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研究課題名(和文)眼炎症疾患発症におけるmiRNAの関与

研究課題名(英文)Roles of miRNA in the development of ocular inflammatory diseases

研究代表者

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研究成果の概要(和文)：BALB/cマウスをブタクサとアラムで全身感作し、10日後ブタクサを点眼することによりアレルギー性結膜炎を誘導した。ブタクサ点眼24時間後、結膜を採取し、RNAを抽出した。全身感作したマウスから、ブタクサを点眼する直前に採取した結膜を対照とした。microarray法により、miRNAの発現を比較した。その結果、結膜炎誘導によりmiRNAの発現倍率が1/2倍未満となった遺伝子は44個、発現倍率が2倍以上となったmiRNAが74個同定された。さらに、統合評価を行い、Lrp2 binding proteinやlactoperoxidaseなどの標的遺伝子を同定した。

研究成果の概要(英文)：Balb/c mice were immunized with ragweed in alum. Ten days later, the mice were challenged with ragweed in eye drops to induce allergic conjunctivitis. Twenty-four hours after the challenge, conjunctivas were harvested to extract RNA. As a control, conjunctivas were collected from immunized mice just prior to ragweed challenge. Expression of miRNA were compared between the two groups by using microarray analysis. Forty-four miRNAs were identified to be downregulated to be less than half by induction of conjunctivitis, while 74 miRNAs were identified to be upregulated to be more than 2-fold by induction of conjunctivitis. Combined evaluation identified the target genes of miRNAs such as Lrp2 binding protein and lactoperoxidase.

研究分野：医歯薬学

科研費の分科・細目：外科系臨床医学・眼科学

キーワード：眼炎症 アレルギー性結膜炎 マイクロRNA ocular inflammation allergic conjunctivitis miRNA

様式 C - 19、F - 19、Z - 19 (共通)

1. 研究開始当初の背景

ぶどう膜炎、アレルギー性結膜疾患などの眼炎症疾患の発症には免疫系細胞を中心に産生されるサイトカインが重要な役割を果たす。

サイトカイン産生は細胞内あるいは細胞外からのシグナルにより制御される。近年、細胞内に存在する長さ 20 から 25 塩基ほどの 1 本鎖ノンコーディング RNA (microRNA) がサイトカインをはじめ種々の遺伝子の発現を調節する機能を有することが明らかとなった。

2. 研究の目的

本研究の目的は、ぶどう膜炎、アレルギー性結膜疾患の発症に microRNA がどのように関与しているかを検討することである。

3. 研究の方法

ぶどう膜炎、アレルギー性結膜疾患の動物モデルにおける microRNA 発現の経時的変化を網膜ならびに結膜で評価する (microarray 法、定量的 RT-PCR(reverse transcription-polymerase chain reaction)法)。

4. 研究成果

(1) BALB/c マウスをブタクサとアラムで全身感作し、10 日後ブタクサを点眼することによりアレルギー性結膜炎を誘導した。ブタクサ点眼 24 時間後、アレルギー性結膜炎を誘導したマウスから結膜を採取し、RNA を抽出した(n=3)。全身感作したマウスから、ブタクサを点眼する直前に採取した結膜から抽出した RNA を対照とした(n=3)。microarray 法により、miRNA の発現を比較した。

(2) その結果、結膜炎誘導により miRNA の発現倍率が 1/2 倍未満となった遺伝子は 44 個、発現倍率が 2 倍以上となった miRNA が 7 4 個同定された。さらに、統合評価を行い、miRNA 発現変化にともない、発現の変動が予測される遺伝子を検索した。

(3) その結果、miRNA が増加し遺伝子発現を抑制したと考えられる遺伝子群 (Lrp2 binding protein, mediator of RNA polymerase II transcription, subunit 12 homolog (yeast)-like, immunoglobulin superfamily, member 1, potassium voltage gated channel, Shaw-related subfamily, member 3, zinc finger, CCHC domain containing 16 など) と、miRNA が減少し遺伝子発現が増加したと考えられる遺伝子群 (lactoperoxidase, aryl hydrocarbon receptor nuclear translocator 2, DnaJ (Hsp40) homolog, subfamily C, member 27, olfactory receptor 54, sarcoglycan zeta など) を同定した。

結膜炎の誘導により発現が減少した遺伝子と発現が上昇した miRNA 関連遺伝子における共通 44 遺伝子リスト

no	発現が減少した遺伝子名	結膜炎誘導後の減少倍率変化 (fold change)	発現が増加した miRNA	結膜炎誘導後の増加倍率変化 (fold change)
1	Lrp2 binding protein	4.975	mmu-miR-494	2.396
2	Lrp2 binding protein	4.975	mmu-miR-499	6.239
3	mediator of RNA polymerase II transcription, subunit 12 homolog (yeast)-like	4.587	mmu-miR-302c	6.922
4	immunoglobulin superfamily, member 1	4.196	mmu-miR-499	6.239
5	potassium voltage gated channel, Shaw-related subfamily, member 3	4.175	mmu-miR-760-3p	4.256
6	zinc finger, CCHC domain containing 16	4.145	mmu-miR-34c	6.107
7	ankyrin repeat and sterile alpha motif domain containing 1B	3.680	mmu-miR-494	2.396
8	methionine adenosyltransferase I, alpha	3.650	mmu-miR-760-3p	4.256
9	solute carrier family 1 (glial high affinity glutamate transporter), member 2	3.631	mmu-miR-494	2.396
10	solute carrier family 26, member 3	3.517	mmu-miR-494	2.396
11	kinesin family member 5A	3.465	mmu-miR-466j	29.948
12	nuclear receptor subfamily 1, group 1, member 2	3.379	mmu-miR-532-3p	4.280
13	synaptonemal complex protein 2	3.349	mmu-miR-302c	6.922
14	synaptonemal complex protein 2	3.349	mmu-miR-499	6.239
15	leucine-rich repeats and guanylate kinase domain containing	3.314	mmu-miR-466j	29.948
16	sulfatase modifying factor 2	3.281	mmu-miR-532-3p	4.280
17	cylicin, basic protein of sperm head cytoskeleton 2	3.250	mmu-miR-499	6.239
18	interleukin 20	3.180	mmu-miR-764-5p	34.150
19	maltase-glucoamylase	3.122	mmu-miR-302c	6.922
20	coiled-coil domain containing 67	3.102	mmu-miR-532-3p	4.280
21	CD8 antigen, alpha chain	3.076	mmu-miR-712	4.253
22	FYVE, RhoGEF and PH domain containing 4	3.075	mmu-miR-760-3p	4.256
23	poly(A) binding protein, cytoplasmic 5	3.044	mmu-miR-302c	6.922
24	Fc receptor-like 6	2.877	mmu-miR-760-3p	4.256
25	serine palmitoyltransferase, long chain base subunit 3	2.803	mmu-miR-764-5p	34.150
26	ankyrin repeat domain 55	2.744	mmu-miR-494	2.396
27	ankyrin repeat domain 55	2.744	mmu-miR-499	6.239
28	placenta-specific 1-like	2.613	mmu-miR-494	2.396
29	regulating synaptic membrane exocytosis 2	2.555	mmu-miR-760-3p	4.256
30	deoxyribonuclease II beta	2.518	mmu-miR-764-5p	34.150
31	growth differentiation factor 2	2.510	mmu-miR-34c	6.107
32	growth differentiation factor 2	2.510	mmu-miR-712	4.253
33	canopy 1 homolog (zebrafish)	2.479	mmu-miR-499	6.239
34	ubiquitin associated and SH3 domain containing, A	2.187	mmu-miR-34c	6.107
35	Na ⁺ /K ⁺ transporting ATPase interacting 2	2.159	mmu-miR-34c	6.107
36	Na ⁺ /K ⁺ transporting ATPase interacting 2	2.159	mmu-miR-466j	29.948
37	G protein-coupled receptor 17	2.150	mmu-miR-760-3p	4.256
38	histone cluster 3, H2bb, pseudogene	2.133	mmu-miR-760-3p	4.256
39	histone cluster 1, H1d	2.112	mmu-miR-760-3p	4.256
40	5,10-methylenetetrahydrofolate reductase	2.091	mmu-miR-764-5p	34.150
41	tumor necrosis factor receptor superfamily, member 8	2.077	mmu-miR-302c	6.922
42	neuronal PAS domain protein 3	2.061	mmu-miR-466j	29.948
43	urocortin 2	2.014	mmu-miR-34c	6.107
44	mitogen-activated protein kinase 10	2.012	mmu-miR-466j	29.948

結膜炎の誘導により発現が増加した遺伝子と発現が減少した miRNA 関連遺伝子における共通 74 遺伝子リスト

no	発現が上昇した遺伝子名	結膜炎誘導後の増加倍率変化 (fold change)	発現が減少した miRNA	結膜炎誘導後の減少倍率変化 (fold change)
1	lactoperoxidase	6.567	mmu-miR-680	66.411
2	aryl hydrocarbon receptor nuclear translocator 2	4.209	mmu-miR-294	6.955
3	DnaJ (Hsp40) homolog, subfamily C, member 27	4.145	mmu-miR-342-5p	4.116
4	olfactory receptor 54	3.819	mmu-miR-294	6.955
5	sarcoglycan zeta	3.599	mmu-miR-1188	4.569
6	G protein-coupled receptor 123	3.445	mmu-miR-874	455.641
7	5-hydroxytryptamine (serotonin) receptor 2A	3.381	mmu-miR-680	66.411

8	collagen, type VI, alpha 6	3.345	mmu-miR-138	4.591
9	regulatory factor X, 4 (influences HLA class II expression)	3.335	mmu-miR-1188	4.569
10	collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase	3.228	mmu-miR-702	3.677
11	ubiquitin interaction motif containing 1	3.166	mmu-miR-138	4.591
12	pyruvate kinase liver and red blood cell	3.104	mmu-miR-342-5p	4.116
13	gap junction protein, beta 5	3.070	mmu-miR-702	3.677
14	fibrinogen alpha chain	3.069	mmu-miR-680	66.411
15	bassoon	3.040	mmu-miR-680	66.411
16	sideroflexin 5	3.039	mmu-miR-342-5p	4.116
17	sideroflexin 5	3.039	mmu-miR-680	66.411
18	sideroflexin 5	3.039	mmu-miR-874	455.641
19	membrane-spanning 4-domains, subfamily A, member 1	3.038	mmu-miR-146a	2.204
20	membrane-spanning 4-domains, subfamily A, member 1	3.038	mmu-miR-680	66.411
21	polyhomeotic-like 3 (Drosophila)	3.019	mmu-miR-146a	2.204
22	polyhomeotic-like 3 (Drosophila)	3.019	mmu-miR-294	6.955
23	TRAF-interacting protein with forkhead-associated domain, family member B	2.953	mmu-miR-342-5p	4.116
24	cell death-inducing DFFA-like effector c	2.928	mmu-miR-342-5p	4.116
25	hypoxia inducible factor 3, alpha subunit	2.868	mmu-miR-138	4.591
26	collagen, type V, alpha 3	2.823	mmu-miR-874	455.641
27	sclerostin	2.801	mmu-miR-702	3.677
28	chordin-like 1	2.790	mmu-miR-680	66.411
29	family with sequence similarity 155, member A	2.779	mmu-miR-1188	4.569
30	leucine rich repeat containing 3	2.779	mmu-miR-294	6.955
31	leucine-rich repeat, immunoglobulin-like and transmembrane domains 1	2.765	mmu-miR-294	6.955
32	metallothionein-like 5, testis-specific (tesmin)	2.763	mmu-miR-702	3.677
33	transmembrane and coiled-coil domains 3	2.745	mmu-miR-125a-3p	4.035
34	sodium channel, voltage-gated, type II, alpha 1	2.745	mmu-miR-680	66.411
35	NFKB activating protein-like	2.706	mmu-miR-294	6.955
36	DnaJ (Hsp40) homolog, subfamily C, member 5 beta	2.669	mmu-miR-294	6.955
37	nuclear receptor coactivator 7	2.654	mmu-miR-1188	4.569
38	nuclear receptor coactivator 7	2.654	mmu-miR-294	6.955
39	nuclear receptor coactivator 7	2.654	mmu-miR-680	66.411
40	growth differentiation factor 5	2.639	mmu-miR-146a	2.204
41	mutS homolog 5 (E. coli)	2.633	mmu-miR-146a	2.204
42	protocadherin beta 3	2.601	mmu-miR-125a-3p	4.035
43	forkhead box N4	2.584	mmu-miR-342-5p	4.116
44	T-box 4	2.576	mmu-miR-342-5p	4.116
45	tripartite motif-containing 14	2.573	mmu-miR-702	3.677
46	forkhead box E3	2.572	mmu-miR-125a-3p	4.035
47	surfactant associated protein B	2.562	mmu-miR-874	455.641
48	solute carrier family 8 (sodium/calcium exchanger), member 1	2.521	mmu-miR-125a-3p	4.035
49	solute carrier family 14 (urea transporter), member 1	2.463	mmu-miR-138	4.591
50	CCCTC-binding factor (zinc finger protein)-like	2.418	mmu-miR-342-5p	4.116
51	adaptor-related protein complex AP-4, epsilon 1	2.407	mmu-miR-125a-3p	4.035
52	glial fibrillary acidic protein	2.365	mmu-miR-138	4.591
53	glial fibrillary acidic protein	2.365	mmu-miR-702	3.677
54	alpha disintegrin-like and metalloproteinase (reprolysin type) with thrombospondin type 1 motif, 6	2.322	mmu-miR-1188	4.569
55	ubiquitin carboxy-terminal hydrolase L1	2.314	mmu-miR-294	6.955
56	ribonuclease, RNase A family, 13 (non-active)	2.309	mmu-miR-1188	4.569
57	solute carrier family 38, member 1	2.301	mmu-miR-702	3.677
58	MKL/myocardin-like 2	2.297	mmu-miR-680	66.411
59	sorting nexin 31	2.259	mmu-miR-702	3.677
60	potassium channel, subfamily K, member 10	2.234	mmu-miR-146a	2.204
61	testis-specific protein, Y-encoded-like 5	2.221	mmu-miR-702	3.677
62	testis-specific protein, Y-encoded-like 5	2.221	mmu-miR-874	455.641
63	connector enhancer of kinase suppressor of Ras 2	2.169	mmu-miR-680	66.411
64	schlafen 5	2.151	mmu-miR-874	455.641
65	NOL1/NOP2/Sun domain family, member 7	2.146	mmu-miR-138	4.591
66	chordin-like 1	2.140	mmu-miR-680	66.411
67	transformed mouse 3T3 cell double minute 1	2.095	mmu-miR-702	3.677

68	potassium channel tetramerisation domain containing 7	2.088	mmu-miR-125a-3p	4.035
69	glypican 5	2.081	mmu-miR-680	66.411
70	fatty acid desaturase domain family, member 6	2.060	mmu-miR-125a-3p	4.035
71	chemokine (C-X3-C) receptor 1	2.037	mmu-miR-874	455.641
72	solute carrier family 35, member F1	2.034	mmu-miR-1188	4.569
73	solute carrier family 35, member F1	2.034	mmu-miR-138	4.591
74	glutamyl aminopeptidase	2.010	mmu-miR-680	66.411

その後、発現レベルを定量的 RT-PCR 法で評価した。定量的 RT-PCR 法ではマイクロアレイで確認された各 miRNA 発現の変動(アレルギー性結膜炎の誘導による)を再現できなかった。

5. 主な発表論文等

(研究代表者、研究分担者及び連携研究者には下線)

(雑誌論文)(計7件)

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