Chapter

Noncoding RNAs as Predictive Biomarkers of Therapeutic Response to Tyrosine Kinase Inhibitors in Metastatic Cancer

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Abstract

Since their discovery, noncoding RNAs have acquired extensive attention due to their eminent role in the regulation of gene expression and thus also in the pathogenesis of many diseases. Currently, strong evidence is showing that noncoding RNAs are integral parts of key cancer-related cellular pathways, and the deregulation of their levels is pathogenetic on one hand but feasible as a biomarker of pathogenesis itself on the other hand. In cancer, diagnosis, prognosis, and prediction of therapy outcome can be derived from levels of various noncoding RNAs. This chapter is focused on potential application of noncoding RNAs in prediction of therapeutic response to tyrosine kinase inhibitors commonly used as targeted therapy in a wide range of metastatic cancers.

Keywords: biomarker, response, ncRNA, tyrosine kinase inhibitors

1. Introduction

Since the 1980s there was some spare evidence of low-molecular RNAs being able to bind complementarily to bigger RNA molecules and having a role in chromatin organization. Small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs) [1–4] were the early discoveries in noncoding RNA field besides tRNAs, and at first, it looked like an exotic exception in rather binary world of protein-coding sequences and the rest of the genome which considered to be "junk" DNA. At the time some mechanisms of regulation of gene expression were known, and overall picture seemed to be complete, give or take a few details. Although it was known that mRNA is a vital part of gene expression and central dogma of molecular biology, the only functional product arising from genetic information, as it was commonly believed, is protein. As the genomic era was just about to come, there was no reason to think that most RNA transcripts are not translated.

Such remarks were first made in 1995 with H19. Expression of this lncRNA correlated with bladder carcinoma caused by loss of H19 imprinting pattern [5]. Further evidence was provided after discovery of other noncoding transcripts, for example, growth arrest-specific 5 (GAS5) [6] and, most importantly, prostate cancer antigen 3 (PCA3/DD3) highly overexpressed in prostate tumor tissue [7].

The beginning of the millennium was marked by the discovery of RNA interference and new short noncoding RNAs regulating gene expression and thus developmental timing in *Caenorhabditis elegans* [8–13]. MicroRNA (miRNA) was coined as the name for this new group of RNAs, and followed by diligent hunt for more, many other microRNAs were identified. Like miRNAs which were discovered first—lin-4 and let-7—many miRNAs were time- or site-specific, meaning they serve their function in some periods of life or only in some cell types [14, 15]. Targets of these RNAs were found in more than 60% of human protein-coding genes [16]. Together with their specific level necessary for fulfilling their job, it was inevitable to notice possible role of ncRNAs in the development of various diseases.

Of all ncRNAs known so far, miRNAs occupy exceptional position, considering the amount of knowledge on their role in pathogenesis of cancer; therefore, their biogenesis, function, and predictive potential will be discussed in the subsequent lines. Following will be lncRNAs, for their potential to be used as a biomarker has been studied extensively in recent years, even though their association with cancer has been outlined already in the very first publications on lncRNAs [5].

This chapter is therefore focused on the potential application of noncoding RNAs in prediction of therapeutic response to tyrosine kinase inhibitors commonly used as targeted therapy in a wide range of metastatic cancers.

2. Noncoding RNAs and their role in cancer

2.1 Classification

Noncoding RNAs (ncRNAs) are usually divided into two groups according to their length. The term small ncRNA (sncRNA) is reserved for diverse group of transcripts shorter than 200 nucleotides. Longer transcripts above 200 nucleotides of length are called long ncRNA (lncRNA). Both short and long ncRNAs usually do not possess any protein-coding capacity [17] which is the main difference from mRNA; there are, however, some cases of cryptic reading frames in longer ncRNAs [18] and even translation of short functional micropeptides from transcripts formerly annotated as noncoding [19].

In contrast to sncRNA, spectrum of lncRNAs is much broader in possible length and thus also in sequence, structure, and function; therefore, similarities with protein-coding mRNA are highly variable with many exceptions among numerous types of noncoding transcripts [20]. Classification of lncRNAs is now more than imperfect due to limited understanding of this group with many structural and functional families unknown yet [21].

For some types of ncRNA, known sequences and their annotations [22] are gathered in online databases. miRbase.org has been established in 2006 as a first noncoding RNA registry for microRNA [23] following the formation of a unified nomenclature for miRNA.

Catalog of lncRNAs has been created much later, in 2012, under the domain mitranscriptome.org and contains data acquired with high-throughput RNA sequencing [24], combining results from several published sources such as The Cancer Genome Atlas [25] or the GENCODE project [21, 24].

2.2 Biogenesis

2.2.1 microRNA

miRNAs are 19–24 nucleotides long endogenously produced regulatory RNAs. Canonical pathway starts with RNA polymerase II which typically transcribes

miRNA sequences, creating capped and polyadenylated primary miRNAs (pri-miRNAs) several hundred nucleotides long. Future mature miRNA sequence resides in the stem region of the secondary hairpin structure of pri-miRNA.

In the next step, pri-miRNA is spliced by a microprocessor complex to one or several hairpins each containing one future mature miRNA sequence—precursor miRNA (pre-miRNA) with its characteristic 5′phosphate and overhang of two nucleotides at 3′OH end. The microprocessor complex comprises mainly of RNase III enzyme Drosha [26] and dimer of protein DiGeorge critical region 8 (DGCR8 or known as Pasha in flies) able to bind double-stranded RNA (dsRNA) [27, 28].

Pre-miRNA is further processed in the cytoplasm, and to get there, it is bound by nuclear transporter protein Exportin 5 [29] and transferred out of the nucleus. In the cytoplasm, pre-miRNA is cleaved by another RNAse III-type enzyme Dicer in cooperation with other proteins depending on species; in humans, for example, it is *trans*-activation-responsive RNA-binding protein (TRBP) [30]. Pre-miRNA is cleaved at stem sequence close to the terminal loop, creating double-stranded RNA intermediate. Depending on several factors such as thermodynamic stability, one of the strands is then recruited into an RNA-induced silencing complex (RISC) by binding with protein Argonaute (AGO) [30], such strand is termed leading. The other, which is thermodynamically more stable, called passenger strand, is usually discarded but can also act in complex with Ago as functional miRNA [31].

Canonical pathway, however, can be overcome, and miRNAs can be produced in alternative, noncanonical ways [32]. Alternative routes independent on various parts of the canonical biogenesis have been described before [33–35], and it is known that they give rise to some other types of sncRNA such as snoRNA or endogenous short hairpin RNAs (shRNAs).

2.2.2 Long noncoding RNA

Due to their highly variable structure and function, it is difficult to outline a general biogenesis pathway for lncRNA. At least part of the biogenesis is shared among lncRNAs and protein-coding mRNAs [21], including transcription by RNA polymerase II and chromatin modifications as those seen during transcription of protein-coding sequences, for example, methylation and acetylation of histones in active promoters [36]. The main differences lie in fewer but usually longer exons in lncRNAs [21], more tissue-specific expression [20], and abundance in the nucleus rather than the cytoplasm [36, 37].

Enormous variability of noncoding RNAs is achieved more on posttranscriptional level than by individual transcriptional mechanisms. Besides standard processes such as polyadenylation, capping, and splicing, nascent ncRNAs undergo modifications that are not typical for mRNAs. Cleaving of 3′end by RNAse P is a typical modification in the biogenesis of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) while creating short tRNA-like transcript (MATAL1-associated small cytoplasmic RNA—mascRNA) and mature lncRNA. Another variation of standard pre-mRNA splicing is the back-splicing of previously spliced transcript creating a circular lncRNA (circRNA). Spliced-out introns can also gain lncRNA status when they escape degradation and then function as lariat-shaped circular RNAs [38, 39]. After all, even miRNAs, as much as other sncRNAs, arise from primary long transcripts which are classifiable as lncRNA but are processed by following miRNA biogenesis pathway [39]. Evidence also suggests that transcriptional apparatus of miRNAs is somehow involved in expression of lncRNAs, too, as knockout of Dicer leads to downregulation of not only miRNAs but also lncRNAs as a class [40].

2.3 Cellular functions and roles in cancer

Distinct length of miRNA predestines them for a specific cellular function. The so-called seed region of miRNA sequence recognizes its target mRNA and binds complementary to its 3′untranslated region. miRNA-mRNA interaction leads to repression of the translation by destabilization of the target mRNA or by recruiting the mRNA degradation factors. As a result, expression of the target is decreased [41]. As the seed region of miRNA is only eight nucleotides long, recognized sequence will not be very specific—many different target mRNAs can contain identical eight-nucleotide combination. miRNAs are therefore pleiotropic in their effect, creating an intertwined posttranscriptional regulatory network. sncRNAs however expand their impact beyond posttranscriptional downregulation of expression. Other types of sncRNAs such as PiWi-interacting RNAs or siRNAs facilitate various cellular functions through pathway of RNA interference and its components. Transposon gene silencing, maturation of rRNA or histone pre-mRNA, and guiding of various complexes to a certain site are only some of very specific functions of short transcripts in cell [42].

In lncRNAs, the range of cellular roles is considerably wider, affecting processes spanning from transcription to epigenetic modification.

LncRNAs regulate transcription in *cis* (genes on the same chromosome) or *trans* (genes on another chromosome) manner acting through transcriptional interference, for example, by overlapping promoters or by binding to transcription factors [43, 44].

Of posttranscriptional modifications, lncRNAs are involved in pre-mRNA capping and polyadenylation, necessary for proper mRNA translation and mRNA splicing, the processes indispensable for diverse protein products from rather small choice of protein-coding sequences in higher eukaryotes [43]. lncRNAs are involved also in epigenetic regulation by loss of imprinting or changes in methylation patterns of cytosine residues in CpG dinucleotide islands. Chromatin remodeling is facilitated by lncRNA, too, as they can recruit chromatin-remodeling and histone-modifying enzymes [43, 45].

Like miRNA, lncRNA can affect mRNA half-life and its stability, consequently triggering mRNA decay or repression of translation by imperfect pairing; on the contrary, perfect pairing can protect the target mRNA from degradation. Moreover, lncRNAs can affect miRNA network by acting as miRNA decoys or cause forming of endogenous siRNAs [43, 46].

The processes stated above are just few of many cellular actions affected by ncRNAs. Mere expression of a gene, protein-coding or not, is only a first step in a working cellular environment which is achieved by fine tuning and multiple layers of control facilitated by ncRNAs on transcriptional and posttranscriptional level. Although different from their targets, ncRNAs suffer from the same errors and damages as protein-coding sequences. Deregulated levels of ncRNAs are mostly observed either because given ncRNA is a target of upstream mutated or epigenetically deregulated effector oncogene, as a result of mutation in ncRNA sequence or defects in transcription and posttranscriptional editing and splicing. Either way, disruption of this network can add to imbalances in critical nodes such as DNA damage repair, cell division, and response to mitogenic and proapoptotic signals, thus shifting cells to precancerous phenotypes.

The genome-wide studies to localize miRNA genes in human genome found that miRNAs are frequently localized at fragile sites, minimal regions of heterozygous loss or amplification, or common breakpoint regions in human cancer [45]. Besides the structural and genetic alterations, the epigenetic silencing of miRNAs genes by DNA promoter hypermethylation or histone hypoacetylation has been described in

some solid tumors and hematologic malignancies. Whole-genome miRNA expression analysis clearly showed that the aberrant miRNA expression patterns present a common feature in the various tumor types. Based on these studies, deregulation of miRNAs was declared to be an important event in the initiation and progression of many cancers. Considering the network of targeted mRNAs and miRNA expression changes, miRNA can be classified as oncogenic miRNA or tumor-suppressive miRNA; some miRNAs may exhibit both features dependently on the cellular context in various cancers [45].

3. Noncoding RNAs as predictive biomarkers of therapeutic response

Drug resistance, either primary or developed secondary, is a crucial factor in tumor recurrence and poor outcome. Administration of the best of current therapies to a group of patients with similar symptoms and seemingly identical diagnosis has shown itself to be inefficient as there is almost always a subgroup of patients not benefiting from the treatment. With ever more precise options in molecular description of patients, it has become evident that cancer is not a single disease, but large family of heterogenous diseases asking for an individual approach. Even after onset of targeted therapy, incomparably more specific than conventional chemo- and radiotherapy, the problem of non-responding subgroups of patients remained. Histological classification was insufficient in prediction of what would be the most effective treatment for a given patient.

To be considered a feasible biomarker, a molecule needs to meet several criteria. Its expression must be cell-type- or tissue-specific and significantly altered during the disease or studied condition compared to normal state. Predictive biomarker then should provide an information on therapeutic outcome in a given patient before the treatment administration, and it could manifest itself in a form of up- and downregulations of RNA or protein expression level, gene copies, mutations, and signaling signatures either downstream or in parallel and can be derived retrospectively or prospectively [47]. For obvious reasons, before ncRNAs, various proteins in the blood and tissue, gene mutations, and later mRNA transcripts were prominent candidates as predictive biomarkers. Up to now, several genetic variants (e.g., SNPs in VEGF-A, VEGF-R1, VEGF-R3, and FGF-R2; [48]) were associated, for example, with response to sunitinib, pazopanib, sorafenib, or axitinib response. Histological and molecular features are also potential biomarkers, in addition to other such as protein expression and immune response activation (e.g., differential levels of some cytokines like IL-6 were observed in patients with progressive disease, although with insignificant results). Also, epigenetic factors such as methylation status were studied; for example, hypermethylation of cystatin-M gene (CST6) and leukocyte adhesion deficiency-1 (LAD1) were observed in patients with shorter PFS on TKI therapy (all reviewed in [48]). Although many molecules have been considered as biomarker candidates, only a few of them have really made it to clinical practice mostly due to lack of proper validation on significant cohorts and study design discrepancies. Also, in some cases, it is not clear whether given molecule has prognostic or rather predictive character.

With the discovery of miRNA and their regulatory impact, attention has been turned to ncRNAs. Concerning miRNAs, the first attempts in finding cancer-specific ncRNA biomarkers were made in Carlo Croce's research group in 2002 [49]. A team of researchers discovered that miR-15 and miR-16 sequences lie in a region frequently deleted in chronic lymphocytic leukemia (CLL) and this deletion leads to downregulation of these miRNAs. Further investigation revealed that many microRNA genes are located at fragile genomic regions and that microRNA

profiles show specific patterns correlating with distinct clinical subtypes of CLL [50, 51]. In this case, microRNAs were the first ncRNAs tested for biomarker potential, but many more different kinds of noncoding RNAs emerged throughout the years, mostly after next-generation sequencing was introduced. Advances in high-throughput profiling technologies led to discovery of over 1900 mature human miRNAs from more than 1500 miRNA gene loci [23] and were followed by numerous studies focused on application of ncRNAs as diagnostic, prognostic, and predictive markers or therapeutic targets. To name just a few of many examples of promising biomarkers [52, 53], long intergenic RNA named HOX transcript antisense RNA (HOTAIR) is known to be metastasis-associated in breast cancer and playing active role in modulating cancer epigenome [54]. Choosing from sncRNAs, one example out of many could be miR-126 which has been shown to be involved in VEGF/PI3K/Akt/MRP1 signaling pathway as a principal player directly binding to vascular endothelial growth factor A (VEGF-A) [55]. Another one is miR-31, a potent factor in the development of various tumors with many target genes [56] which has been shown in many studies to be a reliable biomarker of response to anti-EGFR therapy. Recent large randomized trials proved low expression of miR-31 is an indicator of longer response and overall survival of patients with advanced colorectal carcinoma and wild-type allele of KRAS [57–59]. As for therapeutical applications, phase I study of MRX34, a liposomal miR-34a mimic, has been finished in 2017 with promising results in treatment of various solid tumors [60].

Reasons for extensive biomarker research on ncRNAs are their unique attributes beating proteins and mRNAs as biomarkers. In comparison, ncRNAs often manifest higher tissue-specific expression patterns which are necessary for precise distinction between different molecular subtypes of the disease and avoiding false-positive or false-negative results. Among the important characteristics of promising biomarkers is their detection in samples obtained noninvasively. Overall trend is to get as many and as detailed information with minimal burden for patients. Although ncRNAs are easily detectable in tissue samples (either fresh frozen or formalin-fixed paraffin-embedded), they are released and circulating in body fluids such as the blood, plasma, saliva, or urine as well which is incomparably less painful and faster to obtain than tissue specimens. This would be of tremendous value, for example, for patients with lung cancer who are routinely recommended for molecular testing for mutational status of epidermal growth factor receptor (EGFR) and anaplastic lymphoma receptor tyrosine kinase (ALK) in order to identify patients with superior response to TKIs so that they can avoid conventional chemotherapy. However, to obtain accurate lung biopsies for such testing, patients experience severe discomfort during difficult invasive procedures. Liquid biopsies would be thus much convenient option [61].

Replicative nature of ncRNAs makes them easy to detect by polymerase chain reaction and various modifications of this method, also microarrays and sequencing. ncRNAs vary in stability according to their length, secondary structure, association with proteins, or protection by exosomes; however, there is a consensus that relative to DNA or mRNA, shorter ncRNAs are more stable and less likely to be cleaved by RNAses or to be degraded by environmental agents such as storing temperature [62]. There are however some limits and variability between different types of sample handling and storage [63].

Based on many functions and pleiotropic character of ncRNAs, their involvement in progression of cancer and in modulation of therapeutic response is not surprising. In the following lines, we provide an overview (**Table 1**) of ncRNAs currently known to play a role in the development of resistance to TKIs, and what is more, their level seems to be an indicator of such resistance.

Drug	ncRNA	Deregulation in drug-resistant patients	Diagnosis	Technological platform	Study
Sunitinib	miR-1307-3p + miR-425-5p	Up	RCC (all)	NGS, qPCR	García-Donas et al. [64]
	miR-942 + miR-133 model	Up		qPCR	Kovacova et al. [65]
	miR-942	Up		qPCR array	Prior et al. [66]
	miR-628-5p miR-23b miR-27b	Down		qPCR	Puente et al. [67]
	miR-99-5p	Down		NGS, qPCR	Lukamowicz-Rajska et al. [68]
	miR-9-5p	Up		TaqMan-MicroRNA Cards, qPcr, digital PCR	Ralla et al. [69]
	miR-141	Down		qPCR array	Berkers et al. [70]
	miR-424c	Down		Microarray, qPCR	Gámez-Pozo et al. [71]
	miR-1 + miR-597 model	Up		qPCR array	Khella et al. [72]
	miR-155 miR-484	Up		qPCR array	Merhautova et al. [73]
Sorafenib	SRLR	Up	RCC	Microarray, qPCR	Xu et al. [84]
	miR-425-3p	Down	HCC	TaqMan low density array, qPCR	Vaira et al. [83]
Gefitinib	miR-21	Up	NSCLC	Microarray qPCR	Shen et al. [74]
	EGFR-AS1	Up	HNSC	qPCR, NanoString panel	Tan et al. [85]
	miR-630	Down	LUAD	qPCR	Wu et al. [88]
	miR-200c	Down	NSCLC	qPCR	Li et al. [75]
	MiG6-miR-200 ratio	Up	NSCLC, BC	qPCR	Izumchenko et al. [76]
Erlotinib	miR-630	Down	LUAD	qPCR	Wu et al. [77]
	miR-223	Up	NSCLC	Microarray, FirePlex	Joerger et al. [78]
	miR-200c	Down	NSCLC	qPCR	Li et al. [75]
	EGFR-AS1	Up	HNSC	qPCR, NanoString panel	Tan et al. [85]
	MiG6-miR-200 ratio	Up	NSCLC, BC	qPCR	Izumchenko et al. [76]

Drug	ncRNA	Deregulation in drug-resistant patients	Diagnosis	Technological platform	Study
Lapatinib	miR-16	Down	BRCA, STAD	Microarray, qPCR	Venturutti et al. [86]
	miR-630	Down	BRCA	qPCRC	Corcoran et al. [79]
Nintedanib	miR-200 family	Down	LUAD	qPCR array	Nishijima et al. [80]
Neratinib	miR-630	Down	BRCA	qPCRC	Corcoran et al. [79]
Afatinib	miR-630	Down	BRCA	qPCRC	Corcoran et al. [79]

mRCC, metastatic renal cell carcinoma; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung carcinoma; HNSC, head and neck squamous carcinoma; LUAD, lung adenocarcinoma; BRCA, breast invasive carcinoma; BC, breast carcinoma; STAD, stomach adenocarcinoma.

Table 1.
Overview of potential ncRNA biomarkers of response to tyrosine kinase inhibitors.

3.1 Sunitinib

Most studies on prediction of response by miRNA levels have been carried out on renal cell carcinoma and sunitinib as a prominent treatment choice in patients with clear cell renal cell carcinoma. Ten papers have been published so far on prediction of sunitinib response in metastatic renal cell carcinoma (mRCC). Though there are some discrepancies in experimental design, mainly in samples and technologies used in explorative phase, most of the studies are carried out on a rather small cohort; there is some overlap in results. miR-484, miR-221/222, miR-942, miR-133a, miR-628-5p, and miR-155-5p were successfully validated by more than one study; however, none of them turned out to be significantly deregulated in all the studies [64–73].

It is useful to have some information about mechanistic impact of predictive miRNAs, because usually their deregulation is somehow connected with the development of therapy resistance. For example, in the work of Puente et al., two of three significantly deregulated miRNAs, miR-23b and miR-27b, are known to inhibit Notch1 and c-Met pointing on potential involvement of Notch pathway in sunitinib response, serving as solid base for future research. In some cases, however, the targets of predictive miRNAs are waiting to be characterized and subjected to a further functional analysis of mechanistic connection of a given miRNA with response to sunitinib. In other work [77], miR-99b-5p has been discovered to be significantly lower in patients with shorter progression-free survival; unfortunately they did not manage to validate it in an independent cohort by RT-qPCR with sufficient statistical significance. However, miRNAs from miR-99 family are possibly tumor suppressors not only in RCC; there is evidence of their involvement in OSCC in regulation of IGF1R [81].

3.2 Sorafenib

Primarily used for treatment of RCC, sorafenib is ineffective in patients with initial resistance, which can be predicted by expression levels of sorafenib resistance-associated lncRNA (SRLR) identified by Xu et al. [82]. lncRNA-SRLR level has been

correlated with sorafenib therapy response in RCC patients, and clear connection has been demonstrated. Manipulation with its expression leads to changes in response of RCC cell lines. According to recent findings, SRLR acts through IL-6/STAT3 pathway and by binding to NF-κB promotes IL-6 transcription and activation of STAT3, in the end causing the development of sorafenib resistance [82]. To prove that, researchers introduced STAT3 inhibitor and IL-6-receptor antagonist, which restored response to the treatment.

In another study on patients with hepatocellular carcinoma [83], six miRNAs have been significantly associated with progression-free survival (PFS); however, only miR-425-3p was successfully validated. Higher levels of this miRNA indicated longer PFS. In vitro tests have shown reduced cell motility and increased cell death in HCC cell lines when miR-425-3p was added which indicates that miR-425-3p probably acts as tumor suppressor [83].

3.2.1 Gefitinib, erlotinib, and nintedanib

There are known some mutations in epidermal growth factor receptor (EGFR) which are reliable indicator of response to EGFR-targeting TKIs. However, these mutations are minor, and for patients with wild-type EGFR, there is no biomarker of response to the treatment [84].

Quite robust has been a study of non-small cellular lung carcinoma (NSCLC) patients treated with gefitinib [74], where miR-21 has been proven to be a potent biomarker of response. The study has been carried out on 128 radically resected patients in explorative phase compared to 32 healthy controls; results have been validated on 201 EGFR-mutated patients. In patients with better therapy outcome, miR-21 has been significantly reduced.

Tan et al. [85] showed interesting case report of two patients with exceptional response to gefitinib, diagnosed with head and neck squamous cell carcinoma. Silent mutation in lncRNA epidermal growth factor receptor—associated 1 (EGFR-AS1)—led to destabilization of this lncRNA which in turn shifted splicing of EGFR to isoform D and noncanonical EGFR addiction, thus affecting its sensitivity to tyrosine kinase inhibitors.

Gefitinib and erlotinib are frequently used in EGFR-mutated lung adenocarcinoma where they reach better results and longer progression-free survival than in wild-type-EGFR lung adenocarcinoma patients. However, in both cases the development of resistance to treatment is inevitable; still its mechanism remains uncovered. The first information on the development of resistance was shown by Wu et al. [77]. miR-630 and one of its target transcripts, YAP1, create a feedback loop with ERK and are suspected to be responsible for the resistance in EGFR-mutated adenocarcinoma cells. Further they showed that low level of miR-630 indicates future resistance to TKIs in EGFR-mutated patients with lung adenocarcinoma.

Erlotinib alone has been studied in phase II clinical trial of Swiss Group for Clinical Cancer Research (SAKK) on blood samples of NSCLC patients treated with first-line combination of bevacizumab and erlotinib followed by chemotherapy. The study was focused on circulating miRNAs, and their main objective was to find prognostic miRNAs, but they identified also some predictive miRNAs both for targeted therapy and chemotherapy. miR-223 expression was shown to have the highest predictive value for disease stabilization and time to progression, with higher expression being associated with worse outcome [78].

Among other miRNAs, miR-200 family seems to have extensive impact on response to nintedanib, gefitinib, and erlotinib. Nintedanib is a multi-targeted angiokinase inhibitor prescribed for idiopathic pulmonary fibrosis and advanced

NSCLC [80]. It has been shown in work on lung cancer cell lines (5 nintedanib-resistant/5 nintedanib-sensitive) that some miRNAs belonging to miR-200 family (miR-200, miR-200a, and miR-141) are significantly lower in nintedanib-resistant cells. Induction of miR-200 and miR-141 has led to restored treatment sensitivity in resistant cells. miR-200/ZEB axis might play a role in resistance to treatment and serves as a potential biomarker of response to nintedanib. The work also proved some role of this family in EMT transition which has been outlined before in Izumchenko et al. [76] where miR-200 has been suggested to play a role in TGFβ-miR200-MIG6 axis. According to their findings, authors concluded that this pathway creates an EMT-associated switch-inducing resistance to EGFR-targeting drugs. Further, they observed that the ratio of MIG6 versus miR-200 expression indicates response to erlotinib.

Yet another work connected miR-200c with response to erlotinib and gefitinib in patients with NSCLC. When upregulated, miR-200c correlates with sensitivity to gefitinib in EGFR wild-type cell lines. Besides other pathways leading to EMT, in this work it has been shown that miR-200c regulates EMT also through PI3K/AKT pathway and MEK/WRK. One hundred fifty patients treated with gefitinib or erlotinib as a second- or third-line treatment were tested in this study, and in 66 NSCLC patients with wild-type EGFR, high levels of miR-200c expression were associated with higher disease control rate (DCR), longer progression-free survival (PFS), and longer overall survival (OS) than low miR-200c expression subgroup [75].

3.2.2 Lapatinib

MiR-16 mediates trastuzumab and lapatinib response, as shown on trastuzumab- and lapatinib-resistant breast and gastric cancer cell cultures [86]. Artificial increase of miR-16 expression had an inhibitory effect on cell growth in vitro, and it is speculated that expression of miR-16 is regulated by phosphatidylinositol 3 kinase (PI3K)/AKT pathway starting at extracellular signal regulated kinases 1/2 (ERK1/2) which are blocked by trastuzumab and lapatinib. Probably due to inhibition of c-Myc which is downregulated by PI3K/AKT, the level of miR-16 is then upregulated to normal level and inhibits proliferation of both breast cancer and gastric cancer cells. The same effect was achieved by artificial increase of miR-16, as stated above, indicating that miR-16 is not only a biomarker but possible therapeutic target, too.

3.2.3 HER-targeting drugs

miR-630, as mentioned above, has been linked also to response to HER-targeting drugs, namely, lapatinib, neratinib, and afatinib, used in breast and lung cancer. The same problem as elsewhere repeats itself also in these diagnosis—targeting of HER in HER2 overexpressing patients is mostly effective, except in patients with primary or secondary resistance. Response to these drugs is mediated by IGF1R which is targeted by miR-630. Work of Corcoran et al. [79] shows that an artificial increase of miR-630 in cells with primary or secondary resistance to anti-HER therapy leads to restored efficacy of such drugs. Blocking of miR-630 leads to the development of resistance. Results were validated also on set of tumor and non-tumor tissue. According to current knowledge, miR-630 plays a dual role in apoptosis and drug resistance, because depending on cell type, it serves as a tumor suppressor in breast carcinoma [87] and hepatocellular carcinoma [88] or as an oncogene in renal cell carcinoma [89].

4. Conclusions

Noncoding RNAs gained extensive attention in recent years for their unique features as endogenous regulators of gene expression, potential biomarkers, and therapeutic targets. Tissue specificity, stability, and detectability in all types of tissues and body fluids predestine them to become very promising biomarkers applicable in personalized medicine. Major attention has been devoted to miRNAs; less is known about involvement of lncRNAs. Although studies on profiling and feasibility of various ncRNAs as diagnostic, prognostic, and predictive biomarkers are accumulating, none have made it to real clinical practice so far. Here we provide an overview of current knowledge on possible biomarkers of response to tyrosine kinase inhibitors, a breakthrough targeted therapy of several solid tumors. Currently, besides studies focused on sunitinib, there are rather solitary results acquired on small cohorts of less than 100 patients; therefore, it is difficult to come up with any conclusions. Even if there are more studies on response prediction of one therapeutic agent, inter-study discrepancies in validated biomarkers are significant, and results overlap sparsely. This can be ascribed to differences in study design such as type of samples, technology, normalization, statistical analysis, thresholds, and cutoff values set as criteria for stratification of patients and many more. Out of all TKI, sunitinib is much more ahead in terms of number of biomarker studies, study design similarity, and partial overlap of the results.

In spite all of that, miR-200 family, miR-221/222, miR-484, miR-221/222, miR-942, miR-133a, miR-628-5p, miR-155-5p, and miR-630 seem to have significant biomarker potential indicated by several studies. However, independent prospective validation on larger cohorts taking utmost account of study design in previous relevant studies is necessary for future clinical application of miRNA-based biomarker technology to TKIs' therapeutic response prediction.

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Conflict of interest

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