

## miRNAs and CANCER

- miRNAs (miRNAs) are noncoding RNAs approximately 18-25 base pairs in size that have been shown to be involved in tissue development, cell differentiation, and gene regulation.
- Genes encoding miRNAs may play key roles in cancer development by acting as tumor suppressors.
- The microRNA *let-7* shows reduced expression in human lung cancers and correlates with shortened survival following surgical resection (Takamizawa *et al.* 2004).
- let-7* has also been implicated as a potential regulator of human *RAS* gene expression (Johnson *et al.* 2005).
- Human miRNA genes are often found near cancer-associated genomic regions and fragile sites (Calin *et al.* 2004)
- miRNA genes (*mir-15a*, *mir-16a*) have been shown to be down-regulated or deleted in a majority of chronic lymphocytic leukemia cases (Calin *et al.* 2004).

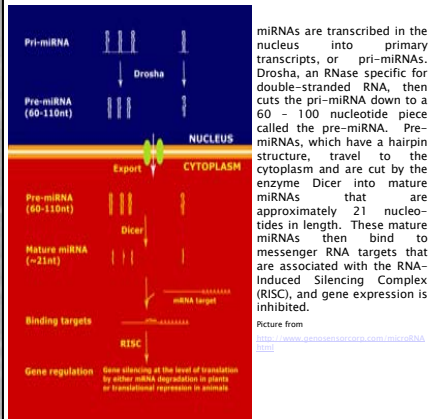
## ABSTRACT

MicroRNAs (miRNAs) are small (18-25 nucleotide), noncoding RNAs that have been identified as potential regulators of gene expression, cell differentiation, and tissue development. Decreased expression of some miRNAs has been found in several human cancers, including lung cancer and chronic lymphocytic leukemia. A high percentage of miRNA genes have also been located in cancer-associated genomic regions and fragile sites, further indicating a possible relationship between miRNA expression and oncogenic events. We performed global expression analysis of miRNAs in human malignant glioma cells using microarrays containing human precursor and mature miRNA probes to 226 miRNAs. To identify miRNAs that potentially confer therapy resistance to glioma cells *in vivo* via constitutive expression, we analyzed miRNA expression differences between cells from primary and recurrent gliomas from the same patient. To identify miRNAs that are induced in resistant cells following therapy, we analyzed miRNA expression differences between the same primary/recurrent tumor cell pairs following *in vitro* selection for resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and Temozolomide. Finally, to examine the time course of expression of miRNAs involved in drug-resistance pathways, expression differences were examined at various timepoints after *in vitro* drug treatment. These parameters allowed us to characterize differences in induced and constitutive expression of miRNAs in cells from primary and recurrent gliomas prior to, and following, *in vitro* selection for therapy resistance. Preliminary data based on  $\geq 2$ -fold expression difference in cells selected for BCNU resistance indicates that four miRNAs (*hsa-let-7b*, *hsa-mir-125b-2*, and *hsa-mir133a-1*, and *hsa-mir-183*) are differentially-regulated in both primary vs recurrent tumors and BCNU-sensitive vs -resistant cells. Expression of two of these miRNAs, *hsa-mir-125b-2* and *hsa-mir133a-1* are novel discoveries in human cells. Additionally, *hsa-let-7b* is of particular note because it has been shown to potentially be involved in colorectal cancer. Further, the gene encoding this miRNA has been mapped to 22q12, a region previously found by our laboratory to be involved in a translocation with chromosome 11q in cells from recurrent tumors. These data suggest a novel role for miRNAs in conferring therapy-resistance to malignant glioma cells and may provide new targets for the treatment of this tumor.

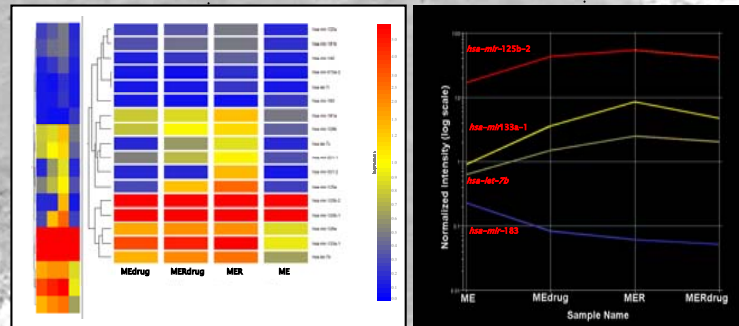
## METHOD

- Cells from primary and recurrent tumor from the same patient were selected for drug resistance *in vitro* and harvested 7 days following treatment. RNA was isolated from these cells, labeled, and hybridized to a microarray containing probes for 226 human precursor and mature miRNAs (Genosensor Corp.).
- Signal values were normalized to tRNA methionine and those miRNAs showing  $\geq 2$ -fold differential expression between samples were identified.

## MECHANISM OF GENE SILENCING BY miRNAs



## RESULTS

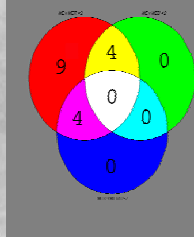


The above dendrogram shows miRNA expression across samples. Each row represents a different miRNA, and each column represents a different cell line. Colors represent normalized miRNA expression according to the color legend on the right side of the figure. The branches on the left side of the figure cluster expression across samples according to standard correlation.

This graph shows the expression of those 4 miRNAs identified to be  $\geq 2$ -fold differentially expressed between BCNU-resistant cells and BCNU-sensitive cells and between cells from primary and recurrent tumor from the same patient. The x-axis shows the different tumor samples and the y-axis shows a logarithmic scale of the normalized intensity, which correlates with overall expression.

## EXPERIMENTAL BACKGROUND

- Human malignant gliomas are aggressive tumors that often recur despite surgical resection, radiation, and chemotherapy treatments. Furthermore, cells from recurrent tumors are more resistant to therapy than cells from primary tumors.
- To identify miRNAs that could potentially be involved in therapy resistance in human malignant glioma cells, global expression analysis of miRNAs was performed using microarrays with human precursor and mature miRNA probes to 226 miRNAs (Genosensor, Inc.).
- miRNA expression was analyzed in cells from primary (ME) and recurrent (MER) gliomas from the same patient to identify miRNAs that are correlated with the constitutive expression of genes involved in therapy resistance *in vivo*.
- miRNA expression was also analyzed in primary and recurrent tumor cell pairs following *in vitro* selection (ME vs. ME drug) for 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) to identify those miRNAs that are induced in resistant cells following chemotherapy.



The Venn diagram to the left shows the number of miRNAs  $\geq 2$ -fold differentially expressed between given cell lines. Seventeen miRNAs are constitutively differentially expressed (ME v MER). Four miRNAs are differentially expressed between cells selected for resistance to BCNU and cells sensitive to BCNU from the primary tumor (ME drug vs. ME). Four separate miRNAs are differentially expressed between recurrent BCNU-resistant cells and recurrent BCNU-sensitive cells (MER drug vs. MER). Each of these sets of four miRNAs are subsets of the 17 constitutively differentially expressed miRNAs.

## CONCLUSIONS

- Based on  $\geq 2$ -fold expression difference in cells selected for BCNU resistance, four miRNAs (*hsa-let-7b*, *hsa-mir-125b-2*, *hsa-mir133a-1*, and *hsa-mir-183*) are differentially-expressed in both primary vs. recurrent tumors and BCNU-sensitive vs. BCNU-resistant cells.
- The miRNA *hsa-let-7b* is of particular interest because it has been implicated as a potential regulator of human *RAS* gene expression and it has shown reduced expression in human lung cancers.
- The gene for *hsa-let-7b* has been mapped to 22q12, a region found by our laboratory to be involved in a translocation with chromosome 11q in cells from recurrent tumors.
- Expression of the miRNAs *hsa-mir-125b-2* and *hsa-mir133a-1* are novel discoveries in human cells.

## FUTURE WORK

- Experimental validation of the four miRNAs found to be differentially expressed in this study. miRNA isolation, probe construction, and detection will be performed with Ambion's *mirvana*™ PARIS™ Kit, *mirvana*™ Probe Construction Kit, and *mirvana*™ miRNA Detection Kit, respectively.
- Analysis of expression differences of miRNAs at various time points following *in vitro* selection for therapy resistance to determine the time course of expression of miRNAs in drug-resistance pathways. (Underway.)
- Continuation of global miRNA expression analysis in other malignant glioma cell lines and in cells selected for resistance to the chemotherapeutic agent Temozolomide.

## FURTHER READING

- Calin, G., *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**, 2999-3004.
- Calin, G., *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101**, 11755-11760.
- Johnson, S., *et al.* (2005) *Cell* **120**, 635-647.
- Takamizawa, J., *et al.* (2004) *Cancer Research* **64**, 3753-3756.