation by RB/E2F	1022	2.23E-308	Chronic myelogenous leukemia	94	6.95E-29	Cancer	146	1.12E-44
hsa-miR-520b	182	690	Transcriptional regulation by RB/E2F	1022	2.23E-308	Chronic myelogenous leukemia	94	6.95E-29	Cancer	146	1.12E-44
hsa-miR-520c-3p	182	690	Transcriptional regulation by RB/E2F	1022	2.23E-308	Chronic myelogenous leukemia	93	9.28E-29	Cancer	145	1.77E-44
hsa-miR-520d-3p	183	690	Transcriptional regulation by RB/E2F	1022	2.23E-308	Chronic myelogenous leukemia	94	6.95E-29	Cancer	146	1.12E-44
hsa-miR-520e	184	690	Transcriptional regulation by RB/E2F	1022	2.23E-308	Chronic myelogenous leukemia	94	6.95E-29	Cancer	146	1.12E-44
hsa-miR-603	252	1150	Transcriptional regulation by Ets-domain family	344	3.26E-104	Multiple myeloma	84	4.36E-26	Cancer	161	4.24E-49

Among 1,223 human miRNAs examined, Diana-microT 3.0 predicted reliable targets from 273 miRNAs. Among them, KeyMolnet extracted molecular networks from 232 miRNAs. The generated network was compared side by side with human canonical networks of the KeyMolnet library, composed of 430 pathways, 885 diseases, and 208 pathological events. The canonical pathways, diseases, and pathological events with the most statistically significant contribution to the extracted network are shown. The table contains only the large-scale miRNA target networks generated by importing targets greater than 100 per individual miRNA into KeyMolnet. See Additional file 1 for all the information on 232 miRNAs and their target networks.

efficient inactivation of the whole molecular network governed by the hub gene c-myb. These results suggest a collaborative regulation of gene expression at both transcriptional and posttranscriptional levels that involve coordinated regulation by miRNAs and transcription factors.

The retinoblastoma protein Rb/E2F pathway acts as a gatekeeper for G1/S transition in the cell cycle. The Rb/E2F-regulated G1 checkpoint control is often disrupted in cancer cells. A recent study showed that miR-106b is directly involved in posttranscriptional regulation of E2F1 [18]. E2F1 activates transcription of miR-106b, while miR-

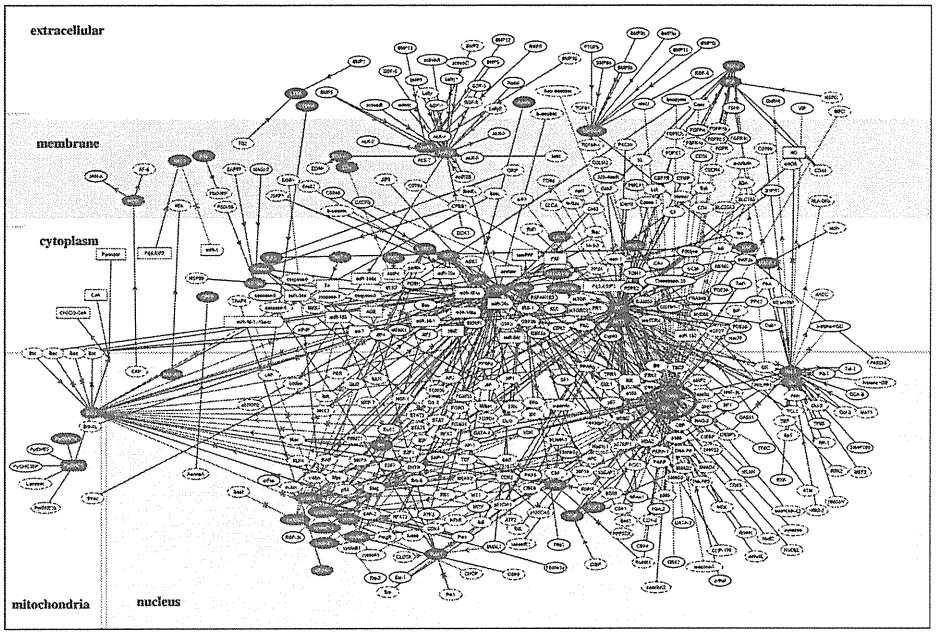

Figure 2 Molecular network of miR-15a targets. By the "neighboring" network-search algorithm, KeyMolnet illustrated a highly complex network of miR-15a targets that has the most statistically significant relationship with the pathway of 'transcriptional regulation by myb'. Red nodes represent miR-15a direct target molecules predicted by Diana-microT 3.0, whereas 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 transcription factor myb is highlighted by a blue circle.

106b targets E2F1, serving as a miRNA-directed negative feedback loop in gastric cancer cells [18]. Supporting these findings, we identified 'transcriptional regulation by Rb/E2F' as the most relevant pathway to the miR-106b target network (the score = 854; the score p-value = 7.21E-258) (Figure 3, Table 1 and Additional file 1). The relationship between miR-106b and Rb/E2F would provide another example of coordinated regulation of gene expression by miRNAs and transcription factors.

We found 'transcriptional regulation by p53' as the most relevant pathway to the target network of all let-7 family members except for let-7d (Table 1). It is worthy to note that the tumor suppressor p53 regulates the expression of components of the miRNA-processing machinery, such as Drosha, DGCR8, Dicer, and TARBP2, all of which have p53-reponsive elements in their promoters [19]. Furthermore, Dicer and TARBP2, along with p53, serve as a target of the let-7 family miRNAs, suggesting a close link between p53 and let-7 in miRNA biogenesis [19]. The expression of let-7 family members was greatly reduced in certain cancer cells [20].

The micropthalmia associated transcription factor (MITF), a basic helix-loop-helix zipper (bHLH-Zip) transcription factor, acts as not only a master regulator of melanocyte differentiation but also an oncogene promoting survival of melanoma. Recent studies indicate that MITF is a direct target of both miR-137 and miR-148b [21,22]. Again, we identified 'transcriptional regulation by MITF family' as the most relevant pathway to both miR-137 (the score = 339; the score p-value = 1.19E-102) and miR-

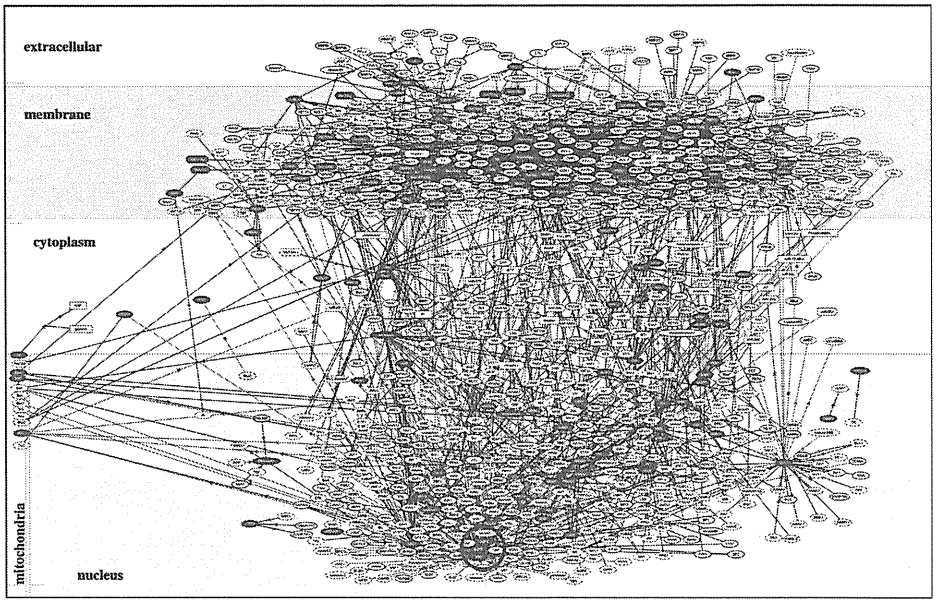

Figure 3 Molecular network of miR-106b targets. By the "neighboring" network-search algorithm, KeyMolnet illustrated a highly complex network of miR-106b targets that has the most statistically significant relationship with the pathway of 'transcriptional regulation by Rb/E2F'. Red nodes represent miR-106b direct target molecules predicted by Diana-microT 3.0, whereas 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 transcription factor E2F is highlighted by a blue circle.

148b (the score = 40; the score p-value = 3.91E-142) target networks (Table 1 and Additional file 1).

Cellular responsiveness to glucocorticoids (GCs) is regulated by the delicate balance of the glucocorticoid receptor (GR) protein, GR coactivators and corepressors, GR splice variants and isoforms, and regulators of GR retrograde transport to the nucleus. A recent study showed that miR-18a targets the GR protein, and thereby inhibits GR-mediated biological events in neuronal cells [23]. Consistent with this, we found 'transcriptional regulation by GR' as the most relevant pathway to the miR-18a target network (the score = 1022; the score p-value = 2.23E-308) (Additional file 1).

Zinc finger transcription factors ZEB1 and ZEB2 act as a transcriptional repressor of E-cadherin. A recent study showed that the expression of miR-200b, which targets both ZEB1 and ZEB2, was downregulated in the cells that undergo TGF-beta-induced epithelial to mesenchymal transition (EMT), and was lost in invasive breast cancer cells [24]. We identified 'transcriptional regulation by ZEB' as the third-rank significant pathway (the score = 155; the score p-value = 1.88E-47) and 'EMT' as the third-rank significant pathological event relevant to the miR-200b target network (the score = 61; the score p-value = 4.15E-19) (Additional file 1).

Discussion

In general, a single miRNA concurrently downregulates hundreds of target mRNAs by binding to the corresponding 3'UTR of mRNA via either perfect or imperfect sequence complementarity [3]. Such fuzzy mRNA-miRNA interactions result in the redundancy

of miRNA-recognized targets. By targeting multiple transcripts and affecting expression of numerous proteins at one time, miRNAs regulate a wide range of cellular functions, such as development, differentiation, proliferation, apoptosis and metabolism. Therefore, we have the question whether a set of miRNA target genes regulated by an individual miRNA generally constitute the biological network of functionally-associated molecules or simply reflect a random set of functionally-independent genes. If the former is the case, what kind of biological networks does the human microRNAome most actively regulates?

To address these questions, first we identified the set of credible target genes for all individual human miRNAs by using the Diana-microT 3.0 program. Then, we investigated miRNA target networks by applying them to KeyMolnet, a bioinformatics tool for analyzing molecular interactions on the comprehensive knowledgebase. Diana-microT 3.0 identified highly reliable targets from 273 miRNAs out of 1,223 all human miRNAs. Previous studies showed that the list of predicted targets for each miRNA varies among different miRNA target prediction programs armed with distinct algorithms, such as TargetScan 5.1 http://www.targetscan.org, PicTar (pictar.mdc-berlin.de), miRanda http://www.microrna.org and Diana-microT 3.0 [25]. Therefore, miRNA target networks are to some extent flexible, depending on the target prediction program employed. Among the programs described above, we have chosen Diana-microT 3.0 because of the highest ratio of correctly predicted targets over other prediction tools and the simplicity of setting a cut-off point for detection of reliable miRNA-target interactions based on the miTG score [11].

Here we found that highly reliable targets of substantial numbers of human miRNAs actually constructed biologically meaningful molecular networks. These observations strongly supported the theoretical view that miRNA target genes regulated by an individual miRNA in the whole human microRNAome generally constitute the biological network of functionally-associated molecules. A recent study showed that interacting proteins in the human PPI network tend to share restricted miRNA target-site types than random pairs, being consistent with our observations [26].

We also found that there exists a coordinated regulation of gene expression at the transcriptional level by transcription factors and at the posttranscriptional level by miRNAs in miRNA target networks. Recently, Cui et al. investigated the relationship between miRNA and transcription factors in gene regulation [27]. Importantly, they found that the genes with more transcription factor-binding sites have a higher probability of being targeted by miRNAs and have more miRNA-binding sites.

A recent study by miRNA expression profiling of thousands of human tissue samples revealed that diverse miRNAs constitute a complex network composed of coordinately regulated miRNA subnetworks in both normal and cancer tissues, and they are often disorganized in solid tumors and leukemias [28]. During carcinogenesis, various miRNAs play a central role, acting as either oncogenes named oncomir or tumor suppressors termed anti-oncomir, by targeting key molecules involved in apoptosis, cell cycle, cell adhesion and migration, chromosome stability, and DNA repair [5]. Many miRNA gene loci are clustered in cancer-associated genomic regions [29]. Furthermore, miRNA expression signatures well discriminate different types of cancers with distinct clinical prognoses [30]. In the present study, KeyMolnet analysis of miRNA target networks showed that the most relevant pathological event is 'cancer', when top three

pathological events were overall cumulated. Furthermore, the highly relevant diseases include 'adult T cell lymphoma/leukemia', 'chronic myelogenous leukemia', and 'hepatocellular carcinoma'. These observations suggest that the human microRNAome plays a more specialized role in regulation of oncogenesis. Therefore, the miRNA-based therapy directed to targeting multiple cancer-associated pathways simultaneously might serve as the most effective approach to suppressing the oncogenic potential of a wide range of cancers.

Conclusion

The reliable targets predicted by Diana microT 3.0 derived from approximately 20% of all human miRNAs constructed biologically meaningful molecular networks by KeyMolnet. These observations support the view that miRNA target genes regulated by an individual miRNA in the whole human microRNAome generally constitute the biological network of functionally-associated molecules. In the human miRNA target networks, the most relevant pathway is transcriptional regulation by transcription factors RB/E2F, the disease is adult T cell lymphoma/leukemia, and the pathological event is cancer. In miRNA target networks, there exists a coordinated regulation of gene expression at the transcriptional level by transcription factors and at the posttranscriptional level by miRNAs.

Additional material

Additional file 1: KeyMolnet identifies microRNA target networks in 232 human miRNAs. The prediction of target genes of individual miRNA was performed by Diana-microT 3.0. Entrez Gene IDs of miRNA target genes were uploaded onto KeyMolnet. The generated network was compared side by side with human canonical networks composed of 430 pathways, 885 diseases, and 208 pathological events of the KeyMolnet library. Top-three pathways, diseases, and pathological events with the statistically significant contribution to the extracted network are shown.

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Authors' contributions
JS designed the methods, analyzed the data, and drafted the manuscript. HT helped the data analysis. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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多発性硬化症・視神経脊髄炎に対する免疫療法

Immunotherapy for Multiple Sclerosis and Neuromyelitis Optica

荒浪利昌
Toshimasa Aranami

多発性硬化症（MS）は，中枢神経系に広く病変が多発し，髄鞘抗原特異的T細胞やB細胞が介在する自己免疫疾患であると考えられており，病原性細胞としてTh1細胞とともにTh17細胞が注目されている．一方，視神経と脊髄が冒される視神経脊髄炎（NMO）には，血中にアクアポリン4に対する自己抗体（抗AQP4抗体）が存在し，病態形成に関与すると考えられている．MS治療の中心であるIFN-βは，Th1細胞の働きが亢進している病態では効果が期待されるが，Th17細胞が優位な状況では無効である可能性がある．NMOに対する新たな免疫療法として，抗CD20抗体などによる液性免疫を標的とする治療の有効性が示唆されている．

key words　多発性硬化症，視神経脊髄炎，免疫療法

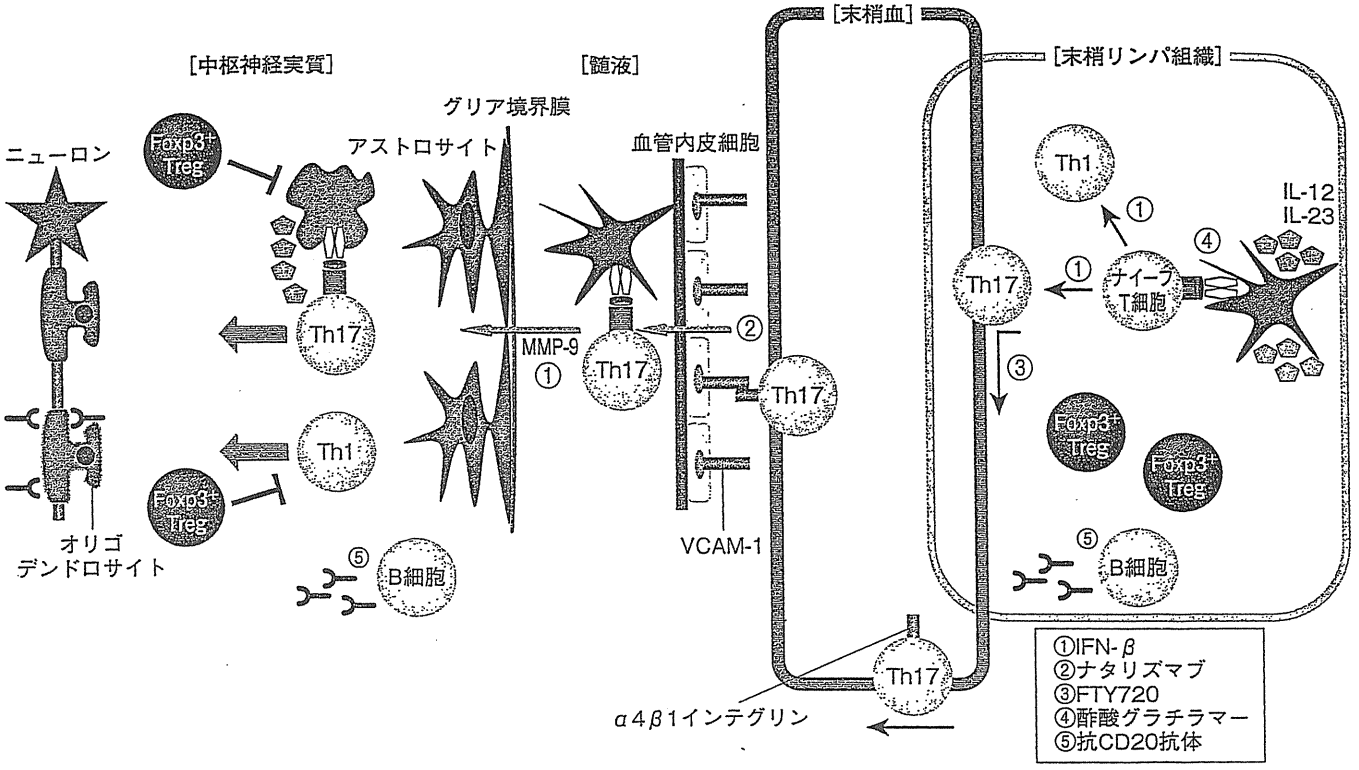
はじめに

多発性硬化症（multiple sclerosis；MS）は，髄鞘抗原特異的なT細胞やB細胞が髄鞘を傷害することにより脱髄病変が生じる中枢神経系の自己免疫疾患であると考えられている．病変は中枢神経系に広く分布し，再発・寛解を繰り返し，視力低下や運動麻痺などの症状が長期にわたる．一方，視神経脊髄炎（neuromyelitis optica；NMO）は，病変が比較的視神経と脊髄に限局している．以前はMSの一病型と考えられていたが，患者末梢血中にアクアポリン4（AQP4）という水チャネル分子に対する自己抗体（抗AQP4抗体）が存在することが発見されて以来[1]，MSとは異なる病態であるとの考えが優勢である．本稿では，MSとNMOの免疫病態およびそれぞれに対する免疫療法の進歩を概説する．

Ⅰ　MSおよびNMOの病態

ヘルパーT（Th）細胞は活性化とともに，特定のサイトカイン産生細胞へと分化するが，従来より自己免疫疾患においてはTh1細胞の過剰な働きが関与するとの考えが優勢であった．しかし近年，インターロイキン（IL）-17を産生するT細胞（Th17細胞）が新たなTh細胞分画として提唱され，MSやその動物モデルである実験的自己免疫性脳脊髄炎（experimental autoimmune encephalomyelitis；EAE）への関与が示唆されている[2]．当初，Th17細胞は，Th1細胞とは異なるT細胞分画であり，EAE病態においてTh1細胞よりも高い病原性を有する分画として提唱されたが，近年IL-17を産生しているT細胞が，後にIFN-γ産生細胞へと変化すること，いわゆる可塑性（plasticity）を有することが示唆されている[3]．また，MSの末梢血や髄液においてもIL-17産生細胞の増加が報告されている[4]．その一方で，Th1およびTh17細胞の分化あるいは病原性の獲得を抑制することが期待されたIL-12とIL-23の中和抗体は，乾癬には有効であったが，MSにおいては再発抑制効果を認めなかった[5]．以上から，MS病態におけるTh1細胞およびTh17細胞の重要性については，いまだ結論は得られていない．また，MS病態においては，$CD4^+Foxp3^+$制御性T細胞（Treg）などのTreg機能の減弱も報告されており[6]，病原性細胞とTregのバランスにより，再発寛解型MSの病態が形成される可能性がある．

一方，NMOの免疫病態の特徴の1つは抗AQP4抗体の存在である．AQP4分子は中枢神経系グリア細胞アストロサイトの足突起などに特に豊富に存在している．NMO患者血清はアストロサイトを補体依存性に傷害し，EAEを誘導したマウスにNMO患者IgGを投与したところ，NMOに酷似した病理像が得られ，臨床症状も増悪したことから，抗AQP4抗体はNMO病態マーカーと言うだけでなく，病態形成に深く関わっているという考え方が有力である．当研究部の千原らは，NMOとMS，健常者の末梢血B細胞分画の頻度を比較し，他の2群と比べ，NMOにおいて形質芽細胞（プラズマブラスト）が有意に増加していることを見いだした．

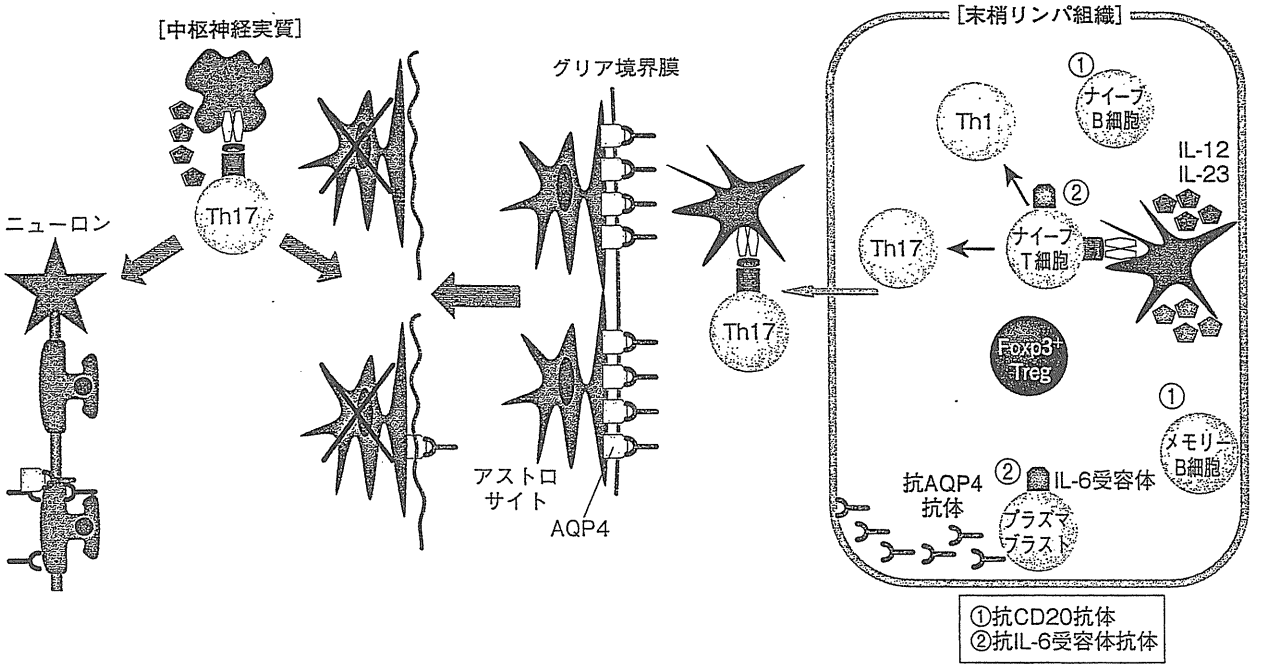


■図1 MS病態と免疫療法
MS病態への関与が示唆される病原性細胞およびTregと免疫療法の想定される作用点(①～⑤)を示す.

さらに,プラズマブラストが末梢血中の主たる抗AQP4抗体産生細胞であり,プラズマブラストの生存および抗AQP4抗体産生にIL-6が重要である可能性を示した[7].また,脳病変がほとんどなく,脊髄と視神経のみが選択的に冒されるMSが本邦を含むアジア諸国に多く,以前はOSMS(opticospinal MS)と呼ばれていたが,OSMSの30～60%は抗AQP4抗体陽性であることが明らかとなり,その病態形成機構はNMOと類似していると考えられている.そのOSMSの髄液においてはIL-17, IL-8, G-CSF(granulocyte colony-stimulating factor)といったIL-17ファミリーとそれにより誘導されるサイトカインの増加が認められる[8].また,NMOの髄液ではマウスのTh17細胞分化に重要なサイトカインであるIL-6も増加している.これらの報告から,NMOの病態形成に液性免疫とともにTh17細胞やIL-6が関与している可能性が示唆される.

Ⅱ MSとNMOの免疫療法

MSおよびNMOの急性増悪時の治療法としては,メチルプレドニゾロンなどのステロイド大量点滴静注療法(パルス療法)や血漿交換療法が用いられる.それとともに,MSおよびNMOの治療においては,再発を予防する治療が長期的な神経機能的予後への影響を考えるうえで最も重要である.IFN-βは再発寛解型MSの再発予防療法として最も広く用いられている.それとともにMS患者の10～50%がIFN-βノンレスポンダー(無効群)であると報告されている.IFN-βの効果発現機構として,Th1細胞やTh17細胞への分化の抑制,あるいは炎症性細胞浸潤に重要なMMP-9(matrix metalloproteinase-9)の抑制など,様々な効果が指摘されている.近年,MS病態におけるIFN-βの治療効果発現機構について新たな知見があった.Axtellらは,髄鞘抗原特異的Th1細胞で誘導したEAEにはIFN-βが有効であり,逆に髄鞘抗原特異的Th17細胞で誘導したEAEはIFN-β投与により症状の増悪が見られることを報告した.Th1細胞を移入して誘導したEAEでは,IFN-β投与により脾臓でのIFN-γ産生の減少と,抑制性サイトカインIL-10産生の著明な増加が認められた.このようなIL-10産生亢進はIFN-γノックアウトマウスでは観察されなかったことから,IFN-βが有効性を発揮するためにはIFN-γシグナルが必要と考えられ



■図2 NMO病態と免疫療法

NMO病態への関与が示唆される病原性細胞およびTregとステロイド以外の免疫療法の想定される作用点(①～②)を示す.

た. 一方, Th17介在性EAEでは, IFN-β投与により脾臓でのIL-17産生は低下するが, IL-10産生に変化がなく, 脊髄でIL-17産生細胞がむしろ増加していたことから, IFN-γ産生の亢進がなければIFN-βによるIL-10産生誘導が生じず, 治療効果が見られないと考えられた. さらに, IFN-βノンレスポンダー中には, 治療前の血清中IL-17FとIFN-β濃度がレスポンダー(有効群)と比べて高い一群が存在し, マウスの実験系と同じく, Th17に偏倚(へんい)している状態では, IFN-β治療が無効であることが示唆された[9]. このように, MSの病態にはTh1に偏倚している状態とTh17に偏倚している状態が存在する可能性がある. 前者にはIFN-β治療が有効であるが, 後者ではむしろ病態を悪化させてしまう可能性があり, 検討が必要である.

MSの再発抑制療法としては, その他にも各種免疫抑制剤のほか, MS病態に即した免疫修飾薬が欧米を中心に用いられている. (1) 中枢神経系への免疫細胞の遊走を抑制する薬剤. (2) 自己反応性T細胞による髄鞘抗原の認識を阻害する薬剤. (3) 生体よりB細胞を除去する薬剤, などである.

(1) としては, ナタリズマブやFTY720がある. ナタリズマブはヒト化抗α4インテグリンモノクローナル抗体であり, 免疫系細胞に広く発現するα4β1インテグリンとVCAM-1 (vascular cell adhesion molecule-1) という接着分子の結合を阻害する働きがあり, 中枢神経系への炎症性細胞浸潤を阻害する機能を有する. MSの再発率を有意に低下させ, 欧米で再発寛解型MSの治療薬として使用されている. FTY720は, リンパ節や二次リンパ組織からのリンパ球の流出に必要なシグナルを阻害する新規化合物である. リンパ節からのリンパ球の流出には, スフィンゴシン1リン酸(S1P)がリンパ球上のS1P受容体に結合することが必要だが, FTY720は体内でリン酸化された後, S1P1受容体にアゴニストとして結合する. これによりS1P1受容体の細胞内へのinternalizationを誘導し, 結果としてS1Pに結合できるS1P1受容体数が減少し, リンパ球はS1P不応性となる. その結果, T細胞はリンパ節に留められることになり, 神経組織に浸潤するリンパ球が減少し, 炎症も抑制されると考えられる[10].

(2) としては酢酸グラチラマー(copolymer-1)がある. これは髄鞘タンパク質の1つであるミエリン塩基性タンパク質(myelin basic protein ; MBP)と同じ頻度で混合された4種類のアミノ酸のランダムポリマーである. 酢酸グラチラマーはマウスおよびヒトの各種MHCクラスⅡ分子に高いアフィニティーで結合することにより, MBPなどの髄鞘抗原がクラスⅡ分子に結合することを競合的に阻害する.

(3) は抗CD20抗体であり, 生体よりB細胞を除去することで, やはりMSの再発率を有意に低下させることが示され

ている（図1）．

NMOの免疫療法に関して，MSとの違いはIFN-βに対する反応性である．NMO患者へのIFN-β治療において，かえって重症化した例が報告されたため，現在ではNMOと判定されれば，IFN-βは使用しない．IFN-βがNMOに対して無効である原因は不明であるが，IFN-β投与患者において，血清中BAFF（B cell-activating factor belonging to the TNF family）濃度が上昇することが報告されている．BAFFはB細胞生存や分化を促進する因子として重要なため，NMOにおいては抗AQP4抗体産生など液性免疫を刺激して，症状の悪化を引き起こす可能性が考えられる[11]．NMOの再発抑制療法としては，現在，経口ステロイド剤が中心であるが，MS同様，抗CD20抗体によるB細胞除去療法が有効であると報告されている．これは，NMO病態における液性免疫の重要性と符合する結果である．また，当研究部では現在，前記の研究結果に基づき，NMOに対する抗IL-6受容体抗体療法の臨床研究を計画している（図2）．

おわりに

MSにはIFN-βが有効な一群と無効な一群が存在し，後者にはTh17細胞やIFN-βが病態形成に関与している可能性がある．また，NMOにおいてもTh17細胞と液性免疫の重要性が示唆されており，やはりIFN-βは無効である可能性が高い．以上より，MS，NMOの免疫療法においては，個々の患者における病態機序を検討したうえで，治療法を選択することが重要であると考えられる．

PROFILE 荒浪利昌
■ 国立精神・神経医療研究センター 神経研究所
■ E-mail：aranami@ncnp.go.jp
1993年北海道大学医学部卒業．北海道大学大学院免疫科学研究所にて基礎免疫学を学ぶ．卒業後，国立精神・神経センター神経研究所免疫研究部研究員として多発性硬化症の病態解析研究に従事．2006年より同研究部室長．

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ナチュラルキラーT (natural killer T: NKT) 細胞の分化と機能
—前編—

岩渕　和也

北里大学医学部免疫学

NKT細胞は，主にCD1d拘束性に脂質抗原を認識するT細胞亜群であり，NKマーカーをも発現するために，呼称にNKが入る。通常のT細胞が，胸腺皮質上皮細胞に発現する主要組織適合抗原(MHC)＋ペプチド抗原による正の選択を経て生成するのに対し，NKT細胞は胸腺細胞上のCD1d＋脂質抗原による正の選択により生成する。骨髄由来細胞による選択の結果，NKT細胞は自然免疫系エフェクター様の迅速な機能発現を呈し，自然－獲得免疫間のギャップを橋渡しする。迅速・大量・多彩な液性因子産生で，炎症・免疫応答を調節し，関連疾患の進展や転帰にさまざまな影響を与え得る。本総説では，NKT細胞のユニークな特性と疾患制御の標的となる可能性について紹介したい。

Key words: 自然リンパ球，NKT細胞，CD1，胸腺内分化，脂質リガンド

はじめに

ナチュラルキラーT (NKT) 細胞は，ナチュラルキラー (NK) 細胞のマーカーを発現してはいるが，遺伝子再構成で生じたT細胞抗原受容体 (T-cell antigen receptor: TCR) を有するT細胞の一亜群である[1]。狭義には，CD1d拘束性に非ペプチド性 (多くはスフィンゴ／グリセロ糖脂質やリン脂質) 抗原を認識し，応答するT細胞である。主要組織適合抗原複合体 (major histocompatibility complex: MHC) 拘束性にペプチド抗原 (すなわちタンパク質) を認識するメインストリームT細胞 (以下，T細胞) とは認識抗原レパートリーに明確な差異があり，代替不可能である。「狭義には」と断ったのは，CD1dと同様MHC class I様分子であるMR1 (MHC-related protein 1) 拘束性の粘膜関連インバリアントT細胞 (mucosa-associated invariant T cell: MAIT)[2]もNKマーカーを発現する自然リンパ球であり，NKT細胞亜群として統合的に分類可能であるためである (Figure 1)。ただし，依然としてMAITとして独立のカテゴリーの細胞群として捉える場合も少なくないため，本稿では主にCD1d拘束性のNKT細胞のみを扱い，以下，単にNKT細胞と記す。

NKT細胞は，Vβ鎖としてVβ8を偏用するCD4$^-$8$^-$ (double negative: DN) 細胞[3]あるいは，CD4$^+$亜群[4]で，IL-4高産生性[5-7]，肝[8]など組織特異的に分布することが，マウスを用いた初期の研究で見出された。その後，

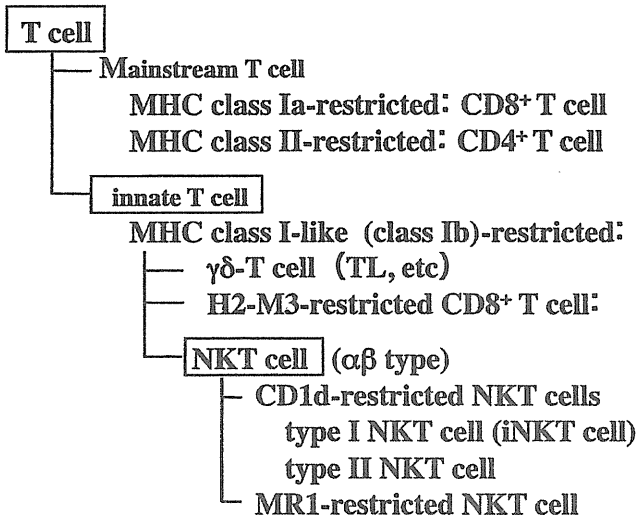

Figure 1. Two major subsets of T-lineage cells

T cells are basically classified as mainstream T cells and innate T cells. Mainstream T cells contain class I-restricted CD8$^+$ T cells and class II-restricted CD4$^+$ T cells. Innate T cells that are class Ib-restricted contain γδ - type T cells, H2-M3-restricted CD8$^+$ T cells, and NKT cells. NKTcells (mostly αβ type) contain CD1d-restricted type I (iNKT) and type II NKT cells, and MR1-restricted NKT cells as known as mucosa-associated invariant T (MAIT) cells.

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連絡先: 岩渕和也 (北里大学医学部免疫学)
〒252-0374 神奈川県相模原市南区北里1-15-1
E-mail: akimari@kitasato-u.ac.jp

NKT細胞の代表的なVα鎖としてVα14Jα281 (現在ではVα14 Jα18と呼ばれる) が明らかにされた[9-11]。すなわち，Vαが同一 (不変＝インバリアント) であり，Vβ鎖もVβ8 (あるいは7，2) に偏って使用されているので，大多数のNKT細胞は (セミ) インバリアントTCR (iTCR＝Vα14Jα18/Vβ8，7，2) を発現するT細胞 (αβ型) 亜群と考えてよい。このタイプのNKT (iNKT) 細胞は特にその後，後述する拘束分子，プロトタイプリガンド，検出・同定用の試薬としてマルチマーや単クローン抗体などが発見・開発された。これらを使用することで，そのユニークな特性や疾患との関連が次々と明らかにされ，現在では腫瘍・感染症・自己免疫疾患の治療への応用が期待されるに至っている[12]。

ただ，NKT細胞を扱う研究者以外には，未だ単に希少な細胞亜群に過ぎない。本稿ではNKT細胞の胸腺内選択や抗原認識，多彩な機能，各種疾患の発症や進展に対する役割，NKT細胞の特性を利用した細胞治療の可能性について前・後編に分けて述べ，認知度向上を図りたい。

1. NKT細胞の胸腺内分化と成熟

(1) 前駆細胞

T細胞亜群であるがユニークな特質を有するNKT細胞は，どのような分化経路を辿るのであろうか。現在大まかに2つの考え方がある。すなわち，T細胞と同じ過程を進み，ランダムな遺伝子再構成でiTCRを発現し，対応リガンドで選択されたものがNKT細胞になるという考え方 (セレクションモデル) と，特別な前駆細胞がT細胞分化の途中から分岐する別経路を通るとする考え方 (プレコミットメントモデル) である。NKT細胞分化に必須の遺伝子 (Runx1，RORγt) をCD4$^+$8$^+$ (double positive: DP) 特異的にノックアウト (KO) すると，NKT細胞が分化出来ないことは，ランダムに生じたDPプールから選択されてNKT細胞は生じることを示しており，セレクションモデルを支持している[13]。一方，DPよりさらに未熟な分画であるDN (CD4$^-$8$^-$/CD44$^-$25$^-$，lineage markers (Lin)$^-$分画であるDN4[14]や，さらに表現型としてはDN1様に見えるCD4$^-$8$^-$/CD44$^+$25$^-$/Lin$^-$亜分画) の中にNKT細胞を生ずる前駆細胞が検出出来るのは，プレコミットメントモデルを支持する結果である。両モデルが相互排除的であるのか，NKT細胞がいずれの経路からも生成し得るのか，興味が持たれている。

(2) 胸腺内分化と正の選択に関わる抗原

NKT細胞も，メインストリームT細胞と同様その生成に胸腺が必要である[15]。NKT細胞は通常CD4$^+$8$^-$ (およびCD4$^-$8$^-$) フェノタイプを有する細胞であるが，胸腺内選択は主にclass II MHC分子ではなく[16]，逆にclass I MHCの発現障害を来すβ$_2$-ミクログロブリン (β$_2$m) KOマウスで分化出来なくなることから[17]，セレクター (正の選択に関る抗原/抗原提示分子複合体－ポジティブセレクターを単にセレクターとした) はβ$_2$m会合性分子－すなわちclass I MHCあるいはclass Ib分子－であることがまず判明した。さらに，放射線骨髄キメラマウスの実験からβ$_2$m会合分子が，宿主側胸腺の放射線抵抗性

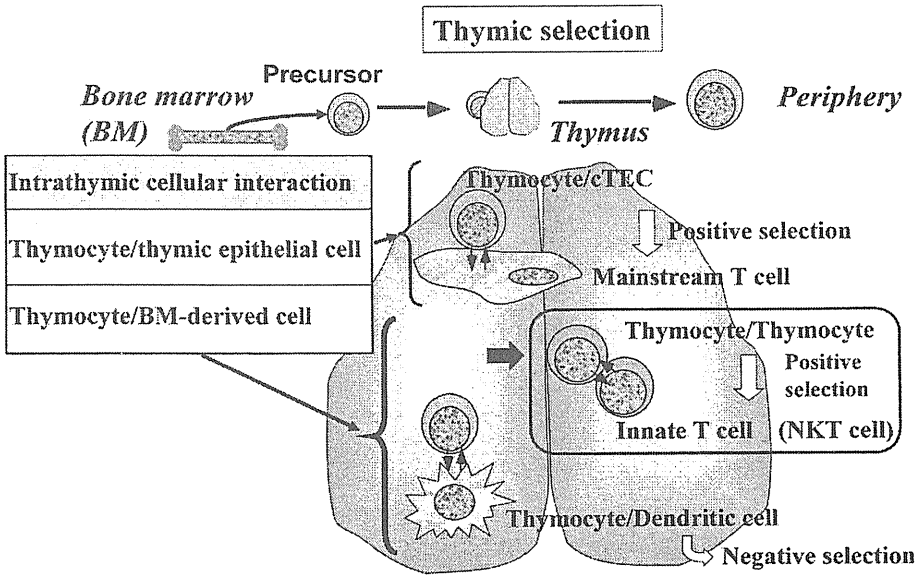


Figure 2. Intrathymic T cell selection

T cell precursors interact either with cortical thymic epithelial cells (cTEC) or bone marrow-derived cells, such as CD4$^+$8$^+$ (double positive; DP) thymocytes or dendritic cells in the thymus to be positively or negatively selected. Mainstream T cells are positively selected with cTEC and NKT cells are positively selected with DP thymocytes. Negative selection could basically be conveyed by any type of cells but efficiently be executed by dendritic cells.

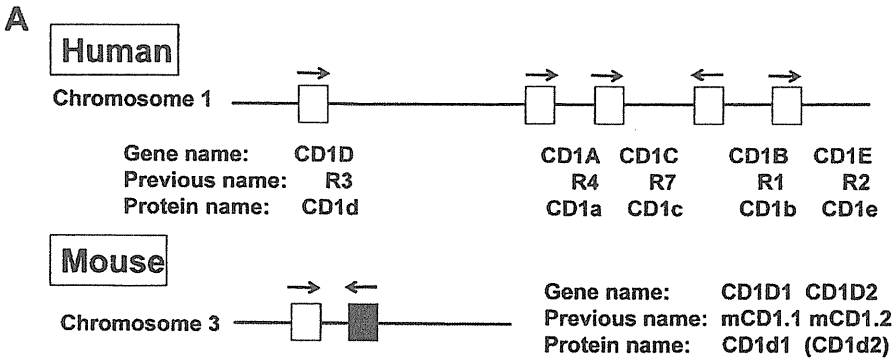
細胞にではなく，ドナー側にある時に正の選択を受けることが分かった (Figure 2)[18]。これはT細胞が，胸腺皮質上皮細胞上のclass I ($CD8^+$) あるいはclass II ($CD4^+$) MHC抗原により，正の選択を受け生成するという分化様式とは正反対で，特筆すべきことである。

その後，この骨髄由来細胞上に提示されるβ_2m会合分子はCD1dであり[19]，NKT細胞はCD1d拘束性に抗原を認識するT細胞であることが判明した。CD1dはCD1ファミリーに属する非古典的CD1分子である (Figure 3A)[20]。より正確を期するならば，DP胸腺細胞上のCD1d分子により選択される[21]。生成した$NK1.1^+$胸腺細胞もDPまで分化が進行する遺伝子改変マウス (TCRα KO) 胸腺細胞で刺激した場合はIL-2を産生するが，DP以前までしか分化が進行しないマウス胸腺細胞 (lck KO，TCRβ KO) との培養ではIL-2を産生しない。なお，CD1ファミリーの古典的CD1 (CD1a〜c) は，ヒトには存在するが，マウスでは欠損している。古典的CD1についても，各CD1拘束性T細胞が存在し，$\alpha\beta$型，$\gamma\delta$型の両T細胞を含むが，これらはNKT細胞と呼ばれない。

(3) セレクターリガンドとNKT細胞リガンド

NKT (前駆) 細胞は，CD1dにより正の選択を受け分化する際，セレクターであるCD1d上に提示されている何らかのリガンド (セレクターリガンドと記す) を同時に認識していることになる。このことは，CD1d上に脂質抗原を提示するための分子—microsomal triglyceride transfer protein (MTP)[22]，saposinをプロセッシングするcathepsin L[23]のKOでは，胸腺におけるNKT細胞分化が障害されることからもその存在が支持される。セレクターリガンドは未だ不明であるが，まず先に同定された成熟NKT細胞の活性化リガンド (以下単にリガンドとも) について簡単に述べる。セレクターリガンドとリガンドは必ずしも同一である必要はなく，T細胞では異なる (ペプチド) と考えられているが，NKT細胞でどのような関係にあるかは解明されていない。

成熟NKT細胞のリガンドは，抗腫瘍効果を指標に海洋天然物から探索されたKRN7000 (agelasphin: (2*S*, 3*S*, 4*R*)-1-*O*-(α-*D*-galactopyranosyl)-2-(*N*-hexacosanoylamino)-1, 3, 4- octadecanetriol = α-GalCer) がCD1dに結合し，NKT細胞を活性化出来る化合物として発見された[24] (Figure 4)。α-GalCerはそれ自体すぐれたリガンドであるが，リード化合物として，OCH (Th2型偏倚リガンド)[25]，α-*C*-GalCer (C-glycoside: マウスではTh1型偏倚リガンド)[26]，α-carba-GalCer (RCAI-56: Th1



B

CD1	human	Ligands/function	mouse
CD1a	+	Dermal wax (*Moody DB*), Dideoxymycobactin (DDM)	-
CD1b	+	Glucose monomycolate (GMM)	-
CD1c	+	Mannosyl phosphomycoketide	-
CD1d	+	Sphingo/glycero glycolipids･phospholipids	+
CD1e	+	Lipid chaperone	-

Figure 3. CD1 gene loci in human and mouse and the ligands for CD1 molecules

A. Gene loci of human and mouse CD1. Human CD1 loci contain three sets of classical CD1 molecules, CD1a, CD1b, and CD1c, and two sets of non-classical CD1 molecules, CD1d and CD1e.[20] Mouse possesses only CD1d molecules, CD1d1 and CD1d2. In B6 background, CD1d2 contains premature stop codon and is not expressed. In other strains, such as BALB/c, AKR, NOD, and 129, CD1d2 has (C→W) mutation in α2 domain that affects surface expression, suggesting that the function could be limited.
B. Ligands/function for CDI molecules.