Research Paper

MicroRNA profiling in human diploid fibroblasts uncovers miR-519 role in replicative senescence

Bernard S. Marasa[1,2], Subramanya Srikantan[1], Jennifer L. Martindale[1], Mihee M. Kim[1], Eun Kyung Lee[1], Myriam Gorospe[1], and Kotb Abdelmohsen[1]

[1] Laboratory of Cellular and Molecular Biology, NIA-IRP, NIH, Baltimore, MD 21224, USA
[2] Department of Biology, The Catholic University of America, Washington, DC 20064, USA

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Abbreviations: *CR, coding region; HDF, human diploid fibroblasts; RISC, RNA-induced silencing complex; RBP, RNA-binding protein; SA, senescence-associated; UTR, untranslated region*
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Correspondence: *abdelmohsenk@grc.nia.nih.gov*

Abstract: MicroRNAs (miRNAs) are short non-coding RNAs that regulate diverse biological processes by controlling the pattern of expressed proteins. In mammalian cells, miRNAs partially complement their target sequences leading to mRNA degradation and/or decreased mRNA translation. Here, we have analyzed transcriptome-wide changes in miRNAs in senescent relative to early-passage WI-38 human diploid fibroblasts (HDFs). Among the miRNAs downregulated with senescence were members of the let-7 family, while upregulated miRNAs included miR-1204, miR-663 and miR-519. miR-519 was recently found to reduce tumor growth at least in part by lowering the abundance of the RNA-binding protein HuR. Overexpression of miR-519a in either WI-38 or human cervical carcinoma HeLa cells triggered senescence, as measured by monitoring β-galactosidase activity and other senescence markers. These data suggest that miR-519 can suppress tumor growth by triggering senescence and that miR-519 elicits these actions by repressing HuR expression.

INTRODUCTION

MicroRNAs are short (~ 22-nt) RNA molecules that modulate changes in gene expression [1,2]. They are generated from precursor transcripts (primary microRNAs) which are exported to the cytoplasm and are cleaved by Dicer; mature miRNAs then assemble into ribonucleoprotein silencing complexes (RISC) that are recruited to specific mRNAs [3]. MicroRNAs function primarily as repressors of mRNA stability and translation [4]. Through their influence on the patterns of expressed genes, microRNAs have been implicated in numerous physiologic processes, such as development of the muscular, immune, neuronal, epithelial and other systems, and in pathologies including neurodegeneration and cancer [5-8]. The latter studies have revealed a number of miRNAs that can function as tumor suppressors (TS-miRNAs) or tumor promoters (oncomiRs) [9].

Cellular senescence is achieved when cells reach the end of their replicative lifespan [10,11]. It is believed to represent a tumor-suppressive mechanism and a contributing factor in aging [12,13]. MicroRNAs have been implicated in replicative senescence, since loss of miRNA biogenesis through Dicer ablation causes senescence in primary cells [14]. Several specific miRNAs were reported to be differentially expressed in senescent cells compared to young, proliferating cells. For example, miRNA-146a and miR-146b are up-regulated in senescent cells and modulate inflammatory responses by suppressing secretion of IL-6 and IL-8 and by downregulating IRAK1 [15].

Recently, four microRNAs (miR-15b, miR-24, miR-25, and miR-141) that jointly lower expression of the kinase MKK4 were found to decline during replicative senescence and to contribute to the senescence process [16]. miR-24 was also found to regulate translation of the cyclin-dependent kinase inhibitor p16, thereby allowing increased p16 expression in senescent cells [17].

Several miRNAs differentially expressed with aging have also been identified. For example, miR-17, miR-19b, miR-20a, and miR-106a were less abundant in cells from older humans [18]. Reduced expression of miR-103, miR-107, miR-128, miR-130a, miR-155, miR-24, miR-221, miR-496, and miR-1538 in older individuals was also recently reported [19]. Age-regulated changes in the expression of microRNAs were also found in mouse liver and brain [20,21]. MicroRNA changes in Ames dwarf mouse liver led to the identification of microRNAs that might delay aging [22]. Studies in *Caenorhabditis elegans* revealed that the microRNA *lin-4* represses *lin-14* transcripts and lin-14 protein to extend lifespan by reducing DAF-16; miRNA profiling in *C elegans* provided evidence that microRNAs may potently influence the biology of aging [23-25].

Many studies have focused on the role of microRNAs in tumorigenesis and age-related diseases. Here, we have studied changes in expressed microRNAs during replicative senescence of WI-38 human diploid fibroblasts (HDFs). We identified subsets of microRNAs that were differentially expressed in young compared with senescent WI-38 cells. miR-519, a microRNA that suppresses tumorigenesis and lowers expression of RNA-binding protein HuR, was upregulated in senescent cells. Overexpression of miR-519 induced senescence in WI-38 and HeLa cells. Our data support the hypothesis that senescence-associated changes in microRNA expression patterns can affect the susceptibility to age-related diseases such as cancer.

RESULTS

Global changes in microRNAs between early-passage and senescent WI-38 human diploid fibroblasts

Compared with early-passage, 'young' proliferating [Y, at population doubling (pdl) 22] WI-38 cells, the senescent (S, pdl 52) WI-38 cells displayed a flattened morphology and senescence-associated (SA) β-galactosidase (SA-β-gal) activity, a widely used senescence marker [26,27] (Figure 1A). Western blot analysis also revealed that senescent cells expressed lower levels of SIRT1 and HuR, whereas p16 and p53 were upregulated (Figure 1B), in keeping with reported literature [28-30].

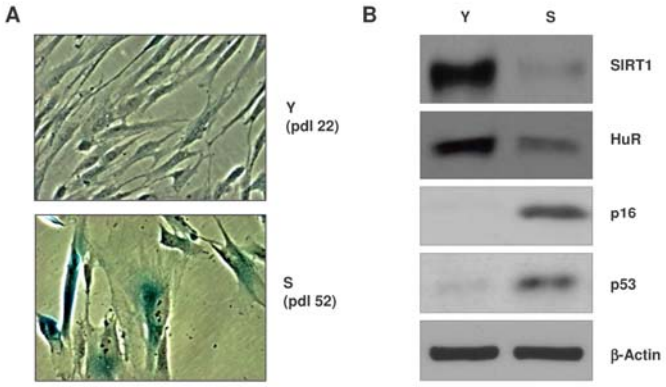

Figure 1. Characterization of early-passage and senescent WI-38 cells. (A) Micrographs illustrating β-galactosidase activity in young (Y) early-passage (pdl 22) and senescent (S), late-passage (pdl 52) WI-38 cells. (**B**) Western blot analysis of the proteins indicated in whole-cell lysates prepared from Y and S WI-38 populations; β-actin served as a loading control.

To test how the pattern of expressed microRNAs is affected by replicative senescence, we studied transcriptome-wide changes in microRNAs using miRNome arrays (not shown); we then validated individual microRNAs by reverse transcription (RT) followed by real-time, quantitative (q)PCR amplification (see Materials and Methods). Depicted in Figures 2 and 3 and in supplemental Table S1 are all of the microRNAs validated using sequence-specific qPCR primers. As shown in Figure 2, several microRNAs were markedly more abundant in senescent cells (e.g., miR-1204, miR-663, miR-548b-3p and miR-431). Other microRNAs were expressed at lower levels in senescent cells [e.g., miR-24, miR-141, and miR-10a (Figure 3, Supplemental Table S1)]. MicroRNAs changing less than twofold with senescence are listed in the supplemental Figure S1.

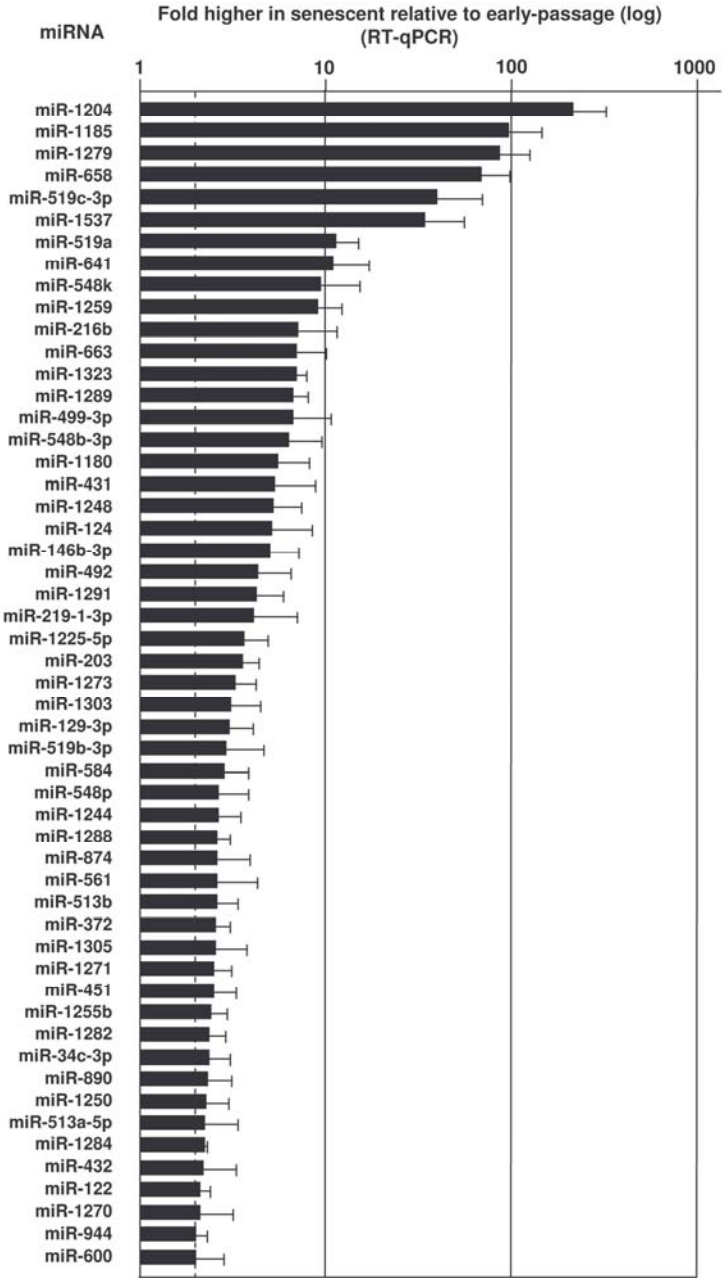

Figure 2. MicroRNAs upregulated in senescent cells. RNA extracted from Y (pdl 22-25) and S (pdl 50-55) WI-38 cells was used to measure the levels of the microRNAs listed, using RT-qPCR (Materials and Methods). MicroRNA abundance was normalized to U1 snRNA levels. Data are the means and S.D. from three independent experiments.

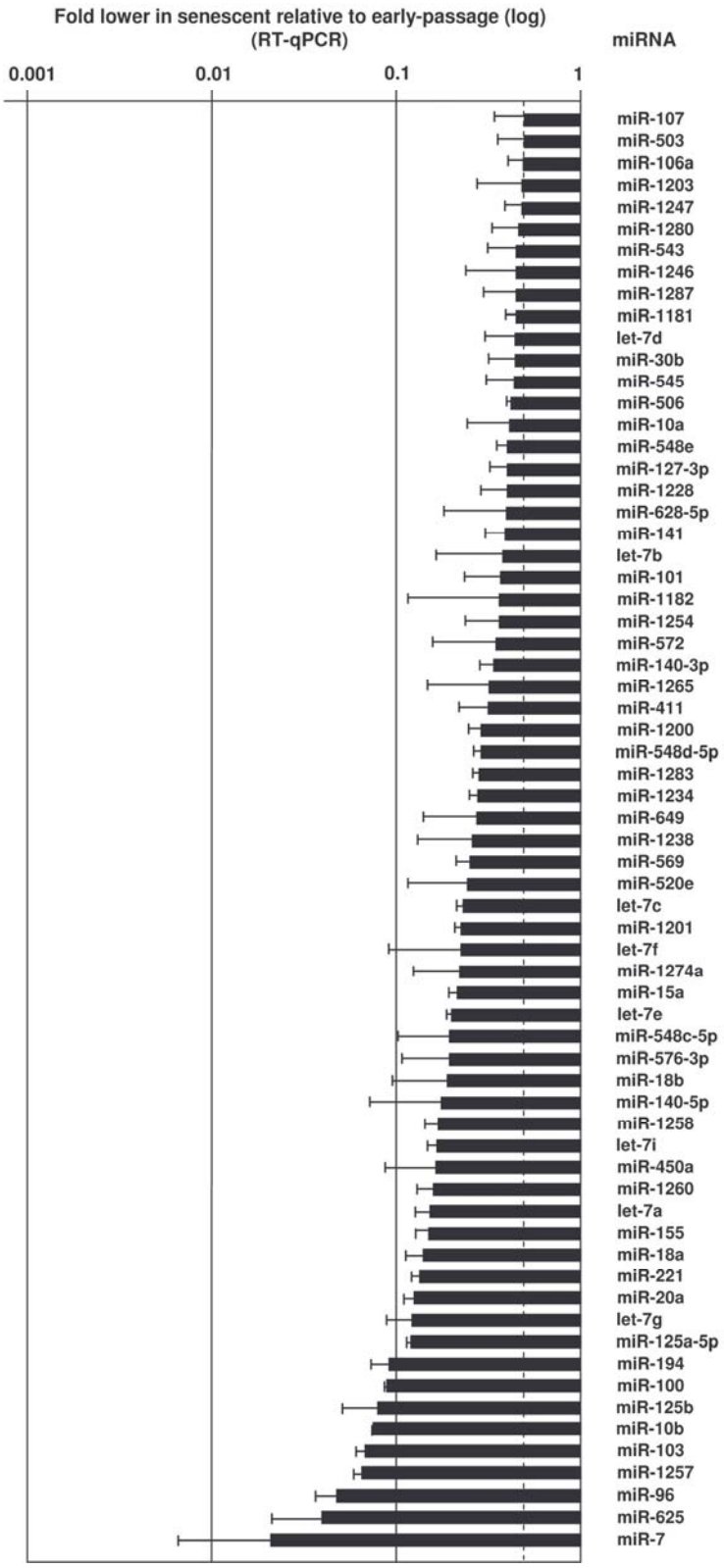

Figure 3. MicroRNAs downregulated in senescent cells. RNA was extracted and analyzed as explained in the legend of Figure 2. Data show the means and S.D. from three independent experiments.

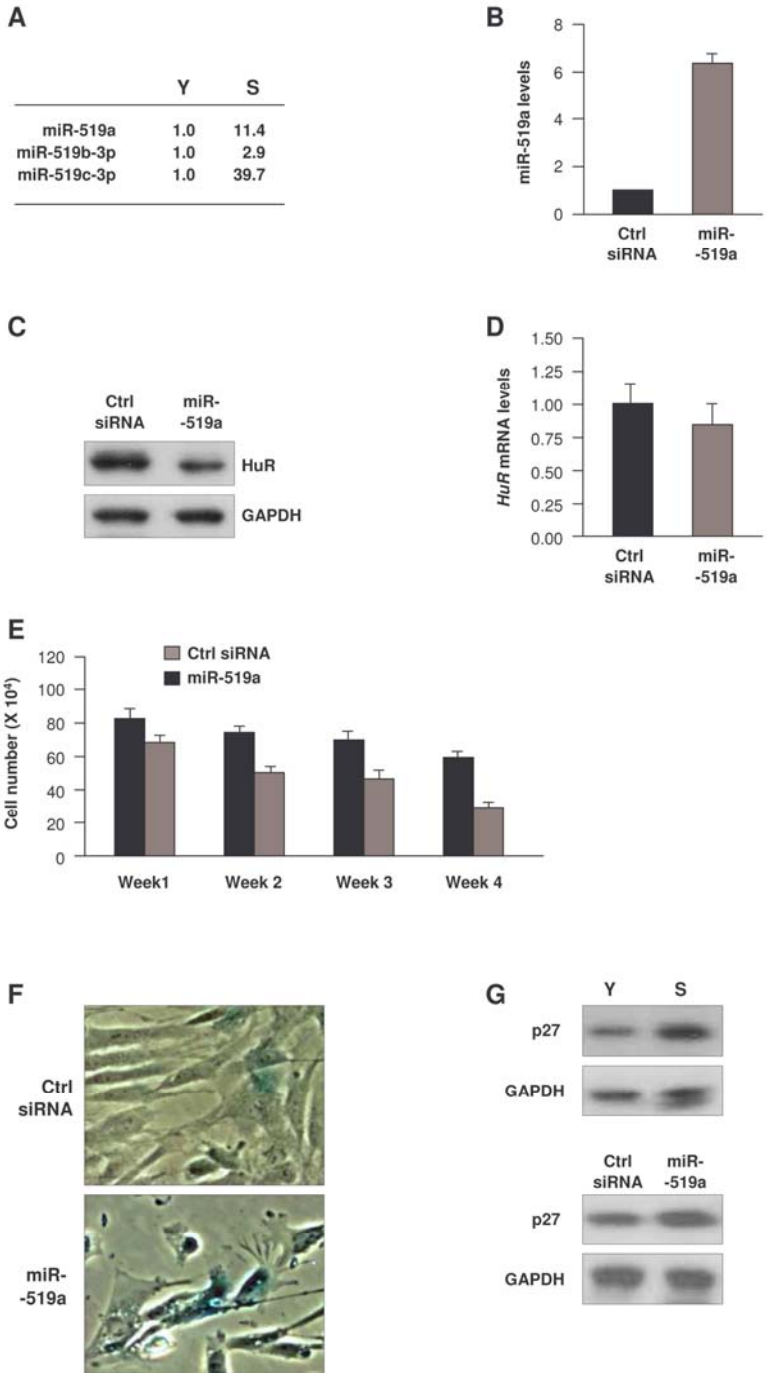

Figure 4. Influence of miR-519 on WI-38 senescence. **(A)** Fold differences in miR-519 expression in S relative to Y cells, calculated as explained in the legend of Figure 2. **(B)** Forty-eight h after transfection of either control (Ctrl) siRNA or miR-519a, the levels of miR-519a were measured by RT-qPCR. **(C,D)** In cells transfected as explained in panel (B), the levels of HuR protein and loading control GAPDH were assessed by Western blot analysis (C), and the levels of *HuR* mRNA and normalization control 18S rRNA were measured by RT-qPCR (D). **(E)** WI-38 cell numbers in cells transfected as in (B) were counted every 7 days. **(F)** SA-β-galactosidase activity in WI-38 cells by week 4 after sequential transfection (every 7 days) of either Ctrl siRNA or miR-519a. **(G)** Western blot analysis of p27 and loading control GAPDH in Y and S WI-38 cells (*top*) or in WI-38 cells by week 4 after transfection as explained in (E) (*bottom*). The data in B,D,E represent the means and S.D. from three independent experiments.

miR-519-induced senescence in HDFs

We were particularly interested in the miR-519 family. miR-519 was recently found to inhibit translation of the RNA-binding protein HuR through its interaction with the HuR coding region [31]. In a separate study, miR-519 suppressed the growth of tumor xenografts in an HuR-dependent manner [32]. Given that HuR promotes cell proliferation and decreases senescence [33,34], we hypothesized that the elevated miR-519 in senescent cells (Figure 4A) might lower HuR expression in WI-38 HDFs, and hence promote senescence. To test this possibility, we overexpressed miR-519a in young-HDFs (Figure 4B); western blot analysis confirmed that miR-519a overexpression repressed HuR (Figure 4C). In keeping with earlier results [31], miR-519a did not influence the levels of *HuR* mRNA (Figure 4D), in agreement with the view that miR-519a inhibited *HuR* mRNA translation without affecting *HuR* mRNA stability. Moreover, sustained miR-519a overexpression for 4 weeks caused a marked reduction in cell number as compared to control transfection groups (Figure 4E). miR-519a-overexpressing cells also showed increased SA-β-gal activity (Figure 4F) and elevated expression of the senescence marker p27 [35,36] (Figure 4G, *bottom*). Together, these data indicate that miR-519a induced cellular senescence and inhibited cell proliferation, resulting in accelerated senescence. They further suggest that miR-519a-induced senescence may be mediated in part by repression of HuR.

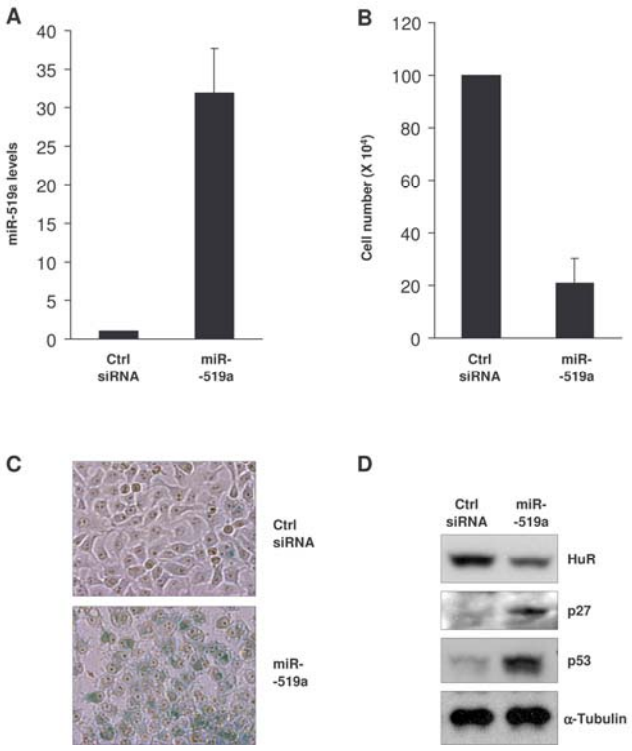

Figure 5. Influence of miR-519 on the senescent phenotype of HeLa cells. (A) Forty-eight h after transfection of HeLa cells with either control (Ctrl) siRNA or miR-519a, miR-519a levels were measured by RT-qPCR. **(B)** Number of HeLa cells remaining by 72 h after transfection of Ctrl siRNA or miR-519a as explained in (**A**). **(C)** β-galactosidase activity in HeLa cells 5 days after transfection with either Ctrl siRNA or miR-519a. **(D)** Seventy-two hours after transfection as indicated in (A), the levels of the proteins shown were assessed by Western blot analysis. The data in A,B represent the means and S.D. from three independent experiments.

miR-519-induced senescence in HeLa cells

As indicated above, miR-519 was found to suppress tumor growth [32]. Since cellular senescence is considered to be an anti-tumorigenic process, we examined the effect of miR-519 on the senescent phenotype of cancer cells. Upon miR-519a overexpression (Figure 5A), HeLa cell numbers declined significantly (Figure 5B). Five days after transfection of miR-519a, cells showed a strong increase in SA-β-gal activity compared to the control transfection group (Figure 5C); in addition, miR-519-induced senescence in HeLa cells was accompanied by increased levels of the senescence markers p53 and p27 (Figure 5D). Together, these data indicate that miR-519 reduced HeLa cell proliferation and promoted HeLa cell senescence. Accordingly, we postulate that one of the mechanisms by which miR-519 suppress tumor growth is by inducing senescence, and further propose that miR-519 triggers senescence –at least in part– by reducing HuR levels.

DISCUSSION

Cells become senescent as a result of factors such as the accumulation of reactive oxygen species, DNA damage, erosion of telomeres, and oncogenic activation. Collectively, these triggers cause cells to undergo morphological changes, to become unable to replicate DNA and to display altered gene expression patterns [10-13]. Here, we investigated microRNA levels in WI-38 human diploid fibroblasts by comparing microRNA patterns in senescent relative to young, proliferating cells. Among the microRNAs showing increasing abundance with senescence, miR-519 was of particular interest because it was shown to inhibit translation of HuR and to diminish tumor growth [31,32]. Through its influence on the expression of many genes, HuR plays a key role in cell proliferation, tumorigenesis, and senescence [37,38]. We found that overexpression of miR-519a decreased HuR levels, lowered cell proliferation, and promoted replicative senescence in both WI-38 and HeLa cells.

microRNAs and senescence

We previously used miRNA microarrays to identify changes in a limited number of microRNAs in senescent cells [16]. Here, we have expanded this analysis and have verified many individual microRNAs whose abundance changes with replicative senescence. Many of them target key proteins implicated in senescence and cancer. For example, miR-146b is upregulated in senescent cells (Figure 2), in keeping with earlier findings that miR-146a and miR-146b increased with senescence and repressed the senescence-associated inflammatory mediators IL-6 and IL-8 [15]. miR-34a regulates SIRT1 expression and induced senescence of cancer cells [39-41]; here, we observed higher miR-34c (not miR-34a) in senescent cells, likely a reflection of the variability and complexity of the senescence process. Several let-7 members were also upregulated in senescent cells (Figure 2 and Supplemental Figure S1); this observation supports the view that the ability of let-7 microRNAs can suppress tumor growth [reviewed in 42], which could contribute to the senescence process. Similarly, upregulation of miR-20 in senescence cells correlates with the ability of miR-20 to inhibit proliferation of K562 human erythromyeloblastoid leukemia cells [43]. In conjunction with the finding that miR-519 reduced tumorigenesis in a xenograft model [32], we propose that the coordinated action of senescence-upregulated microRNAs can suppress tumor growth by reducing the levels of oncogenes or tumor promoters.

Conversely, many microRNAs were downregulated in senescent cells (Figure 3). Among the myriad of senescence-associated proteins that they might regulate, these microRNAs likely repress several tumor suppressors. In this regard, as miR-21 has been shown to lower expression of the tumor suppressor PTEN [44], the downregulated of miR-21 in senescent cells (Figure 3) could allow increased PTEN expression, in turn reducing tumor cell proliferation, migration, and invasion [45].

miR-519a-induced senescence by lowering HuR

We previously reported that miR-519 represses the production of HuR, an RNA-binding protein which is highly abundant in cancer cells and is low in untransformed cells [11,38]. HuR overexpression delays the senescent phenotype while the loss of HuR enhances it [11]. Moreover, while HuR levels are high in tumors and low in normal tissues, miR-519 levels are high in normal tissues and low in cancer tissues [32]. Since HuR potently enhances the expression of cancer-promoting proteins, and reducing HuR levels promotes HDF senescence [11,38], we propose that miR-519 represses tumor growth at least in part, by lowering HuR and thereby promoting senescence (Figs. 4 and 5). Additionally, miR-519 could further repress tumor growth by lowering the expression of other genes, such as ABCG2 or HIF-1α [46,47].

In summary, we have identified collections of microRNAs displaying altered abundance with replicative senescence. As shown here for miR-519, we postulate that these changes help to meet the needs of

senescent cells in eliciting tumor suppression and growth arrest. Future studies will help to recognize more fully the proteins and processes modulated by senescence-regulated microRNAs.

MATERIAL AND METHODS

Cell culture, transfections, and β-galactosidase staining. Early-passage, proliferating ('young', ~20 to 30 pdl) and late-passage, senescent (~50 to 55 pdl) WI-38 human diploid fibroblasts (HDFs; Coriell Cell Repositories) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum and 0.1 mM nonessential amino acids (Invitrogen). HeLa cells were cultured in DMEM supplemented with 10% FBS and antibiotics. miR-519a (Ambion) or control siRNA (AATTCTCCGAACGTGTCACGT, Qiagen) were transfected at a final concentration of 100 nM using Lipofectamine 2000 (Invitrogen). Where indicated, transfections were performed every 7 days for 4 weeks. WI-38 HDFs and HeLa cells were stained with a senescence-associated β-galactosidase (Cell Signaling Technology) detection kit, according to the manufacturer's protocol.

RNA isolation and miRNA profiling. Total cellular RNA was isolated using Trizol (Invitrogen). Isolated RNA was used to measure miRNA levels in young and senescent cells with a 7900HT real-time PCR instrument (Applied Biosystems). All microRNAs were measured and validated using miRNA-specific forward primers (Supplemental Table S2) and a universal reverse primer (System Biosciences, SBI), according to the manufacturer's protocol. The levels of U1 snRNA, used for normalization, were determined using the specific forward primer CGACTGCATAATTTGTGGTAGTGG.

Protein analysis. Whole-cell lysates were prepared with RIPA buffer [10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 0.1% SDS, and 1 mM dithiothreitol]. Proteins were resolved by SDS–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Invitrogen). After incubation with primary antibodies recognizing SIRT1, HuR, p16, p53, p27, GAPDH (all from Santa Cruz Biotechnology) or β-actin (Abcam), blots were incubated with the appropriate secondary antibodies and the signals were detected by ECL Plus (GE Healthcare).

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CONFLICT OF INTERESTS STATEMENT

The authors of this manuscript have no conflict of interests to declare.

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SUPPLEMETAL TABLES

miRNA	FC	SD	miRNA	FC	SD	miRNA	FC	SD
miR-605	1.98	0.61	miR-504	1.25	0.35	miR-129-5p	0.98	0.06
miR-1231	1.97	0.10	miR-508-3p	1.24	0.40	miR-518c	0.95	0.40
miR-1256	1.95	0.55	miR-1262	1.24	0.30	miR-640	0.93	0.20
miR-128	1.79	0.26	miR-509-3-5p	1.24	0.32	miR-1243	0.91	0.31
miR-562	1.79	1.11	miR-509-5p	1.23	0.40	miR-653	0.88	0.43
miR-548l	1.79	0.76	miR-511	1.23	0.30	miR-1263	0.83	0.48
miR-520b	1.75	0.59	miR-512-5p	1.23	0.50	miR-618	0.82	0.60
miR-373	1.72	0.75	miR-515-3p	1.22	0.31	miR-106b	0.82	0.60
miR-483-5p	1.71	1.01	miR-513c	1.22	0.41	miR-135a	0.81	0.51
miR-550	1.71	1.07	miR-516b	1.22	0.22	miR-548d-3p	0.81	0.13
miR-25	1.70	0.75	miR-516a-3p	1.22	0.21	miR-520a-3p	0.80	0.44
miR-518e	1.68	0.53	miR-517b	1.22	0.51	miR-651	0.79	0.35
miR-1224-3p	1.66	0.52	miR-518a-3p	1.21	0.24	miR-133b	0.78	0.17
miR-876-5p	1.66	0.52	miR-518b	1.21	0.36	miR-1202	0.78	0.07
miR-507	1.64	1.04	miR-518d-3p	1.21	0.51	miR-1197	0.78	0.28
miR-1266	1.64	0.57	miR-520a-5p	1.17	0.40	miR-548i	0.77	0.15
miR-577	1.64	0.13	miR-494	1.16	0.20	miR-548c-3p	0.77	0.19
miR-1304	1.62	0.76	miR-520g	1.15	0.31	miR-520d-3p	0.75	0.14
miR-1253	1.59	0.41	miR-523	1.15	0.30	miR-34a	0.75	0.38
miR-760	1.58	1.14	miR-521	1.15	0.29	miR-664	0.74	0.17
miR-519e	1.58	1.02	miR-524-5p	1.15	0.50	miR-1208	0.73	0.13
miR-206	1.57	1.07	miR-525-5p	1.15	0.46	miR-9	0.73	0.13
miR-127-5p	1.54	0.75	miR-526b	1.15	0.44	miR-1268	0.72	0.15
miR-1227	1.50	0.11	miR-532-3p	1.15	0.43	miR-217	0.72	0.02
miR-370	1.50	0.45	miR-539	1.14	0.33	miR-1237	0.70	0.04
miR-371-5p	1.49	0.51	miR-542-3p	1.14	0.63	miR-758	0.70	0.15
miR-1207-3p	1.48	0.11	miR-548b-5p	1.12	0.23	miR-548h	0.70	0.15
miR-510	1.48	0.32	miR-633	1.11	0.40	miR-495	0.69	0.13
miR-888	1.47	1.10	miR-208a	1.10	0.07	miR-1229	0.67	0.10
miR-376a	1.46	0.71	miR-548g	1.10	0.20	miR-1236	0.66	0.13
miR-376c	1.46	0.52	miR-1276	1.10	0.14	miR-320c	0.65	0.10
miR-378	1.46	0.76	miR-1269	1.10	0.52	miR-1179	0.65	0.07
miR-1272	1.46	0.08	miR-508-5p	1.10	0.44	miR-24	0.64	0.21
miR-380	1.46	0.39	miR-549	1.10	0.32	miR-1281	0.63	0.13
miR-553	1.46	0.18	miR-1826	1.09	0.68	miR-548a-5p	0.62	0.13
miR-382	1.45	0.60	miR-1266	1.09	0.20	miR-212	0.61	0.13
miR-1226	1.43	0.10	miR-554	1.09	0.20	miR-450b-5p	0.60	0.17
miR-384	1.42	0.23	miR-552	1.09	0.19	miR-587	0.60	0.08
miR-409-5p	1.41	0.42	miR-622	1.09	0.18	miR-1255a	0.59	0.19
miR-421	1.41	0.51	miR-556-3p	1.09	0.25	miR-1249	0.59	0.15
miR-1278	1.40	0.45	miR-557	1.08	0.42	miR-375	0.59	0.14
miR-423-3p	1.40	0.41	miR-559	1.08	0.34	miR-335	0.59	0.15
miR-551a	1.40	0.52	miR-1233	1.07	0.51	miR-1275	0.58	0.12
miR-424	1.39	0.38	miR-544	1.06	0.20	miR-365	0.57	0.13
miR-429	1.38	0.40	miR-564	1.06	0.27	miR-626	0.57	0.04
miR-330-3p	1.38	0.37	miR-567	1.06	0.37	miR-1296	0.56	0.09
miR-296-5p	1.38	0.13	miR-571	1.06	0.33	miR-1207-5p	0.54	0.15
miR-1184	1.38	0.50	miR-573	1.06	0.43	miR-1264	0.54	0.15
miR-448	1.37	0.41	miR-574-5p	1.05	0.51	miR-132	0.54	0.15
miR-449b	1.37	0.39	miR-577	1.05	0.20	miR-423-5p	0.53	0.24
miR-548j	1.34	0.59	miR-579	1.05	0.24	miR-607	0.53	0.17
miR-450b-3p	1.34	0.50	miR-616	1.05	0.02	miR-320d	0.53	0.21
miR-613	1.33	0.57	miR-135b	1.04	0.48	miR-520c-3p	0.52	0.16
miR-105	1.32	0.60	miR-338-5p	1.04	0.22			
miR-1224-5p	1.32	0.30	miR-125a-3p	1.04	0.03			
miR-1261	1.32	0.45	miR-522	1.03	0.04			
miR-453	1.31	0.50	miR-584	1.03	0.23			
miR-371-3p	1.31	0.65	miR-582-5p	1.03	0.12			
miR-455-3p	1.30	0.51	miR-581	1.03	0.10			
miR-483-3p	1.30	0.31	miR-21	1.03	0.50			
miR-484	1.30	0.40	miR-586	1.03	0.21			
miR-1245	1.28	0.02	miR-1205	1.02	0.19			
miR-486-5p	1.28	0.37	miR-588	1.02	0.16			
miR-485-5p	1.28	0.31	miR-590-3p	1.02	0.21			
miR-487b	1.28	0.41	miR-591	1.01	0.42			
miR-489	1.28	0.51	miR-383	1.01	0.02			
miR-490-5p	1.28	0.29	miR-593	1.01	0.3			
miR-491-5p	1.27	0.41	miR-596	1.00	0.41			
miR-493	1.27	0.50	miR-604	1.00	0.30			
miR-497	1.27	0.27	miR-602	1.00	0.42			
miR-548a-3p	1.26	0.45	miR-598	1.00	0.28			
miR-500	1.26	0.50	miR-1286	0.98	0.32			
miR-501-5p	1.26	0.42	miR-1285	0.98	0.38			
miR-502-5p	1.25	0.39	miR-1267	0.98	0.15			

Supplemental Table S1. MicroRNAs showing less than twofold differences in abundance in senescent relative to early-passage cells. RNA extracted from Y (pdl 22-25) and S (pdl 50-55) WI-38 cells was used to measure the levels of the microRNAs listed, using RT-qPCR (Materials and Methods). microRNA abundance was normalized to U1 snRNA levels. Data are the means and S.D. from three independent experiments.

miR name	miRBase Acc No.	Primer Sequence	miR name	miRBase Acc No.	Primer Sequence
miR-96	MIMAT0000095	TTTGGCACTAGCACATTTTTGCT	miR-206	MIMAT0000462	TGGAATGTAAGGAAGTGTGTGG
miR-944	MIMAT0004987	AAATTATTGTACATCGGATGAG	miR-203	MIMAT0000264	GTGAAATGTTTAGGACCACTAG
miR-9	MIMAT0000441	UCUUUGGUUAUCUAGCUGUAUGA	miR-194	MIMAT0000460	TGTAACAGCAACTCCATGTGGA
miR-890	MIMAT0004912	TACTTGGAAAGGCATCAGTTG	miR-18b	MIMAT0001412	TAAGGTGCATCTAGTGCAGTTAG
miR-888	MIMAT0004916	TACTCAAAAAGCTGTCAGTCA	miR-18a	MIMAT0000072	TAAGGTGCATCTAGTGCAGATAG
miR-876-5p	MIMAT0004924	TGGATTTCTTTGTGAATCACCA	miR-1826	MIMAT0006766	ATTGATCATCGACACTTCGAACGCAAT
miR-874	MIMAT0004911	CTGCCCTGGCCCGAGGGACCGA	miR-15a	MIMAT0000068	TAGCAGCACATAATGGTTTGTG
miR-760	MIMAT0004957	CGGCTCTGGGTCTGTGGGGA	miR-155	MIMAT0000646	TTAATGCTAATCGTGATAGGGGT
miR-758	MIMAT0003879	UUUGUGACCUGGUCCACUAACC	miR-1537	MIMAT0007399	AAAACCGTCTAGTTACAGTTGT
miR-7	MIMAT0000252	TGGAAGACTAGTGATTTTGTTGT	miR-146b-3p	MIMAT0004766	TGCCCTGTGGACTCAGTTCTGG
miR-664	MIMAT0005949	UAUUCAUUUAUCCCCAGCCUACA	miR-141	MIMAT0000432	TAACACTGTCTGGTAAAGATGG
miR-663	MIMAT0003326	AGGCGGGGCGCCGCGGGACCGC	miR-140-5p	MIMAT0000431	CAGTGGTTTTACCCTATGGTAG
miR-658	MIMAT0003336	GGCGGAGGGAAGTAGGTCCGTTGGT	miR-140-3p	MIMAT0004597	TACCACAGGGTAGAACCACGG
miR-653	MIMAT0003328	GTGTTGAAACAATCTCTACTG	miR-135b	MIMAT0000758	TATGGCTTTTCATTCCTATGTGA
miR-651	MIMAT0003321	TTTAGGATAAGCTTGACTTTTG	miR-135a	MIMAT0000428	TATGGCTTTTTATTCCTATGTGA
miR-649	MIMAT0003319	AAACCTGTGTTGTTCAAGAGTC	miR-133b	MIMAT0000770	TTTGGTCCCCTTCAACCAGCTA
miR-641	MIMAT0003311	AAAGACATAGGATAGAGTCACCTC	miR-1323	MIMAT0005795	TCAAAACTGAGGGGCATTTTCT
miR-640	MIMAT0003310	ATGATCCAGGAACCTGCCTCT	miR-132	MIMAT0000426	TAACAGTCTACAGCCATGGTCG
miR-633	MIMAT0003303	CTAATAGTATCTACCACAATAAA	miR-1305	MIMAT0005893	TTTTCAACTCTAATGGGAGAGA
miR-628-5p	MIMAT0004809	ATGCTGACATATTTACTAGAGG	miR-1304	MIMAT0005892	TTTGAGGCTACAGTGAGATGTG
miR-626	MIMAT0003295	AGCUGUCUGAAAAUGUCUU	miR-1303	MIMAT0005891	TTTAGAGACGGGGTCTTGCTCT
miR-625	MIMAT0003294	AGGGGGAAAGTTCTATAGTCC	miR-1296	MIMAT0005794	TTAGGGCCCTGGCTCCATCTCC
miR-622	MIMAT0003291	ACAGTCTGCTGAGGTTGGAGC	miR-129-5p	MIMAT0000242	CTTTTGCGGTCTGGGCTTGC
miR-618	MIMAT0003287	AAACTCTACTTGTCCTTCTGAGT	miR-129-3p	MIMAT0004605	AAGCCCTTACCCCAAAAAGCAT
miR-616	MIMAT0004805	AGTCATTGGAGGGTTTGAGCAG	miR-1291	MIMAT0005881	TGGCCCTGACTGAAGACCAGCAGT
miR-613	MIMAT0003281	AGGAATGTTCCTTCTTTGCC	miR-1289	MIMAT0005879	TGGAGTCCAGGAATCTGCATTTT
miR-607	MIMAT0003275	GTTCAAATCCAGATCTATAAC	miR-1288	MIMAT0005942	TGGACTGCCCTGATCTGGAGA
miR-605	MIMAT0003273	TAAATCCCATGGTGCCTTCTCCT	miR-1287	MIMAT0005878	TGCTGGATCAGTGGTTCGAGTC
miR-600	MIMAT0003268	ACTTACAGACAAGAGCCTTGCTC	miR-1286	MIMAT0005877	TGCAGGACCAAGATGAGCCCT
miR-587	MIMAT0003253	UUUCCAUAGGUGAUGAGUCAC	miR-1285	MIMAT0005876	TCTGGGCAACAAAGTGAGACCT
miR-584	MIMAT0003249	TTATGGTTTGCCTGGGACTGAG	miR-1284	MIMAT0005941	TCTATACAGACCCTGGCTTTTC
miR-577	MIMAT0003242	TAGATAAAATATTGGTACCTG	miR-1283	MIMAT0005799	TCTACAAAGGAAAGCGCTTTCT
miR-576-3p	MIMAT0004796	AAGATGTGGAAAAATTGGAATC	miR-1282	MIMAT0005940	TCGTTTGCCTTTTTCTGCTT
miR-572	MIMAT0003237	GTCCGCTCGGCGGTGGCCCA	miR-1281	MIMAT0005939	UCGCCUCCUCCUCUCCC
miR-569	MIMAT0003234	AGTTAATGAATCCTGGAAAGT	miR-1280	MIMAT0005946	TCCCACCGCTGCCACCC
miR-562	MIMAT0003226	AAAGTAGCTGTACCATTTGC	miR-128	MIMAT0000424	TCACAGTGAACCGGTCTCTTT
miR-561	MIMAT0003225	CAAAGTTTAAGATCCTTGAAGT	miR-1279	MIMAT0005937	TCATATTGCTTCTTTCT
miR-553	MIMAT0003216	AAAACGGTGAGATTTTGTTTT	miR-1278	MIMAT0005936	TAGTACTGTGCATATCATCTAT
miR-551a	MIMAT0003214	GCGACCCACTCTTGGTTTCCA	miR-1276	MIMAT0005930	TAAAGAGCCCTGTGGAGACA
miR-550	MIMAT0004800	AGTGCCTGAGGGAGTAAGAGCCC	miR-127-5p	MIMAT0004604	CTGAAGCTCAGAGGGCTCTGAT
miR-548p	MIMAT0005934	TAGCAAAAACTGCAGTTACTTT	miR-1275	MIMAT0005929	GUGGGGGAGAGGCUGUC
miR-548l	MIMAT0005935	AAAAGTAATTGCGGATTTTGCC	miR-1274a	MIMAT0005927	GTCCCTGTTCAGGCGCCA
miR-548k	MIMAT0005882	AAAAGTACTTGCGGATTTTGCT	miR-127-3p	MIMAT0000446	TCGGATCCGTCTGAGCTTGGCT
miR-548j	MIMAT0005875	AAAAGTAATTGCGGTCTTTGGT	miR-1273	MIMAT0005926	GGGCGACAAAGCAAGACTCTTTCTT
miR-548i	MIMAT0005935	AAAAGUAAUUGCGGAUUUUGCC	miR-1272	MIMAT0005925	GATGATGATGGCAGCAAATTCTGAAA
miR-548h	MIMAT0005928	AAAAGUAAUCGCGGUUUUUGUC	miR-1271	MIMAT0005796	CTTGGCACCTAGCAAGCACTCA
miR-548e	MIMAT0005874	AAAAACTGAGACTACTTTTGCA	miR-1270	MIMAT0005924	CTGGAGATATGGAAGAGCTGTGT
miR-548d-5p	MIMAT0005799	TCTACAAAGGAAAGCGCTTTCT	miR-1269	MIMAT0005923	CTGGACTGAGCCGTGCTACTGG
miR-548d-3p	MIMAT0003323	CAAAAACCACAGTTTCTTTTGC	miR-1268	MIMAT0005922	CGGGCGUGGUGGUGGGGG
miR-548c-5p	MIMAT0004806	AAAAGTAATTGCGGTTTTTGCC	miR-1267	MIMAT0005921	CCTGTTGAAGTGTAATCCCCA
miR-548c-3p	MIMAT0003285	CAAAAAUCUCAAUUACUUUUGC	miR-1266	MIMAT0005920	CCTCAGGGCTGTAGAACAGGGCT
miR-548b-3p	MIMAT0003254	CAAGAACCTCAGTTGCTTTTGT	miR-1265	MIMAT0005918	CAGGATGTGGTCAAGTGTTGTT
miR-548a-5p	MIMAT0004803	AAAAGUAAUUGCGAGUUUUACC	miR-1264	MIMAT0005791	CAAGTCTTATTTGAGCACCTGTT
miR-548a-3p	MIMAT0003251	CAAAACTGGCAATTACTTTTGC	miR-1263	MIMAT0005915	ATGGTACCCTGGCATACTGAGT
miR-545	MIMAT0003165	TCAGCAAACATTTATTGTGTGC	miR-1262	MIMAT0005914	ATGGGTGAATTTGTAGAAGGAT
miR-544	MIMAT0003164	ATTCTGCATTTTTAGCAAGTTC	miR-1261	MIMAT0005913	ATGGATAAGGCTTTGGCTT
miR-543	MIMAT0004954	AAACATTCGCGGTGCACTTCTT	miR-1260	MIMAT0005911	ATCCCACCTCTGCCACCA
miR-522	MIMAT0002868	AAAATGGTTCCCTTTAGAGTGT	miR-125b	MIMAT0000423	TCCCTGAGACCCTAACTTGTGA
miR-520e	MIMAT0002825	AAAGTGCTTCCTTTTGAGGG	miR-125a-5p	MIMAT0000443	TCCCTGAGACCCTTTAACCTGTGA
miR-520d-3p	MIMAT0002856	AAAGUGCUUCUCUUUGGUGGGU	miR-125a-3p	MIMAT0004602	ACAGGTGAGGTTCTTGGGAGCC
miR-520c-3p	MIMAT0002846	AAAGTGCTTCCTTTTAGAGGGT	miR-1259	MIMAT0005910	ATATATGATGACTTAGCTTTT
miR-520b	MIMAT0002843	AAAGTGCTTCCTTTTAGAGGG	miR-1258	MIMAT0005909	AGTTAGGATTAGGTCGTGGAA
miR-520a-3p	MIMAT0002834	AAAGTGCTTCCCTTTGGACTGT	miR-1257	MIMAT0005908	AGTGAATGATGGGTTCTGACC
miR-519e	MIMAT0002829	AAGTGCCTCCTTTTAGAGTGTT	miR-1256	MIMAT0005907	AGGCATTGACTTCTCACTAGCT
miR-519c-3p	MIMAT0002832	AAAGTGCATCTTTTTAGAGGAT	miR-1255b	MIMAT0005945	CGGATGAGCAAAGAAAGTGGTT
miR-519b-3p	MIMAT0002837	AAAGTGCATCCTTTTAGAGGTT	miR-1255a	MIMAT0005906	AGGAUGAGCAAAGAAAGUAGAUU
miR-519a	MIMAT0002869	AAAGTGCATCCTTTTAGAGTGT	miR-1254	MIMAT0005905	AGCCTGGAAGCTGGAGCCTGCAGT
miR-518e	MIMAT0002861	AAAGCGCTTCCCTTCAGAGTG	miR-1253	MIMAT0005904	AGAGAAGAAGATCAGCCTGCA
miR-518d-3p	MIMAT0002864	CAAAGCGCTTCCCTTTGGAGC	miR-1250	MIMAT0005902	ACGGTGCTGGATGTGGCCTTT
miR-518c	MIMAT0002848	CAAAGCGCTTCTCTTTAGAGTGT	miR-1249	MIMAT0005901	ACGCCCUUCCCCCCCUUCUUCA
miR-518b	MIMAT0002844	CAAAGCGCTCCCCTTTAGAGGT	miR-1248	MIMAT0005900	ACCTTCTTGTATAAGCACTGTGCTAAA
miR-518a-3p	MIMAT0002863	GAAAGCGCTTCCCTTTGCTGGA	miR-1247	MIMAT0005899	ACCCGTCCCGTTCGTCCCCGGA
miR-517b	MIMAT0002857	TCGTGCATCCCTTTAGAGTGTT	miR-1246	MIMAT0005898	AATGGATTTTTGGAGCAGG
miR-516b	MIMAT0002859	ATCTGGAGGTAAGAAGCACTTT	miR-1245	MIMAT0005897	AAGTGATCTAAAGGCCTACAT

Supplemental Table S2. Primers used for RT-qPCR detection of the microRNAs in Figures 2 and 3 and in supplemental Figure S1.