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MicroRNA Expression, Survival, and Response to Interferon in Liver Cancer

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ABSTRACT

BACKGROUND

Hepatocellular carcinoma is a common and aggressive cancer that occurs mainly in men. We examined microRNA expression patterns, survival, and response to interferon alfa in both men and women with the disease.

METHODS

We analyzed three independent cohorts that included a total of 455 patients with hepatocellular carcinoma who had undergone radical tumor resection between 1999 and 2003. MicroRNA-expression profiling was performed in a cohort of 241 patients with hepatocellular carcinoma to identify tumor-related microRNAs and determine their association with survival in men and women. In addition, to validate our findings, we used quantitative reverse-transcriptase–polymerase-chain-reaction assays to measure microRNAs and assess their association with survival and response to therapy with interferon alfa in 214 patients from two independent, prospective, randomized, controlled trials of adjuvant interferon therapy.

RESULTS

In patients with hepatocellular carcinoma, the expression of miR-26a and miR-26b in nontumor liver tissue was higher in women than in men. Tumors had reduced levels of miR-26 expression, as compared with paired noncancerous tissues, which indicated that the level of miR-26 expression was also associated with hepatocellular carcinoma. Moreover, tumors with reduced miR-26 expression had a distinct transcriptomic pattern, and analyses of gene networks revealed that activation of signaling pathways between nuclear factor κB and interleukin-6 might play a role in tumor development. Patients whose tumors had low miR-26 expression had shorter overall survival but a better response to interferon therapy than did patients whose tumors had high expression of the microRNA.

CONCLUSIONS

The expression patterns of microRNAs in liver tissue differ between men and women with hepatocellular carcinoma. The miR-26 expression status of such patients is associated with survival and response to adjuvant therapy with interferon alfa.

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HEPATOCELLULAR CARCINOMA IS COM-
mon globally, with dismal outcomes and
an increasing incidence in the United
States.[1] To date, surgery remains the most effec-
tive treatment with curative potential. However,
only about 10 to 20% of patients with hepatocel-
lular carcinoma are currently eligible for surgical
intervention. In addition, patients who undergo
curative resection often have a high rate of re-
lapse. Therapies such as transcatheter arterial
chemoembolization (TACE) and interferon alfa
may prolong survival in some patients. However,
the response is often not satisfactory because of
the limited ability to identify patients who are
most likely to benefit from such targeted adjuvant
therapies.[2-5] The recent findings of the Sorafenib
Hepatocellular Carcinoma Assessment Random-
ized Protocol (SHARP) trial (ClinicalTrials.gov
number, NCT00105443)[6] were encouraging with
respect to the use of sorafenib as a therapeutic
agent. However, the survival benefit was modest.
There is a need for molecular tools that can be
used to stratify patients with respect to progno-
sis and response to therapy.

One of the key features of hepatocellular car-
cinoma is the higher incidence in men than in
women, by a factor of two to six among various
ethnic groups.[7] Classic in vivo carcinogenesis ex-
periments also reveal a higher susceptibility to
hepatocellular carcinoma in male rodents.[8-11]
Moreover, women with this disease tend to have
longer survival than men.[12-14] These findings
suggest that the biologic features of the tumor
and the host microenvironment may differ sig-
nificantly between the sexes and that sex-related
factors may be associated with the prognosis.
However, the underlying reasons for the lower
incidence of hepatocellular carcinoma in women
remain unclear. In a recent study, investigators
found that one contributing factor may be that
estrogen inhibits the induction of interleukin-6
produced by Kupffer cells.[11] The serum level of
interleukin-6 is elevated in several cancers, includ-
ing hepatocellular carcinoma.[15,16]

MicroRNAs, small noncoding RNAs that regu-
late the translation of many genes, are excellent
biomarkers for cancer diagnosis and prognosis.[17-21]
Given the therapeutic and prognostic potential
of microRNAs as biomarkers in hepatocellular
carcinoma, we evaluated microRNA profiles of
specimens of hepatocellular carcinomas and
paired specimens of noncancerous tissue to
search for microRNAs that are associated with
sex and with hepatocellular carcinoma.

METHODS

STUDY OVERSIGHT

The study was approved by the institutional re-
view board at each study center. All patients from
whom tissue samples were obtained provided
written informed consent.

CLINICAL SPECIMENS

We obtained snap-frozen or paraffin-embedded
specimens of tumors and surrounding nontumor
hepatic tissues from 455 patients with hepatocel-
lular carcinoma who had undergone radical re-
section between 1999 and 2003 at the Liver Can-
cer Institute of Fudan University in Shanghai
(376 patients) or at the University of Hong Kong
Medical Centre in Hong Kong (79 patients).[4] Sam-
ples of normal liver tissue were obtained from
eight disease-free liver donors.[22]

Our analyses involved three separate cohorts
of patients. Cohort 1 consisted of 241 patients
with hepatocellular carcinoma (test cohort) for
whom microRNA microarray data were available,
as described previously.[21] Data from these pa-
tients were analyzed to search for microRNAs
that were associated with sex and survival. We
were able to analyze miR-26 expression in 224
patients and survival in 217 patients. Cohorts 2
and 3 consisted of 214 patients from prospective,
randomized, controlled trials of adjuvant therapy
with interferon alfa in patients with hepatocellu-
lar carcinoma.[3,4] In cohort 2, data from 135
patients were evaluated in an independent vali-
dation analysis to measure microRNA expres-
sion with the use of quantitative reverse-tran-
scriptase–polymerase-chain-reaction (qRT-PCR)
assay. We were able to evaluate both miR-26 ex-
pression and survival in 118 patients. In cohort
3, data from 79 patients (40 in the control group
and 39 in the group that received interferon alfa)
were evaluated to validate the association be-
tween miR-26 expression and the response to
interferon alfa therapy.

Additional methods (including statistical analy-
sis) are described in detail in the Supplementary
Appendix, available with the full text of this ar-
ticle at NEJM.org.

RESULTS

CHARACTERISTICS OF THE PATIENTS

Most of the patients in the three cohorts were men (85.1%), were long-term carriers of hepatitis B virus (HBV) (90.5%), and had cirrhosis (88.0%) and an elevated serum level of alpha-fetoprotein (62.2%); 84.4% of the patients had a single tumor nodule at the time of resection (Table 1, and Table 1 in the Supplementary Appendix). Clinical variables were similar between the test and validation cohorts, with the exception of the serum alanine aminotransferase (ALT) level, tumor–node–metastasis (TNM) stage, and status with respect to adjuvant therapy. Liver-inflammation activity in the patients with HBV-related hepatocellular carcinoma, as indicated by ALT levels, was significantly lower in patients in cohort 2 than in those in either cohort 1 or cohort 3, and more patients in cohort 2 had early-stage tumors. In addition, 39 patients in cohort 1 had received prophylactic adjuvant therapies, but the difference in response did not appear to be significant (P=0.90 by the log-rank test). In contrast, 53.3% of patients in cohort 2 and 49.4% in cohort 3 had received "intention-to-cure" adjuvant therapy with interferon alfa, which improved overall survival.[3,4]

ASSOCIATION OF MicroRNAs WITH SEX AND CLINICAL OUTCOME

To search for differences in the expression of microRNAs between liver samples from men and those from women, we globally analyzed the microRNA expression profiles of 241 patients in cohort 1, in which both tumor and nontumor microRNA microarray data were available (Gene Expression Omnibus [GEO] accession number, GSE6857).[21] To avoid potential confounding factors, an age-matched and balanced case set was used to identify microRNAs with different expression levels in men and women, consisting of all 30 women and two age-matched control groups of men, each consisting of 31 patients (groups G1 and G2). The clinical characteristics of the women and the men in the two control groups were similar (Table 2 in the Supplementary Appendix).

Class-comparison analysis of nontumor liver tissues revealed that 15 microRNAs were differentially expressed in the comparison between the 30 women and the first control group of men (G1), whereas 45 microRNAs differed in the comparison between the women and the second control group of men (G2). Seven microRNAs showed differential expression between women and men in both comparisons. In contrast, we identified only one microRNA, miR-129-2, that had differential expression in tumor tissue from women and from men in both comparisons (Table 3 in the Supplementary Appendix). Thus, there were more consistent differences in microRNA expression in nontumor hepatic tissue than in tumors.

In humans, there are three miR-26 members: miR-26a-1, miR-26a-2, and miR-26b; miR-26a-1 was chosen for further analysis, since the difference in its level between sexes was most significant, and it was most abundant. Analyses of data from 224 patients in cohort 1 showed that miR-26a-1 expression in normal hepatic tissue was significantly higher in women than in men (Fig. 1A). This finding was validated by the determination of mature miR-26 expression in 26 women and in 56 age-matched men with the use of qRT-PCR (Fig. 1B).

We then reasoned that miR-26 may act as a tumor-suppressor gene, and if so, the silencing of miR-26 would be a frequent event in tumors. Analyses showed that a significant reduction in miR-26a-1 expression in tumors, as compared with that in nontumor samples, was observed in patients with low levels of miR-26 expression (P<0.001) but not in those with high levels of miR-26 expression (P=0.23) (Fig. 1C). The median factor change in the expression in tumors, as compared with nontumors, was 0.37 in patients with low miR-26a-1 expression and 0.98 in patients with high miR-26a-1 expression, which suggested that the silencing of miR-26 expression was associated only with low miR-26 expression. Moreover, low miR-26 expression, as compared with high expression, was associated with reduced survival (Fig. 1D). The expression patterns of all three miR-26 members and their associations with survival were similar (Fig. 1 in the Supplementary Appendix).

TUMORS WITH LOW MiR-26 EXPRESSION

To test the hypothesis that tumors with low miR-26 expression may be biologically distinct, we analyzed the 224 matched patients in cohort 1 with available microRNA and messenger RNA (mRNA) microarray data. The mRNA microarray

Table 1. Clinical Characteristics of the Patients.*

| Variable	Cohort 1 (N = 241)	Cohort 2 (N = 135)	P Value†
Sex — no. (%)			0.87
Female	30 (12.4)	14 (10.4)	
Male	211 (87.6)	111 (82.2)	
Missing data	0	10 (7.4)	
Age — yr			0.29
Median	50	50	
Range	13–83	20–77	
Alanine aminotransferase — no. (%)			<0.001
Normal, ≤50 U/liter	145 (60.2)	107 (79.3)	
Elevated, >50 U/liter	96 (39.8)	16 (11.9)	
Missing data	0	12 (8.9)	
Hepatitis B virus — no. (%)			0.64
Negative	16 (6.6)	6 (4.4)	
Positive	224 (92.9)	118 (87.4)	
Missing data	1 (0.4)	11 (8.1)	
Tumor size — no. (%)			0.91
≤3 cm	88 (36.5)	46 (34.1)	
>3 cm	153 (63.5)	78 (57.8)	
Missing data	0	11 (8.1)	
Multinodular tumor — no. (%)			0.50
No	214 (88.8)	107 (79.3)	
Yes	27 (11.2)	17 (12.6)	
Missing data	0	11 (8.1)	
Cirrhosis — no. (%)			0.08
No	17 (7.1)	16 (11.9)	
Yes	223 (92.5)	108 (80.0)	
Missing data	1 (0.4)	11 (8.1)	
TNM stage — no. (%)			<0.001
I	97 (40.2)	81 (60.0)	
II	90 (37.3)	29 (21.5)	
III or IV	54 (22.4)	14 (10.4)	
Missing data	0	11 (8.1)	
Alpha-fetoprotein — no. (%)			0.20
Negative, ≤20 ng/ml	77 (32.0)	49 (36.3)	
Positive, >20 ng/ml	162 (67.2)	75 (55.6)	
Missing data	2 (0.8)	11 (8.1)	
Adjuvant therapy — no. (%)‡			<0.001
Yes	39 (16.2)	72 (53.3)	
No	202 (83.8)	63 (46.7)	
Survival (mo)			0.89
Median	>60	67	
Range	2–67	2–82	

* The clinical characteristics of the 79 patients in the validation cohort for interferon alfa therapy (cohort 3) are listed in Table 1 in the Supplementary Appendix. TNM denotes tumor–node–metastasis.
† A P value of less than 0.05 was considered to indicate statistical significance. P values were calculated with the use of Fisher's exact test, except for age, which was calculated with the unpaired t-test; TNM, which was calculated with the chi-square test; and survival, which was calculated with the log-rank test.
‡ In cohort 1, 34 patients underwent transcatheter arterial chemoembolization, 3 received chemotherapy, 1 received interferon alfa therapy, and 1 received lymphokine-activated killer-cell therapy; in cohort 2, 72 patients underwent interferon alfa therapy.

Figure 1. Expression of miR-26 in Hepatic Tumors and Noncancerous Tissue in Men and Women.

Panel A shows miR-26a-1 expression in noncancerous hepatic tissue from 30 women and 194 men, as determined by microarray analysis with the use of an unpaired t-test. Panel B shows miR-26a expression in noncancerous hepatic tissue obtained from 26 women and 56 age-matched men, as determined by quantitative reverse-transcriptase–polymerase-chain-reaction assay with the use of an unpaired t-test. In Panels A and B, the horizontal lines in the box plots represent the median, the boxes represent the interquartile range, and the whiskers represent the 2.5th and 97.5th percentiles. Panel C shows comparisons of relative levels of miR-26a-1 expression in paired tumor and nontumor samples from 224 patients, according to miR-26 status, with the use of paired t-tests. The data in Panels A through C are $\log_2$ relative expression levels, normalized to the values in disease-free samples from eight control subjects. Panel D shows survival according to the level of miR-26a-1 expression in tumor samples, as determined by microarray analysis, with the use of the log-rank test. The median expression level was used as the cutoff. Reduced miR-26 expression in 106 patients was classified as values below the 50th percentile (with a mean reduction in tumor tissue, as compared with nontumor tissue, by a factor of 2.69). High miR-26 expression in 111 patients was classified as values at or above the 50th percentile (with a mean reduction in tumor tissue, as compared with nontumor tissue, by a factor of 0.98).
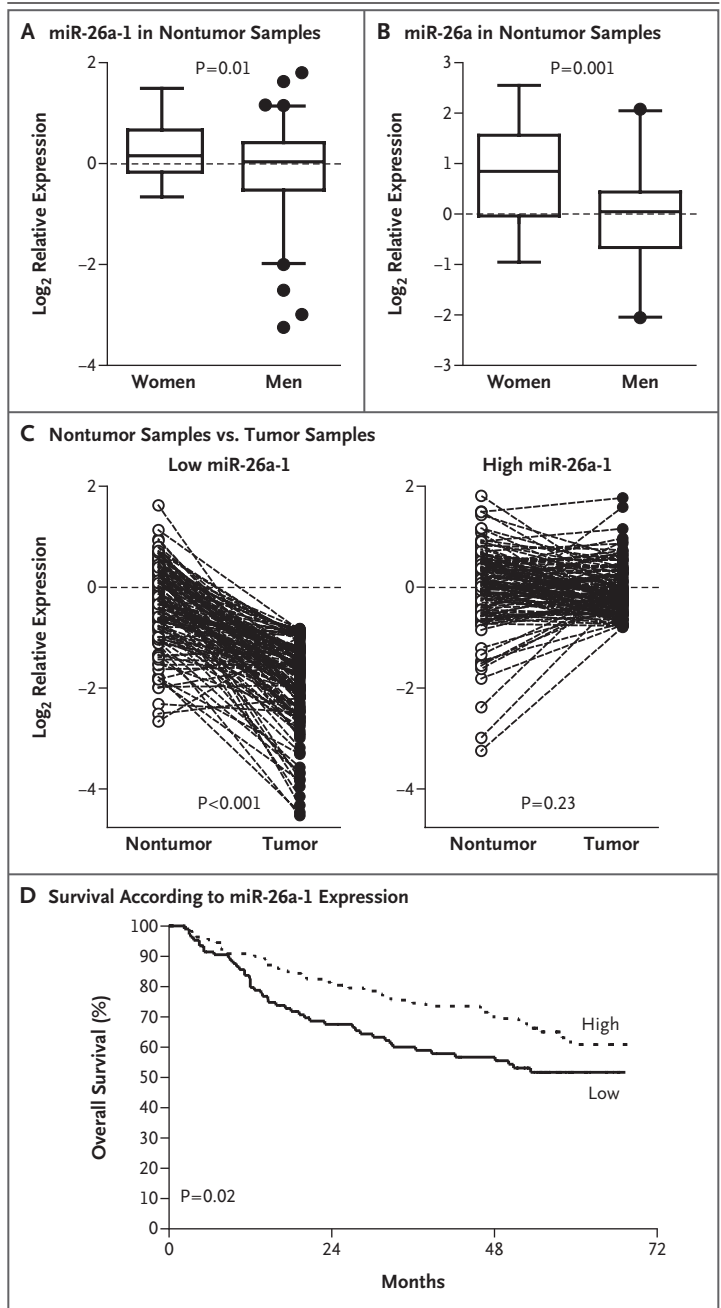
data were based on the expression of approximately 21,000 mRNA genes (GEO accession number, GSE5975).[23] Multidimensional scaling analysis on the basis of the first three principal components of all genes revealed that a majority of patients with low miR-26 expression clustered separately from those with high miR-26 expression (Fig. 2A), according to the dichotomized expression status of the three miR-26 genes (Fig. 2A in the Supplementary Appendix). Class-comparison analysis showed that the expression of a significant number of genes differed between tumors with low miR-26 expression and those with high miR-26 expression; the two groups of tumors had 915 significant genes in common (Fig. 2B in the Supplementary Appendix). *SLC2A6* and *S100P* were selected among the differentially expressed genes for validation by means of qRT-PCR (Fig. 3 in the Supplementary Appendix). Furthermore, multivariate analysis showed that mRNA signatures could significantly predict the stratification of patients according to miR-26 expression, with 80.3% overall accuracy. Thus, patients whose tumors had low miR-26 expression

had genetic-expression patterns that were distinct from those of tumors in patients with high miR-26 expression.

Among the 915 overlapping genes, 770 were overexpressed in patients with low miR-26 expression. Gene-network analyses with the use of these 770 genes revealed a series of putative tumorigenesis networks that had a high score (>10) (for details, see Table 4 in the Supplemen-
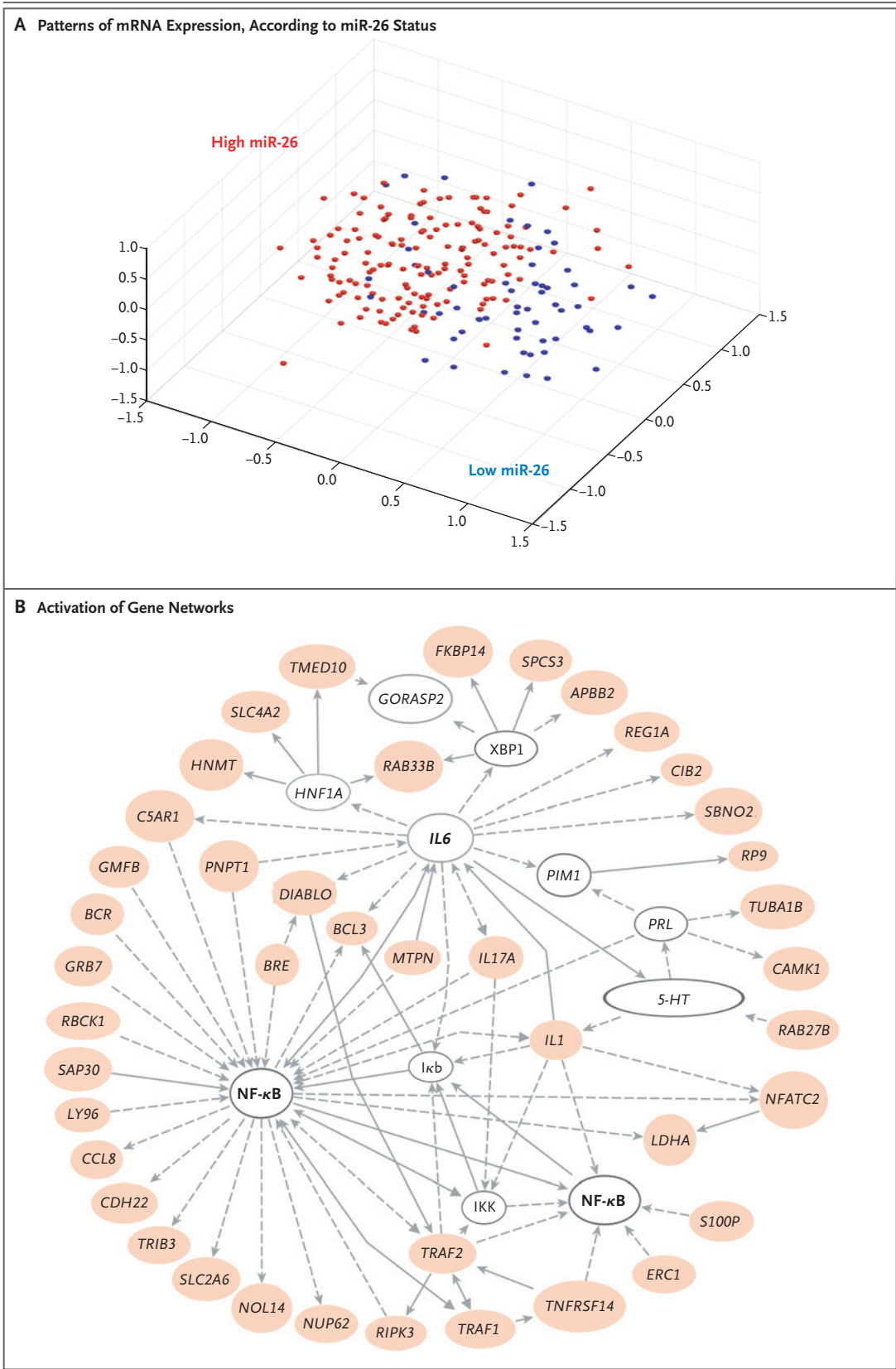
A Patterns of mRNA Expression, According to miR-26 Status

B Activation of Gene Networks

tary Appendix). Examination of the enriched genes in various categories revealed several important signaling networks, the most striking of which showed predominant activation of the signaling pathway between nuclear factor κB (NF-κB) and interleukin-6 in patients with low miR-26 expression (Fig. 2B). To validate which genes were associated with this signaling pathway, we measured the level of the NF-κB target gene, *IL6,* by qRT-PCR, since it was also related to hepatocellular carcinoma and the disparity in miR-26 expression levels between the sexes. Most of the patients with hepatocellular carcinoma who had reduced miR-26 expression had a concomitant elevation in interleukin-6 expression (Fig. 4 in the Supplementary Appendix). With these findings taken together, we concluded that tumors with low miR-26 expression had a distinct genetic profile.

VALIDATION WITH INDEPENDENT COHORTS

To validate the association between the sex-dependent expression of miR-26 and survival, we detected mature miR-26 expression by qRT-PCR in tumor and nontumor tissues obtained from patients in cohort 2. Since adjuvant therapy with interferon alfa altered the survival outcome, we analyzed the control group. Consistent with cohort 1, miR-26 expression was more abundant in nontumor tissues obtained from women, but a significant reduction was observed in tumors, regardless of sex (Fig. 5 in the Supplementary Appendix). Moreover, reduced miR-26 expression in tumors was significantly associated with shorter survival (Fig. 3A, and Fig. 6A in the Supplementary Appendix). The results were similar in another independent cohort (cohort 3) (Fig. 3B, and Fig. 6B in the Supplementary Appendix).

We used Cox proportional-hazards regression to further evaluate the association between miR-26 expression and prognosis in control subjects in cohort 2 (Table 5 in the Supplementary Appendix). In univariate analysis, the level of miR-26a expression in tumors and TNM stage were significantly associated with the prognosis. The final multivariate model revealed that reduced miR-26a expression in tumors was an independent predictor of shorter survival. A similar trend was found for miR-26b. Thus, the dichotomized values for miR-26 expression were independent predictors of prognosis.

EXPRESSION OF MiR-26 AND RESPONSE TO INTERFERON ALFA

In cohort 2, we analyzed associations between miR-26 expression and the response to interferon alfa. Patients with reduced miR-26a expression in tumors had a significant improvement in overall survival after receiving adjuvant therapy with interferon alfa, as compared with those in the control group (P=0.003) (Fig. 3C). This finding was validated in cohort 3 (Fig. 3D). In contrast, patients with high miR-26a expression in both cohorts did not have a response to interferon alfa (Fig. 3E and 3F). Similar results were obtained with respect to miR-26b expression (Fig. 6C through 6F in the Supplementary Appendix).

We used Cox proportional-hazards regression to evaluate the effect of treatment on survival in patients in cohort 2 who had low miR-26 expression (Table 2). In both univariate and multivariate analyses, interferon alfa was associated with significant improvement in survival in patients with reduced miR-26 expression. A significant interaction was observed between miR-26 expression and interferon alfa therapy with respect to the effect on survival (miR-26a, P=0.004 for interaction; miR-26b, P=0.02 for interaction). Thus, miR-26 expression emerged as an independent predictor of the response to interferon alfa.
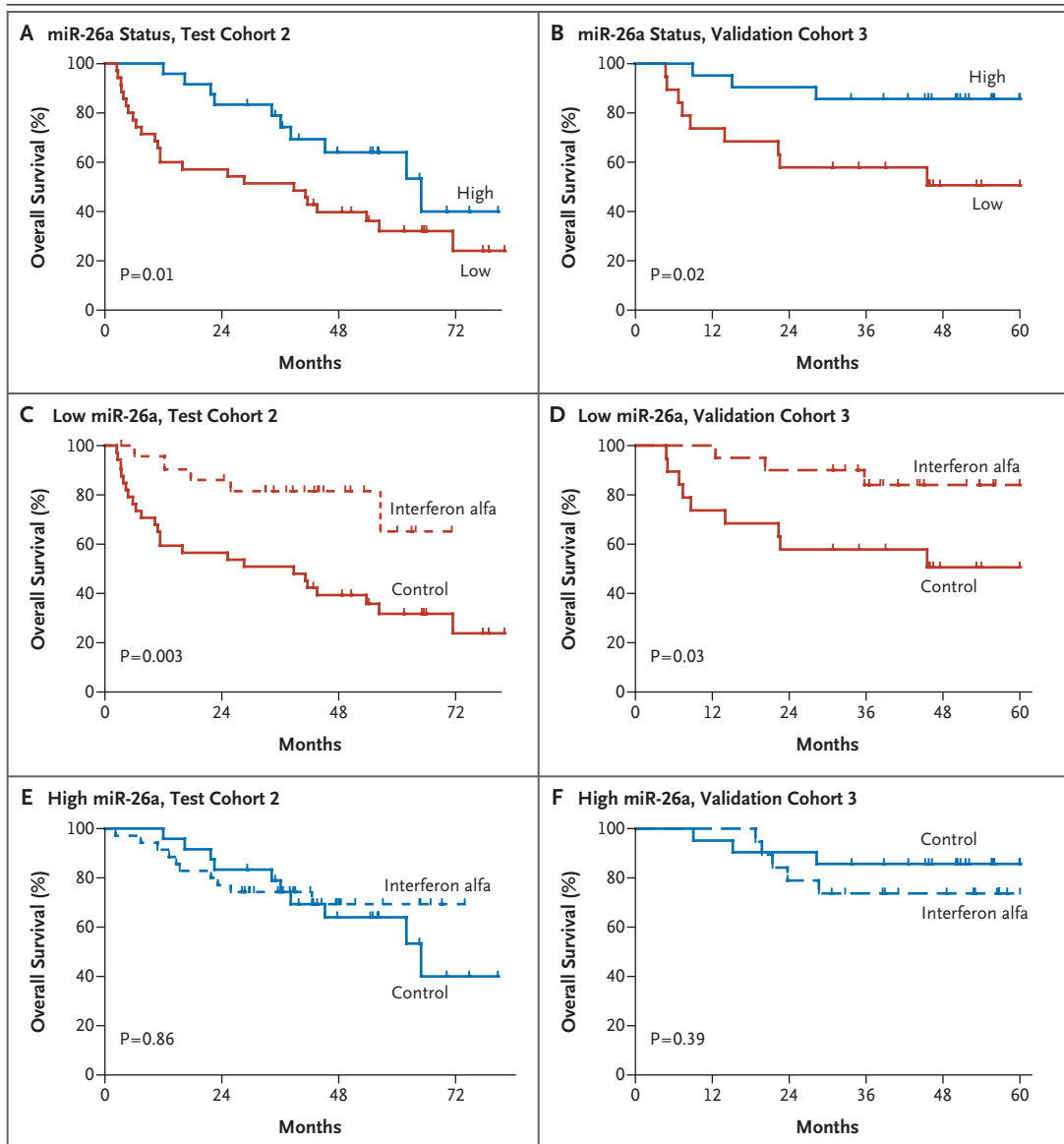
Figure 3. Association between miR-26a Expression in Tumors and Overall Survival in Two Trials of Interferon Alfa.

Panels A and B show the association between miR-26a expression and overall survival in patients with hepatocellular carcinoma from two control groups (cohort 2 in Panel A and cohort 3 in Panel B). In cohort 2, 24 patients had high miR-26a expression and 35 patients had low miR-26a expression. In cohort 3, 21 patients had high miR-26a expression and 19 patients had low miR-26a expression. Panels C and D show the association between adjuvant therapy with interferon alfa and overall survival in patients with tumors with low miR-26a expression. In cohort 2, there were 24 patients in the group that received interferon alfa and 35 patients in the control group. In cohort 3, there were 20 patients in the group that received interferon alfa and 19 patients in the control group. Panels E and F show the association between adjuvant therapy with interferon alfa and overall survival in patients with tumors that had high miR-26a expression. In cohort 2, there were 35 patients in the group that received interferon alfa and 24 patients in the control group. In cohort 3, there were 19 patients in the group that received interferon alfa and 21 patients in the control group.

Table 2. Hazard Ratios for Death among Patients with Low Expression of miR-26a and Patients with Low Expression of miR-26b, According to Univariate and Multivariate Analyses.*

Variable	Reduced miR-26a Expression (N=59)		Reduced miR-26b Expression (N=58)	
	Hazard Ratio (95% CI)	P Value†	Hazard Ratio (95% CI)	P Value†
Univariate analysis				
Interferon alfa vs. control	0.2 (0.1–0.6)	0.003	0.4 (0.2–0.9)	0.04
Age (≤50 yr vs. >50 yr)	1.1 (0.6–2.2)	0.77	0.9 (0.5–1.8)	0.81
Sex (male vs. female)	1.3 (0.4–3.7)	0.65	1.4 (0.4–4.7)	0.57
Alpha-fetoprotein (>20 ng/ml vs. ≤20ng/ml)	1.8 (0.8–4.1)	0.15	1.9 (0.9–4.0)	0.10
Cirrhosis (yes vs. no)	0.7 (0.3–2.1)	0.57	0.7 (0.3–1.8)	0.43
Alanine aminotransferase (>50 U/liter vs. ≤50 U/liter)	1.1 (0.4–3.0)	0.79	1.9 (0.8–4.6)	0.17
Tumor size (>3 cm vs. ≤3 cm)	1.7 (0.8–3.6)	0.15	2.0 (1.0–4.3)	0.06
Tumor encapsulation (no vs. yes)	1.8 (0.9–3.6)	0.12	1.6 (0.8–3.1)	0.20
Multinodular tumor (yes vs. no)	0.9 (0.3–2.3)	0.82	1.1 (0.4–2.6)	0.87
TNM staging (II or III vs. I)	2.7 (1.3–5.5)	0.005	2.7 (1.4–5.3)	0.004
Multivariate analysis				
Interferon alfa vs. control	0.3 (0.1–0.7)	0.005	0.4 (0.2–0.9)	0.04
TNM staging (II or III vs. I)	2.4 (1.2–4.9)	0.02	2.6 (1.3–5.1)	0.007
Sex (male vs. female)	1.4 (0.5–4.0)	0.55	1.5 (0.5–5.1)	0.48

* The analyses were performed with the use of Cox proportional-hazards regression on data from patients with a low level of miR-26 expression in cohort 2.
† A P value of less than 0.05 was considered to indicate statistical significance.

DISCUSSION

We analyzed microRNA profiles in predominantly HBV-related hepatocellular carcinomas in men and women and the association of microRNA expression levels with survival and response to therapy with interferon alfa in three independent cohorts. We found that the expression of miR-26a and miR-26b in nontumor hepatic tissues was higher in women than in men, but the expression was significantly down-regulated in tumor samples, as compared with paired samples of noncancerous tissues, regardless of sex. Tumors with reduced miR-26 expression had a distinct gene-expression profile, and patients whose tumors had low miR-26 expression had shorter survival but were more likely to have a response to interferon alfa, as compared with patients whose tumors had high miR-26 expression.

Our results suggest that miR-26 may be a tumor suppressor and that miR-26 silencing in hepatocytes may contribute to the development of a more aggressive form of hepatocellular carcinoma in men. These hypotheses are supported by the following findings: miR-26 was expressed at higher levels in women than in men in the liver, where presumably more anticarcinogenic activities exist in women; miR-26 expression is silenced in a subgroup of patients who have reduced survival; and genes that are activated in tumors with reduced miR-26 expression are selectively enriched in signaling pathways between NF-κB and interleukin-6. It was recently reported that MyD8-dependent interleukin-6 induction by NF-κB differed between male and female mice.[11] Intriguingly, estrogens inhibit interleukin-6 promoter activity, which may contribute to a decreased susceptibility to hepatocellular carcinoma

in women. Whether such a link is functionally relevant to liver cancer in humans remains to be determined.[24] Our results are consistent with these findings, since interleukin-6 expression was inversely correlated with miR-26 expression.

Our analyses revealed that miR-26 expression was an independent predictor of survival. However, when outcomes of therapy with interferon alfa were stratified, only patients whose tumors had reduced miR-26 expression had a favorable response to interferon alfa in two independent, prospective, randomized, controlled trials. These results indicate that miR-26 status in tumors may be a useful tool in estimating prognosis in patients with hepatocellular carcinoma and in assisting in the selection of patients who are likely to benefit from adjuvant therapy with interferon alfa to prevent relapse. In an evaluation of the effects of adjuvant interferon alfa therapy after liver resection or tumor ablation in seven randomized, controlled trials, Clavien[5] concluded that all the trials showed modest beneficial effects of the drug. In addition, among multiple experimental agents, only a modest survival benefit has been observed with sorafenib.[6] The poor efficacy of current systemic agents may stem from an inability to identify a subpopulation of patients who may have a favorable response to a particular therapy. Prospective studies will be necessary to determine whether interferon alfa might be used as a first-line therapy for patients with hepatocellular carcinoma who have undergone resection and who have tumors with reduced miR-26 expression.

It should be noted that our results involved Chinese patients with a high rate of HBV positivity (90.5%). Our findings therefore need to be confirmed by studies of tumors from non-Asian patients and tumors arising from other underlying liver diseases, such as hepatitis C, or associated with alcohol abuse.

The mechanisms behind the sensitivity of tumors with reduced miR-26 expression to therapy with interferon alfa are unclear. One plausible model is that such tumors have specific activation of the signaling pathway that is responsive to interferon alfa. Consistently, tumors with low miR-26 expression were distinct from those with high miR-26 expression in transcriptomic activities and were associated with poor survival. Many of the genes that are overexpressed in tumors with reduced miR-26 expression are related to cell immunity, such as those encoding proinflammatory and antiinflammatory cytokines (i.e., interleukin-1, interleukin-2, interleukin-10, and interleukin-17). Moreover, many signaling networks that are activated in tumors with low miR-26 expression are immune-associated, such as NF-κB–interleukin-6, interleukin-10, STAT3, and interferon alfa inducible factor signaling networks (Table 4 in the Supplementary Appendix). Tumors with low miR-26 expression may be characterized by a unique activation of interferon alfa signaling, possibly through the NF-κB–interleukin-6 signaling pathway, and thus may be sensitive to growth inhibition mediated by interferon alfa through interleukin-6–STAT3 signaling.[25] These hypotheses require evaluation.

In conclusion, we identified systematic differences in patterns of microRNA expression between liver tissues obtained from men and women with hepatocellular carcinoma. Tumors with low miR-26 expression were biologically distinct from those with high expression and were associated with reduced survival but had a favorable response to adjuvant therapy with interferon alfa.

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