Cholinesterase-targeting microRNAs identified in silico affect specific biological processes

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INTRODUCTION

MicroRNAs (miRs) have emerged as important gene silencers affecting many target mRNAs. Here, we report the identification of 244 miRs that target the 3′-untranslated regions of different cholinesterase transcripts: 116 for butyrylcholinesterase (BChE), 47 for the synaptic acetylcholinesterase (AChE-S) splice variant, and 81 for the normally rare splice variant AChE-R. Of these, 11 and 6 miRs target both AChE-S and AChE-R, and AChE-R and BChE transcripts, respectively. BChE and AChE-S showed no overlapping miRs, attesting to their distinct modes of miR regulation. Generally, miRs can suppress a number of targets; thereby controlling an entire battery of functions. To evaluate the importance of the cholinesterase-targeted miRs in other specific biological processes we searched for their other experimentally validated target transcripts and analyzed the gene ontology enriched biological processes these transcripts are involved in. Interestingly, a number of the resulting categories are also related to cholinesterases. They include, for BChE, response to glucocorticoid stimulus, and for AChE, response to wounding and two child terms of neuron development: regulation of axonogenesis and regulation of dendrite morphogenesis. Importantly, all of the AChE-targeting miRs found to be related to these selected processes were directed against the normally rare AChE-R splice variant, with three of them, including the neurogenesis regulator miR-132, also directed against AChE-S. Our findings point at the synaptic acetylcholinesterase (AChE-S) splice variant, and 81 for the normally rare splice variant AChE-R. Of these, 11 and 6 miRs target both AChE-S and AChE-R, and AChE-R and BChE transcripts, respectively. BChE and AChE-S showed no overlapping miRs, attesting to their distinct modes of miR regulation. Generally, miRs can suppress a number of targets; thereby controlling an entire battery of functions. To evaluate the importance of the cholinesterase-targeted miRs in other specific biological processes we searched for their other experimentally validated target transcripts and analyzed the gene ontology enriched biological processes these transcripts are involved in. Interestingly, a number of the resulting categories are also related to cholinesterases. They include, for BChE, response to glucocorticoid stimulus, and for AChE, response to wounding and two child terms of neuron development: regulation of axonogenesis and regulation of dendrite morphogenesis. Importantly, all of the AChE-targeting miRs found to be related to these selected processes were directed against the normally rare AChE-R splice variant, with three of them, including the neurogenesis regulator miR-132, also directed against AChE-S. Our findings point at the AChE-R splice variant as particularly susceptible to miR regulation, highlight those biological functions of cholinesterases that are likely to be subject to miR post-transcriptional control, demonstrate the selectivity of miRs in regulating specific biological processes, and open new venues for targeted interference with these specific processes.

Keywords: AChE, BChE, microRNA

MATERIALS AND METHODS

MicroRNA candidates were identified on each of the 3′-UTR sequences of AChE and BChE, which are 235, 1030, and 478 nucleotides long for BChE, the major “synaptic” AChE-S variant and the stress-inducible AChE-R variant, respectively (Figure 2A). We used the PicTar1, miRanda2, miRbase3, and microCosm4 algorithms to identify these transcript-specific miRs. All predictions ensured a threshold P-value < 0.05, and analysis specifications allowed both evolutionarily conserved and non-conserved miRs, which enabled us to include primate-targeting miRs as well.

Validation of miR-target interactions generally involved a 3′UTR luciferase assay. In some cases, it was complemented by protein blots, real-time RT-qPCR, microarrays, transgenic technology, β-galactosidase, or GFP-tagged targets. See, for example

1www.pictar.mdc-berlin.de
2www.microRNA.org
3www.mirbase.org
4http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/
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FIGURE 1 | The study’s flow chart. MicroRNAs complementary to the 3′-UTR domains of AChE and BCHE transcripts were identified using several algorithms and other validated targets for those miRs were searched for and analyzed for common biological processes in which both these miR targets and cholinesterases are involved.

the Shaked et al. (2009) report for several of the latter technologies used to explore the miR-132 target AChE, and (Hansen et al., 2010) for the “classical” 3′-UTR and transgenic approaches, in exploring p250GAP which is also a miR-132 target.

To search for gene ontology (GO) categories which are also relevant for the other mRNA targets of cholinesterase-related miRs, we used the DAVID functional annotation clustering tool. For each of the miRs identified as targeting one of the cholinesterases we searched for other experimentally validated targets; and we then used the lists of the other validated targets as gene lists for the DAVID search. Each list was normalized to the entire human genome, which served as a background.

RESULTS

We identified 116, 81, and 47 miRs (24, 8, and 20 miRs/100 nucleotides) that are complementary to the 3′-UTR domains of the BCHE, AChE-R, and AChE-S transcripts, respectively. Of these, 6 miRs target both BCHE and AChE-R whereas 11 miRs are common to both AChE-R and AChE-S, but BCHE and AChE-S do not share any miR (Figure 2B). Positions of the identified miRs are presented in Figure 2C, with miR-132 targeting a similar seed domain localized at the very 3′-end of the 3′-UTR in both the AChE-S and AChE-R transcripts. Of the cholinesterase-targeting miRs, seven had multiple binding sites to the target AChE-S, nine to AChE-R, and seven to the BCHE transcript, suggesting that they have a higher prospect for being functional (John et al., 2004).

Compatible with the different conceptual principles on which each of the algorithms employed is based, only 8.6, 17, and 13.7% (7/81), (8/47), (16/116) of the miRs identified as targeting AChE-R, AChE-S, and BCHE, respectively, were predicted by more than one of the algorithms. For AChE-R, these are hsa-miR-28-5p, -423-3p, -484, -483-5p, -663, -582-3p, -380*. For AChE-S, hsa-miR-194, -939, -658, -608, -615-5p, -423-5p, -920, and let-7f-2* and for BCHE, hsa-miR-203, -218, -221, -222, -181a, -181b, -181c, -181d, -494, -200b, -200c, -576-3p, -16-2*, -625, -195*, -889.

These cholinesterase-targeting miRs and their other validated non-cholinesterase targets are listed in Tables 1–3 with the corresponding functions attributed to these other targets. The relevant citations appear in Tables A1–A4 in Appendix. Of note, numerous cholinesterase-targeting miRs have no experimentally validated targets at this time, yet others have more than one validated target and associate with more than one biological function. Examples include miR-124 which targets both the AChE-S and IQGAP1 (Furuta et al., 2010), a GTPase activating protein which promotes neurite outgrowth (Table 1). Additionally miR-152 and miR-148a, which target AChE-R, also target the calmodulin regulating kinase CaMKIIα (Liu et al., 2010; Table 2). Lastly, the BCHE-targeting cluster of miRs-222 and -221 also target the neuronal early immediate protein c-fos (Ichimura et al., 2010; Table 3).

3http://david.abcc.ncifcrf.gov/
### Table 1 | Additional targets of AChE-S targeting microRNAs.

| miR ID       | Validated targets                                                                 |
|--------------|----------------------------------------------------------------------------------|
| hsa-miR-491-5p | Bcl-X(L; cell death)                                                             |
| hsa-miR-605  | Mdm2 (ubiquitination)                                                            |
| hsa-miR-608  | CD44 (cell–cell/cell–matrix interaction)                                         |
| hsa-miR-124  | Glucocorticoid receptor                                                           |
|              | NeuroD1 (neurogenic differentiation 1)                                            |
|              | Mtpn (mitogen-activated protein kinase 14)                                        |
|              | CDK2 (cyclin-dependent kinase 2)                                                 |
|              | MCP1 (monocyte chemoattractant protein 1)                                         |
|              | Itgb1 (integrin 1)                                                               |
|              | SC1p (synaptonemal filaments)                                                   |
| hsa-let-7g   | C-Myc (transcription)                                                            |
| hsa-miR-196a | HOX-B7 (transcription)                                                           |
|              | S100A9 (calcium-binding protein A9)                                               |
| hsa-miR-542-3p|                                                                                   |
| hsa-miR-525-5p|                                                                                   |

### Table 2 | Additional targets of microRNAs targeting AChE-R.

| miR ID              | Validated targets                                                                 |
|---------------------|----------------------------------------------------------------------------------|
| Hsa-miR-708         | MPL (thrombopoietin receptor; Girardot et al., 2010)                             |
| Hsa-miR-28-5p       | MPL (thrombopoietin receptor; Girardot et al., 2010)                             |
|                     | N4BP1 (NEDD4 binding protein 1; Girardot et al., 2010)                           |
| hsa-miR-503         | ANLN (actin-binding protein anillin)                                             |
|                     | EIF2C1 (argonaute1)                                                              |
|                     | CCNE1 (cyclin E1)                                                                |
|                     | CCND1 (cyclin D1)                                                                |
| hsa-miR-148a        | CaMKIIa (CNS kinase; Liu et al., 2010)                                           |
| hsa-miR-152         | DNMT1 (DNA methyltransferase 1)                                                   |
|                     | CCKBR (modulates anxiety and neuroleptic activity)                               |
|                     | POMC (pro-opiomelanocortin)                                                      |
|                      | TNFa (tumor necrosis factor α)                                                   |
|                      | IRF4 (interferon regulatory factor 4)                                            |
|                     | Blimp1 (zinc finger protein)                                                     |
|                     | Vdr (vitamin D receptor)                                                         |
|                     | CYP2A4A1 (cytochrome P450 family 24A)                                            |
|                     | IGF2 (insulin-like growth factor 2)                                              |
|                     | LIN28 (translational enhancer)                                                   |
| hsa-miR-125-5p      | CaMKIIa (CNS kinase; Liu et al., 2010)                                           |
|                     | DNMT1 (DNA methyltransferase 1)                                                   |
|                     | ERBB2 (erythroblast leukemia viral oncogene homolog 2)                           |
|                     | ERBB3 (erythroblast leukemia viral oncogene homolog 3)                           |
|                     | TEF (thyrotroph embryonic factor)                                                |
|                     | MUC1 (adhesion)                                                                 |
|                     | p53 (tumor suppressor)                                                           |
|                     | Survivin                                                                        |

(Continued)
### Table 2 | Continued

| miR ID       | Validated targets                                        |
|--------------|----------------------------------------------------------|
| hsa-miR-125a-5p | LIN28 (translational enhancer) T-TrkC (neurotrophic tyrosine kinase receptor 3) HuR (cell growth) |
| p53 (tumor suppressor) | KLF13 (transcription factor) AT-rich interactive domain 3B (transcription) |
| PDNP 9 (actin organization) | Bak1 (pro-apoptotic Bcl2 antagonist killer 1) |
| N-ras (oncogene) | MEK3 (phosphorylation of MAP kinase) |
| hsa-miR-214 | SrGAP1 (neuronal migration) EzH2 (stem cell identity) N-ras (oncogene) |
| JNK1 (MAPK8) | PTEN (tumor suppressor) MEK3 (phosphorylation) |
| hsa-miR-199a-5p | Hif-1α (Hypoxia-inducible factor 1) Sirt1 (apoptosis) |
| hsa-miR-31 | ICAM-1 (leukocyte adhesion protein) Fgf13 (fibroblast growth factor 13) Dkk-1 (canonical Wnt signaling) |
| DACT3 (epigenetic regulator of Wnt) | E-selectin (inflammation) p16Ink4a (cell cycle) |
| LAT52 (tumor suppression) | PPP2R2A (signal transduction) Krt16 (keratin 16) |
| Krt17 (keratin 17) | Dlx3 (development of ventral forebrain) E2F6 (cell cycle) |
| TIA1 (T-cell lymphoma invasion and metastasis 1) | M-RIP (regulation of actin) MMP16 (blood vessels matrix remodeling) RDX (actin filaments binding to plasma membrane) |
| M-RIP (regulation of actin) | SATB2 (upper-layer neurons initiation) PROX1 (CNS development) |
| hsa-miR-185 | Sox1 (signal transduction) WAVE3 (signal transmission) |
| hsa-miR-193b | Estrogen receptor α Mcl-1 (myeloid cell leukemia sequence 1) ETS-1 (oncogene) uPA (urokinase-type plasminogen activator) CCND1 (cyclin D1) |
| hsa-miR-7 | Alpha-synuclein (SNCA) LSH (lymphoid-specific helicase) SFRS1 (splicing) ERF (cell proliferation) |
| Associated cdc42 kinase 1 | DAP (cell death-associated protein) MRP1 (human multidrug resistance-associated protein 1) |
| CD98 (sodium transport) | Yan (cell differentiation) EGFR (epidermal growth factor receptor) |
| hsa-miR-483-5p | Socs-3 (cytokine signaling) BBC3/PUMA (apoptosis) IGF1R (insulin-like growth factor 1 receptor) |
| hsa-miR-663 | TGFβ1 (proliferation) JunB (jun B proto-oncogene) JunD (jun D proto-oncogene) |
| hsa-miR-765 | TRK3 (neurotrophic tyrosine kinase) |
| hsa-miR-146b-3p | IRAK1 (IL1 receptor-associated kinase 1) EGFR (epidermal growth factor receptor) MMP16 (degrades extracellular matrix) |

### Table 3 | Additional targets of BChE-targeting microRNAs.

| miR ID       | Validated target                                        |
|--------------|----------------------------------------------------------|
| hsa-miR-203 | SOCS-3 (cytokine signaling) LeF1 (lymphoid enhancer-binding factor) p63 (transcription) |
| ABL1 (cell growth) | BarX1 (transcription) CKAP2 (cytoskeleton associated protein 2) |
| LASP1 (cytoskeletal activities) | BIRC5 (regulator of mitosis) WASF1 (signal transmission) |
| ASAP1 (membrane trafficking) | RUNX2 (runt-related transcription factor 2) |
| hsa-miR-340 | MITF (microphthalmia-associated transcription factor) |
| hsa-miR-218 | IKK-γ (NFκB activation) ROBO1 (roundabout, axon guidance receptor, homolog 1) BIRC5 (mitosis) |
| GJA1 (gap junction protein, α1) | ROBO2 (roundabout, axon guidance receptor homolog 2) GLCE (glucuronic acid epimerase) |
| PNX (paxillin, cytoskeletal protein) | |

(Continued)
| miR ID    | ERα (estrogen receptor α) p57 (cyclin-dependent kinase inhibitor 1C) PTEN (tumor suppressor) Bmf (apoptosis) | Validated target                                                                 | p27 (cell cycle) TIMP3 (TIMP metallopeptidase inhibitor 3) PUMA (apoptosis) C-fos (cell proliferation; Ichimura et al., 2010) CDKN1B (cyclin-dependent kinase inhibitor 1B) |
|-----------|----------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| hsa-miR-221| ICAM-1 (leukocyte adhesion protein) DNA damage-inducible transcript 4 (DDIT4)                      |                                                                                  |                                                                                                                                                                                                                                                                                                                                 |
| hsa-miR-222| p27 (cell cycle)                                                                                   |                                                                                  |                                                                                                                                                                                                                                                                                                                                 |
| hsa-miR-181a| SIRT1 (apoptosis)                                                                                  |                                                                                  | Hox-A11 (transcription)                                                                                                                                                                                                                                                                                                      |
| hsa-miR-181b| p27(cell cycle)                                                                                   |                                                                                  | BCL2 (B-cell CLL/lymphoma 2; apoptosis) OPN (osteopontin)                                                                                                                                                                                                                                                                       |
| hsa-miR-181c| IL2 (immune response)                                                                             |                                                                                  | NOTCH4 (transcriptional activator)                                                                                                                                                                                                                                                                                           |
| hsa-miR-181d| BCL2 (B-cell CLL/lymphoma 2; apoptosis)                                                            |                                                                                  |                                                                                                                                                                                                                                                                                                                                 |
| hsa-miR-494| CalMKIIs (CNS kinase)                                                                             |                                                                                  | LIF [leukemia inhibitory factor (cholinergic differentiation factor)]                                                                                                                                                                                                                                                                                                               |
| hsa-miR-129-5p| CAMTA1 (calmodulin binding transcription activator 1)                                               |                                                                                  | GALNT1 (oligosaccharide biosynthesis)                                                                                                                                                                                                                                                                                       |
| hsa-miR-30d| Galphai2 (G protein, α inhibiting activity polypeptide 2)                                           |                                                                                  |                                                                                                                                                                                                                                                                                                                                 |
| hsa-miR-30c| Runx1 (run-related transcription factor 1)                                                          |                                                                                  | CTGF (connective tissue growth factor)                                                                                                                                                                                                                                                                                      |
| hsa-miR-30a| SOD2 (superoxide dismutase 2)                                                                     |                                                                                  | BDNF (brain-derived neurotrophic factor)                                                                                                                                                                                                                                                                                     |
| hsa-miR-30e| Ubc9 (ubiquitin-conjugating enzyme E2I)                                                            |                                                                                  | Beclin 1 (autophagy)                                                                                                                                                                                                                                                                                                           |
| hsa-miR-320a| HSP27 (heat-shock protein 27)                                                                     |                                                                                  |                                                                                                                                                                                                                                                                                                                                 |
| hsa-miR-140-5p| Smad3 (transcription)                                                                            |                                                                                  | HDAC4 (histone deacetylase 4)                                                                                                                                                                                                                                                                                               |
| hsa-miR-519c-3p| HIF-1α (hypoxia-inducible factor 1α)                                                               |                                                                                  | ABCG2 (exclusion of xenobiotics from the brain)                                                                                                                                                                                                                                                                              |
| hsa-miR-489| PTPN11 (signal transduction)                                                                     |                                                                                  | ROCK-1 (actin assembly)                                                                                                                                                                                                                                                                                                      |

We focused our survey on those functions of those miRs for which experimental validation is available. Table 4 presents these miRs which are shared for AChE-R and AChE-S or AChE-R and BChE and some of their additional targets, highlighting the multitude of miR targets with predicted regulatory functions (e.g., the chromatin modulator zinc finger proteins ZEB1 and ZEB2 targeted by miR-200b, miR-200c, and miR-429 that are also directed to both AChE-R and AChE-S; Gregory et al., 2008). Likewise, the AChE-S-targeted miR-132 (Shaked et al., 2009; Soreq and Wolf, 2011) also targets the GT-Pase regulator p250GAP.
Table 4 | Additional targets of ChE-targeting miRs (common to more than one ChE).

| miR ID       | Validated target common to ACHE-R and AChE-S | miR ID       | Validated targets common to ACHE-R and BChE |
|--------------|--------------------------------------------|--------------|--------------------------------------------|
| hsa-miR-186  | Pro-apoptotic P2 x 7 purinergic receptor     | hsa-miR-24   | SOD1 (superoxide dismutase 1)               |
| hsa-miR-199b-5p | Dyrk1a (brain development)                 | MKK4 (survival signal in T cells)           |
| hsa-miR-429  | ZEB1 (transcriptional repression of IL2; Gregory et al., 2008) | FA1 (apoptosis) | |
|              | RERE (apoptosis)                           |              | |
| hsa-miR-200b | ZEB1 (transcriptional repression of IL2; Gregory et al., 2008) | Serca2 (sarcoplasmic reticulum Ca2+ ATPase) |
|              |                                            |              | |
| hsa-miR-200c | ZEB1 (transcriptional repression of IL2; Gregory et al., 2008) | VEGF (angiogenesis) | |
|              |                                            | KLF13 (transcription factor)               | |

The process-regulation hypothesis of miR function predicts the existence of biological functions in which both cholinesterases, and those other targets which share miRs with cholinesterases, would be involved. To challenge this hypothesis, we first identified the GO categories in which AChE and BChE are involved, and found 24 and 11 biological processes for these two proteins, respectively. Twenty-three, 13, and 18 enriched biological processes emerged as shared processes for the other validated targets of AChE-R, AChE-S, and BChE-targeting miRs, respectively (P-value threshold < 0.05).

Out of over 20 ontology categories attributed to AChE, only two are shared with the categories attributed to the other validated targets of the cholinesterase-targeting miRs. These are: Response to wounding (GO: 0006911; 68 transcripts) and Neuron development (GO: 0048666), and specifically its AChE-relevant child terms Regulation of axonogenesis (GO: 0050770; 78 transcripts) and regulation of dendrite morphogenesis (GO: 0048814; 27 transcripts). Surprisingly, all 10 miRs that regulate Response to wounding and Neuron development selectively target the normally rare, stress-responsive AChE-R transcript, (miR-186, -125b, -200c, -199a-5p, -199b-5p, -125a, -214, -7, -663, -31, and -148a) whereas only three of these miRs also target the prevalent AChE-S mRNA (miR-194, -24, and -132). For BChE, we found only one shared category out of 11 relevant ontology groups: Response to glucocorticoid stimulus (GO: 0051384; 119 transcripts), and no overlap with the AChE-relevant categories (Figures 3A,B).

**DISCUSSION**

Using a variety of available algorithms, we found a plethora of cholinesterase-targeted miRs. Some of these were already validated as functionally capable of silencing other mRNA transcripts. A study of the functionally relevant biological processes in which these other targets are involved revealed a highly focused overlap with only few of the biological processes in which cholinesterases participate. Given that miRs regulate targets which share biological
processes, cholinesterases appear to be primarily subject to miR regulation when involved in neuronal development, response to wounding, and glucocorticoid stimulus; and specific cholinergic processes are regulated by miRs targeting both AChE and other targets participating in the same biological process.

Several limitations should be considered in the context of this study. First, the currently available search algorithms for miR candidates appear to differ substantially, which casts a shadow on the veracity of such identification. Second, research bias has focused much of the efforts in the miR field toward cancer research, whereas neuroscience-focused miRs were relatively neglected. Therefore, we might have overlooked important miRs simply because they have not yet been validated experimentally. This being said, that many of the biological functions in which cholinesterases are involved show no relevant cholinesterase-targeting miR sequences suggests other modes of regulation of cholinesterase levels for most of these functions (e.g., transcriptional (Hill and Treisman, 1995), epigenetic (Allshire and Karpen, 2008), or post-translational processes (Fukushima et al., 2009)). Alternatively, or in addition, miRs might exist which control these functions, but have no role in cancer biology and are therefore not yet characterized. MiR regulation of cholinesterase functions will therefore need to be re-inspected in the near future.

ACKNOWLEDGMENTS

The authors are grateful to E. R. Bennett, Jerusalem, for critical evaluation of this manuscript. This work was supported by the Legacy Heritage Biomedical Science Partnership Program of the Israel Science Foundation (Grant No. 1876/08, to Hermon Soreq).

REFERENCES

Allshire, R. C., and Karpen, G. H. (2008). Epigenetic regulation of centromeric chromatin: old dogs, new tricks? Nat. Rev. Genet. 9, 923–937.

Bartel, D. P. (2009). MicroRNAs: target recognition and regulatory functions. Cell 136, 215–223.

Fukushima, N., Furuta, D., Hidaka, Y., Moriyama, R., and Tsujiuchi, T. (2009). Post-translational modifications of tubulin in the nervous system. J. Neurochem. 109, 683–693.

Furuta, M., Kozaki, K. I., Tanaka, S., Arii, S., Iimoto, I., and Inazawa, J. (2010). miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. Carcinogenesis 31, 766–776.

Girardot, M., Pecquet, C., Boukour, S., Knoops, L., Ferrant, A., Vainchenker, W., Giraudier, S., and Constans, S. N. (2010). miR-28 is a thrombopoietin receptor target.

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FIGURE 3 | MiR regulators of biological processes shared by cholinesterases and validated targets of these miRs. (A) miRs targeting transcripts participating in the AChe-S and AChe-R relevant response to wounding (yellow) and neuron development processes (blue) or both categories (green). (B) miRs targeting transcripts participating in the BChE-relevant response to glucocorticoid stimulus category.

Barry, S. C., Tsykin, A., Farshid, G., Vadas, M. A., Khev-Goodall, Y., and Goodall, G. J. (2008). The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat. Cell Biol. 10, 593–601.

Hansen, K. E., Sakamoto, K., Wayman, G. A., Impy, S., and Obrietan, K. (2010). Transgenic miR132 alters neuronal spine density and impairs novel object recognition memory. PLoS ONE 5, e15497. doi: 10.1371/journal.pone.0015497

Hill, C. S., and Treisman, R. (1993). Transcriptional regulation by extra-cellular signals: mechanisms and specificity. Cell 80, 199–211.

Ichimura, A., Ruike, Y., Terasawa, K., Shimizu, K., and Tsujimoto, G. (2010). MicroRNA-34a inhibits cell proliferation by repressing mitogen-activated protein kinase kinase 1 (MEK1). J. Neurochem. 109, 683–693.

Liu, X., Zhan, Z., Xu, L., Ma, F., Li, D., Guo, Z., Li, N., and Cao, X. (2010). MicroRNA-148/152 impair innate response and antigen presentation of TLR-triggered dendritic cells by targeting C/EBPalpha. J. Immunol. 185, 7244–7251.

Massoulié, J. (2002). The origin of the molecular diversity and functional anchoring of cholinesterases. Neuron 31, 130–143.

Shaked, I., Meerson, A., Wolf, Y., Avni, R., Greenberg, D., Gilboa-Geffen, A., and Soreq, H. (2009). MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. Immunity 31, 965–973.

Soreq, H., and Seidman, S. (2001). Acetylcholinesterase – new roles for an old actor. Nat. Rev. Neurosci. 2, 294–302.

Soreq, H., and Wolf, Y. (2011). Neurim-miRs: micro-RNAs in the neuroimmunological interface. Trends Mol. Med. doi: 10.1016/j.molmed.2011.06.009. [Epub ahead of print].

Received: 25 July 2011; paper pending publication: 23 August 2011; accepted: 14 September 2011; published online: 05 October 2011.

Citation: Hanin G and Soreq H (2011) Cholinesterase-targeting microRNAs identified in silico affect specific biological processes. Front. Mol. Neurosci. 4:28. doi: 10.3389/fnmol.2011.00028

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### APPENDIX

**Table A1 | Additional targets of AChE-S targeting microRNAs.**

| miR ID       | Validated targets                                                                 |
|--------------|-----------------------------------------------------------------------------------|
| hsa-miR-491-5p | Bcl-X (L; cell death; Nakano et al., 2010)                                        |
| hsa-miR-605   | Mdm2 (ubiquitination; Xiao et al., 2011)                                          |
| hsa-miR-608   | CD44 (cell–cell/cell–matrix interaction; Jeyapalan et al., 2011)                 |
| hsa-miR-124   | Glucocorticoid receptor (Mreugdenhil et al., 2009)                                |
|               | NeuroD1 (neurogenic differentiation 1; Liu et al., 2011)                          |
|               | Mtpn (myotrophin; Krek et al., 2005)                                              |
|               | Mapk14 (mitogen activated protein kinase 14; Krek et al., 2005)                   |
|               | CDK2 (cyclin-dependent kinase 2; Nakamachi et al., 2009)                          |
|               | MCP-1 (monocyte chemoattractant protein 1; Nakamachi et al., 2009)                |
|               | Itgb1 (integrin 1; Cao et al., 2007)                                              |
| hsa-let-7g    | SCP1 (synaptonemal filaments; Cao et al., 2007)                                   |
| hsa-miR-196a  | C-Myc (transcription factor; Lan et al., 2011)                                     |
| hsa-miR-542-3p| Survivin (roon et al., 2010)                                                      |
| hsa-miR-525-5p| VPAC1 (vasoactive intestinal peptide receptor 1; Cocco et al., 2010)              |
| hsa-miR-520   | Bcl-XL (cell death; Shimizu et al., 2010)                                         |
| hsa-miR-519   | Collagen alpha2 (COL1A2; Ji et al., 2010)                                         |
| hsa-miR-519   | SPRR2C (small proline-rich protein 2C; Maru et al., 2009)                        |
| hsa-miR-519   | KRT5 (keratin 5; Maru et al., 2009)                                               |
| hsa-miR-519   | HOXC8 (transcription factor; Kim et al., 2009a)                                   |

*miRs without validated targets: hsa-miR-920-5p, -506-2*, -541-2*, -658, -423-5p, -615-5p, -25*, -4688, -4778-3p, -868, -3613-5p, -4700-5p, -718, let-7f-2*, -455-3p, -633, -554, -524-3p, -636, -1025-3p, -611, let-7e*, -4283, -4329, -4278, -3400, -3184, -149*.
### Table A2 | Additional targets of microRNAs targeting AChE-R.

| miR ID       | Validated targets                                                                 |
|--------------|----------------------------------------------------------------------------------|
| hsa-miR-708  | MPL (thrombopoietin receptor; Girardot et al., 2010)                            |
| hsa-miR-28-5p| MPL (thrombopoietin receptor; Girardot et al., 2010)                            |
| hsa-miR-503  | N4BP1 (NEDD4 binding protein 1; Girardot et al., 2010)                          |
| hsa-miR-148a | OTUB1 (immune system transcription; Girardot et al., 2010)                      |
| hsa-miR-125a-5p| MAPK1 (megakaryocyte differentiation; Girardot et al., 2010)                     |
| hsa-miR-125b | MAPK1 (megakaryocyte differentiation; Girardot et al., 2010)                     |
| hsa-miR-125a-5p| MAPK1 (megakaryocyte differentiation; Girardot et al., 2010)                     |

(Continued)
### Table A2 | Continued

| miR ID    | Validated targets                                                                 |
|-----------|-----------------------------------------------------------------------------------|
| hsa-miR-214 | SrGAP1 (neuronal migration; Zhang et al., 2011a)                                    |
|           | N-ras (oncogene; Liu et al., 2010b)                                               |
| hsa-miR-199a-5p | Hif-1α (hypoxia-inducible factor 1; Rane et al., 2009)                             |
|           | DDR1 (discoidin domain receptor 1; Shen et al., 2010)                               |
| hsa-miR-31 | ICAM-1 (leukocyte adhesion protein; Suarez et al., 2010)                            |
|           | p16Ink4a (cell cycle; Malhas et al., 2010)                                          |
| hsa-miR-185 | Six1 (limb development; Imam et al., 2010)                                         |
| hsa-miR-193b | Estrogen receptor α (Leivonen et al., 2009)                                        |
|           | CCND1 (cyclin D1; Xu et al., 2010a)                                                |
| hsa-miR-7   | Associated cdc42 kinase 1 (Saydam et al., 2011)                                     |
|           | EGF (epidermal growth factor receptor; Kefas et al., 2008)                          |
| hsa-miR-483-5p | TRK3 (neurotrophic tyrosine kinase; Guidi et al., 2010)                          |
|           | EGFR (epidermal growth factor receptor; Shao et al., 2011)                          |

miRs without validated targets that are predicted to target AChE-R: hsa-miR-590-3p, -148b, -193a-3p, -182*, -4298, -4644, -4739, -1224-3p, -4769-5p, –582-3p, -380, -1825, -892b, -1275, -3165, -765, -3119, -3139, -563, -92b*, -1321, -4283, -1228*, -4323, -4319, -761, -767-5p, -224, -522, -4271, -1226*, -3179, -92a-1*, -3202, -20b, -4303, -4306, -3065-5p, -4297, -4329, -3148, -3163, -22*, -4302, -513a-5p, -542-5p, -377, -1908, -92a-2*, -608, -626.
### Table A3 | Additional targets of BChE-targeting microRNAs.

| miR ID | Validated target |
|--------|------------------|
| hsa-miR-203 | **SOCS-3** (cytokine signaling; Wei et al., 2010) | Lef1 (lymphoid enhancer-binding factor; Thatcher et al., 2008) | p63 (transcriptional activator or repressor; Yi et al., 2008) |
|         | ABL1 (cell growth; Bueno et al., 2008) | Barx1 (transcription factor; Kim et al., 2011) | CKAP2 (cytoskeleton associated protein 2; Viticchie et al., 2011) |
|         | LASP1 (cytoskeletal activities; Viticchie et al., 2011) | BIRC5 (regulator of mitosis; Viticchie et al., 2011) | WASF1 (signal transmission; Viticchie et al., 2011) |
|         | ASAP1 (membrane trafficking; Viticchie et al., 2011) | RUNX2 (runt-related transcription factor 2; Viticchie et al., 2011) |

| hsa-miR-340 | MIF (microphthalmia-associated transcription factor; Goswami et al., 2010) | MITF (microphthalmia-associated transcription factor; Garofalo et al., 2009) |

| hsa-miR-218 | IKK-β (cytokine-activated intracellular signaling pathway; Song et al., 2010) | ROBO1 (roundabout, axon guidance receptor, homolog 1; Alajez et al., 2011) |
|             | GJA1 (gap junction protein, α1; Alajez et al., 2011) | ROBO2 (roundabout, axon guidance receptor homolog 2; Alajez et al., 2011) |
|             | PTEN (tumor suppressor; Garofalo et al., 2009) | C-fos (cell proliferation; Ichimura et al., 2010) |

| hsa-miR-221 | ERα (estrogen receptor α; Zhou et al., 2008) | ICAM-1 (leukocyte adhesion protein; Hu et al., 2010) |
|             | p57 (cyclin-dependent kinase inhibitor 1C; Kim et al., 2009b) | DNA damage-inducible transcript 4 (DDIT4; Pineau et al., 2010) |
|             | PTEN (tumor suppressor; Garofalo et al., 2009) | PUMA (apoptosis; Zhang et al., 2010) |
|             | Bmf (apoptosis; Gramantieri et al., 2009) | Mdm2 (ubiquitination; Kim et al., 2010) |

| hsa-miR-222 | ERα (estrogen receptor α; Zhou et al., 2008) | p27 (cell cycle; Garofalo et al., 2009) |
|             | STAT5A (signal transducer and activator of transcription 5; Dentelli et al., 2010) | TIMP3 (TIMP metallopeptidase inhibitor 3; Garofalo et al., 2009) |
|             | Bim (apoptosis; Terasawa et al., 2009) | PUMA (apoptosis; Zhang et al., 2010) |
|             | PPP2R2A (protein phosphatase 2A subunit B; Wong et al., 2010) | C-fos (cell proliferation; Ichimura et al., 2010) |
|             | MMP1 (cleaves collagen; Liu et al., 2009) | ICAM-1 (Ueda et al., 2009) |

| hsa-miR-181a | Sirt1 (apoptosis, muscle differentiation; Saunders et al., 2010) | Ataxia telangiectasia mutated (ATM; cell cycle; Wang et al., 2011) |
|             | p27 (cell cycle; Cuesta et al., 2009) | PLAG1 (transcription factor; Pallasch et al., 2009) |
|             | Bim (apoptosis; Lwin et al., 2010) | Tcl1 (cell proliferation; Pekarsky et al., 2006) |

| hsa-miR-181b | AID (activation-induced cytidine deaminase; RNA-editing; De Yebenes et al., 2008) | Ataxia telangiectasia mutated (ATM; cell cycle; Wang et al., 2011) |
|             | TIMP3 (TIMP metallopeptidase inhibitor 3; Wang et al., 2010a) | PLAG1 (transcription factor; Pallasch et al., 2009) |
|             | ZNF37A (transcriptional regulation; Huang et al., 2010) | BCL2 (B-cell CLL/Lymphoma 2; apoptosis; Zhu et al., 2010) |
|             | Mcl-1 (myeloid cell leukemia-1; apoptosis; Zimmerman et al., 2010) | Sirt1 (apoptosis, muscle differentiation; Saunders et al., 2010) |

| hsa-miR-181c | IL2 (immune response; Xue et al., 2011) | BCL2 (B-cell CLL/Lymphoma 2; apoptosis; Zhu et al., 2010) |

*(Continued)*
### Table A3 | Continued

| miR ID   | Validated target                                                                 |
|----------|----------------------------------------------------------------------------------|
| hsa-miR-181d | KRAS (GTPase activity; Hashimoto et al., 2010)                                   |
| hsa-miR-494  | CaMKII (CNS kinase; Wang et al., 2010b)                                           |
| hsa-miR-129-5p | PTEN (phosphatase and tensin homolog; Wang et al., 2010b)                        |
| hsa-miR-30d  | CAMTA1 (calmodulin binding transcription activator 1; Liao et al., 2008)          |
| hsa-miR-30c  | SOD2 (superoxide dismutase 2; Xia et al., 2006)                                   |
| hsa-miR-30a  | Xlim1/Lhx1 (transcription factor; Agrawal et al., 2009)                          |
| hsa-miR-30e  | Ubc9 (ubiquitin-conjugating enzyme E21; Wu et al., 2009)                         |
| hsa-miR-320a | Hsp20 heat-shock protein 20; Ren et al., 2009                                    |
| hsa-miR-140-5p | The transferrin receptor 1 (TfR-1; CD71; development of erythrocytes and the nervous system; Schaar et al., 2009) |
| hsa-miR-519c-3p | HIF-1α (hypoxia-inducible factor 1α; Cha et al., 2010)                         |
| hsa-miR-584  | NXA1 (exocytosis; Luthra et al., 2008)                                           |

**miRs without validated targets:** hsa-miR-147b, -532-5p, -508-3p, -989, -325, -573, -196, -567, -1938r, -625, -16-2, -576-1p, -190b, -518r, -518a, -518d-5p, -147-320d, -320c, -320b, -875-5p, -758, -30b, -1279, -3145, -1183, -664, -4261, -4262, -1237, -1972, -3146, let-7a-2, let-7g, -1911, -2052, -15a, -3148, -555, -656, -636, -3182, -513a-3p, -501-3p, -502-3p, -579, -4316, -4312, -1294, -142-5p, -3128, -30a, -30r, -30r, -4268, -3137-20r, -651, -32, -362-5p, -400b, -501-5p, -1976, -449c, -1224-5p, -302r, -1248, -99r, -99r, -369-3p, -1256, -629, -187, -514b-3p, -378, -1305, -331-tp, -1200, -4272, -4260, -493, -582-5p, -4255, -3133, -4273, -19r, -19b-1, -19b-2, -4277, -19b', -1826.
### Table A4 | Additional targets of ChE-targeting miRs (common to more than one ChE).

| miR ID       | Validated target common to ACHE-R and AChE-S                                                                 |
|--------------|-------------------------------------------------------------------------------------------------------------|
| **Hsa-miR-186** | Pro-apoptotic P2 x 7 purinergic receptor (Zhou et al., 2008)                                               |
|              | AKAP12 (tumor suppressor; Goeppert et al., 2010)                                                           |
| **Hsa-miR-199b-5p** | Dyrk1a (brain development; Da Costa Martins et al., 2010)                                                |
|              | HES1 (transcriptional repressor; Garzia et al., 2009)                                                     |
| **Hsa-miR-429** | ZEB1 (transcriptional repression of IL2; Gregory et al., 2008)                                            |
|              | ZEB2a (SIP1; zinc finger protein; Gregory et al., 2008)                                                    |
|              | RERE (apoptosis; Karres et al., 2007)                                                                       |
| **Hsa-miR-200b** | ZEB1 (transcriptional repression of IL2; Gregory et al., 2008)                                            |
|              | ZEB2 (SIP1; zinc finger protein; Gregory et al., 2008)                                                      |
|              | SIRT1 (apoptosis; Strum et al., 2009)                                                                        |
|              | PLCγ1a (apoptosis; Uhlmann et al., 2010)                                                                    |
| **Hsa-miR-200c** | ZEB1 (transcriptional repression of IL2; Gregory et al., 2008)                                            |
|              | ZEB2 (SIP1; zinc finger protein; Gregory et al., 2008)                                                      |
|              | Serca2 (sarco/endoplasmic reticulum Ca2⁺-ATPase; Salomonis et al., 2010)                                   |
|              | PLCγ1a (apoptosis; Uhlmann et al., 2010)                                                                    |
|              | OREBP (osmotic response element; Huang et al., 2011b)                                                       |
|              | Cyclin D1 (Xia et al., 2010)                                                                                |
|              | VEGF (angiogenesis; Liu et al., 2010a)                                                                       |
|              | TUBB3 (neurogenesis and axon guidance; Cochrane et al., 2009)                                              |
|              | KLF13 (transcription factor; Li et al., 2009a)                                                               |
|              | MBNL2 (muscleblind-like protein 2; Li et al., 2009a)                                                         |

| miR ID       | Validated targets common to ACHE-R and BChE                                                                 |
|--------------|-------------------------------------------------------------------------------------------------------------|
| **Hsa-miR-24** | SOD1 (superoxide dismutase 1; Papaioannou et al., 2011)                                                   |
|              | ALK4 (transducer of activin; Wang et al., 2006)                                                            |
|              | E2F2 (cell cycle; Lal et al., 2009a)                                                                        |
| **Hsa-miR-212** | DHFR (dihydrofolate reductase; Mishra et al., 2009)                                                        |
|              | DHFR (dihydrofolate reductase; Misra et al., 2009)                                                          |
|              | DN1 (miRNA-mediated gene suppression; Liu et al., 2010c)                                                    |
| **Hsa-miR-132** | MeCP2 (interaction with histone deacetylase; Im et al., 2010)                                              |
|              | MYC (transcription; Xu et al., 2010b)                                                                       |
|              | P250GAP (neuron-associated GTPase; Vo et al., 2005)                                                          |
|              | Per1 (circadian clock; Cheng et al., 2007)                                                                  |
|              | MeCP2 (modification of eukaryotic genomes; Klein et al., 2007)                                             |
|              | p300 (chromatin remodeling; Lagos et al., 2010)                                                              |
|              | Jarid1a (histone demethylase; Alvarez-Saavedra et al., 2011)                                               |
|              | Bjg2 (cell cycle; Alvarez-Saavedra et al., 2011)                                                             |
|              | p120RasGAP (angiogenesis; Anand et al., 2010)                                                                |
| **Hsa-miR-198** | Cyclin T1 (Xu et al., 2010b)                                                                               |
|              | Cyclin T1 (Xu et al., 2010b)                                                                               |
|              | Rac1 (GTP-binding protein; Venugopal et al., 2010)                                                          |
|              | Per family (circadian; Nagel et al., 2009)                                                                  |
| **Hsa-miR-194** | MDM2 (p53 negative regulator; Pichiorri et al., 2010)                                                      |
|              | EP300 (transcriptional co-activator; Mees et al., 2010)                                                      |

miRs without validated targets: hsa-miR-423-3p, -484, -4729-3p, -939, -484, -4729-3p.
REFERENCES

Agrawal, R., Tran, U., and Wessely, O. (2009). The miR-30 miRNA family regulates Xenopus pronephros development and targets the transcription factor Xlim1/Lhx1. Development 136, 3927–3936.

Alajez, N. M., Lenarduzzi, M., Ito, E., Hui, A. B., Shi, W., Bruce, J., Sue, S., Huang, S. H., Xu, W., Waldrum, J., O’ Sullivan, B., and Liu, F. F. (2011). MiR-218 suppresses nasopharyngeal cancer progression through downregulation of the SLIT2-ROBO1 pathway. Cancer Res. 71, 2381–2391.

Alvarez-Saavedra, M., Antoun, G., Yanagiy, A., Oliva-Hernandez, R., Cornejo-Palma, D., Perez-Iratxeta, C., Sonenberg, N., and Cheng, H. Y. (2011). MiRNA-132 orchestrates chromatin remodeling and translational control of the circadian clock. Hum. Mol. Genet. 20, 731–751.

Anand, S., Majeti, B. K., Acevedo, L. Ben-Ami, O., Pencovich, N., Lotem, J., Baroukh, N., Ravier, M. A., Loder, M. (2010). Downregulation of miR-106, 238–243.

Anant, S., Majeti, B. K., Acevedo, L. Ben-Ami, O., Pencovich, N., Lotem, J., Baroukh, N., Ravier, M. A., Loder, M. (2010). Downregulation of miR-106, 238–243.

Arvanitis, D. N., Jungas, T., Behar, A., Aprelikova, O., Yu, X., Palla, J., Wei, B. R., Anand, S., Majeti, B. K., Acevedo, L. Ben-Ami, O., Pencovich, N., Lotem, J., Baroukh, N., Ravier, M. A., Loder, M. (2010). Downregulation of miR-106, 238–243.

Arvanitis, D. N., Jungas, T., Behar, A., Aprelikova, O., Yu, X., Palla, J., Wei, B. R., Anand, S., Majeti, B. K., Acevedo, L. Ben-Ami, O., Pencovich, N., Lotem, J., Baroukh, N., Ravier, M. A., Loder, M. (2010). Downregulation of miR-106, 238–243.

Arvanitis, D. N., Jungas, T., Behar, A., Aprelikova, O., Yu, X., Palla, J., Wei, B. R., Anand, S., Majeti, B. K., Acevedo, L. Ben-Ami, O., Pencovich, N., Lotem, J., Baroukh, N., Ravier, M. A., Loder, M. (2010). Downregulation of miR-106, 238–243.

Arvanitis, D. N., Jungas, T., Behar, A., Aprelikova, O., Yu, X., Palla, J., Wei, B. R., Anand, S., Majeti, B. K., Acevedo, L. Ben-Ami, O., Pencovich, N., Lotem, J., Baroukh, N., Ravier, M. A., Loder, M. (2010). Downregulation of miR-106, 238–243.
Garofalo, M., Quintavalle, C., Di Leva, Fujita, Y., Kojima, K., Ohhashi, R., Forrest, A. R., Kanamori-Katayama, M., Edbauer, D., Neilson, J. R., Foster, K. A., Ferretti, E., De Smaele, E., Miele, E., Hanin and Soreq Cholinesterase-targeting microRNAs

Y., Hume, D. A., and Suzuki, H., A., Sato, A., Kondo, S., Kojima, T., Wang, C. F., Seeburg, D. P., Batterton, Deguchi, T., and Ito, M. (2010). MicroRNA profil- ing reveals that miR-125a controls the contribution of actin distribution and cell shape in fibroblasts. PLoS ONE 5, e17169. doi:10.1371/journal.pone.0011769

Hackanson, B., Bennett, K. L., Brena, R. M., Jiang, J., Claus, R., Chen, S. S., Blagilo-Dorfs, N., Maharry, K., Whitman, S. P., Schmittgen, T. D., Lubbert, M., Marcucci, G., Bloomfield, C. D., and Plass, C. (2008). Epigenetic modification of CCAAT/enhancer binding protein alpha expression in acute myeloid leukemia. Cancer Res. 68, 3142–3151.

Hafidlodottir, B. S., Bergsteinsson, D., Kastrinakis, C., and Steingrim- son, E. (2010). miR-148 regulates Mitf in melanoma cells. PLoS ONE 5, e11574. doi:10.1371/journal.pone.0011574

Hashimoto, A., Yakiyama, Y., Otsubo, T., Shimada, S., and Yusa, Y. (2010). Involvement of epigenet- ically silenced microRNA-181c in gastric carcinogenesis. Cancerogene- sis 31, 777–784.

Hu, G., Gong, A. Y., Liu, J., Zhou, R., Deng, C., and Chen, X. M. (2010). miR-212 suppresses ICAM-1 translation and regulates interferon-gamma-induced ICAM-1 expression in human cholangiocytes. Am. J. Physiol. Gastrointest. Liver Physiol. 298, G542–G550.

Huang, L., Luo, J., Cai, Q., Pan, Q., Zeng, H., Guo, Z., Dong, W., Huang, J., and Lin, T. (2011a). MicroRNA-125b suppresses the development of bladder cancer by targeting E2F3. Int. J. Cancer 128, 1758–1769.

Huang, W., Liu, H., Wang, T., Zhang, T., Kang, L., Luo, Y., Chung, S. S., Yuan, L., and Yang, J. Y. (2011b). Toxicity- responsive microRNAs contribute to the maximal induction of osmoregulatory transcription factor OREBP in response to high-NaCl hyper- toxicity. Nucleic Acids Res. 39, 475–485.

Huang, S., Wu, S., Ding, J., Lin, J., Wei, L., Gu, J., and He, X. (2010). MicroRNA-181a modulates gene expression of zinc finger family members by directly targeting their coding regions. Nucleic Acids Res. 38, 7212–7218.

Iliopoulos, D., Lindahl-Alten, M., Poly- tarchou, C., Hirsch, H. A., Tisch- lis, P. N., and Struhl, K. (2010). Loss of miR-200 inhibition of Suz12 leads to polycistron-mediated repression required for the formation and maintenance of cancer stem cells. Mol. Cell 39, 761–772.

Ilnytskyy, Y., Zemp, F. J., Kotur- bash, I., and Kovacchuk, O. (2008). Alk5-mediated SMAD3 activity in irradiated hematopoietic tis- sues suggest a sex-specific protective mechanism. Biochem. Biophys. Res. Commun. 377, 41–45.

Im, H. I., Hollander, J. A., Bali, P., and Kenny, P. J. (2010). MeCP2 controls BDNF expression and cocaine intake through homeostatic interac- tions with microRNA-212. Nat. Neurosci. 13, 1120–1127.

Imam, J. S., Buddhavarapu, K., Lee, Chang, J. S., Ganapathy, S., Camosy, C., Chen, Y., and Rao, M. K. (2010). MicroRNA-185 suppresses tumor growth and progression by targeting the Six1 oncomgene in human cancers. Oncogene 29, 4971–4979.

Incorniato, M., Garafolo, M., Urso, L., Romano, G., Quintavalle, C., Zanca, C., Iaboni, M., Nuovo, G., Croce, C. M., and Condorelli, G. (2010). miR-212 increases tumor necro- sis factor-related apoptosis-inducing ligand sensitivity in non-small cell lung cancer by targeting the anti- apoptotic protein PED. Cancer Res. 70, 3638–3646.

Jeyapalan, Z., Deng, Z., Shatseva, T., Fang, L., He, C., and Yang, B. B. (2011). Expression of CD44 3′-untranslated region regulates endogenous microRNA functions in tumorigenesis and angiogenesis. Nucleic Acids Res. 39, 3026–3041.

Ji, L., Zhao, L., Budiu, A., Forgues, M., Jia, H. L., Qin, L. X., Ye, Q. H., Yu, J., Shi, X., Tang, Z. Y., and Wang, X. W. (2010). Let-7g targets collagen type I alpha2a and inhibits cell migra- tion in hepatocellular carcinoma. J. Hepatol. 52, 690–697.

Jiang, L., Liu, X., Chen, Z., Jin, Y., Heidbreder, C. E., Kolokythas, A., Wang, A., Dai, Y., and Zhou, X. (2010). MicroRNA-7 targets IGFIIR (insulin-like growth factor 1 recep- tor) in tongue squamous cell carci- noma cells. Biochem. J. 432, 199–205.
Ko, H. Y., Lee, D. S., and Kim, S. (2009). Noninvasive imaging of microRNA124-mediated repression of the chromosome 14 ORF 24 gene during neurogenesis. *FEBS J.* 276, 4854–4862.

Komagata, S., Nakajima, M., Takagi, S., Mohri, T., Taniya, T., and Yokoi, T. (2009). Human CYP24 catalyzing the inactivation of calcitriol is post-transcriptionally regulated by miR-125b. *Mol. Pharmacol.* 76, 702–709.

Kotani, A., Ha, D., Hisch, J., Rao, Y. K., Schotte, D., Den Boer, M. L., Armstrong, S. A., and Lodish, H. F. (2009). mir-128b is a potent glucocorticoid sensitizer in MLL-AF4 acute lymphocytic leukemia cells and exerts concerted effects with miR-212. *Blood* 114, 4146–4178.

Krek, A., Grun, D., Poy, M. N., Wolf, R., Rosenberg, L., Epstein, E. J., Macmechan, P., Castelo-Rankin, S., Coo, K. T., Stoffel, M., and Rajewsky, N. (2005). Combinatorial microRNA target predictions. *Nat. Genet.* 37, 495–500.

Lagos, D., Pollara, G., Henderson, S., Gratrix, F., Fabani, M., Milne, R. S., Gotch, F., and Boshoff, C. (2010). miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. *Nat. Cell Biol.* 12, 513–519.

Lal, A., Navarro, F., Mahur, C. A., Maliszewski, L. E., Yan, N., O'Day, E., Chowdhury, D., Dykhdoorn, D. M., Tsai, P., Hofmann, O., Becker, K. G., Gorospe, M., Hide, W., and Lieberman, J. (2009a). miR-274 inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to "seedless" 3′UTR microRNA recognition elements. *Mol. Cell* 35, 610–625.

Lal, A., Pan, Y., Navarro, F., Dykhdoorn, D. M., Moreau, L., Meire, E., Ben-twizh, L., Lieberman, J., and Chowdhury, D. (2009b). miR-24-mediated downregulation of HAX2 suppresses DNA repair in terminally differentiated blood cells. *Nat. Struct. Mol. Biol.* 16, 492–498.

Lan, F. F., Wang, H., Chen, Y. C., Chan, C. Y., Ng, S. S., Li, K., Xie, D., He, M. L., Lim, C. M., and Kung, H. F. (2011). Hsa-let-7g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-Myc and upregulation of p16/INK4A. *Int. J. Cancer* 128, 319–331.

Le, M. T., Teh, C., Shyh-Chang, N., Xie, H., Zhou, B., Korzh, V., Lodish, H. F., and Lim, B. (2009). MicroRNA-125b is a novel negative regulator of p53. *Genes Dev.* 23, 862–876.

Leivonen, S. K., Makela, R., Ostling, P., Kohonen, P., Haapa-Paananen, S., Kleivi, K., Enery, A., Aakula, A., Hellstrom, K., Sahlin, N., Kristensen, V. N., Borresen-Dale, A. L., Saviranta, P., Perala, M., and Kallioniemi, O. (2009). Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. *Oncogene* 28, 3926–3936.

Li, M. H., Hou, D. X., Guo, Y. L., Yang, J. W., Liu, Y., Zhang, C. Y., and Zen, K. (2011). Role of microRNA-214-targeting phosphate and tensin homolog in advanced glycation end product-induced apoptosis delay in monocytes. *J. Immunol.* 186, 2533–2536.

Li, S. S., Yu, S. L., Kao, L. P., Tsai, Z. Y., Singh, S., Chen, B. Z., Ho, B. C., Liu, Y. H., and Yang, P. C. (2009a). Target identification of microRNAs expressed highly in human embryonic stem cells. *J. Cell. Biochem.* 106, 1020–1030.

Li, Y. K., Li, P. J., and Shao, Z. M. (2009b). Downregulation of miR-193b contributes to enhance urokinase-type plasminogen activator (uPA) expression and tumor progression and invasion in human breast cancer. *Oncogene* 28, 3937–3948.

Li, X., and Carthey, R. W. (2005). A microRNA mediates EGF receptor signaling and promotes photoreceptor differentiation in the *Drosophila* eye. *Cell* 123, 1267–1277.

Liao, R., Sun, J., Zhang, L., Lou, G., Chen, M., Zhou, D., Chen, Z., and Zhang, S. (2008). MicroRNAs play a role in the development of human hematopoietic stem cells. *J. Cell. Biochem.* 104, 803–817.

Liu, H., Brannon, A. R., Reddy, A. R., Alexe, G., Seiler, M. W., Arroela, A., Oza, J. H., Yao, M., Juan, D., Liu, S. S., Ganesan, S., Levine, A. J., Rathmell, W. K., and Bhanot, G. V. (2010a). Identifying microRNA targets of microRNA dysregulated in cancer can aid to clear cell renal cell carcinoma. *RMC Syol. Biol.* 4, 51, doi:10.1186/1745-0949-4-1.

Liu, J., Luo, X. I., Xiong, A. W., Zhang, Z. D., Yue, S., Zhu, M. S., and Cheng, S. Y. (2010b). MicroRNA-214 promotes myogenic differentiation by facilitating exit from mitosis via down-regulation of proto-oncogene N-RAS. *J. Biol. Chem.* 285, 26599–26607.

Liu, X., Sempere, L. F., Ouyang, H., Memoli, V. A., Andrew, A. S., Luo, Y., Dimenidko, E., Korc, M., Shi, W., Preis, M., Dragnev, K. H., Li, H., Drenko, J., Bak, M., Freeman, S. J., Kauppinen, S., and Dmitrovsky, E. (2010c). MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. *J. Clin. Invest.* 120, 1298–1309.

Liu, X., Wang, A., Heidbreder, C. E., Jiang, L., Yu, J., Kolokythas, A., Huang, L., Dai, Y., and Zhou, X. (2010d). MicroRNA-24 targeting RNA-binding protein DND1 in tongue squamous cell carcinoma. *FEBS Lett.* 584, 4115–4120.

Liu, X., Zhan, Z., Xu, L., Ma, F., Li, D., Guo, Z., Li, N., and Cao, X. (2010e). MicroRNA-148-3p impairs innate response and antigen presentation of TLR-triggered dendritic cells by targeting CaMKIIalpha. *J. Immunol.* 185, 7244–7251.

Liu, K., Liu, Y., Mo, W., Qiu, R., Wang, X., Wu, J. Y., and He, R. (2011). miR-124 regulates early neurogenesis in the optic vesicle and forebrain, targeting NeuroD1. *Nucleic Acids Res.* 39, 2869–2879.

Liu, X., Yu, J., Jiang, L., Wang, A., Shi, F., Ye, H., and Zhou, X. (2009). miR-128a-22 regulates cell invasion by targeting matrix metalloproteinase 1 (MMP1) and manganese superoxide dimutase 2 (SOD2) in tongue squamous cell carcinoma cells. *Cancer Genomics Proteomics 6*, 131–139.

Luna, C., Li, G., Qiu, J., Epstein, D. L., and Gonzalez, P. (2011). MicroRNA-24 regulates the processing of latent TGFbeta1 during cyclic mechanical stress in human trabecular meshwork cells through direct targeting of FURIN. *J. Cell. Physiol.* 226, 1407–1414.

Luthra, R., Singh, R. R., Luthra, M. G., Li, Y. X., Hannah, C., Romans, A. M., Barkoh, B. A., Chen, S. S., Enser, J., Maru, D. M., Broadus, R. R., Rashid, A., and Albarracin, C. T. (2008). MicroRNA-196a targets annexin A1: a microRNA-mediated mechanism of annexin A1 down-regulation in cancers. *Oncogene* 27, 6667–6678.

Lwin, T., Lin, J., Choi, Y. S., Zhang, X., Moschel, R. C., Weight, V. L., Sotomayor, E. M., Dalton, W. S., and Tao, J. (2010). Follicular dendritic cell-dependent drug resistance of non-Hodgkin lymphoma involves cell adhesion-mediated Bim down-regulation through induction of microRNA-181a. *Blood* 116, 5228–5236.

Ma, N., Wang, X., Qiao, X., Li, F., Hui, X., Zou, C., Jin, J., Li, G., Peng, Y., Wang, L., Huang, H., Zhou, L., Zheng, X., and Gao, X. (2011). Coexpression of an intronic microRNA and its host gene reveals a potential role for miR-483-5p as an IPO2 partner. *Mol. Cell. Endocrinol.* 333, 96–101.

Makeyev, E. V., Zhang, J., Carnas, M. A., and Maniatis, T. (2007). The microRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol. Cell.* 27, 435–448.
Mishra, P. J., Song, B., Wang, Y., Hume-Mees, S. T., Mardin, W. A., Wendel, Maru, D. M., Singh, R. R., Hannah, C., Hanin and Soreq Cholinesterase-targeting microRNAs is regulated independent of p53 and through a target site polymorphism. PloS ONE 4, e6445. doi:10.1371/journal.pone.0008445

Munos-Gimeno, M., Espinosa-Parrilla, Y., Gui, M., Kagerbauer, B., Sipila, T., Maron, E., Pettai, K., Kanaan, L., Navines, R., Martin-Santos, R., Gratacos, M., Metspalu, A., Hovatta, I., and Estivill, X. (2011). Human microRNAs mir-22, mir-138-2, mir-148a, and mir-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biochim. Biophys. Acta 1805, 526–533.

Murata, T., Takayama, K., Katayama, S., Urano, T., Horie-Inoue, K., Ikeda, K., Takahashi, S., Kawazu, C., Hasegawa, A., Ouchi, T., Homma, Y., Hayashizaki, Y., and Inoue, S. (2010). miR-1912 is an age-dependent mRNA promoter that regulates LNCaP prostate cell growth by repressing its target CANDI expression. Prostate Cancer Prostatic Dis. 13, 356–361.

Nagel, R., Clijsters, L., and Agami, R. (2009). The miRNA-192-194 cluster regulates the period gene family and the circadian clock. FEBS J. 276, 5447–5455.

Naguibneva, I., Ameyar-Zazoua, M., Poleskaya, A., Ait-Si-Ali, S., Grosisman, R., Soudi, M., Cuvelier, S., and Harel-Bellan, A. (2006). The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. Nat. Cell Biol. 8, 278–284.

Nakamachi, Y., Kawano, S., Takenokuchi, M., Nishimura, K., Sakai, Y., Chin, T., Saura, R., Kuroasaka, M., and Kumagai, S. (2009). MicroRNA-124a is a key regulator of proliferation and monocye chemoattractant protein 1 secretion in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Rheum. (Munch.) 60, 1294–1304.

Nakano, H., Miyazawa, T., Kinosita, K., Yamada, Y., and Yoshida, T. (2010). Functional screening identifies a microRNA, miR-491 that induces apoptosis by targeting Bel-X(L) in colorectal cancer cells. Int. J. Cancer 127, 1072–1080.

Nguyen, H. T., Dalmasso, G., Yan, Y., Larouhi, H., Dahan, S., Mayer, L., Sitaraman, S. V., and Merlin, D. (2010). MicroRNA-7 modulates CD98 expression during intestinal epithelial cell differentiation. J. Biol. Chem. 285, 1479–1489.

Pais, H., Nicolas, F. E., Soond, S. M., Swingle, T. E., Clark, J. M., Chantry, A., Moulton, V., and Dalmay, T. (2010). Analyzing mRNA expression and through a target site polymorphism. PloS ONE 4, e6445. doi:10.1371/journal.pone.0008445

Pallasci, C. P., Patz, M., Park, V. J., Haggard, S., Eggle, D., Claus, R., Debeypascher, S., Schulz, A., Frenzel, L. P., Claessen, J., Kutsch, N., Krause, G., May, C., Rosenwald, A., Plass, C., Schultze, J. L., Hallek, M., and Wendtner, C. M. (2009). miRNA deregulation by epigenetic silencing disrupts suppression of the oncogene PLAG1 in chronic lymphocytic leukemia. Blood 114, 3255–3264.

Pan, W., Zhu, S., Yuan, M., Cui, H., Wang, L., Luo, X., Li, J., Zhou, H., Tang, Y., and Shen, N. (2010). MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lusup CD4+ T cells by directly and inducibly targeting DNA methyltransferase 1. J. Immunol. 184, 6773–6781.

Papaioannou, M. D., Lagarrigue, M., Veijar, C. E., Rolland, A. D., Khune, F., Aubry, F., Schaal, O., Fort, A., Descombes, P., Neerman-Arbez, M., Guillou, F., Zdolbnev, E. M., Pineau, C., and Nef, S. (2011). Loss of Dicer in Sertoli cells has a major impact on the testicular pro tease activity of mice. Mol. Cell. Proteomics 10, M900587MCP900200.

Park, J. K., Henry, J. C., Jiang, J., Esau, C., Gusey, Y., Lerner, M. R., Poster, R. G., Brackett, D. J., and Schmittgen, D. T. (2011). miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochem. Biophys. Res. Commun. 406, 518–523.

Pedrioli, D. M., Karpanen, T., Dabouras, V., Jurisic, G., Van De Hoek, G., Papaioannou, M. D., Lagarrigue, M., Veijar, C. E., Rolland, A. D., Khune, F., Aubry, F., Schaal, O., Fort, A., Descombes, P., Neerman-Arbez, M., Guillou, F., Zdolbnev, E. M., Pineau, C., and Nef, S. (2011). Loss of Dicer in Sertoli cells has a major impact on the testicular pro tease activity of mice. Mol. Cell. Proteomics 10, M900587MCP900200.

Park, J. K., Henry, J. C., Jiang, J., Esau, C., Gusey, Y., Lerner, M. R., Poster, R. G., Brackett, D. J., and Schmittgen, D. T. (2011). miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochem. Biophys. Res. Commun. 406, 518–523.

Pedrioli, D. M., Karpanen, T., Dabouras, V., Jurisic, G., Van De Hoek, G., Papaioannou, M. D., Lagarrigue, M., Veijar, C. E., Rolland, A. D., Khune, F., Aubry, F., Schaal, O., Fort, A., Descombes, P., Neerman-Arbez, M., Guillou, F., Zdolbnev, E. M., Pineau, C., and Nef, S. (2011). Loss of Dicer in Sertoli cells has a major impact on the testicular pro tease activity of mice. Mol. Cell. Proteomics 10, M900587MCP900200.

Park, J. K., Henry, J. C., Jiang, J., Esau, C., Gusey, Y., Lerner, M. R., Poster, R. G., Brackett, D. J., and Schmittgen, D. T. (2011). miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochem. Biophys. Res. Commun. 406, 518–523.

Pedrioli, D. M., Karpanen, T., Dabouras, V., Jurisic, G., Van De Hoek, G., Papaioannou, M. D., Lagarrigue, M., Veijar, C. E., Rolland, A. D., Khune, F., Aubry, F., Schaal, O., Fort, A., Descombes, P., Neerman-Arbez, M., Guillou, F., Zdolbnev, E. M., Pineau, C., and Nef, S. (2011). Loss of Dicer in Sertoli cells has a major impact on the testicular pro tease activity of mice. Mol. Cell. Proteomics 10, M900587MCP900200.
Saatrom, P., Biesinger, J. L., Smith, D., Thomas, L. F., Majoub, K., Rivas, G. E., Allum, J., Rossi, J. J., Krontiris, T. G., Weitzen, D., Daly, M. B., Benson, A. B., Kirkwood, J. M., O'Dwyer, P. J., Sutphen, R., Stewart, J. A., Johnson, D., and Larson, G. P. (2009). A risk variant in an miR-125b binding site in BMPR1B is associated with breast cancer penetrance. Cancer Res. 69, 7459–7467.

Salomonis, N., Schieve, C. R., Pereira, L., Waloquist, C., Colas, A., Zambon, A. C., Vanizan, K., Spindler, M. J., Pico, A. R., Cline, M. S., Clark, T. A., Williams, A., Blume, J. E., Samal, E., Mercola, M., Merrill, B. J., and Conklin, B. R. (2010). Alternative splicing regulates mouse embryonic stem cell pluripotency and differentiation. Proc. Natl. Acad. Sci. U.S.A. 107, 10514–10519.

Saunders, L. R., Sharma, A. D., Tawney, J., Nakagawa, M., Okita, K., Yamanaka, S., Willenbring, H., and Verdin, E. (2010). miRNAs regulate SIRT1 expression during mouse embryonic stem cell differentiation and in adult mouse tissues. Aging (Albany NY) 2, 415–431.

Saydam, O., Senol, O., Wurdinger, T., Mirzak, A., Ozdener, G. B., Stemmer-Rachamimov, A. O., Yi, M., Ozdener, G. B., Stemmer-Rachamimov, A. O., and Yamanaka, S. (2011). The cartilage-specific microRNA-1 targets Sox9 in chondrocytes. Proc. Natl. Acad. Sci. U.S.A. 108, 29223–29230.

Shen, Q., Cincirici, V. R., Zhang, X., Foss, S., Weher, F., Worringer, G. C., Radlake, A., Lu, M., Paul, A., Gerken, G., and Beckebaum, S. (2010). Role of microRNA-199a-5p and discoidin domain receptor 1 in human hepatocellular carcinoma invasion. Mol. Cancer 9, 227.

Shimizu, S., Takehara, T., Hikita, H., Kodama, T., Miyagi, T., Hossi, A., Tatsumi, T., Ishida, H., Noda, T., Nagano, H., Doki, Y., Mori, M., and Hayashi, N. (2010). The let-7 family of microRNAs inhibits Bcl-xl expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma. J. Hepatol. 52, 699–704.

Smallford, A., Rozen, L. B., Rajagopalan, K. N., Wang, S., and Olson, E. N. (2010). MicroRNA-218 regulates vascular patterning by modulation of Slit-Robo signaling. Circ. Res. 107, 1336–1344.

Smirnov, D. A., and Cheung, V. G. (2008). ATM gene mutations result in both recessive and dominant expression phenotypes of genes and microRNAs. Am. J. Hum. Genet. 83, 243–253.

Sober, S., Lahn, M., and Annilio, T. (2010). MicroRNAs miR-124 and miR-135a are potential regulators of the mineralocorticoid receptor gene (NR3C2) expression. Biochem. Biophys. Res. Commun. 391, 727–732.

Song, L., Huang, Q., Chen, K., Liu, L., Lin, C., Dai, T., Yu, C., Wu, Z., and Li, J. (2010). miR-218 inhibits the invasive ability of glioma cells by direct downregulation of IKK-beta. Biochem. Biophys. Res. Commun. 402, 135–140.

Sossey-Alaoui, K., Downs-Kelly, E., Lyszkiewicz, M., Krueger, A., Ganser, A., Scherr, M., and Eder, M. (2011). Enforced expression of miR-125b affects myelopoesis by targeting multiple signaling pathways. Blood 117, 4338–4348.

Takahav, K., Boldin, M. P., Chang, K. J., and Baltimore, D. (2006). NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. U.S.A. 103, 12481–12486.

Tagaki, S., Nakajima, M., Kid, K., Yamaura, Y., Fukami, T., and Yokoi, T. (2010). MicroRNAs regulate human hepatocyte nuclear factor 4alpha, modulating the expression of metabolic enzymes and cell cycle. J. Biol. Chem. 285, 4415–4422.

Takahav, S., Nakajima, M., Mohri, T., and Yokoi, T. (2008). Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. J. Biol. Chem. 283, 9674–9680.

Tan, Z., Randall, G., Fan, J., Camoretti-Mercado, B., Brockman-Schneider, R., Pan, L., Solwaj, J., Gern, J. E., Lemanske, R. E., Nicolae, D., and Ober, C. (2007). Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am. J. Hum. Genet. 81, 829–834.

Terawasa, K., Ichimura, A., Sato, F., Shimizu, K., and Tsujimoto, G. (2009). Sustained activation of ERK1/2 by NGF induces microRNA-221 and 222 in PC12 cells. FEBS J. 276, 3269–3276.

Thatcher, E. J., Paydar, I., Anderson, K. A., Ueno, K., Hirata, H., Shahryari, V., Chen, Y., Zaman, M. S., Singh, K., Tabatabai, Z. L., Hinoda, Y., and Dahiy, R. (2011). Tumour suppressor microRNA-144 directly targets oncogene Rock-1 and decreases invasion ability in human clear cell renal cell carcinoma. Br. J. Cancer 104, 308–315.

Uhlmann, S., Zhang, J. D., Schwager, A., Mannsperger, H., Riazalhosseini, Y., Burmester, S., Ward, A., Korf, U., Wiemann, S., and Sahin, O. (2010). Dicer-regulated microRNAs 222 and 339 promote resistance of cancer cells to cytotoxic T-lymphocytes by down-regulation of ICAM-1. Proc. Natl. Acad. Sci. U.S.A. 106, 10746–10751.

Ueno, K., Hirata, H., Shahryari, V., Chen, Y., Zaman, M. S., Singh, K., Tabatabai, Z. L., Hinoda, Y., and Dahiy, R. (2011). Tumour suppressor microRNA-144 directly targets oncogene Rock-1 and decreases invasion ability in human clear cell renal cell carcinoma. Br. J. Cancer 104, 308–315.

Valastyan, S., Reinhardt, F., Benaich, N., Calogrias, D., Szasz, A. M., Wang, Z. C., Brock, J. E., Richardson, A. L., and Weinberg, R. A. (2009). A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 137, 1032–1046.

Venugopal, S. K., Jiang, J., Kim, T. H., Li, Y., Wang, S. S., Torok, N. J., Wu, L., and Zern, M. A. (2010). Liver fibrosis causes downregulation of miRNA-150 and miRNA-194 in hepatic stellate cells, and their overexpression causes decreased stellate cell activation. Am. J. Physiol. Gastrointest. Liver Physiol. 298, G101–G106.
Veronese, A., Lupini, L., Consiglio, J., Visone, R., Ferracini, M., Fornari, F., Zanesi, N., Alder, H., D’ella, G., Gramantieri, L., Bolondi, L., Lanza, G., Querzoli, P., Angioni, A., Croce, C. M., and Negrini, M. (2010). Oncogenic role of miR-483-3p at the IGF2/483 locus. Cancer Res. 70, 3140–3149.

Villeneuve, L. M., Kato, M., Reddy, A. M., Wang, M., Mangan, L., and Natarajan, R. (2010). Enhanced levels of microRNA-125b in vascular smooth muscle cells of diabetic db/db mice lead to increased inflammatory gene expression by targeting the histone methyltransferase Suv39h1. Diabetes 59, 2904–2915.

Vitichie, G., Lena, A. M., Latina, A., Formentini, L., Barbosa, A. H., Bernardini, S., Mauriello, A., Miano, R., Spagnoli, L. G., Knight, R. A., Candi, E., and Melino, G. (2011). MiR-203 controls proliferation, migration and invasion potential of prostate cancer cell lines. Cell Cycle 10, 1121–1123.

Vreugdenhil, E., Verissimo, C. S., Mari- n, K., Kamphorst, J. T., Barbosa, J. S., Zweers, T., Champagne, D. L., Schouten, T., Meijer, O. C., De Kloe, E. R., and Fitisimons, C. P. (2009). MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. Endocrinology 150, 2220–2228.

Wang, B., Hsu, S. H., Majumder, S., Kutay, H., Huang, W., Jacob, S. T., Barbosa, R., Kamphorst, J. T., Barbosa, F., Zanesi, N., Alder, H., D’elia, G., Formosa, A., Gregersen, L. H., Lund, J., Schouten, T., Meijer, O. C., De Kloe, E. R., and Fitisimons, C. P. (2009). MicroRNA and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. Endocrinology 150, 2220–2228.

Wang, B., Hsu, S. H., Majumder, S., Kutay, H., Huang, W., Jacob, S. T., Barbosa, R., Kamphorst, J. T., Barbosa, F., Zanesi, N., Alder, H., D’elia, G., Formosa, A., Gregersen, L. H., Lund, J., Schouten, T., Meijer, O. C., De Kloe, E. R., and Fitisimons, C. P. (2009). MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. Endocrinology 150, 2220–2228.

Wei, T., Orlandinis, K., Xu, N., Jans- "Kin, P., Stahle, M., Pivarcsi, A., and Sonkoly, E. (2010). The expression of microRNA-203 during human skin morphogenesis. Exp. Dermatol. 19, 854–856.

Wong, Q. W., Cling, A. K., Chan, A. W., Choy, K. W., To, K. F., Lai, P. B., and Wong, N. (2010). MiR-222 overexpression confers cell migratory advantages in hepatocellular carcinoma through enhancing AKT signaling. Clin. Cancer Res. 16, 867–875.

Wu, D. W., Cheng, Y. W., Wang, J., Chen, C. Y., and Lee, H. (2010a). Paxillin predicts survival and relapse in non-small cell lung cancer by microRNA-218 targeting. Cancer Res. 70, 10392–10401.

Wu, S. H., Chen, J., Tsai, G. Y., Xie, B., Krausner, A. R., and Zhu, J. (2010b). A splicing-independent function of SF2/ASF in microRNA processing. Mol. Cell 38, 67–77.

Wu, F., Zhu, S., Ding, Y., Beck, W. T., and Mo, Y. Y. (2009). MicroRNA-mediated regulation of Ubc9 expression in cancer cells. Clin. Cancer Res. 15, 1530–1537.

Wu, L., and Belasco, J. G. (2005). MicroRNA regulation of the mammalian lin-28 gene during neuronal differentiation of embryonal carcinoma cells. Mol. Cell. Biol. 25, 9198–9208.

Xi, S., Yang, M., Tao, Y., Xu, H., Shan, J., Inchauste, S., Zhang, S., Chen, T., Wan, J., and Schrpm, D. S. (2010). Gigarette smoke induces C/EBP-beta-mediated activation of miR-31 in normal human respiratory epithelium and lung cancer cells. PLoS ONE 5, e15764. doi:10.1371/jou-

Yi, R., Poy, M. N., Stoffel, M., and Fuchs, E. (2008). A skin microRNA promotes differentiation by repressing ‘stemness.’ Nature 452, 225–229.

Yoo, A. S., Staahl, B. T., Chen, L., and Crabtree, G. R. (2009). MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460, 642–646.

Yu, M., Ashoor, R., Cui, X., Li, N., and Te, S. (2011). Transforming growth factor-beta regulates the sphere-initiating stem cell-like feature in breast cancer through mirRNA-124 and ATM. Oncogene 30, 1470–1480.

Yi, R., Poy, M. N., Stoffel, M., and Fuchs, E. (2008). A skin microRNA promotes differentiation by repressing ‘stemness.’ Nature 452, 225–229.

Zhang, C. Z., Zheng, S. J., Zhao, J. H., Zhao, W., Zheng, L. F., Zhao, D., Li, J. M., Zhang, X. F., Chen, Z. B., and Yi, X. N. (2011a). MicroRNAs 144, 145, and 214 are down-regulated in primary neurons responding to sciatric nerve transection. Brain Res. 1383, 65–72.

Zhang, L., Stokes, N., Polak, L., and Fuchs, E. (2011b). Specific MicroRNAs are preferentially expressed by skin stem cells to balance self-renewal and early lineage commitment. Cell Stem Cell 8, 294–308.

Zhao, S., Wang, B., Yang, G., Meng, Y. L., Jia, L. T., Wang, I., Yao, B. L., Jin, B. Q., Wang, T., and Yang, A. G. (2011). Human activated CD4(+) T lymphocytes increase IL-2 expression by downregulating microRNA-181C. Mol. Immunol. 48, 592–599.

Zhao, Y., Zhang, B., Zhao, S., Cui, H., Wang, Z., Chen, S., Luan, X., Li, Y., Liu, M., Li, X., Liu, T., and Tang, H. (2009). MicroRNA-214 is aberrantly expressed in cervical cancers and inhibits the growth of HeLa cells. URBMB Life 61, 1075–1082.

Zhao, J., Liang, L., Huang, S., Ding, J., Tan, N., Zhao, Y., Yan, M., Ge, C., Zhang, Z., Chen, T., Wan, D., Yao, M., Li, J., Gu, J., and He, X. (2010). MicroRNA-30d promotes tumor invasion and metastasis by targeting Golphin2 in hepatocellular carcinoma. Hepatology 51, 846–856.

Yi, R., Poy, M. N., Stoffel, M., and Fuchs, E. (2008). A skin microRNA promotes differentiation by repressing ‘stemness.’ Nature 452, 225–229.

Yoo, A. S., Staahl, B. T., Chen, L., and Crabtree, G. R. (2009). MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. Nature 460, 642–646.

Yoon, S., Choi, Y. C., Lee, S., Jeong, Y., Yoon, J., and Baek, K. (2010). Induction of growth arrest by miR-342-3p that targets survivin. FEBS Lett. 584, 4048–4052.

Yu, J. Y., Reynolds, S. H., Hatfield, S. D., Scherberata, H. R., Fischer, K. A., Ward, E. J., Long, D., Ding, Y., and Ruohola-Baker, H. (2009). Dicer-
Zhou, M., Liu, Z., Zhao, Y., Ding, Y., Liu, H., Xi, Y., Xiong, W., Li, G., Lu, J., Fodstad, O., Riker, A. I., and Tan, M. (2010). MicroRNA-125b confers the resistance of breast cancer cells to paclitaxel through suppression of pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) expression. *J. Biol. Chem.* 285, 21496–21507.

Zhu, H., Wu, H., Liu, X., Li, B., Chen, Y., Ren, X., Liu, C. G., and Yang, J. M. (2009). Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy* 5, 816–823.

Zhu, N., Zhang, D., Chen, S., Liu, X., Lin, L., Huang, X., Guo, Z., Liu, J., Wang, Y., Yuan, W., and Qin, Y. (2011). Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. *Atherosclerosis* 215, 286–293.

Zhu, W., Shan, X., Wang, T., Shu, Y., and Liu, P. (2010). miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. *Int. J. Cancer* 127, 2520–2529.

Zimmerman, E. L., Dollins, C. M., Crawford, M., Grant, S., Nana-Sinkam, S. P., Richards, K. L., Hammond, S. M., and Graves, L. M. (2010). Lyn kinase-dependent regulation of miR181 and myeloid cell leukemia-1 expression: implications for drug resistance in myelogenous leukemia. *Mol. Pharmacol.* 78, 811–817.