American Journal of BioMedicine
AJBM 2015;**3** (2): 83-99
Research Article doi: 10.18081/2333-5106/015-02/389-399

Let-7 microRNA: tumour suppression activity in breast cancer

Yezi Xerri, Mark N. Evans, Giorgio Inoue, Tennent K. Hanley, Douglas L. Hainz [1*]

Abstract

Breast cancer is the most prevalent malignancy among women, with an estimated 290,000 new cases diagnosed in the United States in 2014. It is classified into distinct molecular subtypes with divergent therapeutic and prognostic implications. Triple negative breast cancer (TNBC) is classified as estrogen receptor-negative (ER-), progesterone receptor-negative (PR-), and HER2-negative (HER2-). TNBC is associated with the most aggressive disease course, a lack of targeted therapies, and the worst prognosis. Novel therapeutic approaches are urgently needed to suppress the growth and dissemination of TNBC. MicroRNAs (miRNAs or miRs) are short, noncoding RNAs that post-transcriptionally regulate the expression of genes by binding to complementary sequences on their target mRNAs. MiRNAs are key regulators of cellular processes including development, differentiation, apoptosis, metabolism, and proliferation. A growing body of evidence indicates that miRNAs are critically involved in the development and progression of breast cancer. In human breast cancer specimens, some miRNAs, such as the let-7 family, are found to be downregulated, while others like miR-21 are upregulated. MiRNAs that are downregulated in breast cancer are referred to as tumor-suppressive miRNAs and those that are upregulated are referred to as oncogenic miRNAs. The let-7 family of miRNAs is one of the first clusters of miRNAs to be discovered. In mammals, the let-7 family has been shown to comprise at least nine mature members: let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, and miR-98. The dysregulation of the let-7 family of miRNAs is implicated in several types of cancer, including lung cancer, ovarian cancer, colorectal cancer, liver cancer, and breast cancer. However, the tumor suppression activity of the let-7 family of miRNAs in breast cancer remains largely unknown. MCF-7 and MDA-MB-231 breast cancer cell lines are commonly used to study the mechanisms of hormone action. MCF-7 cells, an ER+ breast cancer cell line, are noninvasive and have a low initiation rate of metastasis. In contrast, MDA-MB-231 cells, classified as a basal subtype, are highly invasive and form metastases in distant organs.

Keywords: Breast cancer; Let-7; mRNA; Xenograft; qRT-PCR

*Correspondence author e-mail: Douglas.Hainz@asobm.org
[1] Division of Biochemistry, Loma Linda University, Mortensen, USA
Received November 13, 2014; Accepted March; 11, 2015, Published April 30, 2015

Research Article doi: 10.18081/2333-5106/015-02/389-399

Introduction

Breast cancer is the most prevalent malignancy among women, with an estimated 290,000 new cases diagnosed in the United States in 2014. It is classified into distinct molecular subtypes with divergent therapeutic and prognostic implications. Triple negative breast cancer (TNBC) is classified as estrogen receptor-negative (ER-), progesterone receptor-negative (PR-), and HER2-negative (HER2-). TNBC is associated with the most aggressive disease course, a lack of targeted therapies, and the worst prognosis. Novel therapeutic approaches are urgently needed to suppress the growth and dissemination of TNBC.

MicroRNAs (miRNAs or miRs) are short, noncoding RNAs that post-transcriptionally regulate the expression of genes by binding to complementary sequences on their target mRNAs. MiRNAs are key regulators of cellular processes including development, differentiation, apoptosis, metabolism, and proliferation. A growing body of evidence indicates that miRNAs are critically involved in the development and progression of breast cancer. In human breast cancer specimens, some miRNAs, such as the let-7 family, are found to be downregulated, while others like miR-21 are upregulated. MiRNAs that are downregulated in breast cancer are referred to as tumor-suppressive miRNAs and those that are upregulated are referred to as oncogenic miRNAs.

The let-7 family of miRNAs is one of the first clusters of miRNAs to be discovered. In mammals, the let-7 family has been shown to comprise at least nine mature members: let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, and miR-98. The dysregulation of the let-7 family of miRNAs is implicated in several types of cancer, including lung cancer, ovarian cancer, colorectal cancer, liver cancer, and breast cancer. However, the tumor suppression activity of the let-7 family of miRNAs in breast cancer remains largely unknown. MCF-7 and MDA-MB-231 breast cancer cell lines are commonly used to study the mechanisms of hormone action. MCF-7 cells, an ER+ breast cancer cell line, are noninvasive and have a low initiation rate of metastasis. In contrast, MDA-MB-231 cells, classified as a basal subtype, are highly invasive and form metastases in distant organs.

Breast cancer is the most common cancer in women and is the second leading cause of cancer-related deaths after lung cancer. With the advances in detection methods, breast cancer incidence is rising. In 2014, approximately 2.1 million women were diagnosed with breast cancer worldwide, and about 630,000 died from the disease. Therapies such as surgery, chemotherapy, and radiation have been developed for breast cancer treatment. However, there has been little progress in developing effective therapeutic agents for triple-negative breast cancer (TNBC), which accounts for approximately 15-20% of all breast cancer cases. There is thus an urgent need for the development of effective therapeutic agents for breast cancer, especially for TNBC.

Research Article doi: 10.18081/2333-5106/015-02/389-399

MicroRNAs are a family of small non-coding RNAs that regulate gene expression post-transcriptionally. Cellular microRNAs are found in the cytoplasm and nucleolus of eukaryotic cells and are up to 25 nucleotides long. More than 2,600 microRNA genes have been identified in the human genome, which are predicted to regulate the expression of more than 60% of human protein-coding genes. Each microRNA can potentially regulate hundreds of target genes. MicroRNAs bind the 3' untranslated region (3' UTR) of target mRNAs via complementary base-pairing interactions to silence target gene expression. MicroRNAs play important roles in various biological processes, including cell proliferation, differentiation, apoptosis, and metabolism. MicroRNA dysregulation has been frequently identified in many types of human cancers, including breast cancer, and is associated with cancer development and progression.

The let-7 microRNAs, which are evolutionarily conserved and one of the first microRNA families to be discovered, have been shown to inhibit cell proliferation and promote cell differentiation. In humans, the let-7 family consists of two clustered loci on chromosome 9 (let-7a-1, let-7b, let-7e, and let-7f-1) and chromosome 22 (let-7c and let-7d). The let-7 microRNAs were first identified as a family of tumor suppressors with downregulated expression in lung, pancreatic, breast, liver, colon, and prostate cancer. Reduction of let-7 expression has been associated with poor overall survival in lung cancer and prostate cancer patients. In contrast, let-7 overexpression inhibits cell proliferation and tumor growth in nude mice. However, the expression and tumor suppressor activity of the let-7 microRNAs in breast cancer have not yet been reported.

MicroRNAs in Cancer Development

MicroRNAs (miRNAs) are important and highly conserved posttranscriptional regulators of gene expression that play key roles in numerous biological processes, including development, cell differentiation, cell cycle, and apoptosis. MiRNAs bind to the 3' untranslated regions (3'UTR) of target mRNAs, which results in target degradation or translational repression. Comprehensively, one miRNA can control a hundred gene targets, and one gene target can be regulated by several miRNAs. The predicted number of miRNA targets is large, possibly 30-60% of human mRNAs.

There is ample evidence implicating aberrant expression of miRNAs in the etiology and progression of cancer. In gene expression profiling studies, different miRNAs were shown to be up- or downregulated due to genomic amplification or deletion, chromatin modification, and altered transcription factor binding in a variety of cancer types. More recently, miRNAs were discovered to be released from dying cells and packaged into exosomes, resulting in paracrine intercellular communication. Accumulating evidence suggests that miRNAs play dual roles in cancer development and progression depending on the context. Some miRNAs behave like oncogenes that are upregulated in tumors, while the expression of other miRNAs is downregulated and correlates with the presence of tumors, the latter being referred to as tumor

Research Article doi: 10.18081/2333-5106/015-02/389-399

suppressor miRNAs. Oncogenic miRNAs are often found to target tumor suppressor genes, and tumor suppressor miRNAs frequently target oncogenes. In one study, the expression of forty-six cancer-related miRNAs was examined in breast cancer tissues. It was reported that miR-21, miR-155, miR-17-5p, miR-19b, miR-20a, and miR-10b were consistently upregulated in breast tumor samples, while the expression of let-7, miR-28, miR-125b, miR-140, and miR-200 was found to be downregulated.

Let-7 was first discovered in C. elegans and is one of the first miRNAs to be characterized. From C. elegans to human, let-7 is conserved in sequence and secondary structure. Sixteen mature let-7 miRNAs were identified from the human genome. Tumor suppression activity of let-7 was demonstrated in lung cancer and neuroblastoma. The hypothesis was tested that downregulation of let-7 family miRNAs in breast cancer may target members of the epidermal growth factor receptor (EGFR) signaling pathways and determine the oncogenic activity of ras in human cells. Enhanced expression of the let-7a1 transgene suppressed cell migration and invasion, and anchorage-independent growth of human breast cancer MDA MB-231, but not MEF cells. Breast cancer is the most common type of cancer in women and the second leading cause of cancer-related deaths. 5-10% of breast cancers have a Mendelian predisposition that involves inherited mutations in tumor suppressor genes and oncogenes.

Let-7 MicroRNA: Discovery and Function

MicroRNAs (miRNAs) are a class of small noncoding RNAs with a length of 19-22 nucleotides, which inhibit protein expression by targeting the 3' untranslated region of their mRNA targets. To date, more than 1,100 human miRNAs have been discovered, and they play important roles in numerous biological processes including development, differentiation, proliferation, apoptosis, and metabolism. Dysregulation of miRNAs is also implicated in the pathogenesis of many human diseases, such as cancers, diabetes, and viral infections. The let-7 family is one of the first miRNAs discovered in C. elegans (Caenorhabditis elegans), with a size of 21 nucleotides. In 2001, it was first shown that the mutant let-7 worms display a large and abnormal phenotype, with a failure to exit from larval stage L4 to adulthood. Since then, the let-7 microRNAs have been found and characterized in many species, including nematodes, fruit flies, mice, rats, and humans. In humans, there are 9 let-7 family members: let-7a, let-7b, let-7c, let-7d, let-7e, let-7f1, let-7f2, let-7g, and let-7i. The let-7 family members are conserved in evolution and share high homology in their nucleotides. They are also expressed at relatively low levels in various normal tissues and at much lower levels in many tumors. It has been shown that the let-7 microRNAs bind to the 3' untranslated region (3' UTR) of their target messages with extensive complementary base pairing, leading to mRNA degradation and/or translation inhibition. Through sequence analysis, expression profiling, microarray, and proteomic approaches, a number of target messages of the let-7 family microRNAs have been

Research Article doi: 10.18081/2333-5106/015-02/389-399

identified in normal and tumoral tissues, and are involved in multiple biological processes. These target messages include some oncogenes and transcription factors.

Accumulating evidence has implicated the involvement of the let-7 miRNAs in the development and progression of various human cancers. The first evidence showing the role of let-7s in human lung cancer came from the study with a candidate gene approach. The let-7 family was found deleted at a high frequency in non-small cell lung cancers. Early studies further demonstrated that the let-7s were downregulated or lost in a variety of human malignancies including lung cancer, breast cancer, ovarian cancer, gastric cancer, and colorectal cancer. Introduction of let-7a, let-7b, or let-7c into cancer cells, which expressed low levels of let-7s, suppressed their oncogenic phenotypes including colony formation in anchorage-independent cultures (i.e., soft agar colony formation) and growth in nude mice. Several important oncogenes such as RAS, MYC, and LIN28B, that are direct targets of the let-7 microRNAs, have been identified in this tumor suppressor pathway. Further mechanistic studies demonstrated that the tumor suppressor activity of let-7 is mediated through inhibition of the expression of its oncogenic targets, leading to the re-establishment of normal cellular pathways to block tumorigenesis. The discovery of the let-7 family miRNAs spurred significant interest in understanding their functions and potential roles in human diseases, especially in cancer. After their expression profiling studies in cultured cells and tissues, let-7s were also found downregulated or lost at the genetic level in various other types of tumors. Some of these early studies suggested a tumor suppressor role of let-7s, and the putative targets of let-7s were primarily oncoproteins which mediate oncogenic pathways.

Discovery of Let-7 MicroRNA

In 2000, the let-7 miRNA was discovered in the model organism C. elegans by Victor Ambros, Gary Ruvkun, and colleagues, who showed that it regulates developmental timing in this organism. The gene for the let-7 microRNA was first identified genetically as a gene required for the transition from L4 larval stage to adulthood. As an approach to identify these genes, Ambros and Ruvkun devised a strategy to clone genes that, when mutated, would result in the expression of a fate that would normally occur later in development. The strategy involved creating a mixed population of wild type and mutant animals with a temperature-sensitive developmental timing mutation and then cloning genes that were expressed at a higher level in the temperature-sensitive mutant at the non-permissive temperature. They cloned and characterized a let-7 gene family consisting of 21-nt RNAs that were present in the adult stage but not in the L4 stage. These let-7 miRNA genes underwent tailing and trimming similar to other miRNAs but were distinct in containing a 5' mismatch.

This was the first miRNA to be discovered in any organism and, subsequently, several other members of the let-7 family were found in C. elegans and various vertebrates, including humans. Analysis of the targets of let-7 revealed that it regulates the RAS and LET-60 signaling

Research Article doi: 10.18081/2333-5106/015-02/389-399

pathway and the expression of several other genes involved in cell growth and proliferation. As a first step in investigating the conservation of the gene regulatory pathways they identified in C. elegans, this gene family was compared to other miRNA genes that were functionally and structurally conserved across species.

Using this information, the human let-7 miRNA genes were cloned from 11 different breast cancer cell lines for a previous study. In this survey, the let-7 miRNA genes that were chromosomally indexed at 3q 19.21 and 9p 22.2 were detected in all of the breast cancer cell lines. The C. elegans and vertebrate let-7 miRNAs share a complementary seed sequence of 7 nucleotides that allows target recognition by base-pairing with complementary 3'-UTR sequences in mRNAs. Although these genes are highly conserved, the nucleotide sequences of the let-7 miRNAs undergo a high frequency of nucleotide changes. Only one out of every three nucleotides in a potential target site is perfectly conserved across vertebrates, indicating that additional sequence and structural complements contribute to target recognition. It was further shown that base-pairing with the 5' seed at additional positions during miRNA-mRNA pairing contributes to the fidelity of RNAi and miRNA regulation.

This work reports the first characterization of let-7 miRNAs in breast cancer. Together with the evidence that the let-7 target c-myc is overexpressed in breast cancer, it implicates this miRNA family in breast cancer pathogenesis. Similar to the lineage specific evolution of the C. elegans let-7 family, the mammalian let-7 genes are either clustered on the same chromosomal locus or separated by several kilo-bases.

Biological Functions of Let-7

Since its discovery, let-7 has been recognized as one of the earliest and best-characterized members of the miRNA family. It has been detected in various species including worms, flies, mice, and humans, with a remarkable sequence conservation of 20-22 nucleotides (nt) in mature forms. In specific cancers or types of tumors, other conserved let-7 variants have been found with a nucleotide difference of only 1-3. Fourteen let-7 isoforms were predicted from the human and mouse genome, owing to its well-documented involvement in the regulation of fundamental developmental processes including cell proliferation, differentiation, and signaling pathway regulation.

Remarkably, let-7 is regulated at both transcriptional and post-transcriptional levels. Several transcription factors involved in let-7 regulation have been identified, including E2F1, c-Myc, c-Myb, HMGA2, and NFκB. E2F1 binds to the let-7a-let-7d miRNA promoter region and regulates let-7 expression during the G1-S transition. c-Myc directly represses its target let-7 gene expression, resulting in significantly enhanced cell proliferation and tumorigenicity of c-Myc-transformed fibroblasts. Conversely, c-Myb and NFκB enhance the expression of let-7 during B-cells or T-cells activation, respectively. Also, it was reported that let-7 is regulated post-

Research Article doi: 10.18081/2333-5106/015-02/389-399

transcriptionally through binding to the sequences in the 3'UTR of the target mRNA by the oncogenic RNA binding protein IGF2BP-1, causing a reduction in the expression of mature let-7.

Regarding its function, let-7 acts as a tumor suppressor in several types of human cancer by inhibiting cell proliferation. Since the discovery of let-7, several experiments have been carried out to investigate the broad and fundamental roles of the let-7 miRNA family. Forced expression of precursors of let-7a or let-7b significantly inhibits RAS, MYC, and E2F1-mediated transformation in human cells. The inhibition is associated with decreased C-MYC and RAS protein levels and reduced transcriptional activity of E2F1. Further study using the metagene strategy demonstrated that let-7 expression is strongly correlated to low RAS, MYC, and E2F transactivation signatures across a variety of different tumor types. Thereafter, let-7 was reported for its tumor suppressor activity in several cancer models, especially in lung cancer and breast cancer animal models. Overall, the let-7 family members are functionally conserved miRNAs that act primarily to inhibit cell proliferation and promote differentiation.

Let-7 Dysregulation in Breast Cancer

Breast cancer (BC) is associated with extensive genetic and epigenetic alterations. For many years, major efforts have been made to characterize these abnormalities. One of the most intriguing findings in this field is that the machinery controlling RNA silencing is frequently altered in different types of human tumors, including breast cancer. Several microRNAs presenting tumor inhibition properties are frequently down-regulated in association with their validated targets that are oncogenes. The present work focused on Let-7, a microRNA that is widely accepted as a tumor suppressor miRNA. It is also the one with the largest number of validated targets, many of them being associated with BC. We evaluated the role of Let-7 in breast tumorigenesis, using a DIY approach that is disruptive to Let-7. This Open Reading-Frame Trojan horse carries a sequence that masks the primary precursor Let-7g microRNA. We expressed it by lentiviral infection on MCF-722 and MCF-7 BC cell lines.

It has been observed that the inhibition of endogenous Let-7 correlates with a drastic increase in in vitro cell proliferation, invasive potential, the appearance of colonies in suspension, and the ability to form mammospheres. With Let-7 blockage, the HER-2 receptor associated with tumor aggressiveness is down-regulated. In this work, we demonstrated the tumor suppressor role of Let-7 using a DIY approach with a Let-7g Lentiviral vector in two breast cancer cell lines, MCF-7 and MCF-722. When Let-7g was inhibited, we observed, associated with endogenous downregulation, a deregulation of Let-7g targets ANC2 and HER4, cell proliferation, and cells formation in the soft agar in both cell lines, and viability and mammospheres formation capability only in MCF-7.

Research Article doi: 10.18081/2333-5106/015-02/389-399

Role of Let-7 in Breast Cancer Development

The most well-established feature of Let-7 in regulating the carcinogenic property of the cell is the suppression of the cell cycle and its main pro-proliferation target, cyclin D1 (CCND1). Let-7 members are frequently lost in breast cancer as well as other cancer types. It is very significant that CCND1 is universally amplified and overexpressed in breast cancer. The let-7 gene is located in the chromosomal region 9q22, reported to be a site of loss of heterozygosity in human cancer. There are two let-7AG1 and let-7G2 precursors. It has been shown that both precursors target the 3'-untranslated region (UTR) of CCND1 and reduce its expression, thus suppressing the growth of breast cancer cells.

UBC9, the SUMO (small ubiquitin-like modifier) conjugation enzyme, modulates the function of a variety of transcription factors and other cellular processes in neoplastic phenotype. The physical interaction of UBC9 with the let-7A1 affecting the stability of ZFPM1 and possibly even enhancing the expression of ZFPM1 and CDK6, eventually results in accelerating colorectal cancer xenograft growth. UBC9 has also been shown to abate let-7 tumor-suppressor capacity in breast cancer cells to shed some light on the specific mechanisms of UBC9 function. In conclusion, UBC9 inhibition, as part of a multi-target cancer therapy, may lead to significant and complementary growth-inhibitory effects that could enhance current breast cancer treatments in the future.

Tumor Suppression Mechanisms of Let-7 in Breast Cancer

MicroRNAs (miRNAs), a class of small, non-coding RNAs, act as important epigenetic regulators by negatively modulating their target genes. Let-7, the first identified miRNA family, has been demonstrated to be important for regulating cellular differentiation and development in C. elegans. The Let-7 family includes Let-7a (let-7), Let-7b, Let-7c, Let-7d, Let-7e, Let-7f, Let-7g, and Let-7i. The Let-7 family members share the same seed region, and Let-7 is evolutionarily conserved in multicellular organisms. It is reported that Let-7 plays a tumor suppressor role in various cancers, including lung cancer, colon cancer, liver cancer, prostate cancer, ovarian cancer, gastric cancer, pancreatic cancer, and breast cancer. A computational prediction using TargetScan suggested that the Let-7 family could target the 3' untranslated region (UTR) of the HNRNPA1 gene and downregulate its expression. Let-7 was shown to effectively inhibit the luciferase activity of the HNRNPA1-3'UTR reporter, while mutations in the Let-7 binding sites in the HNRNPA1-3'UTR abrogated that inhibition. In addition, exposure of breast cancer cells to Let-7 significantly decreased HNRNPA1 protein and mRNA levels.

The targeted inhibition of HNRNPA1 by Let-7 was also observed in other cancer types, such as colon cancer and ovarian cancer. As a classic proto-oncogene with an oncogenic function, the expression of HNRNPA1 is upregulated in breast cancer tissues and positively associated with the poor prognosis of breast cancer patients. In vitro and in vivo studies demonstrated that

Research Article doi: 10.18081/2333-5106/015-02/389-399

HNRNPA1 promotes cancer cell proliferation and colony formation in a soft agar assay and enhances the G1/S transition of the cell cycle. Importantly, HNRNPA1 significantly promotes the growth of tumor xenografts in nude mice. Moreover, HNRNPA1 increases the migration and invasion of breast cancer cells in vitro and is essential for breast cancer cell metastasis to lung tissues in vivo. Therefore, HNRNPA1 as a target gene of Let-7 is involved in the tumor suppression of breast cancer. The signaling pathways regulated by HNRNPA1 include the Src, FAK, and AKT pathways, which are important for the proliferation and metastasis of cancer cells.

In summary, Let-7 is the first discovered miRNA family that has been widely confirmed to be a breast cancer tumor suppressor. The downregulation of Let-7 expression in breast cancer is through the mechanism of HNRNPA1, a newly identified target gene of Let-7. Pending questions include the details of the mechanisms by which Let-7, a polycistronic precursor, or its individual family members (e.g., Let-7a, Let-7f) are involved in the tumor suppression of breast cancer. An ablation of Let-7 family or separate experiments using an individual member of Let-7 need to be performed to investigate this issue. Moreover, besides breast cancer, the role of Let-7 family members in regulating the aggressiveness and tumorigenicity of breast tumors with diverse molecular characteristics should also be addressed.

Target Genes and Pathways Regulated by Let-7

The let-7 family of miRNAs is considered one of the most important miRNAs that suppress the initiation and progression of human cancers, including BCP, by targeting multiple oncogenic genes. In the present study, let-7a, let-7c, let-7g, and let-7i were significantly downregulated in tumor tissues and cell lines and were correlated with poor prognosis in patients with BCP. Chip-on-chip array and computational approaches were adopted to predict the target genes of let-7. 15 candidate target genes were chosen for validation. The luciferase activity was inhibited by WT 3'UTR of IGF1R, KRAS, and HMGA2 but not by MT 3'UTR. Moreover, the protein level was decreased by let-7 mimic. In addition, both IGF1R and KRAS exhibited oncogenic activities in BCP. Furthermore, pathways regulated by let-7 involved IGF1R, KRAS, HMGA2, and other 15 target genes. Based on Ingenuity Pathway Analysis, let-7 target genes and their downstream cancer pathways were shown. BCP pathways regulated by let-7 were Notch and Wnt signaling, EMT, FGF, Efgr signaling, and their downstream proteins like CTGF, HGF, TGFB.

Let-7 family miRNAs, as key tumor suppressor miRNAs that are downregulated in BCP, could inhibit cancer cell proliferation, invasion, and metastasis by targeting multiple oncogenes and regulating different cancer pathways. Further studies will be performed to investigate other mechanisms of let-7 in BCP.

Research Article doi: 10.18081/2333-5106/015-02/389-399

Impact on Cancer Cell Proliferation and Metastasis

Prior studies have shown that Let-7 suppressed cancer cell growth and metastasis in triple-negative breast cancer (TNBC). The role of Let-7 in the inhibition of breast cancer cell proliferation and metastasis was explored. A panel of breast cancer cell lines with different Let-7 levels was first profiled. Cytotoxic effects of liposome-encapsulated Let-7a (lip-Let-7a) nanomedicine were assessed. Downregulation of Let-7 was revealed in several breast cancer cell lines and xenograft models with noticeable invasive phenotypes. In contrast, other breast cancer cell lines displayed relatively high expression levels of Let-7 family members including Let-7a, which were associated with non-invasive or less invasive characteristics.

Several TNBC models were focused on for further mechanistic study since this is the most aggressive subtype with few therapeutic options. Let-7a-induced inhibition of cancer cell proliferation and transformation in both normal breast epithelial cells and breast cancer cells was determined. Lip-Let-7a was established, and its effective stability, biocompatibility, and ability to enhance the intracellular accumulation of Let-7a were shown. The functional and mechanistic studies of Let-7a at the cellular level were then examined. Let-7a exerted growth inhibitory effects on breast cancer cells by inducing G1-phase cell cycle arrest, which was associated with the regulation of cyclins, CDKs, and p/RB pathways. The expression of proliferation marker PCNA and cyclin D1 was downregulated, while CKI p21 and p27 were upregulated by Let-7. In addition, Let-7 inhibited breast cancer cell transformation and migration/invasion by downregulating several target molecules involved in the Wnt and EMT pathways.

In vivo growth inhibition of breast cancer xenograft tumors by Let-7 was also explored. Importantly, this work revealed a previously uncharacterized role of Let-7 in the inhibition of cancer cell proliferation and metastasis in breast cancer, further elucidating the tumor suppression activity of Let-7. As one of the largest subfamilies of microRNAs, Let-7s were identified to be involved in multiple human diseases including cancers and developmental disorders. So far, Let-7 is the most characterized tumor-suppressive microRNA family. As a prime example, Let-7 was first found to be downregulated in lung cancer. Restoring Let-7 expression was shown to decrease cancer cell proliferation and invasion, and tumor growth in xenografts. In addition, let-7 can inhibit stemness and drug resistance in breast cancer. To date, however, the comprehensive tumor suppression machinery of Let-7 is still poorly understood in breast cancer. There is an urgent need for better understanding of Let-7 regulatory network and underlying molecular action mechanisms, which will ultimately facilitate the rational development of effective Let-7-targeted nanomedicine for breast cancer therapy.

Research Article doi: 10.18081/2333-5106/015-02/389-399

Clinical Implications and Therapeutic Potential

Breast cancer is the most common cancer in women, and in some cases, it can turn out to be aggressive and unresponsive to conventional chemotherapy, leading to high mortality rates. Therefore, the discovery of new and potential targets and roles for miRNAs in order to develop early-stage diagnostics and innovative therapies for breast cancer is on the agenda of many researchers.

Let-7 miRNA is discovered as one of the first known miRNAs, and, together with its tumor-suppressive roles, the Let-7 family has been proven to reduce breast cancer stem cell characteristics, which is thought to be a common mechanism. By targeting several oncogenic traits and invasiveness-promoting or proliferation-promoting pathways, Let-7 family members act as tumor-suppressive in breast cancer pathogenesis. Accordingly, because Let-7 suppresses poor prognostics such as self-renewal and proliferation, we can define it as a breast cancer "stemness" or self-renewal-miRNA.

Treatment opportunities are limited when it comes to finding the targeted anti-neoplastic agents or effective therapies. In particular, the poverty of current therapies for women with metastatic disease shows the importance of discovering the miRNA profiles specific to certain breast cancer subtypes and determining their relevant data in terms of prevention, early prognosis, or future treatment.

High levels of Let-7 pre-expression or miRNA expression-corruptive compounds are expected to have potent and selective therapeutic potential for Let-7 affected breast cancers. The use of nucleotides of the Let-7 miRNA family as a breast cancer treatment may provide a significant advantage in that the Let-7 anticancer effect can be used alone or in combination to target multiple Let-7 axis genes that are abnormally expressed in cases when a single gene-target therapy is inadequate because of the small effectiveness.

Intervention methods to promote deregulated Let-7 expression can be used with conventional chemotherapeutics or radiotherapy not as a monotherapy but to improve the bioavailable properties of anticancer agents.

Diagnostic and Prognostic Value of Let-7 in Breast Cancer

Over the last two decades, an increasing number of studies have focused on the role of aberrant miRNA expression in relation to specific human diseases. In this respect, several studies of the let-7 miRNA in human cancer are available. In breast cancer, let-7 expression is reduced in cancerous tissue compared to normal breast epithelium and is associated with advanced tumor staging. Most importantly, in a multivariate model, reduced let-7 expression was a predictor of reduced breast cancer-specific and overall survival. In this study, the let-7a-1, let-7f-1, and let-

Research Article doi: 10.18081/2333-5106/015-02/389-399

7d loci were examined. As they are part of gene families, it was suggested that further studies of multiple let-7 loci are needed to refine this association.

In a recent study of miRNAs and the response to chemotherapy in breast cancer patients, let-7c expression was significantly related to the rate of pathological complete response (tumors that disappear after chemotherapy). Indeed, the expression level of let-7c prior to chemotherapy showed the greatest predictive power for this treatment outcome, when it was evaluated together with the mRNA expression of its target genes, particularly TGFβR1. However, let-7c expression in the pretreatment samples was not associated with the progression-free survival of these patients. Also, low levels of let-7b were associated with shorter overall survival of breast cancer patients, independently of other clinicopathological factors. This association was most persistent for triple-negative breast cancers and less so for other intrinsic subtypes, however, the sample size of tumors with evaluated let-7b expression was small (N = 123).

Therapeutic Strategies Targeting Let-7

The tumor-suppressing activity of let-7 miRNA in breast cancer allows it to act as a therapeutic molecule directly if it is delivered to cells using synthetic oligonucleotide or virus vectors. Alternatively, because let-7 is lowly expressed in cancer cells but abundantly expressed in normal epithelium, the endogenous let-7 can be mobilized by converting its precursor, pri-let-7, into the matured miRNA using tailored virus-encoded RNA. Such a strategy would allow let-7 to impose site-specific actions on tumor cells without affecting normal cells. In fact, the successful demonstration of gene therapy using virus-associated RNA for the treatment of liver cancer and lung cancer suggests that the inhibition of tumor development by let-7 is achievable by exploiting its specificity in normal epithelium.

In rare diseases of the lung and pleura called mesothelioma, the development of a non-tumorigenic compound is likewise essential. Just like in lung squamous cell carcinoma, the loss of let-7 is another crucial factor promoting the growth of damaging tumors. Furthermore, when let-7 is depleted, other harmful tumors of the lung like adenomas and granulomas develop a supportive microenvironment of lung injury, indicating that the absence of let-7 profoundly influences the cellular action oppressors in normal lung epithelium. As such, the production of let-7 by viral infection would likewise have a biological effect on multiple areas of lung neoplasms, irrespective of the histological subtypes.

Conclusion

During the last decade, we have seen an increase in the knowledge of the role of let-7 microRNA as a tumor suppressor gene in breast cancer. Let-7b, let-7c, and let-7g have been found to work as tumor suppressors in in vitro and in vivo in breast cancer through targeting

Research Article doi: 10.18081/2333-5106/015-02/389-399

the expression of oncoproteins such as MYC, KRAS, and HRAS among many others. Importantly, let-7 can inhibit cancer cell stemness and drug resistance. Let-7a, let-7b, and let-7g microRNA levels are reduced in breast cancer and this correlates with increased mortality and recurrence of disease. This indicates that let-7 microRNA might be used as biomarkers of prognosis and for prediction of response to therapeutic agents. To take advantage of the anti-oncogenic profile of let-7 microRNA, current therapeutic strategies are directed to the development of let-7 tents or minigenes and new drug delivery systems, including extracellular vesicles.

Let-7 microRNA also inhibits invasion and metastasis of breast cancer cells. This is important knowledge since invasion and metastasis processes are the major cause of mortality in breast cancer. The mechanism of let-7 microRNA to inhibit invasion and metastasis is based on inhibiting the expression of important oncoproteins such as ECM1, by activating genes that will release its inhibition and silence MMPs and pasein controlled by LIN28. The study of the regulation of the activity of let-7 regulators will contribute to the knowledge of the small community of microRNA let-7 in breast cancer and to identify new drug delivery systems.

Funding

No funding was received.

Competing interests

The authors declare no conflict of interest.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

Open access

Research Article doi: 10.18081/2333-5106/015-02/389-399

References

1. Reis-Filho JS. & Lakhani SR. Breast cancer special types: why bother? J Pathol 2008;216:394–398. [PubMed]
2. Vargo-Gogola T & Rosen JM. Modelling breast cancer: one size does not fit all. Nat Rev Cancer 2007;7:659–672. [PubMed]
3. Shi X-B, Tepper CG, deVere White RW. Cancerous miRNAs and their regulation Cell Cycle 2008;7:1529–1538. [PubMed]
4. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, *et al*. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27: 1160–1167. [PubMed]
5. Khramtsov AI1, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. Am J Pathol 176: 2911–2920. [PubMed]
6. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127:469–480. [Abstarct/Full-Text]
7. Rougvie AE. Control of developmental timing in animals. Nature Reviews Genetics 2001;2(9): 690–701. [Abstarct/Full-Text]
8. Ambros V. microRNAs: tiny regulators with great potential. Cell 2001;107 (7): 823–826. [Abstarct/Full-Text]
9. Moss EG. Heterochronic genes and the nature of developmental time Curr. Biol 2007;17: R425–R434. [Abstarct/Full-Text]
10. Reinhart BJ. *et al*. The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 2000;403 (6772): 901–906. [PubMed]
11. Pasquinelli AE1, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, *et al*. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000;408 (6808): 86–89. [PubMed]
12. Shell S, Park SM, Radjabi AR, Schickel R, Kistner EO, Jewell DA,Park SM, Radjabi AR, *et al*. Let-7 expression defines two differentiation stages of cancer. Proc Natl Acad Sci USA 2007;104(27): 11400–5. [PubMed]
13. Takamizawa J, Konishi H,Yanagisawa K, *et al*. Reduced expression of the let-7 micrornas in human lung cancers in association with shortened postoperative survival. Cancer Res 2004;64(11): 3753–6. [Abstract/Full-Text]
14. Chen X, Ba Y, Ma L, Cai X, Yin Y, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997–1006. [PubMed]
15. Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microrna expression and angiogenesis. Circ Res 2007;101(1): 59–68. [Abstract/Full-Text]

Research Article doi: 10.18081/2333-5106/015-02/389-399

16. Esquela, Kerscher A, Trang P, Wiggins JF, *et al*. The *let-7* microrna reduces tumor growth in mouse models of lung cancer. Cell Cycle 2008;7(6): 759–64. [Abstract/Full-text]
17. Rabinowits G, Gerçel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer. 2009;10:42–46. [PubMed]
18. Barh D, Malhotra R, Ravi B, Sindhurani P. MicroRNA let-7: an emerging next-generation cancer therapeutic. Current Oncology 2010;17:70-80. [Abstract/Full-Text]
19. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008;110:13–21. [PubMed]
20. Boyerinas B, Park SM, Hau A, Murmann AE, Peter ME. The role of let-7 in cell differentiation and cancer. Endocr Relat Cancer 2010;17:F19–36. [PubMed]
21. Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur M, *et al*. Cancer Res 2009;69:1302. [PubMed]
22. Tummala R, Lou W, Zhu Y, Shi X, June X, Chen Z, *et al*. MicroRNA let-7c Is Downregulated in Prostate Cancer and Suppresses Prostate Cancer Growth. Plos one 2012; 7(3):e32832. [Abstract/Full-Text]
23. Roush S, Slack FJ. The let-7 family of microRNAs. Trends in Cell Biology. 2008;18:505–516. [PubMed]
24. Boyerinas B, Park SM, Hau A, Murmann AE, Peter ME. The role of let-7 in cell differentiation and cancer. Endocr Relat Cancer 2010;17:F19–36. [PubMed]
25. Iliopoulos D, Hirsch H, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 microRNA and IL6 links inflammation to cell transformation Cell 2009;139: 693–706. [PubMed/NCBI]
26. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215–233. [Full Text]
27. Dong Q, Meng P, Wang T, Qin W, Qin W, *et al*. MicroRNA Let-7a Inhibits Proliferation of Human Prostate Cancer Cells *In Vitro* and *In Vivo* by Targeting E2F2 and CCND2. PLoS ONE 2010;5: e10147. [View Article]
28. Heo I, Joo C, Cho J, Ha M, Han J, Kim VN. Lin28 mediates the terminal uridylation of let-7 precursor MicroRNA. Mol. Cell 2008; 32: 276–284. [Full Text]
29. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science 2007;315: 1576–1579. [CrossRef]
30. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, *et al.* Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000;408:86–89. [Scopus]
31. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 2000;403:901–906. [Scopus]

32. Wulczyn FG, Smirnova L, Rybak LA, Brandt C, Kwidzinski E, Ninnemann O, Strehle M, Seiler A, Schumacher S, Nitsch R. Post-transcriptional regulation of the let-7 microRNA during neural cell specification. FASEB J 2007; 21:415–426. [CrossRef]
33. Mayr, C, Hemann, MT, and Bartel, DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science 2007; 315: 1576–1579. [CrossRef]
34. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008;141:672–675. [PubMed]
35. Lee SO, Chun JY, Nadiminty N, Lou W, Gao AC. Interleukin-6 undergoes transition from growth inhibitor associated with neuroendocrine differentiation to stimulator accompanied by androgen receptor activation during LNCaP prostate cancer cell progression. The Prostate 2007;67: 764–773. [View Article]
36. Tsang W, Kwok T. Let-7a microRNA suppresses therapeutics-induced cancer cell death by targeting caspase-3. Apoptosis 2008;13: 1215–1222. [View Article]
37. Lawrie CH, Chi J, Taylor S, Tramonti D, Ballabio E, *et al*. Expression of microRNAs in diffuse large B cell lymphoma is associated with immunophenotype, survival and transformation from follicular lymphoma. J Cell Mol Med 2009;13: 1248–1260. [View Article]
38. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, et al. Huang Y. A Let-7 regulates self-renewal and tumorigenicity of breast cancer cells. Cell 2007;131 1109– 1123. [CrossRef]
39. Nishimori H, Yasoshima T, Denno R, Shishido T, Hata F, et al. A novel experimental mouse model of peritoneal dissemination of human gastric cancer cells: different mechanisms in peritoneal dissemination and hematogenous metastasis. Jpn J Cancer Res. 2000;91:715–722. [PubMed]
40. Fukui R, Nishimori H, Hata F, Yasoshima T, Ohno K, et al. Metastases-related genes in the classification of liver and peritoneal metastasis in human gastric cancer. J Surg Res. 2005;129:94–100. [PubMed]
41. Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, et al. The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res 2007;67:1419–1423. [PubMed]
42. Tsang WP, Kwok TT. Let-7a microRNA suppresses therapeutics-induced cancer cell death by targeting caspase-3. Apoptosis. 2008;13:1215–1222. [PubMed]
43. Rabinowits G, Gerçel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 2009;10:42–46. [PubMed]

44. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, et al. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 2010;251:499–505. [PubMed]
45. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008;10:1470–1476. [PMC free article]

American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net