Chapter 6
Role of tRNAs in Breast Cancer Regulation

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Abstract Increased proliferation and protein synthesis are characteristics of transformed and tumor cells. Although the components of the translation machinery are often dysregulated in cancer, the role of tRNAs in cancer cells has not been well studied. Nevertheless, the number of related studies has recently started increasing. With the development of high throughput technologies such as next-generation sequencing, genome-wide differential tRNA expression patterns in breast cancer–derived cell lines and breast tumors have been investigated. The genome-wide transcriptomics analyses have been linked with many studies for functional and phenotypic characterization, whereby tRNAs or tRNA-related fragments have been shown to play important roles in breast cancer regulation and as promising prognostic biomarkers. Here, we review their expression patterns, functions, prognostic value, and potential therapeutic use as well as related technologies.

Keywords tRNA · tRNA-derived fragments · tRFs · tRNA modifications · miRNAs · piRNAs

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6.1 Introduction

Transfer RNAs (tRNAs), aminoacyl-tRNA synthetases (ARSs), and amino acids are essential elements for protein synthesis. ARSs ligate tRNAs with cognate amino acids, after which aminoacyl-tRNAs participate in translation by transporting precursor amino acids to the ribosome [1]. Although a tRNA is usually charged with only one of the 20 different amino acids, the human tRNAome is very complicated and consists of >500 interspersed tRNA genes and 51 anticodon families, constituting 4–10% of total cellular RNA (Fig. 6.1) [2, 3]. According to the nomenclature of tRNAs, $tRNA^{Leu}$ refers to the tRNA type to be charged with Leucine (Leu). When $tRNA^{Leu}$ is aminoacylated with Leu, it is represented as Leu-$tRNA^{Leu}$. Most tRNA types incorporate isoacceptors that are charged with the same type of amino acid but have different anti-codons. For example, $tRNA^{Leu}$(CAG) and $tRNA^{Leu}$(UAG) are isoacceptors of each other, which recognize CTG and CTA codons in a messenger RNA (mRNA) and incorporate Leu into the growing polypeptides, respectively, during translation. In addition, functional equivalence or expression patterns of tRNAs have been revealed to be irrespective of their sequence similarity [4, 5].

For a long time, tRNAs had been considered as mere house-keeping RNAs; however, recent studies have suggested that tRNAs and their fragments may have diverse roles. For example, Mey et al. reported that several tRNAs can bind to cytochrome C, inhibiting caspase activation and apoptosis upon apoptotic stimuli [6]. Initiator tRNA ($tRNA_i^{Met}$) is unique in the sense that it can initiate translation; overexpression of $tRNA_i^{Met}$ has been reported to change the translational efficiency of specific genes and alter the global tRNA expression, resulting in various cellular responses such as proliferation, enhanced invasion, and metastasis [7, 8]. Various stimuli have been reported to cause digestion of tRNAs, generating small tRNA-derived fragments (tRFs) [9]. These fragments can be derived from precursor tRNAs (pre-tRNAs) or mature tRNAs and are similar in size to microRNAs. Our

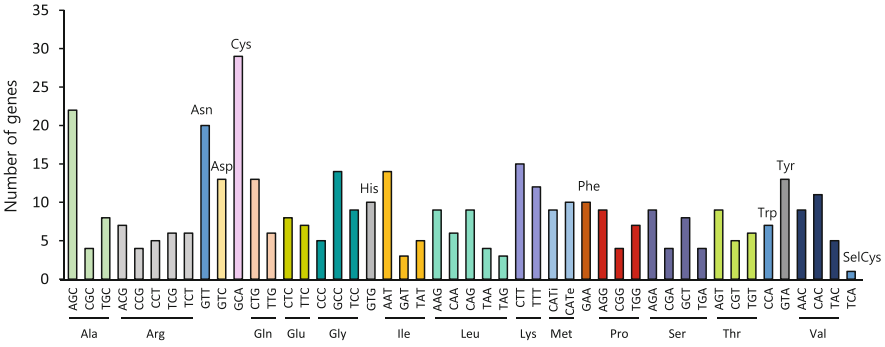

Fig. 6.1 Number of tRNA (transfer RNA) genes in the human genome. Numbers of tRNA genes per amino acid are presented. Different anticodons per amino acid mean isoacceptors of tRNA. Data were based on the high confident set of *Homo sapiens* (GRCh37/hg19) chromosome, in tRNA database (http://gtrnadb.ucsc.edu/) with a total of 416 tRNAs with a selenocysteine (SelCys) tRNA

understanding of the diverse functions of tRFs has recently improved. They are now known to participate in translational regulation, neuroprotection, cell proliferation, tumorigenesis, and RNA silencing like microRNAs [10–14].

In addition, overexpression of tRNAs has been observed in various cancer cell lines and tissues, although their biogenesis and translational requirements remain obscure [5, 15, 16]. Given that tRNA abundance is correlated with protein synthesis [17], it has been hypothesized that tRNA content may affect the rate of translation globally or for a subset of proteins based on codon usage [18]. A recent study has revealed that breast cancer metastasis is promoted by $tRNA^{Glu}$(UUC) and $tRNA^{Arg}$(CCG) (Table 6.1) [5]. This study has demonstrated that overexpression of specific tRNAs can modulate protein expression in a codon-dependent manner, resulting in metastatic behavior. It has also shed light on the importance of quantitative changes in tRNAs. However, it is still debated whether tRNA abundance and codon usage are under concerted regulation of translation rate and efficiency [19–21]. In fact, several studies have suggested that preferentially used codons are not translated faster, and that tRNA variation might play an adaptive role in coping with environmental changes. Analyses of human tRNA expression patterns using microarrays have revealed that tRNA expression is modulated according to the cell cycle, such as during proliferation and differentiation [21, 22]. Taken together, these observations suggest that more studies are required for understanding the relationship between tRNAs and the translational need.

Even with this uncertainty, many reports have suggested important roles of tRNAs and tRFs in cancer as translational and signaling modulators as well as possible biomarkers. Here, we review tRNAs and tRFs reported in breast cancer and their potential as biomarkers, and discuss the future prospects.

6.2 Review of Past Studies

6.2.1 Expressional Analysis of tRNAs and tRFs

6.2.1.1 Generation of tRNAs and tRFs

In eukaryotic cells, tRNA genes are transcribed by RNA polymerase III (RNA Pol III), and pre-tRNAs undergo further processing to generate mature tRNAs. During this process, RNase P and RNase Z remove the 5′ leader and 3′ trailer sequences, respectively, and then CCA trinucleotide is added to the 3′ end of the tRNA for maturation (Fig. 6.2) [23].

tRFs can be generated from pre-tRNAs as well as mature tRNAs (Fig. 6.2). While 3′ trailer of pre-tRNAs is called tRF-1 and identified in itself, 5′ leader is not observed as an independent tRF. Two groups of tRNA halves, namely 5′ and 3′ halves, can be created by digestion of the anticodon loop by RNase T2 or RNase A superfamily, which is released by stress stimuli [24, 25]. It is known that endogenous 5’ tRNA halves generally inhibit translation via diverse mechanisms. In addition,

Table 6.1 Functions of representative tRNAs and tRFs in breast cancer

tRNA	Type	Function or characteristics	Naming in the original article	References
$tRNA^{Leu}$	Mature tRNA	Association between estrogen receptor alpha (ERα) and Brf1 in ER-positive breast cancer	–	[53]
$tRNA^{Leu}$, $tRNA^{Tyr}$	Pre-transcript	Positive correlation with the expression of telomerase reverse transcriptase (TERT)	–	[50, 51]
$tRNA^{Leu}$	Mature tRNA	Enhanced proliferation of ErbB2-positive breast cancer	–	[54]
$tRNA^{Arg}$(UCU), $tRNA^{Arg}$(CCU), $tRNA^{Thr}$(CGU), $tRNA^{Ser}$(CGA), $tRNA^{Tyr}$(GUA)	Mature tRNA	Overexpression in breast cancer	–	[15]
$tRNA^{Ser}$, $tRNA^{Arg}$, $tRNA^{Glu}$, $tRNA^{Gly}$	Mature tRNA	Differential expression in breast cancer and correlation with overall or recurrence-free survival	–	[34]
$tRNA^{Glu}$(UUC), $tRNA^{Arg}$(CCG)	Mature tRNA	Enhanced ribosome occupancy and stability of transcripts enriched with their cognate codons for Glu and Arg to enhance metastasis	–	[5]
$tRNA^{Val}$(CAC), $tRNA^{Val}$(ACC), $tRNA^{Gly}$(GCC), $tRNA^{Gly}$(CCC), $tRNA^{Glu}$(CUC), $tRNA^{Lys}$(CUU), $tRNA^{His}$(GUG)	Mature tRNA	High expression in triple-negative breast cancer cells	–	[52]
$tRNA^{Ser}$	Mature tRNA	Less expression in basal-like 1 subtype of triple-negative breast cancer cells	–	[52]
$tRNAi^{Met}$(CAU)	Precursor tRNA	Target of tumor suppressive miR-34a	–	[48]
$tRNA^{Glu}$(Y*UC), $tRNA^{Asp}$(GUC), $tRNA^{Gly}$(UCC)	i-tRF	Suppression of cell proliferation and cancer metastasis via destabilization of YBX-1-bound oncogenic transcripts	tRF^{GluYTC}, tRF^{AspGTC}, tRF^{GlyTCC}	[27, 62]
$tRNA^{Tyr}$(GUA)	Intron region		tRF^{TyrGTA}	[27, 62]
$tRNA^{Asp}$(GUC), $tRNA^{His}$(GUG), $tRNA^{Lys}$(CUU)	tRF-5	Promotion of cell proliferation via sex hormone-dependent induction	5'-SHOT-RNA^{AspGUC}, 5'-SHOT-	[12, 62]

(continued)

Table 6.1 (continued)

tRNA	Type	Function or characteristics	Naming in the original article	References
			RNA^{HisGUG}, 5'-SHOT-RNA^{LysCUU}	
$tRNA^{Asp}$(GUC)	i-tRF	High expression in cancer	i-tRF from the AspGTC anticodon	[62, 84]
$tRNA^{His}$(GUG), $tRNA^{Arg}$(UCG)	tRF-1	Upregulation by mutations in the oncogenic KRAS, or PIK3CA	Ts-46, and ts-47	[62, 63]
$mtRNA^{Asp}$		Alteration of mtRNA metabolism by the mutation of T7581C in $mtRNA^{Asp}$	Mt-$tRNA^{Asp}$	[42]
$tRNA^{Thr}$, $tRNA^{Lys}$, $tRNA^{Lys}$, $tRNA^{Leu}$		High level in extracellular vesicles of breast cancer cells	miR-720, miR-1274a, miR-1274b, and miR-1260	[58, 59]
$tRNA^{Thr}$, $tRNA^{Leu}$	tRF-3	High level in the blood from patients of ER+/HER2—Breast cancer	miR-720, miR-1260 and miR-1280	[61]
$tRNA^{Cys}$(GCA)	i-tRF	Significant increase in trastuzumab-resistant breast cancer	tRF-30-JZOYJE22RR33, tRF-27-ZDXPHO53KSN	[81]

*Y in $tRNA^{Glu}$(YUC) represents C or U, that is, $tRNA^{Glu}$(CUC) and $tRNA^{Glu}$(UUC)

many tRFs are induced by sex hormones in breast cancer [12]. To date, 3 types of tRFs originating from mature tRNAs have been identified: tRF-5s, tRF-3s, and i-tRFs, which correspond to 5′, 3′, and internal fragments of tRNAs, respectively. tRF-5 s and tRF-3 s are generated by cleavage of tRNAs by Dicer and/or members of the RNase A superfamily. The anticodon loop is usually contained in i-tRFs, which were first identified in breast cancer cells, but biogenesis of i-tRFs is not entirely clear [26]. While the mechanism of tRF-mediated regulation of gene expression remains elusive, involvement of tRFs in the regulation of transcript stability and signaling pathways has been suggested. These assumptions are supported by the fact that tRFs have been shown to associate with Argonautes as siRNAs and miRNAs do, and tRFs interact with several transcription-regulating and RNA-binding proteins [27].

6.2.1.2 Detection of tRNAs and tRFs

The size of a tRNA and tRF ranges from 76 to 90 nucleotides and 14 to 50 nucleotides, respectively. The most conventional detection method for tRNAs and tRFs is

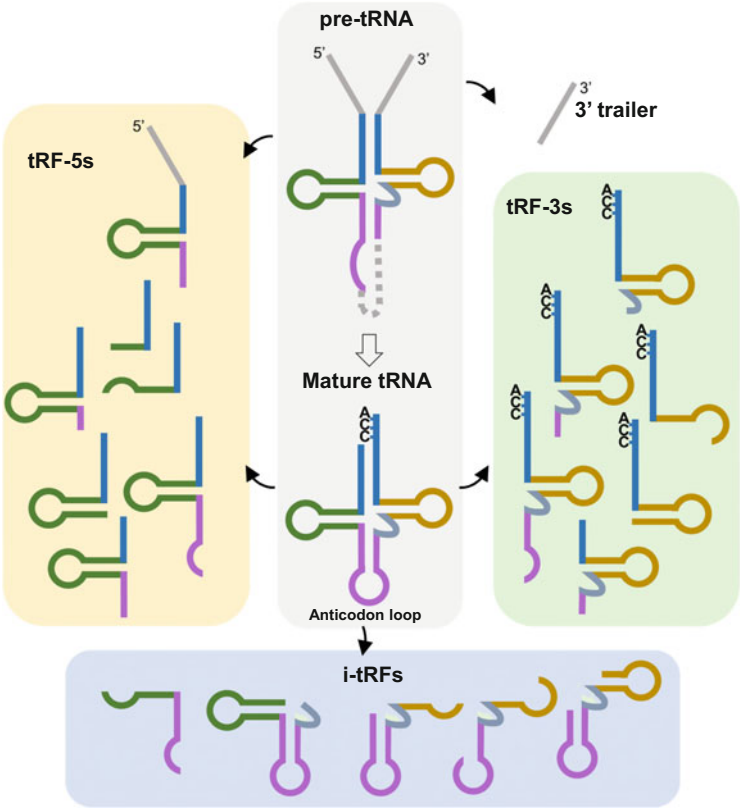

Fig. 6.2 tRNA processing and generation of tRFs (tRNA-derived fragments). Mature tRNAs are generated from precursor tRNA (pre-tRNA) transcripts by digestion of the 5′ leader and 3′ trailer, and then CCA is added to the 3′-end by CCA enzymes. Several kinds of tRFs, tRF-1 (*3′ trailer* of tRNA*)*, tRF-5 (5′ fragment of tRNA), tRF-3 (3′ fragment of tRNA), and i-tRF (internal fragment of tRNA) can be generated by cleavage of pre-mature or mature tRNAs under stimuli such as stress response. Intronic sequences, depicted as the dotted gray line in the pre-tRNA, exist in several tRNAs such as $tRNA^{Tyr}$, $tRNA^{Leu}$, $tRNA^{Ile}$, $tRNA^{Pro}$, and $tRNA^{Arg}$, and part of them are also identified as tRFs

northern blotting. By using specific nucleotide probes labeled radioactively or non-radioactively [28], the size of tRNAs and tRFs can be identified, and even tRNAs loaded with an amino-acid can be distinguished from the unloaded tRNAs based on size [29]. However, northern blotting is a labor-intensive and quantitatively imperfect procedure, which makes northern blotting be considered inadequate to analyze huge amount of samples. To overcome these limitations, Pavon-Eternod et al., for the first time, developed a microarray platform to profile tRNAs in breast cancer [15]. This microarray platform enabled simultaneous analysis of tRNAs in multiplex conditions, but it is still labor-intensive and difficult to be generalized because it is still based on specialized probing techniques. Owing to the

revolutionary development of next-generation sequencing (NGS) techniques, numerous small non-coding RNAs, including tRNAs and tRFs, can be massively analyzed in large and complex datasets at single nucleotide resolution in a rather unbiased way [30]. Recently, several NGS methodologies have been developed to find the optimal conditions for the analysis of mature tRNAs and/or tRFs [31]. Consequently, the sequences of numerous tRFs detected in human samples in various contexts are currently available in several databases [9, 32, 33]. For example, tRFinCancer shows the expression patterns of tRFs in multiple cancer types [32], tRFdb is a relational database of tRFs and other tRNA-related RNA fragments [9], and MINTbase is a database for tRFs of mitochondrial or nuclear origin [33].

6.2.1.3 tRNA Overexpression in Breast Cancer

Pavon-Eternod et al. analyzed the expression levels of individual tRNAs in breast cancer cells using a microarray platform and revealed an unexpected selectivity that is based on cognate amino acid properties and isoacceptor identities [15]. Each breast cancer cell line generates unique tRNA profiles that are markedly different from that of non-cancer breast epithelial cell lines. Overall, the results of Pavon-Eternod et al. highlight the potential of using both genomic DNA- and mitochondrial DNA-encoded tRNAs as biomarkers for malignancy, tumor type, or tumor progression. Remarkably, $tRNA^{Arg}$(CCU), $tRNA^{Ser}$(GCU), $tRNA^{Thr}$(CGU), and $tRNA^{Tyr}$(GUA) are among the most overexpressed tRNAs in the breast cancer cell lines and breast tumors analyzed (Table 6.1). Since the amino acid residues Ser, Thr, and Tyr are targets for protein kinases and phosphatases, this observation suggests that these tRNAs might be part of a potential mechanism for potentiating post-translational regulation of proteins involved in signal transduction. Significant differences in the relative expression levels of tRNA isoacceptors have also been observed. For example, $tRNA^{Arg}$(CCU) and $tRNA^{Lys}$(UUU) were more overexpressed than $tRNA^{Arg}$(ICG) and $tRNA^{Lys}$(CUU). Differential expression of tRNA isoacceptors may provide an additional level of translational regulation for key genes involved in tumorigenesis. Initiator $tRNA^{Met}$ has been found overexpressed in all cancer-derived breast cell lines compared with the healthy controls. However, $tRNA_i^{Met}$ is not overexpressed as much as a few other tRNAs, such as $tRNA^{Ser}$, $tRNA^{Thr}$, and $tRNA^{Tyr}$ in the breast cancer cells. Therefore, further studies are needed to elucidate the regulatory relationship between tRNA expression and cancer.

Krishnan et al., for the first time, investigated the differential expression patterns of tRNAs in breast tumor tissues using NGS to determine if these patterns had any prognostic significance for breast cancer [34]. They profiled 571 tRNAs from 11 normal breast and 104 breast tumor tissues and found that 76 tRNAs were differentially expressed, among which several tRNAs, including $tRNA^{Ser}$, $tRNA^{Arg}$, $tRNA^{Glu}$, and $tRNA^{Gly}$, showed a positive correlation with the overall or recurrence-free survival (Table 6.1). Although the analysis results were dependent on the

controls used, this observation suggests the global tRNA upregulation and differentially expressed tRNAs as potential novel prognostic markers in breast cancer.

6.2.1.4 tRF Detection in Breast Cancer

It is known that 321 tRNA genes out of 625 total human tRNA genes generate diverse forms of tRFs, and the most common form is tRF-3, which consists of the C-terminal half of a tRNA (http://genome.bioch.virginia.edu/trfdb/statistics.php). Various kinds of tRFs have been identified in breast cancer cells and tissues, and they seem to be involved in breast cancer regulation and progression. An interesting report has indicated that levels of several tRFs may be associated with racial disparities in triple negative breast cancer, which is characterized by marked differences between white and black/African-American women [35]. These tRFs include nuclear $tRNA^{Gly}$ and $tRNA^{Leu}$, and mitochondrial $tRNA^{Val}$ and $tRNA^{Pro}$. The functions of tRFs identified in breast cancer will be discussed later.

Small noncoding RNAs circulating in the blood may serve as signaling molecules because of their ability to carry out a variety of cellular functions. Dhahbi et al. have previously described tRFs and other small RNAs circulating as components of larger complexes in the blood of humans and mice, implying that these small RNAs may specifically be processed, secreted, and regulated [36]. Recently, deep sequencing and informatics analysis revealed that 5′ tRNA halves were abundant and significantly different in the serum of clinicopathologic breast cancer patients, showing the potentials of 5′ tRNA halves as circulating biomarkers of breast cancer. Larger studies with multiple types of cancer are needed to adequately evaluate their potential use for the development of noninvasive cancer screening.

6.2.2 Modifications of tRNAs in Breast Cancer

6.2.2.1 Genetic Alterations of tRNAs

In addition to genomic 625 tRNA genes, mitochondrial DNA encodes its own 22 mitochondrial tRNA (mtRNA) genes. mtDNAs are known to be more vulnerable to mutation than their genomic counterparts due to the lack of protective histones, introns, and efficient DNA repair mechanisms [37]. Polymorphism or mutations of mtRNAs, therefore, are more frequently reported to be associated with various diseases than those in genomic tRNAs. There have been indications that mitochondrial function and polymorphisms are involved in the carcinogenic process and increased risk of cancer [38].

tRNA genes do not appear to be hot spots in breast cancer given that trials to find any changes in chromosomal tRNA genes have not revealed any mutations [38, 39]. However, depletion and mutation of mtRNA have been reported in the increased tumorigenic and invasive phenotype [40–42]. An example would be the

case of mtRNA^{Asp} mutation which has been shown to be involved in the carcinogenesis of breast cancer (Table 6.1) [42]. The mutation of T7581C in mtRNA^{Asp} gene creates a new conserved base-pairing (G4-C69), which presumably causes a failure in mtRNA^{Asp} metabolism. It implies that mutations may cause alterations in the tertiary structure of mtRNAs resulting in impairment of mitochondrial protein synthesis.

Other polymorphisms in mtRNAs have also been identified in breast cancer patients [38]. The authors have analyzed all the 22 genes encoding mtRNAs in breast cancer carcinoma as well as blood. Polymorphism of mtRNA^{Asp}, mtRNA^{Lys}, mtRNA^{Gly}, mtRNA^{Arg}, mtRNA^{Leu}, and mtRNA^{Thr} have been found in 6–12% of patients. Distinguishing the polymorphisms or mutations in mt-tRNA genes is still puzzling for the clinicians and geneticists when confronted with breast cancer. Although it is unclear whether these polymorphisms are connected with the pathology or not, it cannot be excluded that mutations in tRNA genes in breast cancer may impact the cell physiology, and cause its dysfunction.

6.2.2.2 tRNA Modifications in Breast Cancer

On average, 13 bases in a tRNA molecule are modified after transcription (Fig. 6.3) [43]. These modifications play multifaceted roles in decoding genetic information as well as in other cellular processes. Abundance, modification, and aminoacylation levels of tRNAs contribute to the translation and differ in different cell types and/or cellular environment [44]. To date, a complete compilation of tRNA modifications and the corresponding modification enzymes have not been determined. Among the predicted and known human tRNA modification enzymes, those linked to breast cancer are listed in Table 6.2 [44].

In fact, base modification itself and the enzymes in charge of tRNA modifications play an important role in the pathogenesis of breast cancer [26, 40]. Studies have indicated that increased tRNA modifications in anticodon swinging bases enhance the translational efficiency due to the increased decoding power of the tRNA [45]. Methyltransferase Misu (NSUN2) and tRNA methyltransferase homolog 12 (TRMT12) have been shown to be significantly increased in breast cancer cell lines and tissues, and they are presumably involved in the proliferation of cancer cells [26, 46]. In human breast cancer, the elevated expression of U34-modifying enzymes directly promotes the translation of oncoprotein DEK, which in turn increases the translation of the oncogenic LEF-1 (lymphoid enhancer binding factor 1) mRNA, promoting the invasion and metastasis of breast cancer cells [47]. Given that extensive base modifications in tRNAs are crucial for their function, future studies should address the potential role of tRNA modifications in breast cancer.

Recent studies suggest that tRNA modifications can increase the stability of tRNAs. Wang et al. observed that miRNA-34a targets pre-tRNA$_i^{Met}$ and induces Argonaute 2 (AGO2)-mediated degradation resulting in reduction of mature tRNA$_i^{Met}$ [48]. Overexpression of tRNA$_i^{Met}$ promotes proliferation and cell cycle transition. Given that mature tRNA$_i^{Met}$ is not a substrate for miRNA-34a-mediated degradation, modification of mature tRNA$_i^{Met}$ may protect it from AGO2-mediated

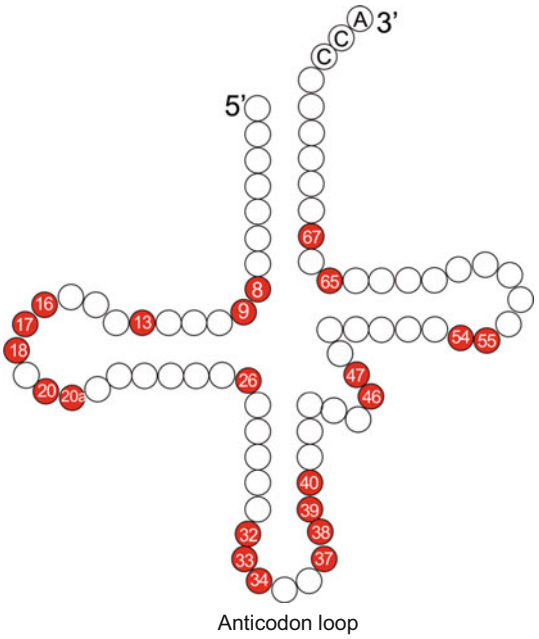

Fig. 6.3 Modification site of tRNAs. Representative modification sites and the number of bases in tRNAs are depicted in red. Base modifications usually happen after the removal of the 5′- and 3′-ends from pre-tRNAs and before the splicing

Table 6.2 tRNA modification genes known or predicted to be linked with breast cancer

Enzyme	Modification	References
THUMPD1 (THUMP domain containing 1)	ac^4C	[85]
METTL6 (methyltransferase like 6)	m^3C	[86]
NSUN2 (NOP2/Sun RNA methyltransferase 2)	m^5C	[46, 87, 88]
ELP3 (elongator complex protein 3)	cm^5U, ncm^5U, mcm^5U, mcm^5s^2U	[47]
CTU1 (cytosolic thiouridylase subunit 1)	s^2U, mcm^5s^2U	[47]
CTU2 (cytosolic thiouridylase subunit 2)	s^2U, mcm^5s^2U	[47]
TRMT12 (tRNA methyltransferase 12 homolog)	o_2yW, yW	[89]
CDKAL1 (CDK5 regulatory subunit associated protein 1 like 1)	ms^2t^6A	[90]
TRMT2A (tRNA methyltransferase 2 homolog A)	m^5U	[91]
MTO1 (mitochondrial tRNA translation optimization 1)	tm^5U	[92]
TRIT1 (tRNA isopentenyltransferase, mitochondrial)	i^6A	[93]
TRMT61B (tRNA methyltransferase 61B)	m^1A	[94]

Most enzymes are expressed in the cytotosol. TRIT1 and TRMT61 are in mitochondria. Ac^4C, N4-acetylcytidine; m^3C, 3-methylcytosine; m^5C, 5-methylcytosine; cm^5U, 5-carboxymethyluridine; ncm^5U, 5-carbamoylmethyluridine; mcm^5U, 5-methoxycarbonylmethyluridine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; s^2U, 2-thiouridine; o_2yW, peroxywybutosine; yW, wybutosine; ms^2t^6A, 2-methylthio-N6-threonyl carbamoyladenosine; m^5U, 5-methyluridine; tm^5U, 5-taurinomethyluridine; i^6A, N6-isopentenyladenosine; and m^1A, 1-methyladenosine

degradation. It has also been reported that BCDIN3D (bicoid interacting 3 domain containing RNA methyltransferase) monomethylated 5′ monophosphate of cytoplasmic $tRNA^{His}$ in vivo and in vitro [49]. BCDIN3D is highly overexpressed in breast cancer and is associated with poor prognosis. BCDIN3D specifically modified cytoplasmic $tRNA^{His}$, without affecting the aminoacylation of $tRNA^{His}$ by histidyl-tRNA synthetase. The exact function of $tRNA^{His}$ in breast cancer was not investigated in this study, but it suggests another link between tRNA modifications with the tumorigenic phenotype of breast cancer beyond translation.

6.2.3 Functions of tRNAs in Breast Cancer

Since tRNAs are principally involved in protein synthesis, their abundance, modification, and mutation are all closely related to protein expression. Synthesis of tRNA is controlled by many oncogenes and tumor suppressors, such as Ras, c-myc, Rb, and p53, all of which affect RNA Pol III-mediated transcription, causing serious dysregulation of tRNA levels [40]. Due to this relation, the alteration of proteins regulating RNA Pol III-mediated transcription also affects the level of tRNAs. In addition, tRNAs can bind to other proteins containing RNA-binding domains and control the function of these proteins they bind to. Accumulating evidence has identified that certain tRNAs and tRFs are involved in the control of proliferation, metastasis, and angiogenesis in human cancers, including breast cancer.

6.2.3.1 tRNA Over-expression in the Subtypes of Breast Cancer

It seems that there are specific tRNA expression patterns, depending on the subtype of breast cancer. In triple-negative breast cancer (TNBC), there is a positive correlation between the expression of telomerase reverse transcriptase (TERT) and pre-transcripts of $tRNA^{Leu}$ and $tRNA^{Tyr}$ in the aggressiveness of cancer (Table 6.1) [50, 51]. In another report, 7 tRNAs, $tRNA^{Val}$(CAC), $tRNA^{Val}$(ACC), $tRNA^{Gly}$(GCC), $tRNA^{Gly}$(CCC) $tRNA^{Glu}$(CUC), $tRNA^{Lys}$(CUU), and $tRNA^{His}$(GUG), have been found to be highly expressed in 26 TNBC cells [52]. All these tRNA types are equally proportional in all the TNBC subtypes, while $tRNA^{Ser}$ is significantly less expressed in the basal-like 1 subtype. It has been reported that $tRNA^{Leu}$ is regulated by the interaction between estrogen receptor alpha (ERα) and Brf1 in estrogen receptor (ER)-positive breast cancer (Table 6.1) [53]. Additionally, it has been suggested that $tRNA^{Leu}$ plays a role in the proliferation of erythroblastic oncogene B (ERBB2)-positive breast cancer (Table 6.1) [54]. Kwon et al. showed that overexpressed $tRNA^{Leu}$ interacted with EBP1 (ERBB3-binding protein 1), reinforcing ERBB2/ERBB3 signaling pathway and enhancing phosphorylation of RSK1 (ribosomal S6 kinase 1) and MSK2 (mitogen-and stress-activated protein kinase 2) [54]. These results suggest that overexpression of any type of $tRNA^{Leu}$ isoacceptors can improve cell proliferation

and apoptotic resistance, showing the possible link between $tRNA^{Leu}$ overexpression and several signaling pathways, such as the RSK1, MSK2, and ERBB2/ERBB3 pathways. All these results suggest that tRNA expression patterns differ in different contexts of breast cancer.

It has been reported that increased $tRNA_i^{Met}$(CAU) levels in carcinoma-associated fibroblasts promote tumor growth and angiogenesis [40, 55]. According to Clarke et al., increased levels of $tRNA_i^{Met}$(CAU) promote growth and angiogenesis of melanoma and lung cancer allografts. They used a mouse model that expressed additional copies of the $tRNA_i^{Met}$(CAU) gene and observed that growth and vascularization of subcutaneous tumor allografts were enhanced in the mice compared with wild-type littermate controls. Elevated expression of $tRNA_i^{Met}$(CAU) was also investigated in the breast cancer–associated fibroblasts obtained from patients; however, due to the small number of samples, the high expression of $tRNA_i^{Met}$(CAU) level was not validated in the breast cancer–associated fibroblasts. The function of $tRNA_i^{Met}$(CAU) in the stroma of breast cancer needs to be studied further. Although the link between upregulation of $tRNA_i^{Met}$(CAU) and breast cancer is obscure at this point, this research shows that $tRNA_i^{Met}$(CAU) may have the ability to generate pro-migratory extracellular matrix for cancer growth and invasion.

6.2.3.2 tRNA Over-expression Promotes Breast Cancer Metastasis

Goodarzi et al. found that specific tRNAs were upregulated in human breast cancer cells resulting in increased metastasis [5]. They found that $tRNA^{Glu}$(UUC) and $tRNA^{Arg}$(CCG) were promoters of breast cancer metastasis, and this observation was corroborated by loss-of-function and gain-of-function analyses as well as clinical-association studies (Table 6.1). Upregulation of these tRNAs enhances the ribosome occupancy and stability of transcripts enriched with the cognate codons of these tRNAs for Glu and Arg. Expression of $tRNA^{Glu}$(UUC) directly upregulates EXOSC2 (exosome component 2) and GRIPAP1 (glutamate receptor-interacting protein 1-associated protein 1), which have high Glu contents. Reduced levels of $tRNA^{Glu}$(UUC) and $tRNA^{Arg}$(CCG) exhibited significantly reduced colonization in the lungs in mice. Consistently, higher levels of these tRNAs were detected in patients with metastatic breast cancer compared with that in the patients without metastasis. These observations suggest that specific tRNAs can induce specific pathways where proteins enriched for their cognate codons are actively involved. Such target transcripts become stabilized in the context of their favored tRNAs and can be more effectively translated, resulting in a greater protein output. Thus, it appears that tRNAs can dynamically regulate gene expression, and the tRNA codon landscape can specifically affect disease progression.

6.2.4 Functions of tRFs in Breast Cancer

6.2.4.1 Tumor Suppressive Roles of tRFs

While overexpression of tRNAs usually shows a positive correlation with poor prognosis of breast cancer [5, 50, 51, 53, 54], tRFs show more diverse effects than tRNAs in many cases. It may be due to the characteristics of full-length tRNA, which support translation and are required under nutritious conditions. Fragmentation of tRNAs can be induced in periods of cellular stress, such as when cells cannot be supported for global translation anymore. Under such conditions, cells should sense the status of their environment, and tRFs may work as regulators to suppress the cell growth since there are already plenty full-length tRNAs that can be processed to generate additional regulators. Of course, there are several clues that tRFs may modulate cancer progression via inhibition of global translation. Thomson and Parker have proposed several possible roles of tRNA halves [56]: [1] translation inhibition via GCN2-mediated stress response activated by nicked tRNAs, [2] formation of a repression complex with other unknown binding partners that should be investigated further, [3] guiding small RNA-mediated translational repression or mRNA destabilization by interacting with Argonaute or PIWI proteins, resulting in silencing of specific transcripts, and [4] guiding mRNA destabilization by interacting with tRNA processing enzymes, such as RNase Z or RNase P [56]. These functions may be linked to breast cancer regulation.

tRFs can also control cancer independently of translation. Upon exposure to stress, tRNAs are enzymatically degraded, yielding distinct classes of tRFs. A novel class of tRFs, derived from $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Tyr}$, shares a common motif that matches the oncogenic RNA-binding protein YBX1 (Y-box binding protein 1) recognition sequence (Table 6.1) [27]. YBX1 is expressed in various kinds of cancers and stabilizes diverse oncogenic transcripts. The fragments derived from $tRNA^{Glu}$, $tRNA^{Asp}$, and $tRNA^{Gly}$ appear to be i-tRFs since they map to the anticodon loops, whereas the $tRNA^{Tyr}$-derived fragment matches to the intronic region (Fig. 6.2). Association of these tRFs with YBX1 displaces the 3′ UTRs of oncogenic transcripts, such as HMGA1 (high mobility group AT-hook 1), CD151 (cluster of differentiation 151), CD97, and TIMP3 (tissue inhibitor of metalloproteinases-3) from YBX1, destabilizing multiple oncogenic transcripts in breast cancer cells. These tRFs are upregulated under hypoxic conditions suppressing breast cancer metastasis. Loss-of-function and gain-of-function studies by using antisense locked nucleic acids (LNAs) and synthetic RNA mimics, respectively, have revealed that these fragments suppress cell growth under serum-starvation, cancer cell invasion, and metastasis of breast cancer cells in vivo. Interestingly, highly metastatic breast cancer cells do not show significant overexpression of these tRFs, implying that a mechanism to attenuate induction of these tRFs exists to evade the tRF-mediated modulation of cancer metastasis. These findings have revealed a tumor-suppressive role of specific tRFs, which can be expanded to other tRFs, non-coding RNAs, or small RNAs.

6.2.4.2 Tumor Proliferative Roles of tRFs

There are also several tRFs which are positively involved in tumorigenesis. Honda et al. reported that a novel type of tRFs that was responsive to sex hormones [12]. These tRFs are specifically and abundantly expressed in ER-positive breast cancer as well as androgen receptor (AR)-positive prostate cancer cell lines. The authors also observed that these tRFs are abundant in human patient tissues, designating these tRFs as sex-hormone-dependent tRNA-derived RNAs (SHOT-RNAs). As expected, SHOT-RNAs are not abundant in other hormone-insensitive cancers, including ER-negative breast cancer and AR-negative prostate cancer, among many others. These SHOT-RNAs are largely identified as the 5′ halves of mature tRNAs by a sort of specific RNA sequencing method. These 5′ halves are generated by angiogenin, a type of RNase A family enzyme, and they increase cell proliferation, strongly suggesting a novel pathway that engages tRNA halves in the development and growth of sex hormone-dependent cancers.

6.2.4.3 MicroRNA (miRNA)-like Role of tRFs

Due to the rapid release of new data from NGS sequencing, numerous novel small non-coding RNAs have been identified expanding our understanding of their characteristics and functions. Yet, experimental data to verify this information are still scarce, causing mis-annotation of some small non-coding RNAs. In fact, there are several small non-coding RNAs that were first recognized as miRNAs but finally proven to be tRFs [57]. Some examples of these RNAs are listed in Table 6.3 (Table 6.3). Among these mis-annotated miRNAs, several of them have been reported to be linked to breast cancer.

Extracellular vesicles (EV), such as exosomes and membrane-shed vesicles, have been implicated in inter-cellular communication. Additionally, their possible use as biomarkers has been being pursued. Guzman et al. investigated the small RNAs in the EVs derived from the breast cancer cell line MCF7 and non-cancerous cell line MCF10A and observed unique miRNA profiles in these secreted vesicles [58]. There was a high abundance of "miRNA-like" tRFs specifically in the EVs of MCF7 but not in the EVs of MCF10A. Whereas the cellular levels of miR-125b, miR-100, and let-7a were correlatively mirrored in the EVs, several small RNAs were only detected in the MCF7 EVs. Interestingly these small RNAs comprised 65% of the total number of small RNAs in MCF7 EVs. The authors reported the four most abundant MCF7 EV miRNAs, such as miR-720, miR-1274a, miR-1274b, and miR-1260 (also known as miR-1260a), which share high sequence homology with $tRNA^{Thr}$, $tRNA^{Lys}$, $tRNA^{Lys}$, and $tRNA^{Leu}$, respectively (Tables 6.1 and 6.3) [58, 59]. Among them, miR-720, miR-1274a, and miR-1274b have been withdrawn from the miRNA database (miRBase) since they are now regarded to originate from the corresponding tRNAs (Table 6.3). It has been reported that tRFs can be induced and secreted under starvation conditions [60], but the small RNA-containing EVs mentioned above were identified under nutritious conditions [58]. Therefore, the

Table 6.3 Probable mis-annotation of human miRNA genes and the corresponding tRNAs

miRNA	Sequence	tRNA	tRF	References
miR-720	UCUCGCUGGGGCCUCCA	Human $tRNA^{Thr}$(UGU)	tRF-3	[57]
miR-1260	AUCCCACCUCU*GCCACCA	Human $tRNA^{Leu}$(AAG)	tRF-3	[95]
miR-1260b	AUCCCACCACUGCCACCAU**	Human $tRNA^{Leu}$(UAG)	tRF-3	[95]
miR-1274a	GUCCCUGUUCAGGCGCCA	Human $tRNA^{Lys}$(UUU)	tRF-3	[57]
miR-1274b	UCCCUGUUCGGGCGCCA	Human $tRNA^{Lys}$(UUU)	tRF-3	[57]
miR-1280	UCCCACCGCUGCCACCC	Human $tRNA^{Leu}$(AAG)	tRF-3	[57]
miR-1308	GCAUGGGUGGUUCAGUGG	Human $tRNA^{Gly}$(GCC)	tRF-5	[57]
miR-3182	GCUUCUGUAGUGUAGUC*	Human $tRNA^{Val}$(CAC)	tRF-5	[96]
miR-4286	ACCCCACUCCUGGUACC	Human $tRNA^{Leu}$(UAA)	tRF-3	
miR-4284	GGGCUCACAUCACCCCAU	Human $mtRNA^{Phe}$	tRF-3	[96]

miR-1260 is also known as miR-1260a. *There are single-base mismatches. Both U and C in miR-1260 and miR-3182 are G in the corresponding tRNA sequences, **U in the miR-1260b does not exist in the corresponding tRNA sequence

mechanisms underlying induction and secretion of the miRNA-derived tRFs detected in this study [58] may be different than those in the study of Lee et al. [60]. These observations imply that high tRF content of tumor-derived EVs alongside the tumor-specific miRNA signatures in them can be used to distinguish these EVs from those of other sources in the circulation.

Another study has also observed that miR-720, miR-1260, and miR-1280 are upregulated in the blood of patients with ER-positive/HER2-negative breast cancer [61]. As mentioned above, miR-720 and miR-1260 are tRF-3s processed from $tRNA^{Thr}$ and $tRNA^{Leu}$, respectively. Additionally, miR-1280 is also a mis-annotated miRNA, and it is actually a tRF-3 derived from $tRNA^{Leu}$ (Table 6.3). In particular, the miR-1280 level is significantly elevated in breast cancer patients, and it is positively correlated with the severity of the disease; the level is the highest in metastatic breast cancer, reduced after systemic treatment. These observations suggest that circulating tRFs, such as miR-1280, may serve as biomarkers for ER-positive breast cancer.

6.2.4.4 Mutations of tRFs in Breast Cancer

Several tRF mutations and their roles have been identified in other cancers. For example, ts-53 and ts-101 are often found to be mutated in chronic lymphocytic

leukemia and lung cancer samples suggesting a key role of these tRFs in tumorigenesis [62]. They are derived from $tRNA^{Thr}$(AGU) and $tRNA^{Ser}$(GCU) but mis-annotated as miR-3676 and miR-4521, respectively. These tRFs associate with PIWI-2 protein to form PIWI-ribonucleoprotein complexes, but the mutations hamper this association. Additionally, these mutations are located in a region required for the interaction of the tRFs with the promoter of ZAP-70 (Zeta-chain–associated protein kinase 70). Consequently, these mutations impair targeting of ZAP-70 promoter by PIWI like protein 2 [63, 64].

It seems that there are no tRFs whose mutations have been identified in breast cancer. However, it has been suggested that tRFs can be key effectors in the pathways regulated by oncogenic mutations. In the MCF7 and MDA-MB-231 breast cancer cells carrying oncogenic mutants of *HRAS*, *KRAS*, or *PIK3CA* genes, the $tRNA^{Arg}$(UCG)-derived tRF, ts-47, is upregulated in *KRAS* mutant cells, and the $tRNA^{His}$(GUG)-derived tRF, ts-46, is upregulated in *PIK3CA* mutant cells (Table 6.1) [63]. Since mutations of *KRAS* and *PIK3CA* have pivotal roles in carcinogenesis, [65–67], tRFs might also function as key effectors in these pathways. Future research is expected to reveal the types and functions of tRF mutants in breast cancer.

6.3 Current Evidence and Concepts

6.3.1 Global Upregulation of tRNA Levels in Cancer

Recently, Zhang et al. have analyzed expression of tRNAs in the uniquely comprehensive data resource from The Cancer Genome Atlas [68]. According to the analysis, almost all cancers express similar overall average expression levels and patterns of tRNAs, while the expression levels of tRNA for each amino acid varies greatly. Among the tRNAs, $tRNA^{His}$ is the most highly expressed, and $tRNA^{Trp}$, $tRNA^{Leu}$, $tRNA^{Phe}$, $tRNA^{Asn}$, or $tRNA^{Sec}$ are not included in the high-expression cluster. Breast cancer is among the 9 cancers that show predominant upregulation of tRNAs across the 31 cancer types analyzed. This study suggests that tRNA overexpression in tumors might increase the translational efficiency in favor of cancer development. They also analyzed other molecules related to tRNAs including ARSs, tRNA-modifying enzymes, and translation factors, including ribosomes. It seems that overexpressed tRNAs may be stabilized by overexpressed tRNA-modifying enzymes, and the increased level of ARSs and translational factors may accelerate the translation in cancers. The merit of this study is that it provides the groundwork for an integrated functional interpretation by covering a broad set of various cancers. By doing so, the authors found that $tRNA^{Arg}$ was overexpressed in multiple cancer types in addition to breast cancer, where $tRNA^{Arg}$ had been reported to promote breast cancer metastasis [5].

6.3.2 Function of tRFs as miRNAs or piRNAs (PIWI-Interacting RNAs)

Since tRFs were first detected in the urine and serum of patients with cancers in the 1970s [62, 69–71], various tRFs have been identified, expanding the roles of tRFs as regulators but not as mere by-products of tRNA degradation. Many reports have suggested the involvement of tRNAs and tRFs in the regulation of transcription, translation, proliferation, cell cycle, apoptosis, and cell signaling. Breast cancer is one of the major cancers where the important implication of tRFs in the regulation of cancer has been shown. As mentioned before, several tRFs have been mis-annotated as miRNAs, suggesting that tRFs may work like miRNAs. However, this relationship between tRFs and miRNAs has not conclusively been clarified. A recent study has shown the interaction of tRFs with the miRNA- and piRNA-related proteins via meta-analysis [72]. Kumar et al. analyzed 50 small RNA datasets and found that tRFs might play a major role in RNA silencing via a microRNA-like mechanism. It is worth noting that tRFs appear to be an abundant class of small RNAs with a distinct biogenesis mechanism different from that of miRNAs. Similarly, several studies have demonstrated that tRFs can also function as piRNAs [64, 73]. A few tRFs have been found in the complexes containing Argonaute proteins, such as AGO1 and AGO2, as well as in complexes containing PIWI proteins. Unlike tRFs, miRNAs are only loaded onto protein complexes containing AGO1 and AGO2. This finding supports that some tRFs could act as piRNAs involved in the epigenetic and post-transcriptional control, such as histone methylation. More evidence, supporting the role of tRFs as an independent group of small non-coding RNAs may come from the breast cancer field with deep mechanistic analyses of the biogenesis and function of tRFs.

6.3.3 tRNAs as Substrates of miRNAs

While tRFs may work as miRNAs or piRNAs do, an interesting report has suggested that pre-tRNAs can be substrates of miRNAs. Wang et al. demonstrated that a tumor-suppressive miRNA, miR-34a, degraded the precursor of $tRNA_i^{Met}$ through AGO2-mediated destabilization [48]. The reduced level of $tRNA_i^{Met}$ inhibited proliferation of breast cancer cells and induced cell cycle arrest resulting in apoptosis. The expression level of miR-34a shows an inverse correlation with that of $tRNA_i^{Met}$ in breast cancer cells, and the cell phenotypes induced by miR-34a are restored by overexpression of $tRNA_i^{Met}$. These observations suggest that $tRNA_i^{Met}$ precursor is a functional target of miR-34a. Accordingly, this study supports the pro-oncogenic role of $tRNA_i^{Met}$ as reported elsewhere [55] and also suggests the protective role of $tRNA_i^{Met}$ modification against cleavage by reducing the interaction of mature $tRNA_i^{Met}$ with miR-34a and AGO2.

6.3.4 Progress in the Detection Methods for tRNAs and tRFs

As mentioned before, recent studies have used NGS to detect small RNAs including tRNAs and tRFs, rather than conventional methods such as northern blotting. However, tRNAs and tRFs have their own characteristics which make their detection more challenging. First, tRNAs and tRFs are post-translationally modified (Fig. 6.3) making the mapping of their deep sequencing reads more challenging [74, 75]. Second, their strong folding characteristics decrease their hybridization onto DNA chips. Overcoming these features can increase the curative and correct detection and interpretation of tRNAs and related fragments. Currently, one of the most reliable approaches for measuring tRNA levels is by DNA chips designed specifically for this purpose by Prof. Tao Pan [15, 76]. Recent studies have utilized the sequencing methods specialized for tRNAs. Several methods have been suggested to overcome the strong self-hybridization tendency of tRNAs via employing novel ligation strategies. For example, a two-step ligation strategy [77], addition of a poly-A tail to the deacylated 3′-ends of mature tRNAs for RT-PCR (real-time-PCR) amplification of tRNAs [78], and Y-shaped adapter application [31]. Furthermore, a DM-tRNA-seq (demethylase tRNA-seq) is intended to reduce the sequence bias from tRNA post-transcriptional methylations by treating tRNAs with AlkB demethylase, followed by a template-switching reaction of thermostable group II intron reverse transcriptase for adapter attachment to tRNAs [79]. There are other methods available for measuring tRNA levels, such as liquid chromatography-mass spectrometry and signature digestion products [80].

6.3.5 The Diagnostic Potential of tRNAs

There is increasing evidence that the expression levels of tRNAs and tRFs may be implicated in disease progression including cancer since their expression is changed or dysregulated in the specific context of diseases.

Recently, several papers have suggested tRFs as predictive markers for breast cancer. Sun et al. investigated tRF profiles in trastuzumab-sensitive and trastuzumab-resistant breast cancer cells via high-throughput sequencing and qRT-PCR and found that two tRFs originated from $tRNA^{Cys}$(GCA) were significantly upregulated in trastuzumab-resistant patients with a positive correlation of ROC (receiver operating characteristic) curve with trastuzumab resistance (Table 6.1) [81].

There are still several challenging points to be considered for the development of tRNAs and tRFs as diagnostic markers. First, robust and efficient approaches to measure tRNA and tRF levels are required. Recent developments in the tools to detect them may shed a light on this field. It is worth noting that tRNAs are relatively stable than other RNAs owing to their self-folding characteristics. This feature

protects tRNAs from being digested by RNA-degrading enzymes and can be advantageous considering that sample-processing time is usually the limiting factor.

Second, there are >500 interspersed tRNA genes, and some of them share the same mature tRNA sequence despite the difference in pre-tRNA sequence. If a tRNA transcribed from a specific locus of a chromosome is to be used as a diagnostic marker, there should be a strategy to differentiate it from the other copies. In addition, a diagnostic tool should consider the adaptation of the mutations or single nucleotide polymorphisms to the human tRNA pool [75].

6.4 Future Research Direction

Based on the reports that have been published in the field of cancer and tRNAs, major studies have focused on the expression levels of these RNA molecules in a specific context of cancer, showing their positive correlations. Deeper and more thoroughly done studies are required to solve the biogenesis and functions of tRNAs and tRFs in breast cancer. It should not be fragmental but comprehensive to give a concrete understanding of tRNAs and tRFs to be used for therapeutic or diagnostic uses. tRNAs and their derivatives are abundant in human body fluids, including serum [62, 69–71, 82]. Therefore, detection of tRNAs and tRFs from EVs in body fluids from cancer patients can be performed via minimally invasive methods. Since they can work as regulatory molecules, widely involved in the pathogenesis of cancers, application of tRNAs and tRF-based non-invasive biomarkers in tumor diagnosis is expected to have broad prospects [83].

There are several things to be solved in basic research. First, the nomenclature of tRFs is still inconsistent. There are >500 tRNA genes in the human genome, and theoretically, all the tRNAs could be cleaved by different types of ribonucleases to produce various tRFs. However, these tRFs have not been categorized with a unified name yet. Many factors should be considered for the unification: the origins and types of tRFs, their chromosomal locations, and inclusion of intron sequences. Li et al. proposed a naming scheme in the form of X-tsRNA$^{AA\text{-}NNN}$, where tsRNA represents the species; X represents the subtypes of tsRNAs based on the mapped location of tRNAs; superscript AA represents the abbreviation of amino acid carried by the mapped tRNAs; superscript NNN represents the anticodon of the mapped tRNAs. For example, 5'-tiRNA and 3a-tRF derived from tRNA$^{Glu\text{-}CTC}$ can be named as 5'-tiRNA$^{Glu\text{-}CTC}$ and 3a-tRF$^{Glu\text{-}CTC}$, respectively [3]. This proposal can be considered as an option before a consensus among the researchers is reached.

Second, the biogenesis process of tRFs is not clearly understood. RNase families and Dicer are known to be involved in the biogenesis of tRFs [3, 26]; however, the understanding of ribonucleases is not very comprehensive. Therefore, the exact biogenesis mechanism of many tRFs remains elusive.

Third, animal models focusing on tRNAs or tRFs would aid to understand the function of tRNAs and tRFs as well as to investigate the phenotypic significance of these RNAs [83]. Animal models are promising tools for analyzing the function and

effect of targets on diseases; therefore, animal model studies with specific tRNAs and tRFs can decrease the gap between in vitro and in vivo studies. Transgenic mice expressing additional copies of $tRNA_i^{Met}$(CAU) will be a good example showing the importance of the animal models [55], where the pro-oncogenic function of $tRNA_i^{Met}$(CAU) for the tumor growth and angiogenesis can be successfully validated.

6.5 Summary

6.5.1 The Bench

An increasing number of reports have revealed that tRNAs and tRFs are involved in various biological processes, such as transcription, translation, proliferation, apoptosis, and metastasis. tRFs are small RNAs working as miRNA and piRNAs do, but they have different biogenesis mechanisms as an independent pool of cell-regulating small RNAs. However, information regarding their expression profiles is fragmented, and the molecular basis behind their biogenesis and function remains still elusive. In accordance with the informatics-based studies, more mechanistic studies will be required to understand the diverse role of tRNAs and tRFs.

6.5.2 Translation and the Bedside

There is growing evidence that tRNAs and tRFs may work as diagnostic markers. The involvement of tRFs and tRNAs in cancers provides fresh perspectives for the exploration and development of new biomarkers and novel therapeutic strategies. The stage of tRNA-based translational research is just at the conceptual step; therefore, active translational research will be on full track in future in accordance with deeper studies.

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