Cancer Epidemiology, Biomarkers & Prevention

MiniReview

The Oncogenic Role of miR-155 in Breast Cancer

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Abstract

miR-155 is an oncogenic miRNA with well described roles in leukemia. However, additional roles of miR-155 in breast cancer progression have recently been described. A thorough literature search was conducted to review all published data to date, examining the role of miR-155 in breast cancer. Data on all validated miR-155 target genes was collated to identify biologic pathways relevant to miR-155 and breast cancer progression. Publications describing the clinical relevance, functional characterization, and regulation of expression of miR-155 in the context of breast cancer are reviewed. A total of 147 validated miR-155 target genes were identified from the literature. Pathway analysis of these genes identified likely roles in apoptosis, differentiation, angiogenesis, proliferation, and epithelial–mesenchymal transition. The large number of validated miR-155 targets presented here provide many avenues of interest as to the clinical potential of miR-155. Further investigation of these target genes will be required to elucidate the specific mechanisms and functions of miR-155 in breast cancer. This is the first review examining the role of miR-155 in breast cancer progression. The collated data of target genes and biologic pathways of miR-155 identified in this review suggest new avenues of research for this oncogenic miRNA. *Cancer Epidemiol Biomarkers Prev; 21(8); 1236–43. ©2012 AACR.*

Introduction

miRNAs are small noncoding RNAs that control expression of target genes by either inhibiting protein translation or directly targeting mRNA transcripts of target genes for degradation (1). Each miRNA has a specific seed sequence 7 to 8 nucleotides long, which directly binds to complementary sequences in regulatory regions of target genes. These binding regions are often in the 3′-untranslated region (3′-UTR) of target genes, but increasingly are being reported in other noncoding regions such as promoter or intronic regions (2). The short length of the seed sequence facilitates the targeting of many transcripts by a single miRNA (3). Some estimates suggest that 30% of all eukaryotic genes are regulated by miRNAs (4, 5). miR-155, a miRNA widely reported to be involved in lymphoma, is also now emerging to have a role in the progression of solid cancers (6). This review will focus on the miRNA miR-155, and its role in breast cancer.

miRNAs were discovered in 1993 when the *C. elegans lin-4* gene, which is transcribed but not translated, was found to regulate levels of LIN-14 protein (7, 8). Since this discovery, there have been over 500 miRNAs described, regulating a wide range of genes and cellular processes, although the total predicted number of unique miRNAs encoded by the human genome is estimated to be more than 1,000 (9). Many of these miRNAs are organized as gene clusters and transcribed as multicistronic messages—for example, the *MIRH1* gene encodes 6 different miRNAs (10). The transcription and processing of miRNAs has been well characterized and is depicted in Fig. 1 using miR-155 as an example. miRNAs originate from an approximately 70 nucleotide RNA hairpin pre-miRNA processed from the RNA transcript of the host gene (11); in the case of miR-155, the host gene *BIC*. The pre-miRNA is typically cleaved by the Drosha and Dicer exonucleases into an approximately 22 nucleotide RNA duplex. One strand of the duplex becomes the mature miRNA and is usually the functional, regulatory unit (12, 13), whereas the other is designated miR* and is usually degraded. The mature miRNA is loaded into Argonaute proteins, forming the RNA Induced Silencing Complex (RISC). The mature miRNA may then bind to its target by partial complementarity of target gene mRNA and either inhibit translation or cause degradation of the mRNA.

The miR-155 host gene, *BIC*, was first described in 1989 and postulated to be involved in the progression of lymphoma (14). In 2002, Lagos-Quintana and colleagues identified miR-155 as a regulatory RNA (15). Subsequently, studies have focused on the roles of miR-155 in lymphoma (16–19) and also in viral infection, cardiovascular disease, and solid cancers (6, 20–22). miR-155 has more than 400 predicted gene targets (23) and more than 100

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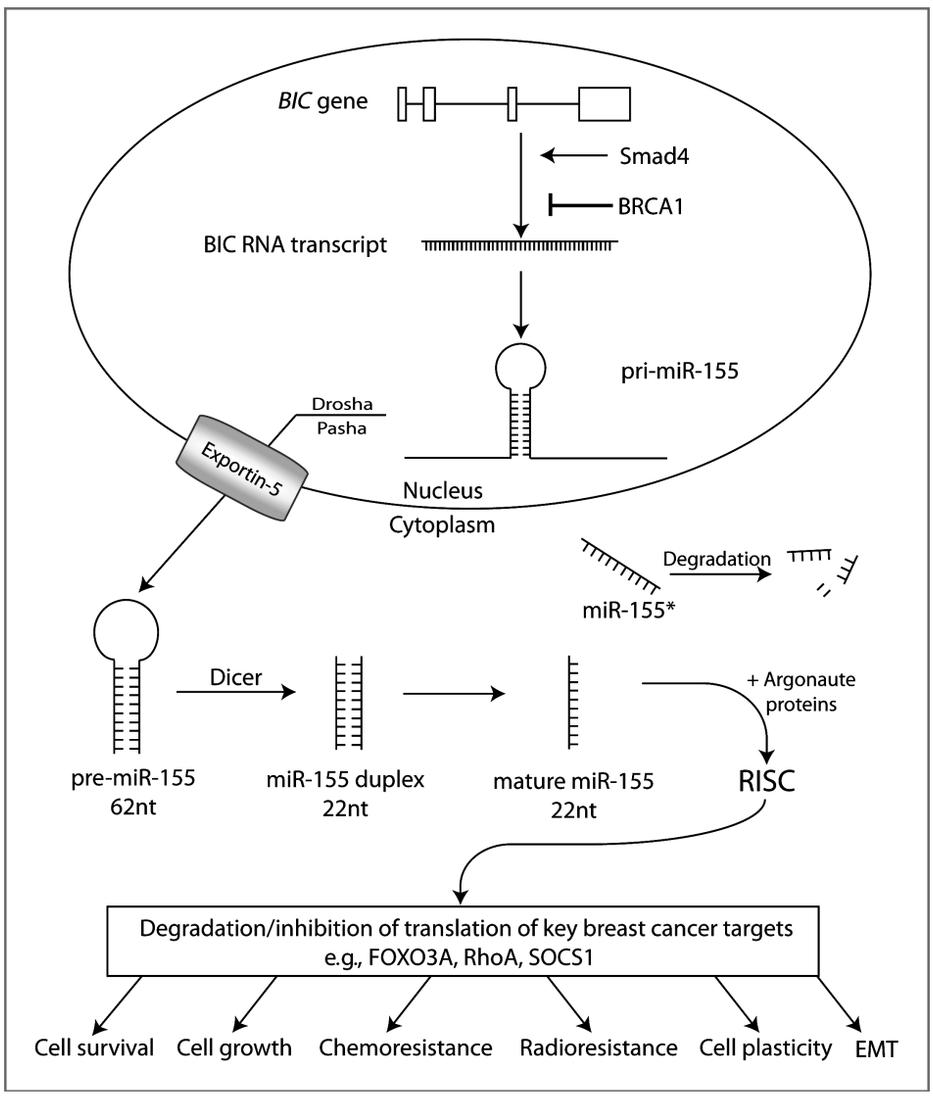

Figure 1. Cellular processing and downstream effects of miR-155 in breast cancer. The pri-miR-155 RNA hairpin transcript is processed from the RNA transcript of the *BIC* gene. Transcription of *BIC* is promoted by Smad4 and inhibited by BRCA1. After processing by Drosha, Pasha, Exportin-5, and Dicer, the mature miR-155 forms a complex with Argonaute proteins called the RISC, to inhibit the translation of miR-155 target mRNAs, such as RhoA, FOXO3A, and SOCS1. The inhibition of target genes by miR-155 in breast cancer can cause such effects as an increase in EMT, cell plasticity, cell survival, growth, chemoresistance, and radioresistance.

confirmed *bona fide* targets. There is now an emerging role of miR-155 in breast cancer progression (20, 21, 24), which is the focus of this review.

Clinical Relevance of miR-155 in Breast Cancer

Studies show the expression level of miR-155 is upregulated in breast cancer with high levels of miR-155 associated with clinicopathologic markers, tumor subtype, and poor survival rates, summarized in Table 1 (21, 25–34). Of 29 miRNAs found to be dysregulated in breast cancer, the majority were downregulated, with only miR-155 and miR-21 significantly upregulated (25). Expression levels of 15 of these dysregulated miRNAs independently predict the invasive potential of breast tissue samples (25). A small microarray study of 8 fresh breast tumor samples found miR-155 was upregulated in the breast tumors compared with normal adjacent tissue (34). In a larger study, 62 breast carcinomas were analyzed to determine miR-155 levels. Of 17 noninvasive tumors, only 2 (12%) exhibited a high level of miR-155 expression. Conversely, 41 of the 45 invasive tumors (91%) displayed miR-155 upregulation (32). In a further study, expression levels of *FOXO3A*, a miR-155 target gene, was determined in 77 primary breast tumors, 38 recurrent tumors, and 11 normal tissue samples. Results showed that miR-155 was upregulated and FOXO3A downregulated in a majority of primary tumors, and also that high miR-155 and low FOXO3A expression was associated with recurrent tumors after radiotherapy or chemotherapy (21). These studies linked miR-155 expression to both invasiveness and recurrence of breast tumors and showed that expression levels of miR-155 and its specific target genes are of potential clinical prognostic value.

Table 1. Summary studies examining miR-155 expression in breast cancer

miR-155	Tissue type	Ref.
↑ in breast cancer	76 Breast tumor 10 Normal breast	(25)
↑ in breast cancer	363 Breast tumor 177 Normal breast	(26)
↑ in ER^- tumors	93 Breast tumor 5 Normal breast	(27)
↑ in malignant breast tissue	34 Breast tumor 6 Normal breast	(28)
↑ in PR+ tumors	Serum–13 breast cancer patients, 8 healthy patients	(29)
↑ in grade II and III tumors	Tumor, normal adjacent tissue, and serum from 68 breast cancer patients	(30)
↑ in ER^- PR^- tumors	Tissue and serum from 40 healthy patients	
Associated with higher tumor grade, advanced tumor stage, lymph node metastasis	92 Breast tumor and normal adjacent tissue	(31)
↑ in 41 of 45 invasive	45 Invasive breast tumor	(32)
↑ 2 of 17 noninvasive tumors	17 Noninvasive breast tumor	
↑ in 55 breast tumors	77 breast tumor	(21)
↑ 31 recurrent tumors	11 Normal breast 38 Recurrent breast tumor	
↑ in breast metastases	13 Breast tumor and paired metastasis	(33)
↑ in tumors	8 Breast tumor and normal adjacent tissue	(34)

In a robust study of lung, stomach, prostate, colon, pancreatic tumors, and 363 breast tumors, Volinia and colleagues globally compared miRNA expression levels in multiple tumor and pooled normal tissue samples to identify dysregulated miRNAs in tumor samples. Comparisons of normal and tumor tissue derived from each individual tissue showed that miR-155 expression was upregulated in breast, colon, and lung cancers. Interestingly, miR-155 was one of only 2 miRNAs (the other being the miR-200 family) found to be upregulated in both breast and lung cancer, implying that these miRNAs may be part of a common mechanism in the development of cancer in these organs (26).

miR-155 expression levels have been shown to be associated with metastasis events and invasive properties of breast cancer. In one study, increased miR-155 expression was associated with high tumor grade, advanced stage, and lymph node metastasis (31). Disease-free and overall survival were also negatively correlated with miR-155 levels, further showing the potential of miR-155 as a miRNA of clinical interest. These findings were further supported by 2 studies involving microarray analyses of formalin-fixed, paraffin-embedded breast cancer samples, which found that miR-155 expression was upregulated in metastases (28, 33).

Because miR-155 is associated with poor prognosis and/or metastasis, a correlation of miR-155 levels with breast cancer clinicopathologic markers would be expected. Analysis of 93 breast cancers for both miRNA levels alongside mRNA levels, to classify tumor subtypes, showed miR-155 levels were significantly upregulated in basal-like tumors and in estrogen receptor negative (ER^-) tumors (27). The correlation with basal-like tumors has particular clinical relevance because of the poor prognosis of this tumor subtype.

Studies have investigated whether serum samples could be used to identify aberrant miRNA expression levels in breast cancer patients. In a small study of 21 patients, Zhu and colleagues found that multiple miRNAs could be detected in sera, and the miRNA levels correlated with the levels in tissue samples (29). The expression of miR-155 was higher in the serum of PR^+ breast cancer patients than in the serum of PR^- patients (29). Further studies confirmed these findings, with a significant correlation ($R^2 = 0.853$) between miRNA levels in fresh breast cancer tissue and matched serum samples (30). They confirmed that miR-155 was upregulated in breast cancer and also that high miR-155 was associated with grade II and III tumors and ER^- and PR^- tumors (30). The detection of miR-155 expression levels in serum is a potential clinical prognostic indicator of tumor grade and hormone receptor status. The relationship of PR status and miR-155 expression is unresolved, with 2 studies reporting contradictory results (27, 29). The topic of serum miRNAs is also somewhat controversial, with some studies suggesting that serum miRNA levels are robust (35, 36), and others claiming that the miRNAs often used as normalization controls are highly variable in sera samples and thus miRNA quantification in sera is not reproducible (37). This suggests analysis of serum alone is not sufficient to determine whether miR-155 is differentially expressed.

Because the number of samples in these studies is generally low, resolution requires a more robust study.

Taken together, these studies showed that miR-155 expression is upregulated in breast cancer, consistent with its status as an oncomiR, and is associated with more aggressive breast tumors. However, the relationships between miR-155 and clinicopathologic markers, such as ER and PR status and tumor subtype, is inconsistent, probably because of small sample sizes and methodologic aspects. For example, the upregulation of miR-155 expression in PR^+ tumors was only identified in one study of a small number of samples (29). Further studies are required to confirm and elucidate the basis of the relationship between miR-155 and hormone receptor status.

Functional characterization of miR-155 oncogenic activities in breast cancer

An important step in determining the clinical significance of miR-155 is to determine whether high expression levels are causally related to the development of breast cancer. *In vitro* effects of altering miR-155 expression levels were assessed in a panel of breast cancer cell lines (21). miR-155 expression was inhibited by anti-miR in HS578T cells. An anti-miR is a 2′-O-methyl oligoribonucleotide that inhibits the action of an miRNA. One proposed mechanism for anti-miR action is antisense binding to the mature miRNA positioned in the RISC (38). The HS578T cell line expresses high levels of endogenous miR-155, and anti-miR-155 application resulted in cell-cycle arrest and induction of apoptosis, implicating miR-155 in these processes (21). Conversely, ectopic overexpression of miR-155 in BT474 cells, which express very low levels of endogenous miR-155, promoted cell proliferation and survival and also improved chemoresistance (21). Taken together, these findings showed that miR-155 has a role in cell proliferation and apoptosis, 2 cellular processes frequently aberrant in cancer. Similar results have also been reported in breast cancer cell lines MDA-MB-231 and MCF-7 in which ectopic miR-155 overexpression increased proliferation, whereas inhibition of miR-155 expression by a specific anti-miR inhibits proliferation and increases radiosensitivity of cells *in vitro* (20, 31).

Xenografted human breast cancer cells in immunodeficient mice provide *in vivo* confirmation of miR-155 as an oncomiR. Xenografts of MDA-MB-231 cells showed reduced tumor volumes compared with control xenografts when anti-miR-155 is expressed, whereas overexpression of miR-155 accelerated tumor growth (20). Similarly, a xenograft of MDA-MB-468 cells, with low endogenous miR-155 expression, showed accelerated tumor growth when miR-155 was overexpressed (24). In the same study, knockdown of miR-155 in an orthotopically transplantated mouse tumor cell line inhibited tumor growth (24). Contrary to this, a recent study using the 4T1 mouse mammary model showed that miR-155 had no effect on growth of the primary tumor (39).

Although numerous studies show that miR-155 is upregulated in human breast cancer, the cause of aberrant miR-155 levels is not well characterized. TGFβ treatment of NMuMG cells results in significant upregulation of miR-155 and an epithelial to mesenchymal transition (EMT; ref. 32). TGFβ is known to drive EMT, in which immobile epithelial cells alter their morphology to become motile mesenchymal cells to promote invasion (40) and, consequently, cancer progression (41, 42). In NMuMG cells, Smad4, a key signaling molecule in the TGFβ pathway, can bind to the *BIC* promoter and enrich miR-155 expression levels, thereby augmenting the TGFβ EMT process (32). Knockdown of miR-155 in NMuMG cells by anti-miR suppressed and ectopic overexpression of miR-155 enhanced TGFβ-mediated EMT (32). Furthermore, a key molecule in EMT, *RhoA*, is a target of miR-155, and expression of *RhoA* is reduced when miR-155 is ectopically expressed. When *RhoA* was expressed without its 3′-UTR (containing the miR-155 seed sequence), the EMT phenotype caused by miR-155 was abrogated (32). The ability to reverse a severe phenotypical change by reexpressing just one of the targets of miR-155 alludes to a potential therapeutic approach. Many miRNAs are known to have a role in metastasis and EMT (43); so in light of these findings, it is plausible that the basis of miR-155 in promoting breast cancer, in particular, the higher grade invasive breast cancers, is from the promotion of EMT. However, the findings from the 4T1 mouse model (39) contradict the findings in the NMuMG cell line (32). Unfortunately, both of the cell lines are of mouse origin. A miR-155 target gene in a mouse model will not necessarily be a target gene in humans, as the 3′-UTR region of transcripts is a common location for miRNA seed sequences, and is not highly conserved between mice and humans. These conflicting results call into question the suitability of using a mouse-specific model for an miRNA study.

Regulation of miR-155 expression

Perhaps the most remarkable recent finding in relation to the role of miR-155 in breast cancer is the involvement with *BRCA1*. *BRCA1*, the breast cancer susceptibility gene, is involved in DNA damage repair and cell-cycle progression. Mutations of *BRCA1* are associated with a high risk of developing breast cancer (24). In a recent study, mouse embryonic stem cells expressing the R1699Q BRCA1 underwent spontaneous differentiation. The mutant cells displayed high levels of miR-155, and overexpression of miR-155 in *BRCA1* wild-type cells gave a similar phenotype to the mutant, indicating that *BRCA1* was acting through miR-155 (24). In mice, a loss of functional BRCA1 protein results in miR-155 upregulation. These results were recapitulated in human cell lines, in which *BRCA1* deficient cells have 50-fold higher miR-155 levels compared with those with functional *BRCA1* (24). Furthermore, the transient overexpression of BRCA1 protein reduces expression of miR-155. In clinical samples, it was found that miR-155 levels were 2- to 6-fold

higher in *BRCA1*-mutant tumors (24). The mechanism of *BRCA1* regulation of miR-155 was through direct binding of BRCA1 protein to the miR-155 promoter. This, in turn, recruits histone deacetylase (HDAC) to repress the expression of *BIC* and thus miR-155 (24). This close association with the breast cancer susceptibility gene reinforces the importance of miR-155 in breast cancer.

Target genes of miR-155

The function of miRNAs are limited to inhibition of their target mRNA and consequent effects on cellular processes. miR-155 clearly has a role in breast cancer, and understanding this role requires the identification of critical miR-155 target genes.

Targetscan is an *in silico* prediction software commonly used to identify putative target genes of particular miRNAs by alignment of the 7 or 8 nucleotide seed sequence with the 3′-UTR of 30,858 human transcripts based on conservation between human and mouse sequences (23). Targetscan version 6 predicts 440 miR-155 targets (23, 44) based on sequence homology and conservation. Confirmation of these potential targets requires validation *in vitro*. To this end, we conducted a literature search to identify published validated miR-155 target genes. A target was defined as validated when there was a specific luciferase 3′-UTR reporter assay, which defines whether miR-155 directly targets the transcript, together with at least one other quantitative method, such as quantitative reverse transcriptase PCR or Western blot analysis, to assess the repression of the expression levels of the endogenous target gene.

Supplementary Table S1 displays a comprehensive list of 147 validated target genes identified in a wide range of miR-155 studies (45–87), and their prediction status by Targetscan. A total of 103 target genes (including 11 target genes validated in other studies) were identified in a single high-throughput next-generation sequencing study and validated by luciferase reporter assay (50). The remaining 44 target genes and their method of validation are displayed in Supplementary Table S2. Of the validated miR-155 target genes, approximately half (48%) were predicted by Targetscan software (23, 44). This highlights the drawbacks in relying on *in silico* prediction tools to investigate potential miRNA targets. The discrepancy between predicted and observed miR-155 binding sites is affected by miR-155 targeting nonconserved sites in target genes, as Targetscan by default searches for seed sequences conserved between human and mouse. Carrying out a Targetscan search irrespective of site conservation predicts 2,390 potential miR-155 targets and encompasses all but 9 validated target genes. This is the first comprehensive collation of all known miR-155 target genes and will be a valuable resource for future reference and research.

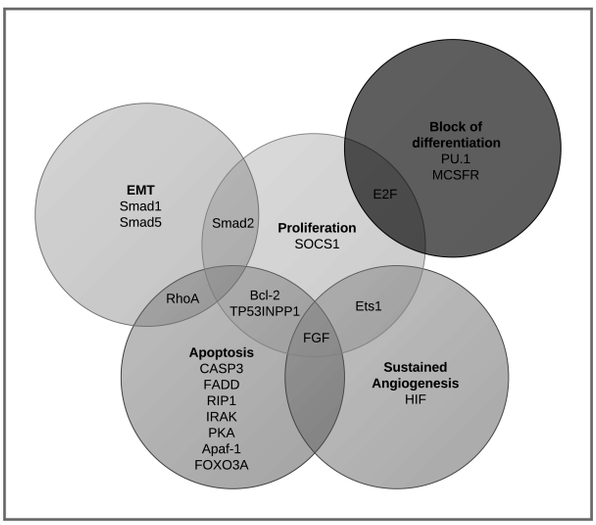

Figure 2. miR-155 target genes involved in cancer-related pathways. Validated miR-155 target genes are present in multiple pathways associated with cancer and cancer progression, including but not limited to EMT, proliferation, block of differentiation, apoptosis, sustained angiogenesis. Pathway analysis was completed using DAVID bioinformatics resource (v 6.7).

Although only a fraction of validated miR-155 target genes have a confirmed role in breast cancer, a number of the targets are involved in cancer-related pathways such as apoptosis, proliferation and EMT (20, 21, 32, 88, 89), as shown in Fig. 2. The presence of validated miR-155 targets in these pathways highlight the importance of miR-155 in cancer progression.

Conclusion

As an oncomiR, expression levels of miR-155 are consistently upregulated in breast tumor samples. Studies have defined the clinical significance of miR-155 in breast cancer with an association with clinical markers, more aggressive tumors, and decreased survival. However, there are some contradictory findings reported, for instance, the varied association of miR-155 with hormone receptor status. It is also unclear as to whether miR-155 functions initiate cancer or predominantly promotes tumor progression. In a mouse model, miR-155 has been shown to transform B cells (90) but in breast cells has only been shown to enhance cancerous properties of tumor cells. More investigation is required to fully understand the significance of aberrantly high levels of miR-155 in breast cancer.

Exploration of the function of miR-155 in breast cancer cell lines and xenograft models shows that miR-155 enhances tumor growth, promotes cell proliferation, inhibits apoptosis, and acts as a mediator of TGFβ-driven EMT. In particular, the role of miR-155 in EMT has promising therapeutic potential, given that miR-155 levels have been shown to be elevated in invasive tumors and in breast tumor metastases. The large number of validated miR-155 targets presented in Supplementary Table S1 provide many avenues of further investigation as to the clinical potential of miR-155. The further investigation of these targets will be required to confirm the mechanistic

and regulatory actions of miR-155 and their contribution to breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Mattiske, R.J. Suetani
Development of methodology: S. Mattiske
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Mattiske
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Mattiske, R.J. Suetani
Writing, review, and/or revision of the manuscript: S. Mattiske, R.J. Suetani, P.M. Neilsen, D.F. Callen
Study supervision: R.J. Suetani, P.M. Neilsen, D.F. Callen

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References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
2. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215–33.
3. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. Nat Genet 2005;37:495–500.
4. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003;115:787–98.
5. Yu Z, Jian Z, Shen SH, Purisima E, Wang E. Global analysis of microRNA target gene expression reveals that miRNA targets are lower expressed in mature mouse and *Drosophila* tissues than in the embryos. Nucleic Acids Res 2007;35:152–64.
6. Faraoni I, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: a typical multifunctional microRNA. Biochim Biophys Acta 2009;1792:497–505.
7. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 1993;75:855–62.
8. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843–54.
9. Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RH, Cuppen E. Phylogenetic shadowing and computational identification of human microRNA genes. Cell 2005;120:21–4.
10. Rao E, Jiang C, Ji M, Huang X, Iqbal J, Lenz G, et al. The miRNA-17 approximately 92 cluster mediates chemoresistance and enhances tumor growth in mantle cell lymphoma via PI3K/AKT pathway activation. Leukemia 2012;26:1064–72.
11. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. EMBO J 2002;21:4663–70.
12. Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. Science 2002;297:2056–60.
13. Pratt AJ, MacRae IJ. The RNA-induced silencing complex: a versatile gene-silencing machine. J Biol Chem 2009;284:17897–901.
14. Clurman BE, Hayward WS. Multiple proto-oncogene activations in avian leukosis virus-induced lymphomas: evidence for stage-specific events. Mol Cell Biol 1989;9:2657–64.
15. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002;12:735–9.
16. Eis PS, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, et al. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc Natl Acad Sci U S A 2005;102:3627–32.
17. Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, et al. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. J Pathol 2005;207:243–9.
18. Kluiver J, Haralambieva E, de Jong D, Blokzijl T, Jacobs S, Kroesen BJ, et al. Lack of BIC and microRNA miR-155 expression in primary cases of Burkitt lymphoma. Genes Chromosomes Cancer 2006;45:147–53.
19. Kluiver J, van den Berg A, de Jong D, Blokzijl T, Harms G, Bouwman E, et al. Regulation of pri-microRNA BIC transcription and processing in Burkitt lymphoma. Oncogene 2007;26:3769–76.
20. Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, et al. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer 2010;70:3119–27.
21. Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D, et al. MicroRNA-155 regulates cell survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. J Biol Chem 2010;285:17869–79.
22. O'Day E, Lal A. MicroRNAs and their target gene networks in breast cancer. Breast 2010;12:201.
23. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005;120:15–20.
24. Chang S, Wang RH, Akagi K, Kim KA, Martin BK, Cavallone L, et al. Tumor suppressor BRCA1 epigenetically controls oncogenic microRNA-155. Nat Med 2011;17:1275–82.
25. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005;65:7065–70.
26. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257–61.
27. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol 2007;8:R214.
28. Hui AB, Shi W, Boutros PC, Miller N, Pintilie M, Fyles T, et al. Robust global micro-RNA profiling with formalin-fixed paraffin-embedded breast cancer tissues. Lab Invest 2009;89:597–606.
29. Zhu W, Qin W, Atasoy U, Sauter ER. Circulating microRNAs in breast cancer and healthy subjects. BMC Res Notes 2009;2:89.
30. Wang F, Zheng Z, Guo J, Ding X. Correlation and quantitation of microRNA aberrant expression in tissues and sera from patients with breast tumor. Gynecol 2010;119:586–93.
31. Chen J, Wang BC, Tang JH. Clinical significance of MicoRNA-155 expression in human breast cancer. J Surg Oncol 2011 Nov 21. [Epub ahead of print].
32. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, et al. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol 2008;28:6773–84.
33. Baffa R, Fassan M, Volinia S, O'Hara B, Liu CG, Palazzo JP, et al. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. J Pathol 2009;219:214–21.
34. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, et al. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008;14:2348–60.
35. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, et al. Serum microRNAs are promising novel biomarkers. PLoS One 2008;3:e3148.
36. Fang C, Zhu DX, Dong HJ, Zhou ZJ, Wang YH, Liu L, et al. Serum microRNAs are promising novel biomarkers for diffuse large B cell lymphoma. Ann Hematol 2011;91:553–9

37. Appaiah HN, Goswami CP, Mina LA, Badve S, Sledge GWJr, Liu Y, et al. Persistent upregulation of U6:SNORD44 small RNA ratio in the serum of breast cancer patients. Breast Cancer Res 2011;13:R86.
38. Meister G, Landthaler M, Dorsett Y, Tuschl T. Sequence-specific inhibition of microRNA- and siRNA-induced RNA silencing. RNA 2004;10:544–50.
39. Xiang X, Zhuang X, Ju S, Zhang S, Jiang H, Mu J, et al. miR-155 promotes macroscopic tumor formation yet inhibits tumor dissemination from mammary fat pads to the lung by preventing EMT. Oncogene 2011;30:3440–53
40. Wendt MK, Allington TM, Schiemann WP. Mechanisms of the epithelial-mesenchymal transition by TGF-beta. Future Oncol 2009;5:1145–68.
41. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 2008;14:818–29.
42. Trimboli AJ, Fukino K, de Bruin A, Wei G, Shen L, Tanner SM, et al. Direct evidence for epithelial-mesenchymal transitions in breast cancer. Cancer Res 2008;68:937–45.
43. Bracken CP, Gregory PA, Khew-Goodall Y, Goodall GJ. The role of microRNAs in metastasis and epithelial-mesenchymal transition. Cell Mol Life Sci 2009;66:1682–99.
44. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19:92–105.
45. Martin MM, Lee EJ, Buckenberger JA, Schmittgen TD, Elton TS. MicroRNA-155 regulates human angiotensin II type 1 receptor expression in fibroblasts. J Biol Chem 2006;281:18277–84.
46. Cheng W, Liu T, Jiang F, Liu C, Zhao X, Gao Y, et al. microRNA-155 regulates angiotensin II type 1 receptor expression in umbilical vein endothelial cells from severely pre-eclamptic pregnant women. Int J Mol Med 2011;27:393–9.
47. Zheng L, Xu CC, Chen WD, Shen WL, Ruan CC, Zhu LM, et al. MicroRNA-155 regulates angiotensin II type 1 receptor expression and phenotypic differentiation in vascular adventitial fibroblasts. Biochem 2010;400:483–8.
48. Zhu N, Zhang D, Chen S, Liu X, Lin L, Huang X, et al. Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. Atherosclerosis 2011;215:286–93
49. Borchert GM, Holton NW, Larson ED. Repression of human activation induced cytidine deaminase by miR-93 and miR-155. BMC Cancer 2011;11:347.
50. Xu G, Fewell C, Taylor C, Deng N, Hedges D, Wang X, et al. Transcriptome and targetome analysis in MIR155 expressing cells using RNA-seq. RNA 2010;16:1610–22.
51. Yin Q, McBride J, Fewell C, Lacey M, Wang X, Lin Z, et al. MicroRNA-155 is an Epstein-Barr virus-induced gene that modulates Epstein-Barr virus-regulated gene expression pathways. J Virol 2008;82:5295–306.
52. O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, Nicoll J, et al. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med 2008;205:585–94.
53. Willimott S, Wagner SD. MiR-125b and miR-155 contribute to BCL2 repression and proliferation in response to CD40 ligand (CD154) in human leukemic B-cells. J Biol Chem 2011;287:2608–17.
54. Wang HQ, Yu XD, Liu ZH, Cheng X, Samartzis D, Jia LT, et al. Deregulated miR-155 promotes Fas-mediated apoptosis in human intervertebral disc degeneration by targeting FADD and caspase-3. J Pathol 2011;225:232–42.
55. He M, Xu Z, Ding T, Kuang DM, Zheng L. MicroRNA-155 regulates inflammatory cytokine production in tumor-associated macrophages via targeting C/EBPbeta. Cell Mol Immunol 2009;6:343–52.
56. Worm J, Stenvang J, Petri A, Frederiksen KS, Obad S, Elmen J, et al. Silencing of microRNA-155 in mice during acute inflammatory response leads to derepression of c/ebp Beta and down-regulation of G-CSF. Nucleic Acids Res 2009;37:5784–92.
57. Yin Q, Wang X, Fewell C, Cameron J, Zhu H, Baddoo M, et al. MicroRNA miR-155 inhibits bone morphogenetic protein (BMP) signaling and BMP-mediated Epstein-Barr virus reactivation. J Virol 2010;84:6318–27.
58. Lossner C, Meier J, Warnken U, Rogers MA, Lichter P, Pscherer A, et al. Quantitative proteomics identify novel miR-155 target proteins. PLoS One 2011;6:e22146.
59. Liu S, Yang Y, Wu J. TNFalpha-induced up-regulation of miR-155 inhibits adipogenesis by down-regulating early adipogenic transcription factors. Biochem 2011;414:618–24.
60. Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, et al. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. J Allergy Clin Immunol 2010;126:581–9.e1–20.
61. Tili E, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol 2007;179:5082–9.
62. Pottier N, Maurin T, Chevalier B, Puissegur MP, Lebrigand K, Robbe-Sermesant K, et al. Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. PLoS One 2009;4:e6718.
63. Yamamoto M, Kondo E, Takeuchi M, Harashima A, Otani T, Tsuji-Takayama K, et al. miR-155, a modulator of FOXO3a protein expression, is underexpressed and cannot be upregulated by stimulation of HOZOT, a line of multifunctional Treg. PLoS One 2011;6:e16841.
64. Dagan LN, Jiang X, Bhatt S, Cubedo E, Rajewsky K, Lossos IS. miR-155 regulates HGAL expression and increases lymphoma cell motility. Blood 2011;119:513–20.
65. Lu F, Weidmer A, Liu CG, Volinia S, Croce CM, Lieberman PM. Epstein-Barr virus-induced miR-155 attenuates NF-kappaB signaling and stabilizes latent virus persistence. J Virol 2008;82:10436–43.
66. Martinez-Nunez RT, Louafi F, Sanchez-Elsner T. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor alpha1 (IL13Ralpha1). J Biol Chem 2011;286:1786–94.
67. Bhattacharyya S, Balakathiresan NS, Dalgard C, Gutti U, Armistead D, Jozwik C, et al. Elevated miR-155 promotes inflammation in cystic fibrosis by driving hyper-expression of interleukin-8. J Biol Chem 2011;286:11604–15.
68. O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci U S A 2009;106:7113–8.
69. Cremer TJ, Ravneberg DH, Clay CD, Piper-Hunter MG, Marsh CB, Elton TS, et al. MiR-155 induction by *F. novicida* but not the virulent *F. tularensis* results in SHIP down-regulation and enhanced pro-inflammatory cytokine response. PLoS One 2009;4:e8508.
70. Pedersen IM, Otero D, Kao E, Miletic AV, Hother C, Ralfkiaer E, et al. Onco-miR-155 targets SHIP1 to promote TNFalpha-dependent growth of B cell lymphomas. EMBO Mol Med 2009;1:288–95.
71. Costinean S, Sandhu SK, Pedersen IM, Tili E, Trotta R, Perrotti D, et al. Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice. Blood 2009;114:1374–82.
72. Zhou H, Huang X, Cui H, Luo X, Tang Y, Chen S, et al. miR-155 and its star-form partner miR-155* cooperatively regulate type I interferon production by human plasmacytoid dendritic cells. Blood 2010;116:5885–94.
73. Bolisetty MT, Dy G, Tam W, Beemon KL. Reticuloendotheliosis virus strain T induces miR-155, which targets JARID2 and promotes cell survival. J Virol 2009;83:12009–17.
74. Tang B, Xiao B, Liu Z, Li N, Zhu ED, Li BS, et al. Identification of MyD88 as a novel target of miR-155, involved in negative regulation of Helicobacter pylori-induced inflammation. FEBS Lett 2010;584:1481–6.
75. Lu C, Huang X, Zhang X, Roensch K, Cao Q, Nakayama KI, et al. MiR-221 and miR-155 regulate human dendritic cell development, apoptosis and IL-12 production through targeting of p27kip1, KPC1 and SOCS-1. Blood 2011;117:4293–303.
76. McInnes N, Sadlon TJ, Brown CY, Pederson S, Beyer M, Schultze JL, et al. FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells. Oncogene 2011;31:1045–54

77. Levati L, Pagani E, Romani S, Castiglia D, Piccinni E, Covaciu C, et al. MicroRNA-155 targets the SKI gene in human melanoma cell lines. Pigment Cell Melanoma Res 2011;24:538–50.
78. Louafi F, Martinez-Nunez RT, Sanchez-Elsner T. MicroRNA-155 targets SMAD2 and modulates the response of macrophages to transforming growth factor-β. J Biol Chem 2010;285:41328–36.
79. Rai D, Kim SW, McKeller MR, Dahia PL, Aguiar RC. Targeting of SMAD5 links microRNA-155 to the TGF-beta pathway and lymphomagenesis. Proc Natl Acad Sci U S A 2010;107:3111–6.
80. Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity 2009;30:80–91.
81. Xie Q, Chen X, Lu F, Zhang T, Hao M, Wang Y, et al. Aberrant expression of microRNA 155 may accelerate cell proliferation by targeting sex-determining region Y box 6 in hepatocellular carcinoma. Cancer 2012;118:2431–42.
82. Martinez-Nunez RT, Louafi F, Friedmann PS, Sanchez-Elsner T. MicroRNA-155 modulates the pathogen binding ability of dendritic cells (DCs) by down-regulation of DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN). J Biol Chem 2009;284:16334–42.
83. Imaizumi T, Tanaka H, Tajima A, Yokono Y, Matsumiya T, Yoshida H, et al. IFN-gamma and TNF-alpha synergistically induce microRNA-155 which regulates TAB2/IP-10 expression in human mesangial cells. Am J Nephrol 2010;32:462–8.
84. Ceppi M, Pereira PM, Dunand-Sauthier I, Barras E, Reith W, Santos MA, et al. MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. Proc Natl Acad Sci U S A 2009;106:2735–40.
85. Wang Y, Scheiber MN, Neumann C, Calin GA, Zhou D. MicroRNA regulation of ionizing radiation-induced premature senescence. Int J Radiat Oncol Biol Phys 2010;81:839–48
86. Gironella M, Seux M, Xie MJ, Cano C, Tomasini R, Gommeaux J, et al. Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. Proc Natl Acad Sci U S A 2007;104:16170–5.
87. Butz H, Liko I, Czirjak S, Igaz P, Khan MM, Zivkovic V, et al. Down-regulation of Wee1 kinase by a specific subset of microRNA in human sporadic pituitary adenomas. J Clin Endocrinol Metab 2010;95:E181–91.
88. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.
89. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1–13.
90. Costinean S, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci U S A 2006;103:7024–9.