MicroRNA Classifiers for Predicting Prognosis of Squamous Cell Lung Cancer

Mitch Raponi, Lesley Dossey, Tim Jatkoe, Xiaoying Wu, Guoan Chen, Hongtao Fan, and David G. Beer

1Centocor Research and Development, Radnor, Pennsylvania; 2Veridex, LLC, a Johnson & Johnson Company, San Diego, California; and 3University of Michigan, Department of Surgery, Ann Arbor, Michigan

Abstract

Non–small cell lung cancer (NSCLC), which is comprised mainly of adenocarcinoma and squamous cell carcinoma (SCC), is the cause of 80% of all lung cancer deaths in the United States. NSCLC is also associated with a high rate of relapse after clinical treatment and, therefore, requires robust prognostic markers to better manage therapy options. The aim of this study was to identify microRNA (miRNA) expression profiles in SCC of the lung that would better predict prognosis. Total RNA from 61 SCC samples and 10 matched normal lung samples was processed for small RNA species and profiled on MirVana miRNA Bioarrays (version 2, Ambion). We identified 15 miRNAs that were differentially expressed between normal lung and SCC, including members of the miR-17-92 cluster and its paralogues. We also identified miRNAs, including miR-155 and let-7, which had previously been shown to have prognostic value in adenocarcinoma. Based on cross-fold validation analyses, miR-146b alone was found to have the strongest prediction accuracy for stratifying prognostic groups at ~78%. The miRNA signatures were superior in predicting overall survival than a previously described 50-gene prognostic signature. Whereas there was no overlap between the miRNAs targeted by the prognostic miRNAs and the 50-gene expression signature, there was a significant overlap in the corresponding biological pathways, including fibroblast growth factor and interleukin-6 signaling. Our data indicate that miRNAs may have greater clinical utility in predicting the prognosis of patients with squamous cell lung carcinomas than mRNA-based signatures.

Introduction

Lung cancer is the most common cause of cancer-related deaths worldwide, whereas non–small cell lung cancers (NSCLC) represent the most frequent type of broncogenic carcinomas (1). NSCLC is the cause of 80% of all lung cancer deaths in the United States and is composed primarily of adenocarcinoma, squamous cell carcinoma (SCC), and to a lesser extent large-cell lung cancer (1). Despite potentially curative surgery, ~40% of patients will relapse within 5 years (2). Genomic profiling of NSCLC has recently provided insight into predicting the prognosis of patients with this disease (3–8). These classifiers can contain up to several hundred genes for the identification of patients with early-stage NSCLC, who might benefit from chemotherapy in addition to surgical resection. Recently, a five-gene signature was reported for predicting survival of patients with NSCLC (9). This classifier can be assessed on a reverse transcription–PCR (RT-PCR)–based platform and could lead to a more immediate clinical adoption compared with microarray-based diagnostics.

A complementary approach to performing gene expression profiling in NSCLC is to analyze microRNA (miRNA) expression signatures. miRNAs are short noncoding RNAs that control protein expression through several mechanisms (10). Their altered expression has been shown to be associated with various cancers, including NSCLC (11–15). MiR-155 and let-7 miRNAs have been shown to predict prognosis in lung adenocarcinoma (12, 14). Functional analysis of the let-7 miRNA indicates that it directly regulates Ras and HMGA2, both of which act as oncogenes in many cancers (16, 17). In addition, Yu and colleagues have recently described a 5-miRNA classifier for predicting survival in NSCLC (18). It has been predicted that any one miRNA has the potential to regulate the activity of hundreds of downstream targets (19). Therefore, aberrant expression of a small number of miRNAs can have a potentially dramatic effect on normal cellular activity.

In this report, we describe the miRNA expression profiles of a subset of previously characterized lung SCC and matched normal lung tissue (5). Using the Ambion mirVana Bioarray platform, miRNAs that are differentially expressed in lung SCC were identified, in addition to miRNAs that were significantly associated with prognosis. MiR-146b was the most robust predictor of overall survival and was further validated by ABI Taqman assays. We also validate the prognostic utility of miR-155 in SCC. The predictive accuracy of the miRNAs was superior to our previously identified 50-gene mRNA prognostic signature. Bioinformatic analyses showed that there was no significant intersection of the predicted miRNA targets and our previously reported 50-gene prognostic signature (5); however, both data sets enrich for the same signaling pathways. Due to the relatively stable nature of miRNAs and the requirement of fewer analytes in the classifier, we suggest that testing for miR-146b expression may provide an improved method for predicting prognosis in squamous cell lung cancer patients over current approaches.

Materials and Methods

Clinical samples. In total, 61 snap-frozen lung SCC and 10 matched normal adjacent lung tissue samples were evaluated for miRNA expression. These samples were collected from patients from University of Michigan Hospital between October 1991 and July 2002 with patient consent and institutional review board approval. Samples chosen for analysis contained
miRNA Profiles in Squamous Cell Lung Carcinoma

Cox score was used to determine each patient's risk of survival. This is defined as the sum of the linear combination of weighted log expression intensity with the average standardized Cox regression coefficient from 400 bootstrapping samples as the weight. The threshold for risk stratification was determined in another round of 5-fold cross-validations to test a series of cutoffs (percentile of risk index for the patients in the training set), and the optimal cutoff was chosen to give the minimum overall error rate. Patients whose scores were ≥0 were classified in the high risk of survival group, whereas patients whose scores were <0 were predicted as the low risk of survival group. Kaplan-Meier survival plots and log-rank tests were used to assess the differences in survival of the predicted high-risk and low-risk groups. Patients were censored from statistical analysis if they were alive but had <3 y of clinical follow up. Sensitivity was defined as the percentage of the patients who died within 3 y, which were predicted correctly by the gene expression signature, and specificity was defined as the percentage of the patients who survived for at least 3 y, which was predicted correctly by the classifier. All the statistical analyses were done using S-Plus 6 software (Insightful). Kaplan-Meier plots were generated using GraphPad Prism (GraphPad Software, Inc., version 4.03).

Pathway analysis. Pathway analysis was undertaken as described previously (23). miRNA prediction analysis was done using the open access software tool MAMI, which is a combination of several prediction algorithms. Except for mir-146b, all target predictions using MAMI were undertaken using 0.68 sensitivity and 0.5 specificity (the reported levels of sensitivity and specificity were calculated on a set of in vivo validated miRNA/target gene pairs). For mir-146b, sensitivity and specificity of 0.91 and 0.22 were used, respectively. To exclude genes that were identified simply by chance, a statistical procedure known as gene set enrichment was undertaken. To this end, the functional annotation of Gene Ontology (GO) biological process terms level 5 was done using the open access software DAVID (24). The Fisher exact test was used to select pathways that were significantly enriched with miRNA targets (25). To further evaluate the specific functional profiles of genes from the broad GO categories that are targeted by miRNAs, a more detailed functional analysis using Ingenuity Pathway Analysis (IPA 5.0 Ingenuity System) was done. The set of pathways were also compared with the reference set of genes that are associated to lung cancer (565 genes; Supplementary Table S1). Fisher exact test was used to select pathways that were significantly enriched with miRNA targets. Finally, pathways that were significantly enriched using the Affymetrix gene set were compared with pathways that were significantly enriched with miRNAs associated with prognosis.

Results

Identification of miRNAs differentially expressed in lung SCC. We initially profiled 61 lung SCC and 10 matched adjacent normal lung tissue samples using the Ambion mirVana Bioarray (v2) that contains 328 human miRNA probes (from Version 8.0 of miRBase Sequence Database). A total of 15 unique miRNAs were differentially expressed in SCC (Table 2). The majority of these were more highly expressed in tumors including members of the miR-17-92 cluster and its paralogue clusters miR-106a-363 and miR-93-106b, which have previously been identified as oncogenic in lung and other cancer types (26–32). Other miRNAs upregulated in lung SCC which have previously been identified as oncogenic in lung and other cancer types (26–32). Other miRNAs upregulated in lung SCC which have previously been identified as oncogenic in lung and other cancer types (26–32).

Identification of miRNAs associated with lung SCC patient outcome. We next identified miRNAs that were associated with prognosis in 54 lung SCC samples using the SAM survival algorithm. A total of 20 miRNAs were identified as having a significant association with overall survival (Table 3). Using a 5-fold cross-validation, it was found that the highest mean value for predicting overall survival
Table 1. Clinical characteristics of 57 patients examined for prognosis

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<td>62.2</td>
<td>Dead</td>
<td>IIb</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Poor</td>
<td>50</td>
<td>M</td>
</tr>
<tr>
<td>LS-65</td>
<td>SCC</td>
<td>8/24/1992</td>
<td>59.6</td>
<td>Alive</td>
<td>IIb</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Poor</td>
<td>69</td>
<td>M</td>
</tr>
<tr>
<td>LS-66</td>
<td>SCC</td>
<td>8/26/1992</td>
<td>94.5</td>
<td>Dead</td>
<td>IIb</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Poor</td>
<td>83</td>
<td>M</td>
</tr>
<tr>
<td>LS-67</td>
<td>SCC</td>
<td>3/10/1993</td>
<td>64.1</td>
<td>Alive</td>
<td>IIb</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Mod</td>
<td>42</td>
<td>M</td>
</tr>
<tr>
<td>LS-68</td>
<td>SCC</td>
<td>6/2/1993</td>
<td>131.5</td>
<td>Dead</td>
<td>IIb</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Mod</td>
<td>62</td>
<td>F</td>
</tr>
<tr>
<td>LS-69</td>
<td>SCC</td>
<td>8/26/1996</td>
<td>78.9</td>
<td>Alive</td>
<td>Ib</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Mod</td>
<td>82</td>
<td>M</td>
</tr>
<tr>
<td>LS-70</td>
<td>SCC</td>
<td>6/14/1995</td>
<td>80.9</td>
<td>Dead</td>
<td>Ia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Poor</td>
<td>72</td>
<td>M</td>
</tr>
<tr>
<td>LS-71</td>
<td>SCC</td>
<td>11/24/1997</td>
<td>78.1</td>
<td>Dead</td>
<td>IIb</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>Mod</td>
<td>82</td>
<td>F</td>
</tr>
<tr>
<td>LS-72</td>
<td>SCC</td>
<td>3/9/1998</td>
<td>7.4</td>
<td>Alive</td>
<td>Ib</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Poor</td>
<td>63</td>
<td>M</td>
</tr>
<tr>
<td>LS-75</td>
<td>SCC</td>
<td>10/23/1998</td>
<td>50.6</td>
<td>Alive</td>
<td>IIb</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>Mod-poor</td>
<td>67</td>
<td>F</td>
</tr>
</tbody>
</table>

Abbreviations: T, tumor; N, lymph nodes; M, metastasis.
within 3 years was 78% when using miR-146b alone (Supplementary Fig. S3). The predictive accuracy dropped but stabilized at 68% when three or more miR-146b were added in a linear fashion. When the median expression of miR-146b was used to stratify patients, the group with high miR-146b expression had significantly worse overall survival (26 months) compared with the low miR-146b group (95 months). The associated hazard ratio (HR) was 2.7 [95% confidence interval (CI) 1.4–5.7, log-rank P = 0.0035; Fig. 1A].

MiR-155, which has previously been shown to predict prognosis in lung adenocarcinoma, was also associated with overall survival in lung SCC (HR 2.3, CI 1.0–5.6, P = 0.06; Fig. 1B). Expression of miR-146b and miR-155 by qPCR significantly correlated with expression identified using the Ambion array (R = 0.65–0.72, P < 0.001; Fig. 1).

Pathway analysis of lung SCC miRNAs. We next used a bioinformatic approach to investigate what signaling processes were associated with miRNA dysregulation in lung SCC. To this end, a total of 2282 target genes were predicted from the 15 miRNAs identified between normal lung and lung SCC. Of these, 1,286 genes were determined by GO enrichment analysis to be associated with 40 known signaling pathways (Supplementary Table S2). We also compared these sets of pathways with a reference set of genes that have been characterized in lung cancer. All 40 pathways were significantly correlated with expression identified using the Ambion array (R = 0.65–0.72, P < 0.001; Fig. 1).

Table 2. miRNAs differentially expressed between normal lung and lung SCC (>6 intensity (log2) in at least one group)

<table>
<thead>
<tr>
<th>PSID</th>
<th>P</th>
<th>Mean N</th>
<th>Mean T</th>
<th>Direction</th>
<th>Location</th>
<th>miRNA cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-mir-210</td>
<td>1.28E-10</td>
<td>6.0</td>
<td>7.7</td>
<td>Up</td>
<td>11p15.5</td>
<td></td>
</tr>
<tr>
<td>hsa-mir-200c</td>
<td>1.06E-09</td>
<td>9.5</td>
<td>10.8</td>
<td>Up</td>
<td>12p13.31</td>
<td>miR-141, miR-200c</td>
</tr>
<tr>
<td>hsa-mir-17-5p</td>
<td>1.49E-13</td>
<td>6.4</td>
<td>7.5</td>
<td>Up</td>
<td>13q31.3</td>
<td>miR-17-92</td>
</tr>
<tr>
<td>hsa-mir-20a</td>
<td>5.15E-09</td>
<td>5.6</td>
<td>6.1</td>
<td>Up</td>
<td>13q31.3</td>
<td>miR-17-92</td>
</tr>
<tr>
<td>hsa-mir-203</td>
<td>4.39E-09</td>
<td>5.2</td>
<td>6.3</td>
<td>Up</td>
<td>14q23.32</td>
<td></td>
</tr>
<tr>
<td>hsa-mir-125a</td>
<td>6.52E-13</td>
<td>10.2</td>
<td>8.9</td>
<td>Down</td>
<td>19q13.41</td>
<td>miR-99b, miR-125a, let-7e</td>
</tr>
<tr>
<td>hsa-mir-25</td>
<td>3.10E-07</td>
<td>9.5</td>
<td>9.1</td>
<td>Down</td>
<td>19q13.41</td>
<td>miR-99b, miR-125a, let-7e</td>
</tr>
<tr>
<td>hsa-mir-200a</td>
<td>2.50E-07</td>
<td>5.7</td>
<td>7.0</td>
<td>Up</td>
<td>1p31.3</td>
<td></td>
</tr>
<tr>
<td>hsa-mir-16b</td>
<td>2.00E-11</td>
<td>5.9</td>
<td>6.9</td>
<td>Up</td>
<td>1p36.3</td>
<td></td>
</tr>
<tr>
<td>hsa-mir-93</td>
<td>2.97E-08</td>
<td>7.5</td>
<td>8.8</td>
<td>Up</td>
<td>1p36.3</td>
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</tr>
<tr>
<td>hsa-mir-162</td>
<td>9.71E-19</td>
<td>5.6</td>
<td>6.8</td>
<td>Up</td>
<td>1p36.3</td>
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<tr>
<td>hsa-mir-183</td>
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<td>5.6</td>
<td>6.3</td>
<td>Up</td>
<td>1p36.3</td>
<td></td>
</tr>
<tr>
<td>hsa-mir-106a</td>
<td>1.54E-18</td>
<td>6.3</td>
<td>7.7</td>
<td>Up</td>
<td>Xp22.33</td>
<td>miR-106-363</td>
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<tr>
<td>hsa-mir-20b</td>
<td>8.66E-16</td>
<td>5.2</td>
<td>6.1</td>
<td>Up</td>
<td>Xq26.17</td>
<td>miR-106-363</td>
</tr>
<tr>
<td>hsa-mir-224</td>
<td>1.03E-16</td>
<td>5.5</td>
<td>6.9</td>
<td>Up</td>
<td>Xq28</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: miRNAs in bold indicate those that are part of clusters which are represented multiple times. Abbreviations: PSID, probe set identification code; N, lung normal signal intensity (log 2); T, lung cancer signal intensity (log 2).
significantly associated with genes known to be involved in lung cancer signaling processes. The top processes included extracellular signal-regulated kinase/mitogen-activated protein kinase (MAPK) signaling and axonal guidance.

**Pathway analysis of prognostic miRNAs.** We next extended this approach to the 20 prognostic miRNAs, which were predicted to target 1408 genes. Four hundred fifty-seven of these genes were associated with 54 signaling pathways based on GO analysis (Supplementary Table S3). Each of these pathways was also significantly associated with genes known to be associated with lung cancer pathogenesis. The top signaling pathways associated with the prognostic miRNAs included B-cell receptor and WNT/β-catenin signaling pathways, which are also present in the top 5 pathways associated with lung SCC miRNAs defined above. The top prognostic miRNA, miR-146b, was predicted to target a total of 195 genes. Of these, 103 genes were associated with eight known signaling pathways, five of which intersected with the top 20 prognostic miRNA targeted pathways (Table 4).

**Comparison of pathways associated with miRNA and mRNA prognostic signatures.** We previously identified a set of 50 genes associated with prognosis in the same sample set, using the Affymetrix U133A gene chips (5). There was no significant overlap between these 50 genes and the predicted targets of the 20 prognostic miRNAs. With the availability of a matched mRNA/miRNA data set, we then investigated the correlations between miRNAs and predicted target gene expression. Whereas there were no significant correlations between the expression of any one miRNA and all genes measured on the Affymetrix microarray, there were shifts toward a negative correlation for a subset of miRNAs (such as miR-155) when only the predicted mRNA targets were analyzed (Fig. 2). This negative shift would be expected if the miRNAs were reducing the steady-state level of targeted mRNAs. However, not all miRNAs
We have profiled global miRNA expression in the largest set of lung SCC samples to date. As expected, members of the miR-17-92 cluster were highly expressed in lung SCC. The miR-17-92 cluster has been found to be overexpressed in cancers, including lung cancer, and has been implicated as an oncogene (oncoMir; refs. 26, 27, 32). MiR-106a, miR-20b, miR-93, and miR-106b, which are paralogues of the miR-17-92 cluster, were also highly expressed in lung SCC and have been previously found to be up-regulated in leukemias and associated with oncogenesis (33, 34). In addition, we found let-7e (and its cluster member miR-125a) to be lowly expressed in lung SCC, which is consistent with previous reports (14, 16, 17, 35).

We compared our results with that of Yanahaira and colleagues, who profiled miRNA expression in NSCLC samples using an alternative array containing 190 human miRNAs (12). They found 16 miRNAs differentially expressed between 39 SCC and 39 matched normal lung tissues. Five of the 16 miRNAs were in common between their data set and ours with four of the five (miR-210, miR-203, miR-125a, and miR-106a) showing the same direction of differential expression (12). Yanahaira and colleagues further showed that high miR-155 and low let-7a expression were associated with poor prognosis in lung adenocarcinoma. We also found that high miR-155 expression was associated with poor outcome in lung SCC. Therefore, miR-155 seems to be a valid marker of survival in both lung adenocarcinoma and SCC. We were unable to test the miRNAs identified in our study using the Yanahaira data set because their clinical outcome data are not publicly available.

We identified a total of 20 miRNAs that were associated with overall survival in patients with lung SCC. In addition to miR-155, we found several other miRNAs that have also been associated with prognosis in a variety of cancers, including miR-21 in NSCLC, colorectal, pancreatic, and breast cancer, miR-191 in acute myeloid leukemia and miR-15a in chronic lymphocytic leukemia (12, 36–41). However, the most robust classifier for predicting prognosis in lung SCC was miR-146b alone. MiR-146b has recently been shown to be associated with thyroid cancer (42, 43); however, it has not been previously shown to be associated with clinical outcome in any cancer setting. Lindsay and colleagues have described the up-regulation of miR-146b in response to lipopolysaccharide using the lung adenocarcinoma A549 cell line model (44). In addition, miR-146b expression is induced by lipopolysaccharide in primary macrophages, which are the primary immune surveillance cells in the lung (45). Because it has been found that miRNAs can be actively transported out of the cell via the exosome (46, 47) and that chronic inflammation can lead to lung cancer (48), it is interesting to speculate whether an inflammatory microenvironment leads to the interstitial transport of miR-146b and other miRNAs from alveolar macrophages into lung epithelial cells, which could eventually lead to the initiation of tumor pathogenesis. This hypothesis remains to be tested.

Yu and colleagues recently described a 5-miRNA prognostic signature for NSCLC (18). However, this classifier did not significantly stratify patient outcome in our data set (data not shown). Importantly, their training data set only consisted of 10 lung SCC samples and was assayed using Taqman qPCR assays that were normalized to U6 RNA. Interestingly, we found that U6 RNA may not be the optimal normalization control in our lung cancer data set because there was a significant association with survival (data not shown). Others have also shown that U6 may not be an appropriate normalization control for miRNA profiling (49). Because Yu and colleagues have only provided U6-normalized data in the public databases, we did not attempt to test our signatures in their data set.

We have previously described a 50-gene signature that predicts prognosis in lung SCC. Interestingly, when the Affymetrix miRNA data from the samples from the current study were interrogated, no significant stratification of prognostic groups could be found when 20 genes or less were used in the classifier (data not shown; ref. 5). However, the miRNAs provided stable predictive utility at 68% when the classifier contained four or more miRNAs. Moreover, miR-146b alone provided the best predictive accuracy at 78%. This indicated that the miRNA signature was more informative than the gene expression-based predictive classifier. This is likely due, in large part, to miRNAs being upstream regulators of gene expression.

### Table 4. Pathway analysis of miR-146b targets

<table>
<thead>
<tr>
<th>Pathway</th>
<th>$P$</th>
<th>Target molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor signaling</td>
<td>7.59E-03</td>
<td>POLR2G, POLR2A, HNRPD, TAF9B</td>
</tr>
<tr>
<td>Huntington's disease signaling</td>
<td>1.91E-02</td>
<td>POLR2G, POLR2A, PRKCE, ZDHHC17, TAF9B</td>
</tr>
<tr>
<td>Nuclear factor-R-B signaling</td>
<td>2.00E-02</td>
<td>TRAF6, CARD10, MAP3K8, IRAK1</td>
</tr>
<tr>
<td>Regulation of actin-based motility by Rho</td>
<td>2.19E-02</td>
<td>ROCK1, PIP4K2B, PIP5K1B</td>
</tr>
<tr>
<td>Nucleotide excision repair pathway</td>
<td>2.51E-02</td>
<td>POLR2G, POLR2A</td>
</tr>
<tr>
<td>p38 MAPK signaling</td>
<td>3.09E-02</td>
<td>TRAF6, PLA2G12B, IRAK1</td>
</tr>
<tr>
<td>Glucocorticoid receptor signaling</td>
<td>3.47E-02</td>
<td>TRAF6, POLR2G, POLR2A, SMAD4, TAF9B</td>
</tr>
<tr>
<td>Toll-like receptor signaling</td>
<td>4.90E-02</td>
<td>TRAF6, IRAK1</td>
</tr>
</tbody>
</table>

Yanahaira and colleagues further showed that high miR-155 expression was associated with poor prognosis in lung SCC. Therefore, miR-155 seems to be a valid marker of survival in both lung adenocarcinoma and SCC. We were unable to test the miRNAs identified in our study using the Yanahaira data set because their clinical outcome data are not publicly available.
and that they have hundreds of downstream targets (50). Furthermore, miRNAs are more stable than mRNA, which has enabled them to be readily detected in clinically relevant specimens, such as formalin-fixed paraffin-embedded tissue, and in cell-free plasma or serum (51, 52).

Examination of the biological pathways targeted by the prognostic miRNAs indicated that there were seven major signaling processes associated with the clinical outcome of lung SCC. Although there was no significant overlap between the 50 genes and the predicted targets of the 20 prognostic miRNAs, six of the seven pathways were in common. These included chemokine signaling, IL-6, and FGF pathways. We previously showed that down-regulation of FGFR2 is associated with poor overall survival in the same set of lung SCC tumors (5). Therefore, two separate sets of genomic data have identified the same biological pathways as being associated with clinical outcome in this disease. The lack of common genes between the 50-gene signature and the 20 prognostic miRNA targets may, in part, be explained if the miRNAs are predominantly functioning through a translational repression mechanism. This may be the case because the correlation analysis indicated that the majority of miRNAs are not affecting the steady-state level of their target mRNAs.

In summary, we have shown that distinct miRNA profiles exist in lung SCC compared with normal lung tissue and that miRNA-based classification may be robust predictors of prognosis in this disease. The biological pathways that are targeted by these miRNAs are similar to those enriched by independent prognostic gene expression signatures. MiR-146h alone was the most robust predictor of overall survival. These miRNAs may provide a unique method for diagnosing and predicting prognosis in lung cancer, in addition to providing novel targets for developing therapeutic options in this disease.

Disclosure of Potential Conflicts of Interest

M. Raponi, L. Dossey, T. Jak toe, and H. Fan are employed by companies that are in the business of commercializing diagnostics and therapeutics for cancer management.

The other authors disclosed no potential conflicts of interest.

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References

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