Resistance to targeted therapies: a role for microRNAs?

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Highlights

Resistance represents the major limit to targeted therapy efficacy.
miRNAs can sustain resistance by targeting critical signal transducers.
Selected miRNAs can be exploited as biomarkers of resistance.

The discovery of oncogene addiction dramatically changed the therapeutic approach for cancer treatment, and many drugs targeting specific molecular alterations are now in clinics. Despite the big success of these new compounds, the main limit to their efficacy is represented by resistance to therapy. The alteration of the activity or of the expression of many proteins has already been linked to the onset of resistance, but recent evidence indicates a role of microRNAs (miRNAs) as well. In this context, the idea of exploiting miRNAs as predictors of response or resistance to cancer therapy represents an intriguing possibility. The purpose of this review is to address the relationship between miRNAs and targeted therapies response and resistance.

Keywords

- miRNAs;
- sensitivity to therapy;
- mechanisms of resistance;
- molecular therapies

How miRNAs can modulate resistance to targeted therapies

One of the major goals of cancer research has been the identification of ‘druggable’ and effective protein targets to be used in therapy. The effective molecular target is a protein that plays a critical role in promoting growth (or in preventing apoptosis) of tumor cells and on whose constitutive activity cancer cells rely for their survival. In other words, a drug is effective when it blocks the gene to which cancer cells are addicted to, thus leading them to death. The concept of ‘oncogene addiction’ has contributed strong pressure to the development and the introduction of many new molecular drugs in clinics. Tyrosine kinase inhibitors (TKIs; see Glossary) and monoclonal antibodies (mAbs) are now widely used to treat patients affected by tumors of different histological origins, alone or in combination with standard chemotherapies [1].

From experience gained in clinical practice, we know that only a percentage of tumors respond to targeted therapy; this condition is referred to as ‘innate’ or ‘primary resistance’. The major mechanisms of resistance to target therapies are described in Box 1. The criteria commonly used to enroll or exclude patients from a specific treatment are the evaluation of the expression of the target and the genetic status

(amplification/mutation) of the target or of its key downstream transducers (http://www.nccn.org). However, even the adoption of the most stringent available criteria does not allow proper identification of responsive patients. Moreover, responding patients almost invariably develop resistance to therapy during treatment, a situation defined as 'secondary' or 'acquired resistance'. Understanding if a tumor cell is resistant to therapy is mandatory to properly select patients that will benefit from a specific pharmacological treatment or, on the contrary, to avoid treatment of those who are predicted to be non-responders. The final aim is to avoid, or at least delay, the onset of resistance or to circumvent it when already present.

Box 1.

Mechanisms of resistance to targeted therapies

The main mechanisms of resistance to targeted therapies are:

(i) Mutation of the target

TKIs are developed to bind and block the target kinase in the wild type status or in the presence of a sensitizing mutation. To escape from blockage, cancer cells can acquire secondary mutations that hamper the interaction between the small molecule and the target, allowing ATP binding and receptor activation. The *EGFR* T790M mutation in patients treated with first generation anti-EGFR compounds (Erlotinib and Gefitinib) represents a paradigmatic event: this mutation decreases the affinity for the drug and increases the affinity for the ATP, leading to EGFR pathway activation in the presence of the drug [76].

Resistance to mAb treatment can also be due to the mutation onset in the target: a recent report by Montagut and colleagues [77] showed the presence of a mutation in the extracellular portion of EGFR that impairs Cetuximab binding. Interestingly, Panitumumab remains effective in inhibiting EGFR and represents an alternative clinical strategy for CRC patients who become resistant to Cetuximab treatment.

(ii) Activation of downstream signal transducers

The activation of molecules downstream of the target is a system exploited by cancer cells to circumvent treatment efficacy. One paradigmatic example is observed in CRC, where activation of downstream proteins has been reported both as a primary and a secondary mechanism of resistance to the anti-EGFR mAbs Cetuximab and Panitumumab. These mAbs are indeed ineffective in CRC patients harboring *KRAS* mutations (primary resistance 78, 79 and 80), and the same genetic alterations have also been shown as a mechanism of secondary resistance [81].

(iii) Activation of parallel pathways

The blockage of signaling pathways important for cell proliferation can be overcome by the activation of parallel pathways. A typical example is the activity of anti-EGFR drugs hampered by the activation of the MET pathway. In fact, amplification of the *MET* tyrosine kinase receptor has been shown as a mechanism of both primary and secondary resistance to anti-EGFR drugs in CRC and NSCLC 82 and 83. MET-mediated resistance can also be due to the increase of hepatocyte growth factor (HGF) availability, leading to constitutive MET activation: this non-cell autonomous mechanism of resistance to targeted therapies is important to understand, because growing evidence indicates the stroma as a source of resistance 24 and 71.

Additional criteria for selecting patients eligible for treatment with targeted therapies are currently under investigation. Modulation in the expression of specific miRNAs or of miRNA families could be one of such criteria (general features of miRNAs are depicted in Box 2). In fact, miRNAs can modulate drug response in

several ways. It has been shown that miRNAs can behave as tumor suppressors 2, 3 and 4 or oncogenes 5,6 and 7, controlling the expression of key components of signal transduction pathways. Deregulation of key miRNAs can lead to the generation of alternative and compensatory signals (parallel or downstream to those blocked by the drug), which can sustain resistance. The activation/inactivation of miRNAs can be transcriptional, due to the presence/absence of specific transcription factors controlling their expression5 and 8, or epigenetic, through the control of the methylation status of miRNA promoters or histone acetylation 9 and 10 (Figure 1A). Interestingly, mutations either in the miRNAs or in the 3′ untranslated regions (UTRs) of miRNA targets have been described: in both cases the miRNA/target recognition is altered and thus new targets can be hit or, vice versa, the negative control for the expression of some key targets is lost 11 and 12 (Figure 1B). Finally, other genetic alterations such as genomic amplifications or loss of heterozygosity (LOH) can involve miRNA loci and alter levels of miRNAs and of their targets 11, 13 and 14(Figure 1C).

Box 2.

General features of miRNAs

miRNAs are small endogenous noncoding RNAs responsible for the post-transcriptional regulation of target messenger RNAs (mRNAs). miRNAs, when processed in the mature form, are incorporated in the RNA-induced silencing complex (RISC) and bind preferentially the 3′ UTR of mRNAs with imperfect base pairing. Protein synthesis from targeted mRNAs is prevented either by mRNA degradation or, more frequently, by inhibition of translation.

miRNAs are located in almost all regions of the genome, including repeats. They can be intronic or exonic, and can be positioned in noncoding regions between annotated genes (intergenic) or in coding regions; they may be grouped into clusters or be single. Regulation of miRNA expression is still poorly understood: miRNAs can be transcribed by the promoter of the gene in which they are inserted or they can have their own promoter.

A peculiar feature of miRNA regulation is their ability to control different targets at the same time, either allowing widespread action on different pathways or increasing their effect by operating at different levels in the same pathway. This is particularly important in embryogenesis, where miRNA activity can orchestrate the complexity of developmental processes.

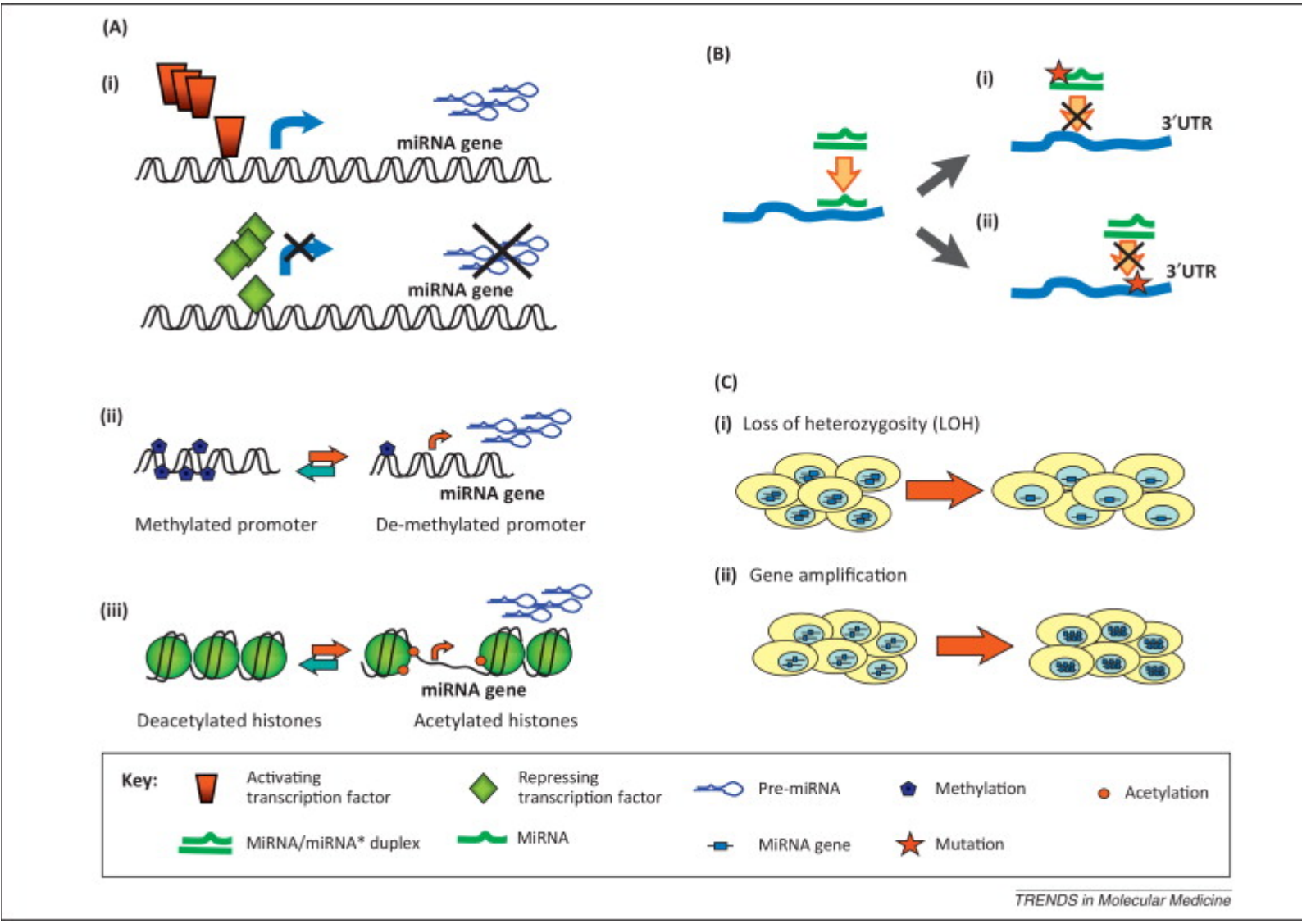

Figure 1.

Mechanisms of microRNA (miRNA) deregulation. miRNA alterations in cancer can lead to resistance to targeted therapies for several reasons. **(A)** Altered transcriptional control: (i) increase/decrease of specific transcription factors acting as activators or repressors of miRNA transcription; (ii) increase/decrease of the methylation status of miRNA promoters; and (iii) change in the histone acetylation in the genomic region codifying miRNAs. In all of these conditions the final effect is an altered level of the miRNA in the cell. **(B)** Mutations: (i) presence of mutations in the miRNA or (ii) in the target mRNA 3′ untranslated region sequences, altering the proper recognition and control of the target by the miRNA. The final outcome is a change in the level of the target. **(C)** Amplifications/deletions: change in miRNA copy number due to: (i) loss of heterozygosity (LOH) of a specific miRNA gene or (ii) miRNA gene amplification. These genetic events modify the amount of miRNA present in the cell.

The literature on the role of miRNAs in drug response/resistance is just at the beginning: the first examples of miRNA involvement in the response to therapy or in the onset/modulation of resistance to antineoplastic drugs have recently been reported. An overview of miRNAs involved in resistance to targeted therapies is reported in Table 1 and Figure 2. The following sections recapitulate the main findings related to the role of miRNAs in sustaining resistance to molecular therapies in different tumors.

Table 1.

miRNAs involved in response/resistance to targeted therapies

miRNA	Drug	Tumor	Refs
Let-7	Gefitinib	NSCLC[a]	Zhong *et al.* [17]
	Sorafenib	HCC[a]	Shimizu *et al.* [65]
	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
	ADT[b]	PC[a]	Nadiminty *et al.* [45]
miRNA-15a	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
miRNA-21	Tamoxifen	BC[a]	Gong *et al.* [40]
	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
	ADT[b]	PC[a]	Ribas *et al.* [47]
miRNA-27a	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
miRNA-30b/c	Gefitinib	NSCLC[a]	Garofalo *et al.* [20]
miRNA-31	ADT[b]	PC[a]	Lin *et al.* [44]
miRNA-122	Sorafenib	HCC[a]	Bai *et al.* [64]
miRNA-125b	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
miRNA-126	Gefitinib	NSCLC[a]	Zhong *et al.* [17]
miRNA-128b	Gefitinib	NSCLC[a]	Weiss *et al.* [16]
miRNA-144	Imatinib	CML[a]	Liu *et al.* [58]
miRNA-145	Gefitinib	NSCLC[a]	Zhong *et al.* [17]
	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
miRNA-146a	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
miRNA-155	Tamoxifen/Fulvestrant	BC[a]	Nam *et al.* [37]
miRNA-200b	Cetuximab	CRC[a]	Mekenkamp *et al.* [29]
miRNA-200c	Erlotinib	NSCLC[a]	Bryant *et al.* [19]
miRNA-210	Trastuzumab	BC[a]	Jung *et al.* [70]
miRNA-212	Cetuximab	HNSCC[a]	Hatakeyama *et al.* [23]
miRNA-214	Gefitinib	NSCLC[a]	Wang *et al.* [18]
miRNA-216/7	Sorafenib	HCC[a]	Xia *et al.* [66]
miRNA-221/2	Gefitinib	NSCLC[a]	Garofalo *et al.* [20]
	Tamoxifen	BC[a]	Zhao *et al.* [36], Nam *et al.* [37]
	ADT[b]	PC[a]	Sun *et al.* [49]
miRNA-342	Tamoxifen	BC[a]	Cittelly *et al.* [33]
miRNA-375	Tamoxifen	BC[a]	Ward *et al.* [31]
miRNA-451	Tamoxifen	BC[a]	Bergamaschi *et al.* [34]
	Imatinib	CML[a]	Lopotovà *et al.* [56], Scholl *et al.* [57], Liu *et al.* [58]
miR-488*	ADT[b]	PC[a]	Sikand *et al.* [46]
miR-616	ADT[b]	PC[a]	Ma *et al.* [52]

a

NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; CRC, colorectal cancer; BC, breast cancer; CML, chronic myeloid leukemia; HCC, hepatocellular carcinoma; PC, prostate cancer.

b

ADT, androgen deprivation therapy.

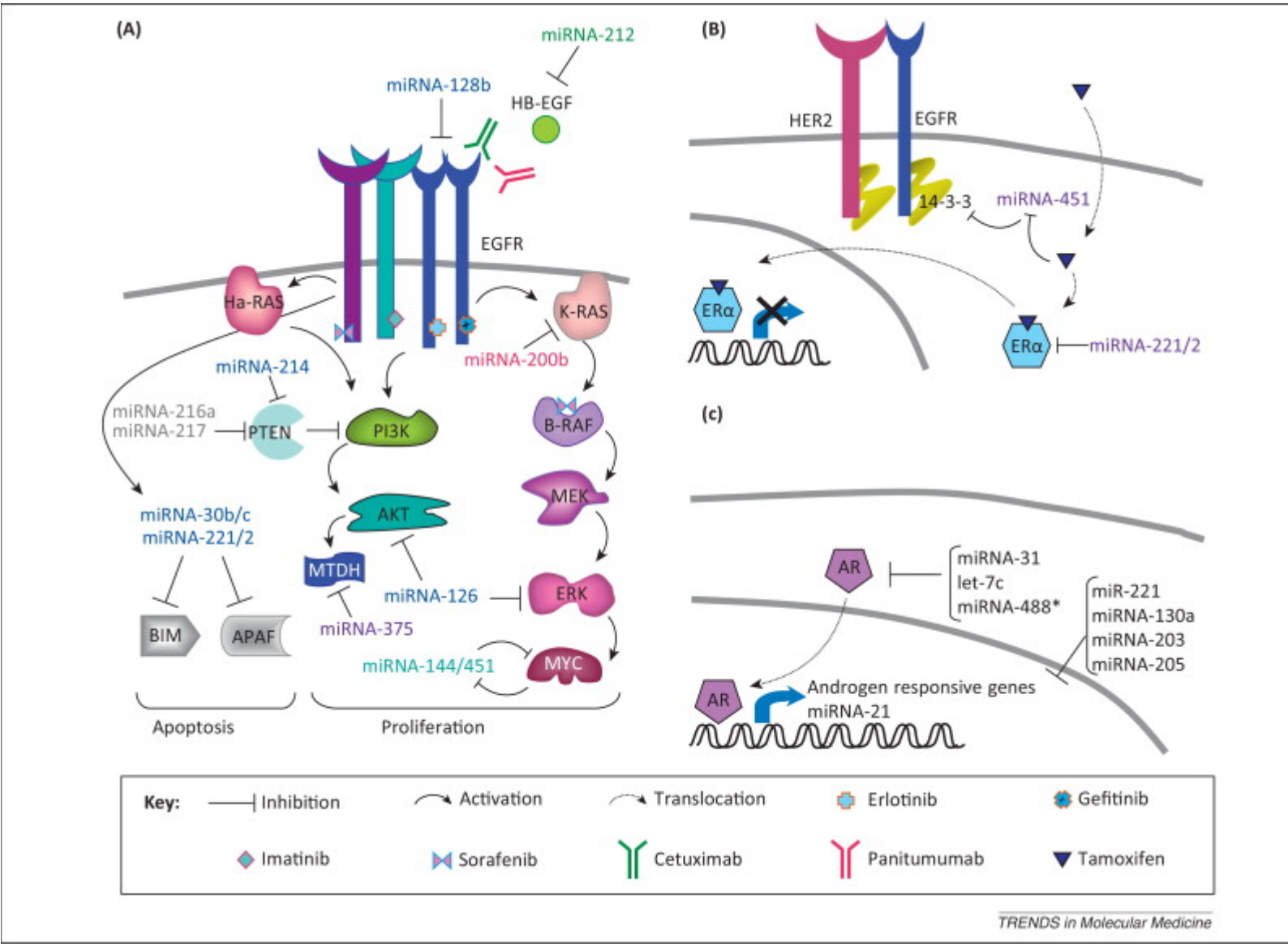

Figure 2.

MicroRNAs (miRNAs) modulate pathways commonly activated in cancer. miRNAs can control key pathways often activated in cancer: **(A)** Tyrosine kinase receptors (RTKs) promote proliferation by activating PI3K–AKT and K-RAS–ERK pathways and block apoptosis by inhibiting BIM and APAF pathways. Both RTKs and downstream transducers are good candidates for targeted therapy and many of these key proteins are under the control of miRNAs. miRNAs deregulated in: non-small cell lung cancer = blue; head and neck squamous cell carcinoma = green; colorectal carcinoma = pink; chronic myeloid leukemia = light blue; breast cancer = violet; hepatocellular carcinoma = gray. **(B)** Hormone responsive breast cancer becomes resistant to Tamoxifen. ERα, estrogen receptor α. **(C)** ADT (androgen deprivation therapy) resistant tumors. AR, androgen receptor. Abbreviations: HB-EGF, heparin binding-EGF-like growth factor; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; MTDH, methaderin; MEK, MAP/ERK kinase; ERK, extracellular signal-regulated kinase; APAF, apoptotic protein activating factor; BIM, BCL-2 interacting mediator of cell death.

Non-small cell lung cancer (NSCLC)

NSCLC accounts for 80–85% of all lung cancer cases [15]. The main focus of targeted therapy in NSCLC patients is the tyrosine kinase receptor for epidermal growth factor (EGFR); the most widely used inhibitors

of EGFR in NSCLC are currently the two TKIs Erlotinib and Gefitinib, even if new and more potent drugs are under study.

The first paper investigating the role of miRNAs in modulating the response to targeted therapies was by Weiss *et al.* [16], who hypothesized that a miRNA able to regulate EGFR could be a predictor of response to EGFR–TKIs (Figure 2A). In fact, genomic loss of an *EGFR* targeting miRNA would increase the EGFR level, offering a target for EGFR–TKI. Thus, they looked for miRNAs predicted to regulate EGFR that were located in chromosomal regions frequently lost in lung cancer: this led to the identification of miRNA-128b, located on chromosome 3p (lost in 96% of lung cancers and 78% of preneoplastic or preinvasive lung epithelial samples [16]). Interestingly, the authors found that the LOH for miRNA-128b correlated with an improved response to Gefitinib. The major limits of this work are the relatively small number of patients studied (n = 58) and the retrospective type of analysis. Moreover, as stated by the authors themselves, the analysis of miRNA-128b in a cohort of advanced NSCLC patients not receiving EGFR–TKI treatment is required to determine whether miRNA-128b LOH is also a prognostic factor or only a predictor of survival for patients receiving EGFR–TKI therapy.

Starting from the identification of miRNA alterations with a predictive prognostic value in lung adenocarcinoma patients, Zhong *et al.* [17] aimed at investigating whether the restoration of three tumor suppressive miRNAs (let-7, miRNA-126, and miRNA-145) elicits an inhibitory growth response and overcomes the cellular resistance to Gefitinib *in vitro*. They showed that forced expression of these miRNAs contributed to Gefitinib cytotoxicity in lung cancer cells; in particular, miRNA-126 expression increased Gefitinib sensitivity sixfold and resulted in the inhibition of AKT and extracellular signal-regulated kinase (ERK) pathways.

A role for miRNA-mediated modulation of the AKT pathway in regulating acquired resistance to Gefitinib was also found by Wang *et al.* [18]. The authors observed that miRNA-214 was significantly upregulated in a lung cancer cell line made resistant to Gefitinib and showed that this miRNA and its target PTEN were inversely expressed in resistant cells. Knockdown of miRNA-214 altered the expression of PTEN and the activation of AKT and resensitized HCC827 cells to Gefitinib. Taken together, these data show that miRNA-214 may contribute to the acquired resistance to Gefitinib via modulation of the PTEN/AKT signaling pathway.

The first example of the role of miRNAs as predictors of response to the anti-EGFR compound Erlotinib was published by Bryant *et al.* [19], who hypothesized that miRNA expression patterns in samples (cell lines or tumors) with different responses to EGFR inhibition could provide biological insights into the mechanism of sensitivity and could function as biomarkers of response to therapy [19]. They identified a signature of 13 miRNAs predicting a response to EGFR inhibition in cancer cell lines and tumors. Notably, this signature was able to separate primary from metastatic tumor samples using unsupervised clustering methods. Because ontological annotation of the potential targets of the 13 miRNAs revealed enrichment in components of epithelial-to-mesenchymal transition (EMT), they hypothesized that members of the signature could mediate both EMT and response to Erlotinib. As a proof of concept, they ectopically expressed a representative member of these miRNAs, miRNA-200c, and confirmed the alteration of both the expression of EMT proteins and the sensitivity to Erlotinib.

Finally, Garofalo *et al.* [20] showed that MET, the receptor for hepatocyte growth factor, and EGFR control Gefitinib-induced apoptosis and EMT by modulating specific miRNAs. In particular, they found that miRNA-

30b/c and miRNA-221/2 control the expression of genes involved in the apoptotic response and thus in sensitivity to Gefitinib, suggesting that these miRNAs are involved in TKI resistance.

Despite the presence of these interesting and provocative reports, none of the identified miRNAs is ready for a direct clinical application and further validations are required in order to exploit these findings in everyday clinical practice. Moreover, no report tackles the modulation of resistance/sensitivity in response to newer anti-EGFR drugs that have already entered the advanced phases of clinical trials: Afatinib and Dacomitinib, two irreversible inhibitors of the whole EGFR family (anti-pan-HER), and AZD8931, a reversible pan-HER inhibitor.

Head and neck squamous cell carcinomas (HNSCCs)

Ninety per cent of HNSCCs overexpresses EGFR and the level of expression correlates with a negative prognosis [21]. Cetuximab (an anti-EGFR mAb) is the only molecular targeted agent showing significant survival benefits in HNSCC patients as monotherapy or in combination with radiation and/or chemotherapy[22]. Despite the huge cohort of patients that could benefit from an anti-EGFR therapy, only a small subset responds to the treatment and even the responders soon develop resistance. However, the mechanisms of response and resistance to anti-EGFR therapy in HNSCC tumors are largely unknown.

An interesting work by Hatakeyama *et al.* [23] hypothesized a role for miRNA-212 in Cetuximab response in HNSCCs. Analyzing 33 HNSCCs and keratinocyte cell lines, the authors correlated miRNA-212 downregulation with the increase of its target, the heparin-binding EGF-like growth factor (HB-EGF), one of the EGFR ligands. They hypothesized that HB-EGF increase (also detected in Cetuximab-resistant cells and in plasma of HNSCC patients) could compete with Cetuximab for EGFR binding, leading to resistance. Although certainly many other mechanisms of resistance coexist and cooperate to assure survival and growth of HNSCC tumors, this work highlights the role of ligands for tyrosine kinase receptors in response to targeted drugs and reveals the possible involvement of miRNAs in controlling their expression. An increasing number of papers, in fact, have underlined how paracrine/autocrine production of ligands can confer both primary and secondary resistance to targeted therapies, reactivating inhibited pathways or activating parallel pathways that join common key downstream players [24].

Colorectal carcinoma (CRC)

Recent advances in understanding the molecular mechanisms underlying CRC progression led to the introduction of anti-EGFR targeted therapies in clinics, with the approval of the EGFR mAbs Cetuximab and Panitumumab [25]. Treatment with these mAbs, both as monotherapy and in combination with chemotherapy, has shown a survival benefit in metastatic CRC patients with *KRAS* wild type tumors [26]. Tumors harboring a *KRAS* codon 12 or 13 mutation are resistant to anti-EGFR therapy and therefore are not eligible for this treatment [27]. Mutations of *BRAF*, phosphoinositide 3-kinase (*PI3K*), phosphatase and tensin homolog (*PTEN*), and amplification of *HER2* have recently been identified as negative predictors of response to anti-EGFR therapies [28]. However, 10–15% of tumors, negative for all these genetic lesions, do not respond to anti-EGFR treatment, suggesting the need for additional predictive markers [28].

In this context, evidence of the deregulation of a specific *KRAS* targeting miRNA (miRNA-200b) has been reported in patients treated with Cetuximab in combination with other drugs: increase of this miRNA

correlated with improved progression free survival (PFS) in *KRAS* mutated tumors [29]. This report is of particular interest because, as stated above, *KRAS* mutated patients fail to respond to Cetuximab. From the work of Mekenkamp *et al.* [29], it appears that not only the genetic status of *KRAS* but also the level of *KRAS* post-transcriptional regulators can be important for Cetuximab response in CRC. The deregulation of *KRAS* targeting miRNAs may be one of the reasons why a small fraction of patients harboring a mutation in the *KRAS* gene may still benefit from anti-EGFR therapy. However, the mechanisms underlying Cetuximab response in CRC patients remain complex: in fact, the same report also describes the downregulation of another *KRAS* targeting miRNA (miRNA-143), which also correlates with a better PFS.

Although the proposed new miRNA-based molecular markers have potentially important clinical implications, they still remain strictly linked to already known mechanisms of resistance, involving KRAS activation in cancer cells. Deeper investigations are needed to find new prognostic markers of resistance to EGFR-targeted therapy, working independently from KRAS/BRAF/PI3K genetic status: this is a challenging goal, mandatory to explain why a high percentage (10–15%) of patients without the already known genetic alterations predictive of lack of response is not responsive to anti-EGFR therapy [28].

Breast cancer

Breast cancer appears as a heterogeneous disease, characterized by the presence/absence of molecular targets that determine the choice of the therapeutic strategy [30]. Breast cancer patients expressing hormone receptors (HRs, i.e., receptors for estrogen and/or progesterone) in the tumor undergo anti-estrogenic therapy and are generally treated with either Tamoxifen or Fulvestrant [two estrogen receptor (ER) antagonists] or aromatase inhibitors (interfering with the synthesis of estrogen). HER2 positive patients (displaying *HER2* gene amplification) receive in most cases Trastuzumab (a humanized anti-HER2 mAb) or Lapatinib (a dual anti-HER2/EGFR TKI). Because primary and secondary resistance to targeted treatments are commonly observed in both HR and HER2 positive cases, the mechanisms of resistance and the role of miRNAs have been investigated.

Ward and colleagues [31] observed features of EMT and identified a strong downregulation of miRNA-375 in MCF-7 breast cancer cells resistant to Tamoxifen. Interestingly, miRNA-375 restoration recovered sensitivity to Tamoxifen and partially reversed EMT. One of the direct targets of miRNA-375 is Metadherin (MTDH), an oncogenic protein downstream to Ha-RAS and c-MYC, often overexpressed in human tumors[32]. MTDH silencing partially phenocopied the effect of miRNA-375 expression, increasing the sensitivity to Tamoxifen and reversing EMT. The authors also observed a shorter disease-free survival and a higher risk of relapse in Tamoxifen-treated patients with higher expression of MTDH. However, whether or not miRNA-375 alone can predict onset of resistance in Tamoxifen-treated patients remains unclear. Cittelly *et al.* [33] used the same cellular model and observed miRNA-342 downregulation in Tamoxifen-resistant versus parental cells. Interestingly, they detected a reduced expression of miRNA-342 in Tamoxifen refractory human breast tumors, opening the possibility of exploitation of these findings to predict patient outcome during Tamoxifen therapy. Bergamaschi and Katzenellenbogen [34] identified a role of miRNA-451 in Tamoxifen-associated development of endocrine resistance. In fact, they found that Tamoxifen downregulates miRNA-451 and upregulates 14-3-3ζ and that 14-3-3ζ is a target of miRNA-451. 14-3-3ζ belongs to the family of 14-3-3 proteins that bind and stabilize key signal transducers, such as EGFR, HER2, and protein kinase C (PKC).

Tamoxifen upregulation of 14-3-3ζ results from its ability to prevent miRNA-451 transcription. In Tamoxifen-resistant breast cancer cells, the levels of 14-3-3ζ were high indeed, whereas those of miRNA-451 were greatly reduced. They also showed that Tamoxifen, but not Raloxifene, was able to regulate miRNA-451 and 14-3-3ζ, paving the way to understand the differences in activity of these two drugs [35].

Interestingly, Zhao *et al.* [36] found that miRNA-221/2 are involved in the resistance to Tamoxifen, besides their role in mediating resistance to Gefitinib, as stated above. They reported that these miRNAs are frequently upregulated in ERα-negative breast cancer cell lines and primary tumors. The increased expression of these miRNAs reduces ERα levels as a consequence of post-transcriptional negative regulation, whereas their knockdown restores ERα expression and Tamoxifen sensitivity.

In line with this report, Nam *et al.* [37] confirmed the role of miRNA-221/2 in anti-estrogen therapy resistance and highlighted new network clusters involving miRNAs-146a, -27a,-145, -21, -155, -15a, -125b, and let-7s. Analyzing MCF-7 cells resistant to Tamoxifen and Fulvestrant, the authors identified potentially interesting interactions between miRNAs and important targets implicated in the resistance to these targeted therapies. This work represents a starting point for further validation *in vitro* and, more importantly, in clinics.

Finally, miRNA-21 is involved in chemoresistance 38 and 39 and in Trastuzumab resistance in breast cancer[40], where its upregulation is observed in tumor biopsies from patients treated with Trastuzumab and is associated with poor response. Interestingly, miRNA-21 is regulated by NF-κB [41] and targets the tumor suppressor PTEN.

Prostate cancer

Prostate cancer starts as a hormone-dependent disease. Androgen receptor (AR) expression and activity are crucial for prostate cancer growth and maintenance. This evidence led to the introduction of androgen deprivation therapy (ADT) as standard first line treatment of hormone responsive tumors [42]. Unfortunately, prostate cancer cells after some time become androgen-independent and thus resistant to ADT. Very often, the cause of ADT resistance is strictly linked to AR: in fact, recent studies have shown that *AR* can be mutated or amplified in ADT-resistant tumors [43]. For these reasons, the control of *AR* levels is crucial for prostate cancer cells and thus miRNAs targeting *AR* can impinge on hormone responsiveness.

In this context, Lin *et al.* [44] described a new mechanism of regulation of AR mediated by miRNA-31, as AR and miRNA-31 mutually control each other. Interestingly, miRNA-31 is often downregulated in advanced prostate cancer, thus favoring *AR* overexpression and androgen independency. Moreover, miRNA-31 binds*AR* mRNA in the coding region, in a position often mutated in prostate cancer. This work highlights the importance of miRNA control of key pathways as the loss of miRNA-31 may impact on AR levels and, thus, on the efficacy of ADT.

Another miRNA, namely let-7c, has been shown to control AR levels and thus the responsiveness to ADT. Nadiminty *et al.* [45] reported that let-7c impairs *c-MYC* expression that in turn regulates *AR* transcription. Furthermore, they showed a negative correlation between let-7c and AR in human prostate cancers.

Finally, Sikand *et al.* [46] also identified miRNA-488* as a direct regulator of *AR*. Indeed, they showed that miRNA-488* ectopic expression impairs prostate cancer cell growth and induces apoptosis.

These reports describing new miRNAs targeting AR are very interesting because they link ADT responsiveness to post-transcriptional control of *AR*. Nevertheless, the direct proof of the biological role of these miRNAs in ADT resistance in clinical samples needs further investigations.

A different approach was undertaken by Ribas *et al.* [47], who looked for androgen-regulated miRNAs. They found that AR directly promotes the transcription of miRNA-21, which in turn elicits oncogenic activities promoting androgen-independent growth *in vitro* and enhanced tumor growth and castration resistance *in vivo*.

Sun *et al.* 48 and 49 further proved the role of miRNA-221/2 in resistance to targeted therapy. In particular, after describing the overexpression of these miRNAs in androgen-independent cells, they focused on miRNA-221 and identified HETCD2 and RAB1A as its crucial targets involved in ADT resistance. Interestingly, miRNA-221 also affects the expression of a group of androgen responsive genes without modifying the expression of *AR*.

The involvement of miRNA-221/2 in resistance to different agents, in diverse tissues, represents an interesting circumstance. miRNA-221/2 are transcriptionally regulated by two cellular stress sensors, namely NF-κB [50] and c-JUN [51], which are often activated in cells exposed to oncogenic stress due to the inhibition of a pathway responsible for constitutive promotion of cell growth. This might explain why miRNA-221/2 expression is altered in both lung cancer cells, where EGFR drives proliferation, and breast and prostate cancer cells, where ERα and AR activation, respectively, play a growth promoting role.

Ma *et al.* [52] approached the problem of ADT resistance comparing miRNA profiling in androgen sensitive versus insensitive cells: they described miRNA-616 overexpression in androgen-resistant cell lines and tumor samples. More intriguingly, *in vivo* experiments injecting androgen-sensitive cells ectopically overexpressing miRNA-616 suggested that miRNA-616 expression is sufficient for maintaining an androgen-independent phenotype, because mice castration did not affect tumor growth rate.

Using the same approach, Boll *et al.* [53] identified three miRNAs (miRNA-130a, miRNA-203, and miRNA-205) that are downregulated in prostate cancer and whose restoration mimics androgen deprivation, impairing the expression of AR-dependent genes. From the perspective of better characterizing the shift of prostate cancer cells towards hormone resistance, these findings appear very intriguing even if the authors did not directly address whether or not the loss of these miRNAs is necessary for ADT resistance.

Chronic myeloid leukemia (CML)

CML is characterized by a chromosomal translocation in which part of the *BCR* (breakpoint cluster region) gene from chromosome 22 is fused with the *ABL* (Abelson) gene on chromosome 9, encoding for a cytoplasmic tyrosine kinase. The resulting BCR–ABL fusion protein is a constitutively active kinase that drives proliferation of leukemic cells. Pharmacological inhibition of the BCR–ABL kinase by the targeted drug Imatinib drastically improved the prognosis of CML patients [54]. Nevertheless, even in this perfect scenario of complete addiction of cancer cells to a single defined genetic alteration, targeted therapy may become inefficient to block cancer progression, because resistance to therapy may occur, frequently due to the onset of secondary mutations inhibiting the binding of the drug to the target [55].

In this context, Lopotovà *et al.* [56] and Scholl *et al.* [57] reported a correlation between miRNA-451 level and response to Imatinib treatment: patients with Imatinib-resistant CML have lower levels of miRNA-451 compared with responders, and upon response to Imatinib miRNA-451 levels are increased.

Liu *et al.* [58] described the presence of a reciprocal regulatory loop between c-MYC and miRNA-144/451. Interestingly, c-MYC overexpression, detected in Imatinib-resistant K562 cells, directly repressed miRNA-144/451 transcription, whereas miRNA restoration resensitized cells to the drug. Even if these data are interesting, the link between miRNA-451 level and BCR–ABL activity remains unclear, as well as the possible direct involvement of miRNA-451 in CML pathogenesis.

Finally, after profiling a small number of Imatinib responder and resistant patients, San José-Enériz *et al.* [59]identified a group of 19 miRNAs differentially expressed between the two groups. This set of miRNAs shares predicted targets already involved in different mechanisms of resistance. Surprisingly, miRNA-451 is not among the 19 listed miRNAs.

Hepatocellular carcinoma (HCC)

HCC treatment still represents a challenging issue because effective targeted therapies are not yet available, although molecular mechanisms of liver tumorigenesis have been deeply studied. At present, only Sorafenib [a VEGFR (vascular endothelial growth factor receptor), PDGFR (platelet-derived growth factor receptor), C-RAF and BRAF KI] is approved for HCC treatment [60].

Concerning miRNAs, the peculiarity of the liver is that a single miRNA, namely miRNA-122, accounts for more than 70% of all miRNAs present in hepatic cells [61], explaining the profound interest in its characterization. miRNA-122 is very well conserved among different species [61], it is required for hepatitis C virus (HCV) replication cycle [62] and is downregulated in liver carcinogenesis [63]. From these starting points, Bai *et al.* [64] studied the role of miRNA-122 in liver cancer: they showed that the ectopic expression of miRNA-122 inhibits tumorigenic properties of liver cancer cells and, more interestingly, sensitizes HCC cells to Sorafenib treatment. Even if the molecular mechanisms by which miRNA-122 makes cells sensitive to Sorafenib were not shown, these results provide the rationale for testing a combination therapy of miRNA-122 mimetic and Sorafenib in animal models. In line with this report, Shimizu *et al.* [65] described the ability of the let-7 family to cooperate with Sorafenib in inducing apoptosis in liver cancer cell lines through the downregulation of BCL-XL.

Recently, miRNA expression in recurrent and non-recurrent human HCC tissue samples allowed the identification of miRNA-216a/217 cluster upregulation in recurrent HCCs [66]. The main identified targets were SMAD7 and PTEN, both downregulated in HCC. Moreover, they found that miRNA-216a/217 positively regulate the transforming growth factor (TGF)-β/PI3K/AKT pathway that further contributes to acquired resistance to Sorafenib.

Despite these reports, the clinical translation of these findings remains elusive. More investigations involving animal models and patient-derived specimens are needed to unravel the role of these miRNAs in drug response.

Circulating miRNAs

Recently, the presence of miRNAs in cell free components of body fluids (such as blood, serum, and plasma) has been described. Because miRNAs are very stable, this discovery gave the opportunity of exploiting miRNAs for diagnostic and prognostic purposes.

The first report considering the possibility of evaluating the presence of miRNAs in blood as a noninvasive tool for the diagnosis of cancer was by Lawrie *et al.* [67]: they described higher levels of miRNA-21 in the serum of patients with B cell lymphoma compared with healthy individuals, and correlated miRNA-21 amount with relapse. A few months later, Mitchell *et al.* [68] extended monitoring of circulating miRNAs to solid tumor diagnosis: in their work, serum levels of miRNA-141 could distinguish patients with prostate cancer from healthy controls. These pioneering reports opened the field of research towards the use of circulating miRNAs for diagnostic and prognostic purposes: currently, circulating miRNAs associated with almost every type of cancer have been reported and their role as biomarkers has been proposed [69].
Future studies of the correlation between the presence of miRNAs in plasma/serum and sensitivity/resistance to therapy would prove if circulating miRNAs can represent a powerful noninvasive tool for monitoring tumor response.

In this regard, a very intriguing but preliminary report recently described the association between miRNA-210 plasma levels in breast cancer patients and Trastuzumab sensitivity, tumor presence and lymph node metastases: in particular, high miRNA-210 plasma levels correlated with Trastuzumab resistance, both in a cohort of breast cancer patients ($n = 29$) and in a breast cancer cell line rendered resistant to Trastuzumab[70]. These data highlight once more the value of *in vitro* studies exploiting cell lines rendered resistant to antineoplastic compounds and make more concrete the perspective of monitoring the tumor behavior through circulating miRNA levels. Nevertheless, more studies are needed to make use of circulating miRNA levels as markers of response and resistance to current targeted therapies. The goal for the future is the translation of basic research in every day clinical practice for an earlier diagnosis and a more effective treatment.

Concluding remarks

Over the past few years, cancer treatment drastically changed. Upon the discovery of targeted therapies it appeared that, hitting the right ‘heal’, many tumors could become curable. Since the initial enthusiasm, we discovered that although the introduction of targeted therapies certainly represented a big step forward in the field, the occurrence of resistance strongly limits their efficacy. Many efforts are now directed towards the study of the mechanisms underlying resistance to treatment. The remaining goal is to predict resistance onset before cancer reacquires the initial aggressiveness or, vice versa, to predict tumor response, avoiding wasting time and money in useless treatments. In this scenario, miRNAs can represent a powerful tool: if the levels of defined miRNAs in the blood can foresee tumor behavior, we can offer a precise diagnosis and treatment plan to every individual. From the available studies, it appears that some miRNAs are associated with already established mechanisms of cancer development (such as the activation of stress sensors or the inactivation of key tumor suppressors) and are thus involved in the onset of resistance to different therapies in different contexts. A brand new field is now opening, investigating the role of the microenvironment in inducing resistance of cancer cells to targeted therapies. Many studies, in fact, have shown that the

microenvironment is not an ‘innocent bystander’ but plays an active role in tumor responses to therapies24 and 71. To date, many studies have demonstrated that it acts by producing soluble factors that activate in tumor cells signaling pathways able to confer resistance to molecular drugs. However, some papers have recently shown that the microenvironment can also modulate miRNA expression in neoplastic cells through secretion of soluble molecules [72], cell–cell adhesion 72 and 73, and other yet unknown mechanisms [74]. Moreover, because miRNAs are a potential strategy to suppress the expression of proangiogenic signaling components, their role in resistance to antiangiogenic therapies is still an unexplored field [75].

Overall, miRNA involvement in the onset of resistance to targeted therapies remains in most cases drug- and tissue-specific, rendering its clinical exploitation more difficult. Nevertheless, the first clinical trials evaluating the levels of miRNAs during exposure to targeted therapies are ongoing (http://www.clinicaltrials.gov). Their results will show if miRNA changes can have a predictive function and, provided the demonstration of their causative role in the onset of resistance, will also identify new possible therapeutic targets. Another important aid to advance our knowledge in this field comes from the availability of several bioinformatic tools for the study of molecular determinants of tumor cell behavior and drug sensitivity/resistance. Wide integromics studies will be required to join together biological data, obtained in different preclinical and clinical systems, in order to obtain a more general view of the role of each miRNA in different tumors and in response to different drugs.

In summary, even if the research on the role of miRNAs in sustaining resistance to antineoplastic treatments is promising, many questions are still unanswered: are there miRNAs impinging on general mechanisms of cell survival that are involved in resistance to different drugs? Do they generate the same mechanisms of resistance in different tumors? Can miRNA evaluation help foresee the onset of resistance? Very likely, in the near future some of these questions will be answered and robust validation studies will clarify if the preclinical findings lead to clinical translation.

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References

1. A. Gschwind *et al.*

The discovery of receptor tyrosine kinases: targets for cancer therapy Nat. Rev. Cancer, 4 (2004), pp. 361–370

2 G.A. Calin *et al.*

Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia Proc. Natl. Acad. Sci. U.S.A., 99 (2002), pp. 15524–15529

3. S.M. Johnson *et al.*

RAS is regulated by the let-7 microRNA family Cell, 120 (2005), pp. 635–647

4. M.S. Kumar *et al.*

Suppression of non-small cell lung tumor development by the let-7 microRNA family Proc. Natl. Acad. Sci. U.S.A., 105 (2008), pp. 3903–3908

5. L. He *et al.* **A microRNA polycistron as a potential human oncogene** Nature, 435 (2005), pp. 828–833

6. F. Meng *et al.*
MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer Gastroenterology, 133 (2007), pp. 647–658

7. I.A. Asangani *et al.*
MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer Oncogene, 27 (2008), pp. 2128–2136

8. K.A. O'Donnell *et al.*
c-Myc-regulated microRNAs modulate E2F1 expression Nature, 435 (2005), pp. 839–843

9. Y. Saito *et al.*
Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells Cancer Cell, 9 (2006), pp. 435–443

10. A. Lujambio *et al.*
Genetic unmasking of an epigenetically silenced microRNA in human cancer cells Cancer Res., 67 (2007), pp. 1424–1429

11. G.A. Calin *et al.*
A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia N. Engl. J. Med., 353 (2005), pp. 1793–1801

12. J.F. Abelson *et al.*
Sequence variants in SLITRK1 are associated with Tourette's syndrome Science, 310 (2005), pp. 317–320

13. L. Zhang *et al.*
microRNAs exhibit high frequency genomic alterations in human cancer Proc. Natl. Acad. Sci. U.S.A., 103 (2006), pp. 9136–9141

14. L. Zhang *et al.*
Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer Proc. Natl. Acad. Sci. U.S.A., 105 (2008), pp. 7004–7009

15. A. Jemal *et al.* **Cancer statistics, 2010** CA Cancer J. Clin., 60 (2010), pp. 277–300

16.G.J. Weiss *et al.*
EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines Ann. Oncol., 19 (2008), pp. 1053–1059

17. M. Zhong *et al.*
MicroRNAs reduce tumor growth and contribute to enhance cytotoxicity induced by gefitinib in non-small cell lung cancer Chem. Biol. Interact., 184 (2010), pp. 431–438

18. Y.S. Wang *et al.*

MicroRNA-214 regulates the acquired resistance to gefitinib via the PTEN/AKT pathway in EGFR-mutant cell lines Asian Pac. J. Cancer Prev., 13 (2012), pp. 255–260

19. J.L. Bryant *et al.*
A microRNA gene expression signature predicts response to erlotinib in epithelial cancer cell lines and targets EMT Br. J. Cancer, 106 (2012), pp. 148–156

20. M. Garofalo *et al.*
EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers Nat. Med., 18 (2012), pp. 74–82

21. J.R. Grandis, D.J. Tweardy
Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer Cancer Res., 53 (1993), pp. 3579–3584

22. R. Mehra *et al.*
The role of cetuximab for the treatment of squamous cell carcinoma of the head and neck Clin. Adv. Hematol. Oncol., 6 (2008), pp. 742–750

23. H. Hatakeyama *et al.*
Regulation of heparin-binding EGF-like growth factor by miR-212 and acquired cetuximab-resistance in head and neck squamous cell carcinoma PLoS ONE, 5 (2010), p. e12702

24. T.R. Wilson *et al.*
Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors Nature, 487 (2012), pp. 505–509

25. F. Ciardiello, G. Tortora
EGFR antagonists in cancer treatment N. Engl. J. Med., 358 (2008), pp. 1160–1174

26. J.B. Baker *et al.*
Tumour gene expression predicts response to cetuximab in patients with KRAS wild-type metastatic colorectal cancer Br. J. Cancer, 104 (2011), pp. 488–495

27. C.J. Allegra *et al.*
American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy J. Clin. Oncol., 27 (2009), pp. 2091–2096

28. A. Bardelli, S. Siena
Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer J. Clin. Oncol., 28 (2010), pp. 1254–1261

29. L.J. Mekenkamp *et al.*
Beyond KRAS mutation status: influence of KRAS copy number status and microRNAs on clinical outcome to cetuximab in metastatic colorectal cancer patients BMC Cancer, 12 (2012), p. 292

30. M.J. Higgins, J. Baselga

Targeted therapies for breast cancer J. Clin. Invest., 121 (2011), pp. 3797–3803

31. A. Ward *et al.*
Re-expression of microRNA-375 reverses both tamoxifen resistance and accompanying EMT-like properties in breast cancer Oncogene, 32 (2013), pp. 1173–1182

32. G. Hu *et al.*
The multifaceted role of MTDH/AEG-1 in cancer progression Clin. Cancer Res., 15 (2009), pp. 5615–5620

33. D.M. Cittelly *et al.*
Downregulation of miR-342 is associated with tamoxifen resistant breast tumors Mol. Cancer, 9 (2010), p. 317

34. A. Bergamaschi, B.S. Katzenellenbogen
Tamoxifen downregulation of miR-451 increases 14-3-3ζ and promotes breast cancer cell survival and endocrine resistance Oncogene, 31 (2012), pp. 39–47

35. V.G. Vogel *et al.*
Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial JAMA, 295 (2006), pp. 2727–2741

36. J.J. Zhao *et al.*
MicroRNA-221/222 negatively regulates estrogen receptor α and is associated with tamoxifen resistance in breast cancer J. Biol. Chem., 283 (2008), pp. 31079–31086

37. S. Nam *et al.*
An integrative analysis of cellular contexts, miRNAs and mRNAs reveals network clusters associated with antiestrogen-resistant breast cancer cells BMC Genomics, 13 (2012), p. 732

38. M. Mei *et al.*
Downregulation of miR-21 enhances chemotherapeutic effect of taxol in breast carcinoma cells Technol. Cancer Res. Treat., 9 (2010), pp. 77–86

39. Z.X. Wang *et al.*
MicroRNA-21 modulates chemosensitivity of breast cancer cells to doxorubicin by targeting PTEN Arch. Med. Res., 42 (2011), pp. 281–290

40. C. Gong *et al.*
Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer J. Biol. Chem., 286 (2011), pp. 19127–19137

41. J. Niu *et al.*
DNA damage induces NF-κB-dependent microRNA-21 up-regulation and promotes breast cancer cell invasion J. Biol. Chem., 287 (2012), pp. 21783–21795

42. E.M. Messing *et al.*
Immediate hormonal therapy compared with observation after radical prostatectomy and pelvic lymphadenectomy in men with node-positive prostate cancer N. Engl. J. Med., 341 (1999), pp. 1781–1788

43. K.K. Waltering *et al.*
Androgen receptor (AR) aberrations in castration-resistant prostate cancer Mol. Cell. Endocrinol., 360 (2012), pp. 38–43

44. P.C. Lin *et al.*
Epigenetic repression of miR-31 disrupts androgen receptor homeostasis and contributes to prostate cancer progression Cancer Res., 73 (2013), pp. 1232–1244

45. N. Nadiminty *et al.*
MicroRNA let-7c suppresses androgen receptor expression and activity via regulation of Myc expression in prostate cancer cells J. Biol. Chem., 287 (2012), pp. 1527–1537

46. K. Sikand *et al.*
miR 488* inhibits androgen receptor expression in prostate carcinoma cells Int. J. Cancer, 129 (2011), pp. 810–819

47. J. Ribas *et al.*
miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth Cancer Res., 69 (2009), pp. 7165–7169

48. T. Sun *et al.*
The altered expression of MiR-221/-222 and MiR-23b/-27b is associated with the development of human castration resistant prostate cancer Prostate, 72 (2012), pp. 1093–1103

49. T. Sun *et al.*
MiR-221 promotes the development of androgen independence in prostate cancer cells via downregulation of HECTD2 and RAB1A Oncogene (2013) http://dx.doi.org/10.1038/onc.2013.230

50. S.M. Cortes *et al.*
The role of the NFκB signalling pathway in cancer Clin. Transl. Oncol., 10 (2008), pp. 143–147

51. E. Shaulian
AP-1 – the Jun proteins: oncogenes or tumor suppressors in disguise? Cell. Signal., 22 (2010), pp. 894–899

52. S. Ma *et al.*
MicroRNA-616 induces androgen-independent growth of prostate cancer cells by suppressing expression of tissue factor pathway inhibitor TFPI-2 Cancer Res., 71 (2011), pp. 583–592

53. K. Boll *et al.*
MiR-130a, miR-203 and miR-205 jointly repress key oncogenic pathways and are downregulated in prostate carcinoma Oncogene, 32 (2013), pp. 277–285

54. B.J. Druker *et al.*
Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells Nat. Med., 2 (1996), pp. 561–566

55. P. Oestreicher
Drugs offer new hope for patients with CML who are resistant to imatinib ONS Connect, 22 (2007), pp. 20–21

56. T. Lopotovà *et al.*
MicroRNA-451 in chronic myeloid leukemia: miR-451-BCR-ABL regulatory loop? Leuk. Res., 35 (2011), pp. 974–977

57. V. Scholl *et al.*
miRNA-451: a putative predictor marker of imatinib therapy response in chronic myeloid leukemia Leuk. Res., 36 (2012), pp. 119–121

58. L. Liu *et al.*
Myc induced miR-144/451 contributes to the acquired imatinib resistance in chronic myelogenous leukemia cell K562 Biochem. Biophys. Res. Commun., 425 (2012), pp. 368–373

59. E. San José-Enériz *et al.*
MicroRNA expression profiling in imatinib-resistant chronic myeloid leukemia patients without clinically significant ABL1-mutations Mol. Cancer, 8 (2009), p. 69

60. J.M. Llovet *et al.*
Sorafenib in advanced hepatocellular carcinoma N. Engl. J. Med., 359 (2008), pp. 378–390

61. J. Chang *et al.*
miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1 RNA Biol., 1 (2004), pp. 106–113

62. C.L. Jopling *et al.*
Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA Science, 309 (2005), pp. 1577–1581

63. W.C. Tsai *et al.*
MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis J. Clin. Invest., 122 (2012), pp. 2884–2897

64. S. Bai *et al.*
MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib J. Biol. Chem., 284 (2009), pp. 32015–32027

65. S. Shimizu *et al.*
The let-7 family of microRNAs inhibits Bcl-xL expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma J. Hepatol., 52 (2010), pp. 698–704

66. H. Xia *et al.*
MiR-216a/217-induced epithelial–mesenchymal transition targets PTEN and SMAD7 to promote drug resistance and recurrence of liver cancer Hepatology, 58 (2013), pp. 629–641

67. C.H. Lawrie *et al.*
Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma Br. J. Haematol., 141 (2008), pp. 672–675

68. P.S. Mitchell *et al.*

Circulating microRNAs as stable blood-based markers for cancer detection Proc. Natl. Acad. Sci. U.S.A., 105 (2008), pp. 10513–10518

69. J. Shen *et al.*

MicroRNAs as potential biomarkers in human solid tumors Cancer Lett., 329 (2013), pp. 125–136

70. E.J. Jung *et al.*

Plasma microRNA 210 levels correlate with sensitivity to trastuzumab and tumor presence in breast cancer patients Cancer, 118 (2012), pp. 2603–2614

71. R. Straussman *et al.*

Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion Nature, 487 (2012), pp. 500–504

72. M. Hao *et al.*

Bone marrow stromal cells protect myeloma cells from bortezomib induced apoptosis by suppressing microRNA-15a expression Leuk. Lymphoma, 52 (2011), pp. 1787–1794

73. T. Lwin *et al.*

Follicular dendritic cell-dependent drug resistance of non-Hodgkin lymphoma involves cell adhesion-mediated Bim down-regulation through induction of microRNA-181a Blood, 116 (2010), pp. 5228–5236

74. J.J. Driscoll *et al.*

Microenvironment-induced changes in miRNA levels promote brain metastasis, drug resistance and KRAS expression Proceedings of the 104th Annual Meeting of the American Association for Cancer Research (AACR), April 6–10, 2013, Washington, DC, AACR (2013) Abstract nr LB-277

75. S.M. Weis, D.A. Cheresh

Tumor angiogenesis: molecular pathways and therapeutic targets Nat. Med., 17 (2011), pp. 1359–1370

76. S. Kobayashi *et al.*

EGFR mutation and resistance of non-small-cell lung cancer to gefitinib N. Engl. J. Med., 352 (2005), pp. 786–792

77. C. Montagut *et al.*

Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer Nat. Med., 18 (2012), pp. 221–223

78. A. Lievre *et al.*

KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer Cancer Res., 66 (2006), pp. 3992–3995

79. C.S. Karapetis *et al.*

K-ras mutations and benefit from cetuximab in advanced colorectal cancer N. Engl. J. Med., 359 (2008), pp. 1757–1765

80. R.G. Amado *et al.*
Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer J. Clin. Oncol., 26 (2008), pp. 1626–1634

81. S. Misale *et al.*
Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer Nature, 486 (2012), pp. 532–536

82. J.A. Engelman *et al.*
MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling Science, 316 (2007), pp. 1039–1043

83. A. Bardelli *et al.*
Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer Cancer Discov., 3 (2013), pp. 658–673

Glossary

Monoclonal antibodies (mAbs)

recombinant antibodies that recognize antigens on the cancer cell surface and induce target inactivation or downregulation and/or antibody-dependent cell mediated cytotoxicity (ADCC). Rituximab was the first mAb approved for the treatment of lymphomas and directed against the CD20 antigen expressed on B cells.

Primary (or innate) resistance
mechanism that renders cancer cells refractory to drug treatment from the beginning (patients never benefit from treatment).

Resistance
mechanism exploited by cancer cells to overcome the inhibitory effect of a drug in order to survive and proliferate.

Secondary (or acquired) resistance
mechanism that renders cancer cells refractory to the treatment after a period of time of response (patients initially respond to treatment but become resistant over time).

Tyrosine kinase inhibitors (TKIs)
chemically synthesized small molecules that bind tyrosine kinase proteins with different degrees of specificity and compete with or prevent ATP binding. Tyrosine kinase proteins represent good candidates for cancer treatment because they are key regulators of cell proliferation and are often constitutively active in cancer cells due to overexpression, amplification, or mutations. The first TKI approved by the FDA was Imatinib for the treatment of CML harboring *BCR–ABL*translocation.