RMJ Ramathibodi Medical Journal

Rama Med J | Original Article

MicroRNA Expression of Primary and Metastatic Colorectal and Breast Carcinoma

Thaniya Sricharunrat[1], Artit Jinawath[2], Pattana Sornmayura[2], Sansanee Wongwaisayawan[3], Budsaba Rerkamnuaychoke[2]

[1] Pathology and Forensic Science Department, Chulabhorn Hospital, Chulabhorn Royal Academy, Bangkok, Thailand

[2] Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[3] Department of Medical Science, Faculty of Science, Rangsit University, Pathum Thani, Thailand

Background: Colorectal and breast carcinoma are frequently diagnosed cancers. At advanced stages, cancers metastasize to certain organs resulting in loss of function of these organs, and eventually death. Therefore, there is a specific need for the prognosis of these cancers. Currently, microRNAs (miRNAs), have emerged as a new target of cancer-specific biomarker.

Objective: To examine the expression of miRNA in primary and metastatic breast and colorectal cancers.

Methods: This study investigated the expression of 6 miRNAs (miR-10b, miR-21, miR-145, miR-155, miR-200c, and miR-373) in formalin fixed paraffin embed tissues from pairs of normal tissues with primary and metastatic tumor samples of breast and colorectal carcinoma cases in Ramathibodi Hospital, Thailand by real-time RT-PCR.

Results: Among 6 miRNAs, miR-145 decreased in all samples of primary and metastatic colorectal and breast carcinoma. There was significantly decreased expression of miR-145 in metastatic colorectal carcinoma compared to their primary colorectal carcinoma ($P < .05$). Whereas miR-10b, miR-155, and miR-200c showed a decreased expression; miR-21, and miR-373 showed an increased expression in the majority of cases. Unlike miR-145, other miRNAs showed no significant difference of expression ($P > .05$).

Conclusions: This finding indicates that miR-145 may be the potential metastatic biomarker. Decrease of miR-145 could be applied to the prognosis and target for therapy of breast and colorectal carcinoma.

Keywords: MicroRNA, Breast cancer, Colorectal carcinoma, Metastasis

Rama Med J: doi:10.33165/rmj.2022.45.3.257343

Received: April 15, 2022 **Revised:** August 29, 2022 **Accepted:** September 16, 2022

Corresponding Author:
Budsaba Rerkamnuaychoke
Department of Pathology,
Faculty of Medicine
Ramathibodi Hospital,
Mahidol University,
270 Rama VI Road,
Ratchathewi, Thung Phaya Thai,
Bangkok 10400, Thailand.
Telephone: +66 2201 1369
Fax: +66 2201 1267
E-mail: budsaba.rer@mahidol.ac.th

Introduction

The term cancer refers to a large group of diseases that cause malignant growth or tumor resulting from an uncontrollable cell division. It can occur in almost any organ or tissue of the body. It generally results in partial, or complete dysfunction of the involved organ, and in many cases, death. Cancer is among the leading causes of death worldwide, approximately 10 million deaths in 2020.[1] Worldwide, breast cancer is the most frequent invasive cancer and the fifth leading cause of cancer deaths among women. While colorectal cancer is the second most common in women and the third most common in men worldwide. In certain circumstances, cancers can metastasize and most frequently cancer can spread to the liver and lungs. This tendency may explain the poor prognosis. In addition, metastases are associated with the cause of death in most cancer patients. Therefore, detection of metastatic biomarkers can assist doctors with monitoring and treatment of cancers more effectively.

Interestingly, microRNAs (miRNAs) are now becoming interesting candidates for cancer diagnosis, prognosis and therapy. The miRNAs are endogenous, short noncoding RNA of 18 to 25 nucleotides that do not encode proteins, but regulates gene expression by targeting messenger RNAs (mRNAs).[2] The miRNAs can bind complementarily with the 3′ untranslated region (3′UTR) of targeted mRNA transcripts to stimulate the degradation of mRNAs or prevent the translation of mRNAs, thereby controlling the expression of at the post-transcriptional level. They are linked with several cellular process including cell cycle, growth, and apoptosis. The miRNAs are predictable to regulate human gene expression of 30% to 90% by combining to their target mRNAs.[3] Widespread dysregulation of miRNAs reflected the hallmarks of cancer, including breast and colorectal carcinoma. However, metastatic miRNA of these diseases is still not well-defined.

Among cancer studies, the formalin-fixed, paraffin-embedded (FFPE) tissue samples derived from cancer patients are extremely valuable source of tissue to investigate the diseases. There is evidence to indicate that miRNA expression profile from FFPE tissues faithfully resemble that from fresh tissue,[4] therefore, FFPE tissues can be used as appropriate resources for analyzing the expression of miRNA.

In the present study, we chose FFPE tissues from pairs of primary and metastatic breast and colorectal carcinoma cases at Ramathibodi Hospital, Thailand to investigate the expression of 6 miRNAs (miR-10b, miR-21, miR-145, miR-155, miR-200c and miR-373) using real-time, reverse transcription-polymerase chain reaction (RT-PCR). Based on a literature inspection, we selected 6 miRNA candidates for investigation: (i) miR-10b: known to be involved in cancer progression of several types of cancers, including breast and colon,[5] (ii) miR-21: a classical and best-described oncomiR that is commonly upregulated in a wide range of cancers,[6] (iii) miR-155: a suggested oncomiR that is upregulated in a variety of carcinomas,[6] (iv) miR-145: known to be a tumor suppressor and downregulated in melanoma cells with high metastatic potential,[7] (v) miR-200c: a member of miRNA-200 family which functions as tumor suppressor in most of human carcinoma, including colorectal and breast cancer,[8] and (vi) miR-373: whose either downregulated or upregulated, and involved in tumorigenesis of numerous types of tumors.[9] Our findings highlight the potential metastatic miRNA that may be profitable to precise prognosis of breast and colorectal cancer.

Methods

Case Collection, Database Search, and Inclusion Criteria

We selected FFPE tissue samples from routine surgical pathology service at Department of Pathology, Ramathibodi Hospital. Samples collected ranged from 2003 to 2008. The inclusion criteria were cases that have resected, wide excision or biopsy of metastatic organ that has diameter more than 0.5 cm, with typical histology of carcinoma from the breast or colon primary and no controversy in pathological diagnosis. There were 8 cases of colorectal carcinoma (4 cases with liver metastasis,

3 cases with lung metastasis, and 1 case with both liver and lung metastasis) and 4 cases of breast carcinoma (2 cases with liver metastasis, 1 case with lung metastasis, and 1 case with lung and cerebellar metastasis) (Table 1).

All of each case had primary tumor, normal tissue from primary organ and metastatic tumor (in liver and/or lung or cerebellar tissue). Paraffin blocks of tumor in which the tumor area of 75% or more was chosen or if connected with significant amount of normal tissue, it was trimmed manually to obtain as much as possible of tumor.

Ethics

This study has been reviewed and approved by the Committee on Human Rights Related to Researches Involving in Human Subjects Faculty of Medicine, Ramathibodi Hospital, Mahidol University (No. MURA 2009/1231 on February 19, 2009).

Total RNA Extraction and Poly-(A) Polymerase Reaction

The paraffin block was sectioned with microtome to 20 µm thickness with 4 sections per block. The total RNA extraction was performed using RecoverAll Total Nucleic Acid Isolation Kit (Invitrogen) by following the recommended guidelines. The total RNA yield was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific). Then 4 µg of total RNA from each sample (with exception of some samples with less than 4 µg; overall of the obtained RNA were used) was modified by poly-(A) polymerase using *E. coli* Poly (A) polymerase (New England Biolabs). The poly (A) RNA was purified by performing phenol/chloroform extraction and ethanol precipitation. It was then dissolved with RNase free-water and the quantity measured using the Nanodrop 2000 spectrophotometer.

Reverse Transcription

The poly (A) RNA was used for the reverse transcription reaction using Superscript III reverse transcriptase (Invitrogen). The process was performed to the manufacturer's guidelines. Reaction was prepared in 20 µL, and incubated at 50 ºC for 60 minutes, followed by inactivation of enzyme at 70 ºC for 15 minutes. The reverse primer for reverse transcription was oligo dT adapter primer (5′-GCGAGCACAGAATTAATACGACT CACTATAGGTTTTTTTTTTTTTTTTVN-3′).

Table 1. Characteristic of Cases with Summary of Clinical Information

Case No.	Organ of Primary Tumor	Diagnosis (Type, Differentiation)	Metastatic Organ	Metastasectomy Before or After Chemotherapy
B1	Breast	Invasive ductal carcinoma, grade II	Liver	After
B2	Breast	Invasive ductal carcinoma, grade III	Liver	After
B3	Breast	Invasive ductal carcinoma, grade II	Lung	After
B4	Breast	Invasive ductal carcinoma, grade II	Lung, cerebellum	After
C1	Sigmoid colon	Adenocarcinoma, well differentiated	Lung	Before
C2	Right side colon	Adenocarcinoma, well differentiated	Lung	Before
C4	Sigmoid colon	Adenocarcinoma, moderately differentiated	Lung	After
C5	Rectum	Adenocarcinoma, moderately differentiated	Liver, lung	After
C6	Sigmoid colon	Adenocarcinoma, well differentiated	Liver	After
C7	Descending colon	Adenocarcinoma, well differentiated	Liver	After
C8	Middle rectum	Adenocarcinoma, moderately differentiated	Liver	Before
C9	Rectum	Adenocarcinoma, moderately differentiated	Liver	Before

Real-Time PCR Reaction

The cDNA was diluted according to the poly (A) RNA for 5 ng per each reaction in real-time PCR reaction of total 20 µL, using SsoFast EvaGreen Supermix with low ROX (Bio-Rad Laboratories). The reaction was assembled according to the product manufacturer recommendation.

The F primer was chosen from sequence of mature miRNA (miR-10b, miR-21, miR-145, miR-155, miR-200c, and miR 373) from database of the Sanger (www.sanger.ac.uk) with omitted 1 or 2 bases at the 3′ sequence. The common R primer was used as described elsewhere.[10] Sequences of F primers were listed (Table 2). The final concentration of F or R primer in the reaction is 500 nM.

The real-time PCR was performed using the 7500 Fast real-time PCR system (Applied Biosystems). The sample was initially heated at 95 °C for 2 minutes followed by 35 cycles at 95 °C for 15 seconds and then 61 °C for 1 minute. Following this, the melting curve analysis was done. RNU6B was used as endogenous control gene in each sample. Each sample in duplicate was examined for real-time PCR reaction.

The relative expression was calculated using ddCt method using the matched normal tissue (breast or colon) from each case for normal control. The data was calculated by the software accompanied with the machine.

Table 2. Primers Used in This Study

Primer Name	5′-3′ Primer Sequence
R primer	GCGAGCACAGAATTAATACGA
F primers	
RNU6B	CACGCAAATTCGTGAAGCGTT
miR-10b	TACCCTGTAGAACCGAATTTG
miR-21	TAGCTTATCAGACTGATGTTG
miR-145	GTCCAGTTTTCCCAGGAATC
miR-155	TTAATGCTAATCGTGATAGGGGT
miR-200c	TAATACTGCCGGGTAATGATG
miR-373	GAAGTGCTTCGATTTTGGGGTG

Statistical Analysis

All experiments were conducted in triplicate at a minimum, expressed as mean and standard deviation (SD), and statistical analyses of independent experiments were performed by a paired *t* test, using SPSS statistical software version 24.0 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp; 2016). Results were considered significant at *P* value less than .05 ($P < .05$).

Results

To study the expression of miRNAs, total RNA was extracted from the paraffin block tissues of primary and metastatic breast and colorectal carcinoma from confirmed cases at Ramathibodi Hospital. The specimens included 4 cases of breast carcinoma (2 liver metastasis, 1 lung metastasis, and 1 lung and brain metastasis), and 8 cases of colorectal carcinoma (4 liver metastasis, 3 lung metastasis, and 1 liver and lung metastasis). All cases of breast carcinoma and 4 cases of colorectal carcinoma received chemotherapy prior to resection of metastatic tumors.

RNA of miR-10b, miR-21, miR-145, miR-155, miR-200c, and miR-373 was analyzed using real-time RT-PCR. Relative quantification of cDNA was performed and the RNU6B was used as internal control gene. The relative expression level from each gene to RNU6B was then normalized with their normal tissue of primary organs (breast or colon) and then express as fold of expression relative to the normal tissue. Expression of all genes could be detected in the analyzed samples. Interestingly, the miR-145 showed a significant decrease of gene expression in all samples of both primary and metastatic breast and colorectal carcinoma (Figure 1 and 2). The mean (SD) relative expression levels of miR-145 were 0.09 (0.12) in primary breast cancer, 0.09 (0.07) in metastatic breast cancer, 0.12 (0.09) in primary colorectal cancer, and 0.04 (0.02) in metastatic colorectal cancer (Table 3). In addition, the average expression of miR-145 in metastatic colorectal carcinoma showed decreased expression compared to the primary tumor ($P = .03$) (Figure 2). In breast carcinoma, 2/4 sample of metastatic tumor had

decreased miR-145 expression as compared to the primary tumor (but the average expression did not show significant difference in both groups ($P = .46$) (Figure 1).

On the other hand, the difference in the overall expression of miR-10b, miR-21, miR-155, miR-200c, and miR-373 between the cancerous and control groups was not statistically significant ($P > .05$) (Figure 1 and 2). The relative expression levels of miR-10b was low in both primary and metastatic breast and colorectal carcinoma. The mean (SD) relative expression levels of miR-10b were 0.26 (0.32) in primary breast cancer, 0.10 (0.07) in metastatic breast cancer, 0.37 (0.29) in primary colorectal cancer, and 0.20 (0.13) in metastatic colorectal cancer (Table 3). The miR-10b had decreased expression in 4/4 (100%) and 8/8 (100%) samples of primary breast and colorectal carcinoma, with 4/4 (100%) and 7/7 (100%) samples of metastatic breast and colorectal carcinoma respectively. However, there was no significant difference of miR-10b expression between the primary and metastatic carcinoma tissues in both of breast and colorectal cancer cases (Figure 1 and 2).

For miR-155, the relative expression levels were low in primary and metastatic colorectal carcinoma, and metastatic breast carcinoma. The mean (SD) relative expression levels of miR-155 were 0.83 (0.86) in primary colorectal cancer, 0.65 (0.29) in metastatic colorectal cancer, 1.87 (2.70) in the primary breast cancer, and 0.70 (0.82) in the metastatic breast cancer (Table 3). The miR-155 had decreased expression in 4/6 (33.3%) and 3/4 (75%) samples of primary colorectal and breast carcinoma, with 5/7 (71.4%) and 3/4 (75%) samples of metastatic colorectal and breast carcinoma respectively. However, there was no significant difference of miR-155 expression between the primary and metastatic carcinoma tissues in both of breast and colorectal cancer cases (Figure 1 and 2).

Similar to miR-155, the relative expression levels of miR-200c were low in primary and metastatic colorectal carcinoma. The mean (SD) relative expression levels of miR-200c were 0.79 (0.30) in primary colorectal cancer and 0.62 (0.59) in metastatic colorectal cancer (Table 3). In breast carcinoma, the relative expression level of miR-200c was low in the primary cancer but not in the metastatic cancer. The mean (SD) relative expression levels of miR-200c were 0.50 (0.32) in primary breast cancer and 1.17 (1.07) in metastatic breast cancer (Table 3). The miR-200c had decreased expression in 4/4 (100%) and 5/8 (62.5%) samples of primary breast and colorectal carcinoma, with 3/4 (75%) and 6/8 (75%) samples of metastatic breast and colorectal carcinoma respectively. However, there was no significant difference of miR-200c expression between the primary and metastatic carcinoma tissues in both of breast and colorectal cancer cases (Figure 1 and 2).

For miR-21, the relative expression levels were high in both primary and metastatic breast carcinoma, and metastatic colorectal carcinoma. The mean (SD) relative expression levels of miR-21 were 2.13 (2.08) in primary breast cancer, 2.23 (2.32) in metastatic breast cancer, 0.98 (0.45) in primary colorectal cancer, and 1.20 (1.70) in the metastatic colorectal cancer (Table 3). The miR-21 had increased expression in 2/4 (50%) and 4/8 (50%) samples of primary breast and colorectal carcinoma, with 2/4 (50%) and 2/8 (25%) samples of metastatic breast and colorectal carcinoma respectively. However, there was no significant difference of miR-21 expression between the primary and metastatic carcinoma tissues in both of breast and colorectal cancer cases (Figure 1 and 2).

Lastly, the relative expression levels of miR-373 were high in primary and metastatic colorectal carcinoma, and metastatic breast carcinoma. The mean (SD) relative expression levels of miR-373 were 1.51 (0.89) in primary colorectal cancer, 1.18 (1.15) in metastatic colorectal cancer, 0.65 (0.46) in primary breast cancer, and 1.25 (1.66) in the metastatic breast cancer (Table 3). The miR-373 had increased expression in 5/7 (71.4%) and 1/4 (25%) samples of primary colorectal and breast carcinoma, with 3/7 (42.9%) and 1/4 (25%) samples of metastatic colorectal and breast carcinoma respectively. However, there was no significant difference of miR-373 expression between the primary and metastatic carcinoma tissues in both of breast and colorectal cancer cases (Figure 1 and 2).

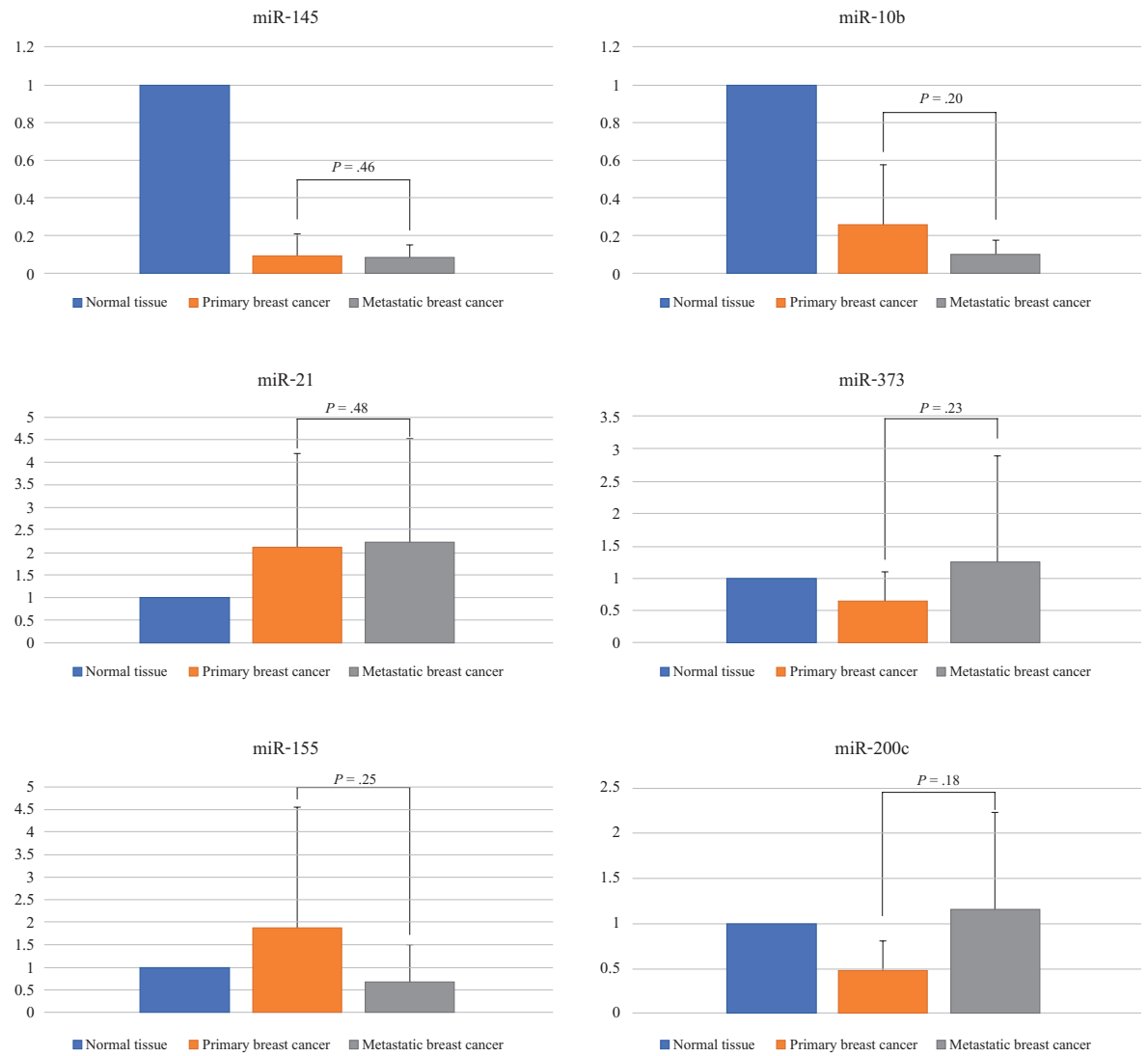
Table 3. Relative Expression Level (Fold Change) of miRNAs in Breast and Colorectal Carcinoma

Sample ID*	Relative Expression Level					
	miR-10b	miR-21	miR-145	miR-155	miR-200c	miR-373
Primary breast carcinoma						
B1-1	0.16	0.98	0.08	0.36	0.53	0.18
B2-1	0.04	1.80	0.02	0.88	0.22	0.34
B3-1	0.10	0.59	0.02	0.35	0.30	0.98
B4-1	0.73	5.16	0.26	5.91	0.93	1.11
Mean (SD)	0.26 (0.32)	2.13 (2.08)	0.09 (0.12)	1.87 (2.70)	0.50 (0.32)	0.65 (0.46)
Metastatic breast carcinoma						
B1-3	0.15	0.56	0.11	0.21	0.95	0.18
B2-3	0.01	1.97	0.01	0.23	0.65	0.37
B3-3	0.16	5.58	0.17	1.92	2.73	3.72
B4-5	0.10	0.81	0.05	0.46	0.36	0.73
Mean (SD)	0.10 (0.07)	2.23 (2.32)	0.09 (0.07)	0.70 (0.82)	1.17 (1.07)	1.25 (1.66)
Primary colorectal carcinoma						
C1-1	0.17	2.27	0.01	0.53	1.66	5.35
C2-1	0.47	1.59	0.10	1.02	0.67	0.99
C4-1	0.09	0.61	0.22	0.35	0.44	1.93
C5-1	0.23	0.94	0.21	NS	0.90	2.66
C6-1	0.11	0.50	0.02	0.13	0.93	1.18
C7-1	0.66	1.05	0.06	2.24	1.16	NS
C8-1	0.82	1.56	0.17	0.40	1.03	2.08
C9-1	0.18	0.61	0.03	NS	0.38	0.20
Mean (SD)	0.37 (0.29)	0.98 (0.45)	0.12 (0.09)	0.83 (0.86)	0.79 (0.30)	1.51 (0.89)
Metastatic colorectal carcinoma						
C1-3	NS	7.68	0.03	6.26	1.59	NS
C2-3	0.04	0.27	0.05	0.43	0.09	0.03
C4-3	0.26	0.90	0.06	0.87	0.36	2.34
C5-3	0.25	0.57	0.03	0.66	0.64	3.07
C6-3	0.12	0.75	0.02	0.39	0.39	0.28
C7-3	0.29	0.28	0.06	0.44	0.29	0.67
C8-3	0.39	5.03	0.08	1.09	1.88	1.47
C9-3	0.07	0.62	0.02	NS	0.72	0.43
Mean (SD)	0.20 (0.13)	1.20 (1.70)	0.04 (0.02)	0.65 (0.29)	0.62 (0.59)	1.18 (1.15)

Abbreviations: NS, no signal; SD, standard deviation

* The letter B is from patients with primary breast carcinoma (4 cases; B1 to B4) and the letter C is from patients with primary colorectal carcinoma (8 cases; C1, C2, and C4 to C9). The sample ID ended with -1 is tissue of primary tumor, -3 is lung or liver tissue of the same case with metastatic carcinoma, and -5 is brain tissue (cerebellum) of the same case with metastatic carcinoma.

Figure 1. miRNA Expression of Primary Breast Cancer Tissues and Paired Metastatic Breast Cancer Tissues



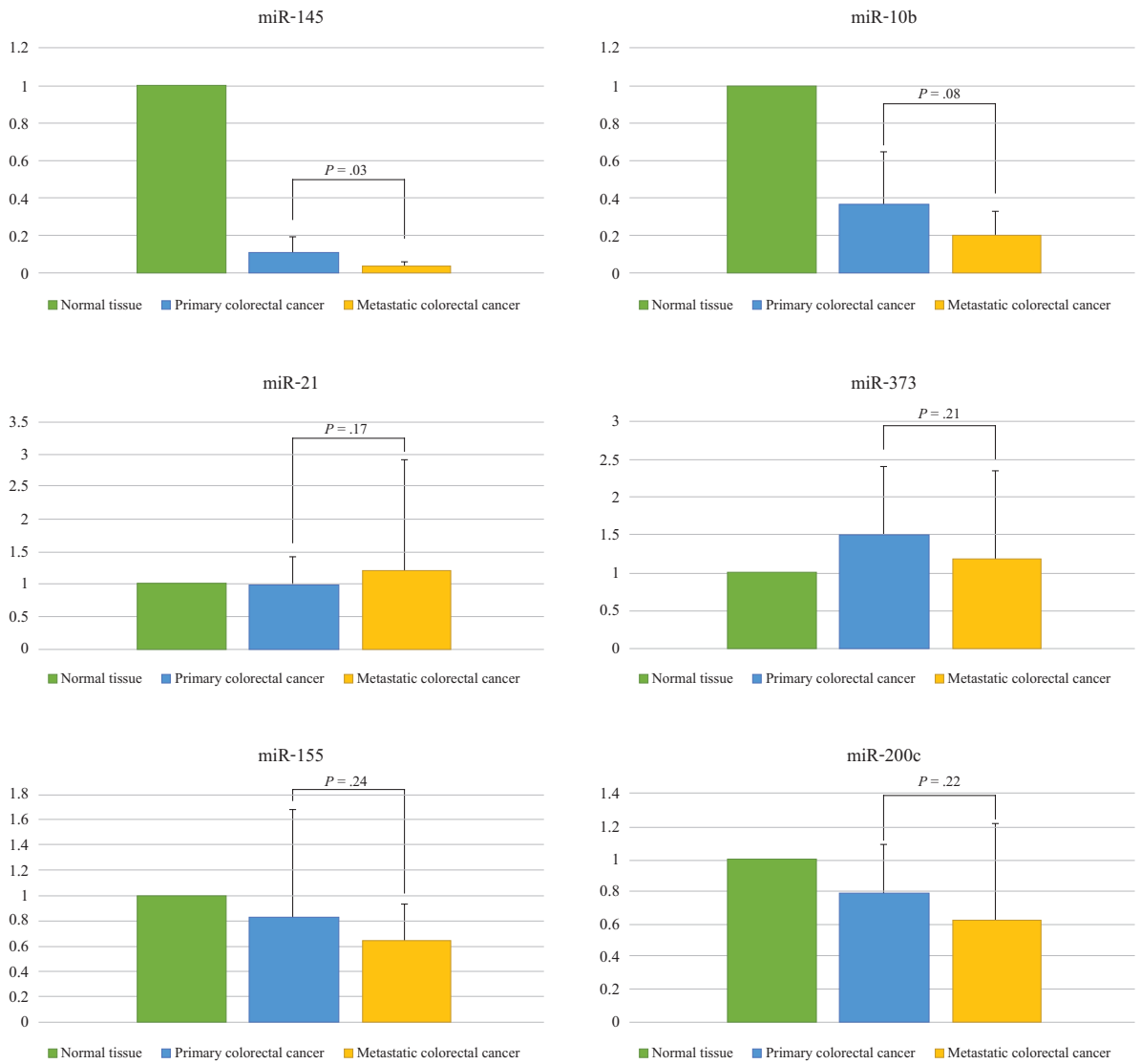
The fold change values indicate the relative change in the expression levels between samples and the normal tissues (n = 4), assuming that the value of the normal tissue of each sample was equal to 1. Each bar is the mean (SD) from experiments.

Discussion

Cancer is a complex group of diseases that has unlimited cell growth and the potentiality to metastasize to other organs of the body. Among cancer diseases occurring worldwide, breast and colorectal carcinoma are 2 of the most frequent causes of cancer-related death.

At present, the purpose of cancer research is attempting to identify and validate miRNA signatures to diagnose and monitor diseases. miRNAs are short, conserved noncoding RNAs that post-transcriptionally control the expression of targeted genes.[2] Alteration in miRNA expression has been shown to be associated with various types of cancers, including colorectal and breast cancers.[11, 12] However, studies conducted to determine the metastatic miRNAs in clinical samples in primary and metastatic breast and colorectal cancer are limited. In the current research, we studied the expression of 6 miRNAs (miR-10b, miR-21, miR-145, miR-155, miR-200c, and miR-373) in paraffin block tissues

Figure 2. miRNA Expression of Primary Colorectal Cancer Tissues and Paired Metastatic Colorectal Cancer Tissues



The fold change values indicate the relative change in the expression levels between samples and the normal tissues (n = 8), assuming that the value of the normal tissue of each sample was equal to 1. Each bar is the mean (SD) from experiments.

from primary and metastatic breast and colorectal carcinoma cases diagnosed at Ramathibodi Hospital. We observed that miR-145 was significantly decreased in all samples of primary and metastatic colorectal and breast carcinoma. Recent meta-analysis studies reported low expression of miR-145 was related with poor overall survival of patients with colorectal cancer, which would indicate that miR-145 plays an inhibitory role in the progression of colorectal and breast carcinoma.[13] In colorectal cancer cells, miR-145 regulates several proteins that are related to the metastasis of cancer such as TWIST1 and LASP1.[14-16] In agreement with several recently published literature, miRNA-145 is often downregulated in many cancers, thus regulating various oncogenes that associated with cellular activities, including the cell proliferation, growth, and apoptosis.[17]

The miR-10b has also been shown to have an involvement with the progression of various cancers, such as breast, pancreas, and central nervous system.[18] Several studies reported that miR-10b is remarkably

expressed in metastatic cancer cells and plays a vital role in metastasis of cancer.[19, 20] Moreover, it investigated the clinical significance of miR-10b in human colorectal cancer.[21] They suggested that miR-10b is a prognostic marker in colorectal cancer and considers resistance to the chemotherapeutic agent 5-fluorouracil in colorectal cancer cells. The increase of miR-10b expression in primary colorectal carcinoma tissue was associated with tumor size, vascular invasion, advanced stage disease, poorer differentiation, and risk of metastasis.[22] It was found that miR-10b displayed decreased expression in liver metastatic tissue as compared to primary colorectal carcinoma tissue.[23] Our study of miR-10b showed a trend of decreased expression in metastatic colorectal carcinoma in most samples as normalized with normal colorectal tissue. Comparison of the expression levels between metastatic and primary colorectal carcinoma showed decreased expression in 4 out of 8 samples, but did not show significant difference (P = .08). It would therefore be interesting to further discover the cause of down expression for miR-10b in these cancers.

Previous studies in breast and colorectal cancer revealed that upregulation of miR-21 is a consistent occurrence and is related to a poor prognosis.[24] Another report observed the overexpression of circulating miR-21 in nasopharyngeal carcinoma and proposed that this may be an event leading to progression of cancer.[25] In the present study, miR-21 was upregulated in 2/4 (50%) and 2/4 (50%) samples of primary and metastatic breast carcinoma, and in 3/8 (37.5%) and 2/8 (25%) cases of primary and metastatic colorectal carcinoma. However, the overall fold change ratio was not significantly diverse when compared to controls.

Similar to miR-21, many studies showed the relative expression of miR-155 was increased in breast cancer and colorectal cancer with high levels of miR-155 related to clinicopathologic markers, tumor subtype, and poor survival rates.[6, 26] However, our observation of miR-155 found that 1/4 (25%) case of primary breast cancer and 1/4 (25%) case of metastatic breast cancer exhibited miR-155 upregulation. Also, 1/8 (12.5%) cases of primary and metastatic colorectal cancer displayed miR-155 upregulation. Further investigation of miRNA-21 and miRNA-155 using a larger sample size would be required to confirm the consistency of our findings.

The expression pattern and function of the miR-200 family has been extensively explored in numerous cancers.[8] This family harbors the tumor-suppressive functions in a wide variety of cancers, including breast and colorectal cancer. It comprises of 5 members, which are miR-200a, miR-200b, miR-200c, miR-429, and miR-141.[27] Among these, the downregulation of miR-200c has been found to be linked with breast and colorectal cancer.[8, 28] In our present study, the expression of miR-200c was suppressed in 3/4 (75%) cases of primary breast cancer, 2/4 (50%) cases of metastatic breast cancer, 3/8 (37.5%) cases of primary colorectal cancer and 5/8 (62.5%) cases of metastatic colorectal cancer. We suggest that further investigation of miRNA-200c using a larger sample size would be beneficial.

The miR-373 was first described as a possible novel oncogene in testicular germ-cell tumors.[29] Subsequently, miR-373 has been validated in several cancers.[9] In HCT-15 colon cancer cells, miR-373 is extremely expressed, and this expression is linked with invasiveness.[30] Our results showed the upregulation of miR-373 in 4/8 (50%) primary and 2/8 (25%) metastatic colorectal cancer was found. In breast cancer, we found upregulation of miR-373 in 1/4 (25%) case of metastatic breast cancer. At present, the roles of miR-373 in breast carcinoma remain controversial. Based on findings from *in vitro* and *in vivo* studies, miR-373 can both induce and prevent metastasis of breast cancer cells, by working in a cell type-specific way. For human breast cancer cell line MCF-7, which is categorized by non-migratory and non-metastatic phenotype. In the case of overexpression of miR-373, this promote the migration and invasion of cancer. However, human breast cancer cell line MDA-MB-435, downregulation of miR-373 considerably inhibited the cell migration and invasion of cancer.[30]

Based on the above, we suggested that miR-373 expression may diverge relying on the origin or type of cancer.

Our data indicate that miR-145 may be the potential metastatic biomarker and treatment target for colorectal and breast cancer. In miR-10b, there is a trend of decreased expression, especially in metastatic colorectal and breast cancer. Therefore, a study with an increased sample size is suggested. Our investigation is restricted to speculation on the potential role of miR-21, miR-155, miR-200c, and miR-373 as such biomarkers. More investigations are warranted. Probably, organization of a panel of appropriate miRNAs is more rational and worthwhile. Furthermore, functional study and target examination may be essential for expounding the roles of miRNAs in the exact underlying mechanisms of the carcinogenesis in breast and colorectal cancer. In addition, from clinical sights, acceptable sensitivity and specificity of miRNAs should be further investigated in well-designed research. Therefore, the findings of this study provide a significance of miRNAs in breast and colorectal cancer, which may have implications for future clinical prognosis and treatment.

Conclusions

The comparison of miRNA expression between cancerous (primary and metastatic breast and colorectal cancer) and non-cancerous tissues revealed that miR-145 could be considered as a potential prognostic biomarker and treatment target for metastasis of colorectal and breast cancer.

Acknowledgments

This work was supported by a research grant (ID 02-52-20), fiscal year 2009, from the Faculty of Medicine Ramathibodi Hospital, Mahidol University.

We wish to thank Robert Dandy for his assistance with proofreading and editing this manuscript for clarity in English.

References

1. World Health Organization. Fact sheets: Cancer. World Health Organization; 2021. Update February 3, 2022. Accessed April 15, 2022. https://www.who.int/news-room/fact-sheets/detail/cancer
2. Heydarzadeh S, Ranjbar M, Karimi F, Seif F, Alivand MR. Overview of host miRNA properties and their association with epigenetics, long non-coding RNAs, and Xeno-infectious factors. *Cell Biosci.* 2021; 11(1):43. doi:10.1186/s13578-021-00552-1
3. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 2009;19(1):92-105. doi:10.1101/gr.082701.108
4. Liu A, Xu X. MicroRNA isolation from formalin-fixed, paraffin-embedded tissues. *Methods Mol Biol.* 2011;724:259-267. doi:10.1007/978-1-61779-055-3_16
5. Nishida N, Yamashita S, Mimori K, et al. MicroRNA-10b is a prognostic indicator in colorectal cancer and confers resistance to the chemotherapeutic agent 5-fluorouracil in colorectal cancer cells. *Ann Surg Oncol.* 2012;19(9):3065-3071. doi:10.1245/s10434-012-2246-1
6. Hunsaker M, Barba G, Kingsley K, Howard KM. Differential microRNA expression of miR-21 and miR-155 within oral cancer extracellular vesicles in response to melatonin. *Dent J (Basel).* 2019;7(2):48. doi:10.3390/dj7020048
7. Dynoodt P, Speeckaert R, De Wever O, et al. miR-145 overexpression suppresses the migration and invasion of metastatic melanoma cells. *Int J Oncol.* 2013;42(4):1443-1451. doi:10.3892/ijo.2013.1823
8. Bojmar L, Karlsson E, Ellegård S, et al. The role of microRNA-200 in progression of human colorectal and breast cancer. *PLoS One.* 2013;8(12):e84815. doi:10.1371/journal.pone.0084815

9. Wei F, Cao C, Xu X, Wang J. Diverse functions of miR-373 in cancer. *J Transl Med*. 2015;13:162. doi:10.1186/s12967-015-0523-z
10. Li S, Fu H, Wang Y, et al. MicroRNA-101 regulates expression of the v-fos FBJ murine osteosarcoma viral oncogene homolog (FOS) oncogene in human hepatocellular carcinoma. *Hepatology*. 2009;49(4):1194-1202. doi:10.1002/hep.22757
11. Li W, Chang J, Tong D, et al. Differential microRNA expression profiling in primary tumors and matched liver metastasis of patients with colorectal cancer. *Oncotarget*. 2017;8(22):35783-35791. doi:10.18632/oncotarget.16206
12. Tsai HP, Huang SF, Li CF, Chien HT, Chen SC. Differential microRNA expression in breast cancer with different onset age. *PLoS One*. 2018;13(1):e0191195. doi:10.1371/journal.pone.0191195
13. Li C, Yan G, Yin L, Liu T, Li C, Wang L. Prognostic roles of microRNA 143 and microRNA 145 in colorectal cancer: a meta-analysis. *Int J Biol Markers*. 2019;34(1):6-14. doi:10.1177/1724600818807492
14. Shen X, Jiang H, Chen Z, et al. MicroRNA-145 inhibits cell migration and invasion in colorectal cancer by targeting TWIST. *Onco Targets Ther*. 2019;12:10799-10809. doi:10.2147/OTT.S216147
15. Wang W, Ji G, Xiao X, et al. Epigenetically regulated miR-145 suppresses colon cancer invasion and metastasis by targeting LASP1. *Oncotarget*. 2016;7(42):68674-68687. doi:10.18632/oncotarget.11919
16. Qin J, Wang F, Jiang H, Xu J, Jiang Y, Wang Z. MicroRNA-145 suppresses cell migration and invasion by targeting paxillin in human colorectal cancer cells. *Int J Clin Exp Pathol*. 2015;8(2):1328-1340.
17. Cui SY, Wang R, Chen LB. MicroRNA-145: a potent tumour suppressor that regulates multiple cellular pathways. *J Cell Mol Med*. 2014;18(10):1913-1926. doi:10.1111/jcmm.12358
18. Huang Q, Song Q, Zhong W, Chen Y, Liang L. MicroRNA-10b and the clinical outcomes of various cancers: a systematic review and meta-analysis. *Clin Chim Acta*. 2017;474:14-22. doi:10.1016/j.cca.2017.08.034
19. Ma H, Marti-Gutierrez N, Park SW, et al. Ma et al. reply. *Nature*. 2018;560(7717):E10-E23. doi:10.1038/s41586-018-0381-y
20. Sheedy P, Medarova Z. The fundamental role of miR-10b in metastatic cancer. *Am J Cancer Res*. 2018;8(9):1674-1688.
21. Nishida N, Yamashita S, Mimori K, et al. MicroRNA-10b is a prognostic indicator in colorectal cancer and confers resistance to the chemotherapeutic agent 5-fluorouracil in colorectal cancer cells. *Ann Surg Oncol*. 2012;19(9):3065-3071. doi:10.1245/s10434-012-2246-1
22. Jiang H, Liu J, Chen Y, Ma C, Li B, Hao T. Up-regulation of mir-10b predicate advanced clinicopathological features and liver metastasis in colorectal cancer. *Cancer Med*. 2016;5(10):2932-2941. doi:10.1002/cam4.789
23. Vychytilova-Faltejskova P, Pesta M, Radova L, et al. Genome-wide microRNA expression profiling in primary tumors and matched liver metastasis of patients with colorectal cancer. *Cancer Genomics Proteomics*. 2016;13(4):311-316.
24. Huang CS, Yu W, Cui H, et al. Increased expression of miR-21 predicts poor prognosis in patients with hepatocellular carcinoma. *Int J Clin Exp Pathol*. 2015;8(6):7234-7238.
25. He Y, Zhang L, Cheng G, et al. Upregulation of circulating miR-21 is associated with poor prognosis of nasopharyngeal carcinoma. *Int J Clin Exp Pathol*. 2017;10(7):7362-7368.
26. Kong W, He L, Richards EJ, et al. Upregulation of miRNA-155 promotes tumour angiogenesis by targeting VHL and is associated with poor prognosis and triple-negative breast cancer. *Oncogene*. 2014;33(6):679-689. doi:10.1038/onc.2012.636

27. Senfter D, Madlener S, Krupitza G, Mader RM. The microRNA-200 family: still much to discover. *Biomol Concepts.* 2016;7(5-6):311-319. doi:10.1515/bmc-016-0020
28. Kumar S, Nag A, Mandal CC. A comprehensive review on miR-200c, a promising cancer biomarker with therapeutic potential. *Curr Drug Targets.* 2015;16(12):1381-1403. doi:10.2174/1389450116666150325231419
29. Voorhoeve PM, le Sage C, Schrier M, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell.* 2006;124(6):1169-1181. doi:10.1016/j.cell.2006.02.037
30. Huang Q, Gumireddy K, Schrier M, et al. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat Cell Biol.* 2008;10(2):202-210. doi:10.1038/ncb1681

RMJ Ramathibodi Medical Journal

Rama Med J | Original Article

การแสดงออกของไมโครอาร์เอ็นเอของมะเร็งเต้านมและมะเร็งลำไส้ปฐมภูมิและมะเร็งระยะแพร่กระจาย

ธนิยะ ศรีจรุณรัตน์[1], อาทิตย์ จินาวัฒน์[2], พัฒนา ศรมยุรา[2], ศันสนีย์ วงศ์ไวศยวรรณ์[3], บุษบา ฤกษ์อำนวยโชค[2]

[1] งานพยาธิวิทยาและนิติเวชศาสตร์ โรงพยาบาลจุฬาภรณ์ ราชวิทยาลัยจุฬาภรณ์ กรุงเทพฯ ประเทศไทย

[2] ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล กรุงเทพฯ ประเทศไทย

[3] ภาควิชาวิทยาศาสตร์การแพทย์ คณะวิทยาศาสตร์ มหาวิทยาลัยรังสิต ปทุมธานี ประเทศไทย

บทนำ: มะเร็งเต้านมและมะเร็งลำไส้ใหญ่เป็นโรคมะเร็งที่พบบ่อย และในระยะลุกลามสามารถแพร่กระจายไปที่อวัยวะอื่นทำให้สูญเสียหน้าที่ และผู้ป่วยอาจเสียชีวิตได้ ปัจจุบันมีการศึกษาเกี่ยวกับไมโครอาร์เอ็นเอ (microRNAs) ในโรคมะเร็งเพิ่มขึ้น

วัตถุประสงค์: เพื่อตรวจการแสดงออกของไมโครอาร์เอ็นเอในมะเร็งเต้านมและมะเร็งลำไส้ใหญ่ปฐมภูมิและมะเร็งระยะแพร่กระจาย

วิธีการศึกษา: การศึกษาการแสดงออกของไมโครอาร์เอ็นเอ 6 ชนิด ได้แก่ miR-10b, miR-21, miR-145, miR-155, miR-200c, และ miR-373 โดยใช้บล็อกชิ้นเนื้อมะเร็งปฐมภูมิและระยะแพร่กระจายเปรียบเทียบกับชิ้นเนื้อปกติของผู้ป่วยมะเร็งเต้านมและมะเร็งลำไส้ใหญ่ จากตัวอย่างผู้ป่วยในโรงพยาบาลรามาธิบดี ประเทศไทย โดยวิธี Real-time RT-PCR

ผลการศึกษา: การแสดงออกของไมโครอาร์เอ็นเอทั้ง 6 ชนิด พบว่า miR-145 มีการแสดงออกลดลงในทุกตัวอย่าง ซึ่งในมะเร็งลำไส้ใหญ่ระยะแพร่กระจายมีการแสดงออกลดลงอย่างมีนัยสำคัญเมื่อเทียบกับมะเร็งปฐมภูมิ ($P < .05$) ส่วน miR-10b, miR-155, และ miR-200c มีการแสดงออกลดลง ขณะที่ miR-21 และ miR-373 มีการแสดงออกเพิ่มขึ้นในเกือบทุกตัวอย่างโดยไม่พบความแตกต่างอย่างมีนัยสำคัญ ($P > .05$)

สรุป: การแสดงออกของไมโครอาร์เอ็นเอชนิด miR-145 สามารถใช้บ่งชี้การแพร่กระจายโรคมะเร็งและอาจนำไปใช้พยากรณ์โรคหรือเป็นเป้าหมายในการรักษามะเร็งเต้านมและมะเร็งลำไส้ใหญ่

คำสำคัญ: ไมโครอาร์เอ็นเอ มะเร็งเต้านม มะเร็งลำไส้ใหญ่ การแพร่กระจาย

Rama Med J: doi:10.33165/rmj.2022.45.3.257343

Received: April 15, 2022 **Revised:** August 29, 2022 **Accepted:** September 16, 2022

Corresponding Author:
บุษบา ฤกษ์อำนวยโชค
ภาควิชาพยาธิวิทยา
คณะแพทยศาสตร์
โรงพยาบาลรามาธิบดี
มหาวิทยาลัยมหิดล
270 ถนนพระรามที่ 6
แขวงทุ่งพญาไท เขตราชเทวี
กรุงเทพฯ 10400 ประเทศไทย
โทรศัพท์ +66 2201 1369
โทรสาร +66 2201 1267
อีเมล budsaba.rer@mahidol.ac.th