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Clinical relevance of microRNA expressions in breast cancer validated using the cancer genome atlas (TCGA)

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Abstract

Background—MicroRNAs (miRNAs) play a critical role in the carcinogenesis and progression of breast cancer. MiRNA-205 has tumor suppressive properties, whereas miRNA-18a has both oncogenic and tumor suppressive roles. MiRNA-744's role in breast cancer is unknown, but is tumor-suppressive *in vitro*. We hypothesize that high expression of all three miRNAs is associated with a better survival based on their known functions in breast cancer.

Methods—All data was obtained from the Cancer Genome Atlas (TCGA). Expression of miRNA-18a, miRNA-205, and miRNA-744 were retrieved from the Genomic Data Commons (GDC) data portal for analyses. After miRNA-specific thresholds were derived and used to group the patients into a high or low expression group, survival data was calculated using the Cox proportional hazard model. Further subanalyses separating the patients based on receptor status and AJCC 7th edition TNM staging were similarly compared.

Results—1052/1097 samples logged in TCGA had clinical data and miRNA-sequence datasets on the miRNAs of interest. High expression of miRNA-18a (p=0.079), miRNA-205 (p=0.034) and miRNA-744 (p=0.0135) was associated with a better survival. On subanalysis, estrogen receptor (ER), progesterone receptor (PR) positive, and lymph node negative disease had a statistically significant survival advantage with miRNA-18a, miRNA-205 and miRNA-744 high expression.

Conclusions—By utilizing a big dataset (TCGA) with sufficient statistical power, we found that high expression of miRNA-18a, miRNA-205, and miRNA-744 in the breast tumor samples were all associated with better overall survival in ER/PR positive, lymph node negative disease supporting their role as a tumor suppressor in breast cancer.

Introduction

MicroRNAs (miRNAs) are short noncoding RNAs approximately 19–25 nucleotides long that function in the epigenetic control of their target genes. Because the dysregulation of miRNAs is implicated in many different types of cancer, there is an increasing amount of research regarding the role miRNAs specifically in carcinogenesis[1]. The expression of miRNAs is regulated by a variety of mechanisms including environmental factors, DNA methylation, and histone acetylation modifications[1]. Once a particular miRNA is transcribed, it gets processed to become a mature miRNA. The mature miRNA binds to the seed region of its target messenger RNA (mRNA) and either prevents the translation of the mRNA or degrades it[2]. Some miRNAs are described as "oncogenic" because their expression leads to properties promoting cell growth, invasion, and proliferation, while others are described as "tumor suppressive" as their expression inhibits tumor growth and migration, and promotes apoptosis. Many researchers are interested in the role and expression patterns of miRNAs since they have the potential to be used as biomarkers to predict prognosis. The problem is that some of the miRNAs demonstrate both oncogenic and tumor suppressive properties depending upon the context, thus, their relevance in the clinical setting necessary.

MiRNA-18a, is thought to be “tumor suppressive” since Guo et al. found it was under-expressed in human breast cancer[3]. This conflicts with the report that it is overexpressed in estrogen receptor (ER) negative breast tumors on 171 breast cancer samples, which implicates that it is “oncogenic”[4]. In animal models, miRNA-18a high expression leads to decreased spontaneous lung metastasis and tumor growth and increased apoptosis *in vitro* in response to hypoxic and acidotic conditions, supporting its role as tumor suppressive[5]. In contrast, Mouw et al. found that miRNA-18a high expression leads to the opposite result in response to different environmental conditions, favoring an oncogenic role[6].

MiRNA-205 expression is decreased in breast cancer and increased in normal breast tissue, which implicates that it is a “tumor suppressive” miRNA. In animal models, high expression of miRNA-205 results in a decrease in spontaneous lung metastasis and inhibition of cell proliferation, anchorage independent growth, and cell invasion[7]. MiRNA-205 is implicated in reducing the invasive and migratory potential in both breast and prostate cancer cells by increasing E cadherin, although they use different targets[89]. However, in endometrial cancer cells, miRNA-205 high expression is associated with tumor progression and worse prognosis by suppressing phosphatase and tensin homolog (PTEN)[10].

In breast cancer cells, over expression of miRNA-744 is described as a “tumor suppressive” miRNA *in vitro*[11]. Similarly, in cervical cancer cells high expression of miRNA-744 decreases the expression of B-cell lymphoma 2 (Bcl2) leading to reduced cellular proliferation *in vitro* and decreased tumor growth in mice[12]. Contrastingly, miRNA-744 is an “oncogenic” miRNA in pancreatic cancer[13]. MiRNA-744 high expression is found to target important negative modulators of the Wnt/β-catenin signaling pathway and leads to an increase in the stem cell-like phenotype in pancreatic cancer cells[13].

The Cancer Genome Atlas (TCGA) is a large dataset with both epigenomic data and robust clinical data from more than one thousand breast cancer patient samples[14]. We hypothesize that with the statistical power and survival data of TCGA, we are able to determine whether expression of miRNAs: miRNA-18a, miRNA-205, and miRNA-744 are associated with an improved survival in breast cancer.

Materials/Methods

Acquiring miRNA-seq and clinical data from TCGA

Both the clinical data and the miRNA-Seq data were acquired from TCGA breast cancer cohort within the Genomic Data Common (GDC) data portal. 1052 of 1097 breast cancer samples logged in TCGA had both the survival data and miRNA-Seq data available for analysis. An Institutional Review Board was not required because all information within TCGA is publicly accessible and de-identified[15]. Samples were collected from patients with untreated breast cancer. Briefly, each frozen tumor sample was paired with normal breast tissue, and processed by the Biospecimen Core Resource[1617]. DNA and RNA extraction was performed using the DNA/RNA All prep kit (Qiagen). The RNA was processed and sent to Genome Characterization Centers (GSCs) and Genome Sequencing Centers (GSCs) where they were sequenced. These results were then sent to the TCGA Research network and

further analyzed, interpreted and made publically available.[16,1718] The breast cancer cohort within TCGA was last updated on 5/31/2016[24].

Survival Analysis

The clinical information from the breast cancer cohort of patients within TCGA was individually reviewed for overall survival (OS) event times. 1052/1097 patient samples, had both survival event data as well as miRNA expression data and were thus used for our analysis. For the patients without an event (death), their censored survival time was calculated based on the last follow-up date.

Using a gene-specific threshold, the expression of each miRNA was classified as “high expression” when the expression levels of the miRNA were above this threshold and “low expression” if it did not meet the threshold. The gene specific thresholds were obtained as described by Ramanathan et al[15]. The Cox proportional hazard model was used to compare the OS in the “high” vs. the “low” expression groups. For the sub-analyses, we also looked at estrogen, progesterone and her2-neu receptor status (ER, PR and Her2), and the TNM stages according to the AJCC 7th edition.

Results

Patient Characteristics

The clinical and pathological characteristics were obtained from the breast cohort within TCGA and listed in Table 1. The majority of the patients were Caucasian (69.7%) and greater than 50 years old (72.9%) (Table 1). Additionally, 75% of the patients had stage I and II breast cancers, with only 1.9% of the patients presenting with stage IV breast cancer (Figure 1). ER was positive in 74%, PR was positive in 64%, and Her2 was positive in 33% of the patients (Table 1).

Selection of the microRNAs

By performing a literature search using Pubmed Central, we chose three microRNAs, miRNA-18a, miRNA-205, and miRNA-744, with conflicting or unknown roles in breast cancer. MiRNA-18a and miRNA-205 have been described as both tumor suppressive and oncogenic in breast cancer, as well as other cancers. MiRNA-744 has been found to be oncogenic in pancreatic cancer and tumor suppressive in breast cancer cells *in vitro*. We then used the clinical and epigenetic information within a large cohort of breast cancer patients logged in the Cancer Genome Atlas (TCGA) to validate whether high expression of these microRNAs was associated with an improved survival or worse survival.

Survival advantage of miRNA-18a was only statistically significant in ER positive, lymph node metastasis negative, distant metastasis-free patients

When evaluating the effect miRNA-18a expression had on survival, a survival advantage was seen in the group that overexpressed miRNA-18a; however, this was not statistically significant (Figure 2a: n-High = 970, n-low = 82; p=0.079). Interestingly, in the sub-analysis, an association with an improved survival with miRNA-18a was statistically significant when looking at ER positive cancers (Figure 2b: n-High = 717, n-low = 62;

p=0.039), as well as PR positive, or Her2 negative patients (data not shown). For Her2 positive and triple negative breast cancers (TNBC), there was an association with a worse survival when miRNA-18a was overexpressed; however, this was not statistically significant (Figure 2c: n-High = 321, n-low = 24; p=0.385)(Figure 2d: n-High = 103, n-low = 7; p=0.61). When the patients did not demonstrate any regional or distant metastases, the high expression of miRNA-18a was also associated with an improved survival (Figure 2e: n-High = 467, n-low = 36; p=0.053)(Figure 2g: n-High = 805, n-low = 72; p=0.023). For the patients with 1–3 positive lymph nodes, the two expression groups seemed to have similar survival patterns (Figure 2f: n-High = 318, n-low = 33, p=0.933), not statistically significant, and intriguingly, all of the patients with distant metastases were within the high expression group (Figure 2h: n-High = 20, n-low = 0, p=n/a). High expression of miRNA-18a seemed to be associated with an improved survival in ER/PR positive, her2 negative, early stage cancers.

High expression of miRNA-205 significantly associated with prolonged overall survival

For miRNA-205, its high expression was associated with a better survival when all patients were analyzed (Figure 3a: n-High = 422, n-low = 630, p=0.034). Sub-analysis revealed that the high expression of miRNA-205 in ER positive patients was associated with an improved survival (Figure 3b: n-High = 312, n-low = 467, p=0.036.) In fact, a positive association with survival and miRNA-205 expression was also seen in Her2 positive and TNBC patients (not statistically significant) (Figure 3c: n-High = 131, n-low = 214, p=0.015) (Figure 3d: n-High = 48, n-low = 62, p=0.286). Similarly to miRNA-18a, in N0 and M0 disease, a positive association with survival was seen with miRNA-205 high expression, although only significant in N0 disease (Figure 3e: n-High = 210, n-low = 293, p=0.017), (Figure 3g: n-High = 366, n-low = 511, p=0.077). There was no association with survival in lymph node positive cancers (Figure 3f: n-High = 139, n-low = 212, p=0.65), and appeared to have a worse survival in metastatic cancers, again not statistically significant (Figure 3h: n-High = 3, n-low = 17, p=0.237).

High expression of miRNA-744 significantly associated with prolonged overall survival

Lastly, high expression of miRNA-744 was found to have an improved survival in breast cancer (Figure 4a: n-High = 318, n-low = 734, p=0.014). This improved survival was seen in ER positive patients (Figure 4b: n-High = 237, n-low = 542, p=0.048), but not Her2 positive patients (Figure 4c: n-High = 118, n-low = 227, p=0.734). There did appear to be a better survival in TNBC when miRNA-744 was expressed high, but our results were not significant (Figure 4d: n-High = 32, n-low = 78, p=0.248). Again, we found a survival advantage when miRNA-744 was overexpressed in cancers that were N0 and M0 (Figure 4e: n-High = 151, n-low = 352, p=0.0139) (Figure 4g: n-High = 101, n-low = 250, p=0.66). In lymph node positive cancers and cancers with metastases, there was no clear association with survival when miRNA-744 was expressed high.

Discussion

By using TCGA, we found that high expression of miRNA-18a and miRNA-205 in breast cancer was associated with an improved survival, especially in earlier stage breast cancers,

and validated a role for miRNA-744 in breast cancer as tumor suppressive in this cohort of 1052 patients.

Many target genes of miRNA-18a have been described including hypoxia-inducible factor 1-α (HIF1-α), homeobox protein A9 (HOXA9) and estrogen receptor α (ER-α)[5619]. MiRNA-18a has been found to suppress these targets in response to different environmental stimuli, which may or may not favor cancer. For example, when cells were exposed to hypoxic and acidotic conditions, ectopic miRNA-18a expression was found to decrease the viability of the cells by suppressing the expression of HIF1α[5]. On the other hand, extracellular matrix stiffness was found to induce miRNA-18a expression leading to suppression of HOXA9 which then led to the suppression of PTEN and enhanced activity of phosphoinositide 3-kinase (Pi3K) *in vitro* leading to a more malignant phenotype in breast cancer cells lines[6]. Additional targets of miRNA-18a include ERα; however, the clinical significance of this interaction in breast cancer is not clear[45]. Guo et al. found that miRNA-18a was underexpressed in ER negative breast cancer tissues, but Yoshimoto et al. found the opposite result[19]. Although our results showed that high expression of miRNA-18a was associated with an improved survival in ER/PR positive,, Her2 negative and earlier stage breast cancers, the exact mechanism for this association is unclear. In order to clarify the role of miRNA-18a in breast cancer patients, further studies looking at the expression patterns of miRNA-18a and its target genes need to be performed.

MiRNA-18a down-regulation was correlated with an improved survival, implying an oncogenic role.[420] Although we do not have any data to explain why our results are different from their observation, one possibility may be a result of different biology of breast cancers that originate from different ethnic backgrounds[21].

MiRNA-205 has been implicated in the progression of early stage cancers. Gregory *et al.* found that *in vitro,* when miRNA-205 was downregulated, TGF-β was able to induce the epithelial to mesenchymal transition (EMT) phenotype, thus changing a cells ability to invade and metastasize, indicating a tumor suppressive role of miRNA-205. Our association with survival in the miRNA-205 high expression group in early stage cancers is in agreement with the notion that miRNA-205 suppresses tumor progression by preventing EMT in breast cancer[22].

We found that high expression of miR-744 was also associated with an improved survival especially in the earlier stages of breast cancer. This is in agreement with what Vislovukh *et al.* found in breast cancer cell lines, as the *in vitro* experiments revealed a decrease in the proliferation of breast cancer cells when miRNA-744 was overexpressed.[11] Similarly miRNA-744 was expressed low in cervical cancer cells. Animal models of miRNA-744 high expression revealed a decrease in the size of the tumors, and *in* vitro experiments showed impaired proliferation of cervical cancers. On the other hand, miRNA-744 appeared to demonstrate an oncogenic phenotype in pancreatic cancer, where its high expression was associated with increased recurrences, lymph node metastasis and poor survival[1323]. We do not have a good explanation for this conflicting result other than the fact that miRNA-744 may function differently in different cancers. Further studies are warranted to elucidate the specific roles of miRNA-744 in each cancer and how they develop the differences.

There are several limitations to this study. The survival advantage seen amongst all three miRNAs-18a, 205, and 744 in early stage ER positive breast cancers was an unexpected finding and may reflect an unknown biology present within early stage ER positive breast cancers causing overexpression of these miRNAs. Further studies within different breast cancer cohorts are warranted. We have only explored one of the databases collecting both genomic and clinical data to validate the roles of the miRNAs in breast cancer. In addition, when performing our sub-analyses, some of the subgroups studied did not have sufficient sample size and thus our results for these groups may not reach statistical significance simply due to less power, especially for the TNBC cohort and patients with metastatic disease. Despite these limitations, we have found that TCGA, as one validation cohort, may improve our knowledge of how different miRNAs are functioning in different cancers.

Conclusion

We used TCGA to verify the roles of miRNA-18a, miRNA-205 and miRNA-744 as tumor suppressive microRNAs in earlier stage breast cancers as their high expression was associated with improved survival. We chose to look at only three different miRNAs with unclear roles in breast cancer; however, as more information regarding the function of varying miRNAs becomes available, we can continue to use the data within TCGA to determine the clinical significance of many different miRNAs. Furthermore, as the roles of miRNAs are more clearly defined as tumor suppressive or oncogenic in breast cancer, we may be able to identify a specific miRNA expression pattern that can be used as a prognostic indicator for survival.

References

1. Piletic K, Kunej T. MicroRNA epigenetic signatures in human disease. Archives of toxicology. Oct; 2016 90(10):2405–2419. [PubMed: 27557899]
2. Bertoli G, Cava C, Castiglioni I. MicroRNAs: New Biomarkers for Diagnosis, Prognosis, Therapy Prediction and Therapeutic Tools for Breast Cancer. Theranostics. 2015; 5(10):1122–1143. [PubMed: 26199650]
3. Guo X, Yang C, Qian X, et al. Estrogen receptor alpha regulates ATM Expression through miRNAs in breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. Sep 15; 2013 19(18):4994–5002. [PubMed: 23857602]
4. Yoshimoto N, Toyama T, Takahashi S, et al. Distinct expressions of microRNAs that directly target estrogen receptor alpha in human breast cancer. Breast cancer research and treatment. Nov; 2011 130(1):331–339. [PubMed: 21755340]
5. Krutilina R, Sun W, Sethuraman A, et al. MicroRNA-18a inhibits hypoxia-inducible factor 1alpha activity and lung metastasis in basal breast cancers. Breast cancer research : BCR. Jul 28.2014 16(4):R78. [PubMed: 25069832]
6. Mouw JK, Yui Y, Damiano L, et al. Tissue mechanics modulate microRNA-dependent PTEN expression to regulate malignant progression. Nature medicine. Apr; 2014 20(4):360–367.
7. Wu H, Zhu S, Mo YY. Suppression of cell growth and invasion by miR-205 in breast cancer. Cell research. Apr; 2009 19(4):439–448. [PubMed: 19238171]
8. Lee JY, Park MK, Park JH, et al. Loss of the polycomb protein Mel-18 enhances the epithelial-mesenchymal transition by ZEB1 and ZEB2 expression through the downregulation of miR-205 in breast cancer. Oncogene. Mar 06; 2014 33(10):1325–1335. [PubMed: 23474752]
9. Gandellini P, Folini M, Longoni N, et al. miR-205 Exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase Cepsilon. Cancer research. Mar 15; 2009 69(6): 2287–2295. [PubMed: 19244118]

10. Zhang G, Hou X, Li Y, Zhao M. MiR-205 inhibits cell apoptosis by targeting phosphatase and tensin homolog deleted on chromosome ten in endometrial cancer Ishikawa cells. BMC cancer. Jun 14.2014 14:440. [PubMed: 24929707]
11. Vislovukh A, Kratassiouk G, Porto E, et al. Proto-oncogenic isoform A2 of eukaryotic translation elongation factor eEF1 is a target of miR-663 and miR-744. British Journal of Cancer. Jun 11; 2013 108(11):2304–2311. [PubMed: 23695020]
12. Chen XF, Liu Y. MicroRNA-744 inhibited cervical cancer growth and progression through apoptosis induction by regulating Bcl-2. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. Jul.2016 81:379–387. [PubMed: 27261616]
13. Zhou W, Li Y, Gou S, et al. MiR-744 increases tumorigenicity of pancreatic cancer by activating Wnt/beta-catenin pathway. Oncotarget. Nov 10; 2015 6(35):37557–37569. [PubMed: 26485754]
14. Chin L, Andersen JN, Futreal PA. Cancer genomics: from discovery science to personalized medicine. Nature medicine. Mar; 2011 17(3):297–303.
15. Ramanathan R, Olex AL, Dozmorov M, Bear HD, Fernandez LJ, Takabe K. Angiopoietin pathway gene expression associated with poor breast cancer survival. Breast cancer research and treatment. Feb; 2017 162(1):191–198. [PubMed: 28062977]
16. Comprehensive molecular portraits of human breast tumours. Nature. Oct 04; 2012 490(7418):61–70. [PubMed: 23000897]
17. Weinstein JN, Collisson EA, Mills GB, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nature genetics. Oct; 2013 45(10):1113–1120. [PubMed: 24071849]
18. Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemporary oncology (Poznan, Poland). 2015; 19(1a):A68–77.
19. Liu WH, Yeh SH, Lu CC, et al. MicroRNA-18a prevents estrogen receptor-alpha expression, promoting proliferation of hepatocellular carcinoma cells. Gastroenterology. Feb; 2009 136(2): 683–693. [PubMed: 19027010]
20. Shidfar A, Costa FF, Scholtens D, et al. Expression of miR-18a and miR-210 in Normal Breast Tissue as Candidate Biomarkers of Breast Cancer Risk. Cancer prevention research (Philadelphia Pa.). Jan; 2017 10(1):89–97.
21. Tsuchida J, Nagahashi M, Rashid OM, Takabe K, Wakai T. At what age should screening mammography be recommended for Asian women? Cancer medicine. Jul; 2015 4(7):1136–1144. [PubMed: 25914223]
22. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nature cell biology. May; 2008 10(5):593–601. [PubMed: 18376396]
23. Miyamae M, Komatsu S, Ichikawa D, et al. Plasma microRNA profiles: identification of miR-744 as a novel diagnostic and prognostic biomarker in pancreatic cancer. British Journal of Cancer. Nov 17; 2015 113(10):1467–1476. [PubMed: 26505678]
24. [Accessed June 5, 2017] TCGA Data Portal. Available at: https://tcga-data.nci.nih.gov/docs/publications/tcga/?

Synopsis

The Cancer Genome Atlas was used as a validation cohort correlating expression patterns of microRNA-18a (miRNA), miRNA-205 and miRNA-744 with survival in breast cancer. High expression of “tumor suppressive miRNAs” miRNA-18a and miRNA-205, and miRNA-744 were associated with improved survival.

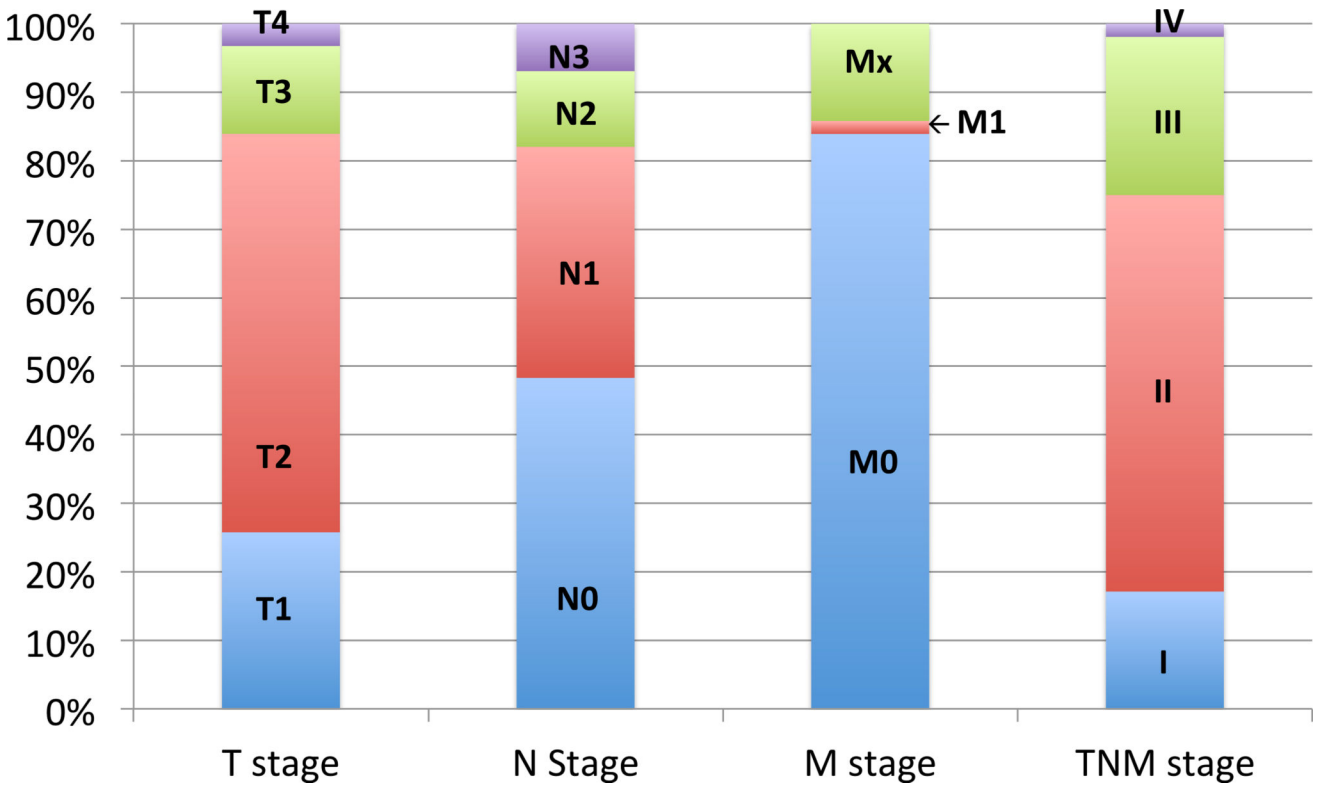

Figure 1. Distribution of TNM stages

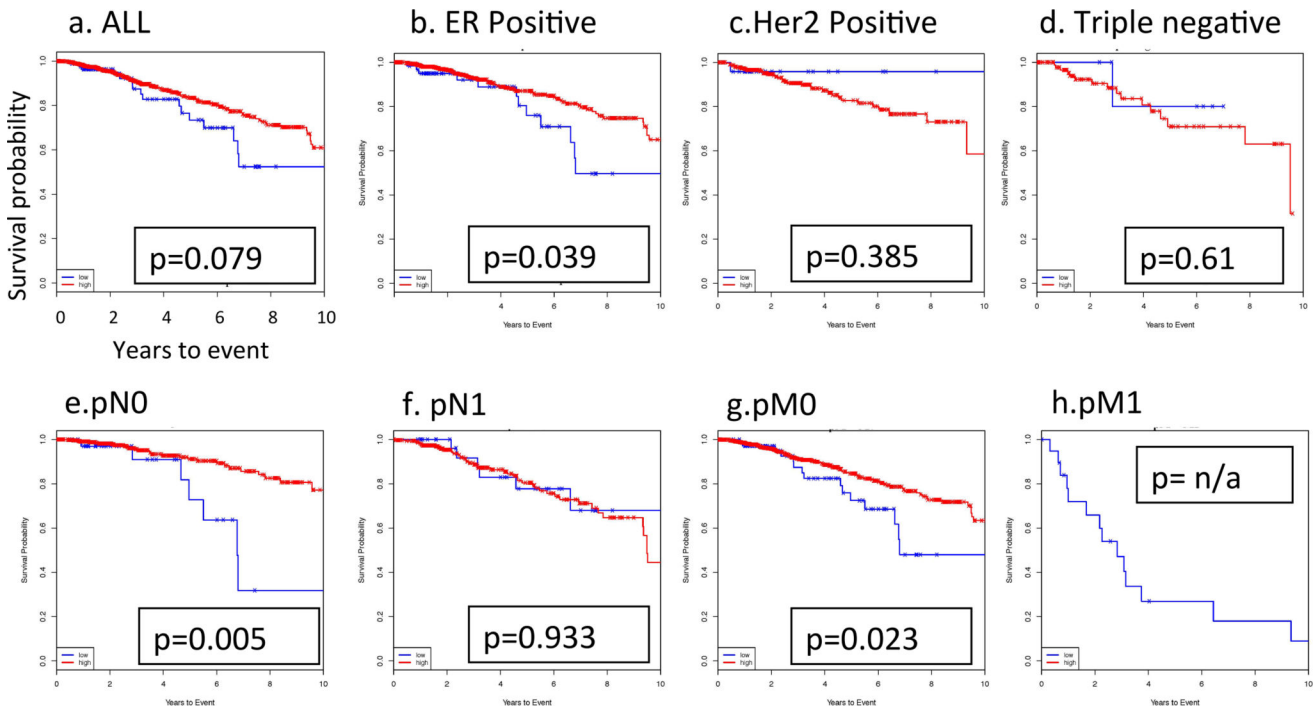

Figure 2.

Survival probability for high expression (red) vs. low expression (blue) of miRNA18a in breast cancer samples retrieved from TCGA.

a. Analysis involving all patients; n-High = 970, n-low = 82.

b. Sub-analysis involving ER positive patients; n-High = 717, n-low = 62.

c. Sub-analysis involving Her2-neu positive patients; n-High = 321, n-low = 24.

d. Sub-analysis involving triple negative breast cancer patients; n-High = 103, n-low = 7.

e. Sub-analysis involving lymph node negative patients; n-High = 467, n-low = 36.

f. Sub-analysis involving patients with 1–3 positive lymph nodes; n-High = 318, n-low = 33.

g. Sub-analysis involving patients without distant metastasis; n-High = 805, n-low = 72.

h. Sub-analysis involving patients with distant metastasis; n-High = 20, n-low = 0.

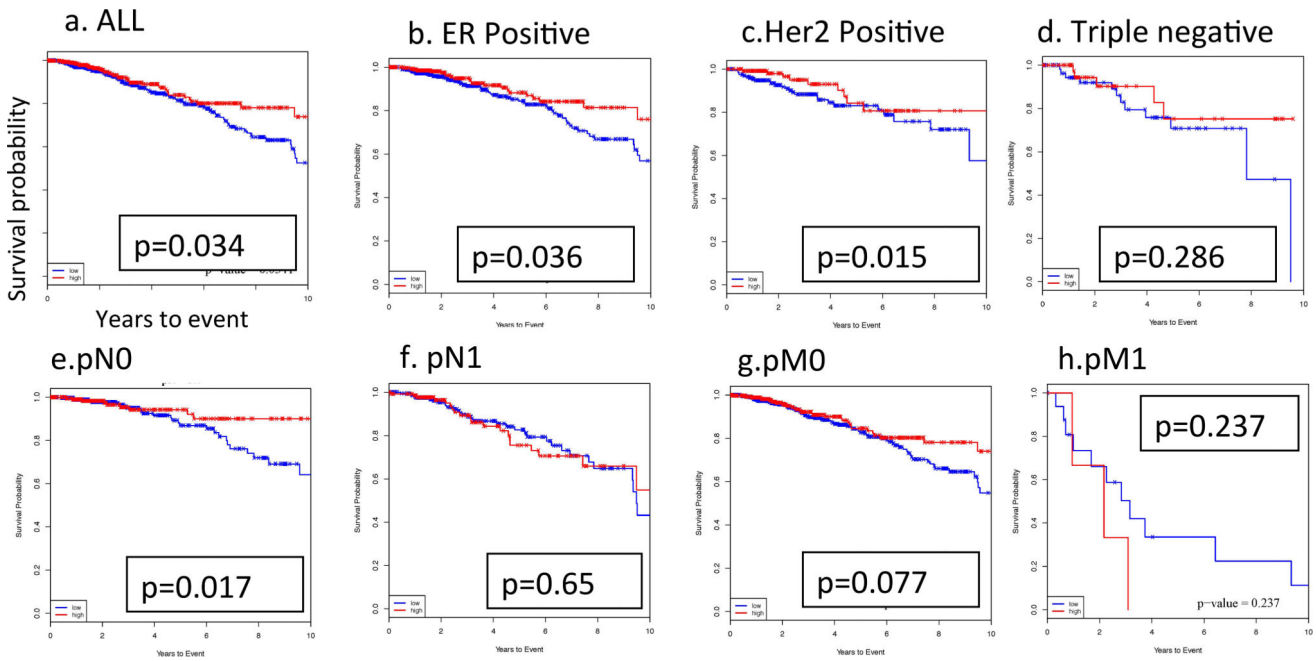

Figure 3.

Survival probability for high expression (red) vs. low expression (blue) of miRNA205 in breast cancer samples retrieved from TCGA.

a. Analysis involving all patients; n-High = 422, n-low = 630.

b. Sub-analysis involving ER positive patients; n-High = 312, n-low = 467.

c. Sub-analysis involving Her2-neu positive patients; n-High = 131, n-low = 214.

d. Sub-analysis involving triple negative breast cancer patients; n-High = 48, n-low = 62.

e. Sub-analysis involving lymph node negative patients; n-High = 210, n-low = 293.

f. Sub-analysis involving patients with 1–3 positive lymph nodes; n-High = 139, n-low = 212.

g. Sub-analysis involving patients without distant metastasis; n-High = 366, n-low = 511.

h. Sub-analysis involving patients with distant metastasis; n-High = 3, n-low = 17.

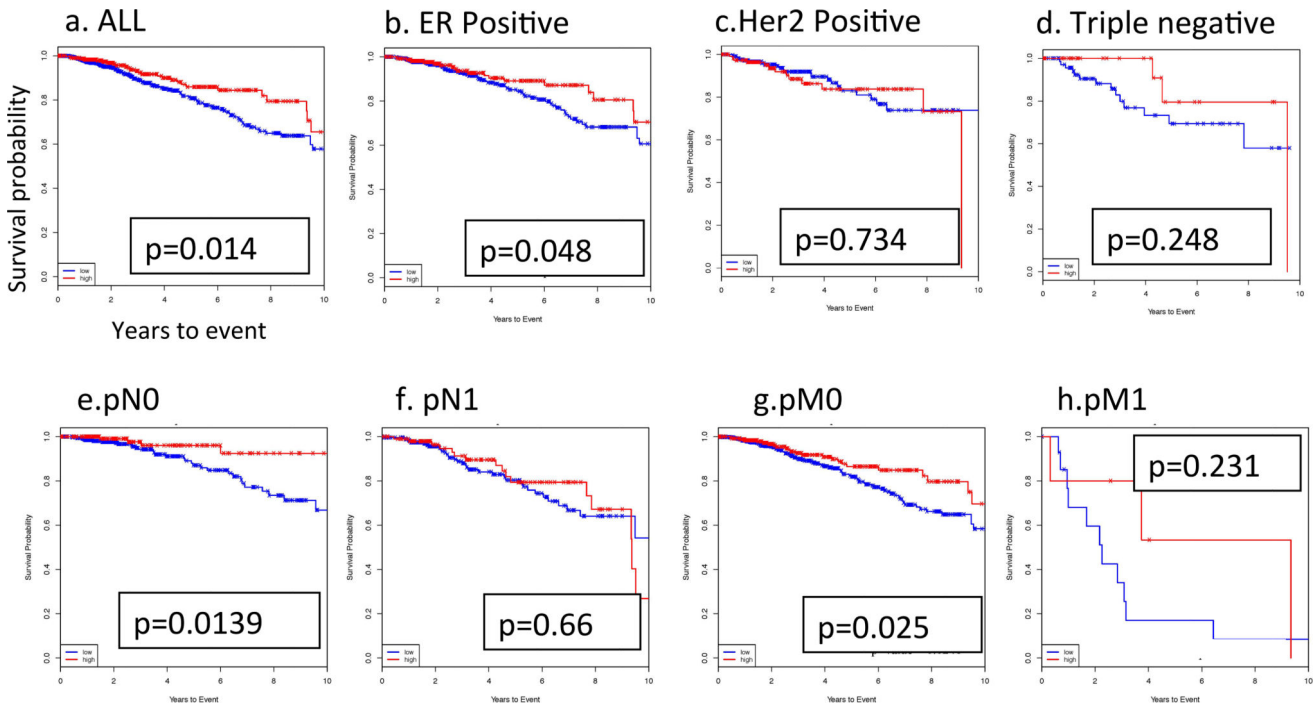

Figure 4.

Survival probability for high expression (red) vs. low expression (blue) of miRNA744 in breast cancer samples retrieved from TCGA.

a. Analysis involving all patients; n-High = 318, n-low = 734.

b. Sub-analysis involving ER positive patients; n-High = 237, n-low = 542.

c. Sub-analysis involving Her2-neu positive patients; n-High = 118, n-low = 227.

d. Sub-analysis involving triple negative breast cancer patients; n-High = 32, n-low = 78.

e. Sub-analysis involving lymph node negative patients; n-High = 151, n-low = 352.

f. Sub-analysis involving patients with 1–3 positive lymph nodes; n-High = 101, n-low = 250.

g. Sub-analysis involving patients without distant metastasis; n-High = 270, n-low = 607.

h. Sub-analysis involving patients with distant metastasis; n-High = 5, n-low = 15.

Table 1

Clinical and pathological characteristics of patients in the breast cohort within TCGA

Age	Number of pts (%)
Less than 40	73 (6.9%)
40–49	212 (20.2%)
50–59	272 (25.9%)
60–69	275 (26.1%)
70 or greater	220 (20.9%)
Ethnicity	
Caucasian	733 (69.7%)
Black or African American	175 (16.6%)
Asian	61 (5.8%)
Native American or Alaskan	1 (0.1%)
n/a	82 (7.8%)

Receptor	Positive	Negative	Not available
Estrogen Receptor	N=779 (74.05%)	N=227 (21.58%)	N=46 (4.37%)
Progesterone Receptor	N=676 (64.26%)	N=329 (31.27%)	N=47 (4.47%)
Her2-neu Receptor	N=345 (32.79%)	N=543 (51.62%)	N=164 (15.6%)