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## ABSTRACT

Measuring the quantity of miRNAs in tissues of different physiological and pathological conditions is an important first step to investigate the functions of miRNAs. Matching samples from normal state provide essential baseline references to analyze the variation of miRNA abundance. We provided expression data of 345 miRNAs in 40 normal human tissues, which identified universally expressed miRNAs, and several groups of miRNAs expressed exclusively or preferentially in certain tissue types. Many miRNAs with co-regulated expression patterns are located in the same genomic clusters. Hierarchical clustering of normal tissues by their miRNA expression profiles basically followed the structure, anatomical locations, and physiological functions of the organs, suggesting that functions of a miRNA could be appreciated by linking to the biologies of the tissues in which it is uniquely expressed. Many predicted target genes of miRNAs that had specific reduced expression in brain and peripheral blood mononuclear cells are required for embryonic development of the nervous and hematopoietic systems based on database search. Up to one third of predicted target genes of three brain-specific miRNAs are either directly or indirectly involved in functions of the immune system and cellular response to inflammation.

## MATERIALS AND METHODS

**miRNA targets:** 345 human miRNAs.

**Total RNA:** Total RNAs of normal tissues were purchased from Ambion and Stratagene.

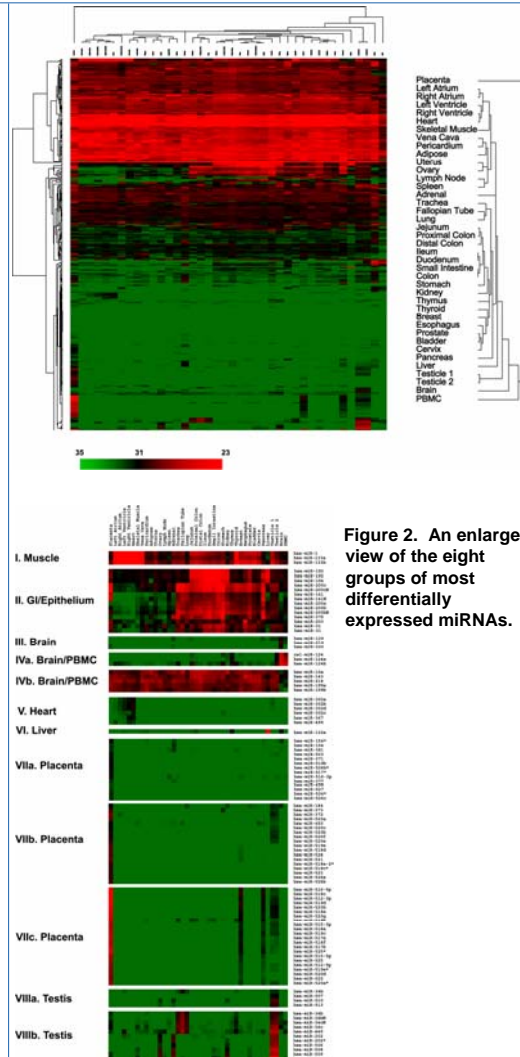
**RT-PCR:** TaqMan® MicroRNA Assays (Applied Biosystems) were performed following the instructions (ref. 1).

**Data analysis:** Agglomerative hierarchical clustering of data was performed using the Cluster and displayed using the TreeView (ref. 2). Prediction of miRNA target genes was based on miRBase (<http://microrna.sanger.ac.uk/cgi-bin/targets/v3/search.pl>).

## RESULTS

**Figure 1. Unsupervised hierarchical clustering of normal human tissues by variations of miRNA abundance.**

Normalized  $C_T$  for each assay was transformed into  $\Delta C_T$  against the average  $C_T$  of all assays examined and clustered without centering the data. A pseudocolor scale outlines the  $C_T$  values represented in the heat map. A detailed view of the clustering patterns of normal tissues is on the right.



**Table 1. Genomic locations of miRNAs in the 8 differentially expressed groups.**

Group	Ch	Start	End	Strand	Group	Ch	Start	End	Strand
<b>Group A</b>					<b>Group VIIa</b>				
Heim-133b	6	52121680	52121738	+	Heim-512a	15	72729815	72729828	+
Heim-133a-1	17	76956657	76956748	+	Heim-520b	19	58877077	58877083	+
Heim-133a-2	17	76956748	76956754	+	Heim-520c	19	58877083	58877089	+
Heim-133a-3	20	60561568	60562028	+	Heim-520f	19	58877225	58877311	+
Heim-133a-4	20	60561568	60561574	+	Heim-520g	19	58880431	58880437	+
Heim-133a-5	20	60561574	60561580	+	Heim-520h	19	58880437	58880443	+
Heim-133a-6	20	60561580	60561586	+	Heim-520i	19	58880443	58880449	+
Heim-133a-7	20	60561586	60561592	+	Heim-520j	19	58880623	58880629	+
Heim-133a-8	20	60561592	60561598	+	Heim-520k	19	58880629	58880635	+
Heim-133a-9	20	60561598	60561604	+	Heim-520l	19	58880635	58880641	+
Heim-133a-10	20	60561604	60561610	+	Heim-520m	19	58880641	58880647	+
Heim-133a-11	20	60561610	60561616	+	Heim-520n	19	58880647	58880653	+
Heim-133a-12	20	60561616	60561622	+	Heim-520o	19	58880653	58880659	+
Heim-133a-13	20	60561622	60561628	+	Heim-520p	19	58880659	58880665	+
Heim-133a-14	20	60561628	60561634	+	Heim-520q	19	58880665	58880671	+
Heim-133a-15	20	60561634	60561640	+	Heim-520r	19	58880671	58880677	+
Heim-133a-16	20	60561640	60561646	+	Heim-520s	19	58880677	58880683	+
Heim-133a-17	20	60561646	60561652	+	Heim-520t	19	58880683	58880689	+
Heim-133a-18	20	60561652	60561658	+	Heim-520u	19	58880689	58880695	+
Heim-133a-19	20	60561658	60561664	+	Heim-520v	19	58880695	58880701	+
Heim-133a-20	20	60561664	60561670	+	Heim-520w	19	58880701	58880707	+
Heim-133a-21	20	60561670	60561676	+	Heim-520x	19	58880707	58880713	+
Heim-133a-22	20	60561676	60561682	+	Heim-520y	19	58880713	58880719	+
Heim-133a-23	20	60561682	60561688	+	Heim-520z	19	58880719	58880725	+
Heim-133a-24	20	60561688	60561694	+	Heim-520aa	19	58880725	58880731	+
Heim-133a-25	20	60561694	60561700	+	Heim-520ab	19	58880731	58880737	+
Heim-133a-26	20	60561700	60561706	+	Heim-520ac	19	58880737	58880743	+
Heim-133a-27	20	60561706	60561712	+	Heim-520ad	19	58880743	58880749	+
Heim-133a-28	20	60561712	60561718	+	Heim-520ae	19	58880749	58880755	+
Heim-133a-29	20	60561718	60561724	+	Heim-520af	19	58880755	58880761	+
Heim-133a-30	20	60561724	60561730	+	Heim-520ag	19	58880761	58880767	+
Heim-133a-31	20	60561730	60561736	+	Heim-520ah	19	58880767	58880773	+
Heim-133a-32	20	60561736	60561742	+	Heim-520ai	19	58880773	58880779	+
Heim-133a-33	20	60561742	60561748	+	Heim-520aj	19	58880779	58880785	+
Heim-133a-34	20	60561748	60561754	+	Heim-520ak	19	58880785	58880791	+
Heim-133a-35	20	60561754	60561760	+	Heim-520al	19	58880791	58880797	+
Heim-133a-36	20	60561760	60561766	+	Heim-520am	19	58880797	58880803	+
Heim-133a-37	20	60561766	60561772	+	Heim-520an	19	58880803	58880809	+
Heim-133a-38	20	60561772	60561778	+	Heim-520ao	19	58880809	58880815	+
Heim-133a-39	20	60561778	60561784	+	Heim-520ap	19	58880815	58880821	+
Heim-133a-40	20	60561784	60561790	+	Heim-520aq	19	58880821	58880827	+
Heim-133a-41	20	60561790	60561796	+	Heim-520ar	19	58880827	58880833	+
Heim-133a-42	20	60561796	60561802	+	Heim-520as	19	58880833	58880839	+
Heim-133a-43	20	60561802	60561808	+	Heim-520at	19	58880839	58880845	+
Heim-133a-44	20	60561808	60561814	+	Heim-520au	19	58880845	58880851	+
Heim-133a-45	20	60561814	60561820	+	Heim-520av	19	58880851	58880857	+
Heim-133a-46	20	60561820	60561826	+	Heim-520aw	19	58880857	58880863	+
Heim-133a-47	20	60561826	60561832	+	Heim-520ax	19	58880863	58880869	+
Heim-133a-48	20	60561832	60561838	+	Heim-520ay	19	58880869	58880875	+
Heim-133a-49	20	60561838	60561844	+	Heim-520az	19	58880875	58880881	+
Heim-133a-50	20	60561844	60561850	+	Heim-520ba	19	58880881	58880887	+
Heim-133a-51	20	60561850	60561856	+	Heim-520bb	19	58880887	58880893	+
Heim-133a-52	20	60561856	60561862	+	Heim-520bc	19	58880893	58880899	+
Heim-133a-53	20	60561862	60561868	+	Heim-520bd	19	58880899	58880905	+
Heim-133a-54	20	60561868	60561874	+	Heim-520be	19	58880905	58880911	+
Heim-133a-55	20	60561874	60561880	+	Heim-520bf	19	58880911	58880917	+
Heim-133a-56	20	60561880	60561886	+	Heim-520bg	19	58880917	58880923	+
Heim-133a-57	20	60561886	60561892	+	Heim-520bh	19	58880923	58880929	+
Heim-133a-58	20	60561892	60561898	+	Heim-520bi	19	58880929	58880935	+
Heim-133a-59	20	60561898	60561904	+	Heim-520bj	19	58880935	58880941	+
Heim-133a-60	20	60561904	60561910	+	Heim-520bk	19	58880941	58880947	+
Heim-133a-61	20	60561910	60561916	+	Heim-520bl	19	58880947	58880953	+
Heim-133a-62	20	60561916	60561922	+	Heim-520bm	19	58880953	58880959	+
Heim-133a-63	20	60561922	60561928	+	Heim-520bn	19	58880959	58880965	+
Heim-133a-64	20	60561928	60561934	+	Heim-520bo	19	58880965	58880971	+
Heim-133a-65	20	60561934	60561940	+	Heim-520bp	19	58880971	58880977	+
Heim-133a-66	20	60561940	60561946	+	Heim-520bq	19	58880977	58880983	+
Heim-133a-67	20	60561946	60561952	+	Heim-520br	19	58880983	58880989	+
Heim-133a-68	20	60561952	60561958	+	Heim-520bs	19	58880989	58880995	+
Heim-133a-69	20	60561958	60561964	+	Heim-520bt	19	58880995	58881001	+
Heim-133a-70	20	60561964	60561970	+	Heim-520bu	19	58881001	58881007	+
Heim-133a-71	20	60561970	60561976	+	Heim-520bv	19	58881007	58881013	+
Heim-133a-72	20	60561976	60561982	+	Heim-520bw	19	58881013	58881019	+
Heim-133a-73	20	60561982	60561988	+	Heim-520bx	19	58881019	58881025	+
Heim-133a-74	20	60561988	60561994	+	Heim-520by	19	58881025	58881031	+
Heim-133a-75	20	60561994	60562000	+	Heim-520bz	19	58881031	58881037	+
Heim-133a-76	20	60562000	60562006	+	Heim-520ca	19	58881037	58881043	+
Heim-133a-77	20	60562006	60562012	+	Heim-520cb	19	58881043	58881049	+
Heim-133a-78	20	60562012	60562018	+	Heim-520cc	19	58881049	58881055	+
Heim-133a-79	20	60562018	60562024	+	Heim-520cd	19	58881055	58881061	+
Heim-133a-80	20	60562024	60562030	+	Heim-520ce	19	58881061	58881067	+
Heim-133a-81	20	60562030	60562036	+	Heim-520cf	19	58881067	58881073	+
Heim-133a-82	20	60562036	60562042	+	Heim-520cg	19	58881073	58881079	+
Heim-133a-83	20	60562042	60562048	+	Heim-520ch	19	58881079	58881085	+
Heim-133a-84	20	60562048	60562054	+	Heim-520ci	19	58881085	58881091	+
Heim-133a-85	20	60562054	60562060	+	Heim-520cj	19	58881091	58881097	+
Heim-133a-86	20	60562060	60562066	+	Heim-520ck	19	58881097	58881103	+
Heim-133a-87	20	60562066	60562072	+	Heim-520cl	19	58881103	58881109	+
Heim-133a-88	20	60562072	60562078	+	Heim-520cm	19	58881109	58881115	+
Heim-133a-89	20	60562078	60562084	+	Heim-520cn	19	58881115	58881121	+
Heim-133a-90	20	60562084	60562090	+	Heim-520co	19	58881121	58881127	+
Heim-133a-91	20	60562090	60562096	+	Heim-520cp	19	58881127	58881133	+
Heim-133a-92	20	60562096	60562102	+	Heim-520cq	19	58881133	58881139	+
Heim-133a-93	20	60562102	60562108	+	Heim-520cr	19	58881139	58881145	+
Heim-133a-94	20	60562108	60562114	+	Heim-520cs	19	58881145	58881151	+
Heim-133a-95	20	60562114	60562120	+	Heim-520ct	19	58881151	58881157	+
Heim-133a-96	20	60562120	60562126	+	Heim-520cu	19	58881157	58881163	+
Heim-133a-97	20	60562126	60562132	+	Heim-520cv	19	58881163	58881169	+
Heim-133a-98	20	60562132	60562138	+	Heim-520cw	19	58881169	58881175	+
Heim-133a-99	20	60562138	60562144	+	Heim-520cx	19	58881175	58881181	+
Heim-133a-100	20	60562144	60562150	+	Heim-520cy	19	58881181	58881187	+
Heim-133a-101	20	60562150	60562156	+	Heim-520cz	19	58881187	58881193	+
Heim-133a-102	20	60562156	60562162	+	Heim-520da	19	58881193	58881199	+
Heim-133a-103	20	60562162	60562168	+	Heim-520db	19	58881199	58881205	+
Heim-133a-104	20	60562168	60562174	+	Heim-520dc	19	58881205	58881211	+
Heim-133a-105	20	60562174	60562180	+	Heim-520dd	19	58881211	58881217	+
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Heim-133a-107	20	60562186	60562192	+	Heim-520df	19	58881223	58881229	+
Heim-133a-108	20	60562192	60562198	+	Heim-520dg	19	58881229	58881235	+
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Heim-133a-110	20	60562204	60562210	+	Heim-520di	19	58881241	58881247	+
Heim-133a-111	20	60562210	60562216	+	Heim-520dj	19	58881247	58881253	+
Heim-133a-112	20	60562216	60562222	+	Heim-520dk	19	58881253	58881259	+
Heim-133a-113	20	60562222	60562228	+	Heim-520dl	19	58881259	58881265	+
Heim-133a-114	20	60562228	60562234	+	Heim-520dm	19	588812		

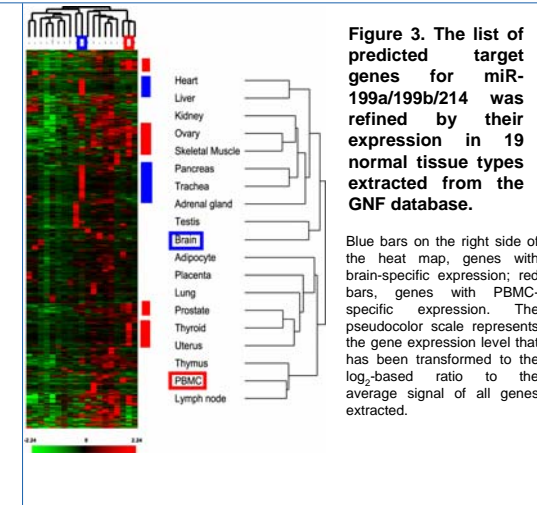
\*Whether each expression group was enriched in miRNAs located in genomic clusters was evaluated by Chi test. miRNAs in different subgroups were analysed together in one test as long as they are located within a genomic cluster.

**Table 2. Correlation of expression patterns in human normal tissues between intronic miRNAs and their host genes.**

[illegible]

\*When multiple clones are available in the database, the clone with the best p value was chosen.

<sup>\*\*\*</sup>Pearson correlation; gray boxes indicate those with p values > 0.05.



**Figure 3.** The list of predicted target genes for miR-199a/199b/214 was refined by their expression in 19 normal tissue types extracted from the GNF database.

Blue bars on the right side of the heat map, genes with brain-specific expression; red bars, genes with PBMC-specific expression. The pseudocolor scale represents the gene expression level that has been transformed to the  $\log_2$ -based ratio to the average signal of all genes extracted.

**Table 3. Classification of identified predicted target genes of 129/219/330 based upon their functions.**

[illegible]

## REFERENCES

1. Chen, C, et al. *Nucleic Acids Res.* 2005. 33(20):e179.
2. Eisen, M, et al. *PNAS.* 1998. 95:14863.

## ACKNOWLEDGEMENTS

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