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Review

Deregulated miRNAs in Triple Negative Breast Cancer

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Abstract

Triple negative breast cancer (TNBC) is defined by its unique characteristics of estrogen receptor (ER) negative, progesterone receptor (PR) negative and human epidermal growth factor receptor 2 (HER2) negative. Therefore it cannot be treated with drugs targeting these receptors, resulting in poor prognosis in general. miRNAs have been implicated in the regulation of a variety of cellular processes, implying that they can function either as oncogenes or tumor suppressors. Recent advances including our own findings demonstrate that some miRNAs play a role in TNBC development and therapeutics. Here, we summarize the roles of miRNAs in the management of TNBC, with a focus on the recently identified deregulated miRNAs harboring diagnostic, prognostic and therapeutic implications.

Key Words: Breast cancer, miRNAs, Biomarker, Triple negative breast cancer TNBC

Introduction

Breast cancer is one of the most common types of cancer, responsible for more than 1,300,000 cases and 450,000 deaths each year worldwide [1]. It is expected that one in 8 women in the United States will develop breast cancer in her lifetime [2]. Triple negative breast cancer (TNBC) is defined as estrogen receptors (ER) negative, progesterone receptors (PR) negative, and human epidermal growth factor receptor 2 (HER2) negative. Therefore, this subtype cannot be treated with specific therapies targeting the

综述

在三阴性乳癌中调节失调的微小核糖核酸（miRNA）

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摘要

三项阴性乳癌（TNBC）的定义是，具有独特特征的雌激素受体（ER）阴性、孕激素受体（PR）阴性和人体表皮生长因子受体 2（HER2）阴性的乳癌。因此，它不能使用靶向这些受体的药物治疗，从而导致总体上预后不良。微小核糖核酸 miRNA）已被证实涉及各种细胞过程的调节，意指它们可作为癌基因或肿癌抑制子而起作用。包括我们自己的发现在内的现化进展显示，在三项阴性乳癌（TNBC）的发展和治疗中，某些微小核糖核酸（miRNA）起着重要作用。在此，我们概述了微小核糖核酸（miRNA）在三项阴性乳癌（TNBC）处理中的作用，其重点在于现代已证实调节失调的微小核糖核酸（miRNA）所包含的诊断、预后和治疗的含意。

关键词：乳癌，微小核糖核酸（miRNA）,生物标记物，三项阴性乳癌（TNBC）

导言

乳癌是最常见癌症类型之一，每年全世界报导病例多于 130 万，而死亡数为 45 万例 [1]，在美国预期是 8 个妇女中有一名将在她一生中发生乳癌 [2] 。三阴性乳癌（TNBC）的定义是雌激素受体（ER）阴性，孕激素受体（PR）阴性和人体表皮生长因子受体 2（HER2）阴性的乳癌。因此，这种癌症亚型不能应用靶向雌激素受体、孕激素受体或人体表皮生 长因子受体 2 的特异性治疗药剂治疗，

ER, PR, or HER2, resulting in poor prognosis [3-5]. Although approximately 50% of TNBC patients respond to conventional chemotherapies, including taxanes, anthracyclines, cyclophosphamide, and platinum salts [6], there is still in need for specific markers for an effective targeted therapy of TNBC, due to its high metastatic nature and its systemic and local recurrence. Extensive research efforts are actively looking for targeted therapies to effectively treat this aggressive disease [4, 7].

从而导致预后不 [3-5]。虽然大概有 50%的三阴性乳癌病人对常规化学治疗药物，包括 Taxanes, Anthracyclines, Cyclophosphamide 以及铂盐 [6] 等有反应，但仍需要一些特殊的标记物作为三项阴性乳癌的有效靶向治疗，因为此癌症具有较高的转移特性，以及系统的和局部的复发之故。目前广泛的研究努力是，积极地找寻有效的治疗这种侵袭性疾病的靶向治疗 [4-7] 。

MicroRNAs (miRNAs) are short (18-24 nucleotides) noncoding single-stranded RNA molecules, which are cleaved from larger, 70-100 nucleotide miRNA precursors. miRNAs regulate gene expression either at the transcriptional or translational level, based on its specific binding to the complementary sequence in the coding or noncoding region of mRNA transcripts. [8]. miRNAs have been implicated in the regulation of a variety of cellular processes, including stemness and metastasis, implying that they can function either as oncogenes or tumor suppressors [9]. Recent findings have revealed that miRNA profiles discriminate malignancies of the breast [10], lung [10, 11], pancreas [10, 12], and liver [13-15] from their counterparts [16]. Cell signaling pathways in both normal and tumor tissues are regulated by different miRNAs [7,8]. Since miRNA dysregulation is highly correlated with cancer, new method for selectively silencing whole families of miRNAs may provide a new paradigm for disease therapy [17].

微小核糖核酸（miRNA）是一种短链的（18-24 个核苷酸）非编码的单股核糖核酸分子，它是由较大的 70-100 核苷酸组成的微小核糖核酸前体物裂解而来，它能在转录水平或翻译水平上调节基因的表达，其基础在于它能在信使核糖核酸（miRNA）转录的编码区域或非编码域内特异性地结合到互补的序列上[8]。微小核糖核酸（miRNA）已被证实涉及到调节已被证实涉及到调节各种细胞过程，包括家系血统（Stemness）和转移，意指它们能作为癌症基因或肿瘤抑制子而发挥作用[9]。近代发现揭示，微小核糖核酸（miRNA）的状况，能区别开乳腺[10]、肺脏[10,11]，胰腺[10,12]和肝脏[13,15]的恶性肿瘤及其相应的正常结构[16]。在正常和肿瘤两种组织中的细胞信号通路，是由不同的微小核糖核酸（miRNA）所调节[7,8]，由于微小核糖核酸（miRNA）失调与癌症高度相关，因此选择性地阻抑整个家族微小核糖核酸（miRNA）的新方法，可能提供癌症治疗的一个新的范例[17]。

Different types of cancers at different developmental stages display unique expression profiles of microRNAs. This suggests the use of these unique microRNA expression patterns as tumor diagnostic and prognostic tools, but also for future microRNA gene therapy [18]. Our review will focus on the deregulated miRNAs in the cell lines and patients' samples in TNBC, which will highlight the potential implications of miRNAs in breast cancer diagnostics, prognosis and treatment strategies [19].

在不同发展阶段的不同类型癌症，显示独特的微小核糖核酸(miRNA)的表达状态，这就提示，可能使用这些独特的微小核糖核酸(miRNA)表达样式来作为肿瘤诊断和预后的工具，而且可作为今后微小核糖核酸(miRNA)基因治疗之用[18]。我们的评述重点将放在三项阴性乳癌(TNBC)细胞株和临床病例的调节失调的微小核糖核酸(miRNA)上，这将强调在乳癌诊断，预后和治疗战略中微小核糖核酸(miRNA)具有潜在的重要含义[19]。

Differentially expressed miRNAs in TNBC cell lines

Cell lines have retained most of the genomic abnormalities of the original tumors. Thus, it is widely used in cancer research. For example, MDA-MB-231 and BT549 cell lines are representative TNBC cell lines [20]. MCF7 and T47D cells represent the luminal A subtype [21]. Unsupervised hierarchical clustering of miRNA expression based on 33 breast cell lines clearly separated breast cancer cell lines from normal breast samples, which suggests that miRNA expression in breast cancer

三阴性乳癌细胞株中不同表达的微小核糖核酸

该细胞株已保留其原始癌瘤的大多数基因异常，因此，它已被广泛用作癌症研究。例如，MDA-MB-231 细胞株和 BT549 细胞株，已用作三项阴性（TNBC）细胞株的代表[20]，MCF7 细胞株和 T47D 细胞株代表腔隙型(Luminal)A 亚型[21]。来自 33 个乳癌细胞株的不受监管大量群集的微小核糖核酸（miRNA）表达，已能清晰地区分开乳癌细胞株和正常乳腺样本。这就提示，在乳癌细胞株中微小核糖核酸（miRNA） 的表达，大部分是管控不良的

cell lines is largely dysregulated, particularly for TNBC cell lines [22]. For example, cluster-miR-17 members, miR-19a, -205 and -146a were upregulated, while miR-451 was down-regulated in TNBC [23].

Several deregulated miRNAs have been identified in TNBC cell lines compared to normal cell lines by using qRT-PCR method (Table 1). miR-205 is significantly down-regulated in TNBC cell lines compared to a normal-like cell line. This miRNA is a novel transcriptional target of p53, and that it exerts a role as tumor suppressor in TNBC, especially in a model representative of the most undifferentiated and mesenchymal subgroup. This is shown to be done primarily through the regulation, at least in part, of the two newly identified target genes, E2F1 and LAMC1, thus resulting in reduced cellular proliferation, both in vitro and in vivo [24]. miR-146a/b-5p are either weakly or not expressed in normal mammary cell lines, while their expression is upregulated in some tumor cell lines, particularly in those which have been classified as basal-like. This study also suggested that the two microRNAs negatively regulating BRCA1 expression [25]. TNBC cell lines (MDA-MB-468 and MDA-MB-231) showed significant miR-203 repression. This signifies that miR-203 could inhibit the proliferation and migration of TNBC cells by directly regulating the expression of BIRC5 and LASP1 [26]. We have recently reported that miR-638 regulates BRCA1 expression in TNBC [27].

Downregulated miRNAs in TNBC

A lot of miRNAs were reported to be downregulated or lost in TNBC cell lines, such as miR-31, miR-19, and miR-200f [28-32]. Augoff K et al detected the mature miR-31 and the pri-miR-31 in non-malignant breast epithelial MCF10A, luminal MCF7, SKBR3 and T47D, and basal MDA-MB-435S, MDA-MB-231 and BT549 BC cell lines. They found that miR-31 expression is lost in aggressive basal-type breast cancer cell lines. Loss of miR-31 expression in TNBC cell lines is attributed to hypermethylation of its promoter associated CpG island [31]. Zhang X, et al found that miR-19 is highly expressed in MCF-7 cells versus MDA-MB-231 cells. Application of the miR-19 inhibitor induces endogenous tissue factor expression in MCF-7 cells, and overexpression of miR-19 down-regulates tissue factor expression in MDA-MB-231 cells. This study demonstrates that miR-19 regulates tissue factor expression in breast cancer cells, providing a molecular basis for the selective expression of the tissue factor gene [32]. Strong downregulation of miR-200f is

失 调节产物，特别是三项阴性乳癌（TNBC）细胞株更是如此[22]。例如，聚集子-微小核糖核酸（miR）-17 组的成员，以及 miR-19a、miR-205 和 miR-146a 都是三项阴性乳癌（TNBC）的升高型调节物，而 miR-451 则是其降低型调节物[23]。

应用 gRT-PCR 方法（附表 1），与正常乳腺细胞株对比，已从三项阴性乳癌（TNBC）细胞株中鉴定出若干种失调节的微小核糖核酸（miRNA）。对比正常乳腺样细胞株，在三项阴性乳癌（TNBC）细胞株中，miR-205 是一种明显的降低型调节物，这种微小核糖核酸（miRNA）是 P53 蛋白质的新颖的转录标靶，它在三项阴性乳癌（TNBC）中作为肿瘤抑制者发挥作用，特别是在最不分化的间充质性亚群的模式代表中更是如此。这就表明它主要是通过调节而致，至少部分是通过两个新近鉴定的标靶基因 E2F1 基因和 LAMC1 基因，因此导致体外和体内的癌细胞两者增殖降低[24]。miR-146a-5P 和 miR-146b-5P 在正常乳腺细胞株中是弱表达者或不表达者，而它们在某此癌瘤细胞株中的表达是增高型调节物，特别是在那些已被归类为基本样式的细胞株中更为如此。这个研究也证实，这两种微小核糖核酸（miRNA）是 BRCA1 表达的阴（负）性调节者[25]。三项阴性乳癌（TNBC）细胞株（MDA-MB-468 株和 MDA-MB-231 株）表明有明显的 miR-203 表达抑制。这就表明 miR-203 能通过直接调节 BIRC5 和 LASP1 的表达，而抑制三项阴性乳癌（TNBC）的增殖和迁移[26]。我们最近已报导，miR-638 能调节三项阴性乳癌（TNBC）中 BRCA1 的表达[27]。

三阴性乳癌中下调的微小核糖核酸

既往报导，已有许多种微小核糖核酸（miRNA）是三项阴性乳癌（TNBC）细胞株中的降解调节者或丢失者，例如 miR-31, miR-19 和 miR-200f [28-32]。Augoff K 等人测试了非恶性乳腺上皮 MCF10A 细胞株，腔隙型（Luminal）MCF7、SKBR3 和 T47D 细胞株，以及基底以及基底型 MDA-MB-435B、MDA-MB-231 和 BT549BC 细胞株中的成年（熟）型 miR-31 和原始型 pri-miR-31。他们发现，在侵袭性的基底型乳癌细胞株中缺乏 miR-31 的表达。三项阴性乳癌细胞株中 miR-31 的表达缺失，是由于与 CpG 岛区相关的其启动区的高度甲基化所致 [31]。Zhang X 等人发现，相对于 MDA-MB-231 的细胞株而言，在 MCF-7 细胞株中 miR-19 有高度表达，应用 miR-19 抑制剂，能导致 MCF-7 细胞株中内生性组织因子的表达，以及 MDA-MB-231 细胞株中 miR-19 降解调节组织因子的过度表达。这个研究显示，在乳癌细胞中 miR-19 负责调节组织因子的表达， 并提供了选择性表达该组织因子基因的分子学基础[32]。 与腔隙型

observed in mesenchymal-like basal B cell lines (MDA-MB-231, BT-549) compared with luminal (MCF7, T47D) and HER2+ (SKBR3, BT474) cell lines. Downregulation of miR-200f and hypermethylation of the miR-200c -141 locus, together with upregulation of EMT (Epithelial–mesenchymal transition) -transcriptional inducers also occur in an in vitro cellular model of spontaneous EMT [33] .

Upregulated miRNAs in TNBC

However, some miRNAs have higher levels of expression in TNBC cell lines. For example, miR-206 expression was upregulated in MDA-MB-231 cells compared with ER-positive MCF-7 cells. miR-206 represses endogenous ER expression in both human MCF-7 and T47D breast cancer cells [34]. In vitro study suggested that miR-182 can promote proliferation and invasion of MDA-MB-231 cells and could negatively regulate the target gene PFN1 expression [35]. In comparison to the expression level in normal breast tissue, miR-221 is upregulated in all the TNBC lines detected (MDA-MB-231, Hs578T, BT-20, and MDA-MB-468), but not in non-TNBC lines, such as T47D, ZR75-1, and MCF-7. miR-221 knockdown not only blocked cell cycle progression, induced cell apoptosis, and inhibited cell proliferation in vitro, but it also inhibited in vivo tumor growth by targeting p27kip1. Furthermore, miR-221 knockdown inhibited cell migration and invasion by altering E-cadherin expression and its regulatory transcription factors Snail and Slug in human TNBC cell lines [36].

Expression of miR-15a, miR-15b, miR-16 and miR-128 were assayed 9 human breast cancer cell lines, MCF-9, T47D, MDA-MB-231, BT549, MDA-MB-436, DU4475, MDA-MB-468, BT474 and SK-BR-3, compared to MCF-10A cells. DU4475 cells showed increased expression of miR-15b, miR-16 and miR-128. BT549 cells exhibited increased expression of miR-15a, miR-15b and miR-16. MDA-MB-436 cells had increased expression of miR-15b, miR-16, and miR-128. Thus, these TNBC cell lines that exhibited Smurf2 downregulation had a tendency to express higher levels of these miRNAs. In contrast, MDA-MB-231 cells, which had high levels of Smurf2 mRNA and protein, showed no major change in the expression of these miRNAs, except for a decrease in

（MCF7 和 T47D）细胞株以及 HER2+型（SKBR3 和 BT474）细胞株相比，在间充质样基底型 B 细胞株（MDA-MB-231 和 BT-549）中可见明显的降解调节的 miR-200f。miR-200f 的降解调节和 miR-200c-141 部位的高度甲基化，与 EMT（上皮-间充质转变）-转录诱发剂的升高调节一起，也见于体外培养的自发性上皮-间充质转变的模型细胞株[33]。

三阴性乳癌中上调的微小核糖核酸

但是，在三项阴性乳癌（TNBC）细胞株中有些微小核糖核酸（miRNA）有较高水平的表达，例如，与雌激素受体（ER）-阳性的 MCF-7 细胞株相比，在 MDA-MB-231 细胞株中，miR-206 微小核糖核酸表达调节增高。在人体 MCF-7 和 T47D 两种乳癌细胞株中，miR-206 抑制了内生性雌激素受体的表达[34]。体外研究证示，miR-182 微小核糖核酸能促进 MDA-MB-231 细胞株的增殖和侵入行为，并能负面（阴性）调节靶基因 PFN1 的表达[35]。与正常乳腺组织的表达水平相比，miR-221 微小核糖核酸，在所有测定的三项阴性乳癌（TNBC）细胞株中(MDA-MB-231,Hs578T,BT20 和 MDA-MB-468)都具有增高调节作用，但是在非三项阴性乳癌细胞株中均并非如此，例如在 T47D、ZR75-1 和 MCF-7 细胞株。已知 miR-221 的降低，不仅能在体外培养时阻抑细胞周期前行，诱发细胞 apoptosis，以及抑制细胞增殖，并且也能通过靶向 p27kip1 而抑制体内癌瘤的生长。因此，miR-221 的降低，在人体三项阴性乳癌（TNBC）细胞株中，通过改变 E-cadherin 表达，以及它的转录因子 Snail 和 Slug, 抑制了细胞迁移和侵入[36]。

miR-15a，miR-15b，miR-16 和 miR-128 曾被 9 株人体乳癌细胞株测试，这些细胞株为 MCF-9，T47D, MDA-231, BT549, MDA-MB-436, DU4475, MDA-MB-468, BT474 和 SK-BR-3, 其对比组为 MCF-10A 细胞株。DU4475 细胞株表明，其 miR-15b, miR-16 和 mir-128 的表达增高。BT549 细胞株，则抑制 miR-15a，miR-15b 和 miR-16 的表达增高。MDA-MB-436 细胞株，则增加 miR-15b, miR-16 和 miR-128 的表达。因此，这些三阴性乳癌（TNBC）细胞株都抑制了 Smurf2 的降解调节，并倾向于表达其较高水平的微小核糖核酸（miRNA）。 相反，MDA-MB-231 细胞株具有较高水平的 Smurf2 微小核糖核酸和蛋白质，除有较低水平的 miR-15a 外，其微小核糖核酸（miRNA）

the level of miR-15a. Also in MCF-7 cells, the levels of miR-15a, miR-15b and miR16 were low, whereas the expression of miR-128 was modestly increased. The study further revealed that miRNAs such as miR-15a, miR-15b, miR-16 and miR-128, downregulate translation of Smurf2 protein in TNBC cells [37].

Based on the cell lines studies, miR-46a/b-5p, miR-19a, miR-205, and miR-146a, miR-206, and miR-221 were upregulated, while miR-205, miR-203, sf-miR-451, miR-31, miR-19, and miR-200f were down regulated in TNBC cell lines. These deregulated miRNAs may be important in maintaining the characteristics of TNBC cells, and may function as oncomiRs or tumor suppressor miRNAs in TNBC cells [26, 36].

的整个表达没有较大的改变。同样，在 MCF-7 细胞株，其 miR-15a，miR-15b 和 miR-16 的表达水平较低，而 miR-128 的表达呈轻度增加。这个研究进一步揭示，在三项阴性乳癌（TNBC）细胞株内，例如 miR-15a, miR-15b, miR-16 和 miR-128 等微小核糖核酸（miRNA），能降解调节 Smurf2 蛋白质的翻译[37]。

根据细胞株的研究获知，在三阴性乳癌（TNBC）细胞株中，miR-46a/b-5p、miR-19a、miR-205、以及 miR-146a、miR-206 和 miR-221 都是增高调节，而 miR-205、miR-203、sf-miR-451、miR-31、miR-19 和 miR-200f 均具有降解调节作用。这些调节失调的微小核糖核酸（miRNA），在维持三项阴性乳癌（TNBC）细胞株的特征方面可能具有重要意义，而且在三项阴性乳癌（TNBC）细胞株内，可能作为癌症微小核糖核酸（OncomiR）或癌瘤抑制子对微小核糖核酸（miRNA）发挥作用[26,36]。

Table 1. Differentially expressed microRNAs and their potential functions in TNBC cell lines
表 1. 三阴性乳癌（TNBC）细胞株内分化表达的微小核糖核酸（miRNA）及它们的潜在功能

Deregulated miRNAs 失调节的微小核糖核酸	Potential Functions / Mechanisms 潜 在 功 能 / 机 制	References 参考文献
miR-206	Represses endogenous ERα expression 抑制内生性雌激素受体 a（Era）的表达	[34]
miR-31	Expression lost is attributed to hypermethylation of its target promoter 表达丢失是由于它的靶向促进子的过度甲基化所致	[31]
miR-200f	Related to EMT in breast cancer 在乳癌中与上皮-间充质转变（EMT）有关	[33]
miR-146a/b-5p	Negatively regulates BRCA1 expression 负面（阴性）调节 BRCA1 的表达	[25]
miR-182	Promotes cell proliferation and invasion and regulates the target gene PFN1 促进细胞增殖和侵入，并调节靶子基因 PFN1	[35]
miR-15b, -16, -128, -15a	Downregulates translation of Smurf2 protein 降解调节 Smurf2 蛋白质的翻译	[37]
miR-221	Functions as an oncomir 作为癌症微小核糖核酸（Oncomir）起作用	[36]
miR-205	May function as a tumor suppressor 可能作为癌瘤抑制子发挥作用	[24]
miR-203	May function as a tumor suppressor 可能作为癌瘤抑制子发挥作用	[26]
miR-19	Regulates tissue factor expression 调节组织因子的表达	[32]

Differentially expressed miRNAs in TNBC tissue

Though the studies in cell lines can provide important information, the comparisons between cell lines and primary tumors show that the cell line collection, as a system, mirrors many but not all biological and genomic properties of primary tumors [20]. Major differences in miRNA expression between primary

在三阴性乳癌组织中不同表达的微小核糖核酸

虽然在细胞株中的许多研究都能提供重要的信息，但是在细胞株和原发性癌瘤之间的对比表明，细胞株的收集可用作一个系统，来反映许多但并非全部原发性癌瘤的生物学和基因学特性 [20]。在主要的人体组织和细胞株之间，存在着微小的核糖核

human tissue and cell lines exist [22]. Therefore, the recognition of miRNAs that are differentially expressed between normal and tumor samples may help identify those that are involved in human cancer and establish the basis to unravel their pathogenic roles [38]. Importantly, it has been demonstrated that the feasibility of miRNA expression profiling analysis using archived FFPE tissues, rich with clinical information, as the means towards miRNA biomarker discovery [39]. Microarray studies on miRNA levels in various human breast cancer tissues have revealed that some miRNAs are up regulated in breast cancer vs. normal breast tissue [38].

miRNA expression signatures can differentiate normal and breast cancer tissues [40]. One study used miRNA microarray technology to evaluate miRNA expression profiles between 76 neoplastic breast tissues and 10 normal tissues. Among the differentially expressed miRNAs, miR-10b, -125b, -145, -21, and -155 emerged as the most consistently deregulated miRNAs in breast cancer. Three of them, miR-10b, -125b, and -145, were down-regulated and the remaining two, miR-21 and -155, were up regulated, suggesting that they may potentially act as tumor suppressor genes and oncogenes, respectively. The overall miRNA expression could clearly separate normal versus cancer tissues, with the most significantly deregulated miRNAs being miR-125b, -145, -21, and -155 [38]. Another study analyzed the expression of 667 miRNAs in 29 tumors and 21 adjacent normal tissues, resulting in 130 differentially expressed miRNAs (17 upregulated and 113 downregulated) [40].

Deregulated miRNA expression signatures can differentiate TNBC from other subgroups

miRNA expression patterns allow an accurate discrimination between different types of breast cancer, and the miRNA signatures may predict ER, PR and HER2 status in breast cancer [42-44]. TNBC was the most distinctive type from other tumor subtypes, and showed the largest number of miRNA dysregulation, such as the mir17-92 cluster (Table 2)[22, 23, 45, 46]. For example, miR-150, -142-3p, -18a, -155, -135b, -126, -100, -99a, -10a, -130a, and -342 were associated with the molecular subtype of the patients with TNBC [22]. Enerly E et al showed that miRNA expression alone is sufficient to distinguish luminal-A from basal-like samples. The top miRNAs with elevated

酸（miRNA）表达的重大区别[22]。因此，识别在正常和癌瘤样本之间分化表达的微小核糖核酸（miRNA），可能有助于鉴定涉及人体癌症的这些物质，并建立基础来阐明它们的病原学作用[38]。更重要的是业已表明，微小核糖核酸（miRNA）的表达，具有对使用所获的 FFPE 组织，内含丰富临床信息，从而进行轮廓描述分析的可行性特点，因为该方法优点是可朝向微小核糖核酸（miRNA）生物标记发现方向发展[39]。在各种人体乳癌组织中微小核糖核酸（miRNA）水平的微量测试研究表明，在乳癌对比正常乳腺组织时，其中某些微小核糖核酸具有增高调节作用[38]。

微小核糖核酸（miRNA）表达的信号，能区分开正常乳腺和乳癌组织[40]。有一研究在 76 例新生的乳癌组织和 10 例正常乳腺组织之间，应用微小核糖核酸（miRNA）微量测试技术，来揭示微小核糖核酸（miRNA）表达的概况。在分化表达的微小核糖核酸（miRNA）之中，miR-10b、miR-125b、miR-145、miR-21 和 miR-155，显示是乳癌中最恒定的失调节作用的微小核糖核酸（miRNA）。其中有三种，即 miR-106、miR-125b 和 miR-145 是降解调节者，而其余两者即 miR-21 和 miR-155 是增高调节者，这就提示它们可能分别以癌瘤抑制子基因和癌症基因而具有潜在性作用。总体来说这些微小核糖核酸（miRNA）的表达，能够清晰地区分正常乳腺组织和乳癌组织，其中最明显的调节失调的微小核糖核酸，是 miR-125b、miR-145、miR-21 和 miR-155 [38]。另一研究在 29 例乳癌组织和 21 例邻近的正常乳腺组织中，分析了 667 种微小核糖核酸（miRNA），结果显示有 130 种分化表达的微小核糖核酸（miRNA）（17 种为增高调节者，113 种为降解调节者[40]。

调节失调的微小核糖核酸（miRNA）表达信号能区分开三项阴性乳癌（TNBC）与其它亚型

微小核糖核酸（miRNA）表达的方式，可允许在不同类型的乳癌之间作出正确的区别，而且这些微小核糖核酸（miRNA）信号，可以预告乳癌中雌激素受体（ER）、孕激素受体（PR）和人体表皮生长因子受体 2（HER2）的状态[42-44]。三项阴性乳癌（TNBC）曾是来自其它癌瘤亚型的最明显的一种类型乳癌而且表明有最大数量的微小核糖核酸（miRNA）调节不良，例如 mir17-92 聚集子（表 2）[22,23,45,46]。举例如下：miR-150、miR-142-3P、miR-18a、miR-155、miR-135b、miR-126、miR-100、miR-99a、miR-10a、miR-130a 和 miR-342,都与 三阴性乳癌 （TNBC）病人 的分子亚型相关[22]。Enerly E 等人表明，仅用一项微小核糖核酸（miRNA）表达，即足以区分开腔隙型-A（Luminal A）和基底型样乳癌样本。在基底型癌症

expression levels in basal-like samples were miR-18a/b and other members of the miR-17-92 cluster (miR-17/17*, -18a/b, -19a, -20a and -106a). Among the prominently downregulated miRNAs in basal-like tumors were the representatives of the miR-29 family along with miR-190b [45]. Cluster-mir-17 member miR-19a, present in ductal cell lines, were actually highly expressed in TNBC compared to non-TNBCs. Special histological TNBCs (atypical medullary, metaplastic, adenoid cystic, and apocrine carcinomas) were differentiated from invasive TNBCs by less abundant miR-224, absent from some ductal cell lines. Oncogenic cluster-mir-17-92 member miR-19a showed approximately 3-fold higher levels in TNBCs, suggesting regulatory potential [23].

Another interesting observation is the presence of several miRs (miR-532-5p, -500, -362-5p, and -502-3p) located at Xp11.23 in cancers with a TN signature and the upregulation of several miR-17 cluster members in ER negative tumors [46]. miR-342 expression was the highest in ER and HER2/neu positive luminal B tumors and lowest in TN tumors. miR-520g expression was elevated in ER and PR negative tumors [47]. Volinia S et al identified differentially expressed miRNA in the IDC subtypes by expression matrix contained measures for 159 miRNAs in 107 samples. Examples are as follows: miR-190 was overexpressed in ER+/HER2− IDC; TN IDC was characterized by activation of the Myc-regulated miR17/92 oncomir cluster, miR-200c, and -128; and miR-145, -143*, -331, and -199b-5p were the most repressed miRNAs in TNBC. Conversely, miR-200c was among the most repressed miRNAs in ER+/HER2+ double-positive breast cancers, together with miR-148a and -96 [42]. Both miR-205 and miR-342 expressions were significantly down regulated in TNBC [48]. But the expressions of miR-210, -181a, -155 in TNBC were significantly higher than in non-TNBC subtypes [49-51].

Highly expressed miRNAs in TNBC include miR-17/17*,-17/92, -18a/b, -19a, -20a , -106a, -146a, -205, -362-5p, -500, -502-3p, and -532-5p . Down-regulated miRNA in TNBC compared with other subtypes were as follows: miR-29 family, -128, -143*, -145, miR-190b, -199b-5p, -200c, -205, -331, -342,

样本中具有表达水平增高的顶尖的微小核糖核酸(miRNA），有 miR-18a/b 和 miR-17-92 聚集子的其它成员(miR-17/17*, miR-18a/b, miR-19a, miR-20a 和 miR-106a)。在基底型样乳癌中明显降解调节的微小核糖核酸（miRNA），有 miR-29 家族的一些代表以及 miR-190b [45]。聚集子(Cluster)-mir-17 家族成员 miR-19a，存在于导管型癌细胞株内，对比非三项阴性乳癌来说，它在三项阴性乳癌（TNBC）中实际上有较高的表达。特殊组织学特征的三项阴性乳癌（TNBC）（具有不典型髓腔，后成质性，腺样囊泡，以及顶浆分泌性肉瘤），是由侵润型三项阴性乳癌（TNBC）通过减少丰富量的 miR-224 分化而来，但后者在某些导管型细胞株内却缺失。而在三项阴性乳癌（TNBC）中，致癌性聚集子（Cluster）-mir-17-92 的成员 miR-19a 的水平大概高三倍，实其具有调节的潜力[23]。

另一个很有意义的观察是，在伴有一个 TN 信号的乳癌细胞的 Xp11.23 部位，存在有若干种微小核糖核酸（miR-532-5P, miR-500, miR-362-5P 和 miR-502-3P）,而在雌激素受体（ER）阴性的乳癌细胞内，具有若干种 miR-17 聚集子成员的增高调节作用[46]。在雌激素受体（ER）和人体表皮生长因子受体 2/神（HER2/neu）阳性的腔隙性 B 型乳癌中，miR-342 的表达最高，而 在 TN 乳癌，其表达最低。在雌激素受体（ER）和孕激素受体（PR）阴性乳癌中，miR-520g 的表达增高[47]。Volinia S 等证实，在 107 例 IDC 亚型乳癌中，分化表达的微小核糖核酸（miRNA），是通过表达含有基质的测量方法测试出 159 种微小核糖核酸。其例证如下：miR-190 的过度表达，见于雌激素受体阳性/人体表皮生长因子受体 2 阴性 IDC 乳癌中(ER+/HER2-IDC）；在 TNIDC 乳癌细胞内的特征，是激活 Myc 调节的 miR17/92 致癌微小核糖核酸聚集子（oncomir cluster），miR-200C 和 miR-128；而在三项阴性乳癌（TNBC）中，miR-145、miR-143*、miR-331 和 miR-199b-5P 是表达最受抑制的微小核糖核酸。相反，在雌激素受体阳性和人体表皮生长因子受体 2 阳性（ER+/HER2+）双项阳性乳癌中，表达最多的微小核糖核酸（miRNA）是 miR-200C,合并一起增多的还有 miR-148a 和 miR-96(42)。在三项阴性乳癌（TNBC）中，miR-205 和 miR-342 两者的表达是明显的降解调节[48]。但是，在三项阴性乳癌（TNBC）中，miR-210、miR-181a 和 miR-155 的表达，要明显高于非三项阴性乳癌（non-TNBC）亚型者[49-51]。

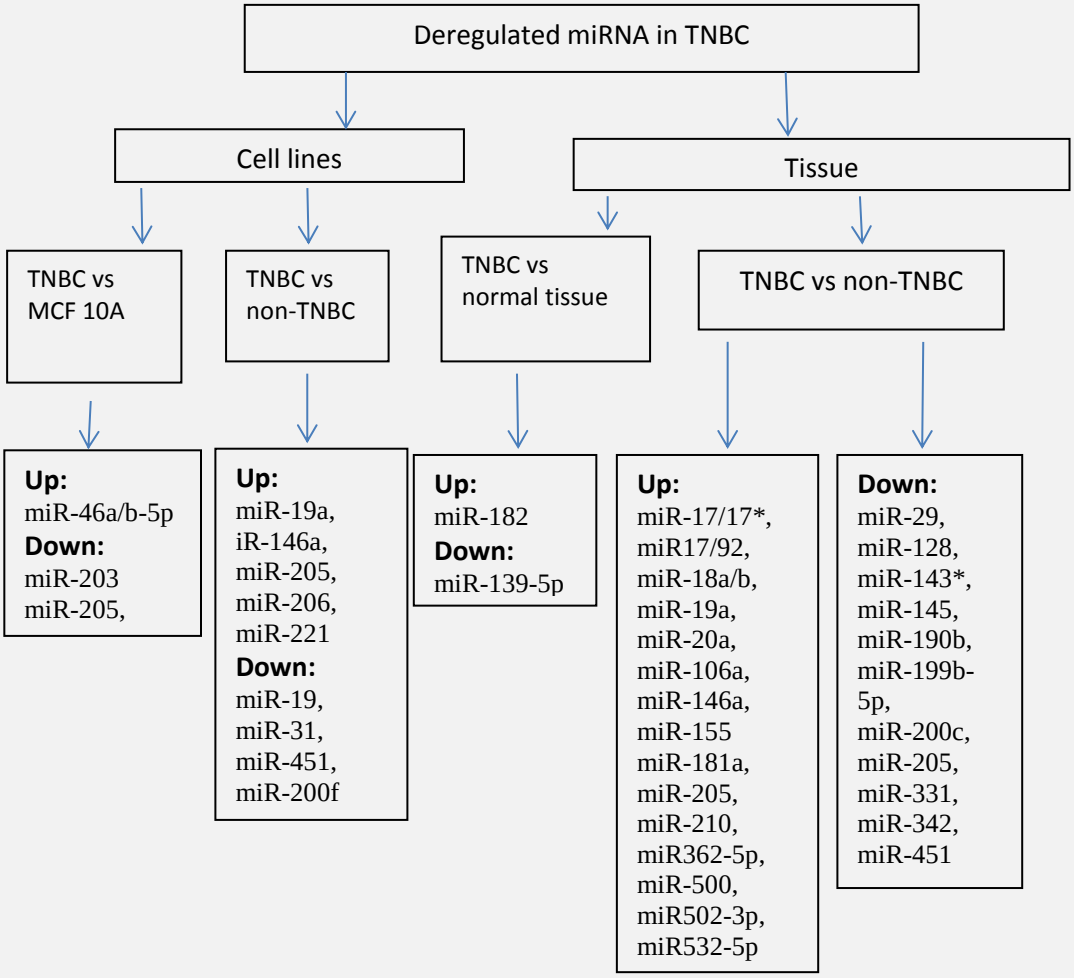
在三阴性乳癌（TNBC）中高度表达的微小核糖核酸,包括 miR-17/17*, miR-17/92, miR-18a/b, miR-19a, miR-20a, miR-106a, miR-146a, miR-205, miR-362-5p, miR-500, miR-502-3p 和 miR-532-5p. 在三项阴性乳癌（TNBC）内与其它亚型对比，其降解调节的微小核糖核酸名称如下： miT-29 家族， miR-128， miR-143*， miR-145， miR-190b, mir-199b-5p, miR-200c, miR-205, miR-331 和 miR-

and -451. These differently expressed miRNA may be useful to distinguish TNBC and non-TNBC. And they can be used to explore the new targets for TNBC specified treatment.

451。这些分化表达的微小核糖核酸（miRNA），可能有助于区分开三项阴性乳癌（TNBC）和非三阴性乳癌，而且它们也能有助于为三阴性乳癌（TNBC）的特定治疗探测新的标靶。

Figure 1. Differentially expressed miRNAs in TNBC

图 1. 三阴性乳癌内不同表达的微小核糖核酸



Prognostic significance of deregulated miRNAs in TNBC

A more accurate means of prognostication in breast cancer will improve the selection of patients for adjuvant systemic therapy, reducing the rate of both overtreatment and undertreatment [52]. miRNA expression signatures can be used to predict TNBC metastasis and survival (Table 2) [18, 42, 45, 53]. One study identified 14 miRNAs associated with prognosis in ER-negative tumors; of these, 7 were also significant in the subset of TNBC tumors, four miRNAs (miR-16-2, -766, -381, -409-5p) showing

三阴性乳癌内失调微小核糖核酸的预后意义

对乳癌的一个更正确的预后方法，将改进对病人辅助治疗的选择，降低过度治疗和治疗不足的两项比率[52]。微小核糖核酸表达信号，能够用来预告三项阴性乳癌（TNBC）的转移和存活（表 2）[18, 42,45, 53]。有一研究证实，在雌激素受体（ER）阴性乳腺肿瘤中，有 14 种微小核 糖 核酸与 预后相关。其中在三项阴性乳癌（TNBC）亚型中，有 7 种微小核糖核酸也表现明显，有 4 种微小核糖核酸（名称是 miR-16-2，miR-766,miR-381 和 miR-409-5p）,在三项阴性乳癌（TNBC）中表现与距离-转移免于存活（DMFS）有 更为 强 有力的相关。

strongest association with distant-metastases free survival (DMFS) in TNBC tumors. They also found that expression levels of 64 miRNAs were associated with DNA copy number changes [54]. Among miRNAs with prognostic value in ER-negative samples, five (miR-150, -342, good prognosis; and miR-210, -144, -27b, poor prognosis) were also related with distant relapse-free survival (DRFS) of TNBC [55].

miR-210 has been analyzed in several studies [42, 49, 56]. The median miR-210 expression level has dichotomized time to distant metastasis (TDM) curves as a function of subgroups in the 69 TNBC specimens studied. Bioinformatic analysis coupled miR-210 to hypoxia/VEGF signaling [56]. A higher expression of miR-210 was an independent factor indicating a worse prognosis, and related with time to metastasis and overall survival [42, 49]. Another similar study found that there was a tendency for high expression of miR-21, -210, -221 and -222 to be associated with worse patient disease-free and overall survival (OS), However, this observation did not reach statistical significance [18].

The dysregulated miRNAs may be related to the OS or disease-free survival (DFS) of the patients (Table 2). The data from 173 TNBC showed that miR-16, -155, -493, and -374a (protective), miR-125b, -30e, and -27a (risk) are correlated with OS [53, 57]. Another study of all 231 patients (including 152 TNBC) suggested a negative correlation between OS and miR-155 expression level [51]. The expression of miR-181a significantly predicted shorter DFS of breast cancer patients whose tumors lacked amplification of the ErbB2 locus [50]. miR-34b expression was negatively correlated with DFS and OS in TNBC patients [58].

Some miRNAs can be a biomarker for lymph node (LN) metastasis. The expression of miR-101 was negatively correlated with the percentage of LNs positive while the negative correlation of let-7b and miR-29c with the percentage of LNs positive approached significance[58]. Elevated miR-155 was significantly associated with late-stage (stage III/IV) and high-grade tumors, lymph node metastasis and TNBC [51].

miR-16, -374a, and -493 might be good prognostic markers, and miR-27a , -30e, -34b, -125b, -181a, and -210 might be poor prognostic markers. These factors can be useful for decision-making regarding treatment in the adjuvant setting in TNBC patients. They can also be the potential target molecules in the therapy of TNBC.

他们也发现，有 64 种微小核糖核酸的表达水平，与 DNA 考贝数量变化相关[54]。在雌激素受体（ER）阴性乳癌标本内，在与预后评估有关的微小核糖核酸（miRNA）中，有 5 种微小核糖核酸（miR-150 和 miR-342 显示良好预后，而 miR-210、miR-144 和 miR-27b 显示预后不良），也显示与三项阴性乳癌（TNBC）的距离-转移免于存活（DMFS）相关[55]。

miR-210 曾被若干研究中分析[42,49,56]。在 69 例三项阴性乳癌（TNBC）研究标本中，应用中位数表达的 miR-210 水平数值分成两部分，针对距离转移时间（TDM）作成曲线，作为各亚型的一个功能指标，并结合 miR-210 对应低氧/VEGF 信号作成对性的生物信息分析（[56]。较高水平 miR-210 的表达，是表明较差预后的一个独立的因素，并与转移和总体存活的时间相关 [42,49]。另一研究发现，高水平表达的 miR-21, miR-210, miR-221 和 miR-222，倾向于与预后较差病人的无病患和总体存活（OS）相关，但是，这一观察结果并未具有统计学意义[18]。

调节失调的微小核糖核酸，可能与乳癌病人的总体存活（OS）或无疾病存活（DFS）相关（表 2）。来自 173 例三项阴性乳癌的资料显示，miR-16、miR-155、miR-493 和 miR-374a（均有防护功能），以及 miR-125b、miR-30e 和 miR-27a（均有危险性），都与总体存活（OS）相关 [57,58]。另一研究共计 231 例乳癌病人（包括 152 例三阴性乳癌）证实，在总体存活（OS）和 miR-155 表达水平之间存在负性相关[51]。miR-181a 的表达，能明显地预告乳癌病人较短的无疾病存活时间（DFS），这些病人的肿瘤缺乏 ErbB2 部位的扩大[50]。miR-34b 表达，与三阴性乳癌病人的无疾病存活（DFS）和总体存活（OS）,具有负性相关[58]。

某些微小核糖核酸，可作为淋巴结（LN）转移的生物标记物。miR-101 的表达，与淋巴结转移阳性的比例存在负性相关，而 let-7b 和 miR-29c 与淋巴结转移阳性比例之间的负性相关，具有统计学意义[58]。miR-155 的表达增高，与晚期阶段（III 期和 IV 期）和高级别癌瘤、淋巴结转移以及三项阴性乳癌(TNBC), 都存在明显相关 [51]。

miR-16、 miR-374a 和 miR-493，可能是预后良好的标记物，而 miR-27a、miR-30e、miR-34b、miR-125b、miR-181a 和 miR-210，可能是预后不良的标记物。这些因素能够有利于对三项阴性乳癌病人辅助治疗设计的策划决定，它们也能作为三项阴性乳癌（TNBC）治疗中的潜在有力的标靶分子。

Table 2. Differentially expressed microRNAs and the prognostic value in TNBC
表 2. 三项阴性乳癌中分化表达的微小核糖核酸（miRNA）和预后的评估

No. of TNBC samples 样本数量	Main findings in miRNAs deregulation 在微小核糖核酸调节失调的主要发现	Prognostic findings 预后发现	References 参考文献
37		miR-150, -342, -210, -144, -27b	[55]
173	116 miRNAs	miR-16, -155, -374a ,-125b	[53]
26	miR-200f		[33]
114		miR-16-2, 766, 381, 409-5p	[54]
15	miR-17/17*, -18a/b, -19a, -20a , -106a, -190b, and miR -29 family		[45]
48	miR-19a, -205, -146a, -451	Cluster-miR-423	[23]
69		miR-210	[56]
5	miR-532-5p, -500, -362-5p, -502-3p		[46]
152	miR-155	miR-155	[51]
18	miR-139-5p	miR-139-5p	[41]
NA	miR-182		[35]
11	miR-342		[47]
49	miR-10b, -145, -205, -122a -21, -210, -221	miR-21, -210, -221, -222	[18]
17	miR-205, -342		[48]
39		miR-34b	[59]
16	miR-181a	miR-181a	[50]
58	miR-210	miR-210	[49]
33	miR17/92 cluster, miR-200c, -128, -145, -143*, -331, -199b-5p	miR-210, -21, -221, -652, -210, -21, -106b*, -197, let-7i	[42]
173		miR-155, -493, -30e, -27a	[57]

miRNA can be predictive biomarkers for chemotherapy

Oncologists are therefore pursuing more personalized therapies. Gene expression analysis has been widely incorporated into these studies, but less is known about epigenetic factors such as miRNAs and their role in tailoring an individual systemic and surgical approach for breast cancer patients [60-62]. More precise identification, before initiation of treatment, of those patients who would benefit from specific chemotherapeutic regimens may improve the response rates, avoid toxicity of ineffective therapy and guide the extent of necessary surgery i.e. breast conservation versus mastectomy [63, 64]. In all 11 TNBC core biopsies 19

微小核糖核酸作为化学治疗预告性生物标记物

因此肿瘤专家正在寻求更加个人化的治疗。基因表达分析已被广泛地结合到这些研究之中，但是很少知道有关乳癌病人中，例如这些微小核糖核酸（miRNA）后生因子及其在制订一个单人系统的和外科的方法中的作用[50-62]。更精确的鉴定，治疗前的激发， 哪些病人能从 特 异性化学治疗进程中获得好处，这些都可能改进反应几率，避免无效治疗的毒性，以及指导必要外科即乳腺保留直到乳房切除术的程度[63,64]）。在总数 11 例三项阴性乳癌核心部位生物活检中，每个病例分析了以下 19 种微小核糖核酸（miRNA）：miR-512, miR-190,miR-200, miR-346, miR-148, miR-449, miR-203, miR-577,

miRNAs per sample: miR-512, -190, -200, -346, -148, -449, -203, -577, -93, -126, -423, -129, -193, -182, -136, -135, -191, -122 and -222 were analyzed. Higher miR-200b-3p, -190a and lower -512-5p expression levels in core biopsies may be associated with better pathologic response to chemotherapy and the increased feasibility of breast conserving surgery in these patients [62]. At the same time, drugs may exert their effects through regulation of miRNA expression levels. When MDA-MB-453 TNBC tumor cells were treated with 5-fluorouracil together with ixabepilone, miR-122a, -145 and -205 were elevated, miR-296 decreased, and miR-221, -210, -21 and -10b were also altered [65]. The predictive miRNAs for chemotherapy need further studies.

Significance of circulating cell-free miRNAs in TNBC

Currently, one of the most important challenges in the management of breast cancer is to discover sensitive and specific, yet minimally invasive, biomarkers that can be exploited to detect breast cancer at an early stage, as well as to monitor the progress of patients with breast cancer and their response to treatments [66]. It is known that the signatures of serum/plasma miRNAs may reflect correlations to physiological or disease conditions [67]. One study measured the relative concentrations of 6 circulating microRNAs (miR-10b, -17, -34a, -93, -155, and -373) in serum samples from 120 patients with primary breast cancer after surgery and before chemotherapy. The concentrations of circulating miR-34a, -93, and -373 were significantly higher in breast cancer patients than in healthy women. The deregulated serum concentrations of miR-17 and -34a were associated with hormonal receptor status. These findings indicate HER2+ patients had significantly lower miR-373 concentrations than women with TNBC [68]. TNBC had significantly higher level of plasma miRNA-30a than other types of breast cancer [66].

Prospects

TNBC is a heterogeneous disease at the molecular, pathologic and clinical levels with poor outcome. Molecular sub-classification of TNBCs is essential for optimal use of current therapies and for development of new drugs [53, 57, 69] . Deregulated miRNA expression is not only a marker for prognosis of TNBC, but it could also present an attractive target for therapeutic intervention [43, 47].

Abbreviations

TNBC: triple negative breast cancer; ER: estrogen receptors; PR: progesterone receptors; HER2: epidermal growth factor receptor 2; miRNAs: MicroRNAs; TDM: time to distant metastasis; OS: overall survival; DFS: disease-free survivl; LN: lymph node.

miR-93, miR-126, miR-423, miR-129, miR-193, miR-182, miR-136, miR-135, miR-191, miR-122 和 miR-222。在核心部位活检组织中，较高水平表达的 miR-200b-3p 和 miR-190a，以及较低水平表达的 miR-512-5p，都可能与这些病人对化学治疗较好的病理学反应，和增加保留乳腺外科手术的可行性相关[62]。在同一时间，通过调节微小核糖核酸表达水平，药物可能存在它们的影响。对 MDA-MB-453 三项阴性乳癌（TNBC）细胞，使用 5-fluororacil 合并 ixabepilone 药物治疗时，其 miR-122a、miR-145 和 miR-205 的表达水平增高，而 miR-296 表达降低，且 miR-221、miR-210、miR-21 和 miR-10b 的表达也发生改变[65]。这些化学治疗中具有预告作用的微小核糖核酸（miRNA），需要进一步研究。

三阴性乳癌内无细胞微小核糖核酸循环的意义

近来，在乳癌管理中最重要的挑战之一，是发现敏感和特异的，也是最小侵入性的生物标记物，这些标记物能开拓来在早期阶段检测乳癌，以及监控乳癌病人的进展和病人对治疗的反应[66]。现在已知的是，血清/血浆内微小核糖核酸（miRNA）的信号，可能反映了对生理学的或疾病的状况[67]。有一研究，已对 120 例原发性乳癌病人外科手术后和化学疗法前的血清样本，测试了 6 个正在循环中的微小核糖核酸（miRNA）（miR-10b, miR-17, miR-34a, miR-93, miR-155 和 miR-373）的相对浓度。在乳癌病人血循环中的 miR-34a, miR-93 和 miR-373 的浓度，明显高于健康妇女。调节失调的 miR-17 和 miR-34a 的血清浓度，与激素受体的状态相关。这些发现揭示，人体表皮生长因子受体 2 阳性（HER2+）乳癌病人 miR-373 的血清浓度，明显低于三项阴性乳癌（TNBC）妇女（68）。三项阴性乳癌（TNBC）病人血浆中的 miRNA-30a 浓度，明显高于其它类型乳癌病人[66]。

展望

三阴性乳癌（TNBC）是一种分子学的，病理学的和临床水平的异质性疾病，后果不良。三项阴性乳癌 的分子亚型分类，对最理想的使用近代的治疗和开发新的药物是很必要的 [57, 58, 69]。调节失调的 微小核糖核酸表达， 不仅 是三阴 性乳癌进展性乳癌进展的一个标志物，而且也能为治疗干预提出一个有吸引力的标靶[43,47]。

缩 写 词

TNBC：三阴性乳癌； ER：雌激素受体； PR：孕激素受体；HER2：人体表皮生长因子受体 2；miRNAs：微小核糖核酸；TDM：距离转移的时间；OS：总体存活； DFS：无疾病存活；LN：淋巴结

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Competing Interests

The authors have declared that no competing interest exists.

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作者们声明本文不存在利益争议。

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