Table S4

miR name	T-miR	N-miR	fold-change	p-value
miR-200a	5.8183	8.5496	0.1506	<0.00001
miR-375	3.7664	6.0955	0.1990	<0.00001
miR-200b	6.7698	9.0919	0.2000	<0.00001
miR-199b-3p	8.6670	10.8304	0.2232	<0.00001
miR-199a-3p	8.7558	10.8862	0.2284	<0.00001
miR-199a-5p	8.5426	10.5858	0.2426	<0.00001
miR-150	6.0219	7.7215	0.3079	<0.00001
miR-10a	6.8784	8.4695	0.3319	<0.00001
miR-424	6.8159	8.3325	0.3495	<0.00001
miR-214	6.9813	8.4080	0.3720	<0.00001
miR-139-5p	4.8902	6.3078	0.3744	<0.00001
miR-451	11.4110	12.7522	0.3947	<0.00001
miR-142-3p	4.3882	5.5899	0.4348	0.00001
miR-142-5p	6.7147	7.8031	0.4703	0.00003
miR-223	8.3237	9.3963	0.4755	<0.00001
miR-146a	6.4679	7.5048	0.4874	0.00001
miR-486-5p	4.9461	5.9425	0.5012	<0.00001
miR-30a*	6.1348	7.1145	0.5071	<0.00001
miR-130a	8.2858	9.2523	0.5118	<0.00001
miR-376c	4.9713	5.8816	0.5321	0.00542
miR-378	8.3006	9.1927	0.5388	<0.00001
miR-125a-5p	8.1841	9.0551	0.5468	<0.00001
miR-195	8.8553	9.7022	0.5560	<0.00001
miR-497	7.3460	8.1444	0.5750	0.00002
miR-422a	7.1003	7.8778	0.5834	<0.00001
miR-342-3p	8.0099	8.6998	0.6199	<0.00001
miR-125b	10.3309	10.9920	0.6324	0.00001
miR-101	6.4174	7.0780	0.6326	0.00005
miR-1249	4.6112	5.2266	0.6527	0.00728
miR-30e*	7.7371	8.3455	0.6559	<0.00001
miR-1238	5.1302	5.7055	0.6711	0.00216
miR-335	6.6345	7.1763	0.6869	0.00812
miR-145	9.6902	10.2310	0.6874	0.00037
miR-455-5p	6.0197	6.5470	0.6939	0.00087
miR-22*	5.4614	5.9578	0.7089	0.00632
miR-99a	10.4387	10.9317	0.7105	0.00047
miR-146b-5p	9.6150	10.0391	0.7453	0.00990
miR-100	10.4659	10.8843	0.7482	0.00039
miR-143	10.8380	11.2255	0.7645	0.00411
let-7g	11.0311	11.4135	0.7672	0.00078
miR-181a	7.5643	7.9462	0.7674	0.00515
miR-99b	7.5207	7.8866	0.7760	0.00058
miR-22	12.1227	12.4560	0.7937	0.00006
miR-30a	10.4827	10.8113	0.7963	0.00034
miR-26a	12.3555	12.6796	0.7988	0.00004
miR-30c	10.9891	11.2535	0.8325	0.00007
let-7c	11.5850	11.8016	0.8606	0.00070
let-7a	12.2072	12.3928	0.8793	0.00395
miR-1280	14.0985	14.2756	0.8845	0.00168

Table S5

miR name	T-miRs	normal	fold-change	p-value	miR name	N-miRs	normal	fold-change	p-value
Up-regulated miRs									
miR-96	4.5990	1.8477	6.7330	0.03489	miR-96	3.4966	1.8477	3.1359	0.04947
miR-222	7.3771	5.1257	4.7614	0.00001	miR-222	6.5574	5.1257	2.6976	0.00939
miR-18b	5.6000	3.4268	4.5102	0.03660	miR-886-3p	7.7848	6.5724	2.3173	0.04503
miR-224	7.1005	5.1620	3.8330	0.04204	miR-146b-5p	10.0391	9.0101	2.0406	0.00038
miR-221	8.9564	7.3352	3.0763	0.00010	miR-199a-3p	10.8862	10.1048	1.7188	0.01696
miR-21	12.0351	10.4628	2.9737	0.00007	miR-181a	7.9462	7.1682	1.7147	0.00651
miR-1469	9.1517	7.7425	2.6558	0.00302	miR-199a-5p	10.5858	9.8103	1.7118	0.01517
miR-362-3p	5.4439	4.1095	2.5217	0.04277	miR-199b-3p	10.8304	10.1247	1.6309	0.02380
miR-663	9.8008	8.5582	2.3663	0.01236	miR-10a	8.4695	7.7943	1.5969	0.03236
miR-106b	9.8617	8.6820	2.2654	0.00023	miR-214	8.4080	7.7728	1.5531	0.03594
miR-34a	9.6314	8.4736	2.2312	0.01399					
miR-25	8.9278	8.2994	1.5459	0.03904					
Down-regulated miRs									
miR-375	3.7664	6.2964	0.1731	0.01715	miR-1238	5.7055	7.0204	0.4020	0.00922
miR-1249	4.6112	6.6075	0.2506	0.00533	miR-296-5p	8.4425	9.6868	0.4221	0.01721
miR-139-5p	4.8902	6.8648	0.2544	0.01140	miR-1228	6.0568	7.1856	0.4573	0.02378
miR-1238	5.1302	7.0204	0.2698	0.00990	miR-1913	8.3088	9.2908	0.5063	0.00631
miR-625*	5.3830	7.0589	0.3130	0.00344	miR-940	6.9842	7.8839	0.5360	0.02220
miR-422a	7.1003	8.7371	0.3216	0.00783	miR-422a	7.8778	8.7371	0.5512	0.01481
miR-486-5p	4.9461	6.4846	0.3442	0.02208	miR-148a	10.3720	11.1663	0.5766	0.03234
miR-378	8.3006	9.8241	0.3478	0.00364	miR-193a-5p	5.7622	6.5466	0.5806	0.02435
miR-296-5p	8.2098	9.6868	0.3592	0.00125	miR-365	7.7796	8.5251	0.5965	0.01594
miR-101	6.4174	7.7477	0.3977	0.01811	miR-101	7.0780	7.7477	0.6286	0.04963
miR-1228	5.9237	7.1856	0.4170	0.01134	miR-1201	9.0958	9.7294	0.6446	0.04848
miR-1913	8.1404	9.2908	0.4505	0.00105	miR-92a	8.5022	8.9180	0.7496	0.04124
miR-193a-5p	5.4028	6.5466	0.4526	0.02306					
miR-30e*	7.7371	8.5683	0.5620	0.04295					
miR-125a-3p	7.5449	8.2814	0.6002	0.04314					

Table S6

	T-miR					N-miR			
miR	HBV(+)	HBV(-)	Diff	p-value*	miR	HBV(+)	HBV(-)	Diff	p-value*
Up-regulated					Up-regulated				
miR-106b	10.3206	9.7715	0.5491	0.0034	miR-24	11.6927	11.5276	0.1651	0.0435
miR-18a	7.4176	5.7149	1.7026	0.0053					
miR-18b	6.9740	5.3297	1.6443	0.0082					
miR-19b	12.0658	11.4107	0.6551	0.0126					
miR-483-3p	6.0029	3.9654	2.0375	0.0157					
miR-92a	9.0847	8.4392	0.6455	0.0167					
miR-19a	10.0270	9.3212	0.7057	0.0195					
miR-146a	7.3693	6.2906	1.0786	0.0298					
miR-375	4.9232	3.5388	1.3844	0.0320					
miR-483-5p	6.3649	4.7003	1.6646	0.0368					
miR-25	9.2463	8.8651	0.3812	0.0408					
miR-320a	8.5294	8.0563	0.4731	0.0484					
miR-96	5.9103	4.3410	1.5692	0.0494					
Down-regulated					Down-regulated				
miR-34b*	4.7227	6.3285	-1.6058	0.0001	miR-204	5.0377	5.8588	-0.8211	0.0035
miR-99b	6.8002	7.6624	-0.8622	0.0006	miR-186	4.9359	5.8206	-0.8847	0.0045
miR-29b	8.9477	9.5701	-0.6223	0.0018	miR-320a	7.5878	7.9429	-0.3550	0.0047
miR-125b	9.3161	10.5306	-1.2145	0.0019	miR-335	6.3978	7.3294	-0.9316	0.0106
miR-99a	9.6034	10.6030	-0.9996	0.0024	miR-486-5p	5.3699	6.0552	-0.6853	0.0121
miR-100	9.7506	10.6066	-0.8560	0.0029	miR-320b	7.6168	7.8877	-0.2709	0.0215
miR-34a	8.9715	9.7613	-0.7898	0.0052	miR-101	6.6977	7.1528	-0.4551	0.0282
miR-365	7.4817	8.3136	-0.8319	0.0052	miR-374a	5.2447	5.8441	-0.5994	0.0294
miR-30e*	7.1643	7.8498	-0.6855	0.0059	miR-361-5p	7.5175	7.7615	-0.2439	0.0305
miR-29c	8.8776	9.5295	-0.6519	0.0102	miR-30e*	8.0976	8.3943	-0.2967	0.0326
miR-193b	8.1940	9.0749	-0.8809	0.0139	miR-28-5p	7.9896	8.5349	-0.5453	0.0366
miR-30a	10.0684	10.5642	-0.4958	0.0156	miR-20a	8.4863	9.2370	-0.7506	0.0381
miR-374b	4.2973	5.4034	-1.1061	0.0201	miR-452	4.2277	5.0494	-0.8217	0.0405
miR-195	8.1499	8.9941	-0.8442	0.0232	miR-126*	6.1436	6.9171	-0.7734	0.0406
miR-362-3p	4.7253	5.5853	-0.8600	0.0290	miR-193a-5p	5.4007	5.8333	-0.4325	0.0425
miR-26b	9.7553	10.2310	-0.4756	0.0306	miR-30a*	6.8183	7.1728	-0.3545	0.0496
miR-361-3p	6.1658	6.6966	-0.5309	0.0319					
miR-192*	4.3472	5.3080	-0.9608	0.0373					
miR-660	6.3082	6.9920	-0.6839	0.0414					
miR-374a	5.0107	5.6917	-0.6810	0.0419					
miR-125a-5p	7.6032	8.2983	-0.6951	0.0485					

Table S7

T-miR					N-miR				
miR	HCV(+)	HCV(-)	Diff	p-value*	miR	HCV(+)	HCV(-)	Difference	p-value*
Up-regulated					Up-regulated				
miR-34b*	6.4265	5.2253	1.2012	0.0003	miR-222	6.8848	5.7984	1.0864	<0.0001
miR-30e*	7.9329	7.2832	0.6497	0.0011	miR-150	8.0739	6.9044	1.1695	<0.0001
miR-192*	5.4894	4.3634	1.1260	0.0021	let-7i	9.2825	8.9220	0.3604	0.0008
miR-455-3p	8.6178	8.0202	0.5977	0.0036	miR-181a	8.0824	7.6304	0.4519	0.0008
miR-193b	9.1567	8.4048	0.7519	0.0092	miR-886-3p	8.0565	7.1551	0.9014	0.0016
miR-215	9.5249	8.9718	0.5531	0.0167	miR-96	3.8601	2.6540	1.2061	0.0030
miR-34a	9.7944	9.2538	0.5406	0.0187	miR-106b	9.1893	8.6924	0.4969	0.0032
miR-30a	10.6001	10.2103	0.3898	0.0188	miR-155	5.7522	4.6028	1.1494	0.0033
miR-365	8.3473	7.7817	0.5656	0.0196	miR-342-3p	8.8314	8.3948	0.4366	0.0033
miR-125a-3p	7.6574	7.2840	0.3735	0.0353	miR-142-5p	8.0579	7.2124	0.8455	0.0048
miR-30c	11.0656	10.8115	0.2541	0.0463	miR-199a-5p	10.7131	10.2907	0.4224	0.0060
miR-26b	10.2583	9.9081	0.3502	0.0494	miR-221	7.9253	7.4180	0.5073	0.0073
					miR-199b-3p	10.9496	10.5542	0.3954	0.0090
					miR-146b-5p	10.1462	9.7907	0.3555	0.0093
					miR-142-3p	5.8838	4.9084	0.9755	0.0102
					miR-362-3p	5.0836	4.2171	0.8665	0.0110
					miR-199a-3p	11.0069	10.6066	0.4003	0.0116
					miR-331-3p	6.3914	5.8842	0.5072	0.0233
					let-7d	12.1539	11.9643	0.1897	0.0267
					miR-16	11.2669	11.0684	0.1985	0.0288
					miR-186	5.8385	5.2965	0.5420	0.0334
					miR-147	5.5457	5.0445	0.5012	0.0406
					miR-140-3p	8.0675	7.8483	0.2191	0.0414
					miR-185	7.5071	7.2301	0.2770	0.0432
					miR-146a	7.6435	7.1835	0.4600	0.0459
Down-regulated					Down-regulated				
miR-146a	6.1684	7.1622	-0.9938	0.0127	miR-296-5p	8.1440	9.1344	-0.9904	<0.0001
miR-24	11.4161	11.6670	-0.2509	0.0161	miR-1913	8.1286	8.7266	-0.5980	0.0004
miR-370	4.9433	5.7049	-0.7615	0.0178	miR-148a	10.1907	10.7925	-0.6019	0.0008
miR-23a	11.8544	12.1104	-0.2560	0.0240	miR-422a	7.7153	8.2547	-0.5394	0.0014
miR-744	7.2950	7.8370	-0.5420	0.0369	miR-1228	5.8350	6.5709	-0.7359	0.0022
miR-1228*	9.6644	10.1021	-0.4377	0.0389	miR-1238	5.4915	6.2016	-0.7101	0.0034
miR-494	9.6574	10.1067	-0.4493	0.0461	miR-1201	8.9610	9.4083	-0.4473	0.0039
miR-575	6.1797	6.6792	-0.4995	0.0493	miR-99a	10.8449	11.1329	-0.2880	0.0068
					miR-625*	5.5356	6.3972	-0.8615	0.0070
					miR-365	7.6609	8.0547	-0.3939	0.0081
					miR-940	6.8369	7.3257	-0.4888	0.0104
					miR-1249	4.9838	5.7895	-0.8058	0.0207
					miR-378	9.0651	9.4886	-0.4235	0.0232
					miR-92a	8.4412	8.6436	-0.2024	0.0420

Table S8

	T-miRs						
miR name	grade1	grade2	grade3	diff (1 vs 2)	diff (1 vs 3)	diff (2 vs 3)	p-value*
miR-191	10.1177	10.5007	10.1918	-0.3831	-0.0742	0.3089	0.0039
miR-126*	7.4668	6.5014	6.3017	0.9654	1.1651	0.1998	0.0074
miR-1915	7.6699	7.3598	6.3638	0.3101	1.3061	0.9960	0.0202
miR-378	8.7756	8.0561	8.4477	0.7194	0.3279	-0.3915	0.0217
miR-455-5p	6.2626	6.0735	4.9452	0.1891	1.3175	1.1284	0.0232
miR-126	12.0775	11.6789	11.7130	0.3985	0.3644	-0.0341	0.0234
miR-486-3p	7.0058	6.4565	5.6443	0.5493	1.3615	0.8122	0.0285
miR-1228	6.1406	5.9577	5.0541	0.1828	1.0864	0.9036	0.0290
miR-20a	9.3135	9.3725	8.0935	-0.0590	1.2199	1.2790	0.0397
miR-923	13.3713	13.9565	13.5210	-0.5852	-0.1497	0.4355	0.0419
miR-106b	9.5858	9.9831	9.9092	-0.3974	-0.3235	0.0739	0.0425
miR-744	7.8564	7.3688	6.8402	0.4876	1.0162	0.5286	0.0456

Table S9

HBV(+) cases (n=12)

T-miRs				N-miRs			
Rank	microRNA	hazard ratio	p-value	Rank	microRNA	hazard ratio	p-value
1	miR-1913	0.0992	0.0057	1	miR-638	0.1125	0.0073
2	miR-663	0.2277	0.0069	2	miR-1202	0.6105	0.0112
3	miR-1909	0.1647	0.0106	3	let-7i	18.629	0.0133
4	miR-575	0.2499	0.0140	4	miR-99b	8.4818	0.0140
5	miR-638	0.2146	0.0167	5	miR-107	15.909	0.0159
6	miR-18b	2.1562	0.0205	6	miR-146b-5p	7.3577	0.0184
7	miR-18a	2.2643	0.0215	7	miR-223	5.9927	0.0188
8	miR-1469	0.2768	0.0243	8	miR-663	0.3757	0.0189
9	miR-1908	0.2849	0.0251	9	miR-130b	2.0950	0.0202
10	miR-20a	3.1284	0.0255	10	miR-96	1.7335	0.0206
11	miR-20b	2.7301	0.0267	11	miR-23a	16.157	0.0211
12	miR-455-3p	2.5900	0.0277	12	miR-484	8.7674	0.0218
13	miR-296-5p	0.2116	0.0277	13	miR-146a	4.9436	0.0220
14	miR-362-3p	2.3579	0.0314	14	miR-1909	0.1095	0.0230
15	miR-17	3.4918	0.0343	15	miR-27a	14.401	0.0269
16	miR-106a	3.1753	0.0346	16	miR-149*	0.2075	0.0292
17	miR-335	2.6876	0.0362	17	miR-1260	0.0136	0.0301
18	miR-92a	3.0776	0.0372	18	miR-122*	0.3034	0.0311
19	miR-29a	0.2359	0.0435	19	miR-23b	10.679	0.0320
20	miR-940	0.2839	0.0449	20	miR-192	0.2663	0.0332

Table S10

HCV(+) cases (n=51)							
T-miRs				N-miRs			
Rank	microRNA	hazard ratio	p-value	Rank	microRNA	hazard ratio	p-value
1	miR-100	0.4810	0.0008	1	miR-27a	6.7460	0.0004
2	miR-99a	0.5821	0.0012	2	miR-486-5p	0.4468	0.0008
3	miR-125b	0.5773	0.0048	3	miR-24	13.869	0.0013
4	miR-92b*	1.6247	0.0131	4	miR-96	1.5296	0.0016
5	miR-30c	0.3833	0.0194	5	miR-21	1.6457	0.0026
6	miR-1268	1.6946	0.0200	6	miR-18a	2.2505	0.0038
7	miR-575	1.5276	0.0210	7	miR-142-3p	1.5871	0.0040
8	miR-1275	1.3010	0.0221	8	miR-23a	5.8634	0.0042
9	miR-30e	0.6382	0.0230	9	miR-148a	0.5434	0.0050
10	miR-130b	0.6909	0.0236	10	miR-1238	0.5584	0.0057
11	miR-1246	1.2356	0.0252	11	miR-191	9.1921	0.0105
12	miR-129-5p	0.7183	0.0261	12	miR-222	1.8491	0.0109
13	miR-148b	0.7898	0.0333	13	miR-296-5p	0.5316	0.0116
14	miR-22	0.5544	0.0347	14	miR-103	6.3409	0.0116
15	miR-103	0.6878	0.0375	15	let-7f	6.3824	0.0124
16	miR-99b	0.6843	0.0411	16	miR-18b	1.6347	0.0144
17	miR-638	1.4609	0.0455	17	miR-107	6.1065	0.0173
18	miR-665	1.2995	0.0485	18	miR-30c	0.4300	0.0179
19				19	miR-146b-5p	2.6044	0.0186
20				20	miR-378	0.6372	0.0187

Table S11

HBV(-) HCV(-) cases (n=14)

T-miRs				N-miRs			
Rank	microRNA	hazard ratio	p-value	Rank	microRNA	hazard ratio	p-value
1	miR-17	30.384	0.0080	1	miR-30b	0.0170	0.0071
2	miR-106a	24.516	0.0109	2	miR-342-3p	0.0068	0.0189
3	miR-20a	29.072	0.0139	3	let-7e	32.803	0.0233
4	miR-93	48.859	0.0143	4	miR-147	147.4	0.0235
5	miR-92a	42.274	0.0148	5	miR-152	12.138	0.0249
6	miR-103	17.182	0.0154	6	miR-1202	0.5826	0.0352
7	miR-92b	16.617	0.0186	7	miR-126*	38.122	0.0384
8	miR-125b	0.0234	0.0191	8	miR-128	31.80	0.0389
9	miR-107	17.215	0.0193	9	miR-17	54.282	0.0416
10	miR-24	0.0780	0.0209	10	miR-93	25542	0.0468
11	miR-19a	8.6331	0.0227	11	miR-146a	0.0050	0.0481
12	miR-140-3p	0.0400	0.0230	12	miR-26b	58.694	0.0484
13	miR-20b	63.800	0.0260	13	miR-335	17.668	0.0492
14	miR-483-3p	1.4653	0.0272	14	miR-374b	8.9145	0.0497
15	miR-98	4.3393	0.0349	15			
16	let-7f	1122.9	0.0357	16			
17	miR-224	1.8818	0.0407	17			
18	miR-148b	6.4607	0.0415	18			
19	miR-30e	3.6760	0.0431	19			
20	miR-125a-5p	0.2555	0.0456	20			

Table S12

Grade 1 (well diff, n=21)				Grade 2 (mod diff, n=45)			
Rank	T-miRs	hazard ratio	p-value	Rank	T-miRs	hazard ratio	p-value
1	miR-224	2.1100	0.0049	1	miR-99a	0.5806	0.0007
2	miR-193b	3.3817	0.0074	2	miR-100	0.5640	0.0015
3	miR-152	0.2691	0.0142	3	miR-378	0.6030	0.0092
4	miR-452	1.6740	0.0219	4	miR-30e*	0.5498	0.0186
5	miR-191	0.1882	0.0252	5	miR-129-5p	0.7036	0.0291
6	miR-20a	2.1191	0.0260	6	miR-140-3p	0.5061	0.0300
7	miR-92b	2.0843	0.0270	7	miR-422a	0.7489	0.0352
8	miR-1228	2.1790	0.0288	8	miR-99b	0.6989	0.0406
9	miR-199a-5p	0.6349	0.0298	9	miR-125b	0.8529	0.0468
10	miR-20b	2.0342	0.0325	10	miR-193b	0.7259	0.0493
11	miR-376c	0.7592	0.0372				
12	miR-145	0.3157	0.0438				
13	miR-199b-3p	0.6717	0.0454				

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MINIREVIEW

MicroRNAs and epigenetics

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MicroRNAs (miRNAs) comprise species of short noncoding RNA that regulate gene expression post-transcriptionally. Recent studies have demonstrated that epigenetic mechanisms, including DNA methylation and histone modification, not only regulate the expression of protein-encoding genes, but also miRNAs, such as let-7a, miR-9, miR-34a, miR-124, miR-137, miR-148 and miR-203. Conversely, another subset of miRNAs controls the expression of important epigenetic regulators, including DNA methyltransferases, histone deacetylases and polycomb group genes. This complicated network of feedback between miRNAs and epigenetic pathways appears to form an epigenetics–miRNA regulatory circuit, and to organize the whole gene expression profile. When this regulatory circuit is disrupted, normal physiological functions are interfered with, contributing to various disease processes. The present minireview details recent discoveries involving the epigenetics–miRNA regulatory circuit, suggesting possible biological insights into gene-regulatory mechanisms that may underlie a variety of diseases.

Introduction

MicroRNAs (miRNA) comprise a class of short noncoding RNAs with 18–25 nucleotides in length that are found in animal and plant cells. In 1993, the first miRNAs were recognized in *Caenorhabditis elegans* by Lee *et al.* [1]. In 2001, various small regulatory RNAs were discovered in plants and mammals [2–4] and designated 'microRNA' [5]. Currently, 1100 human miRNAs are registered in the miRBase database (release 16, September 2010) [6–9]. miRNAs are involved in RNA interference (RNAi) machinery to regulate gene expression post-transcriptionally, and they contribute to diverse physiological and pathophysiological functions, including the regulation of developmental timing and pattern formation [2], restriction of differentiation potential [10], cell signaling [11], cardiovascular diseases [12] and carcinogenesis [13]. The biogenesis and RNAi functions of miRNA (i.e. how miRNAs are generated and processed into a mature form, and how they regulate gene expression) have been intensively investigated and well-described [10]. Furthermore, developments in miRNA-related technologies, such as miRNA expression profiling and synthetic oligoRNA, have contributed to the identification of miRNAs involved in a number of physiological and pathological phenotypes. However, some questions remain largely unanswered, such as how miRNA expression is controlled and which genes are regulated by each miRNA. Recently, accumulating studies have shown that a subgroup of miRNAs is regulated epigenetically. Although epigenetics and miRNAs have been frequently

Abbreviations
DGCR8, DiGeorge syndrome critical region gene 8; DNMT, DNA methyltransferase; EMT, epithelial–mesenchymal transition; HDAC, histone deacetylase; miRNA, microRNA; NF-κB, nuclear factor kappa B; PRC, polycomb repressor complex; RISC, RNA-induced silencer complex; RLC, RISC-loading complex; RNAi, RNA interference; SNP, single nucleotide polymorphism; TGIF2, TGFβ-inducing factor 2; VNTR, variable nucleotide tandem repeat; YY1, Yin Yang 1.

reviewed [14–18], few reviews have focused upon the relationship between epigenetics and miRNA. In the present minireview, we illustrate the current knowledge regarding the epigenetics–miRNA regulatory networks aiming to provide biological insights for a wide range of biomedical researchers.

Biogenesis and RNAi functions of miRNAs

As illustrated in Fig. 1, in the nucleus, mainly RNA polymerase II initially transcribes miRNAs into long segments of coding or noncoding RNA, known as pri-miRNAs, which are usually capped and polyadenylated. Portions in the pri-miRNAs measuring approximately 70–100 nucleotides in length and containing a stem-loop, are captured and extracted from pri-miRNAs by a complex containing RNase type III, Drosha and the dsRNA binding protein DiGeorge syndrome critical region gene 8 (DGCR8) (also called Pasha) [19]. These short stem-loop-shaped RNAs are called pre-miRNAs, and the protein complex of RNase, Drosha and DGCR8 is known as the microprocessor complex. Pre-miRNAs form a complex with exportin-5 and RAN-GTP, and are then exported from the nucleus to the cytoplasm. The pre-miRNAs are further processed to a double-stranded miRNA duplex by a dsRNase type III, Dicer. This double-stranded miRNA duplex is incorporated into a RNA-induced silencer complex (RISC)-loading complex (RLC) in an ATP-dependent manner [20]. Next, one strand (the passenger strand) of the miRNA is removed from the RLC, whereas the other strand (the guide strand) remains in the complex to form a mature RNA-induced silencer complex (mature RISC) and serves as a template for capturing target mRNAs. Under most conditions, the mature RISC represses gene expression post-transcriptionally. For highly complementary target mRNAs, the mature RISC complex cleaves target mRNAs via a catalytic domain (RNase III domain) of Argonaute proteins, a core component of the RISC complex, and degrades them by the SKI complex and XRN1 [21]. For partially complementary targets, the RISC complex decaps and deadenylates target mRNAs via the DCP1-DCP2 and CAF1-CCR4-NOT complexes, respectively, to reduce the stability of the target mRNAs [22]. In addition, the RISC complex also represses the translation of target genes under most conditions. However, not all miRNAs work in translational repression. Under serum-starved conditions, miR-369-3 activates translation of tumor necrosis factor-α by binding to AU-rich elements in the 3′ UTR of the transcript with fragile X mental retardation-related protein 1 [23]. Thus, molecular mechanisms of the RISC in translational regulation remain to be clarified. At the same time, turnover of miRNAs is mediated by the *XRN2* gene in *C. elegans* [24]. However, the mechanisms underlying miRNA turnover in human cells also remain unclear.

Epigenetically-regulated miRNAs

As described above, the biogenesis of miRNA has been intensively studied and is well-described. However, the regulation of miRNA expression remains largely unclear. In early studies, promoter regions had been determined for only a small portion of miRNAs. Although several *in silico* studies attempted to predict the promoter regions of miRNAs [25–27], most of these predicted miRNA promoters were not confirmed in wet-laboratory experiments.

miRNAs can be classified as either 'intragenic' and 'intergenic', according to whether the miRNA is localized in a genome region transcribed by a gene, or not. Our *in silico* analysis (see Materials and methods) revealed that, among 939 miRNAs, 293 (31.2%) of miRNAs were intergenic, whereas 317 (44.4%), 119 (12.7%) and 110 (11.7%) were overlapped by RNA transcripts in the same, opposite and both directions, respectively. Localization of promoters for intergenic and inversely-directed intragenic miRNAs is largely unknown, whereas promoters for overlapping primary genes are considered to be promoters for the intragenic miRNAs that are localized in the same direction as the primary gene. However, some studies have identified that an independent promoter within the intron in which a miRNA is embedded can also regulate miRNA expression [28]. Additionally, as shown in one study [29], a single member of a miRNA cluster, although ordinarily expressed from the same pri-miRNA, can alternatively be regulated independently by its own promoter in certain scenarios. Furthermore, the total amount of miRNAs contained within a given quantity of total RNA can be reduced in cancer cells and rapidly proliferating cells [13,30], a finding for which the underlying mechanism is still unknown. Thus, the means by which miRNA expression is regulated appears somewhat complicated.

Recently, Saito *et al.* [29] established that the expression of miR-127 is regulated epigenetically. In their study, pharmacological unmasking of epigenetically silenced miRNAs activated 17 of 313 miRNAs investigated in the bladder cancer cell line T24 and the normal fibroblast cell line LD419. The gene for miR-127 was upregulated the most in epigenetically unmasked

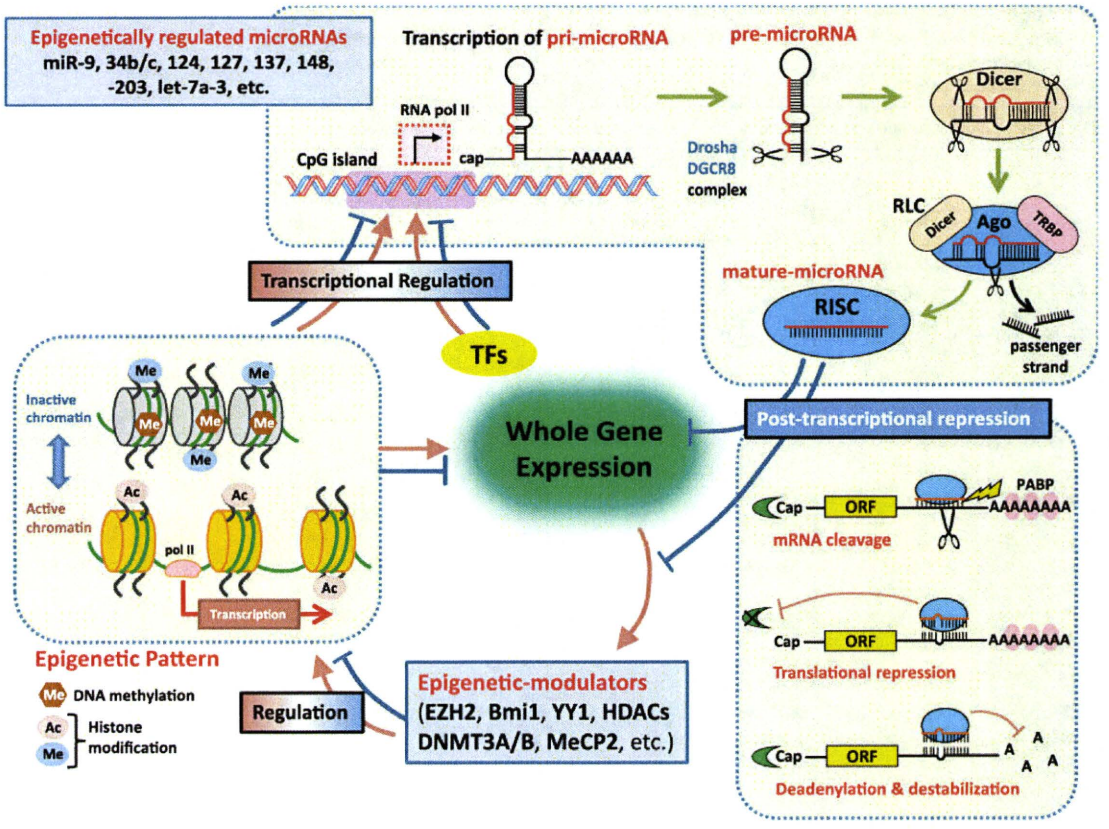

Fig. 1. Epigenetics–miRNA regulatory circuit. Epigenetics and miRNAs regulate whole gene expression pattern transcriptionally and post-transcriptionally, respectively. At the same time, epigenetics and miRNAs controll each other to form a regulatory circuit and to maintain normal physiological functions. A disruption of this regulatory circuit may cause various diseases, such as cardiovascular diseases and cancers. PABP, poly(A) binding protein; TF, transcriptional factors; TRBP, Tar RNA binding protein.

cancer cells. DNA methylation level and histone modification status at identified promoter regions of miR-127 correlated significantly with mature miR-127 expression. Subsequent to this initial report, the number of studies documenting the epigenetic regulation of miRNAs has increased dramatically (Table 1). We summarize the findings regarding some of the more intensively studied miRNAs for which expression is regulated by epigenetic mechanisms.

miR-9

miR-9 is expressed from three genomic loci, miR-9-1, miR-9-2 and miR-9-3, all of which are associated with CpG islands. Hypermethylation of miR-9 loci is observed in various malignant tissues, including breast, lung, colon, head and neck cancers, melanoma and acute lymphoblastic leukemia [31–34]. In breast cancer, the miR-9-1 locus is highly methylated not only in invasive ductal carcinoma, but also in ductal carcinoma *in situ* and the intraductal component of invasive ductal carcinoma [34]. In addition, an *in vitro* experimental study showed that xenoestrogen exposure may induce aberrant epigenetic patterns at various miRNA gene loci, including miR-9-3 [35]. These findings suggest that epigenetic silencing of miR-9 loci constitutes an early event in breast carcinogenesis. Furthermore, the miR-9 DNA methylation signature is correlated with cancer metastasis [33]. Target genes of mature miR-9 responsible for carcinogenesis and cancer metastasis remain largely unknown. However, a recent study demonstrated that mature miR-9 targets nuclear factor kappa B (NF-κB), which is overexpressed in a number of different cancers [36].

Table 1. Epigenetically-regulated miRNAs. The numbers in the 'binding sites' column represent the distance (bp) between the stop codon and binding sites of seed sequences in the miRNAs. The letters 'c' and 'p' with respect to miRNA binding site numbers indicate that the miRNA binding sites are the 'conserved region' and 'poorly conserved region' among vertebrates, according to the TargetScan database (http://www.targetscan.org/).

miRNA genes	Inter-/intra genic	Locus	Host gene	Target genes	Binding sites	References
let-7a-3	Intergenic	22q13.31		IGF2BP1-3	1632c, 1651c, 4269c, 4923c, 5568c	[55,83]
miR-1-1	Intragenic	20q13.33	C20orf166	FoxP1	772c, 819c[a], 965p, 3447p	[84]
				MET	499p, 811c	
				HDAC4	2333c, 3513c[a],3546c[a]	
miR-9-1	Intragenic	1q22	C1orf61	NFKB1	29p[a]	[31–35]
miR-9-2	Intragenic	5q14.3	CR612213			
miR-9-3	Intragenic	15q26.1	FLJ30369			
miR-10a	Intragenic	21q21.32	HOX3B	HOXA3	299c	[31,85]
				HOXD10	276c	
miR-34a	Intragenic	1p36.23	EF570048	CDK6	1087c, 6941p, 9172c	[39]
miR-34b/c	Intragenic Intragenic	11q23.1	BC021736	CDK6	1087c, 6941p, 9172c	[28,31,33,40,41]
				MYC	138p[a]	
				E2F3	2714c	
				CREB	3259p, 3317c	
miR-107	Intragenic	10q23.31	PANK1	CDK6	308c, 1815p	[86]
miR-124-1	Intergenic	8p23.1			1532p, 1647p, 7788p, 8004p	[31,34,44–48]
miR-124-2	Intragenic	8q12.3	AK124256/	CDK6		
miR-124-3	Intergenic	20q13.33	FLJ42262	C/EBPα	283c, 340c, 981c	
				VIM	81c	
				SMYD3	43p	
miR-126	Intragenic	9q34.3	EGFL7			[87]
miR-127	Intergenic	14q32.31		BCL6	584c	[29,47]
miR-129-2	Intragenic	11p11.2	EST			[32]
miR-132/212	Intergenic	17p13.2				[31]
miR-137	Intragenic	1p21.3	AK311400	CDK6	4214p, 7114p, 7133c	[32,40,47]
				E2F6	79c	
				NCOA2	1244c	
miR-148a	Intergenic	7p15.2		TGIF2	159c, 566p[a], 2288c	[33,34]
miR-152	Intragenic	17q21.32	COPZ2			[34]
miR-181a/b-2	Intragenic	9q33.3	NR6A1	PLAG1	391p, 3501c, 4389c	[88]
miR-193a	Intergenic	17q11.2		E2F6	127c	[40,47]
				PTK2	545p	
				MCL1	315c[a]	
miR-196a-2	Intragenic	12q13.13	EST			[89]
miR-196b	Intragenic	7p15.2	EST			[31]
miR-199a*-1	Intragenic	19p13.2	DNM2	MET	1425c[a]	[90]
miR-199a*-2	Intragenic	1q24.3	DNM3			
miR-141/200c	Intergenic	12p13.31		ZEB2	207c, 733p, 774c	[51–53]
miR-200a/b/429	Intergenic	1p36.33		ZEB1	369c, 463c	
				ZEB2	391c[a], 454c[a], 812c, 897c, 1028c, 1362c[a]	
				SOX2	477c[a]	
				KLF4	42c[a]	
miR-203	Intergenic	14q32.33		ABL1	1074c	[31,40,48,51,54]
				BCR-ABL1	1074c	
				Bmi-1	1443c	
miR-342	Intragenic	14q32.2	EVL			[91]
miR-370	Intragenic	14q32.31	EST	MAP3K8	567p	[92]
miR-512-5p	Intergenic	19q13.41		Mcl-1	1631p	[93]
miR-663	Intragenic	20p11.1	BC036544			[34]

[a] SNPs are located within the miRNA binding sites (not only the seed sequence regions, but also an approximately 23 bp region), which may affect the affinity of miRNA with the binding sites.

miR-34 (a and b/c)

The net level of miR-34 reflects the expression of three separate genes for miR-34: miR-34a, miR-34b and miR-34c. miR-34a is monocistronic, whereas miRs-34b/c are polycistronic. Promoter regions of both loci contain p53-binding sites, and are regulated by the p53 signal. Likely as a result of this feature, the expression of mature miR-34a species is induced by DNA damage and oncogenic stress, as well as other p53-related events that control the cell cycle, induce apoptosis and suppress tumor formation [37,38]. The host or 'mother' gene (*FLJ41150*) of miR-34a is associated with a CpG island surrounding its transcriptional start site, which is frequently methylated in various malignancies [39]. The epigenetic mechanism underlying miR-34b/c transcriptional regulation was described in detail by Toyota *et al.* [28]. The miR-34b/c host gene (*BC021736*) contains a CpG island, not within its own promoter region, but also located at the first intron–second exon boundary. The latter CpG island also happens to lie within the promoter region of the oppositely-oriented BTG4 gene, thus exerting bidirectional promoter activity for both the *BTG4* gene and the miR-34b/c polycistoron [28]. Thus, miR-34b/c expression may be regulated by both the promoter of the host gene and the promoter in the latter CpG island. The methylation levels of the CpG island are inversely correlated with mature miR-34b/c expression levels in various cancers [28,31,33,40,41]. In colorectal cancer cell lines, in which the miR-34b/c locus is epigenetically silenced, the p53 signal alone does not induce miR-34b/c expression [28]. This finding suggests that hypermethylation of the CpG island modulates p53-mediated miR-34b/c expression. In terms of the functions of miR-34 species, mature miR-34 miRNAs target various genes related to the cell cycle, oncogenesis and cancer metastasis, including *MYC*, *CDK4*, *CDK6*, *E2F3*, *CREB* and *MET* [33,37,41]. Ectopic expression of miR-34 species induces cell-cycle arrest and apoptosis and suppresses cell growth and metastasis, possibly by silencing these target genes [28,33,37,39–41].

miR-124

Many studies have shown that mature miR-124 is the most abundant miRNA in the adult brain, and that it plays a key role in neurogenesis [42]. Conversely, epigenetic silencing of three miR-124 loci (miR-124-1 to -3) is frequently observed not only in brain tumors, but also in a variety of other cancer types [43–48], such as colon (prevalence: 75%), breast (32–50%), lung (48%), leukemia (36%) and lymphoma (41%). miR-124 loci are also hypermethylated in precancerous lesions. Methylation levels at miR-124 loci in the gastric mucosae of healthy volunteers infected by *Helicobacter pylori* are markedly elevated compared to healthy individuals without *H. pylori* infection [47]. Thus, *H. pylori* infection appears to induce aberrant epigenetic patterns at miRNA loci in normal gastric mucosae, which may contribute to gastric carcinogenesis as a 'field effect'. Targets of mature miR-124 include the 3′ UTR of *CDK6*, an oncogene. Epigenetically masking of miR-124 induces activation of *CDK6* and consequent phosphorylation of Rb at serine residues 807 and 811, the targets of CDK6, resulting in an acceleration of cell growth. Notably, in acute lymphoblastic leukemia, epigenetic silencing of miR-124 loci is linked to both disease-free and overall survival [31].

miR-137

Physiologically, miR-137 is involved in neurogenesis by targeting *CDK6*, analogous to miR-124 [43], as well as in melanocyte function by targeting microphthalmia-associated transcription factor [49]. miR-137 is an intragenic miRNA that is directly overlapped by a CpG island. The CpG island is specifically hypermethylated in cancer tissues [32,40,47]. Overexpression of miR-137 in cancer cells induces cell cycle G1 arrest and apoptosis [40]. Furthermore, a 15 nucleotide variable tandem repeat (VNTR) (5′-TAGCAGCGGC AGCGG-3′) is located just 5′ to pre-miR-137, and extending the length of this VNTR impairs the maturation of miR-137. Specifically, pri-miR-137 with three VNTRs is more efficiently processed to mature miR-137 than is pri-miR-137 with 12 VNTRs. Thus, both genomic and epigenetic variations affect mature miR-137 expression levels and may contribute to disease formation.

miR-148

Lujambio *et al.* [33] screened cancer metastasis-related miRNAs that are epigenetically inactivated, using a pharmacological epigenetic reversal technique in metastatic cancer cell lines, which identified three miRNAs, one of which is miR-148. The miR-148 locus is more heavily methylated in metastatic than in non-metastatic cancer tissues. Cancer cells that stably express exogenous miR-148 exhibit reduced invasiveness, cell motility and metastatic propensity in an *in vivo* model [33]. In addition, miR-148 targets TGFβ-inducing factor 2 (TGIF2), which is overexpressed in highly malignant ovarian cancers [50]. Thus, epigenetic inactivation of miR-148 would be expected to enhance TGIF2

activation. In addition, several isoforms of DNA methytransferase (DNMT)3b are targeted by miR-148 within their coding region (described in detail below). Therefore, although being targeted epigenetically, miR-148 may itself exert effects on DNA methylation in cells.

The miR-200 family

The miR-200 family consists of miR-141, 200a/b/c and 429, which share similar seed sequences. miRs-141/200c and miRs-200a/b/429 comprise multicistronic miRs whose genomic loci are located in close proximity to each other. Several studies have established that the miR-200 family is involved in epithelial–mesenchymal transition (EMT). EMT occurrence in cancer cells comprises a phenomenon in which these cells obtain phenotypes characteristic of mesenchymal cells, such as spindle-shaped morphology, activated cell motility and invasiveness. Therefore, EMT research is important for understanding the molecular mechanisms underlying the malignant potential of cancer cells. Recently, Wellner *et al.* [51] demonstrated that an EMT activator, ZEB1, suppresses miR-200c, whereas miR-200c targets ZEB1. This finding suggests that miR200c and ZEB1 form a feedback loop regulatory mechanism that maintains EMT [51]. Additional studies showed that both the miR-141/200c [52,53] and miR-200a/b/429 [53] clusters are epigenetically regulated. Thus, EMT could conceivably be regulated by epigenetic events targeting the miR-200 family. Table 1 shows that miR-200a/b/429 binding sites in the 3′ UTR of ZEB2 have several single nucleotide polymorphism (SNP) sites. However, to date, no study is available demonstrating the clinical significance of these SNPs.

miR-203

In hematopoietic malignancies, 12% of miRNAs are located in fragile genomic regions that encompass only seven megabases (0.2% of whole genome). miR-203 is one of these regions, and it targets *ABL1* and *BCR-ABL1*, an oncogenic fusion gene generated by the Philadelphia translocation [54]. Epigenetic silencing of miR-203 enhances activation of the *BCR-ABL1* fusion gene, resulting in an elevation of tumor cell growth rate. Epigenetic inactivation of miR-203 is frequently observed in other types of malignancies, including oral cancer, hepatocellular carcinoma, etc. [40,48]. Another candidate target gene of miR-203 is Bmi-1, a member of the polycomb repressor complex 1 [51], which is a histone modifier complex regulating gene expression. Introduction of ectopic miR-203 into cancer cells induces apoptosis and represses cell growth [48], possibly as a result of polycomb-mediated modification in epigenetic patterns.

let-7a-3

Epigenetic control of let-7a-3 expression was discovered by a comparison between parent and DNMT1-3B double-knockout HCT116 colon cancer cells [55]. The let-7a-3 locus is generally methylated in normal tissues but hypomethylated in some types of cancers, such as colon and lung cancer [55]. Methylation levels of let-7a-3 correlate inversely with let-7a-3 pri-miRNA expression levels [55]. However, the effect of let-7a-3 methylation status on mature let-7a expression level is unclear because levels of mature let-7a reflect the expression of three let-7a genes, let-7a-1, let-7a-2 and let-7a-3. Indeed, let-7a-3 methylation levels in ovarian cancer correlate with mature let-7a levels. In the context of miRNA function, let-7a-3 has oncogenic potential. The introduction of let-7a-3 enhanced the colony-forming ability of A549 lung adenocarcinoma cells. In addition, let-7a may regulate IGF-II via targeting of IGF2-binding proteins (IMP-1 and 2). Methylation levels at the let-7a-3 locus correlate inversely with IGF-II levels, and are also linked to the survival of ovarian cancer patients. In general, the let-7 family is considered to comprise tumor suppressor miRNAs [56–58]. Diversity in functions among let-7 family members may cause apparently contradictory observations.

Imprinting and miRNAs

Genomic imprinting is an epigenetic process by which a small proportion of genes (< 1% of all genes in mammals) are expressed in a parent-of-origin-specific manner [59]. In genomic imprinting, DNA methylation and histone modification regulate monoallelic expression. These epigenetic patterns are established in germline cells, and are inherited through somatic cells. For example, at the well-investigated *IGF2/H19* locus, the *IGF2* gene is expressed from the paternal allele, whereas the *H19* gene is expressed from the maternal allele. Abnormal genomic imprinting is associated with several diseases. Some inheritable disorders, such as Prader–Willi syndrome and Angelman syndrome, are caused by aberrant imprinting. Furthermore, the phenomenon known as loss of imprinting, in which the normally inactivated allele becomes reactivated as a result of hypomethylation or histone abnormalities, is frequently observed in cancers [60].

Several miRNAs are located within imprinting-associated regions, including miR-296 and miR-298 at the *GNAS/NESP* locus, miR-483 and miR-675 at the

IGF2/H19 locus, and miR-335, miR-29a and miR-29b at the *MEST/KLF14* locus [61]. However, the imprinting and expression status of such miRNAs remains largely unknown.

miRNAs regulating epigenetic pathway-related genes

miRNAs themselves are capable of targeting genes that control epigenetic pathways. As shown in Table 2, various miRNAs may control chromatin structure by regulating histone modifier molecules, such as polycomb group-related genes and histone deacetylase (HDAC). The polycomb group proteins are transcriptional repressors that regulate lineage choices occurring during development and differentiation. There are two polycomb repressor complexes (PRCs), PRC1 and PRC2. The PRC1 core complex contains Cbx, Mph, Ring, Bmi-1 and Mel18, whereas the PCR2 core complex consists of Ezh2, Suz12 and Eed [62]. In an initial step, PRC2 initiates silencing by catalyzing histone H3 Lysine-27 (H3K27) methylation. Recent studies have advanced our understanding of the means by which epigenomic dysregulation potentially contributes to various diseases.

EZH2

Expression levels of EZH2, a conserved catalytic subunit within PRC2, are elevated in cancers relative to corresponding normal tissues, with the highest EZH2 levels correlating with advanced disease stages and poor prognosis. In some cases, EZH2 overabundance is paralleled by DNA amplification of the gene [63]. A second mechanism of EZH2 overexpression is post-transcriptional regulation by miRNAs. EZH2 expression is controlled by miR-26a, miR-101, miR-205 and miR-214 [64–68]. Cancer-specific downregulation of these miRNAs results in overexpression of EZH2.

Table 2. miRNAs targeting genes that are involved in epigenetic regulatory pathways. The letters 'c' and 'p' with respect to miRNA binding site numbers indicate that the miRNA binding sites are the 'conserved region' and 'poorly conserved region' among vertebrates, according to the TargetScan database (http://www.targetscan.org/).

Target genes	miRNAs	Binding sites	References
EZH2	miR-26a	249c	[64–66,68]
	miR101	58p, 113c[a]	
	miR-214	172p	
Bmi1	miR-128	481c	[51,71]
	miR-203	1443c	
YY1	miR-29b	774c	[73]
HDAC1	miR-449	459p	[94]
HDAC4	miR-1	2333c, 3513c[a], 3546c[a]	[95]
DNMT3A	miR-29	855c	[79,80]
DNMT3B	miR-29	1202c	[79–81]
	miR-148	1424c and 2384c in coding region	
MeCP2	miR-132	6886c	[96]

[a] SNPs are located within the miRNA binding sites (not only the seed sequence regions, but also an approximately 23 bp region), which may affect the affinity of miRNA with the binding sites.

Bmi-1

In a subsequent step, PRC2 and the H3K27 methylation recruit PRC1 binding to chromatin to maintain stable gene silencing. PRC1 catalyzes ubiquitinylation of histone H2A and remains anchored to chromatin after its modification by the cooperation between PRC2 and PRC1. Bmi-1, a component of PRC1, plays an important role in gene silencing and is overexpressed in several cancers, including nonsmall cell lung cancer and colorectal cancer. Bmi-1 overexpression contributes to self-renewal in some types of cancer stem cells, including those of the pancreas [69], breast [70], brain [71] and white blood cell lineage [72]. Downregulation of miR-128 in glioma tissue causes elevated expression of Bmi-1, which consequently enhances self-renewal of the cancer stem cell population via chromatin remodeling [71]. In addition, recently, Wellner *et al.* [51] recently demonstrated that an EMT-related miRNA, miR-203, targets Bmi-1. This finding suggests that EMT mechanisms include the regulation of epigenetic regulators by miRNAs.

Yin Yang 1 (YY1)

YY1 is a transcription factor that contributes to various biological processes, including embryogenesis, the cell cycle, apoptosis, inflammation, carcinogenesis and epigenetics. In the epigenetic context, YY1 is a PRC-binding protein that recruits PRC2 and HDAC to a specific genome locus to induce chromatin remodeling. NF-κB-mediated miR-29b/c repression reactivates YY1 protein expression from post-transcriptional silencing induced by these two miRs. In addition, YY1 also represses miR-29b/c. This NF-κB-miR-29-YY1 regulatory circuit is also involved in myogenesis and tumorigenesis, probably via chromatin remodeling [73].

HDACs

In human cells, PRC2 physically associates with HDACs 1 and 2 [74]. If H3K27 is pre-acetylated, methylation at an H3K27 residue by PRC2 may

require deacetylation by HDACs. Thus, both acetylation and deacetylation of histones is involved in the transcriptional regulation of target genes. In addition, recent studies have demonstrated that HDACs target not only histone proteins, but also nonhistone proteins: p53 and Myo-D are targeted by HDAC-1, whereas Bcl-6, Stat3 and YY1 are targeted by HDAC-2. By regulating both histone and nonhistone proteins, HDACs 1 and 2, classified as class I HDACs, are implicated in cell proliferation, apoptosis and chemoresistance. The expression of HDACs 1 and 2 is elevated in various cancers [75]. However, the mechanism of HDAC overexpression remains unclear. Dysregulation of miRNAs may contribute to the overexpression of HDACs observed in cancer cells. In prostate cancer, HDAC-1 is a direct target of miR-449a, and downregulation of miR-449a causes overexpression of HDAC-1. Thus, aberrant expression of miR-449a may contribute to the abnormal epigenetic patterns occurring in prostate cancer.

DNMT 3A and 3B

DNMTs 1, 3A, and 3B are key DNA methylation enzymes. Recent studies in human cells have demonstrated that PRC2 and DNMTs are physically and functionally linked [76], and that DNMT-mediated DNA methylation lies downstream of PRC2-mediated H3K27 methylation [76,77]. Thus, these two key epigenetic repression systems cooperate in the silencing of target genes. Dysregulation of DNMTs has been linked to various disease processes, including cancer and congenital disorders. These DNMTs are predicted to be potential targets of miRNAs [78]. Fabbri *et al.* [79] showed that members of the miR-29 family directly target DNMTs 3A and 3B, and that exogenous miR-29 species can reactivate methylation-silenced tumor suppressor genes by restoring normal patterns of DNA methylation in nonsmall cell lung cancer cells. Another study reported similar findings in acute myeloid leukemia [80]. Thus, miRNAs may be involved in the establishment and/or maintenance of DNA methylation. In addition, some isoforms of DNMT3B are targeted at the penultimate exon of their coding regions by miR-148 [81]. DNMT3B exhibits several splicing isoforms, of which DNMT3B-1 and -3 are the most abundant. DNMT3B-1 possesses a catalytic domain and a miR-148 target site. Thus, DNMT3B-1 is a miR-148-sensitive isoform. By contrast, DNMT3B-3 lacks a catalytic domain and the miR-148 target site, and remains miR-148 resistant. The biological roles of different DNMT3B isoforms are not yet fully understood. However, this finding indicates that miRNAs can regulate gene expression uniquely among different gene isoforms by targeting a coding exon.

As described above and illustrated in Fig. 1, a number of miRNAs are regulated epigenetically. At the same time, a variety of miRNAs regulate epigenetic pathway-related molecules, most notably polycomb group proteins, HDACs and DNA methyltransferases. Taken together, post-transcriptional regulation by miRNAs and transcriptional control machinery by epigenetics cooperate with each other to organize the whole gene expression profile and to maintain physiological functions in cells. Once this miRNA–epigenetics regulatory circuit is disrupted, normal physiological functions are interfered with, contributing to various disease processes. A comprehensive elucidation of this regulatory network still remains to be completed. Therefore, continual studies on dysregulation of the miRNA–epigenetics regulatory circuitry would be highly beneficial for deepening our understanding of diseases.

Materials and methods

Typing of miRNAs by positional relationship to mRNA transcripts

Information about the localization and strand direction of 939 miRNAs, 35245 Refseq genes and 283708 mRNAs was retrieved from the genome browser of University of California Santa Cruz [82] on 31 January 2011. Because the original data table of refseq genes included miRNA genes, these miRNA data were excluded from the Refseq data set. Using MATLAB, version 2011a (Mathworks, Natick, MA, USA), we compared localization and strand direction between miRNAs and transcripts (Refseq genes and mRNAs). Intragenic and intergenic miRNAs were defined by whether the miRNAs are overlapped by transcripts, or not, respectively. In addition, intragenic miRNAs were divided into three different types, which are overlapped by transcripts only in the same strand direction, only in opposite direction, or in both directions, respectively. The complete results of this typing analysis are provided in Table S1.

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