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Review

miRNA – Therapeutic tool in breast cancer? Where are we now?

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ABSTRACT

Objective: The aim of this study was to review the current knowledge about involvement of microRNAs in breast cancer, and their potential in the clinic, published in scientific journals searched in Pubmed/Medline database until March 2014.

Results: MicroRNAs (miRNAs) are a family of 21–25 nucleotide small RNAs molecules. Currently, it is well known that miRNA plays a key role in all cellular processes of the organism including tumour initiation and progression. Many studies have shown that circulating miRNAs are attractive, easily detectable tumour biomarkers. Breast cancer is one of the most common cancers in the world. It is clinically established that different subtypes may respond differently to therapies, give metastases and present drug resistance. MicroRNAs have a potential as diagnostic, prognostic and therapeutic tools in breast cancer.

Conclusion: Molecular knowledge is crucial for choosing the most effective therapy for individual patients. MicroRNAs holds a great potential in anticancer therapy.

1. Breast cancer – why is it so hard to cure?

Breast cancer is one of the most common cancers in the world with more than 1,300,000 cases worldwide.[1] This very heterogeneous disease is clinically divided by histological types based on expression of specific receptors: estrogen receptor (ER) positive (the most numerous and diverse), progesterone receptor (PR) and HER2 (ERBB2) receptor or the absence of all of them, named triple negative breast cancer (TNBC).[1–3,5,26] Another classification of breast cancer distinguished luminal A, luminal B, basal and HER2 enriched groups.[4] It is clinically established that different subtypes may respond differently to therapies, give metastases and present drug resistance.[1,6] For example, TNBC is associated with high risk of recurrence and distant metastases to the brain, compared to other, receptor positive tumours,[7] and responses only to chemotherapy.[1] Molecular knowledge is crucial for choosing the most suitable therapy for individual patients combined with cost-effectiveness of the treatment.[98] Conventional treatment for breast cancer includes wide local excision, sentinel lymph node biopsy or axillary lymph node dissection, adjuvant medical treatment and radiotherapy to the whole breast.[25,99]

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2. miRNA – briefly about biogenesis, mechanism and function

MicroRNAs (miRNAs) are a family of 21–25 nucleotide small RNAs molecules.[8,9] When in 1993 Victor Ambros et al. found out that gene lin-4 did not code a protein,[10] no one could predict that this small molecule plays such an important role in gene regulation. Currently, it is well known that miRNA plays a key role in all cellular processes of the organism, including among others: development, proliferation, apoptosis, differentiation, organogenesis.[8–12] Therefore, it is not surprising that currently miRNA plays a role in tumour initiation and progression. The number of reports associating miRNA with cancer has risen from 0.002% of total cancer reports in 2002 to 2% at present.[11] The biogenesis of miRNAs has been extensively examined.[13] miRNA are intergenic, intronic or exonic (in exons of coding or non-coding genes) and can be transcribed as a single miRNA from its own promoter (approximately 50%) or several miRNAs as a cluster from a shared promoter.[8,14,15] The transcription of miRNA is driven for the largest part by RNA polymerase II. The long primary transcript (pri-miRNA) can measure more than 1 kb[8,16] and contains a 5′-cap structure and a 3′-poly(A) tail. A region with not perfectly complementary sequence creates a secondary hairpin structure recognized and processed by a complex of RNAses. Pri-miRNA is converted into a pre-form by ribonuclease DROSHA and the DGCR8 cofactor which is a hairpin structure of ~70–80 nucleotides.[16] These stem-loop shaped pre-miRNAs are translocated from nucleus into cytosol via exportin 5.[17] The RNase III endonuclease DICER1 cleaves the pre-form generating a double-stranded miRNA of 18–25 nucleotides in length.[16] The double-strand is unwound and the single strands are incorporated into RNA-Induced Silencing Complex – RISC (Fig. 1). Recent reports show that not only 5′-strand is functional as it was believed in the past, 3′-strand is not always degraded but also can give mature, functional miRNA.[18] As a part of this complex, microRNA is able to regulate gene expression at a post-transcriptional level, bind partial complementary target mRNA, mainly leading to mRNA degradation or translation inhibition.[19] The interactions between miRNA and mRNA are usually located near 5′ terminus miRNA molecule. These ~6–8 nt sequence is highly conserved.[20] It is well known that 3′-UTR mRNA may contain multiple miRNA-binding sites for different miRNAs, and a single miRNA may bind multiple targets.[21,22] The "many targets" hypothesis suggests that ~60% of the mRNAs have one or more evolutionarily conserved sequences and that they are able to interact with miRNA.[21] Computational approaches are used to predict mRNA–miRNA interactions and a number of algorithms have been currently developed and tested for accuracy and precision (using both computational and laboratory techniques).[23,24] Currently, 2024 mature miRNAs have been discovered in humans [http://www.mirbase.org/] and it is estimated that around 30% of human genes are regulated by miRNA.[8]

3. miRNA in tumourogenesis

The first evidence of the involvement of microRNAs in human cancer was described in 2002 by Calin and et al.[27] Studies on chronic lymphocytic leukaemia (CLL) have shown the knock down or knock out of miR-15a and miR-16–1 in approximately 69% of CLLs (deletion of chromosome 13q14). These results pushed the investigators further and they mapped all known microRNA genes.[28] Many of them are located in chromosomal loci prone to deletions or amplifications. In the case when

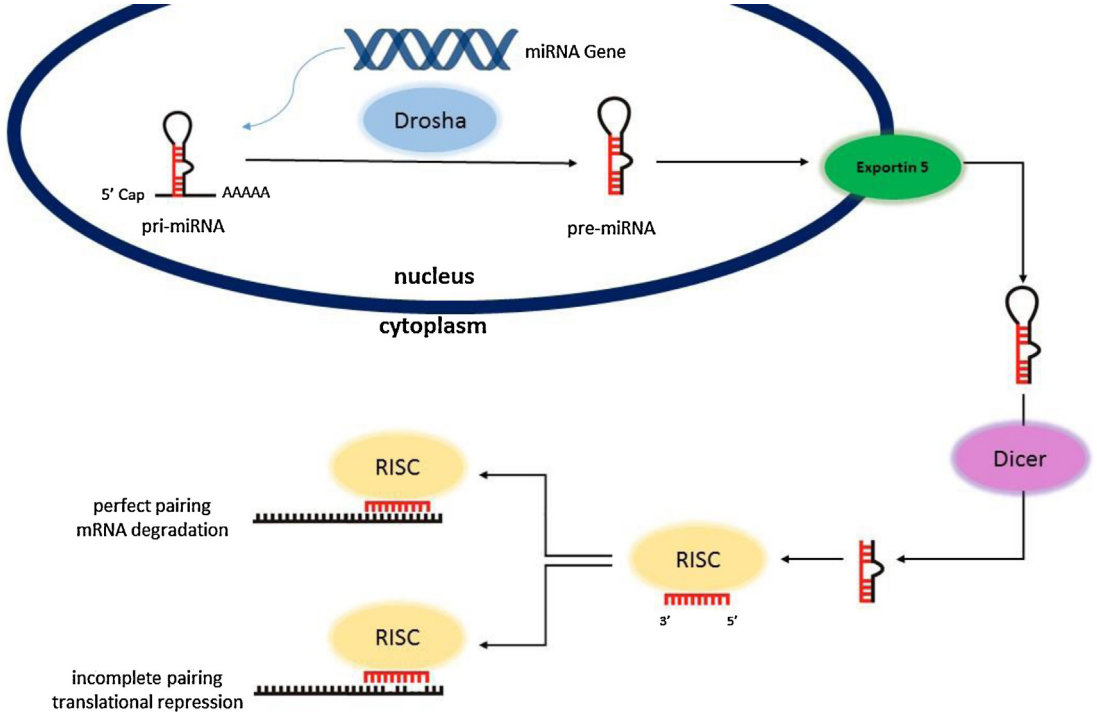

Fig. 1 – Model for microRNA biogenesis.

microRNAs involved in the negative regulation of a transcript encoding a known tumour suppressor gene are amplified, this amplification results in the increased expression of the miRNA and consequent silencing of the tumour suppressor gene. In opposite, miRNAs repressing oncogenes are often located in fragile foci, where deletions or mutations can occur and result in reduced miRNA levels causing overexpression of the target oncogene. Therefore, in human cancer, alterations of microRNA expression represent a rule rather than being exceptional or incidental.

In 2000 Hanahan and Weinberg described the hallmarks of cancer.[63] Below, we present a few examples of how microRNA is involved in the cancerogenesis process.

Uncontrolled proliferation is a special feature of cancer cells. It is well established that Notch signalling maintains between cell proliferation, differentiation and apoptosis, thus, alterations in this signalling pathway are associated with tumourigenesis.[65] Several studies have shown that the miR-34 participates in p53 and Notch pathways regulation in consistency with tumour suppressor activity. Ji et al. reported that human gastric cancer cells with miR-34 restoration reduced the expression of target gene Notch[66] and that Notch-1 and Notch-2 are downstream genes of miR-34 in pancreatic cancer cells.[67] It is worth to mention that they also found that pancreatic cancer stem cells are enriched with tumour-initiating cells or cancer stem cells with high levels of Notch-1/2 and loss of miR-34 which suggests the engagement of miR-34 in pancreatic stem cell self-renewal via modulation of target Notch.

Other miRNA involved in Notch pathway regulation is miR-199. Inhibition of Hes-1 (transcriptional factor) by miR-199b-5p negatively modulated the medulloblastoma (MB) cell growth. Moreover, over-expression of miR-199b-5p causes decreasing of cells with stem-like cells phenotype (CD133+) and blocks expression of several cancer stem-cell genes.[68] It is important that the level of miR-199b-5p in the non-metastatic cases was significantly higher than in the metastatic. Patients from first group (high level) have shown a better overall survival.

MiR-290 cluster can directly target the Retinoblastoma-like 2 protein (Rbl2), affect telomere integrity and telomere-length homeostasis.[69] Small, non-coding RNA plays also an important role in angiogenesis. For example let7-f, miR-27b and miR-130a have been identified as pro-angiogenic players in vitro.[70,71] Lee et al. identified the miR-378 function as an oncogene to enhance tumour cell survival and blood vessel expansion through repression of the expression of tumour suppressors Sufu and Fus-1.[72]

The most common cause of mortality in human cancers are metastasis. It is a series of complicated processes regulated by multiple factors and it allows the outgrowth of metastatic tumours in the new microenvironment.[73] Currently, there is strong evidence that miRNAs coordinate and play important roles in tumour invasion and metastasis.[74,75] Si et al. have found that miR-21 was highly overexpressed in breast tumours compared to the matched normal breast tissues among 157 human miRNAs analyzed. They did transfection of breast cancer MCF-7 cells with anti-miR-21 oligonucleotides and found that anti-miR-21 suppressed both in vitro cell growth and tumour growth in the xenograft mouse model.[76] They also made a two-dimensional differentiation in gel electrophoresis of tumours treated with anti-mir-21 and identified the tumour suppressor tropomyosin 1 (TPM1) as a potential mir-21 target, which can at least in part explain the notion that mir-21 functions as an oncogene.[77] Tumour-suppressor Pdcd4 inhibits transformation and invasion and it is down regulated in cancers. Asangani et al. have shown that miR-21 downregulates Pdcd4 protein and upregulates tumour cell invasion in cultured colon cancer cells. They demonstrated an inverse correlation between miR-21 and Pdcd4-protein.[78]

4. Methods of detection of miRNA in clinical practice

In the clinical practice, it is necessary to use biomarkers for detection and early diagnosis. miRNAs are stable due to their small size, which makes them a better tool for diagnosis. This molecule can be detected and isolated from frozen and paraffin-embedded tissues, blood,[37,38] and different biologic fluids like urine,[39] sputum[40] or saliva.[41] Many studies have shown that circulating miRNAs are attractive, easily detectable tumour biomarkers.[42–45] It is necessary to explore the miRNA topic more deeply because of its potential for being used in tumour diagnosis, prognosis and cure.[29] Many studies have been developed on the global expression of miRNA genes in normal and diseased tissues[30–34,61]. In contrast to mRNA global profiling, miRNA expression signatures (miRNome) allowed to discriminate with high accuracy different types of cancer.[32,35] It is worth to notice that miRNA expression profile is tissue unique, which has foreseeable benefits in aiding clinical diagnosis and treatment.[36]

Currently, there is a possibility to use different techniques and methods to detect miRNA.[24,46] The most important issue is to determine which miRNAs are changing in a specific disease and if the changes are representative to it, and if only to it.[46] Methods and strategies for profiling miRNA expression are described by Kong et al. in the review.[24] When we have a based knowledge about the expression profile that is necessary for results interpretation, we can create and develop new methods to act. Some of miRNAs have already been used in clinical practise, for example, a miRNA-based diagnostic assay approved by FDA.[47] The most popular methods to detect miRNA that can be easily employed in a routine clinic diagnostic are: quantitative-reverse transcription PCR (qRT-PCR), hybridization-based methods and next generation sequencing [review 47]. Wei et al. systematically screened miRNA expression profile using the Solexa deep-sequencing technology.[62] They have created two libraries from T-47D breast cancer cells treated with or without prolactin, identified a number of miRNAs significantly differentially expressed between these two panels, and also detected several new miRNAs associated with prolactin receptor signalling pathway in breast cancer.

Also new technology-based methods have been recommended for the exploration and examination of miRNA, for example electrochemical genosensor that can easily detect miRNA in the serum or other biological samples[48] or nanopore sensor based on the α-haemolysin protein.[49] Unfortunately, there are still considerable limitations to use miRNA expression as routine in the clinic hence more clinical studies are required.

5. miRNA in breast cancer – problem during effective treatment?

Altered miRNA expression in human breast cancer was first described by Iorio et al. in 2005.[50] They analyzed 76 breast cancers, 10 normal breast samples and 14 breast cancer cell lines to identify miRNA whose expression is significantly deregulated in cancer versus normal breast tissues. In this panel there were 29 miRNAs identified with aberrant expression by microarray and Northern blot analyses. MiR-10b, miR-125b, miR-145 (all of them down-regulated), miR-21 and miR-155 (all of them up-regulated) were the most consistently deregulated in breast cancer. This finding suggests that they may act as tumour suppressor (down-regulated) or oncogene (up-regulated) in breast cancer tumourogenesis. It is known that expression of miR-125b is high in differentiated cells and tissues[51] so the decreased level of its expression in breast cancer suggests the impairment of differentiation capabilities of cancer cells.[50] In 2007, Sempere et al. published a paper about miRNas' distribution in breast tumour tissue versus normal tissue from more than 100 patients using hybridization in situ.[52]

As it was mentioned above, miRNAs can inhibit tumourogenesis by repressing oncogenes. Members of the ErbB family play a very important role in development, cellular proliferation and survival in human epithelial malignancies and they are frequently amplified or overexpressed in breast cancer (20–30%); moreover, they are significantly associated with a worse prognosis.[53,54] Scott et al. have examined miR-125a and miR-125b overexpression in SKBR3 cells which decreased ErbB2 protein level of approximately 40–65% and ErbB3 level of around 60–80%.[54] SKBR3 cells with overexpression of miR-125a and miR-125b were impaired in their malignant cell phenotype (reduced migration and invasion capacities). Wang et al. first providing experimental data to demonstrate that miR-125a, miR-15b and miR-205 act in concert to regulate the expression of ErbB2/ErbB3 in breast cancer cells.[55] Because of its role in tumourogenesis, ErbB family is an excellent target for selective anticancer therapies. ErbB-targeted therapies currently used in clinic are divided into two strategies: blocking antibodies (for example transtuzumab targeting ErbB2) and tyrosine kinase inhibitor (for example aslapatinib against EGFR and ErbB2). Because of the lack or low kinase activity ErbB3 receptor can be blocked only using antibody[56] and it has been currently clinically studied (http://www.clinical-trials.gov/). ErbB2 required ErbB3 to promote breast cancer cell proliferation[57] and ErbB3 has an important role in ErbB2-altered breast cancers.[58] The same group reported that ErbB3 contributes to ErbB2-mediated therapeutic resistance to tamoxifen[59] and paclitaxel,[60] thus they proposed a novel approach to target ErbB2/ErbB3 by reducing their protein levels by microRNA rather than inhibiting only the signalling pathways.[55]

Biagioni et al. identified 22 differentially expressed miRNAs in HER2 tumours, 31 in basal-like tumours and 33 in luminal tumours.[64] Two miRNAs: miR-10b* and miR-139-5p were down-regulated and three: miR-425, miR-454 and miR-301a were up-regulated for all three subtypes. The most important finding from this analyses is that the miR-10b* is a master regulator of breast cancer cell proliferation (lower levels of miR-10b* correspond to higher tumour size, which was also confirmed by soft agar colony formation assay in MCF7 breast cancer cells). Further in vivo examination in a xenograft model confirmed a pivotal role of miR-10b* in breast cancer cell proliferation.[64] This experiment can be crucial for exploring the therapeutic potential of miR-10b* in breast cancer.

Zhong et al. demonstrated that an altered miRNA expression pattern is involved in acquiring resistance to adriamycin and docetaxel which are two chemotherapeutic agents commonly used in the treatment of breast cancer. This regulation could be in part via targeting PTEN.[79]

It was mentioned above that miR-21 plays an important role in tumourogenesis. Wang et al. investigated the association of miR-21 expression with the sensitivity of breast cancer cells to doxorubicin. Using TaqMan RT-PCR and Western blot assay to detect the expression of mature miR-21 and tumour suppressor gene (PTEN) protein they found that dysregulation of miR-21 plays a critical role in the doxorubicin resistance of breast cancer.[80]

Breast cancer drug resistance is also combined with deregulation of miRNA-200c. Examination of the miRNA-200c expression in tumour specimens obtained from thirty-nine breast cancer patients who had received neoadjuvant chemotherapy showed that miRNA-200c was down-regulated in non-responders as compared to responders.[81] Another paper reports that miRNA-30c played a pivotal role in chemoresistance via direct targeting of the actin-binding protein Twinfilin 1 which is responsible for the promoting of epithelial-to-mesenchymal transition.[82] Study of miRNA-19 expression levels in three multidrug resistance (MDR) cell lines in comparison with their parent cell line, MCF-7, using a miRNA microarray showed that miRNA-19 was overexpressed in all three MDR cell lines and modulate chemoresistance directly targeting PTEN. Inhibition of miR-19 sensitized MDR cells to chemotherapeutic agents in vitro and in vivo.[83]

Radiotherapy is an effective and well-established cancer treatment, however, currently, little is known of how microRNA may regulate radiation resistance [revived in 85] in breast cancer. Liu et al. showed that miR-95 promotes radiation resistance and development of an aggressive phenotype on prostate and breast cancer.[85] Interestingly, examinations have been performed on different cell line varied with TP53 status, and the results suggest that miR-95 promotes radiation resistance independently of the TP53 function. Xenograft tumour experiments revealed that miR-95 overexpressing tumours have less necrosis and increase proliferation even after irradiation. Also miRNA-21 plays a role in radioresistance acting as a radioresistant miRNA.[86] miRNA-21 expression in breast cancer cells contributes to radiation resistance by compromising cell cycle progression (radiation-induced G2/M arrest). Chen et al. showed that antisense targeting miR-155 could increase the sensitivity of breast cancer cells to irradiation.[87] Liang et al. also suggest that miRNA-302 could be a potential sensitizer to radiotherapy. miRNA-302 sensitizes resistant breast cancer cells to irradiation in vitro and in vivo.[100]

MetastamiRs are microRNAs crucial in metastatic spreading. It was proved in migration, invasion and poor prognosis phenotype. Cloonan et al. found that miR-139-5p is

de-regulated in human triple negative breast cancer samples and it is able to target several invasive pathways and migratory phenotypes of cancer cells.[88] Overexpression of miR-205 inhibits MCF7 cell migration and invasiveness and reduces the growth and colony-formation capacity of MCF7 cells by inducing apoptosis.[89] Li et al. demonstrated that decreased expression of miR-720 was correlated with lymph node metastasis and manifests antimetastatic activity by down-regulating TWIST1.[90] Studies have shown that ectopic overexpression of miR-301a promote breast cancer cell migration, invasion and metastasis both in vitro and in vivo via constitutively activated Wnt/β-catenin signalled by direct targeting PTEN.[91] Also miRNA-155 plays a critical role in breast cancer progression and metastasis. High miR-155 expression was closely correlated with higher tumour grade, advanced tumour stage and lymph node metastasis and it is worth mentioning that relative expression of miR-155 in tumours with lymph node metastasis was significantly higher than that in tumours with no lymph node metastasis.[87] Petrović et al. showed that miR-21 expression levels in invasive with non-invasive component and pure invasive cancers were significantly increased compared with normal tissue. The highest difference between non-invasive and pure invasive cancer samples than in other compared group pairs indicates that miR-21 is a strong specific factor to the process of invasion.[92]

Trastuzumab resistance is a major issue in therapy for $HER2^+$ breast cancers. Gong et al. screened for miRNAs that were differentially expressed in the trastuzumab-resistant breast cancer cells, and identified that miRNA-21 was up-regulated among the reported PTEN-targeting miRNAs. They showed that blocking the action of miR-21 with antisense oligonucleotides re-sensitized the resistant cells to the therapeutic activities of trastuzumab by inducing growth arrest, proliferation inhibition, and G_1-S cell cycle checked in the presence of the antibody.[93]

Mei et al. showed that combined taxol chemotherapy and miR-21 inhibitor treatment via polyamidoamine (PAMAM) dendrimers vector to evaluate the effects of the combination therapy reduced cell viability and invasiveness, thus it might represent a promising novel therapeutic approach for the treatment of breast malignancies.[94]

Another clinical problem in HER2 positive breast cancer treatment is resistance to tamoxifen (the selective oestrogen receptor modulator). Cittelly et al. analyzed multiple cell models of tamoxifen resistance derived from MCF-7 cells to examine the influence of microRNAs (miRNAs) on tamoxifen resistance.[95] They suggest that miR-342 regulates tamoxifen response in breast tumour cell lines via the regulation of expression genes involved and in tumour cell apoptosis and cell cycle progression. Thus, restoring miR-342 expression may be a novel therapeutic approach to sensitizing and suppressing the growth of tamoxifen refractory breast tumours. Another group also demonstrated that tamoxifen resistance in breast cancer is associated with MiR-221/222.[96]

The data published by Kim in 2011, strongly supported that overexpression of miRNA-145 reduces the levels of cancer cell survival factors and inhibits cancer cell growth and metastasis.[97] They demonstrated that miR-145 delivered using an adenoviral vector system showed significant inhibition of tumour growth in breast tumour bearing mice.

U.S. National Institutes of Health approved clinical trial NCT01612871 focuses on the role of miRNAs in resistance and sensitivity in breast cancer. The study concerns women with metastatic invasive breast cancer or locally advanced breast cancer and for whom treatment with tamoxifen or anti aromatase is indicated. This study is currently recruiting participants [http://www.clinicaltrials.gov]. Description of other clinical trials are available online.

As indicated above, deregulation of miRNAs is involved in the development of cancer disease, invasiveness, metastasis and treatment failure. Aberrant miRNA expression has been observed in various types of human tumours playing a role as tumour-suppressor gene or oncogene. miRNA has a great potential in the therapeutic design because of its multiple function in cell homeostasis and one hit-multiple target pathway.

Cited literature indicates that miRNA could be a potential therapeutic tool and promising biomarker in personalized treatment.

Conflict of interest

None declared.

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