Global microRNA Analysis of the NCI-60 Cancer Cell Panel

Rolf Søkilde1, Bogumil Kaczkowski2, Agnieszka Podolska3, Susanna Cirera3, Jan Gorodkin4, Søren Møller1, and Thomas Litman1

Abstract

MicroRNAs (miRNA) are a group of short noncoding RNAs that regulate gene expression at the posttranscriptional level. They are involved in many biological processes, including development, differentiation, apoptosis, and carcinogenesis. Because miRNAs may play a role in the initiation and progression of cancer, they comprise a novel class of promising diagnostic and prognostic molecular markers and potential drug targets. By applying an LNA-enhanced microarray platform, we studied the expression profiles of 955 miRNAs in the NCI-60 cancer cell lines and identified tissue- and cell-type-specific miRNA patterns by unsupervised hierarchical clustering and statistical analysis. A comparison of our data to three previously published miRNA expression studies on the NCI-60 panel showed a remarkably high correlation between the different technical platforms. In addition, the current work contributes expression data for 369 miRNAs that have not previously been profiled. Finally, by matching drug sensitivity data for the NCI-60 cells to their miRNA expression profiles, we found numerous drug-miRNA pairs, for which the miRNA expression and drug sensitivity profiles were highly correlated and thus represent potential candidates for further investigation of drug resistance and sensitivity mechanisms. Mol Cancer Ther; 10(3); 375–84. ©2011 AACR.

Introduction

Twenty years have passed since the NCI-60 cell panel for anticancer drug screening was established in 1990 (1), and today, these cell lines represent the most extensively studied and best characterized set of tumor cells available. The panel comprises 59 human cancer cell lines derived from 9 different tissues of origin: melanoma, leukemia, breast, central nervous system (CNS), colon, lung, ovary, prostate, and kidney. One breast cancer cell line, MB-468, has high identity to MDA-MB-231 (http://www.sanger.ac.uk/genetics/CGP/Genotyping/nci60.shtml) and was excluded from the NCI-60 panel that we received for our analysis. Chemosensitivity profiles of the NCI-60 cells for more than 100,000 compounds have been generated, and together with genomic, transcriptomic, proteomic, epigenomic, and metabolomic data, this information is publicly available via the NCI Developmental Therapeutics Program website (2) or can be mined directly by the CellMiner query tool (3). One type of data, which could be important for understanding the link between genomic and chemosensitivity data, particularly for which there seems to be a lack of correlation between mRNA and protein levels, was recently added to the NCI-60 database, namely, microRNA (miRNA) expression information (4–6).

miRNAs constitute a group of short, noncoding RNAs which repress gene expression at the posttranscriptional level by binding to complementary sequences in the 3′-untranslated region of their target mRNAs (7). They are involved in the regulation of numerous biological processes, including differentiation, proliferation, and apoptosis. Moreover, aberrant miRNA expression has been associated with many diseases, including cancer, in which miRNAs can function both as tumor suppressors and as oncogenes (8). Because miRNAs play a role in cancer pathogenesis, they may have potential as molecular biomarkers, not only for classification purposes but also for prediction of treatment response and for discovery of new targets. Today, 1,100 miRNAs and several thousand putative miRNA targets have been identified in the human genome and are accessible via miRBase, the main miRNA database (9). It is the expression of these miRNAs and a number of viral and novel sequences identified by high-throughput sequencing (10) that we have been studying with the application of an LNA-enhanced microarray platform.

We present here a comprehensive analysis of the NCI-60 cell line panel miRNA landscape, where the expression of 955 miRNAs has been correlated to drug sensitivity, mRNA and protein level, and tissue of origin.
of the cells. In addition, our meta-analysis of the 4 available NCI-60 miRNA data sets [i.e., real-time PCR (RT-PCR) study of Gaur and colleagues (5), custom microarray data of Blower and colleagues (4), Agilent array data of Liu and colleagues (6), and the current LNA microarray analysis] cross-validates the individual platforms where they have overlapping and concordant targets. (At the time of submission of this article, the complete data set was not yet publicly available. Therefore, our analysis is limited to the 365 miRNAs reported to be expressed in at least 10% of the cell lines by Liu and colleagues.)

Materials and Methods

Cell lines

The NCI-60 cell stocks were kindly provided by Dr. Susan Holbeck at the NCI Developmental Therapeutics Program in May 2006, except for the SR lymphoma cell line, which was purchased from American Type Culture Collection (CRL-2262) in 2007. Supplementary Table S1 lists the origin of the cell lines and some of their characteristics. All cells were grown at 37°C in 5% CO₂ in RPMI-1640 medium (Invitrogen), supplemented with 2 mmol/L L-glutamine and 10% FBS (Invitrogen). The tissue origin of each cell line is denoted as follows: BR, breast; CNS, glioma; CO, colon; LE, leukemia; LU, lung; ME, melanoma; OV, ovary; PR, prostate; RE, renal. The cells were grown to approximately 80% confluence and passed twice before RNA extraction.

RNA extraction and quality control

Total RNA was extracted by Trizol reagent (Invitrogen) according to the manufacturer’s instructions. RNA quality was assessed with Bioanalyzer 2100 Nano chips (Agilent) and did not show sign of degradation as judged by the RNA integrity number, which was above 8 for most samples. RNA concentrations were measured on a NanoDrop ND-1000 (Thermo Scientific).

RNA labeling

Our microarray experimental setup used a common reference design as described in detail by Søkilde and colleagues (11). One microgram of total RNA from each sample was labeled using the miRCURY LNA microRNA Power labeling Kit (Exiqon), following the manufacturer’s recommendations. Cell line–specific RNA was labeled with Hy3 fluorophore, whereas the common reference RNA pool was labeled with Hy5.

Microarray hybridization and scanning

For miRNA profiling, we applied the LNA-enhanced miRCURY Dx 9.2 microarray platform, containing quadruplicate probes for 955 mature miRNAs (Exiqon). Labeled RNA samples were hybridized with the arrays for 16 hours at 65°C in a Tecan HS 4800 hybridization station. After washing and drying, the microarrays were scanned in a DNA Microarray Scanner (Agilent) and the resulting images were quantified using ImaGene v. 8.0 (BioDiscovery). Both automatic quality control (i.e., flagging of poor spots) and manual, visual inspection was done to ensure the highest possible data quality.

Data preprocessing and normalization

All low-level analyses, such as importing and preprocessing of the data, were done in R environment by using the LIMMA package (12). After excluding flagged spots from the analysis, the “normexp” background correction method, plus offset = 50, was applied, after which intensities were log₂ transformed and quantile normalized as implemented in LIMMA. Both log₂ intensities (single-channel analysis) and log₂ ratios (dual channel analysis) of 4 intraslide replicates were averaged. All expression data were deposited in the Rosetta Resolver (Rosetta Biosoftware) data management system.

High-level data analysis

To identify miRNAs that were differentially expressed, we applied the empirical Bayes, moderated t-statistics implemented in LIMMA. Linear models were fitted to the data, and comparisons of interest were extracted as contrasts. Unsupervised hierarchical clustering with complete linkage, using both Euclidean and (1 – Pearson correlation) as distance metrics, was applied to cluster the NCI-60 cell lines according to their miRNA expression levels.

Mapping probes from prior data sets

The quantitative PCR (qPCR) data generated by Gaur and colleagues (5) and the array data by Blower and colleagues (4) and Liu and colleagues (6) were matched to the current miRBase release 15.0, in which annotation was kept at the level of MIMAT names (the identifier for mature miRNA sequences; see Supplementary Table S2). Probe designs for the Blower array were downloaded from the ArrayExpress Archive (13), and all probes with a perfect match to a mature miRNA in miRBase 15.0 were kept at the level of MIMAT names (the identifier for mature miRNA sequences; see Supplementary Table S2).

Drug sensitivity correlation analysis

To examine the relationship between drug sensitivity and miRNA expression, we took 3 approaches: First, we downloaded GI₅₀ data (July 2007 release) for the NCI-60 panel from the NCI DTP web site (14). The raw data consisted of GI₅₀ values for 45,857 drugs, which after applying a variance filter (variance > 0; ref. 15) were reduced to 6,081 drugs. After minimum range filtering (GI₅₀_max – GI₅₀_min > 2), 4,442 drugs were left in the data set.

Second, in addition to the aforementioned raw GI₅₀ data, we included a preprocessed data set from the CellMiner database (3). These data consist of 118 drugs with known mechanism of action and are described by Bussey and colleagues (15).

Correlation between miRNA expression and drug sensitivity was calculated in a pairwise manner, for each miRNA–drug combination, wherein each drug was represented by a vector of GI₅₀ measurements and each
miRNA was represented by a vector of \( \log_2 \) Hy3 intensities. Both Spearman and Pearson correlation coefficients were calculated, and the \( P \) values of the correlation significance were adjusted for multiple testing by the Benjamini and Hochberg false discovery rate (FDR) correction method (16).

Finally, a COMPARE analysis (October 2009 release) was run to search for correlations with unfiltered \( G_{50} \) values and resulted in 36,553 correlations with \( P < 0.005 \).

mRNA expression correlation analysis

Global mRNA expression profiles of NCI-60 cells measured on the Affymetrix HG U133A and U133B microarray platform were downloaded from Gene Expression Omnibus (GEO; accession number GSE5720; ref. 17) and normalized using Rosetta Resolver. Correlations between \( \log_2 \) intensities of overlapping miRNA–mRNA pairs [i.e., miRNAs that reside within introns of protein coding genes; the pairs are represented by miRCURY (for miRNA) and Affymetrix (for mRNA) probes] were calculated using both Spearman and Pearson correlation coefficients, and the \( P \) values were adjusted for multiple testing by the Benjamini and Hochberg method with FDR of 0.01 or less. The genomic coordinates of all known human miRNAs were extracted from the human miRNA annotation file downloaded from miRBase release 15.0 (18). Pairs of miRNA and mRNA transcripts were identified by matching the genomic location: chromosome, start, end, and strand.

Molecular targets correlation analysis

A correlation analysis of our miRNA expression data against the Molecular Targets database (September 2008 release; ref. 19) included 43,981 Pearson correlations with a cutoff value of \( P < 0.005 \) (Supplementary Table S3). The Molecular Targets data set contains information on protein levels, RNA expression, DNA mutation status, and enzyme activity for the NCI-60 cell lines.

Quantitative RT-PCR

The expression levels of 20 selected miRNAs and 10 reference genes were validated by quantitative RT-PCR applying the miRCURY LNA microRNA PCR system and SYBR Green master mix, following the manufacturer’s instructions (Exiqon). The results are shown in Supplementary Fig. S3.

Northern blot analysis

To visualize the level and size of 7 candidate miRNAs, we conducted Northern blot analysis on RNA from the SNB-19 (glioma), HCT-15 (colon), and SR (leukemia) cell lines. The method and results are described in the Supplementary Fig. S4.

Results and Discussion

We studied global miRNA expression in the NCI-60 cell line panel by applying a recently described (20), highly sensitive and specific miRCURY microarray platform, which contains LNA-enhanced and melting temperature \( \left( T_{m} \right) \)–normalized capture probes for quantitation of 599 mature human miRNAs, annotated in miRBase v.15.0, and 345 proprietary miRNAs, 29 viral miRNAs, and 10 snoRNAs.

Cross-platform comparison

Three previous studies have reported miRNA expression patterns from the NCI-60 cell lines: Blower and colleagues (4) and Liu and colleagues (6) used oligonucleotide microarrays, whereas Gaur and colleagues (5) applied real-time quantification by TaqMan stem-loop RT-PCR. A comparison of the different platform coverage and the latest miRBase version 15.0 shows that the current work contributes with expression data for 369 miRNAs that have not previously been profiled (Fig. 1A), including 345 miRPlus sequences not yet annotated in miRBase.

To assess correlation between platforms, the 125 miRNAs common to all 4 analyses were identified and the 6 pairwise (interplatform) Pearson correlation coefficients were computed, as summarized in Table 1 (values in bold). (Only 365/727 miRs were reported as expressed by Liu and colleagues. Therefore, the number of expressed miRNAs in common between the 4 studies is 125, and not 158, which is the total number of common miRNAs analyzed.) Taking into consideration that completely independent RNA preparations and assay technologies have been applied, the concordance between the 4 miRNA platforms is remarkably high compared with a similar miRNA analysis on the NCI-60 cell line panel (17). The miRNA correlation values (the same miRNA measured across platforms) ranged from highly correlated (\( r = 0.97 \)) to negative (\( r = -0.29 \)) and with an overall average of 0.48 (SD = 0.28), which is far better than what has been reported previously in an mRNA correlation study comparing oligonucleotide and cDNA microarray measurements on the NCI-60 panel (the Pearson correlation coefficient between the 2 array platforms was close to zero; ref. 21).

Besides obvious methodologic and biological interstudy differences (such as differences in growth conditions, RNA extraction, and analysis method), we have noticed that the 3 previously published studies apply various types of flooring to the data. Although flooring may serve to eliminate negative expression values due to background subtraction from low-intensity values, such an ad hoc threshold does not reflect the experimental measurements and can even lead to artifacts (22). Therefore, we chose to apply the normexp background correction method for stabilizing the variance of low-intensity probes after \( \log_2 \) transformation.

Although there is a good overall consistency between platforms as assessed by the distribution of miRNA correlations (Fig. 1B), we observed an even higher correlation between the cell line–specific signatures (Table 1, values in italics; and Fig. 1C). This suggests
that cell line–specific miRNA profiles are robust and can be identified regardless of platform. Only 2 cell lines showed very low correlation between the different studies, namely, the CNS cell lines SNB-19 and SNB-75, with an average correlation coefficient of 0.18 to 0.31. This could indicate that these cell lines have a phenotype which is sensitive to culturing conditions. Interestingly, the LNA microarray was the only platform to correlate the SNB-19 and U251 cell lines closely together, which agrees with their similar genotypes, as they are derived from the same individual (23).

For further comparison of the platforms, the best correlating of the 125 common miRNAs were used in an unsupervised hierarchical cluster analysis which showed that most of the tissue- and cell-specific expression patterns are retained between the 4 assays (Fig. 2). We selected 62 miRNAs with the highest average correlation ($R > 0.5$) between all combinations of platforms to obtain a

### Table 1. Correlation between studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Gaur</th>
<th>Blower</th>
<th>LNA</th>
<th>Liu</th>
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<tr>
<td>Liu</td>
<td>0.74</td>
<td>0.64</td>
<td>0.69</td>
<td>1</td>
</tr>
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**NOTE:** The table shows pairwise Pearson correlation coefficients between the different miRNA profiling datasets on the basis of either miRNAs (125 in common, in bold) or on the 59 cell lines studied (italics).
core set of “high confidence” miRNAs for clustering with resampling (Fig. 2A). Strong tissue-specific miRNA signatures were identified for cell lines of colon, melanoma, leukemia, and kidney origin (the latter included a single outlier, SN12C). These cell lines clustered together independently of platform and cell line origin, suggesting a strong and consistent tissue-specific signature in these tissues, such as miR-211 expression in melanoma, miR-142 expression in leukemia, miR-192 expression in colon, and miR-138 in kidney. However, we also identified cell lines representing tissue groups, including lung, ovary, and breast, with a more diverse expression pattern. Therefore, we chose to cluster these tissues separately to identify miRNA patterns unique to the individual cell line (Fig. 2B–D).

Again, we found good concordance between the platforms on the basis of “high confidence” set of miRNAs, showing that cell lines, though not displaying tissue-specific expression, retain their miRNA profile between different laboratories and studies. This is promising for the research of miRNA function, because related cell lines studied in different experimental settings will likely display similar miRNA profiles. However, there are exceptions; for example, the lung cancer cell line HOP-62 on the Gaur platform showed a miRNA profile that differed markedly from that of the 3 other platforms (Fig. 2B).

We found that the lung cancer cell lines (Fig. 2B) showed the most intratissue diversity among all the cell lines studied, as these cell lines scattered throughout the full cluster (Fig. 2A). From the clustering of the lung cancer cell lines, the non–small cell lung carcinoma large cell HOP-92, which is extremely slow growing, showed the most distinct miRNA profile.

Two of the ovarian cancer cell lines, OVCAR-8 and NCI/ADR-RES, have similar karyotypes (24). This is reflected in Fig. 2C, as all 4 platforms cluster these cell lines together. Other cell line pairs with high identity, and thus most likely, a common origin, include the CNS cell lines SNB-19 and U251 and the melanoma cell lines M14 and MDA-MB-435.

All platforms agreed on separating the breast cancer cell lines into 2 distinct clusters, representing miRNA expression profiles specific for basal and luminal type breast cancer (Fig. 2D).

In summary, our correlation analysis shows that most miRNA expression profiles are retained across NCI-60 data sets and therefore the data can potentially be used in combination, for example, in a meta-analysis. The
observed interstudy variation may be due to both technology and biology: different assay and probe designs are likely to give different results, besides the expected variation due to differences in growth conditions and handling of the cell lines. For the rest of the article, we focus on the data generated by the use of LNA-enhanced microarrays.

**Tissue- and cell line–specific miRNA expression**

The distribution of tissue-specific miRNAs (i.e., those miRNAs that were preferentially expressed in cell lines originating from one tissue compared with the rest of the cell lines) is summarized in the heatmap shown in Fig. 3.

**Breast cancer**

Because of the heterogeneous nature of the breast cancer cell lines, only 2 miRNAs were identified as breast cancer specific compared with the rest of the cell lines: miR-195 and miR-196a. Both these miRNAs are upregulated in breast cancer patients, and recently, miR-195 has been identified as a serum biomarker diagnostic for breast cancer (25). When looking at the "breast cancer
only” miRNA cluster (Fig. 2D), it seems that the cell lines fall in 2, clinically distinct clusters, representing luminal (MCF7 and T47D) and basal (HS578T, BT549, and MDA-MB-231) breast cancer, in which the latter, basal triple-negative subtype, is the prognostically more unfavorable (26). The luminal cell lines are characterized by hormone receptor expression [estrogen receptor (ESR1), progesterone receptor (PGR), and HER2] and luminal cytokeratins (e.g., CK7), whereas the basal type cancer cell lines are negative for the hormone receptors and have expression of basal cytokeratins (e.g., CK5 and CK6).

Recently, miR-22 was found to target ESR1, which is in agreement with our data, in which low miR-22 expression is observed when ESR1 levels are high (Fig. 3B). A similar inverse relationship between miR-222–miR-221 and ESR1 was found in breast cancer cell lines and patients (27), consistent with our observations.

Among the upregulated miRNAs in ESR-positive cell lines are miR-200a–miR-200b–miR-429 (Fig. 3B), a cluster linked to the regulation of E-cadherin via ZEB1/2 (28). miR-193b has been associated with the regulation of urokinase-type plasminogen activator (uPA), an important prognostic factor in breast cancer development (29). We found that the E-cadherin mRNA level follows the level of miR-200, whereas the uPA transcript levels are negatively correlated with miR-193b in support of the reported associations. On the other hand, miR-23b, which was found to correlate positively with uPA, has been shown to downregulate uPA and MET in hepatocellular carcinomas, although this interaction has not been reported in breast cancer.

Scott and colleagues showed that miR-125a/b regulates ERRB2 at both transcript and protein levels (30), which is in agreement with our observations: miR-125b is expressed at higher levels in the ERRB2-negative breast cancer cell lines than in ERRB2-positive cells (Fig. 3B).

Unexpectedly, we found positive correlation between miR-146 expression and epidermal growth factor receptor mRNA and protein levels (Supplementary Fig. S1). This is unlike the canonical model for miRNA action, in which high levels of miRNA repress their corresponding targets protein levels.

CNS–glioma
The CNS-specific miR-376, miR-377, and miR-654-3p localize to a large cluster of miRNAs on chromosome 14, often referred to as the miR-379–miR-656 cluster, found in an imprinted region of transcripts. A functional role of the cluster has been shown in neuronal development (31); interestingly, the 3 candidates are expressed at higher levels in the 2 estrogen receptor–positive cell lines, suggesting a role in hormone regulation (Fig. 3A).

Two miRNA clusters, miR-100–let-7a-2–125b-1 and miR-23b–27b–24-1, are expressed significantly higher in the brain-derived cells than in the rest of the cell lines. The miR-100 cluster, along with the let-7 family, has been described as brain specific in mammals and conserved expression was also found in zebrafish (32). Interestingly, there are no reports on specific expression of the miR-23b cluster in the brain. The brain-specific miR-124 was undetected in all CNS-derived cell lines. This miRNA is supposedly important for the maturation of neurons and the lack of its expression may be explained by the lack of differentiation of the CNS tumor cell lines. A miRNA reported to be involved in neurogenesis is miR-9; we observed a distinct pattern of miR-9 and miR-9* expression in the cell lines SF-295, SNB-19, and U251, whereas 3 other CNS cell lines, namely, SF-268, SF-539, and SNB-75, seem to be miR-9 and miR-9* negative, suggesting differences in differentiation between these 2 groups.

Colon cancer
Most notably, the cells of colon origin gave rise to many colon-specific miRNAs, due to the homogenous expression pattern observed in these cell lines. Interestingly, SW-620 and COLO-205 do not form tight epithelial cell clusters, which are characteristic of the other colon cancer cell lines in the panel. Instead, they form round bipolar cells with lower cell–surface and cell–cell adhesion (33). Recently, the miR-200 family was reported to be connected to epithelial cluster formation (28), which is why we decided to investigate this circuit in the cell lines. Although we did not identify any differences that could account for the lower cell–cell adhesion properties of SW-620 and COLO-205 (Fig. 3C), we found that miR-142 (both 5p and 3p)—a miRNA characteristic for leukemia and involved in hematopoiesis—was highly expressed in these 2 cell lines. We then looked for possible pathways targeted by miR-142 by applying DIANA-miRPath (34) and found that many adhesion pathways were enriched for target sites for miR-142 (3p and 5p), including TGF-β signaling, adhesion junction, tight junction, regulation of actin cytoskeleton, and extracellular matrix–receptor interaction. Many of the proteins targeted by miR-142 are involved in several of these pathways and are important regulators of adhesion (Supplementary Fig. S2).

We also detected differential expression of miRplus sequences, among which we looked closer at hsa-miRPlus-C1018, a sequence variant of hsa-miR-20a*. We were unable to detect hsa-miR-20a* on the array, and because the miRplus-C1018 and miR-20a signals seem highly correlated ($R^2 = 0.82$), this could indicate a mature miRNA-20a* different from the one annotated in miRBase.

Lung cancer
The lung cancer cell lines displayed very diverse expression profiles. From the clustering of the lung cancer cell lines alone (Fig. 2B), HOP-92 and NCI-H460 clustered furthest away from the other cell lines and have previously been described as large cell undifferentiated carcinomas (35). Another subcluster contained NCI-H322M and EKVX; both are epithelial cell lines with high expression of miR-200 and miR-203. On the other hand, the squamous miRNA marker miR-205 was only high in
miR-223, which is in agreement with the observation that miR-452 was upregulated in the group with high metastatic potential, cell lines. We found miR-452 to be upregulated in the high metastatic potential lung cancer cell lines, which is in agreement with the observation that miR-452 is upregulated in lymph node positive bladder cancer patients.

**Leukemia**

In general, the miRNA expression pattern in the leukemia cell lines is distinct from the other cell lines, which are all derived from solid tumors. Thus, we are able to confirm the "hematologic" signature differentiating nonsolid from solid tumors, as suggested by Gaur and colleagues (5). By applying the same split (into a low and high metastatic potential group), we identified a short list of miRNAs (Table 2) which could separate high from low metastatic potential cell lines. We found miR-452 to be upregulated in the group with high metastastic potential, which is in agreement with the observation that miR-452 is upregulated in lymph node positive bladder cancer patients.

**Melanoma**

The melanoma cell lines displayed a distinct clustering pattern (Fig. 2A), due to their unique expression of miR-204 and miR-211. These miRNAs have a similar seed sequence but differ in 2 nucleotides on positions 17 and 18. miR-211 is located in an intron of transient receptor potential cation channel subfamily M member 1 (TRPM1) and shows high correlation to the expression of TRPM1 mRNA (data not shown). TRPM1 is known to be transcriptionally regulated by microphthalmia-associated transcription factor (MITF), but the function of TRPM1 in melanocytes is still unclear. The role of miR-204 and miR-211 has been investigated in fetal human retinal pigment epithelium, in which they regulate the epithelial barrier functions and target TGF-βR2 and SNAIL2 (40). We also observed high levels of miR-146a and miR-146b in the melanoma cell lines; these miRNAs are involved in inflammation and psoriasis (41), but their function in melanocytes is still unclear. In addition, the melanoma cell lines showed high expression of the testis- and ovary-specific miR-509–miR-514 cluster, which is located in a genomic region (Xq27) known to be highly expressed in melanoma. We investigated (array-based comparative genomic hybridization data; not shown) whether the high miR-509–miR-514 expression could be explained by a similar increase in copy number in the melanoma cell lines, but this was not the case.

We found that the LOX-IMVI cell line differed markedly from the other melanoma cell lines in its miRNA profile. This cell line is amelanotic and thus does not express typical melanocyte genes. LOX-IMVI is characterized by low MITF expression and, in parallel, diminished expression of melanocyte-specific genes and miRNAs, such as miR-211, which is located within TRPM1.

**Ovarian cancer**

As the only cell line in the NCI-60 panel, IGROV1 expresses the miRNA cluster miR-302b–miR-302c–miR-302d–miR-367 (miR-302–miR-367), which is mainly expressed in pluripotent stem cells. Morphologic studies of IGROV1 cells suggest that they are derived from an ovarian carcinoma with multiple differentiations, endometroid to a large extent, together with some serous clear cells and undifferentiated foci (42). We find it likely that such compartments of undifferentiated cells posses a pluripotent precursor, which is reflected in the expression of miR-302–miR-367. In addition, the IGROV1 cell line is only in the NCI-60 panel carrying the BRCA1 mutation, which gives a very strong correlation ($r = 1, P = 4E-68$) between BRCA1 and the miR-302–miR-367 cluster, but whether this is a causal relationship, or just a coincidence, remains to be determined. The $2$ closely related NCI/ADR-RES and OVCAR-8 cell lines both have a deletion in 1p36.33 (15), which is reflected in the lack of expression of the miR-200a-200b-429 cluster, situated in this region.

**Prostate cancer**

The $2$ prostate cancer cell lines both have high expression of miR-31, and $2$ members of the miR-96–182–183 cluster have all been found to be differentially expressed in a recent analysis of prostate cancer and normal adjacent prostate (43). It has been debated whether the $2$ prostate
cancer cell lines PC-3 and DU-145 are androgen receptor positive or negative and whether or not they respond to hormone depletion (44). We therefore surveyed the literature for evidence of miRNA-guided differentiation between androgen-dependent or -independent prostate cancer. Lee and colleagues (45) showed that miR-125b was critical in cell proliferation and expressed at higher levels in PC-3 than in DU-145, whereas Shi and colleagues (46) showed that miR-125b could be upregulated by androgen receptor signaling. We also detect miR-125b at higher levels in PC-3 than in DU-145, indicating that PC-3 could have active androgen signaling. The same pattern is seen for miR-100, which was found to be upregulated in the study by Shi and colleagues (46). Interestingly, Shi and colleagues speculate that the expression of miR-125b comes from mir-125b-2, a precursor located on chromosome 21 in the miR-99a–let-7c–125b-2 cluster. We found that expression from PC-3 originates from another cluster, namely, miR-100–let-7a–2–125b-1 on chromosome 11, as the miR-125b expression data correlate better with this cluster. We detected only low levels of miR-99a, although miR-100 and let-7a are upregulated in PC-3 compared with DU-145.

**Renal cancer**

In the clustering between platforms (Fig. 2A), the renal cancer cell lines all group together except SN12C, which has a distinct miRNA profile. Stinson and colleagues observed that SN12C is the only renal cell line positive for neuronal system–specific enolases (35), which are expressed in neurons but with cross-reactivity to cancers of other origins. The cell line is negative for many of the renal-specific miRNAs, including miR-30a and miR-886. We detected an interesting pattern in the expression of miR-200 in the renal cell lines: All of the miR-200–positive renal cell lines were negative for miR-200c–141, which have been shown to be regulated by DNA methylation (47), suggesting that tissue-specific methylation may play a role in kidney development.

**Correlation with protein expression and with drug sensitivity**

See the Supplementary Results section and Figs. S5 and S6.

**Conclusion**

Several conclusions can be drawn from this study: First, despite major technical differences between the assays used to study miRNA expression (qRT-PCR, spotted arrays, Agilent arrays, LNA-enhanced arrays, total RNA vs. fractionated RNA), the correlation between the 4 methods is high and serves to cross-validate the assays. Where there is a lack of correlation, this can be attributed to the data-trimming methods previously applied, in which low values have been floored, which renders the correlation analysis imperfect.

Second, the biology of the NCI-60 cell lines is reflected in their miRNA landscape, as tissue-specific miRNA signatures are retained across all cell lines. For each histology, we also identified a number of characteristic miRNAs that may explain the regulation of tissue-specific markers; this conclusion, however, is in some cases based on only a few cell lines and should therefore be interpreted with caution.

Third, our miRNA–protein correlation analysis revealed several known (e.g., the miR-200 regulatory pathway) and potentially novel miRNA–protein interactions, most of which are associated with cell adhesion and motility.

Fourth, the correlation between miRNA expression and drug sensitivity (GI50) data can help in the identification of new molecular targets and miRNA-based biomarkers for personalized medication.

In conclusion, the miRNA landscape of the NCI-60 cell lines assayed by our LNA-enhanced microarray platform is in good overall agreement with previous miRNA studies. The miRNA expression data are available in GEO (accession number GSE26375). We envisage that future studies on the NCI-60 cell lines will include ChIP-Seq data, next-generation sequencing, and functional follow-up studies on associated targets.

**Disclosure of Potential Conflicts of Interest**

This study was carried out when R. Søkilde, S. Møller, and T. Litman were employees of Exiqon A/S.

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