A Three-microRNA Signature Predicts Responses to Platinum-Based Doublet Chemotherapy in Patients with Lung Adenocarcinoma

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Abstract

Purpose: To examine the clinical utility of intratumor microRNAs (miRNA) as a biomarker for predicting responses to platinum-based doublet chemotherapy in patients with recurring lung adenocarcinoma (LADC).

Experimental Design: The expression of miRNAs was examined in LADC tissues surgically resected from patients treated with platinum-based doublet chemotherapy at the time of LADC

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recurrence. Microarray-based screening of 904 miRNAs followed by quantitative reverse transcription-PCR–based verification in 40 test cohort samples, including 16 (40.0%) responders, was performed to identify miRNAs that are differentially expressed in chemotherapy responders and nonresponders. Differential expression was confirmed in a validation cohort (n = 63 samples), including 18 (28.6%) responders. An miRNA signature that predicted responses to platinum-based doublet chemotherapy was identified and its accuracy was examined by principal component and support vector machine analyses. Genotype data for the **TP53**-Arg72Pro polymorphism, which is associated with responses to platinum-based doublet chemotherapy, were subsequently incorporated into the prediction analysis.

Results: A signature comprising three miRNAs (miR1290, miR196b, and miR 135a*) enabled the prediction of a chemotherapeutic response (rather than progression-free and overall survival) with high accuracy in both the test and validation cohorts (82.5% and 77.8%). Examination of the latter was performed using miRNAs extracted from archived formalin-fixed paraffin-embedded tissues. Combining this miRNA signature with the **TP53**-Arg72 Propolymorphism genotype marginally improved the predictive power.

Conclusion: The three-miRNA signature in surgically resected primary LADC tissues may by clinically useful for predicting responsiveness to platinum-based doublet chemotherapy in patients with LADC recurrence.

Introduction

Lung adenocarcinoma (LADC) is the most common type of non–small cell lung cancer (NSCLC) and is a leading cause of cancer mortality worldwide (1). Surgical resection is the best curative treatment for NSCLC; however, patients that experience recurrence after surgery and those with advanced disease receive chemotherapy to slow tumor growth and improve survival. LADCs harboring an **EGFR** mutation or an ALK fusion are primarily treated with specific tyrosine kinase inhibitors (TKI), with response rates of approximately 60% (2, 3). Other oncogene aberrations, such as **BRAF, HER2, RET**, and **ROS1**, are also targeted by specific TKIs (4–6), but a major barrier to curative treatment of LADC using TKIs is innate and acquired drug resistance (7, 8). Furthermore, more than 60% of USA/ European and more than 30% of Japanese LADC cases do not harbor the oncogene aberrations listed above (9, 10). Such resistant and oncogene-negative LADC cases are treated with chemotherapy. The standard regimens comprise platinum-based doublets, that is, a combination of platinum and another agent; the drugs paired with platinum (cisplatin or carboplatin) include microtubule-targeted agents (paclitaxel, docetaxel, or vinorelbine) and DNA-damaging agents (gemcitabine or irinotecan). A series of trials in unselected patients revealed that the efficacy of each combination is similar, with response rates of 30% to 40% (11–13); thus, identifying biomarkers that can discriminate between patients that will respond to platinum-based doublet chemotherapy and those who may not before treatment will help improving the efficacy of chemotherapy for LADC.

microRNAs (miRNA) are small noncoding RNAs that posttranscriptionally regulate the translation of target genes; these miRNAs show altered expression in a variety of cancers and can modify the malignant properties of cancer cells, including the response to DNA damage (14–16). In fact, functional studies in LADC cell lines have identified a number of

miRNAs that modulate sensitivity to platinum-based agents (17–23). Furthermore, miRNAs are stable in formalin-fixed paraffin-embedded (FFPE) tissues, that is, materials used for daily pathologic diagnosis (24, 25). Indeed, intratumor miRNA expression is a promising prognostic marker in patients that have undergone surgical resection(26–31), but few studies have examined the utility of miRNAs as a predictor of chemotherapeutic responses (32). To the best of our knowledge, only two such studies have been reported: one shows that a two-miRNA signature (miR149 and miR375) is associated with responses to platinum-based chemotherapy in NSCLC (n = 38), and the other shows that miR92a-2* expression is associated with chemoresistance in small-cell lung cancer (n = 34; refs. 33, 34).

Here, we investigated the utility of intratumor miRNAs as a biomarker for predicting responses to platinum-based doublet chemotherapy. We examined the expression of miRNAs in 103 surgically resected specimens obtained from patients who received platinum-based chemotherapy upon LADC recurrence to ascertain whether miRNA expression in primary tumors could predict responses to platinum-based doublet chemotherapy in patients who experienced LADC recurrence after surgery. First, a two-step screening process involving 904 miRNAs was performed to identify an miRNA signature with predictive value. The first step was performed using a test cohort comprising 40 frozen tumor samples from which RNAs were isolated, and the second step was performed using a validation cohort comprising 63 cases, for which RNAs from FFPE tissues were available. We identified a three-miRNA signature that predicted responses to platinum based doublet chemotherapy with an accuracy of >75%.

Materials and Methods

Materials

Of note, 103 surgically resected LADC tissues were examined in the present study (Fig. 1A). Briefly, 643 Japanese patients with NSCLC received platinum-based doublet chemotherapy at the National Cancer Center Hospital (NCCH; Tokyo, Japan), between 2000 and 2008, and the therapeutic response was evaluated using the Response Evaluation Criteria In Solid Tumors (RECIST) guidelines (35). None of the patients had received prior treatment with platinum-based chemotherapy. Of the 643 cases, 118 were recurrent cases that had undergone surgical resection at NCCH, and all were pathologically diagnosed with adenocarcinoma. Tumor tissues for RNA extraction were available for 103 of 118 cases; these cases were examined in the present study. Information regarding age, gender, pathologic TNM stage (the 7th classification), smoking habits, postoperative chemotherapy regimens and responses to platinum doublet therapy, and performance status (PS) were retrospectively collected. RNAs isolated from fresh-frozen tissues were available for 40 of 103 cases; these were defined as the test cohort. The RNAs from the test cohort were subjected to miRNA microarray analysis followed by verification by quantitative reverse transcriptase-PCR (qRT-PCR) analysis. RNAs from FFPE tissues were available for the remaining 63 cases; these were defined as the validation cohort. In addition, patients were classified into two categories according to RECIST guidelines: those that responded to platinum-based doublet chemotherapy [complete response (CR) or partial response (PR)] and those that did not [stable disease (SD) or progressive disease (PD)].

RNA extraction

RNA was extracted from snap-frozen tissues (test cohort) using TRizol reagent (Thermo Fisher Scientific). The quality and quantity of the RNAs were examined using a Bioanalyzer (Agilent). All RNA samples showed an RNA integrity number RIN >6.0; therefore, they were subjected to microarray analysis. For the validation cohort, RNA was isolated from three unstained FFPE sections (5-mm thick). The area of the carcinoma in the three unstained sections was outlined by referring to a sequential section that was stained with hematoxylin and eosin. Each marked area was macro-dissected using a sterile disposable scalpel and RNA was isolated using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion). Total RNA was quantified using a NanoDrop ND-1000 spectrometer (Thermo Fisher Scientific). The optical density (OD) 260/280 and OD 260/230 ratios were used for quality control.

Microarray experiments

The Human miRNA Microarray Kit release 14 (8×15 K; Agilent Technologies), covering 904 miRNAs, was used to screen for miRNAs in the test cohort samples (**n = 40**). Data were normalized and analyzed using GeneSpring GX software (version 12.5; TOMY Digital Biology). The foldchange in expression was defined as the ratio of expression in responders to that in nonresponders. Normalized and raw expression data were deposited in the Gene Expression Omnibus at the National Center for Biotechnology Information (GSE56264).

Examination of driver oncogene aberrations

All 40 test cohort samples were also screened for oncogene fusions (***EML4*– and *KIF5B*– *ALK, KIF5B*– and *CCDC6*–*RET*; and *CD74*–, *EZR*–, and *SLC34A2*–*ROS1***) by reverse transcription-PCR as previously described (4,36). GenomicDNA was extracted from fresh or frozen samples from all 103 subjects using a QIAamp DNA Mini Kit (QIAGEN) and then analyzed for ***EGFR, KRAS, BRAF***, and HER2 hot spot mutations using the high resolution melting method (37, 38).

qRT-PCR analysis

qRT-PCR of mature miRNA was performed using TaqMan MicroRNA assays (Thermo Fisher Scientific) and the 7900 HT Fast Real-Time PCR System (Thermo Fisher Scientific). cDNA was synthesized using miRNA-specific primers and a TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific). RNA (40ng) was reverse transcribed in a 20 μL reaction containing gene-specific RT probes. All assays were performed in triplicate and investigators were blinded to the clinical outcome. All TaqMan probes were purchased from Thermo Fisher Scientific: hsa-miR135a-3p (ID 002232), hsa-miR196b-5p (ID 002215), hsa-miR1181 (Assay ID 241045_mat), hsa-miR31–5p (ID 002279), hsa-miR31–3p (ID 002113), hsa-miR1290 (ID 002863), hsa-miR598 (ID 001988), hsa-miR1 (ID 002222), hsa-miR144– 5p (ID 002148), hsa-miR628–5p (ID 002433), hsa-miR449a (ID 001030), and hsa-miR34b-3p (ID 002102). RNU66 (ID 001002) was used as a normalization control. Relative expression of miRNAs was calculated using RQ manager 1.2 (Thermo Fisher Scientific).

Statistical analysis

Differences in miRNA expression levels between responders and nonresponders were tested by the Mann–Whitney U test using Graphpad Prism v5.0 (Graphpad Software Inc). Spearman correlation analysis was used to examine the correlation between microarray and qRT-PCR data (Graph-pad Prism v5.0). Linear discriminant analysis was performed for each cohort to distinguish responders from nonresponders (JMP 10 software; SAS Institute) based on miRNA expression (i.e., the miRNA signature). Continuous expression values for a single miRNA or for plural miRNAs, that is, C_t values obtained by qRT-PCR against RNU66, were included as variables in the analysis. Receiver operating characteristic (ROC) curves were generated to evaluate response sensitivity and the are a under the curve(AUC)was calculated (JMP 10). Principal component analysis (PCA) of the expression of three miRNAs (miR1290, miR196b, and miR135a*) was performed using JMP 10.

Results

Sample selection

The aim of this study was to identify biomarkers in patients with metastatic LADC who relapsed following potential curative surgical resection. Therefore, surgically resected primary tumor tissues from 103 LADC patients who were treated with platinum-based doublet chemotherapy up on recurrence were selected form Irna profiling (Fig. 1A). The cases were assigned to a test cohort (n = 40; RNAs from frozen tissue available) or a validation cohort (n = 63; RNAs from FFPE tissues available) according to the availability of tumor tissue samples. Patients in both cohorts were classified as responders (CR and PR) or nonresponders (SD and PD) to platinum-based doublet chemotherapy according to the RECIST criteria (Materials and Methods; Supplementary Table S1). In this study, platinum-based doublet chemotherapy includes several different regimens. The cohorts were similar in terms of clinicopathologic characteristics such as age, gender, smoking habits, pathologic stage, representative oncogene mutations, therapeutic regimen, and therapeutic response (Table 1). The samples were subjected to a two-step screening procedure to identify miRNAs whose expression is associated with responses to platinum-based doublet chemotherapy (Fig. 1B).

Differential expression of miRNAs between responders and nonresponders

First, we used microarray analysis to identify miRNAs in test cohort samples that were differentially expressed in LADC tissues from responders and nonresponders to platinum-based doublet chemotherapy. Fifty-nine miRNAs were identified (P < 0.05; Welch t test; Supplementary Table S2). Of these, 28 were upregulated in responders and 31 were downregulated.

Next, to identify the limited number of miRNAs that can be used to deduce responsiveness in the clinic, we searched for miRNAs showing highly differential expression between responders and nonresponders. Twelve miRNAs (miR135a*, miR196b, miR1181, miR31, miR31, miR1290, miR598, miR1, miR144, miR628–5p, miR449a, and miR34b) showing a >5-fold change in expression were identified as potential candidates and investigated further (Fig. 2A;Supplementary Table S2). The expression of these 12 miRNAs was reanalyzed by

qRT-PCR using the same RNA samples used in the microarray experiments. A good agreement between the qRT-PCR (C_t value) and microarray ($\log_2$ signal) data (as indicated by the Pearson correlation coefficient) was observed for 10 of the 12 miRNAs (excluding miR1181 and miR598; Supplementary Fig. S1). qRT-PCR identified three miRNAs (miR1290, miR196b, and miR135a*) as differentially expressed in responders and non-responders (*P*<0.001, P<0.001, and *P*<0.008, respectively; Fig. 2B; Supplementary Table S3). The fold changes observed in the qRT-PCR experiment were lower than those observed in the microarray experiment. This may be due to the higher sensitivity of the former. Several samples that gave no significant signal (calculated as zero) in the microarray experiment yielded C_t values in the qRT-PCR experiment (Supplementary Fig. S1), leading to a reduction in the fold change values (Supplementary Table S3).

We next used linear discriminant analysis to examine these three miRNAs for their potential to discriminate responders from nonresponders. For this, continuous expression values for a single miRNA or a combination of two or three miRNAs, that is, C_t values obtained by qRT-PCR, were included as variables in the analysis. ROC curves were plotted to examine both sensitivity and specificity. The results showed that a combination of all three miRNAs provided the best discrimination, with an AUC of 0.893 (Fig. 2C). This combination of three miRNAs is, henceforth, referred to as the "three-miRNA signature."

Ability of the three-miRNA signature to predict responses to chemotherapy

To validate the findings in the test cohort, we next examined the expression of the three-miRNA signature in the validation cohort (Supplementary Fig. S2 and Supplementary Table S3). We then performed a linear discriminant analysis to evaluate the potential of the three-miRNA signature as a biomarker. The mean expression levels of the three miRNAs were higher in responders than in nonresponders in the validation cohort, although the difference in the expression of miR135a* did not reach a statistical significance. However, a combination of all three miRNAs again was better able to distinguish responders from nonresponders (AUC = 0.837) than a single miRNA or a combination of two miRNAs (Supplementary Fig. S3).

PCA and support vector machine (SVM) analysis showed a predictive response of 37.5% for the test cohort containing 40% responders and a predictive response of 31.7% for the validation cohort containing 28.6% responders. The signature predicts responders and nonresponders in the test and validation cohorts with an accuracy of 82.5% and 77.8%, respectively (Fig. 3). The sensitivity, specificity, and positive and negative predictive values were similar for both cohorts (Supplementary Table S4). There was no significant difference in the clinical characteristics between true responders/nonresponders and predicted responders/nonresponders (Supplementary Table S1). Taken together, these results show that the predictive ability of the three-miRNA signature was confirmed in the independent validation cohort, and that the signature is still predictive even if archive FFPE tissues are used for analysis. Specificity and negative predictive values greaterthan sensitivity and positive predictive values suggest that the three-miRNA signature predicts nonresponders better than responders (Supplementary Table S4).

Combining the three-miRNA signature with the TP53-Arg72Pro polymorphism genotype

We previously showed in the same study population (i.e., the 640 cases shown in Fig. 1A) that the Arg72Pro polymorphism in the *TP53* gene in noncancerous (germline) DNA is associated with responses to platinum-based doublet chemotherapy: The response rate is higher in those harboring the TP53–72Pro polymorphism (35). Therefore, we combined the three-miRNA signature with the TP53Arg72Pro genotype data to ascertain whether the predictive power of the miRNA signature was enhanced. We dichotomized the study cohorts into two subgroups (patients with theTP53–72 Pro allele and those without) and examined the predictive accuracy of the three-miRNA signature. We found that the predictive accuracy marginally improved in both the test (85.0%) and validation (82.5%) cohorts (Fig. 1B).

The three-miRNA signature predicts responses to chemotherapy irrespective of driver oncogene aberrations and clinical characteristics

Driver oncogene aberrations in LADC are a critical factor that determines the therapeutic strategy for each patient. In addition, such aberrations are associated with clinical characteristics, as represented by the predominance of *EGFR* mutation in females and never-smokers. Therefore, we next addressed whether the ability of the three-miRNA signature to predict responses to chemotherapy was affected by driver oncogene alterations or clinical characteristics. The three miRNA signature failed to predict responses in 21 of 103 cases (21%). The test and validation cohorts contained seven (one with an EGFR mutation, one with a *HER2* mutation, and five aberration-negative cases) and 14 (six with *EGFR* mutations, one with a KRAS mutation, and seven aberration-negative cases) nonpredicted cases, respectively (Fig. 4). The nonprediction rate was 15% (7/47 cases) for patients harboring the *EGFR* mutation, 10% (1/10) for those harboring the KRAS mutation, 33% (1/3) for those harboring the *HER2* mutation, and 32% (12/38) for aberration-negative cases; therefore, there was no significant correlation between driver gene status and nonprediction. Similarly, there was no significant association between clinical factors and nonprediction; thus, the three-miRNA signature may be a useful biomarker for predicting the responses of patients with LADC to chemotherapy, irrespective of driver oncogene aberrations and clinical characteristics.

Discussion

Here, we performed miRNA expression profiling of patients who initially underwent surgical resection for primary LADC and were then treated with platinum-based doublet chemotherapy upon recurrence. We identified a three-miRNA signature (miR1290, miR196b, and miR135a*) that predicts whether patients with recurring LADC respond to and, therefore, will benefit from platinum-based doublet chemotherapy. Even patients with LADC harboring druggable on cogene aberrations (that are resistant to treatment with TKIs) may be treated with platinum-based doublet chemotherapy; therefore, platinum-based doublet chemotherapy is a major therapeutic strategy for almost all patients with LADC(39–41). Personalized therapy, in which a drug with the greatest chance of eliciting a response (i.e., tumor shrinkage) is chosen specifically for each patient, is the first critical step toward improved prognosis for patients with LADC with advanced and recurrent disease; indeed, clinical trials examining the effect of new drugs on NSCLC have set improved response rates

as their primary endpoint (10); thus, response to treatment according to the RECIST criteria ratherthan survival was the outcome measure selected for the present study. The three-miRNA signature will facilitate personalized therapy for LADC and will include platinum-based doublet therapy as an option.

Here, we examined the three-miRNA signature of primary tumors to predict the responsiveness of recurrent tumors. Itis noteworthy that the biologic characteristics of recurrent tumors are not the same as those of primary tumors due to tumor cell heterogeneity in the primary lesions and the accumulation of additional genetic/epigenetic changes during progression. Thus, at present our findings are applicable to the treatment of recurrent tumors for which corresponding primary tumor tissue samples are available. The finding that the three-miRNA signature is predictive in archived primary tumor tissues is an advantage; patients are spared the additional burden of further tissue sampling for genetic analysis. However, it is also worth analyzing recurrent tumors and inoperable advanced tumors to find out whether the three miRNA signature is applicable to patients for whom archived surgical tissues are not available. The finding that the signature can be identified in archived FFPE tissues is also an advantage and will facilitate translation to the clinic.

We also examined the combination of the three-miRNA signature with the TP53-Arg72Pro polymorphism genotype to see whether this provided greater predictive accuracy, as blood cells used for genotyping polymorphisms are easily obtained from patients. However, the improvement was only marginal. Therefore, more polymorphisms associated with responses to platinum-based doublet therapy must be identified if we are to achieve any marked improvement over the three-miRNA signature alone. In addition, we used a fold change >5 as a criterion for identifying candidate miRNAs that are differentially expressed between responders and nonresponders. Using less stringent or other statistical criteria may lead to the identification of more miRNAs that are useful for prediction.

The three-miRNA signature predicted responses irrespective of the presence of driver oncogene aberrations. This is important when we consider that LADCs that have acquired resistance to specific TKIs are treated with platinum-based doublet chemotherapy. However, unfortunately, the present study cohort did not include samples from patients with EGFR- or ALK-positive LADC that received TKIs before platinum-based doublet chemotherapy. Such cases should be examined to address this issue.

This study has several limitations. First, it was retrospective in nature, so the ability of the three-miRNA signature to predict responses needs to be further validated using more samples. Here, different types of tumor tissue for the test and validation sets (bulk frozen and micro dissected FFPE tissues, respectively), which contained patients that had undergone several different chemotherapeutic regimens, were subjected to analysis; therefore, we may have over- or underestimated predictive value. Thus, further studies that use a larger number of samples obtained according to a defined experimental procedure and take factors such as previous treatment regimen, disease stage, and PS into account are required. In addition, prospective studies, for example, studies using samples from patients treated with a single therapeutic regimen, and the analysis of primary and recurrent tumors and inoperable advanced tumors, should be conducted to confirm the utility of the three-

microRNA signature. Second, although the three-miRNA signature was significantly associated with response to chemotherapy, differences in progression-free survival were only suggestive (Supplementary Fig. S4). We chose the response as the primary endpoint of efficacy to identify subgroups for which chemotherapy does work (35). However, treatment is continued after the failure of platinum-based doublet chemotherapy; therefore, clinical response alone would not be enough to improve the outcome. Third, the functional relevance of miR1290, miR196b, and miR135a* to the chemosensitivity of LADC remains unclear. Interestingly, a recent study shows that the expression of miR196b is upregulated in patients with rectal adenocarcinoma that respond to neoadjuvant chemoradiotherapy (capecitabine or 5-fluorouracil), which supports the findings of the present study (42). However, preliminary experiments examining the exogenous expression of the three miRNAs in LADC cell lines did not show increased sensitivity to a platinum agent, cisplatin (CDDP). Therefore, the direct or indirect effects of miRNAs on chemosensitivity should be further investigated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Translational Relevance

The use of biomarkers to identify patients that will respond to platinum-based doublet chemotherapy before treatment is a critical strategy for improving the efficacy of chemotherapy for lung adenocarcinoma (LADC). Here, we report that the expression profile of three miRNAs in surgically resected primary tissues is clinically useful for predicting responsiveness to platinum-based doublet chemotherapy in patients with LADC recurring after initial surgical resection. This three-miRNA signature may be useful for the clinical management of LADC.
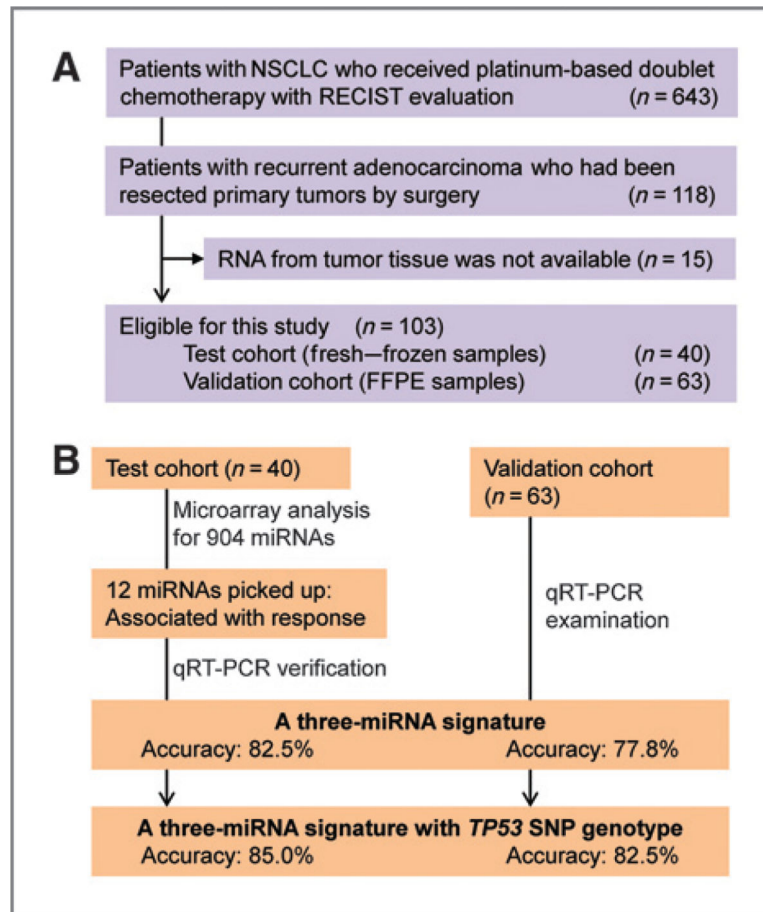
Figure 1.
Patients and treatment strategy. A, selection of eligible cases, that is, 103 surgically resected cases that received platinum-based doublet chemotherapy upon LADC recurrence. B, identification and evaluation of a three-miRNA signature for the prediction of responses to chemotherapy.
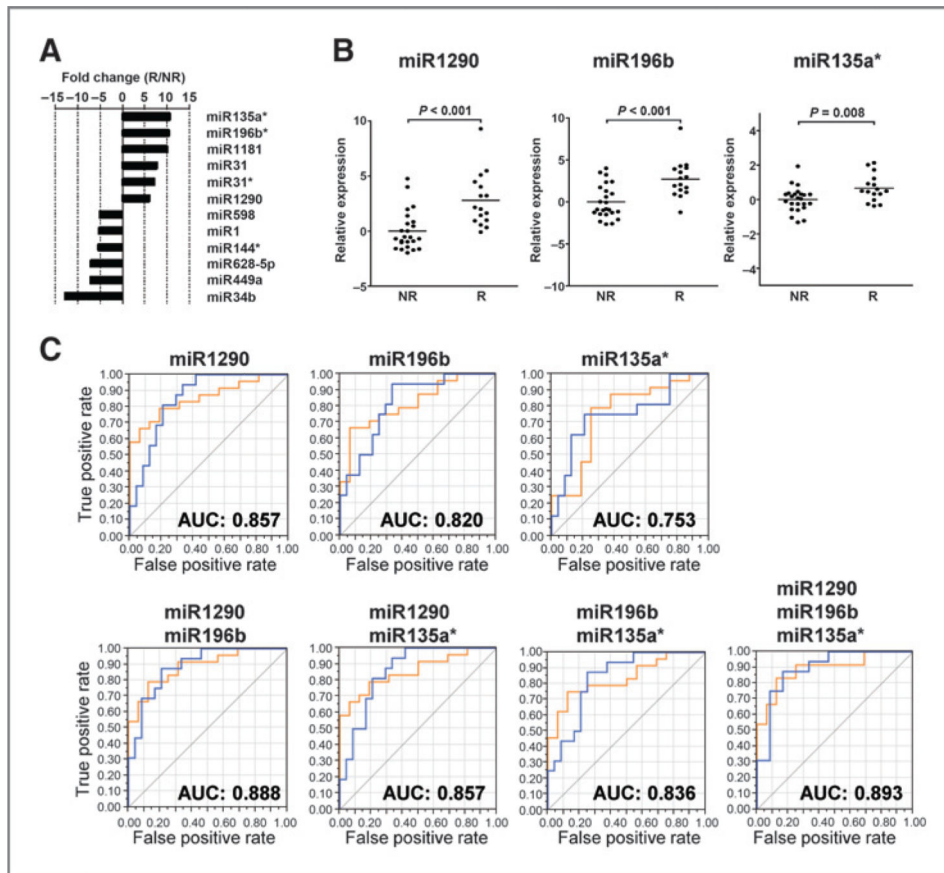
Figure 2.
Selection of the three miRNAs whose expression was associated with responses to platinum-based doublet chemotherapy upon LADC recurrence. A, twelve miRNAs differentially expressed in responders (R) and nonresponders (NR) to platinumbased doublet chemotherapy in the test cohort. The diagram depicts miRNAs showing a >5-fold change in expression and with a *P* value of <0.05. The fold change is represented by the ratio of R to NR derived from the microarray data. B, expression of miRNAs in NR and R in the test cohort, as measured by qRT-PCR. Dot plots, miRNA relative threshold cycle values. Expression was normalized to that of RNU66. Threshold cycle values relative to the mean value in NR are shown on a $\log_2$ scale. Horizontal bars, the mean expression value; *P* values (Mann–Whitney test) are indicated. C, ROC analysis was performed for miR1290, miR196b, and miR135a* in the test cohort. The AUC value is shown. The blue line, the results for responders and the orange line represents the results for NR
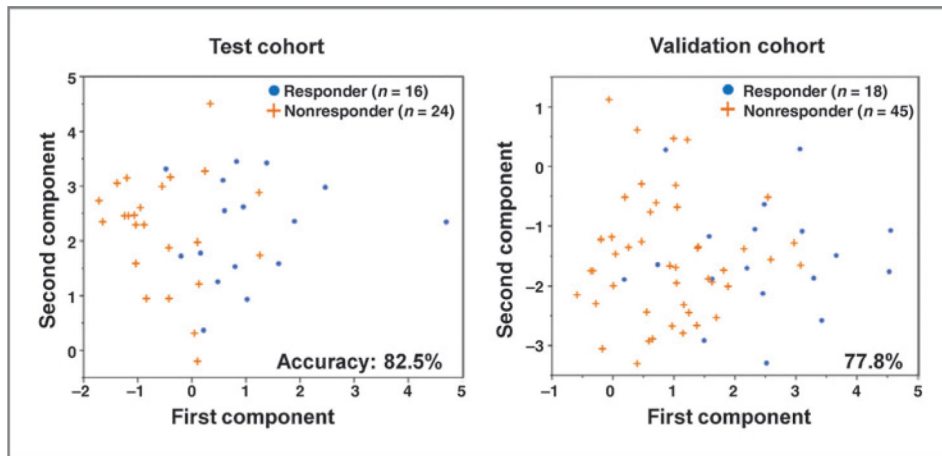
Figure 3.
PCA. A PCA–SVM strategy using three miRNAs (miR1290, miR196b, and miR135a*) was used to construct a classifier, which could distinguish responders from nonresponders. The blue dots represent the responders and the orange crosses represent the nonresponders in the test (left) and validation cohorts (right). The classifier had a predictive accuracy of 82.5% for the test cohort and an accuracy of 77.8% for the validation cohort.
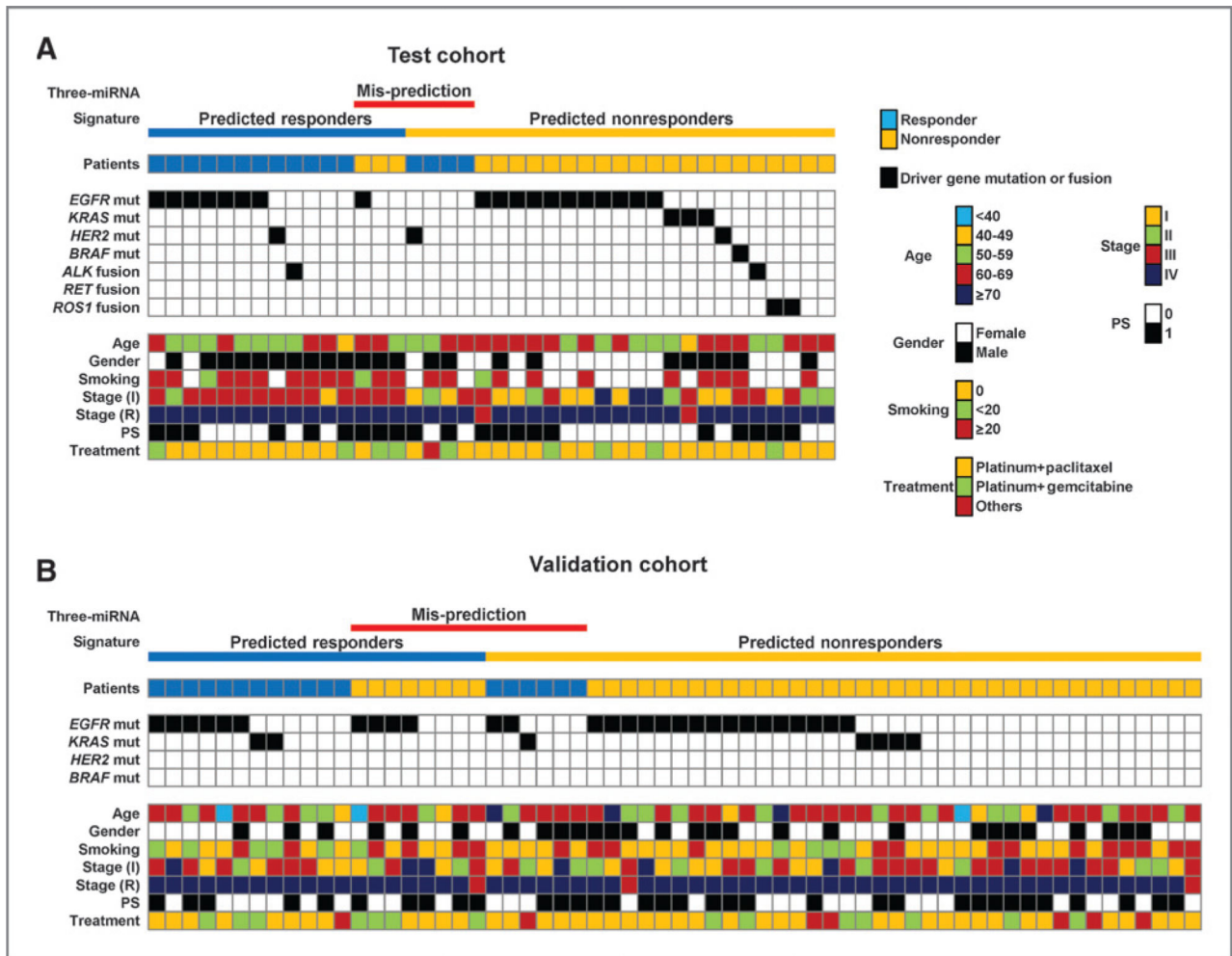
Figure 4.
Response prediction by the three-miRNA signature according to clinicopathologic factors in the test (A) and validation cohorts (B). Driver gene mutations and clinical features are shown: patients (blue, responder; orange, nonresponder); driver gene (black, *EGFR, KRAS, HER2, BRAF* mutation- or *ALK, RET*, or *ROS1* fusion-positive; white, negative); age (blue, <40; orange, 40–49; green, 50–59; red, 60–69; and navy blue, 70); gender (white, female; black, male); smoking (orange, pack years = 0; green, <20; and red, 20); tumor stage at initial diagnosis (I) and at recurrence (R; orange, I; green, II; red, III; and navy blue, VI); PS (white, 0; black, 1); treatment (orange, platinum + paclitaxel; green, platinum + gemcitabine; and red, other).

Table 1.

Clinicopathologic characteristics on surgically resected primary lung ADC

	Test cohort (*n* = 40)	Validation cohort (*n* = 63)	*P*
Age, y			0.20
Mean (range)	59 (47–67)	59 (32–72)	
Gender number (%)			0.23
Male	23 (58)	28 (44)	
Female	17 (42)	35 (56)	
Smoking (pack year) number (%)			0.09
Never smoker	16 (40)	33 (52)	
<20	3 (8)	10 (16)	
20	21 (52)	20 (32)	
TP53 genotype number (%)			0.49
Arg/Arg	20 (50)	24 (38)	
Arg/Pro	16 (40)	33 (52)	
Pro/Pro	4 (10)	6 (10)	
Driver oncogene mutation number (%)			0.34
Negative	14 (35)	28 (44)	
Positive	26 (65)	35 (56)	
EGFR mutation	19	28	
KRAS mutation	3	7	
HER2 mutation	3	0	
BRAF mutation	1	0	
Driver oncogene fusion number (%)			
Negative	36 (90)	NE	
Positive	4 (10)	NE	
ALK fusion	2	NE	
RET fusion	0	NE	
ROS1 fusion	2	NE	
TNM stage at initial diagnosis number (%)			0.83
IA	6 (15)	5 (8)	

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	Test cohort ($n = 40$)	Validation cohort ($n = 63$)	P
IB	5 (13)	12 (19)	
IIA	4 (10)	10 (16)	
IIB	2 (5)	2 (3)	
IIIA	18 (45)	26 (41)	
IIIB	2 (5)	0	
IV	3 (8)	8 (13)	
TNM stage at recurrence number (%)			0.32
IA	0	0	
IB	0	0	
IIA	0	0	
IIB	0	0	
IIIA	0	0	
IIIB	0	0	
IV	0	0	
Recurrent portion			1
Local/regional	2 (5)	3 (5)	
Metastasis	38 (95)	60 (95)	
M1a	22	28	
M1b	16	32	
Platinum-based regimens number (%)			0.44
Platinum + paclitaxel	30 (75)	42 (67)	
Platinum + gemcitabine	9 (23)	14 (22)	
Platinum + docetaxel	0	4 (6)	
Platinum + pemetrexed	0	2 (3)	
Platinum + vinorelbine	1 (2)	1 (2)	
Tumor response number (%)			0.31
Responder	16 (40)	18 (29)	
CR	1	0	
PR	15	18	
Nonresponder	24 (60)	45 (71)	
SD	21	35	

	Test cohort (*n* = 40)	Validation cohort (*n* = 63)	*P*
PD	3	10	

NOTE: The P value from the Fisher's exact test.

Abbreviations: NE, not examined; Platinum, cisplatin or carboplatin.