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Overview of non-coding RNAs in breast cancers

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ABSTRACT

Breast cancer in women is the second most common cancer and the fifth leading cause of cancer death worldwide. Although earlier diagnosis and detection of breast cancer has resulted in lower mortality rates, further advances in prevention, detection, and treatment are needed to improve outcomes and survival for women with breast cancer as well as to offer a personalized therapeutic approach. It is now well-established that non-coding RNAs (ncRNAs) represent 98% of the transcriptome but in-depth knowledge about their involvement in the regulation of gene expression is lacking. A growing body of research indicates that ncRNAs are essential for tumorigenesis by regulating the expression of tumour-related genes. In this review, we focus on their implication in breast cancer genesis but also report the latest knowledge of their theragnostic and therapeutic role. We highlight the need for accurate quantification of circulating ncRNAs which is determinant to develop reliable biomarkers. Further studies are mandatory to finally enter the era of personalized medicine for women with breast cancer.

Introduction

According to the Global Cancer Project 2018, breast cancer in women is the second most common cancer and the fifth leading cause of cancer death worldwide [1]. There are an estimated 2,088,849 new cases of breast cancer per year (11.6% of the 18.1 million new cancer cases), and 626,679 breast cancer-related deaths (6.6% of the 9.6 million annual cancer deaths) occurred worldwide in 2018 [1]. New diagnostic, therapeutic, and prognostic approaches are thus required [2]. In France, 58,000 women are diagnosed with breast cancer each year with an annual death rate of over 11,000.

Breast cancer is the result of abnormal proliferation of the cells lining the mammary glands and ducts. Most malignant lesions of the breast are classified as primary adenocarcinomas [2]. Breast cancer is a highly heterogeneous disease [3,4]. It is also important to note that 25% of cancers detected by screening correspond to *in situ* cancers (ductal carcinoma *in situ*: DCIS) before the invasive stage. Some of these *in situ* cancers often combine both an *in situ* and an invasive component that is often initially overlooked at biopsy and only diagnosed after surgery, exposing these patients to multiple surgeries. In this context, it would be interesting to have a biological marker to estimate the risk of invasive cancer associated with DCIS. This situation is similar for patients with so-called "borderline breast lesions" mainly corresponding to atypical hyperplasia [5].

Breast cancer is a complex disease with multiple steps involved in its pathogenesis including various processes of tumour initiation and growth [4], metastasis and invasion [6], angiogenesis [7], and significant risk of relapse [8]. These processes occur when cellular and molecular signalling pathways in breast cells are disrupted or deregulated [9,10].

The 5-year survival rates for FIGO (International Federation of Gynaecology and Obstetrics) stage I, II, III, and IV breast cancer are 100%, 93%, 72%, and 26%, respectively [11,12]. These survival rates depend on several parameters: the molecular subtype, lymph node status, and existence of metastases both at initial diagnosis and at recurrence.

Although earlier diagnosis and detection of breast cancer has resulted in lower mortality rates, further advances in prevention, detection, and treatment are needed to improve outcomes and survival for patients with breast cancer [13]. Currently, many treatments are available for breast cancer including: surgery [14] which is often followed by

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radiation therapy [15]; hormone therapy (different in pre- and post-menopausal women) [16]; targeted therapies [17]; immunotherapy [18]; and chemotherapies [19]. However, the effectiveness of these therapies can be limited by intrinsic or acquired resistance develop after various genetic and epigenetic modifications [20,21]. Therefore, the crucial challenge is to select therapy in an individualised manner. While some tools have been developed to better adapt therapies (hormone therapy versus chemotherapy) such as Oncotype DX [22], they cannot predict the response to a particular drug. In this context, theragnostic tools are necessary, especially for neoadjuvant therapy.

We now know that only 1-2% of the human genome sequence codes for proteins. Up to fairly recently, it was believed that the rest of the genome was devoid of function. However, it turns out that nearly 90% of this sequence is also transcribed. The resulting non-coding RNAs (ncRNAs) appear to be involved in sophisticated and still little-known mechanisms regulating gene expression. ncRNAs thus represent 98% of the transcriptome. amongst them, ncRNAs of fewer than 50 nucleotides are defined as small RNAs (sncRNAs) and include microRNAs (miRNAs), Piwi interacting RNAs (piRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs), and small interfering RNAs (siRNAs) [23]. A recent study reported the presence of partial sncRNAs derived from tRNAs, such as tRNA halves (tiRNAs) and tRNA fragments (tRFs) [24]. ncRNAs with more than 200 nucleotides are defined as long RNAs (lncRNAs), and include intergenic ncRNAs (lincRNAs), some circular RNAs (circRNAs), and ribosomal RNAs (rRNAs) [25].

We are gradually gaining more knowledge about the biological functions of ncRNAs, such as regulating gene expression at the transcriptional and translational levels, guiding DNA synthesis or gene rearrangement, and protecting the genome from foreign nucleic acids [26]. A growing body of research indicates that ncRNAs are essential for tumorigenesis by regulating the expression of tumour-related genes. Mechanistically, ncRNAs regulate gene expression primarily by acting as transcription factors, regulating chromatin remodelling or participating in post-transcriptional regulation [27]. CircRNAs can regulate gene expression at the epigenetic, transcriptional and post-transcriptional levels (mainly as ceRNAs) [28] of protein-coding mRNAs [29–31]. In addition, some tiRNAs can participate in gene regulation and silencing via complementary binding to target genes using a mechanism like miRNAs [32]. Approximately 70% of studies in this field have evaluated exosomes as the source of choice for ncRNAs as biomarkers [33]. Yuan et al. estimated that mature miRNAs accounted for 40.4% of the total RNA content in exosomes, piwi-interacting RNAs for 40% (piRNAs), pseudogenes for 3.7%, lncRNAs for 2.4%, tRNAs for 2.1%, and mRNAs for 2.1% [34]. In this presentation we will focus on miRNAs, piRNAs and lncRNAs. The aim of this review was to provide an up-to-date perspective on the potential of the non – coding RNAs and their potential in diagnostic, prognostic and therapeutic role in patients with breast cancer.

miRNAs

miRNAs were the first to be identified and analysed in cancers [31, 32]. These small intracellular RNAs are able to induce silencing of gene expression by post-transcriptional regulatory mechanisms, by binding in a targeted manner to the 3′ untranslated region (3′-UTR) of mRNAs, causing a blockage of translation or degradation. However, this mechanism is not exclusive; binding of miRNAs to 5′-UTR is also possible and induces either activation or repression of translation.

It is undisputed that miRNAs remain the best characterized class of sncRNAs. miRNA pathways and functions have been extensively reviewed [35–37] and will only be described briefly here. Sequence analysis has revealed that most miRNAs are transcribed from intergenic regions of the human genome [38,39]. However, some miRNAs are also transcribed from exonic or intronic regions [39,40].

The biogenesis of miRNAs is illustrated in Fig. 1. It is estimated that miRNAs can regulate the expression of more than 60% of the human genes by guiding a diverse set of multiprotein RNA-induced silencing complexes (RISCs) to specifically target mRNAs [41]. RISCs associated with miRNAs consist of the argonaute (Ago) and glycine-tryptophan (GW) protein families as well as other accessory proteins [42,43]. The mode of miRNA-mediated gene expression silencing (mRNA decay or translational repression) is determined by the combinatorial nature of the components of the RISC and the degree of complementarity between the miRNA sequence and the miRNA targeting site of the mRNA [42,43]. In addition to these miRNA-targeting-based "silencing" mechanisms, some miRNAs are complementary to gene promoters and mediate activation and "silencing" through targeting of Ago/GW182-containing complexes to promoter regions [44,45]. Therefore, miRNAs can modulate gene expression via several distinct mechanisms.

Dysregulation of oncogenic and tumour suppressor miRNA expression in cancer can result from multiple pathological mechanisms occurring at the transcriptional or post-transcriptional level. For

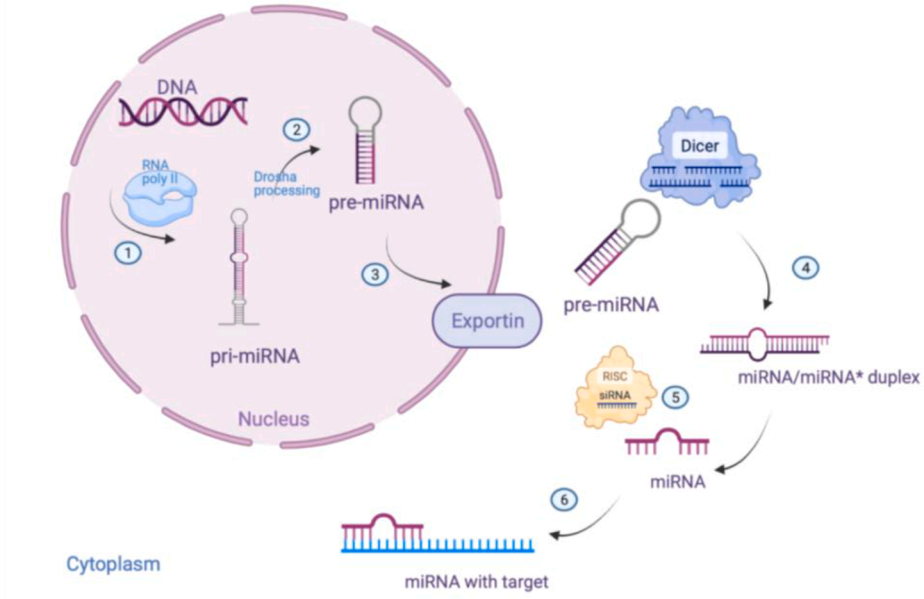

Fig. 1. Biogenesis of miRNAs is a multistep process, beginning with the transcription of primary miRNAs (pri-miRNAs) by RNA polymerase II [35–37]. The pri-miRNAs are transformed into precursor miRNAs (pre-miRNAs, 70 nucleotides long) by the RNase III Drosha-DGCR8-DDX5 microprocessor complex [36,37,46], and are then exported to the cytoplasm by Exportin (a Ran-GFP-dependant transporter) (44-46). In the cytoplasm, pre-miRNAs are cleaved by the RNase Dicer-TAR RNA-binding protein (TRBP) complex, producing mature single-stranded miRNAs 18–23 nucleotides in length [35–37]. Not all miRNAs pass through the canonical miRNA biogenesis pathway. Special miRNAs called mirtrons are produced from spliced introns with structural features like pre-miRNAs and undergo a miRNA processing pathway that bypasses the Drosha-mediated cleavage step [47].

example, DNA hypermethylation of the miRNA promoter has been identified in various cancers, including breast cancer, leading to the silencing of miRNA expression at the transcriptional level [48]. Alterations affecting the functionality of protein regulators involved in pri-miRNA and pre-miRNA processing and miRNA maturation can also lead to deregulation of miRNA expression in cancer.

piRNAs

piRNAs are a recently discovered class of small ncRNAs of 24–32 nucleotides in length that were first identified in Drosophila germ cells [49]. In Drosophila, mature piRNAs are generated from the processing of single-stranded RNAs transcribed from piRNA clusters in the genome, [49]. In addition, piRNAs can be generated from another mechanism in the cytoplasm, called the "ping-pong" cycle, which involves piRNA-directed antisense primary cleavage of transposon transcripts by Aubergine and PIWI proteins [49]. Fewer than 20% of mammalian piRNAs have been identified to date [47]. piRNAs can modulate histone modifications and DNA methylation in a sequence-specific manner, leading to alterations in chromosomal conformation and gene expression regulation [50]. Dysregulation of piRNAs and proteins (e.g. PIWI family proteins) involved in piRNA biogenesis has been found in a variety of cancers, including breast cancer [50,51].

lncRNAs

lncRNAs are a class of ncRNA transcripts that regulate gene expression at the transcriptional, translational, and post-translational levels. However, although lncRNAs are vital for the proper functioning of cellular processes, they do not encode peptides or proteins [52]. lncRNAs act through a variety of mechanisms including mediating inter-chromosomal interactions, serving as sponges for endogenous RNAs, regulating the degradation of mRNAs, and modulating epigenetic components which are redirected to their targets. Therefore, any change in the expression levels of lncRNAs can lead to various diseases [53].

The definition and classification of lncRNAs remains somewhat unclear because the exact mechanisms and pathways of action of these molecules have not been fully elucidated. lncRNAs are composed of more than 200 nucleotides, with most consisting of between 1000 and 10,000. Moreover, this type of ncRNA generates secondary and three-dimensional structures, allowing them to play the dual role of RNA and protein [54,55]. The biogenesis of lncRNAs is similar to that of mRNAs in that they are first transcribed by RNA polymerase II, after which most of the transcripts are spliced. However, lncRNAs are mainly localized in the nucleus and have lower expression levels than mRNAs. In addition, their expression profiles are cell-type specific [54–56].

Four different archetypes of molecular functions of lncRNAs have been described: they act as signals, decoys, guides, and scaffolds. In the first archetype, lncRNAs can act as molecular signals because transcription of a lncRNA occurs at a specific time and location to integrate developmental signals, interpret the cellular context, or respond to various stimuli. In this archetype, lncRNAs can act as markers of significant biological events. In the second archetype, lncRNAs function as a molecular decoy; in this context, lncRNAs bind to their target protein following transcription, and frequently act as negative regulators of effector molecules. In the third archetype, lncRNAs can act as gene expression guides in two ways: as a cis-form in the immediate vicinity of genes, and as a trans-form for distant genes. The lncRNAs bring together components of different complexes and transcription factors that can either repress or activate target genes. In the fourth archetype, lncRNAs act as scaffolds [56]. Different ncRNA domains bind simultaneously to distinct effector molecules, resulting in either activation or repression of transcription. A better understanding of how scaffold complexes are assembled and regulated provides potential new strategies for selecting and using specific signalling components to modify cellular activities.

Role of ncRNAs in breast cancers

Current breast cancer screening protocols are based on mammography. However, lesions found on mammograms do not always have pathognomonic features of breast cancer, which may prevent the radiologist from performing a biopsy or generate false positives. Other limitations of this technique include cumulative radiation exposure and possible overdiagnosis underlining the need for non-invasive biomarkers of breast cancers [57–59].

A growing volume of literature has explained the role of various classes of ncRNAs in cancer progression. Recently, Tomar et al. reviewed the potential role of non-coding RNAs in breast cancer [10] and highlighted their role in various signalling pathways associated with angiogenesis [60], metastasis [61] EMT [62] and cancer stem cells phenotypes [63,64] contributing to increased aggressiveness and metastatic potential.

Role of miRNAs in the transition from normal to malignant breast tissue

Since the first report in 2005 of the role of miRNA deregulation in breast cancer [57], many studies have shown altered expression of miRNAs in breast cancer and consequent transformation of normal breast cells into malignant cells. These breast cancer-associated miRNAs can be subdivided into oncogenic miRNAs (oncomiR) and tumour suppressor miRNAs (tsmiR). OncomiRs are generally upregulated in breast cancer, suppressing the expression of potential tumour suppressor genes and leading to breast malignancy [65]. Conversely, tsmiRs can inhibit the expression of oncogenes that promote breast tumourigenesis [66], and their downregulation may thus lead to breast malignancy [65]. In fact, it is the imbalance between miRNAs with an oncogenic function and miRNAs with a tumour suppressor function that is involved in the development of breast cancer. It is therefore possible that a miRNA signature could reflect tumour transformation, thereby identifying patients who require either biopsy sampling or surgery to remove the tumour, beyond the radiological aspect. Recently, using qRT-PCR on 324 miRNAs, Corcoran et al. identified 30 miRNAs that were dysregulated in breast cancer [67]. To detect breast cancer, an optimised eight-miRNA panel exhibited an AUC, accuracy, sensitivity, and specificity of 0.915, 82.3%, 72.2% and 91.5%, respectively [67].

From the pathophysiological point of view, we know that some of these miRNAs are involved in specific signalling pathways. We also know that these miRNAs are differentially expressed in breast cells according to the various stages of carcinogenesis. As previously specified, some miRNAs are linked to the transformation of normal breast tissue into *in-situ* carcinoma and others in the progression of *in-situ* carcinoma to invasive carcinoma [68–70]. Mei et al. [69] identified key miRNAs involved in the transition from normal breast cells to DCIS and from DCIS to invasive ductal carcinoma (IDC) [69,71]. From their analysis, 66 miRNAs were identified as differentially expressed in DCIS compared with normal breast. This profile of differentially expressed miRNAs was maintained in the DCIS to IDC transition [69]. Therefore, miRNA profile analysis supports the accepted concept that DCIS is a precursor of IDC. Volinia et al. [70] evaluated the expression of 365 miRNAs by qRT-PCR to generate a miRNA expression signature differentiating normal breast epithelium from DCIS. However, only 11 differentially expressed miRNAs were common in both the Volonia et al. and Hannafon et al. studies highlighting discrepancies related to the biological techniques used and the pre-selection of the miRNAs studied. In addition, comparing IDC and DCIS biopsies, Mei et al. [69] identified nine differentially expressed miRNAs in IDC versus CCIS: let-7 d, miR-181 a, -210, and -221 were upregulated, whereas miR-10 b, -126, -218, -335-5 p, and -143 were downregulated. However, the upregulation of let-7 d and downregulation of miR-10b in IDC compared with CCIS are not consistent with their reported functional roles in breast cancer [61,72,73].

Roles of miRNAs in the molecular subtypes of breast cancer

Currently, invasive breast cancer is classified into molecular subtypes – luminal A, luminal B, HER+, triple-negative (also known as basal-like), and claudin-low – based on its phenotype (proliferation), tumour grade, and most importantly, its molecular characteristics; hormone receptors and human EGF-like receptor 2 (HER2) status [74] (Table 1). The four most frequently observed subtypes are luminal A (accounting for 50% to 60% of breast cancers), luminal B (10–20%), HER+ (15–20%), and triple-negative (10–20%) [75,76]. The other molecular subtypes of breast cancer are quantitatively rare.

Beyond the positive diagnosis of breast cancer, Eroles et al. described a panel of miRNAs according to molecular classification that could compensate for the uncertainty of breast cancer type based on initial biopsy and thus guide the therapeutic strategy [77]. The expression of six miRNAs (miR-9, miR-18 a, miR-19 a, miR-93, miR-106 b, miR-210) is upregulated in basal-like breast cancer, whereas the expression of 10 miRNAs (miR-10 b miR-26 b, miR-126, miR-143, miR-193 b, miR-195, miR-326, miR-449 a, miR-449 b, miR497) is downregulated in the same molecular subtype. Deregulation of these 16 miRNAs contributes to the development of an aggressive subtype of breast cancer characterized by high tumour grade, high proliferation rate, increased frequency of recurrence, and the presence of p53 mutations [77].

The role of miRNAs in breast cancer metastasis

In the context of metastases, it is necessary to differentiate locoregional metastases, represented mainly by ipsilateral axillary lymph node invasion, from distant metastases.

Axillary metastases: The lymph node status can be assessed clinically, but with a risk of underestimation in up to 50% of cases. Similarly, the rate of underestimation by ultrasound and cytology can be as high as 20% which negatively impacts outcomes due to a poor initial therapeutic choice [78]. It has been suggested that serum miRNA profiles are correlated with the diagnosis of axillary lymph node metastases before surgery [79]. This is of particular interest for invasive carcinomas because of the risk of false negative cytology. In addition, for patients without an axillary metastasis profile, identification of a serum miRNA signature could avoid an unnecessary sentinel node procedure or lymphadenectomy, which is associated with morbidity in more than a quarter of patients, particularly in the case of associated radiotherapy. A model that includes a combination of two miRNAs (miR-629-3p and miR-4710) and three clinico-pathological factors (T stage, lymphovascular space invasion and ultrasound findings) has been shown to be of diagnostic potential with a sensitivity of 0.88, a specificity of 0.69, and an accuracy of 0.86 [80].

Distant metastasis: Distant metastasis is the leading cause of death from breast cancer. It is secondary to a multistep process involving extensive vascularization at the primary tumour site, mobilization and invasion by tumour cells, dissemination of tumour cells via the bloodstream, and colonization at distant tissue sites. Detection of distant metastases at diagnosis and recurrence relies mainly on the use of tumour markers, such as CA 15-3, and the use of PET-CT. However, CA 15-3 may be normal in more than 50% of patients with metastases [75]. PET-CT is more relevant with a false-negative rate of 10%. However, PET-CT raises some specific concerns due to its availability and cost which means that it is often initially restricted for certain molecular subtypes or advanced stages. This also results in a risk of underestimation of distant metastases in patients with presumed early-stage breast cancer.

Seven miRNAs have been identified to target the crucial factors involved [61]. By targeting HOXD10, miR-10b activates the expression of RhoC, a pro-metastatic factor promoting cell migration and invasion [61]. However, three pieces of conflicting evidence indicate that miR-10b may serve as a metastatic suppressor: (i) miR-10b is not associated with breast cancer metastasis and prognosis, but is inversely correlated with tumour size and grade [81]; (ii) it inhibits tumour cell migration and invasion by targeting TIAM1 (T lymphoma invasion and metastasis 1) [82]; and (iii) its expression is repressed in the DCIS to IDC transition [69]. Moriarty et al. [83] identified miR-335 as a metastatic suppressor that prevents migration and metastasis. miR-29b is overexpressed in luminal breast cancers, and its under-expression increases the risk of metastasis [84]. Chou et al. have reported the various miRNA panels associated with metastatic behaviour of breast cancer according to molecular classification [85].

piRNAs and breast cancer

Although the role of piRNAs has been clearly established in the epigenetic control of many cancers including breast cancer, relatively few data are available about their specific role in breast cancer. For example, dysregulation of piRNAs and proteins (e.g. PIWI family proteins) involved in piRNA biogenesis has been found in breast cancer [50,51,86]. Furthermore, Guo et al. demonstrated that piRNA-36 expression was associated with malignant phenotypes of breast lesions, and that piRNA-36 interacted with miRNAs and RNAs produced by SEPW1P [87]. Recently, Tan et al. have underlined the difficulties in evaluating the exact role of piRNAs in breast cancer linked to piRNA annotations [88].

lncRNAs and breast cancer

Data regarding lncRNAs in breast cancer are very weak. A study by Kärkkäinen et al. found that lncRNAs are involved in the signalling pathways of breast cancer [89]. Flores-Huerta et al. found that the down-regulation of lncRNA Linc00152 suppressed cell viability, invasion, migration, and epithelial to mesenchymal transition in breast tissues [90]. Moreover, Hu et al. demonstrated the involvement of lncUSMycN in the transition from benign ductal lesions to IDC [91]. Amongst the oncogenic lncRNAs, the most important are HOTAIR (Hox transcript antisense intergenic RNA), MALAT1, HULC, AWPPH and ARNILA. amongst these lncRNAs, HOTAIR, a spliced polyadenylated transcript comprising six exons of about 2200 nucleotides in length and located on chromosome 12q13.13, has been shown to play a particular role in the progression of multiple tumours, including pancreatic, colorectal, hepatocellular, gastric, lung, ovarian and breast [92–94]. HOTAIR is oncogenic when upregulated. Its expression levels are thus correlated with patient prognosis, and HOTAIR could be used as a predictive biomarker.

Table 1
Characteristics of breast cancer according to molecular classification.

Molecular subtype	ER status	PR status	HER2 status	Histological grade	Basal marker	Proliferation	Prognosis
Luminal A	+	+	–	Low	–	Low	Good
Luminal B	±	±	±	High	–	High	Intermediate
Triple negative	–	–	–	High	+	High	Poor
HER2+/ER-	–	±	±	High	±	High	Poor
Apocrine	–	–	±	Intermediate/High	±	High	Poor
Claudine-low	–	–	–	High	High	High	Intermediate

ER: estrogen Receptor, PR: Progesterone receptor, HER2: Huamn Epidermal Receptor 2.

Theragnostic and therapeutic role of ncRNAs

The predictive value of miRNAs is crucial because of its direct impact not only on the initial therapeutic choice but also to detect patients under treatment who show signs of early resistance to the drugs and radiotherapy, especially for those receiving chemotherapy. The most frequent drugs used in chemotherapy are anthracyclines (e.g., doxorubicin [95] and epirubicin [96]), taxanes, alkylating agents (cyclophosphamide (CTX) [97], carboplatin [98]), HER2-targeted monoclonal antibodies [98], and anti-metabolites (5-fluorouracil) [97]. These drugs can be used either in a neoadjuvant or adjuvant setting.

Like most other tumour cells, breast cancer cells can develop multidrug resistance (MDR) [99]. MDR in breast cancer is characterized by a combination of mechanisms including P-glycoprotein (P-gp) [100], multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP) [99,101]. Other mechanisms that simultaneously contribute to MDR are: increased aldehyde dehydrogenase (ALDH) activity; upregulation of anti-apoptotic B-cell family-2 (Bcl-2) proteins; and abnormal activation of signalling pathways such as PI3K (phosphatidylinositol 3- kinase)/Akt, Notch, Hedgehog, and Wnt pathways [102–105]. Previous studies have shown that both miR-451 and miR-326 are involved in the regulation of drug resistance by controlling the expression of MDR1 [95,106]. Liang et al. and Bao et al. have shown that decreased expression of miR-298 and increased expression of miR-21 is associated with chemo-resistance of breast cancer cells by targeting MDR1 and PTEN respectively [107,108].

While few data are available to date about the therapeutic value of RNAs, the Covid-19 pandemic has opened up new therapeutic approaches based on their use. Wang et al. have published the results of four trials using miRNAs to treat various cancers [109]. In the specific context of breast cancer, Grimaldi et al. evaluated the contribution of circulating miRNAs as biomarkers to predict response to therapies [110]. In the same way, McAnena et al. underlined the synergistic anticancer effects of piRNA-36 with paclitaxel and doxorubicin [111]. Finally, Gavgani et al. have shown that some lncRNAs are involved in inducing drug resistance, mainly in breast triple negative cancer [92].

The challenges of analysing RNAs in biofluids

Accurate quantification of circulating ncRNAs in body fluids poses several challenges due to their low abundance and small size. However, some tools have been developed to overcome these obstacles, each with advantages and limitations. Real-time qRT-PCR is a widely used and sensitive method that requires only small amounts of ncRNAs [112]. Nevertheless, a major limitation of qRT-PCR is that it is often used to quantify the levels of a defined set of RNAs, and especially miRNAs: it generally includes fewer than 700 miRNAs whereas more than 2600 human miRNAs are currently known. Microarray platforms are an alternative method to detect circulating miRNAs. The advantage of this method is the ability to detect large numbers of circulating miRNAs simultaneously [113], but the downside is that it is of low dynamic range and cannot detect novel (i.e., unannotated) miRNA species. Next-generation sequencing (NGS) is another sequencing-based technology for detecting circulating RNAs [114]. This method has the advantage of being able to detect both annotated and unannotated RNAs but requires analysis by complex bioinformatic tools [115].

The most frequent biofluids used for ncRNA analysis are whole blood, serum, plasma, and saliva. However, analysis of ncRNAs in whole blood, serum and plasma is not easy in routine practice because of restrictive collection, storage, and analysis conditions. Data suggest that whole blood is a poor biological fluid because cancer cells alter the expression levels of sRNAs and miRNAs in the circulation [116]. Overall, there would seem to be a consensus on the value of using plasma and serum [117]. However, different methods of sample preparation, anticoagulation, centrifugation, storage properties, and PCR protocols have contributed to variability and inconsistencies between reported results [112],[118–120]. An alternative is the use of saliva, which has similar ncRNAs and miRNA expression levels as in plasma and serum, but without the technical constraints. Salivary miRNA analysis has been proven to be a useful diagnostic biomarker in many pathologies, but has never been evaluated in breast cancer [121]. Using NGS and bioinformatics, we were able to build a miRNA blood and saliva signature of endometriosis with high accuracy allowing its use in routine practice [122].

Despite numerous studies on the expressions of ncRNAs, and especially miRNAs, as reliable and reproducible clinical biomarkers in breast cancer, we have not yet discovered new biomarkers that can compete with ER, PR, and HER2 receptors for diagnosis, and for determining prognosis and therapeutic strategies. Since the emergence of the molecular era, genomic signatures such as the 21-gene test (Oncotype DX) and the Mammaprint test (70 genes) have helped to refine the routine prescription of chemotherapy and facilitate the personalization of breast cancer treatment [123–127]. The identification and characterization of ncRNA and miRNA expression, which is as reliable and reproducible and less costly than these genomic panels, could constitute important biomarkers [128]. Biomarker signatures, such as the 21-gene and 70-gene assays, all rely on the absolute quantification of gene targets from paraffin-embedded tumour samples. On the other hand, the diagnostic, prognostic, theragnostic, and therapeutic use of ncRNAs can be performed in a peripheral, iterative and non-invasive manner allowing the development of diagnostic signatures (breast cancer diagnosis, DCIS with invasive component, molecular type specific signature, lymph node involvement, initial metastatic involvement), prognostic signatures (recurrence signature, signature of the metastatic risk, signature of the global survival, signature of the survival without recurrence), and theragnostic signatures (response to first-line neoadjuvant chemotherapy, detection of therapeutic resistance before initiation of chemotherapy, evaluation of radiosensitivity to choose between conservative or radical treatment).

Conclusion

In this review, several aspects of the potential role of non-coding RNAs in breast cancer have been underlined. On the pro side, their diversity reflecting the heterogeneity of breast cancer, and the fact they represent the main part of the transcriptome. Besides, the multiple limitations of the “standard” diagnostic and therapeutic tools available highlight the need for such new biomarkers. On the cons side, the challenge represented by their sequencing and most of all analysis, imposing complex bio informatics support to interpret the amount of data available. Overall, large studies should be conducted to better understand the role of ncRNAs in breast cancer with a view to developing personalized therapeutic strategies for all stages of the disease that would improve survival and quality of life and reduce the economic burden.

Data availability statement

The data underlying this article are available in the article.

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Yohann Dabi: Writing – review & editing, Writing – original draft, Conceptualization. **Sofiane Bendifallah:** Methodology, Data curation, Conceptualization, Writing – original draft. **Stéphane Suisse:** Conceptualization. **Julie Haury:** Writing – review & editing, Conceptualization. **Cyril Touboul:** Methodology, Data curation, Writing – original draft. **Anne Puchar:** Writing – review & editing, Writing – original draft.

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Declaration of Competing Interest

None.

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