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The Oncogenic Role of miR-155 in Breast Cancer

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Abstract

miR-155 is an oncogenic microRNA 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 biological pathways relevant to miR-155 and breast cancer progression. Publications describing the clinical relevance, functional characterisation, and regulation of expression of miR-155 in the context of breast cancer are reviewed. 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 EMT. 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 biological pathways of miR-155 identified in this review suggest new avenues of research for this oncogenic microRNA.

Introduction

MicroRNAs (miRNAs) are small noncoding RNAs which 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 7-8 nucleotides long, which directly binds to complementary sequences in regulatory regions of target genes. These binding regions are often in the 3' UTR of target genes, but increasingly are being reported in other non-coding 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). The biological relevance of miRNAs have increasingly been recognised in recent years, with some estimates suggesting that 30% of all eukaryotic genes are regulated by miRNAs (4; 5). miR-155 is a miRNA widely published to be involved in lymphoma, but in more recent years evidence has also emerged for it playing a role in the progression of solid cancers (6). This review will focus on the microRNA 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 over 1000 (9). Many of these miRNAs are organised 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 characterised, and is depicted in Figure 1 using miR-155 as an example. miRNAs originate from a ~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 a ~22 nucleotide RNA duplex. One strand of the duplex becomes the mature miRNA and is usually the functional, regulatory unit (12; 13) while 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 *et al* identified miR-155 as a regulatory RNA (15). Since this discovery, the role of miR-155 has been extensively studied in lymphoma (16-19), and has also been implicated in regulating genes involved in viral infection, cardiovascular disease and solid cancers (6; 20-22). miR-155 has over 400 predicted gene targets (23) and more than 100 confirmed *bona fide* targets involved in pathways such as apoptosis and cancer progression. In recent years, there has been an emerging role of miR-155 in breast cancer progression, as seen by a number of recent studies (20; 21; 24). This article will review the current literature regarding miR-155 and breast cancer, and discuss both predicted and validated target genes of miR-155.

Clinical Relevance of miR-155 in Breast Cancer

The expression level of miR-155 has been found to be upregulated in breast cancer in several studies. In some cases high levels of miR-155 have also been associated with clinicopathological markers, tumour subtype and poor survival rates. These findings are summarised in Table 1.

Numerous studies have found an association with increased miR-155 levels in breast cancer tissue. 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 could be used to independently predict the invasive potential of independently analysed breast tissue samples (25). A small microarray study of 8 fresh breast tumour samples compared to normal adjacent tissue found miR-155 was upregulated in the breast tumours (34). Kong *et al.* conducted two studies investigating the role of miR-155 in breast cancer. Firstly, 62 breast carcinomas were analysed to determine miR-155 levels. Out of 17 non-invasive tumours, only 2 (12%) exhibited a high level of miR-155 expression. Conversely, 41 of the 45 invasive tumours (91%) displayed miR-155 upregulation (32). The second study investigated expression levels of miR-155 target gene FOXO3A in 77 primary breast tumours, 38 recurrent tumours and 11 normal tissue samples. This found that miR-155 was upregulated and FOXO3A downregulated in a majority of primary tumours, and also that high miR-155 and low FOXO3A expression was associated with recurrent tumours after radiotherapy or chemotherapy (21). These studies linked miR-155 expression to both invasiveness and recurrence of breast tumours, and demonstrated that expression levels of miR-155 and its specific target genes are of potential clinical prognostic value.

In a robust study that included 363 breast tumour samples, Volinia *et al.* globally compared miRNA expression levels in multiple tumour and pooled normal tissue samples to identify dysregulated miRNAs in tumour samples. This was performed for a wide range of tumours including lung, breast, stomach, prostate, colon and

pancreatic. Comparisons of normal and tumour tissue derived from each individual tissue were conducted and miR-155 expression was found to be upregulated in breast, colon and lung cancers. Interestingly, miR-155 was one of only two miRNAs (the other being the miR-200 family) found to be upregulated in both breast and lung cancer, implying that these microRNAs may be part of a common mechanism in the development of cancer in these organs (26).

Other studies have found miR-155 expression levels to be associated with metastasis events and invasive properties of breast cancer. One such study found that increased miR-155 expression is associated with high tumour grade, advanced tumour 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 two studies involving microarray analyses of FFPE breast cancer samples, which found that miR-155 expression was upregulated in the malignant breast cancer (28, 33).

Because of the relationship between miR-155 and poor prognosis and/or metastasis, a similar relationship between miR-155 and clinicopathological markers would be expected, and is indeed the case. Numerous studies have found links between hormone receptor status of tumours and miR-155 expression levels. Blenkiron *et al.* analysed miRNA levels alongside mRNA levels, enabling classification of tumour subtype and identification of clinicopathological factors. In 93 breast cancer samples miR-155 levels were significantly upregulated in basal-like tumours and estrogen receptor negative (ER-) tumours (27), although no proposal for the mechanism of this

association is given. The correlation with basal-like tumours has clinical relevance due to the poor prognosis of this tumour subtype.

Similarly, 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 *et al.* found that multiple miRNAs could be detected in sera and the miRNA levels correlated with the levels in tissue samples (29). They also found that miR-155 was expressed at a higher level in the serum of PR+ breast cancer patients than in the serum of PR- patients (29). Following these studies, Wang *et al.* conducted an in-depth study into miRNA levels in breast cancer using both fresh tumour and sera samples. The correlation of miRNA expression between tissue and matched serum samples was significant (R^2=0.853). They confirmed that miR-155 was upregulated in breast cancer, and also found that high miR-155 associated with grade II and III tumours, as well as ER- and PR- tumours (30). The detection of miR-155 expression levels in serum could be a useful clinical prognostic indicator of tumour grade and hormone receptor status. This study reproduced the finding that miR-155 was expressed at higher levels in ER- tumours (27), but contradicted the study showing miR-155 levels to be higher in the serum of progesterone receptor positive (PR+) tumours (29). The topic of serum miRNAs is somewhat controversial, with some studies similarly suggesting that serum miRNA levels are robust (35; 36), and others claiming that miRNAs often used as normalisation controls are highly variable in sera samples, and thus miRNA quantification in sera is not reproducible (37). This might be an indicator that analysis of serum alone is not sufficient to determine whether miR-155 is differentially expressed. Additionally, the low number

of samples used in some of these studies could indicate that a more robust study is required before a conclusion can be made.

Taken together, these studies show that miR-155 expression is upregulated in breast cancer, consistent with its status as an oncomiR. In addition, the association between miR-155 and a more invasive breast tumour has been identified in multiple studies. However, between studies the relationships between miR-155 and clinicopathological markers such as ER and PR status and tumour subtype is somewhat inconsistent. For example, the upregulation of miR-155 expression in PR+ tumours was only identified in one study that analysed a small number of samples (29). The mechanism linking miR-155 and hormone receptors remains unknown and further study is required to elucidate the mechanism and significance of these observations.

Functional characterisation of miR-155 oncogenic activities in breast cancer

An important step in determining the clinical significance of a molecule such as miR-155, is to determine whether high expression levels are causally related to the development of breast cancer. Kong *et al.* assessed the effects of altering miR-155 expression levels in a panel of breast cancer cell lines *in vitro* (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 a 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 demonstrate that miR-155 has a role in cell proliferation and apoptosis, two cellular processes frequently aberrant in cancer. Similar results have also been reported in breast cancer cell lines MDA-MB-231 and MCF7 where ectopic miR-155 overexpression can increase proliferation, while inhibition of miR-155 expression by a specific anti-miR inhibits proliferation and increases radio-sensitivity of cells *in vitro* (20; 31).

The first study of miR-155 in immunodeficient mice xenografted with human breast cancer cells confirmed that miR-155 acts as an oncomiR *in vivo*. Xenograft tumours established with MDA-MB-231 cells expressing anti-miR-155 displayed reduced tumour volume compared to control xenografts. In addition, overexpression of miR-155 in MDA-MB-231 xenografts accelerated tumour growth (20). Similar results were obtained using a xenograft of MDA-MB-468 cells, which are low in endogenous miR-155 expression: tumour growth was accelerated when miR-155 was overexpressed (24). The same study also used an orthotopically transplantated mouse tumour cell line, where knockdown of miR-155 inhibited tumour 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 tumour (39).

Although it has been widely shown that miR-155 is upregulated in breast cancer, the cause of aberrant miR-155 levels is not well characterised. When Kong *et al.* treated NMuMG cells with TGFβ they found miR-155 to be strongly upregulated (32). TGFβ can drive epithelial to mesenchymal transition (EMT), the process by which immobile epithelial cells alter their morphology to become motile mesenchymal cells and promote invasion (40). As EMT can be important in cancer progression (41; 42) they

investigated the role of miR-155 in TGFβ mediated EMT using NMuMG cells. It was found that Smad4, a key signalling 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 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 contest the findings of Kong *et al* in NMuMG . 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 a 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, the R1699Q BRCA1 mutant caused mouse embryonic stem cells to undergo 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 resulted in miR-155 upregulation. These results were recapitulated in human cell lines, with deficient BRCA1 have 50-fold higher miR-155 levels compared to those with functional BRCA1 (24). Furthermore, the transient overexpression of BRCA1 in BRCA1 deficient cell lines reduces expression of miR-155. In clinical samples it was found that miR-155 levels were two to six fold higher in BRCA1 mutant tumours (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

Although miR-155 clearly has a role to play in breast cancer, microRNAs have limited function other than inhibiting expression of their target mRNAs. Therefore, ultimately it is the target genes and their inhibition that has an effect on cellular processes. Critical to understanding the role of miR-155 in breast cancer is identifying miR-155 target genes, specifically those target genes important in breast cancer.

Several i*n silico* prediction software programs are commonly used to identify putative target genes of particular miRNAs. Targetscan is one such commonly used software,

which predicts target genes of 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 sequence (23). Using this program, Targetscan version 6 predicts 440 potential miR-155 targets (conserved) (23; 44). However these targets are only predicted based on sequence homology, and must be tested *in vitro* before being considered true miR-155 target genes. To this end, an extensive literature search was conducted to identify published validated miR-155 target genes. Validation was defined as a specific luciferase 3`UTR reporter assay for the target gene together with at least one other quantitative method to assess the repression of the endogenous expression levels of the target, such as qRT-PCR or Western blot analysis. Validation by luciferase 3`UTR reporter assays was considered an essential criterion for miR-155 targets, to ensure that miR-155 in fact directly targets the transcript for repression. The second criterion, validation by at least one other quantitative method, ensures that miR-155 has an effect on expression of the target gene.

Table 2 displays a comprehensive list of 147 validated target genes identified in a wide range of miR-155 studies, and their prediction status by Targetscan. 103 target genes (including 11 target genes validated in other studies) were identified in a single high throughput next generation sequencing (NGS) study and validated by luciferase reporter assay (50). The remaining 44 target genes were validated by luciferase assay and at least one other method. Of the validated miR-155 target genes, approximately half (48%) were predicted to be targets 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 non-conserved sites in target genes, as TargetScan by default searches for seed sequences conserved between human and mouse. Performing a Targetscan search irrespective of site conservation predicts 2390 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 could be a valuable resource for future reference and research.

Although only a fraction of these miR-155 target genes were validated in the context of breast cancer, a number of the targets are involved in cancer-related pathways such as apoptosis, proliferation and EMT (20; 21; 32; 88; 89). miR-155 target genes involved in cancer-related pathways are shown in Figure 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 tumour samples. Some studies have associated miR-155 with clinical markers, more aggressive tumours and even decreased survival. It is clear from these studies that miR-155 is of clinical significance in breast cancer. However, some contradictory findings have been noted, for instance the varied association of miR-155 with hormone receptor positive/negative tumours. It is also unclear as to whether miR-155 is an initiator of cancer, or if it just promotes tumour 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 tumour 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 both xenograft models of breast cancer and breast cancer cell lines has shown that miR-155 enhances tumour growth, promotes cell proliferation, inhibits apoptosis and acts as a mediator of TGFβ-driven EMT. The role of miR-155 in EMT in particular has promising therapeutic potential, given that miR-155 levels have been shown to be elevated in invasive tumour and in breast tumour metastases. The large number of validated miR-155 targets presented here (Table 2) 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 the contribution of this to breast cancer.

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Tables

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

Table 1 –Studies examining miR-155 expression in breast cancer

Displayed are sample tissue type, number of samples in each study and a brief summary of the miR-155 related findings.

miR-155 Target Gene	Accession No.	Predicted	Not predicted	Not predicted (nonconserved)	Reference
AGTR1 (AT1R)	NM_000685		X		(45-48)
AICDA (AID)	NM_020661.2	X			(49)
ANAPC16 (C10orf104)	NM_173473.2		X		(50)
APAF1	NM_013229.2		X		(50)
ARID2	NM_152641	X			(50; 51)
ARL15	NM_019087.2		X		(50)
ARL6IP5	NM_006407.3		X		(50)
ARMC2	NM_032131.4		X		(50)
ARNT	NM_001668		X	X	(52)
BACH1	NM_206866	X			(50-52)
BCL2	NM_000633.2		X		(53)
BCORL1	NM_021946.4	X			(50)
C10orf26	NM_001083913.1	X			(50)
C16orf62	NM_020314.4		X		(50)
C3orf18	NM_016210.4	X			(50)
C5orf44	NM_001093755.1		X		(50)
CARD11	NM_032415.3	X			(50)
CARHSP1	NM_014316.2	X			(50)
CASP3	NM_004346		X	X	(54)
CCDC41	NM_016122.2		X		(50)
CDC40	NM_015891.2		X		(50)
CEBPB	NM_005194	X			(51; 52; 55-57)
CHD9	NM_025134.4	X			(50)
CIAPIN1	NM_020313.2		X		(50)
CKAP5	NM_001008938.1		X		(58)
CLUAP1	NM_015041.1		X		(50)
CREB1 (CREB)	NM_004379.3	X			(59)
CSF1R (CD115)	NM_005211	X			(52)
CSNK1A1	NM_001025105.1		X		(50)
CTLA4	NM_005214	X			(60)
CUX1 (CUTL1)	NM_181552	X			(52)
CYP2U1	NP_898898.1		X		(50)
DCUN1D2	NM_001014283.1		X		(50)
DET1	NM_017996.3	X			(50)
DHX40	NM_024612.4	X			(50)
E2F2	NM_004091.2	X			(50)
EIF2C4	NM_017629.2	X			(50)
ETS1	NM_001143820	X			(48; 50)
EXOSC2	NM_014285.5		X		(50)
FADD	NM_003824		X	X	(54; 61)
FAM135A	NM_001105531.2	X			(50)
FAM177A1	NM_173607.3		X		(50)
FAM199X (CXorf39)	NM_207318.2		X		(50)
FAM91A1	NM_144963.2		X		(50)

FGF7 (KGF)	NM_002009	X			(62)
FOXO3 (FOXO3A)	NM_001455	X			(21; 63)
GABARAPL1	NM_031412.2		X		(50)
GATM	NM_001482.2		X		(50)
GCET2 (HGAL)	NM_152785.4		X		(64)
GCFC1 (C21orf66)	NM_016631.3	X			(50)
GNAS	NM_016592.2	X			(50)
GOLT1B	NM_016072.4		X		(50)
HBP1	NM_012257.3	X			(50)
HIF1A	NM_001530		X		(52)
HIVEP2	NM_006734	X			(50; 51; 57)
IGJ	NM_144646.3		X		(50)
IKBKE (IKKE)	NM_014002	X			(61; 65)
IL13RA1	NM_001560		X	X	(66)
IL17RB	NM_018725.3		X		(50)
INPP5D (SHIP1)	NM_001017915	X			(67-71)
INPP5F	NM_014937.2		X		(50)
INTS6	NM_012141.2		X		(50)
IRAK3 (IRAKM)	NM_007199		X	X	(72)
JARID2	NM_004973	X			(50; 52; 73)
KBTBD2	NM_015483.2		X		(50)
KIAA0430	NM_014647.3		X		(50)
KIAA1274	NM_014431.2	X			(50)
KLHL5	NM_015990.4		X		(50)
LCORL	NM_153686.7		X		(50)
LNX2	NM_015093	X			(50)
LRIF3 (C1orf103)	NM_018372.3		X	X	(50)
LRRC59	NM_018509.3	X			(50)
LSM14A	NM_001114093.1	X			(50)
MAP3K10	NM_002446.3	X			(50)
MAP3K14	NM_003954.2	X			(50)
MASTL	NM_001172303.1		X		(50)
MBNL3	NM_018388.3	X			(50)
MCM8	NM_032485.4		X		(50)
MED13L	NM_015335.4		X		(50)
MEF2A	NM_005587.2	X			(50)
MORC3	NM_015358.2		X		(50)
MPP5	NM_022474.2		X		(50)
MRPL18	NM_014161.3		X		(50)
MRPS27	NM_015084.2		X		(50)
MYBL1	NM_001080416.2	X			(50)
MYD88	NM_001172567		X		(74)
MYO10	NM_012334	X			(57)
MYO1D	NM_015194.1	X			(50)
MYST3	NM_001099412.1		X	X	(50)
NARS	NM_004539.3		X		(50)

PAK2	NM_002577.4	X			(50)
PCDH9	NM_203487.2	X			(50)
PDCD4	NM_014456.3		X		(50)
PHC2	NM_198040.2	X			(50)
PHF14	NM_014660.3		X		(50)
PHF17	NM_199320.2	X			(50)
PICALM	NM_007166		X		(50; 52)
PKN2	NM_006256.2	X			(50)
POLE3	NM_017443.4		X		(50)
PRKAR1A	NM_002734.3	X			(50)
RAB11FIP2	NM_014904.2	X			(50)
RAC1	NM_006908.4		X		(50)
RAPGEF2	NM_014247.2		X		(50)
RHEB	NM_005614.3	X			(50)
RHOA	NM_001664		X	X	(32)
RIPK1	NM_003804		X	X	(61)
RNF123 (KPC1)	NM_022064	X			(75)
SAP30L	NM_024632.5	X			(50)
SATB1	NM_002971.3	X			(76)
SDCBP	NM_005625.3	X			(50)
SECISBP2	NM_024077.3		X		(50)
SKI	NM_003036	X			(77)
SLA	NM_001045556	X			(52)
SLC33A1	NM_004733.3	X			(50)
SLC35F2	NM_017515.4		X		(50)
SMAD1	NM_005900	X			(57)
SMAD2	NM_005901	X			(50; 78)
SMAD5	NM_005903		X		(51; 57; 79)
SMARCA4	NM_001128849.1	X			(50)
SOCS1	NM_003745	X			(20; 75; 80)
SOX6	NM_017508.2		X		(81)
SPI1 (PU.1)	NM_001080547		X		(52; 82)
TAB 2 (MAP3K7IP2)	NM_015093	X			(50; 72; 83; 84)
TAF5L	NM_014409.3		X		(50)
TBC1D14	NM_020773.2		X		(50)
TBCA	NM_004607.2		X		(50)
TCF12	NM_207036.1		X		(50)
TLE4	NM_007005.3	X			(50)
TOMM20	NM_014765.2	X			(50)
TP53INP1	NM_033285	X			(50; 85; 86)
TRAK1	NM_001042646.1		X		(50)
TRIP13	NM_004237.3		X		(50)
TSGA14	NM_018718.1	X			(50)
TSPAN14	NM_030927.2	X			(50)
UBQLN1	NM_013438.4		X		(50)
VPS18	NM_020857.2	X			(50)

WEE1	NM_003390	X			(50; 87)
WHSC1L1	NM_023034.1		X		(50)
WWC1	NM_001161661.1	X			(50)
ZKSCAN5	NM_014569.3		X		(50)
ZNF248	NM_021045.1	X			(50)
ZNF254	NM_203282.2		X		(50)
ZNF273	NM_021148.2		X		(50)
ZNF28	NM_006969.2		X		(50)
ZNF611	NM_030972.3		X		(50)
ZNF652	NM_001145365.1	X			(50; 51)
ZNF83	NM_001105549.1		X		(50)

Table 2 – Validated miR-155 target genes and prediction status by Targetscan

An extensive literature search identified published validated miR-155 target genes. Validation was defined as luciferase reporter assay and at least one other method. Prediction of miR-155 targeting was determined using Targetscan (v 6.0), searching for conserved sites between human and mouse. Alternative gene names are given in brackets.

Figure Legends

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. Drosha and Pasha proteins process the pri-miR-155 into a 62 nucleotide pre-miR-155, which is exported from the nucleus by Exportin-5. This is subsequently processed by the Dicer protein into a 22 nucleotide miRNA duplex (16). One strand of the duplex (miR-155*) is degraded with the remaining strand becoming the mature miR-155. miR-155 forms a complex with Argonaute proteins called the RNA Induced Silencing Complex (RISC), in order 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.

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 (46; 47). Pathway analysis was completed using DAVID bioinformatics resource (v 6.7).