Characterization of MicroRNA Expression Levels and Their Biological Correlates in Human Cancer Cell Lines

Arti Gaur,[1] David A. Jewell,[2] Yu Liang,[3] Dana Ridzon,[3] Jason H. Moore,[1,2] Caifu Chen,[3] Victor R. Ambros,[2] and Mark A. Israel[1,2]

[1]Norris Cotton Cancer Center and [2]Department of Genetics, Dartmouth Medical School, Lebanon, New Hampshire; and [3]Molecular Cell Biology-R&D, Applied Biosystems, Foster City, California

Abstract

MicroRNAs are small noncoding RNAs that function by regulating target gene expression posttranscriptionally. They play a critical role in developmental and physiologic processes and are implicated in the pathogenesis of several human diseases including cancer. We examined the expression profiles of 241 human microRNAs in normal tissues and the NCI-60 panel of human tumor-derived cell lines. To quantify microRNA expression, we employed a highly sensitive technique that uses stem-loop primers for reverse transcription followed by real-time PCR. Most microRNAs were expressed at lower levels in tumor-derived cell lines compared with the corresponding normal tissue. Agglomerative hierarchical clustering analysis of microRNA expression revealed four groups among the NCI-60 cell lines consisting of hematologic, colon, central nervous system, and melanoma tumor–derived cell lines clustered in a manner that reflected their tissue of origin. We identified specific subsets of microRNAs that provide candidate molecular signatures characteristic of the tumor-derived cell lines belonging to these four clusters. We also identified specific microRNA expression patterns that correlated with the proliferation indices of the NCI-60 cell lines, and we developed evidence for the identification of specific microRNAs as candidate oncogenes and tumor suppressor genes in different tumor types. Our results provide evidence that microRNA expression patterns may mark specific biological characteristics of tumors and/or mediate biological activities important for the pathobiology of malignant tumors. These findings call attention to the potential of microRNAs to provide etiologic insights as well as to serve as both diagnostic markers and therapeutic targets for many different tumor types. [Cancer Res 2007;67(6):2456–68]

Introduction

Molecular biomarkers that help classify the numerous types of human cancers and that correlate with specific biological activities of tumor cells are essential for elucidating the molecular basis of oncogenesis and for effectively treating cancer. More than 300 microRNA genes and an even greater number of predicted microRNA targets have been identified in the human genome. The breadth of genetic regulatory effects potentially mediated by microRNAs and their central role in diverse cellular and developmental processes (1–4) has led to the hypothesis that aberrant expression of microRNA genes could contribute to human disease, including cancer (5–8). A substantial number of microRNA genes are located in genomic regions that are frequently amplified, deleted, or rearranged in cancer, providing further evidence of a role for microRNAs in cancer pathogenesis (9, 10). Deregulated microRNA expression has been documented in diverse cancers including lymphoma (11–14), colorectal cancer (15), lung cancer (16), breast cancer (17), and glioblastoma (18, 19), and specific microRNAs have been shown to target genes which are critical regulators for the development of cancer such as E2F (20) and RAS (21).

The NCI-60 panel of human tumor cell lines provides unique opportunities for identifying the molecular and genetic underpinnings of neoplasia. This set of 59 tumor cell lines is derived from melanoma and from cancers of the gastrointestinal tract, kidney, ovary, breast, prostate, lung, and central nervous system (CNS) as well as from various leukemias. The NCI-60 cell lines have been extensively employed as experimental models of neoplastic disease and are annotated by multiple large-scale data sets, including results of pharmacologic studies that document the sensitivities of these cells to >100,000 different chemical compounds and chemotherapeutics (22–25). In addition, the National Cancer Institute's Developmental Therapeutics Program has assessed the molecular features of these cell lines related to cancer and chemotherapeutic sensitivity (24, 26, 27). To better understand how microRNAs might contribute to malignancy, we evaluated the expression patterns of 241 microRNAs in each of the 59 cell lines that comprise the NCI-60 panel and in a set of corresponding normal tissues. We found that the patterns of microRNA expression among these normal and neoplastic cells suggest the potential activities of specific microRNAs in contributing to the pathobiology of certain types of human tumors.

Materials and Methods

RNA purification from cell lines and normal tissue samples. Frozen pellets of the NCI-60 panel cell lines were received from Dr. Susan Holbeck at the National Cancer Institute Developmental Therapeutics Program. Total RNA was enriched by standard procedures using TRIzol. Human total RNA samples were purchased from Ambion (Austin, TX). RNA from human peripheral blood mononuclear cells was available in the laboratory.

Real-time quantification of 241 microRNAs using stem-loop real-time PCR. The expression profiles of 241 microRNAs were measured as described previously (28). This method uses stem-loop primers for reverse transcription followed by real-time PCR (TaqMan MicroRNA Assays; Applied Biosystems, Foster City, CA). RNA input was normalized using four endogenous controls: 18S rRNA, β2M, glyceraldehyde-3-phosphate dehydrogenase, and β-actin.

Data analyses. Statistical analyses including the leave-one-out sensitivity analyses, Student's t tests, and Spearman rank analysis were carried out using TM4MeV v4.0b software (Institute for Genomic Research, Rockville, MD),

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Mark A. Israel, Norris Cotton Cancer Center, Dartmouth Medical School, Lebanon, NH 03755. Phone: 603-653-3611; Fax: 603-653-9003; E-mail: Mark.A.Israel@dartmouth.edu.

©2007 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-06-2698

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

R statistical package[4] and Microsoft Excel. Experimentally normalized ΔCt values for the microRNA profiles were used to evaluate the NCI-60 cell lines by agglomerative hierarchical clustering using average linkage and correlation similarity and verified for significance by multiscale bootstrap resampling analyses (29).

Target prediction for microRNAs up-regulated in CNS tumor–derived cell lines. Potential targets for microRNAs were predicted using MiRanda associated with the Sanger MIRBASE. Gene expression data describing glioblastomas and normal brain tissue (30) and NCI-60 cell lines (22) were extracted from the Stanford Microarray Database.

Results

We employed a recently described (28), highly sensitive TaqMan MicroRNA assay for quantitative measurement of 241 mature human microRNAs in 13 normal tissues and in 59 cell lines of the NCI-60 panel of human tumor cell. This assay allows the rapid quantification of microRNA levels using as little as 25 pg of total RNA, and exhibits a dynamic range of seven orders of magnitude.

MicroRNA expression patterns characterize human cancer cell lines based on their tissue of origin. Experimentally normalized ΔCt values corresponding to the level of expression of 241 microRNAs were used to evaluate normal tissues and the NCI-60 cell lines by agglomerative hierarchical clustering. This analysis of patterns of microRNA expression revealed potential relationships among the normal tissues and the 59 cell lines. To assess the robustness of these relationships, we conducted a multiscale bootstrap resampling analysis of these same data. The resulting dendrogram describing these relationships had three main branches: one that held all the NCI-60 cell lines and two others which together held all the normal tissue samples (Fig. 1*A*). This bootstrap analysis identified six statistically significant clusters. Two of the six clusters consisted entirely of normal tissues. One of these consisted of normal brain tissue and peripheral blood mononuclear cells, whereas the other cluster included samples of the normal tissues from small intestine, thymus, breast, prostate, kidney, lung, pancreas, spleen, ovary, liver, lymph node, and normal colon (Fig. 1*A, black*). The other four significant clusters consisted entirely of NCI-60 cell lines derived from hematologic, colon, melanoma, or CNS tumors (Fig. 1*A*).

The most prominent feature of the clustered data was that although many microRNAs displayed similar patterns of expression among all samples, the expression levels of most microRNAs were significantly reduced in the cancer cell lines as compared with the normal tissue (Fig. 1*A*). Also, the clusters into which the cell lines segregated generally reflected the tissue of origin of the tumors from which they were derived (Fig. 1*A*). Cell lines derived from hematologic, melanoma, CNS, colon, and renal tumor tissues were clustered into independent terminal branches corresponding to the tissues from which these tumors arose. The exceptions to this trend towards clustering by tissue of origin included cell lines derived from epithelial tumors of lung, ovarian, prostate, and breast, and these distributed in multiple different terminal branches. Overall, these results suggest that the patterns of microRNA expression among the NCI-60 cell lines reflect, in part, the lineage-specific characteristics and the biological, including pathologic, characteristics of these cell lines.

The NCI-60 lines have also been characterized by others based on mRNA expression levels. As microRNAs can regulate mRNA levels, we explored whether the relationships between cell lines that we detected based on microRNA expression patterns (Supplementary Fig. S1) were similar to the relationships between these same cell lines based on mRNA expression patterns. We compared our hierarchical clustering of the NCI-60 microRNA profiles to the clustering analysis of mRNAs in these lines reported in the Stanford cDNA Microarray Database (Supplementary Fig. S1). A comparison of the dendrograms derived from these clustering analyses revealed similar patterns, in which cell lines segregated in a manner that reflected the tissue of origin of the tumors from which they were derived. Similar to the hierarchical clustering based on microRNA expression patterns (Fig. 1*A*), the clustering based on mRNA expression of cell lines derived from hematologic, melanoma, CNS, colon, and renal tumor tissues were found in independent terminal branches, and those derived from lung, ovarian, prostate, and breast tumors were distributed in multiple different terminal branches (Supplementary Fig. S1).

Expression of specific microRNAs is essential for the clustering of tumor cell lines based on their tissue of origin. The multiscale bootstrap analysis characterized the expression levels of 241 different microRNAs and identified four highly significant clusters of cell lines derived from hematologic, colon, melanoma, and CNS tumors, respectively (Fig. 1*A*). To identify those microRNAs in which the expression patterns most significantly distinguished these four cell line clusters, we did a comparative *t* test analysis. For each of the four clusters, the average Ct of each microRNA in the cell lines within the cluster was compared with the average Ct for that same microRNA in all of the NCI-60 cell lines not within that cluster. By this analysis, microRNAs were selected in which the average level differed significantly ($P \leq 0.01$) between a cluster and the rest of the NCI-60 cell lines (Supplementary Table S1). From this evaluation of all microRNAs in each of the four clusters, a total of 81 distinguishing microRNAs were identified for further analysis.

To determine if this set of 81 significant microRNAs was sufficient to define the four significant tumor cell clusters, we conducted a hierarchical cluster analysis of the NCI-60 cell lines using expression data from these 81 microRNAs only. The dendrogram describing the relationship of the cell lines in this analysis (Supplementary Fig. S1) contains distinct clusters of cell lines derived from hematologic, melanoma, CNS, colon, and renal tissues, whereas cell lines derived from lung, ovarian, prostate, and breast tumors were distributed in multiple different terminal branches. This pattern closely parallels the relationships we observed in our initial analysis of all 241 microRNAs (Fig. 1*A*). Moreover, multiscale bootstrap analysis of the dendrogram in Fig. 1*B* revealed the same four significant clusters of cell lines derived from hematologic, melanoma, CNS, and colon tumors as were detected in Fig. 1*A*. These findings confirm that this set of 81 microRNAs is sufficient to define the four clusters of tumor cell lines from hematologic, colon, melanoma, and CNS tissue.

To evaluate the relative contribution of each of the 81 microRNAs to the integrity of the tumor cell line clusters, we determined the effect of omitting each individual microRNA from the multiscale bootstrap analysis shown in Fig. 1*B*. For this evaluation, we first eliminated 39 microRNAs that displayed an expression pattern indistinguishable from the pattern of one of the other microRNAs, based on a Pearson coefficient of correlation of >0.8 at $P \leq 0.05$ (Table 1). The remaining 42 microRNAs (Table 1, *boldface*), each of which is expressed in a unique pattern, were evaluated by a leave-one-out sensitivity analysis. Of these 42 tested, two microRNAs

[4] http://www.r-project.org

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

(mir-375 and mir-211), upon being taken out of the analysis, had no effect; however, when each of the remaining 40 microRNAs were individually removed from the multiscale bootstrap analysis of all the NCI-60 cell lines, the confidence interval for one or more clusters dropped below 95% (corresponding to $P \leq 0.05$) or cluster membership changed by two or more cell lines (Table 1). Thus, among the core set of 81 distinguishing microRNAs that were identified based on t test analyses (Supplementary Table S1), the 40 microRNAs (and the candidates showing correlated expression patterns) play a significant role in maintaining one or more of the four significant NCI-60 cell line clusters in Fig. 1*A*.

Distinctive patterns of microRNA expression levels are associated with cell lines derived from different human tumors. The analysis described above shows that the expression levels of a distinct subset of microRNAs underlies the tissue-specific clustering of tumor cell lines. Suggesting that the expression patterns of specific microRNAs might provide characteristic molecular signatures that could be used to identify specific types of cancers. Candidates for such signature microRNAs would include subsets of the 81 microRNAs, the expression of which was significantly different by t test (Supplementary Table S1).

To assess the expression patterns of the microRNAs identified as being differentially expressed in a specific cell line cluster, we created heat maps in which the expressions of microRNA were found to be significantly different in one cell line cluster when compared with all other cell lines (Fig. 2*A–D*). In these heat maps, the cell lines of the NCI-60 panel were grouped by tissue of origin and aligned across the top of Fig. 2, and microRNAs identified by t test analysis as being differential expressed were displayed on the vertical axis. Indeed, for each of the cell line clusters derived from hematologic, CNS, colon, and melanoma tumors (Fig. 1*A*), the subset of microRNAs key for the establishment of each cluster (Table 1) displays a pattern of expression apparently unique to that cluster (Fig. 2).

In the six hematologic cell lines, 24 microRNAs were expressed at significantly different levels compared with the other NCI-60 cell lines; 8 microRNAs were down-regulated, whereas 16 were up-regulated (Fig. 2*A*). The hematopoietic tumor cell lines examined here were derived from several different tumor types, but were all non–solid tumors, whereas all the other NCI-60 lines were derived from solid tumors. Thus, the pattern of microRNA expression associated with hematologic tumors (Fig. 2*A*) suggests a signature that could differentiate non–solid from solid tumors.

Other microRNAs provide potential signature patterns of expression in each of the cell line clusters examined. In the six CNS tumor–derived lines, a total of 52 microRNAs were expressed at significantly different levels when compared with their level of expression in the other NCI-60 lines. Of these 52 microRNAs, only 6 were expressed at higher levels, whereas 46 microRNAs were down-regulated (Fig. 2*B*). Among the seven colon tumor-derived lines, 30 microRNAs were expressed at significantly different levels. Of these, 5 were down-regulated, whereas the remaining 25 were up-regulated when their expression levels were compared with the other NCI-60 lines (Fig. 2*C*). In the eight melanoma lines, only 15 microRNAs were expressed significantly differently; 4 were up-regulated and 11 down-regulated when compared with levels in the other NCI-60 lines (Fig. 2*D*). Therefore, the down-regulation of specific microRNAs together with the up-regulation of other microRNAs formed a distinctive signature for each of the four cell line clusters examined (Fig. 2*A–D*).

MicroRNAs as candidate tumor suppressors or oncogenes. Cancer is a collection of heterogeneous genetic diseases that arise in association with the accumulation of mutations that activate proto-oncogenes and inactivate tumor suppressor genes. There is considerable literature relevant to the emerging role of microRNAs in tumor development, and others have identified a number of microRNAs as candidate oncogenes and tumor suppressor genes (31–34). We sought to identify microRNAs in which the expression level in specific tumor cell lines was either significantly increased or decreased from that observed in a corresponding normal tissue and therefore suggested that their function was either enhanced or diminished in association with tumorigenesis. We examined the microRNAs expressed in three clusters of cell lines (hematologic, CNS, and colon) as each of these clusters expressed a significantly distinct pattern of microRNAs (Fig. 2), and in each case, there was a sufficient number of normal tissue controls for comparison in a t test analysis.

One hundred and forty-five microRNAs were significantly increased or decreased at least 2-fold in one or more cell line clusters compared with their corresponding normal tissues (Supplementary Table S2). Seventy microRNAs were decreased in all three clusters or in two of the three clusters, compared with their respective normal tissues, and could act as tumor suppressors in more than one type of tumor-derived cell line (Supplementary Table S2). Alternatively, reduction of microRNAs in multiple cell line clusters could reflect other differences between a cultured tumor cell line and a normal tissue specimen, such as changes resulting from the *in vitro* culture of tumor cells. However, if a microRNA is down-regulated in cell lines derived from just a single tumor type, it is less likely that this reduction would result from nonspecific changes associated with *in vitro* growth conditions. Accordingly, in Table 2A and B, we present only those microRNAs that were expressed at a significantly different level in only one of the three clusters we examined. In the hematologic, colon, and CNS tumor–derived cell lines, 4, 7, and 59 potential tumor suppressors, respectively, were identified that satisfied this criterion of tumor cell type specificity (Table 2A). Of these potential tumor suppressor microRNAs, 15 microRNAs (1 in the hematologic, 1 in the colon, and 13 in the CNS tumor–derived cell lines) are of particular interest as they did not display cell type–specific expression among the normal hematologic, colon, and CNS tissue samples (Table 2A). This finding suggests that these microRNAs do not seem to be simply markers of

Figure 1. *A,* hierarchical clustering with bootstrap analysis of 241 microRNA expression profiles in 59 tumor-derived cell lines. Expression profiles (ΔCt values) of 241 microRNAs measured in total RNA from normal tissues and the NCI-60 cell lines were clustered and verified for significance by multiscale bootstrap resampling analyses (1,000 iterations, sampling with replacement). Clusters were scored as statistically significant in cases in which three or more cell lines or normal tissues clustered with a 95% or better confidence interval (corresponding to $P \leq 0.05$), as determined by the bootstrap analysis. *B,* hierarchical clustering of the NCI-60 panel with bootstrap analysis of 81 selected microRNAs. Eighty-one microRNAs which were expressed within one of the four cell line clusters identified in (*A*) at levels significantly different ($P \leq 0.01$) from the expression level in all the other cell lines in a comparative t test analysis (see Materials and Methods and Table 1) were evaluated. The expression of these 81 microRNAs in the NCI-60 cell lines was analyzed by agglomerative hierarchical clustering and verified for significance by multiscale bootstrap resampling analyses. *Columns,* NCI-60 tumor cell lines; *rows,* microRNA expression profiles (*A* and *B*). *Star,* clusters of cell lines having a 95% confidence interval or higher and containing three or more samples as determined by multiscale bootstrap analysis. The cell lines are color-coded based on their tissue of origin.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

hematologic, colonic, or CNS differentiation, providing additional evidence of their potential role in oncogenesis.

We did not identify any microRNAs that were up-regulated in either colon or hematologic lines compared with the corresponding normal tissues. Five microRNAs were expressed at higher levels in the CNS tumor–derived cell lines compared with normal brain tissue, and hence, were candidate oncogenes for CNS cells (Table 2B). Two of these five candidate CNS oncogenes, mir-196a and mir-10b, did not display brain-specific expression in normal tissues (Table 2B). Interestingly, two other microRNAs in which the level of expression was increased in CNS tumor–derived cell lines, mir-10a and mir-196b, behaved like tumor suppressors in hematologic cell lines.

We sought to ascertain whether an association between microRNA expression and copy number changes in the DNA encoding the set of 81 microRNAs that define the four highly significant clusters of tumor cell lines derived from hematologic, colon, melanoma, and CNS tissues (Fig. 1*B*) could be identified. We used spectral karyotyping data[5] combined with single nucleotide polymorphism (SNP) data[6] to calculate the SNP copy number in the specific regions that carry the 81 microRNAs. If a microRNA fell within the 50 kb range of a known SNP copy number (calculations described in Supplementary Table S4A), it was included in our analysis. Based on this criteria, we identified 59 microRNAs in which the SNP copy number could be evaluated and compared that to the level of their corresponding RNA copy number in the 27 cell lines that make up the four distinct clusters. Of these 59, we found 3 microRNAs in which the expression levels were moderately correlated ($P \leq 0.05$) with the DNA copy number of the region in which they are located (Supplementary Table S4A). Mir-182, mir-192, and mir-31 had a Spearman r value of 0.482295482, 0.425824176, and 0.402625153, respectively (Supplementary Table S4B). All three of these microRNAs were highly expressed in cell lines that had excess copies of the region of the genome in which they were located, suggesting that gene amplification might contribute to the high levels of expression we observed.

Potential targets of microRNAs that are up-regulated in CNS tumor–derived cell lines. Although determining the precise contribution of each microRNA in which the level of expression might be altered in a particular tumor type is beyond the scope of this investigation, we sought additional evidence supporting the likelihood that microRNAs expressed at aberrant levels were contributing to the malignant characteristics of the tumors from which the cell lines we examined were derived. We identified putative target genes that might be regulated by microRNAs highly expressed in CNS tumor–derived cell lines. We used the MiRanda target prediction algorithm associated with the Sanger MIRBASE to examine mirs-10a/b, mirs-196a/b, and mir-21 that are overexpressed in CNS tumor–derived cell lines. Six hundred and eighty-nine unique predicted target transcripts for these microRNAs were identified. Four hundred and seventy-four of these have UniGene IDs, and 388 have analyzable mRNA expression profiles in CNS tumors reported in a published microarray data set examining 3 normal brain and 29 glioblastoma specimens (30). We hypothesized that some of the transcripts might encode proteins involved in tumor-suppressive activities in glioblastomas, and hence, should have decreased expression in glioblastomas compared with normal brain. To determine whether the expression of these genes was significantly decreased in glioblastomas, a Student's t test was done on 252 genes for which there were data for at least two specimens of both glioblastoma tissue and normal brain. Among these, we found 23 genes with significantly decreased expressions in glioblastomas compared with normal brain ($P \leq 0.05$; Table 3; Supplementary Table S3). Many genes among these have no currently recognizable relationship to tumorigenesis, however, one extracellular matrix protein, SPOCK1 (35), and two transcription regulators, ZMYND11 (36) and RB1CC1 (ref. 37; Table 3), have known functions that could contribute to tumorigenesis.

MicroRNA expression levels correlate with cell proliferation indices. The work of others, and our findings, suggest that patterns of microRNA expression might be used to classify diverse types of cancers and seems likely to be associated with important biological activities that contribute to tumorigenesis. Cell cycle deregulation leading to uncontrolled cellular proliferation is a key aspect of tumorigenesis. Measurement of S phase fraction and indices of proliferation such as the tumor cell doubling time could be associated with both pathologic and clinical tumor characteristics and thereby serve as a biomarker for disease classification and identification. Therefore, among the 241 microRNAs, we identified microRNAs whose expression patterns correlated with the doubling time of cell lines in the NCI-60 panel (using Spearman rank analysis; $P \leq 0.01$). The heat maps shown in Fig. 3 describe the expression levels of microRNAs that had a significant correlation with doubling time. Two distinct subgroups emerged consisting of five microRNAs in which the expression levels tended to increase with increasing doubling time (Fig. 3*A*) and 16 microRNAs in which the expression levels tended to decrease with increasing doubling time (Fig. 3*B*). The microRNAs in which elevated expression correlates with longer doubling times could be interpreted as candidate antiproliferative microRNAs that act across a broad range of tissue types. Similarly, the microRNAs in which expression correlates with rapid cell cycling represent a set of potentially broadly acting enhancers of proliferation.

Discussion

Knowledge of genetic regulatory mechanisms initiating and maintaining malignancy are essential for understanding malignant cellular transformation, pathologic attributes of cancer, and ultimately, for designing effective strategies for cancer prevention and treatment. Genes encoding microRNAs are numerous and each microRNA potentially regulates a large number of targets (38–40). Understanding the function of microRNAs in tumorigenesis may provide insight into a key conundrum that pervades all cancer research; i.e., how can the limited number of genetic alterations recognized in tumor cells result in the profound physiologic changes that characterize all malignant tissues. Our precise quantification of the expression levels of 241 microRNAs in normal tissues and diverse cancer cell lines provides a dimension of the molecular phenotype of malignancy beyond that provided by conventional mRNA profiles and a powerful data set for genomic analysis of tumorigenesis.

Agglomerative hierarchical clustering followed by multiscale bootstrap resampling analyses identified major features of the microRNA expression patterns in these cells. First, we observed that these patterns were distinctly different in normal and malignant tissues. Normal tissues clustered separately from the NCI-60 cell lines, and the cell lines segregated into major subclusters (Fig. 1*A*). Second, major subclusters reflected their tissue of origin, suggesting

[5] http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi?formtype=submitters
[6] http://dtp.nci.nih.gov/mtargets/download.html

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

Table 1. MicroRNAs required for clustering of tumor cell lines based on tissues of origin

microRNA*	Confidence interval for tumor cell line clusters [†]			
	Hematologic (95) [‡]	Colon (95) [‡]	Melanoma (96) [‡]	CNS (97) [‡]
miR-127, miR-382	95	95	96	0,X
miR-130aN	70	70	81	94
miR-200a, miR-141, miR-141N, miR-200c, miR-200cN, miR-200b, miR-200bN	96	96	92	73,X
miR-100, miR-125b, miR-99a	89	89	94	90
miR-10b	96	96	96	81
miR-106a, miR-17-3p, miR-17-5p, miR-18, miR-19a, miR-19b, miR-20, miR-20N, miR-92N	95	95	95	85
miR-92	94	94	96	95
miR-10a	96	96	96	86
miR-146	94	94	94	87,X
miR-203	95	95	95	88
miR-335	100	88,X	92	96,X
miR-30a-3p	100	94,X	91	91,X
miR-342	100	95,X	93	95
miR-108, miR-185	96	96	95	95
miR-142-3p, miR-142-5p	96	97,X	95	96,X
miR-135b	96	96	95	98
miR-148a	93	93	97	98
miR-204	94	94	98	94
miR-31	95	95,X	97	96
miR-95	94	94	94	92
miR-151	95	95	95	94
miR-153	95	95	95	94
miR-184	94	94	93	95
miR-148b	94	94	95	97
miR-218	94	94	95	96
miR-128a, miR-129, miR-133a, miR-302a, miR-302b, miR-302c, miR-302d, miR-367, miR-326	94	94	94	96
miR-182, miR-183, miR-96	94	94	94	96
miR-378, miR-422a, miR-422b	95	95	94	97
miR-137	95	95	95	96
miR-25, miR-23bN, miR-27b, miR-93, miR-32	95	95	95	96
miR-194, miR-215	92	92	93	97
miR-7, miR-7N	92	92	92	97
miR-224	93	93	95	97
miR-330	93	93	94	96
miR-34b	94	94	93	98
miR-372, miR-373	93	93	93	98
miR-192	90	90	95	99
miR-196b	90	90	95	99
miR-149	91	91	96	96
miR-34a, miR-34aN	91	91	95	96

NOTE: Among the 81 microRNAs that were selected as being sufficient to define the four statistically significant clusters (Fig. 3), 38 microRNAs were determined to be expressed in a pattern similar to that of at least one other microRNA based on a Pearson coefficient of correlation >0.8 at a $P \leq 0.05$, and hence, only one representative of each such group was taken for further analysis. We evaluated the remaining 43 microRNAs (shown in boldface), which were expressed in unique patterns, in a leave-one-out sensitivity analysis. Of the 43 tested, 36 exhibited a critical contribution to the clustering: when any one of these 36 and their correlated microRNAs listed in the table were removed from a multiscale bootstrap/hierarchical cluster analysis of the NCI-60 cell lines, the confidence interval either dropped to <95% or changed the cluster memberships by two or more cell lines. The effect on all four of the clusters in the absence of any one of the 31 microRNAs is shown by a change in the confidence interval or a change in the cell lines making up the clusters. The microRNAs in each of the correlated expression groups are identified and listed together with the microRNAs that were tested in the analysis. Seven microRNAs (highlighted in gray) were those that upon being left out of the analysis, did not cause a decrease or an increase in the confidence interval or change the dissimilarity distance between cell lines.

*MicroRNAs in boldface were tested in the leave-one-out analysis. MicroRNAs with correlated expression patterns are listed adjacent to the ones that were tested.

† MicroRNAs listed here exhibited the following behavior in a leave-one-out multiscale bootstrap resampling analysis (see Materials and Methods): Leaving out the indicated microRNA caused a decrease in the confidence interval assignment, and/or changed the dissimilarity distance between cell lines (indicated by an X) leading to gain or loss of cell line members in a cluster.

‡ Starting confidence interval for the individual clusters as calculated by multiscale bootstrap resampling analyses.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

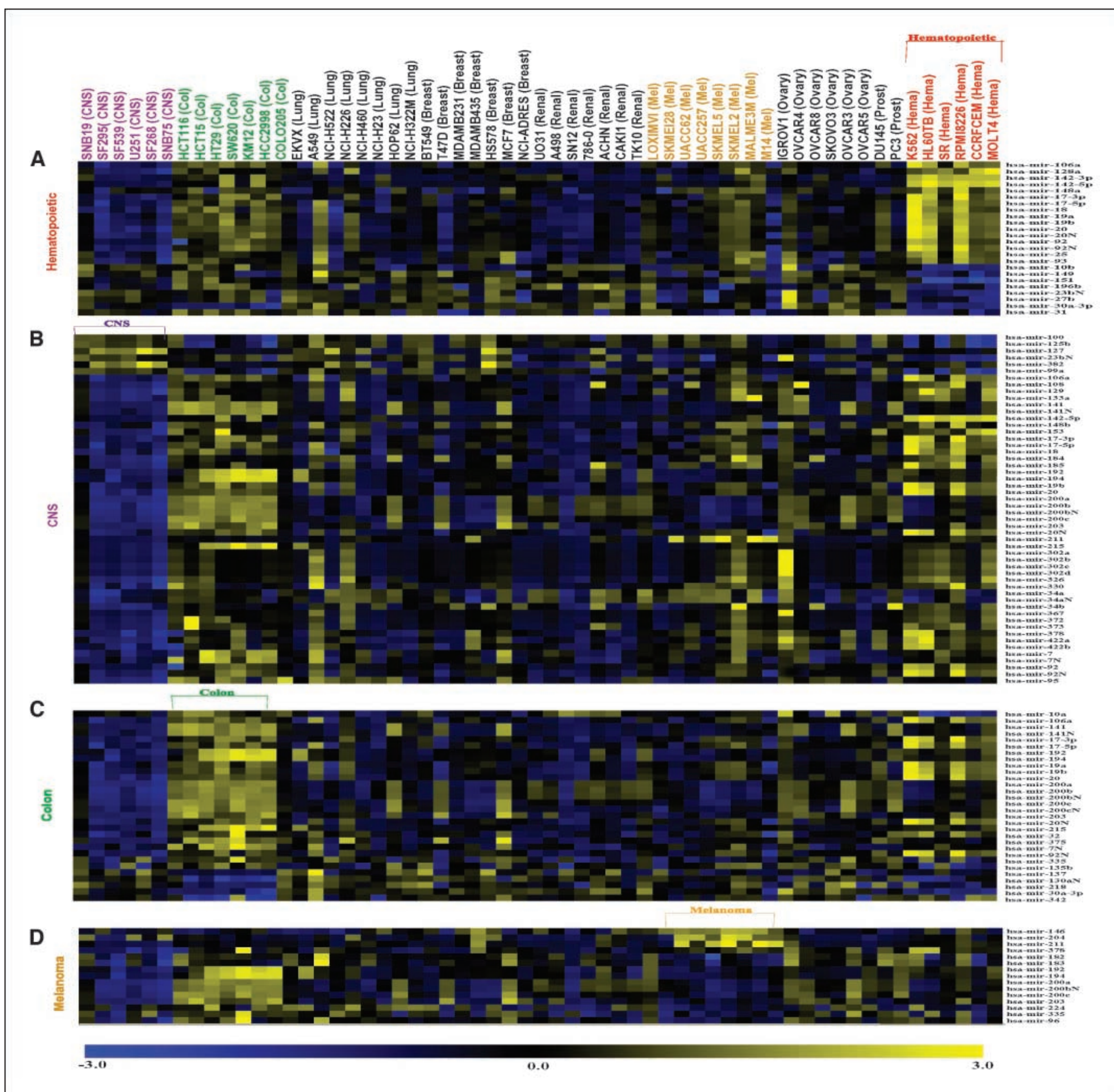

Figure 2. MicroRNA expression signatures of cell lines derived from specific types of human tumors. MicroRNAs that were expressed at significantly different levels in any one of the cell line clusters in Fig. 1*A* and *B* compared with the rest of the NCI-60 lines (see Table 1) are grouped by the cell line cluster that they distinguish. *Rows,* microRNA; columns, NCI-60 cell lines. *A* to *D,* significant microRNAs for the hematologic (*A*), CNS (*B*), colon (*C*), and melanoma lines (*D*). A blue-yellow color scale (−3 to +3) depicts normalized microRNA expression level (in which 3 on the scale corresponds to a Ct value of 21 and −3 to a Ct value of 35).

that the microRNA expression may signify the fundamental properties of these tumor lines. *In vitro* growth conditions including the inclusion of synthetic medium and fetal bovine serum may have an effect on microRNA expression patterns in these human tumor cell lines. However, it seems unlikely that the microRNA expression patterns of the NCI-60 cell lines are merely artifacts of *in vitro* culture conditions. If that were the case, similar changes in microRNA expression throughout the NCI-60 cell lines irrespective of their distinctive biological characteristics would have been observed. Finally, the expression patterns of a limited number of specific microRNAs (Table 1) were found to underlie the tissue-based clustering of some tumor cell lines. Leave-one-out sensitivity analysis showed that the expression of 81 specific microRNAs was sufficient for the tissue-specific clustering of cell lines derived from melanoma and tumors of hematologic, colonic, and CNS origin (Table 1; Fig. 1*B*). The molecular signatures encompassed by this relatively small number of microRNAs (Fig. 1*B*) may reflect the role of these microRNAs in mediating tissue-specific differentiation and/or tumorigenesis, and furthermore, suggest a molecular basis for the development of disease.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

Table 2. MicroRNAs as candidate tumor suppressors or oncogenes

MicroRNAs	Colon			Hematologic			CNS			Studies establishing function in primary tumors
	Copies/cell		Fold decrease	Copies/cell		Fold decrease	Copies/cell		Fold decrease	
	Normal tissue	Tumor lines		Normal tissue	Tumor lines		Normal tissue	Tumor lines		
(A) Candidate tumor suppressor microRNAs										
hsa-miR-214	**1,838***	**0**	**1,838**	—	—	—	—	—	—	
hsa-miR-424	**83**	**5**	**16.6**	—	—	—	—	—	—	
hsa-miR-130a	*501* †	*35*	*14.3*	—	—	—	—	—	—	Down-regulated in colon cancer; Lu et al. (7)
hsa-miR-378	**279**	**21**	**13.3**	—	—	—	—	—	—	
hsa-miR-148a	**1,392**	**206**	**6.75**		—	—	—	—	—	
hsa-miR-15a	**2,652**	**552**	**4.8**	—	—	—	—	—	—	Antiapoptotic, targets BCL2, down-regulated in chronic lymphocyte leukemia; Calin et al. (9)
hsa-miR-422a	**57**	**6**	**9.5**	—	—	—	—	—	—	
hsa-miR-28	—	—	—	**380**	**30**	**12.6**	—	—	—	
hsa-miR-10a	—	—	—	**511**	**40**	**12.8**	‡	‡	‡	
hsa-miR-196b	—	—	—	**147**	15	**9.8**	‡	‡	‡	
hsa-miR-27b	—	—	—	*1,022*	*117*	*8.7*	—	—	—	
hsa-miR-124b	—	—	—	—	—	—	**5,233**	**1**	**5,233**	
hsa-miR-124a	—	—	—	—	—	—	**2,779**	**0**	**2,779**	
hsa-miR-7N	—	—	—	—	—	—	*298*	*1*	*251.3*	
hsa-miR-153	—	—	—	—	—	—	*188*	*1*	*188*	
hsa-miR-7	—	—	—	—	—	—	*797*	*5.1*	*155.4*	
hsa-miR-219	—	—	—	—	—	—	**133**	**0**	**133**	
hsa-miR-383	—	—	—	—	—	—	*83.6*	*1*	*83.6*	
hsa-miR-129	—	—	—	—	—	—	*82*	*1*	*82*	
hsa-miR-128b	—	—	—	—	—	—	**73**	**0**	**73**	Up-regulated in colon, lung and pancreatic cancers; Volinia et al. (41)
hsa-miR-323	—	—	—	—	—	—	**358**	**6**	**59.6**	
hsa-miR-128a	—	—	—	—	—	—	**49**	**0**	**49**	
hsa-miR-346	—	—	—	—	—	—	**72**	**1.7**	**42.3**	
hsa-miR-330	—	—	—	—	—	—	**34**	**0**	**34**	
hsa-miR-340	—	—	—	—	—	—	**80**	**3**	**26.6**	
hsa-miR-34aN	—	—	—	—	—	—	**477**	**18**	**26.5**	
hsa-miR-137	—	—	—	—	—	—	**1,495**	**66**	**22.6**	
hsa-miR-138	—	—	—	—	—	—	**22**	**0**	**22**	
hsa-miR-321	—	—	—	—	—	—	**9,096**	**448**	**20**	
hsa-miR-187	—	—	—	—	—	—	*19.3*	*1*	19.3	
hsa-miR-338	—	—	—	—	—	—	**170**	**9**	**18.8**	
hsa-miR-34a	—	—	—	—	—	—	**56**	**3**	**18.6**	
hsa-miR-149	—	—	—	—	—	—	*926*	*52*	17.7	
hsa-miR-203	—	—	—	—	—	—	*53*	*3*	17.3	Down-regulated in colon cancer; Lu et al. (7)
hsa-miR-328	—	—	—	—	—	—	**480**	**28**	**17**	
hsa-miR-425	—	—	—	—	—	—	**82**	**6**	**13.6**	
hsa-miR-135a	—	—	—	—	—	—	**880**	**70**	**12.5**	
hsa-miR-154*	—	—	—	—	—	—	**11**	**0**	**11**	
hsa-miR-370	—	—	—	—	—	—	**54**	**5**	**10.8**	
hsa-miR-98	—	—	—	—	—	—	**189**	**18**	**10.5**	
hsa-miR-382	—	—	—	—	—	—	*181*	*18*	*9.7*	
hsa-miR-107	—	—	—	—	—	—	**70**	**8**	**8.75**	Up-regulated in colon, lung and pancreatic cancers; Volinia et al. (41)
hsa-miR-361	—	—	—	—	—	—	**228**	**29**	**7.8**	
hsa-let-7g	—	—	—	—	—	—	**3,313**	**449**	**7.3**	
hsa-miR-134	—	—	—	—	—	—	**29**	**4**	**7.25**	
hsa-miR-331	—	—	—	—	—	—	**399**	**59**	**6.7**	
hsa-miR-17-3p	—	—	—	—	—	—	**13**	**2**	**6.5**	B-Cell lymphoma; He (12)

(*Continued on the following page*)

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

Table 2. MicroRNAs as candidate tumor suppressors or oncogenes (Cont'd)

MicroRNAs	Colon			Hematologic			CNS			Studies establishing function in primary tumors
	Copies/cell		Fold decrease	Copies/cell		Fold decrease	Copies/cell		Fold decrease	
	Normal tissue	Tumor lines		Normal tissue	Tumor lines		Normal tissue	Tumor lines		
hsa-miR-181c	—	—	—	—	—	—	**95**	**15**	**6.3**	Down-regulated in glioblastomas; Ciafre et al. (18)
hsa-miR-30c	—	—	—	—	—	—	**5,522**	**876**	**6.3**	Down-regulated in glioblastomas; Ciafre et al. (18)
hsa-miR-212	—	—	—	—	—	—	**65**	**11**	**5.9**	
hsa-let-7d	—	—	—	—	—	—	*365*	*64*	*5.6*	
hsa-miR-103	—	—	—	—	—	—	**1,124**	**208**	**5.4**	
hsa-miR-213	—	—	—	—	—	—	**42**	**8**	**5.2**	Down-regulated in colon cancer; Lu et al. (7)
hsa-miR-148b	—	—	—	—	—	—	*116*	*23*	*5.1*	
hsa-miR-194	—	—	—	—	—	—	**44.9**	**8.8**	**5.1**	
hsa-miR-26b	—	—	—	—	—	—	**3,162**	**657**	**4.8**	
hsa-miR-181a	—	—	—	—	—	—	**1,938**	**410**	**4.7**	Down-regulated in glioblastomas; Ciafre et al. (18)
hsa-miR-192	—	—	—	—	—	—	**67**	**14**	**4.5**	
hsa-miR-191	—	—	—	—	—	—	**1,078**	**246**	**4.4**	
hsa-miR-324-3p	—	—	—	—	—	—	**306**	**74**	**4**	
hsa-miR-324-5p	—	—	—	—	—	—	**339**	**83**	**4**	
hsa-let-7iN	—	—	—	—	—	—	**1,273**	**322**	**3.9**	
hsa-miR-30bN	—	—	—	—	—	—	**623**	**1,585**	**3.9**	
hsa-miR-32	—	—	—	—	—	—	*60*	*17*	*3.5*	
hsa-miR-30b	—	—	—	—	—	—	**1,650**	**483**	**3.4**	
hsa-miR-30d	—	—	—	—	—	—	**350**	**108**	**3.2**	
hsa-miR-181b	—	—	—	—	—	—	**3,055**	**981**	**3.1**	
hsa-miR-345	—	—	—	—	—	—	**161**	**57**	**2.8**	
hsa-miR-197	—	—	—	—	—	—	**306**	**119**	**2.5**	
hsa-miR-20N	—	—	—	—	—	—	*829*	*325*	*2.5*	
(B) Candidate oncogenic microRNAs										
hsa-miR-10a	—	—	—	—	—	—	**0.8***	**108**	**136**	
hsa-miR-196a	—	—	—	—	—	—	*1* †	*64.9*	*64.9*	
hsa-miR-196b	—	—	—	—	—	—	**1**	**36.51**	**36.5**	
hsa-miR-10b	—	—	—	—	—	—	*8*	*1,861*	*23.2*	Up-regulated in glioblastomas
hsa-miR-21	—	—	—	—	—	—	**809**	**14,361**	**17.7**	Up-regulated in glioblastomas, breast, colon, lung, pancreatic, prostate, and stomach cancers; Chan et al. (19)

NOTE: MicroRNAs that were either significantly down-regulated (potential tumor suppressor microRNAs; *A*) or significantly-up-regulated (potential oncogenic microRNAs; *B*) in only one of the hematological, colon, or CNS tumor–derived cell line clusters are shown. Each of the three clusters selected had multiple normal tissue controls ($n > 3$) to which they could be compared. The microRNAs listed showed expression levels in one set of tumor cell lines significantly different from that observed in the corresponding normal tissue. The RNA copy number in the cell lines as well as in normal tissue is listed for each microRNA. The microRNAs are listed in descending order of fold change from the expression level in the corresponding normal tissue.

*MicroRNAs that are in boldface show tissue-specific expression patterns, i.e., these microRNAs vary significantly among corresponding normal tissue comparisons.

† MicroRNAs that are in italics are not tissue-specific, i.e., these microRNAs do not vary significantly among corresponding normal tissue comparisons.

‡ MicroRNAs are down-regulated in either colon or hematologic cancer lines, but are up-regulated in CNS tumor lines compared with normal brain tissue and may act as potential oncogenes (Table 2B).

Further support for the interpretation that microRNA expression patterns reflect bona fide properties of the cells of origin of the NCI-60 cell lines comes from our comparison of the expression patterns of microRNAs to the patterns of mRNA expression in the NCI-60 panel (Supplementary Fig. S1). Specifically, the microRNA and mRNA clustering patterns exhibited both, significantly clustering based largely on the tissue of origin (Supplementary Fig. S1). In particular, cell lines derived from hematologic, melanoma, CNS, colon, and renal tumor tissues were similarly clustered into independent terminal branches in both analyses (Fig. 1*A*; Supplementary Fig. S1). The observation that lung, ovarian, prostate, and breast tumors were distributed throughout

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

many branches in the cluster analysis would seem to be inconsistent with this observation. However, these branches all emanated from the same main secondary branch, perhaps reflecting the likely epithelial tissues of origin.

The pattern of microRNA expression associated with hematologic tumors (Fig. 2*A*) could provide a signature to differentiate non–solid from solid tumors, as these cell lines are all found clustered on a single dendrogram branch that differentiates them from solid tumors (41). Of particular note is the polycistronic set of microRNAs consisting of mir-17, mir-18, mir-19a, mir-19b, and mir-92, which are overexpressed in leukemic lines compared with the other cell lines. These closely linked, coexpressed microRNAs lie within a region (C13orf25v2) of amplification in some leukemias and lymphomas (11, 12). The mir-17-92 cluster is up-regulated in chronic lymphocyte leukemias and chronic lymphocyte leukemia cell lines, as well as many different lymphoma cell lines. Also, overexpression of this cluster of microRNAs in human diffuse large B-cell lymphoma has been described (19). Recently, in a mouse B-cell lymphoma model, overexpression of the mir-17 cluster was found to cooperate with c-myc to accelerate tumor development (12). Our finding that mir-17-92 expression contributes to the signature of hematologic tumor cell lines provides evidence that microRNA expression patterns in NCI-60 cell lines reflects microRNA patterns observed in primary hematologic malignancies.

The overall reduction in the expression level of many microRNAs in cell lines compared with normal tissues (Fig. 1*A*) may reflect of the relatively undifferentiated characteristics of most tumor cells. The lowest levels of microRNA expression are seen in very poorly differentiated tumors (42). Also, in mammalian embryonic stem cells, most microRNAs are induced during cellular maturation in tissue-specific gene expression patterns and play key roles in the maintenance of cell lineage characteristics (43–45). Therefore, the patterns of microRNA expression retained by tumor cells could reflect microRNAs defining the developmental state of the cells in which the tumor originated as well as microRNAs functioning in transformation pathways.

Altered expression of microRNAs could have profound effects on the expression of proteins key for mediating transformation.

Table 3. Potential targets of microRNAs up-regulated in CNS tumors and CNS tumor–derived cell lines

Gene Name and symbol*	Mean normal	Mean Glioblastoma	*P*	MicroRNAs predicted to target the listed gene
Visinin-like 1 (VSNL1)	2.53	−0.32	0.00040	miR-196a
Sparc/osteonectin, cwcv and kazal-like domains proteoglycan1 (SPOCK1)	2.29	−0.29	0.00006	miR_21
Reticulon 4 receptor (RTN4R)	2.19	−0.18	0.00001	miR-10a, miR-10b
EGF-like repeats and discoidin I-like domains 3 (EDIL3)	2.02	−0.11	0.00644	miR-21
Reticulon 4 receptor (RTN4R)	2.01	−0.11	0.00042	miR-10a, miR-10b
ST6 beta-galactosamide alpha-2,6-sialyltranferase1 (ST6GAL1)	1.85	0.44	0.00093	miR-21
Reticulon 4 receptor (RTN4R)	1.79	−0.10	0.00044	miR-10a, miR-10b
Mannan-binding lectin serine peptidase2 (MASP2)	1.68	0.64	0.00176	miR-10a, miR-10b
Fused toes homolog (mouse) FTS	1.55	0.93	0.03596	miR-196a, miR-196b
EGF-like repeats and discoidin I-like domains 3 EDIL3	1.38	−0.20	0.00022	miR-21
Zinc finger, MYND domain containing 11 ZMYND11	1.36	0.52	0.00892	miR-10a
Tropomyosin 1 (alpha) TPM1	1.29	0.14	0.00540	miR-21
Transcriptional adaptor 2 (ADA2 homolog, yeast)-like TADA2L	1.17	0.40	0.00455	miR-21
RUN and SH3 domain containing 2 RUSC2	1.07	−0.04	0.00001	miR-10a, miR-10b
Copine IV CPNE4	0.95	0.21	0.00131	miR-196a, miR-196b
TBP-like 1 TBPL1	0.82	0.30	0.01409	miR-196a, miR-196b
v-ski sarcoma viral oncogene homolog (avian) SKI	0.67	−0.04	0.02134	miR-21
RB1CC1 || RB1-inducible coiled-coil 1 RB1CC1	0.36	−0.24	0.00358	miR-10b
Signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	0.32	−0.24	0.02060	miR-196a
NPC1 (Niemann-Pick disease, type C1, gene)-like 1 NPC1L1	0.18	−0.92	0.00133	miR-196a
RB1CC1 || RB1-inducible coiled-coil 1 RB1CC1	0.17	−0.34	0.00310	miR-10b
Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1) GOT1	0.08	−1.60	0.00001	miR-10b
Jumonji, AT rich interactive domain 2 JARID2	0.00	−0.84	0.00914	miR-10a, miR-10b
NHP2 non-histone chromosome protein 2-like 1 (*S. cerevisiae*) NHP2L1	−0.05	−0.66	0.01998	miR-10a, miR-10b
ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide ATP5B	−0.74	−1.37	0.02689	miR-21
cAMP responsive element binding protein 3-like 1 CREB3L1	−0.44	−1.21	0.03011	miR-196a, miR-196b
Tumor-associated calcium signal transducer 1 TACSTD1	−3.35	−4.38	0.00001	miR-21

NOTE: Potential targets for mirs-10a/b, mirs-196a/b, and mir-21 that are overexpressed in CNS tumor–derived cell lines were predicted using MiRanda associated with the Sanger MIRBASE (http://microrna.sanger.ac.uk/sequences/). Gene expression data describing glioblastomas and normal brain tissue (http://microarray-pubs.stanford.edu/gbm/) and NCI-60 cell lines (http://genome-www.stanford.edu/nci60/) were extracted from the Stanford Microarray Database smd.stanford.edu/cgi-bin/publication/viewPublication.pl?pub_no=443 and smd.stanford.edu/cgi-bin/publication/viewPublication.pl?pub_no=81, respectively). Correlation of expression between two genes was evaluated using the Pearson test, and the means of gene expression between two specimen groups were compared using Student's t test ($P \leq 0.01$). The extracellular matrix protein SPOCK1 and the two transcription regulators, ZMYND11, and RB1CC1, with known functions that may potentially contribute to tumorigenesis are highlighted in gray.

*Replicates of target genes are from different microarray IMAGE clones from which the data were obtained.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

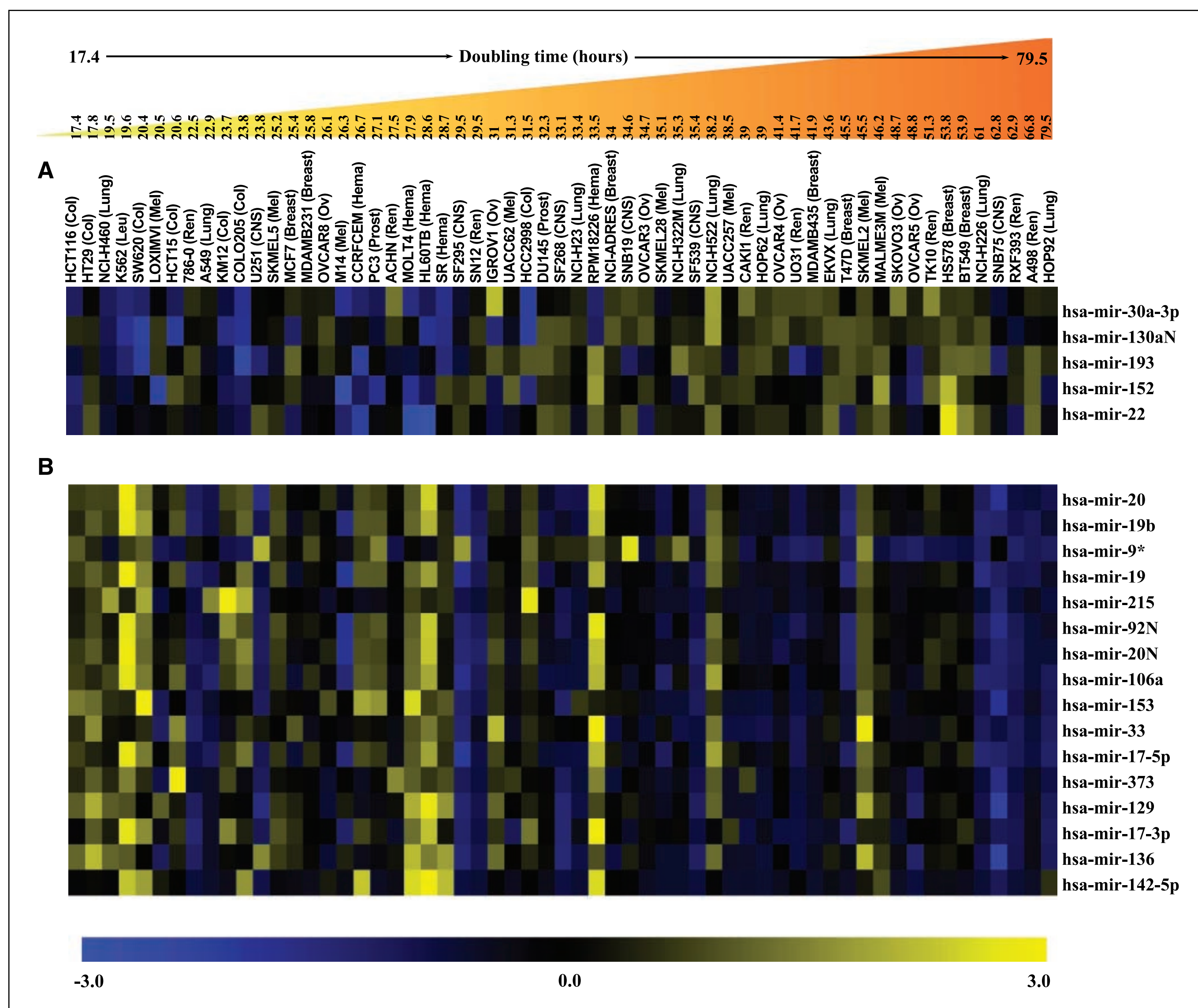

Figure 3. MicroRNA expression levels correlate to doubling time. MicroRNAs in which the expression levels in the NCI-60 cell lines showed a statistically significant correlation with published measurements of doubling time in culture were selected by Spearman rank analysis ($P \leq 0.01$). Cell lines are arranged in columns from the fastest (17.4 h) to slowest (79.5 h) doubling times. The microRNAs that correlate with proliferative index fall into two distinct subgroups: microRNAs in which the expression levels display an increase with increasing doubling time (*A*), and microRNAs in which the expression levels exhibit a decrease with increasing doubling time (*B*). A blue-yellow color scale (−3 to +3) depicts normalized microRNA expression level (in which 3 on the scale corresponds to a Ct value of 23 and −3 to a Ct value of 35).

MicroRNAs that are overexpressed in tumors might contribute to oncogenesis by diminishing the level of expression of targeted tumor suppressor genes. Conversely, microRNAs with diminished expressions in tumors might normally repress one or more oncogenes, such that reduction of the microRNA could lead to enhanced expression of one or more oncogenic mRNA. Accordingly, we identified microRNAs that might contribute to malignancy using the criterion that they were either significantly down-regulated (Table 2A) or significantly up-regulated (Table 2B) in just one of the three major tumor cell line clusters relative to the corresponding normal tissue, and that did not display tissue-specific expression in comparisons among the normal tissue samples. MicroRNAs that were up-regulated or down-regulated in more than one major tumor cell line clusters (Supplementary Table S2) were interpreted as less likely to be tumor-suppressive or oncogenic microRNAs, as their expression might have been altered as a consequence of *in vitro* culture. It is striking that a relatively large number of microRNAs (59) seemed to behave like potential tumor suppressors for CNS (Table 2A). This result suggests that the malignant behavior of CNS tumor cells could result from relatively complex changes in gene expression, compared with the colon or hematopoietic tumors. Alternatively, the widespread reduction of microRNAs in CNS tumor lines relative to normal brain tissue could reflect the widely recognized complexity of cell types that make up brain tissue and which would not be represented in the tumor line being examined. We also compared our data on these potentially tumor-suppressive or oncogenic microRNAs in cell lines to existing literature in tissue from specific tumor types [Table 2A and B; Supplementary Table S2; and reviewed in refs. (33, 46)].

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

Our finding that a number of microRNAs that were highly expressed in selected cell lines exhibited an association between their expression levels and the copy number of adjacent SNPs (Supplementary Table S4) suggests that these microRNAs might be biologically selected during tumorigenesis because of the important functions they serve. Furthermore, the correlation of microRNA expression in malignant tissues and cancer-associated genomic regions (CAGR) identifies microRNAs possibly involved in cancer (47). We examined whether genes for microRNAs up-regulated or down-regulated in NCI-60 cell lines were also known to be located in CAGRs such as fragile sites, minimal regions of loss of heterozygosity, minimal regions of amplification, or common breakpoint regions. Among the candidate tumor-suppressive microRNAs we identified (Table 2A; Supplementary Table S2), mir-125b, mir-34a, mir-143, mir-145, mir-26a, mir-99a, let-7a, let-7d, let-7f, and let-7g were located in fragile sites and regions of loss of heterozygosity/amplification (47). One of the candidate oncogenic microRNAs that we identified (Table 2B), mir-21, was also located in a fragile site/region of amplification (47). Importantly, certain microRNAs that we have identified as potentially tumor-suppressive or oncogenic are located in CAGRs known to be important in various malignancies. For example, mir-125b and let-7a are located in regions known to be altered in lung, breast, ovarian, and cervical cancer, whereas mir-34a, mir-99a, and mir-26a are located in CAGRs involved in breast, lung, and epithelial/nasopharyngeal cancers. Additionally, mir-143 and mir-145 are located in CAGRs involved in the myelodysplastic syndrome and the oncogenic mir-21 in regions critical for neuroblastoma (47). Four of the five potentially oncogenic microRNAs we identified in the CNS tumor–derived cell lines (Table 2B) are located within HOX clusters, many of which are overexpressed in glioblastoma cell lines as well as in primary glioblastoma tumor tissue suggests their potential as transforming genes (48). Mir-10a and mir-196a are located within the HOX B cluster on 17q21, mir-196b is in the HOX C cluster on 12q13, and mir-10b is in the HOX D cluster on 2q31 (10), suggesting the potential involvement of these particular microRNAs in tumorigenesis.

Although a comprehensive analysis of the potential regulatory targets of microRNAs in NCI-60 cell lines is beyond the scope of this study, we found that a set of potential targets decreased in glioblastomas. SPOCK1 is a predicted target of mir-21 in which the level is increased 17.7-fold in CNS tumor–derived lines when compared with normal brain (Table 2B). SPOCK1 is a proteoglycan originally described as being expressed in neurons (35), but its expression is increased in reactive astrocytes, and it inhibits the activities of membrane-type matrix metalloproteinases and cathepsin L, which promote migration of neoplastic astrocytes (49). Mir-10a is up-regulated 64.9-fold in CNS tumor–derived lines compared with brain (Table 2B) and one of its potential targets is ZMYND11, a transcriptional suppressor of the adenovirus E1A protein (36). E1A interacts with tumor suppressor genes (such as RB1). RB1CC1, another known tumor-suppressor is up-regulated 23.2-fold in CNS tumor lines compared with brain, and is a predicted target of mir-10b (Table 2B). RB1CC1 is a key regulator of RB1 (37). Mutations of RB1CC1 occur in 20% of primary breast cancers (50).

The NCI-60 cell line panel has been studied for numerous characteristics related to malignancy including drug sensitivity, gene expression patterns, and molecular and biological alterations of importance for tumorigenesis. Our studies, revealing that microRNA expression correlates to cell proliferation indices, suggests specific microRNAs in which the biological activities may be of importance in regulating proliferation. Importantly, these findings indicate the feasibility of seeking correlations between microRNA expression and the numerous other characteristics of these cell lines reported in public databases. This should further elucidate the pathophysiologic roles of microRNAs in various types of cancers.

Acknowledgments

Received 7/31/2006; revised 11/14/2006; accepted 12/29/2006.

Grant support: Theodora Betz foundation (M. Israel) and NIH grants GM34028 and GM066826 (V. Ambros).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Susan Holbeck for sending us frozen pellets of the NCI-60 panel of cell lines as well as for calculating the SNP copy number in the specific regions that carry the 81 core microRNAs. We are also grateful for the advice and help received from Dr. Eric Posmentier at Dartmouth College in correlating microRNA expression levels to DNA copy number, and for the excellent support in data analyses that we received from Alexis Meng and Carol S. Ringelberg at Dartmouth College.

References

1. Ambros V. The functions of animal microRNAs. Nature 2004;431:350–5.
2. Bartel DP, Chen CZ. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. Nat Rev Genet 2004;5:396–400.
3. Miska EA. How microRNAs control cell division, differentiation and death. Curr Opin Genet Dev 2005;15:563–8.
4. Sevignani C, Calin GA, Siracusa LD, Croce CM. Mammalian microRNAs: a small world for fine-tuning gene expression. Mamm Genome 2006;17:189–202.
5. McManus MT. MicroRNAs and cancer. Semin Cancer Biol 2003;13:253–8.
6. Caldas C, Brenton JD. Sizing up miRNAs as cancer genes. Nat Med 2005;11:712–4.
7. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–8.
8. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. Cell 2005;122:6–7.
9. Calin GA, Dumitru CD, Shimizu M, 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 2002;99:15524–9.
10. Nairz K, Rottig C, Rintelen F, Zdobnov E, Moser M, Hafen E. Overgrowth caused by misexpression of a microRNA with dispensable wild-type function. Dev Biol 2006;291:314–24.
11. Tagawa H, Seto M. A microRNA cluster as a target of genomic amplification in malignant lymphoma. Leukemia 2005;19:2013–6.
12. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. Nature 2005;435:828–33.
13. Costinean S, Zanesi N, Pekarsky Y, et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(μ)-miR155 transgenic mice. Proc Natl Acad Sci U S A 2006;103:7024–9.
14. Kluiver J, Haralambieva E, de Jong D, et al. Lack of BIC and microRNA miR-155 expression in primary cases of Burkitt lymphoma. Genes Chromosomes Cancer 2006;45:147–53.
15. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003;1:882–91.
16. Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17–92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005;65:9628–32.
17. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005;65:7065–70.
18. Ciafre SA, Galardi S, Mangiola A, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun 2005;334:1351–8.
19. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;65:6029–33.
20. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839–43.
21. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635–47.
22. Scherf U, Ross DT, Waltham M, et al. A gene expression database for the molecular pharmacology of cancer. Nat Genet 2000;24:236–44.
23. Jirapech-Umpai T, Aitken S. Feature selection and classification for microarray data analysis: evolutionary methods for identifying predictive genes. BMC Bioinformatics 2005;6:148.
24. Wang X, Wang X. Systematic identification of

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

microRNA functions by combining target prediction and expression profiling. Nucleic Acids Res 2006;34: 1646–52.
25. Garraway LA, Sellers WR. From integrated genomics to tumor lineage dependency. Cancer Res 2006;66: 2506–8.
26. Covell DG, Wallqvist A, Huang R, Thanki N, Rabow AA, Lu XJ. Linking tumor cell cytotoxicity to mechanism of drug action: an integrated analysis of gene expression, small-molecule screening and structural databases. Proteins 2005;59:403–33.
27. Huang R, Wallqvist A, Covell DG. Assessment of *in vitro* and *in vivo* activities in the National Cancer Institute's anticancer screen with respect to chemical structure, target specificity, and mechanism of action. J Med Chem 2006;49:1964–79.
28. Chen C, Ridzon DA, Broomer AJ, et al. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res 2005;33:e179.
29. Suzuki R, Shimodaira H. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. Bioinformatics 2006;22:1540–2.
30. Liang Y, Diehn M, Watson N, et al. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. Proc Natl Acad Sci U S A 2005;102:5814–9.
31. Couzin J. Cancer biology. A new cancer player takes the stage. Science 2005;310:766–7.
32. Hammond SM. MicroRNAs as oncogenes. Curr Opin Genet Dev 2006;16:4–9.
33. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006;94: 776–80.
34. Voorhoeve PM, le Sage C, Schrier M, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 2006; 124:1169–81.
35. Cifuentes-Diaz C, Alliel PM, Charbonnier F, et al. Regulated expression of the proteoglycan SPOCK in the neuromuscular system. Mech Dev 2000;94: 277–82.
36. Ladendorff NE, Wu S, Lipsick JS. BS69, an adenovirus E1A-associated protein, inhibits the transcriptional activity of c-Myb. Oncogene 2001;20:125–32.
37. Chano T, Ikegawa S, Kontani K, Okabe H, Baldini N, Saeki Y. Identification of RB1CC1, a novel human gene that can induce RB1 in various human cells. Oncogene 2002;21:1295–8.
38. Wienholds E, Plasterk RH. MicroRNA function in animal development. FEBS Lett 2005;579:5911–22.
39. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005;132:4653–62.
40. Massirer KB, Pasquinelli AE. The evolving role of microRNAs in animal gene expression. Bioessays 2006; 28:449–52.
41. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103: 2257–61.
42. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006;25:2537–45.
43. Houbaviy HB, Murray MF, Sharp PA. Embryonic stem cell-specific MicroRNAs. Dev Cell 2003;5:351–8.
44. Suh MR, Lee Y, Kim JY, et al. Human embryonic stem cells express a unique set of microRNAs. Dev Biol 2004; 270:488–98.
45. Darr H, Benvenisty N. Factors involved in self-renewal and pluripotency of embryonic stem cells. Handb Exp Pharmacol 2006;174:1–19.
46. Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer 2006;6: 259–69.
47. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999–3004.
48. Abdel-Fattah R, Xiao A, Bomgardner D, Pease CS, Lopes MB, Hussaini IM. Differential expression of HOX genes in neoplastic and non-neoplastic human astrocytes. J Pathol 2006;209:15–24.
49. Colin C, Baeza N, Bartoli C, et al. Identification of genes differentially expressed in glioblastoma versus pilocytic astrocytoma using suppression subtractive hybridization. Oncogene 2006;25:2818–26.
50. Hughes-Davies L, Huntsman D, Ruas M, et al. EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. Cell 2003;115:523–35.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.

AACR American Association for Cancer Research

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Characterization of MicroRNA Expression Levels and Their Biological Correlates in Human Cancer Cell Lines

Arti Gaur, David A. Jewell, Yu Liang, et al.

Cancer Res 2007;67:2456-2468.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/67/6/2456
Supplementary Material	Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2007/03/14/67.6.2456.DC1

Cited articles	This article cites 49 articles, 12 of which you can access for free at: http://cancerres.aacrjournals.org/content/67/6/2456.full#ref-list-1
Citing articles	This article has been cited by 54 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/67/6/2456.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/67/6/2456. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerres.aacrjournals.org on November 13, 2020. © 2007 American Association for Cancer Research.