

■ CANCER AND CELL BIOLOGY

**Unique MicroRNA Molecular Profiles in Lung Cancer
Diagnosis and Prognosis**

Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, and Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis *Cancer Cell* 9: 189–98, 2006.

Lung cancer is the leading cause of cancer deaths in the world, indicating the obvious need for a better understanding of the mechanisms that underlie carcinogenesis in the lung. Although systematic analysis of mRNA and protein expressions has contributed to defining the molecular network of lung carcinogenesis, previously unknown markers such as noncoding RNA gene products may also lend insight into the biology of lung cancer.

MicroRNAs (miRNAs) are small noncoding RNA gene products that are found in diverse organisms and play key roles in regulating the translation and degradation of mRNAs. miRNAs have been implicated in various biological processes, including cell proliferation, cell death, stress resistance, and fat metabolism, through the regulation of mRNA stability and/or translation of multiple target genes. Our understanding of miRNA expression patterns and function in normal or neoplastic human cells is just emerging. Although the precise mechanisms regulating miRNA expression are not yet fully understood, several mechanisms, including genetic and epigenetic alteration, might affect its expression and might lead to alterations in the target genes' expression in cancers. To investigate miRNA involvement in lung carcinogenesis, we examined its expression profiles for lung cancers by using miRNA microarray technology.

The miRNA expression profiles of 104 pairs of primary lung cancers and corresponding noncancerous lung tissues were analyzed. Each pair was obtained from the same patient to eliminate genetic differences between tumor and normal tissues. miRNA microarray analysis identified statistically unique profiles that could discriminate lung cancers from noncancerous lung tissues. When we compared miRNA expression among lung cancer tissues with that of corresponding noncancerous lung tissues, 43 miRNAs showed statistical differences in expression. Several of the miRNAs are located inside fragile sites and/or in the cancer-associated genomic regions, such as frequently deleted or amplified regions in several malignancies. This finding, and the fact that more than 50% of miRNAs are located in cancer-related chromosomal regions, supports the hypothesis that miRNAs play a role as a novel class of oncogenes or tumor suppressor genes. We next asked whether the microarray

data revealed specific molecular signatures for lung cancer subsets that differ in clinical behavior. We identified six miRNAs that were expressed differently in the two most common histological types of non-small-cell lung cancer (NSCLC), adenocarcinoma and squamous cell carcinoma. No miRNAs were identified as differently expressed when classified by age, sex, or race in our data set.

Our next question was, Do the miRNA molecular profiles of lung cancer correlate with patient survival? We found that the miRNA molecular profile of lung adenocarcinoma correlates with patient survival. Furthermore, the miRNA molecular signature of lung adenocarcinomas, including those without evidence of metastasis, also correlates with patient survival. A univariate Cox proportional hazard regression model with a global permutation test indicated that expression of the miRNAs *hsa-mir-155* and *hsa-let-7a-2* was related to adenocarcinoma patient outcome. Kaplan-Meier survival analysis showed that the lung adenocarcinoma patients with either high *hsa-mir-155* (Figure 1) or reduced *hsa-let-7a-2* expression had poor survival. The difference in the prognosis of these two groups was statistically significant for *hsa-mir-155* ($P = 0.006$; log-rank test) and marginally significant for *hsa-let-7a-2* ($P = 0.033$; log-rank test). Subsequently, a multivariate Cox proportional hazard regression analysis indicated that high *hsa-mir-155* expression correlated significantly with an unfavorable prognosis independent of other clinicopathological factors ($P = 0.027$; risk ratio 3.03; 95% CI, 1.13–8.14). The miRNA expression signature associated with outcome was confirmed by real-time RT-PCR analysis of precursor forms for the same miRNAs. Furthermore, we were able to cross-validate the clinical importance of outcome-predictive miRNAs using another independent case of adenocarcinoma. These results indicate that miRNA expression profiles are diagnostic and prognostic markers of lung cancer.

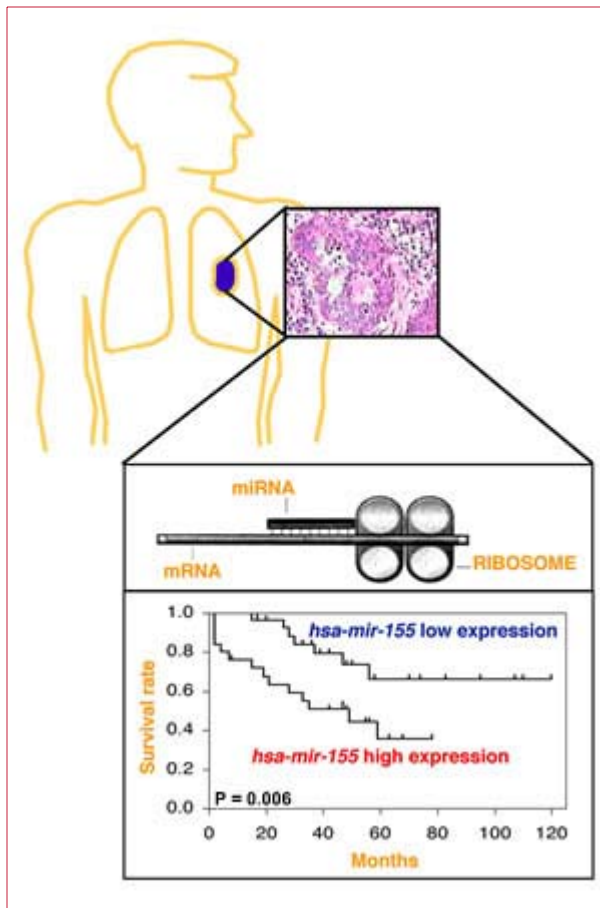


Figure 1. Kaplan-Meier survival analysis showing that the lung adenocarcinoma patients with high *hsa-mir-155* miRNA expression had poor survival.

Although curative resections of patients with early-stage NSCLC are performed, the risk of relapse remains substantial. This may indicate that there are micrometastases that have not been detected by general imaging and/or pathological examinations. Although additional studies confirming our results need to be performed, we anticipate that the miRNA expression signature with other biomarkers will allow the selection of lung cancer patients who may need more aggressive screening and treatment.

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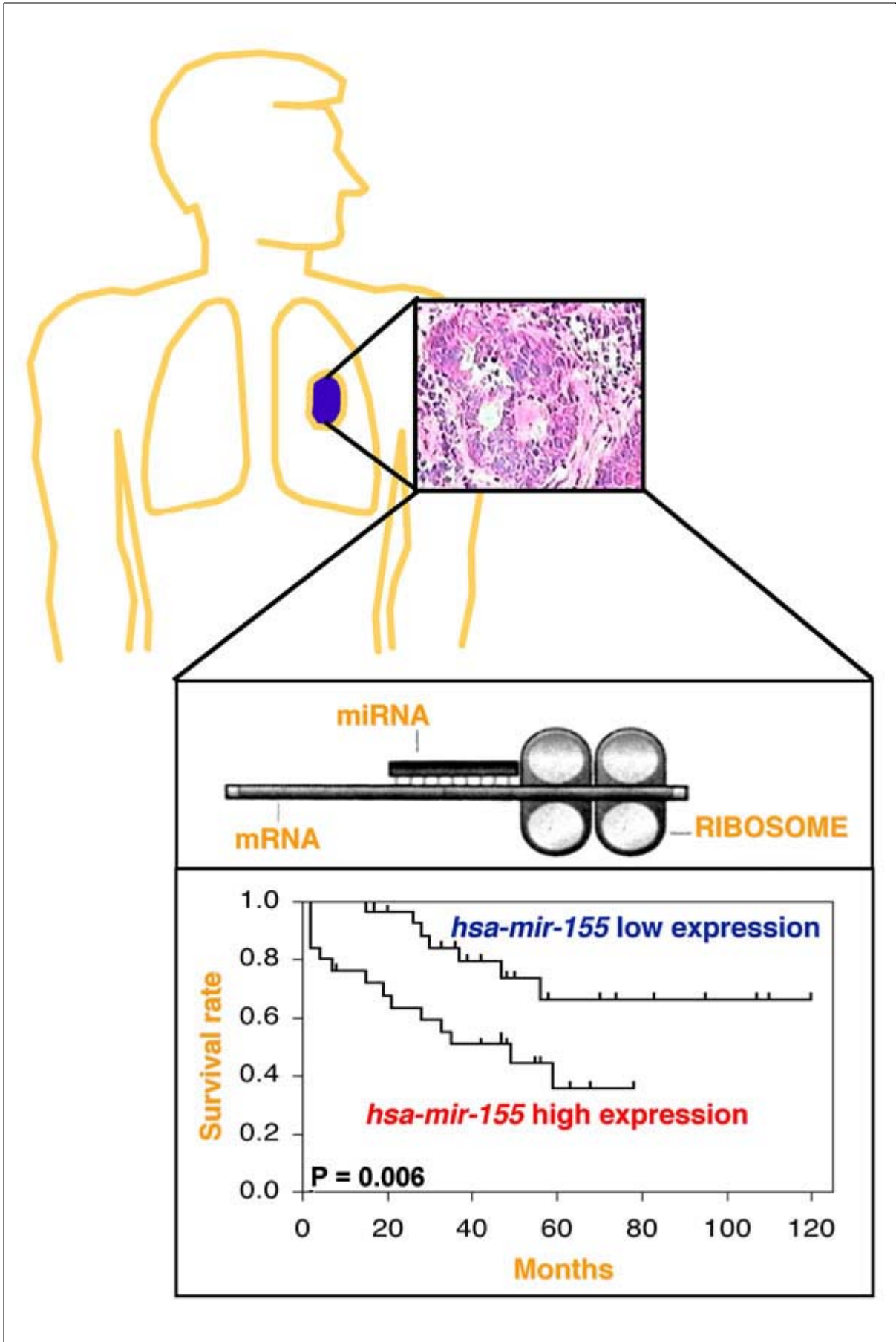


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