10.1148/radiol.2016160024

Appendix E1

Cell Culture

The MatLyLu cell line was kindly provided by John Isaacs, PhD (Johns Hopkins University, Baltimore, Md), and was maintained in RPMI 1640 medium with 10% fetal bovine serum, 2 mmol/L of L-glutamine, 100 U/mL of penicillin, 100 U/mL of streptomycin, and 250 nmol/L of dexamethasone (Sigma 2915, St Louis, Mo) (1). The cells were found to be negative for rodent pathogens (Impact V rat panel, Idexx Bioresearch, Columbia, Mo).

Model System

We sought to overcome two limiting features of existing mouse models of circulating miRNA biomarkers (2): *(a)* low available blood volume, which restricts the number of collections possible, and *(b)* blood collection via a tail vein or submandibular bleeds, which may contaminate samples with skin cells. Therefore, we chose to use a rat model system with surgically implanted jugular vein polyurethane catheters (Charles River Laboratories, Wilmington, Mass). (Of note, we observed that polyurethane catheters remained more patent and showed more resistance to blockage relative to other materials available from the vendor.) This increased the available blood volume by approximately tenfold and enabled serial puncture-free blood collection (thus avoiding contamination by skin tissue, etc). Cannulas were flushed per the vendor protocol by using taurolidine citrate (Access Technologies, Skokie, Ill) as a lock solution to avoid downstream PCR inhibition by heparin. The MatLyLu derivative of the Dunning rat prostate cancer model was selected because of its rapid growth of syngeneic tumors, which prevents the expiration of cannula patency and enables the use of immunocompetent rats (1).

Passive Cavitation Detection and Signal Processing

A miniature flat passive cavitation detector was positioned at the side of the transducer and aligned to detect broadband emissions from inertially collapsing bubbles in the focal area during each focused US pulse. The passive cavitation detector was 3 mm in aperture, with the frequency band of 6.3–14.2 MHz at a 6-dB level (Panametrics XMS-310; Olympus, Waltham, Mass). The signals received by the passive cavitation detector were amplified by 20 dB (Panametrics PR5072; Olympus) and recorded by using a digital oscilloscope.

RNA Isolation

Lysates were thawed on ice, followed by thorough homogenization at room temperature (3). A mixture of three synthetic *C. elegans* miRNA oligoribonucleotides (4) and MS/2 carrier RNA (5) (10165948001; Roche, Basel, Switzerland) formulated in Qiazol (Qiagen, Hilden, Germany) was then added to the samples, and the solution was rehomogenized. Total RNA was isolated from these samples and Qiazol lysates of the MatLyLu cell line by using the miRNeasy kit (Qiagen) with minor modifications as described previously (3) (J.R.C.).

miRNA Profiling and Identification of Candidate miRNA Biomarkers

RNA derived from four samples of untreated rat plasma and the MatLyLu cell line were used for discovery of candidate biomarkers (J.R.C., M.D.G.). Samples were profiled for the relative abundance of 375 miRNAs by using microRNA ready-to-use PCR, human panel I, V2.M RT-qPCR arrays (Exiqon, Vedbaek, Denmark). Thirteen microliters of each sample were reverse transcribed with the miRCURY LNA Universal RT microRNA PCR kit (Exiqon), as directed. Reverse transcription products were combined with SYBR Green master mix and loaded into the 384-well RT-qPCR arrays. Quantitative PCR was performed on a 7900HT instrument (Applied Biosystems, Foster City, Calif). In addition, each RNA sample was separately assayed for the spike-in oligonucleotide *cel*-miR-39 by TaqMan RT-qPCR (Applied Biosystems), as described previously (4). Data from all miRNA RT-qPCR arrays were imported into SDS Enterprise software (V2.2.2; Applied Biosystems), and cycle threshold values were calculated by using automated, assay-specific baseline and threshold settings. The difference between the mean plasma cycle threshold and cell line cycle threshold was calculated for each miRNA, and five of the top 10 miRNAs that showed the greatest difference in cycle threshold values were selected as candidate biomarkers: miR-34c, miR-100, miR-129–5p, miR-196a, and miR-9 (Fig 5). These biomarkers were undetectable or had low abundance in untreated rat plasma and high abundance in the cell line. To select among the top 10 miRNAs, those with previously reported high expression in any blood cell type were excluded (6), and those for which in-house assays were available were given preference. In our laboratory, we have observed that miR-502–5p and miR-582–5p are present in whole blood, neutrophils, and monocytes (6) (data available on request). In the same study, we observed that miR-196b is present in whole blood and neutrophils. A miR-9* assay was not readily available in-house at the time of analysis. We initially sought to include the analysis of miR-129–3p, but preliminary evaluation of the assay for this miRNA showed inconsistent results. Because of this technical concern and the reasonable performance of the assays for the other miRNAs, we chose not to pursue miR-129–3p further.

Individual RT-qPCR Assays

TaqMan assays (Applied Biosystems) for human miRNAs (identical to the rat sequences) *hsa*-miR-16, *hsa*-miR-34c, *hsa*-miR-100, *hsa*-miR-129–5p, and *hsa*-miR-196a, in addition to *C. elegans* miRNA *cel*-miR-39, were obtained from Applied Biosystems. Oligoribonucleotides that corresponded to the mature sequence of each miRNA were synthesized (Integrated DNA Technologies) and serially diluted for standard curves (3). Individual miRNAs were detected by using RT-qPCR, as described previously (4), on a ViiA 7 instrument (Applied Biosystems). Cycle threshold values were calculated by the ViiA 7 signal-processing algorithm (v1.0; Applied Biosystems) to automatically call baseline and threshold with ROX (carboxy-x-rhodamine passive reference dye; Applied Biosystems) normalization. Undetermined cycle thresholds were arbitrarily set to 40 as an estimate of the maximum possible abundance of target. The concentration of analyte was calculated by comparing the experimental cycle threshold to that of the standard curve, and results were presented as the mean of the PCR duplicates. *Cel*-miR-39 normalization was used to correct for variations in RNA recovery during purification, per our standard protocol (4) (J.R.C., M.D.G.).

Statistical Analysis

For the results presented in Figure 6, the data for an individual treatment were compared against those for the mock treatment at the corresponding time point by using one-way analysis of variance (Kruskal-Wallis test), followed by the Dunn multiple-comparisons test. For the results presented in Figure 7, the data for tumor liquefaction with pulsed focused US at each time point were compared with the pretreatment values by using one-way analysis of variance (Friedman test), followed by the Dunn multiple-comparisons test. Nonparametric tests were chosen because of the relatively small sample sizes and the non-Gaussian distribution of data, in addition to providing conservative estimates of the *P* values. All statistical analysis was performed by using Prism 7.0a software (GraphPad Software, La Jolla, Calif) with criteria for significance given as *P* up to .05, *P* up to .01, or *P* up to .001 (J.R.C., T.D.K.).

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Table E1. Identification of Candidate Tumor Biomarkers in the MatLyLu Rat Model System

Rank	Target Name	Plasma Cycle Threshold	MatLyLu Cycle Threshold	Difference in Cycle Threshold Values
1	hsa-miR-129–3p	39.8	21.6	18.2
2	hsa-miR-129–5p†	39.8	23.0	16.8
3	hsa-miR-196a†	38.5	21.7	16.8
4	hsa-miR-9†	38.9	22.7	16.1
5	hsa-miR-34c-5p†	36.2	21.7	14.5
6	hsa-miR-502–5p	37.9	23.7	14.2
7	hsa-miR-100†	38.0	24.9	13.1
8	hsa-miR-582–5p	35.9	23.3	12.6
9	hsa-miR-9*	39.8	27.2	12.5
10	hsa-miR-196b	36.1	23.6	12.5
11	hsa-miR-190	38.0	26.0	12.0
12	hsa-miR-708	37.8	25.8	12.0
13	hsa-miR-221	30.7	19.1	11.7
14	hsa-miR-98	36.6	25.6	11.0
15	hsa-miR-199a-5p	35.3	24.3	11.0
16	hsa-miR-125b	27.3	16.7	10.7

17	hsa-miR-31	30.2	19.5	10.7
18	hsa-miR-34b	34.5	23.9	10.5
19	hsa-miR-424	29.9	19.6	10.4
20	hsa-miR-218	35.3	25.0	10.2
21	hsa-miR-29b	32.3	22.2	10.1
22	hsa-miR-449a	36.0	25.9	10.1
23	hsa-let-7e	32.4	22.5	9.9
24	hsa-miR-153	39.8	30.1	9.7
25	hsa-miR-34a	33.3	23.6	9.7
26	hsa-miR-10a	34.2	24.5	9.7
27	hsa-let-7f	29.8	20.2	9.6
28	hsa-miR-210	29.2	19.8	9.4
29	hsa-miR-450a	32.3	23.0	9.3
30	hsa-miR-21	26.0	16.7	9.3
31	hsa-let-7a	30.6	21.3	9.3
32	hsa-miR-26a-2*	39.2	30.0	9.2
33	hsa-miR-7	35.1	26.0	9.1
34	hsa-miR-31*	31.6	22.6	9.0
35	hsa-let-7i	29.8	20.8	9.0
36	hsa-miR-301b	39.8	31.0	8.8
37	hsa-miR-431	39.1	30.3	8.8
38	hsa-miR-615–3p	34.3	25.6	8.7
39	hsa-miR-125a-5p	30.1	21.4	8.7
40	hsa-miR-542–5p	38.2	29.5	8.7
41	hsa-let-7d	29.7	21.1	8.6
42	hsa-miR-222	30.3	21.9	8.4
43	hsa-miR-665	39.7	31.4	8.3
44	hsa-miR-33b	39.8	31.5	8.3
45	hsa-let-7c	29.1	20.8	8.2
46	hsa-miR-29a	28.4	20.2	8.2
47	hsa-miR-30c-2*	37.5	29.3	8.2
48	hsa-miR-1979-obsolete in V16	29.6	21.4	8.1
49	hsa-miR-130b	34.2	26.1	8.1
50	hsa-miR-211	39.7	31.5	8.1
51	hsa-miR-99b	30.5	22.4	8.1
52	hsa-miR-28–5p	34.8	26.8	8.0
53	hsa-miR-130a	31.8	23.8	8.0
54	hsa-miR-374b	32.6	24.7	8.0
55	hsa-miR-193a-3p	34.9	26.9	8.0
56	hsa-miR-149	30.9	23.2	7.7
57	hsa-miR-151–5p	31.5	23.8	7.7
58	hsa-miR-29c	29.3	21.6	7.7
59	hsa-miR-365	29.2	21.6	7.7
60	hsa-miR-99a	29.7	22.1	7.6
61	hsa-let-7b	27.1	19.5	7.6
62	hsa-miR-493	39.8	32.2	7.6
63	hsa-miR-29b-2*	37.8	30.4	7.5
64	hsa-miR-199b-5p	36.2	28.8	7.4
65	hsa-miR-148b	30.7	23.4	7.4
66	hsa-miR-421	33.8	26.5	7.3

67	hsa-miR-152	30.3	23.0	7.3
68	hsa-miR-27b	26.8	19.6	7.2
69	hsa-miR-501–5p	38.6	31.4	7.2
70	hsa-miR-107	29.5	22.2	7.2
71	hsa-miR-324–5p	31.5	24.3	7.2
72	hsa-miR-23b	27.4	20.3	7.2
73	hsa-miR-27a	26.1	19.0	7.2
74	hsa-miR-181a	29.5	22.4	7.2
75	hsa-miR-449b	38.7	31.6	7.1
76	hsa-miR-602	39.8	32.8	7.0
77	hsa-miR-214	32.2	25.2	7.0
78	hsa-miR-301a	30.1	23.1	7.0
79	hsa-miR-337–3p	34.9	27.9	7.0
80	hsa-miR-30b*	39.8	32.8	7.0
81	hsa-miR-181a*	36.0	29.0	7.0
82	hsa-miR-26a	27.0	20.1	6.9
83	hsa-miR-499–5p	37.6	30.8	6.8
84	hsa-miR-103	28.0	21.2	6.8
85	hsa-miR-629	39.7	32.9	6.7
86	hsa-miR-106b	31.8	25.1	6.7
87	hsa-miR-589	37.3	30.6	6.7
88	hsa-miR-34c-3p	38.6	31.9	6.7
89	hsa-miR-181b	32.1	25.4	6.7
90	hsa-miR-181c	34.7	28.1	6.6
91	hsa-miR-506	39.8	33.2	6.6
92	hsa-miR-199a-3p	30.3	23.8	6.5
93	hsa-miR-23a	25.3	18.8	6.5
94	hsa-miR-361–3p	37.8	31.4	6.4
95	hsa-miR-891a	39.8	33.4	6.4
96	hsa-miR-216a	39.1	32.8	6.3
97	hsa-miR-15b	27.6	21.3	6.3
98	hsa-miR-503	39.1	32.9	6.2
99	hsa-miR-33a	32.8	26.6	6.2
100	hsa-miR-324–3p	31.1	25.0	6.2
101	hsa-miR-514	39.8	33.7	6.1
102	hsa-miR-155	39.3	33.2	6.1
103	hsa-miR-24	24.3	18.2	6.1
104	hsa-miR-425*	37.0	31.1	6.0
105	hsa-miR-15a	27.4	21.4	6.0
106	hsa-let-7 g	26.7	20.7	6.0
107	hsa-miR-662	38.8	32.8	6.0
108	hsa-miR-26b	29.6	23.7	5.9
109	hsa-miR-652	29.8	23.9	5.9
110	hsa-miR-17	30.1	24.3	5.9
111	hsa-miR-18a	30.4	24.6	5.9
112	hsa-miR-22	29.9	24.1	5.8
113	hsa-miR-425	28.9	23.0	5.8
114	hsa-miR-766	39.7	33.9	5.8
115	hsa-miR-940	33.3	27.6	5.7
116	hsa-miR-631	39.8	34.1	5.7

117	hsa-miR-219–5p	32.6	26.9	5.7
118	hsa-miR-19a	30.8	25.1	5.7
119	hsa-miR-532–5p	33.6	28.0	5.6
120	hsa-miR-516b	39.8	34.2	5.6
121	hsa-miR-663	34.2	28.6	5.6
122	hsa-miR-106a	26.0	20.4	5.6
123	hsa-miR-30b	25.7	20.1	5.6
124	hsa-miR-30a	30.2	24.6	5.6
125	hsa-miR-30d	29.1	23.6	5.6
126	hsa-miR-671–5p	34.3	28.7	5.6
127	hsa-miR-99a*	39.8	34.2	5.6
128	hsa-miR-373*	37.4	31.8	5.6
129	hsa-miR-185*	39.3	33.8	5.6
130	hsa-miR-518f	39.8	34.3	5.5
131	hsa-miR-101	29.0	23.4	5.5
132	hsa-miR-483–3p	38.5	32.9	5.5
133	hsa-miR-20a	25.5	20.0	5.5
134	hsa-miR-186	29.8	24.3	5.5
135	hsa-miR-362–5p	38.8	33.3	5.5
136	hsa-miR-550a	39.8	34.4	5.4
137	hsa-miR-30c	26.2	20.8	5.4
138	hsa-miR-18b	29.0	23.6	5.3
139	hsa-miR-331–3p	31.2	25.9	5.3
140	hsa-miR-335	33.9	28.6	5.3
141	hsa-miR-93	26.3	21.0	5.3
142	hsa-miR-216b	39.8	34.5	5.3
143	hsa-miR-10b	32.6	27.3	5.3
144	hsa-miR-19b	25.0	19.7	5.3
145	hsa-miR-188–5p	36.1	30.8	5.3
146	hsa-miR-181d	32.2	26.9	5.3
147	hsa-miR-146b-5p	34.3	29.0	5.3
148	hsa-miR-628–3p	39.8	34.5	5.3
149	hsa-miR-143	29.5	24.3	5.2
150	hsa-miR-518c*	39.8	34.6	5.1
151	hsa-let-7d*	27.1	22.0	5.1
152	hsa-miR-760	36.7	31.6	5.1
153	hsa-miR-191	26.2	21.2	5.0
154	hsa-miR-193b	30.3	25.3	5.0
155	hsa-miR-132	32.8	27.8	4.9
156	hsa-miR-18a*	32.0	27.1	4.9
157	hsa-miR-887	39.8	34.9	4.9
158	hsa-miR-744	31.2	26.3	4.9
159	hsa-miR-608	39.8	34.9	4.9
160	hsa-miR-32	32.7	27.8	4.9
161	hsa-miR-127–5p	39.7	34.9	4.9
162	hsa-miR-30e*	31.1	26.3	4.8
163	hsa-miR-410	39.8	35.0	4.8
164	hsa-miR-509–3p	38.0	33.2	4.8
165	hsa-miR-151–3p	39.8	35.0	4.8
166	hsa-miR-873	39.8	35.1	4.7

167	hsa-miR-21*	39.8	35.1	4.7
168	hsa-miR-596	38.8	34.2	4.7
169	hsa-miR-22*	31.6	27.0	4.6
170	hsa-miR-411	39.6	35.0	4.5
171	hsa-miR-320a	26.0	21.5	4.5
172	hsa-miR-298	39.8	35.3	4.5
173	hsa-miR-423–5p	30.6	26.1	4.5
174	hsa-miR-720	24.6	20.1	4.5
175	hsa-miR-25	26.9	22.5	4.5
176	hsa-miR-510	39.8	35.3	4.4
177	hsa-miR-423–3p	28.1	23.7	4.4
178	hsa-miR-877	35.1	30.7	4.4
179	hsa-miR-572	39.3	34.9	4.4
180	hsa-miR-204	33.8	29.4	4.4
181	hsa-miR-202	39.7	35.4	4.4
182	hsa-miR-371–5p	39.2	34.8	4.4
183	hsa-miR-193a-5p	39.8	35.4	4.4
184	hsa-miR-185	29.0	24.7	4.4
185	hsa-miR-30e	30.8	26.6	4.2
186	hsa-miR-370	39.0	34.8	4.2
187	hsa-miR-345	39.8	35.6	4.2
188	hsa-miR-326	30.7	26.5	4.1
189	hsa-miR-187*	38.8	34.8	4.0
190	hsa-miR-433	39.2	35.3	3.9
191	hsa-miR-16	23.7	19.8	3.9
192	hsa-miR-302b	39.8	36.0	3.8
193	hsa-miR-886–5p-obsolete in V16	38.2	34.4	3.8
194	hsa-miR-187	34.2	30.4	3.8
195	hsa-miR-147b	33.4	29.6	3.7
196	hsa-miR-484	28.2	24.5	3.7
197	hsa-miR-140–5p	28.7	25.1	3.7
198	hsa-miR-455–5p	31.5	27.9	3.6
199	hsa-miR-617	39.8	36.2	3.6
200	hsa-miR-885–5p	39.8	36.3	3.5
201	hsa-miR-518e	39.8	36.3	3.5
202	hsa-miR-505	30.0	26.6	3.5
203	hsa-miR-328	27.5	24.1	3.4
204	hsa-miR-517a	39.8	36.4	3.4
205	hsa-miR-194	31.3	27.9	3.4
206	hsa-miR-765	38.5	35.2	3.3
207	hsa-miR-128	29.7	26.3	3.3
208	hsa-miR-888	37.2	33.9	3.3
209	hsa-miR-342–3p	28.1	24.8	3.3
210	hsa-miR-339–5p	29.4	26.1	3.2
211	hsa-miR-92a	24.8	21.6	3.2
212	hsa-miR-20b*	34.4	31.2	3.1
213	hsa-miR-487b	39.1	36.0	3.1
214	hsa-miR-376c	39.8	36.7	3.1
215	hsa-miR-584	39.8	36.7	3.1
216	hsa-miR-409–3p	38.8	35.7	3.1

217	hsa-miR-96	39.0	36.0	3.0
218	hsa-miR-374b*	39.8	36.8	3.0
219	hsa-miR-376b	39.8	36.9	2.9
220	hsa-miR-668	39.1	36.3	2.8
221	hsa-miR-147	36.3	33.6	2.7
222	hsa-miR-369–5p	39.8	37.1	2.7
223	hsa-miR-124	36.2	33.5	2.7
224	hsa-miR-623	39.8	37.2	2.6
225	hsa-miR-198	39.8	37.2	2.6
226	hsa-miR-374a	39.8	37.2	2.6
227	hsa-miR-138	31.4	28.9	2.5
228	hsa-miR-491–5p	37.8	35.3	2.5
229	hsa-miR-497	32.3	29.8	2.5
230	hsa-miR-595	39.5	37.1	2.4
231	hsa-miR-184	39.6	37.2	2.3
232	hsa-miR-574–3p	28.6	26.3	2.3
233	hsa-miR-361–5p	39.8	37.5	2.3
234	hsa-miR-154*	39.8	37.5	2.3
235	hsa-miR-570	39.8	37.5	2.3
236	hsa-miR-548b-3p	39.4	37.1	2.3
237	hsa-miR-145	26.0	23.8	2.2
238	hsa-miR-654–5p	39.8	37.6	2.2
239	hsa-miR-576–3p	39.8	37.6	2.2
240	hsa-miR-302d*	39.8	37.7	2.1
241	hsa-miR-299–3p	39.8	37.8	2.0
242	hsa-miR-323–3p	39.1	37.0	2.0
243	hsa-miR-452	37.7	35.8	1.9
244	hsa-miR-329	38.1	36.3	1.8
245	hsa-miR-217	39.8	38.0	1.8
246	hsa-miR-92b	34.0	32.3	1.7
247	hsa-miR-627	39.8	38.1	1.7
248	hsa-miR-302a	37.6	36.0	1.6
249	hsa-miR-377	39.8	38.2	1.6
250	hsa-miR-183	37.1	35.6	1.5
251	hsa-miR-299–5p	38.7	37.3	1.5
252	hsa-miR-127–3p	37.2	35.7	1.4
253	hsa-miR-524–5p	36.6	35.2	1.4
254	hsa-miR-381	38.4	37.0	1.4
255	hsa-miR-518c	39.8	38.4	1.3
256	hsa-miR-154	35.8	34.4	1.3
257	hsa-miR-583	39.8	38.5	1.3
258	hsa-miR-525–5p	37.7	36.4	1.3
259	hsa-miR-126	26.5	25.3	1.2
260	hsa-miR-302c*	38.9	37.8	1.2
261	hsa-miR-212	33.6	32.5	1.1
262	hsa-miR-517c	39.0	38.1	1.0
263	hsa-miR-520d-5p	39.7	38.7	1.0
264	hsa-miR-182	35.3	34.4	0.8
265	hsa-miR-382	35.5	34.7	0.8
266	hsa-miR-518a-3p	39.8	39.0	0.8

267	hsa-miR-508–3p	39.8	39.1	0.7
268	hsa-miR-379	39.0	38.3	0.7
269	hsa-miR-376a	37.9	37.2	0.7
270	hsa-miR-105	39.8	39.1	0.6
271	hsa-miR-140–3p	25.7	25.1	0.6
272	hsa-miR-195	29.7	29.1	0.6
273	hsa-miR-490–3p	35.2	34.7	0.6
274	hsa-miR-622	34.1	33.6	0.6
275	hsa-miR-513a-5p	39.8	39.3	0.5
276	hsa-miR-95	39.8	39.3	0.5
277	hsa-miR-637	39.8	39.4	0.4
278	hsa-miR-432	39.6	39.2	0.4
279	hsa-miR-524–3p	39.8	39.5	0.3
280	hsa-miR-598	35.9	35.7	0.2
281	hsa-miR-134	33.6	33.5	0.2
282	hsa-miR-136	38.4	38.3	0.1
283	hsa-miR-934	32.3	32.2	0.0
284	hsa-miR-137	36.9	37.0	–0.1
285	hsa-miR-526b	39.8	40.0	–0.2
286	hsa-miR-422a	39.8	40.0	–0.2
287	hsa-miR-330–3p	39.8	40.0	–0.2
288	hsa-miR-551b	39.8	40.0	–0.2
289	hsa-miR-557	39.8	40.0	–0.2
290	hsa-miR-202*	39.8	40.0	–0.2
291	hsa-miR-539	39.8	40.0	–0.2
292	hsa-miR-520c-3p	39.8	40.0	–0.2
293	hsa-miR-509–3-5p	39.8	40.0	–0.2
294	hsa-miR-890	39.8	40.0	–0.2
295	hsa-miR-620	39.8	40.0	–0.2
296	hsa-miR-371–3p	39.8	40.0	–0.2
297	hsa-miR-642a	39.8	40.0	–0.2
298	hsa-miR-660	39.8	40.0	–0.2
299	hsa-miR-519d	39.8	40.0	–0.2
300	hsa-miR-454	39.8	40.0	–0.2
301	hsa-miR-96*	39.8	40.0	–0.2
302	hsa-miR-325	39.8	40.0	–0.2
303	hsa-miR-921	39.8	40.0	–0.2
304	hsa-miR-625*	39.8	40.0	–0.2
305	hsa-miR-220a-obsolete in V16	39.8	40.0	–0.2
306	hsa-miR-302c	39.8	40.0	–0.2
307	hsa-miR-302d	39.8	40.0	–0.2
308	hsa-miR-363*	39.8	40.0	–0.2
309	hsa-miR-372	39.8	40.0	–0.2
310	hsa-miR-373	39.8	40.0	–0.2
311	hsa-miR-429	39.8	40.0	–0.2
312	hsa-miR-488	39.8	40.0	–0.2
313	hsa-miR-492	39.8	40.0	–0.2
314	hsa-miR-500a	39.8	40.0	–0.2
315	hsa-miR-512–5p	39.8	40.0	–0.2
316	hsa-miR-516a-5p	39.8	40.0	–0.2

317	hsa-miR-519a	39.8	40.0	–0.2
318	hsa-miR-521	39.8	40.0	–0.2
319	hsa-miR-548c-3p	39.8	40.0	–0.2
320	hsa-miR-549	39.8	40.0	–0.2
321	hsa-miR-576–5p	39.8	40.0	–0.2
322	hsa-miR-590–5p	39.8	40.0	–0.2
323	hsa-miR-651	39.8	40.0	–0.2
324	hsa-miR-886–3p-obsolete in V16	39.8	40.0	–0.2
325	hsa-miR-92a-1*	39.8	40.0	–0.2
326	hsa-miR-933	39.8	40.0	–0.2
327	hsa-miR-337–5p	39.8	40.0	–0.2
328	hsa-miR-126*	28.4	28.7	–0.3
329	hsa-miR-141	32.9	33.3	–0.3
330	hsa-miR-224	36.1	36.4	–0.3
331	hsa-miR-346	34.9	35.3	–0.4
332	hsa-miR-597	39.6	40.0	–0.4
333	hsa-miR-200c	31.8	32.3	–0.5
334	hsa-miR-215	27.4	28.0	–0.5
335	hsa-miR-192	26.4	27.0	–0.6
336	hsa-miR-494	39.4	40.0	–0.6
337	hsa-miR-142–3p	26.7	27.4	–0.7
338	hsa-miR-658	39.3	40.0	–0.7
339	hsa-miR-498	39.2	40.0	–0.8
340	hsa-miR-491–3p	38.1	38.9	–0.9
341	hsa-miR-545	38.1	39.0	–0.9
342	hsa-miR-338–3p	34.2	35.1	–0.9
343	hsa-miR-135a	39.0	40.0	–1.0
344	hsa-miR-383	39.0	40.0	–1.0
345	hsa-miR-135b	38.9	40.0	–1.1
346	hsa-miR-367	38.9	40.0	–1.1
347	hsa-miR-518b	38.8	40.0	–1.2
348	hsa-miR-495	36.9	38.1	–1.2
349	hsa-miR-197	38.7	40.0	–1.3
350	hsa-miR-523	38.7	40.0	–1.3
351	hsa-miR-206	38.7	40.0	–1.3
352	hsa-miR-486–5p	24.9	27.0	–2.0
353	hsa-miR-20b	35.1	37.2	–2.1
354	hsa-miR-340	37.6	40.0	–2.4
355	hsa-miR-146a	30.2	32.9	–2.7
356	hsa-miR-1	32.0	35.4	–3.5
357	hsa-miR-150	24.7	28.3	–3.6
358	hsa-miR-378	27.9	31.6	–3.6
359	hsa-miR-142–5p	29.3	33.7	–4.4
360	hsa-miR-133b	29.9	34.4	–4.5
361	hsa-miR-133a	31.3	36.0	–4.7
362	hsa-miR-363	34.7	40.0	–5.3
363	hsa-miR-296–5p	34.5	40.0	–5.5
364	hsa-miR-200b	31.9	37.5	–5.6
365	hsa-miR-139–5p	31.1	36.8	–5.7
366	hsa-miR-802	34.2	40.0	–5.8

367	hsa-miR-203	33.8	40.0	−6.2
368	hsa-miR-375	29.2	35.5	−6.3
369	hsa-miR-451	24.1	31.6	−7.6
370	hsa-miR-144	27.6	35.4	−7.8
371	hsa-miR-200a	31.7	40.0	−8.3
372	hsa-miR-122	24.5	33.7	−9.3
373	hsa-miR-205	30.0	40.0	−10.0
374	hsa-miR-223	22.9	33.9	−11.0
375	hsa-miR-148a	28.8	40.0	−11.2

Note.—Numerical data correspond to the graphs displayed in Figure 5. Higher relative abundance is indicated by lower relative cycle threshold values, and lower relative abundance is indicated by higher relative cycle threshold values. miRNAs were ranked according to the difference in cycle threshold values, and then five of the top 10 miRNAs were selected as candidate biomarkers.

† Selected as a candidate biomarker.