IN THE SPOTLIGHT

Discovery in Context: Leveraging Multidimensional Glioblastoma Datasets to Identify Targetable Regulatory Networks

Ivan Babic and Paul S. Mischel

Summary: The Cancer Genome Atlas (TCGA) promises to transform the treatment of patients with cancer by identifying new drug targets. However, extracting mechanistically insightful, therapeutically actionable information from complex multidimensional datasets remains a significant challenge. In this issue of *Cancer Discovery*, Genovese and colleagues apply a context-dependent modeling algorithm to the glioblastoma TCGA datasets and couple it with functional genetic screens and experimental validation to identify a novel, and potentially targetable, microRNA-mediated regulatory pathway. *Cancer Discov; 2(8); 676–8. ©2012 AACR.*

Commentary on Genovese et al., p. 736 (9).

High-throughput genomic technologies are transforming the understanding of human cancer, promising to deliver a pipeline of new cancer drug targets. Where are we now with regard to that goal? In glioblastoma, in-depth genomic analyses have revealed novel mutations (1) and defined molecularly distinct glioblastoma subtypes (proneural, mesenchymal, neural, and classical) that may differ in their biology, and thus, potentially in their drug targets (2). This new molecular taxonomy provides a basis for subsequent studies that have revealed salient aspects of the transcriptional, epigenetic, and proteomic architecture of glioblastoma subclasses (3–7). However, extracting biologically relevant regulatory networks and new drug targets from complex multidimensional cancer datasets remains a significant challenge. Developing context-specific approaches toward mining *in silico* data, coupled to functional genetic screens and experimental preclinical models, remains a central priority for new cancer target discovery.

Glioblastoma is now one of the best genomically characterized types of human cancer. The glioblastoma dataset of The Cancer Genome Atlas (TCGA) includes both coding and noncoding microRNA (miR) expression. MiRs are a class of small noncoding RNA of 19–23 nucleotides that bind to partly complementary base pairs in the 3′-untranslated regions (UTR) of target mRNA. They function in regulating gene expression by either blocking translation of the target mRNA or stimulating the degradation of the transcript. It is estimated that miRs can fine-tune expression of nearly 50% of protein-coding genes. MiRs have been reported to have oncogenic capacity, tumor-suppressive ability, and may also potentially define biologically meaningful disease subsets (8). MiRs can significantly impact regulatory networks and can exert control over gene expression indirectly through regulation of transcription factors. This amplifies/expands the number of genes regulated by miR expression and can drive tumorigenesis.

Most current approaches for identifying miR targets and regulatory networks involve sequence-based computational methods to predict physical association. Such approaches to identify miR–mRNA connections relevant to cancer are based on quantification of linear dependencies between pairwise variables. Important functional regulatory relationships between pairwise variables, including miRs and mRNAs, may not be linear. Sequence prediction–based approaches miss a significant number of critical regulatory interactions, which are mediated through indirect effects. MiR-regulated networks can be expanded by targeting transcription factors, chromatin modifiers, molecules involved in signal transduction, and molecules involved in miR biogenesis and protein translation. Thus, integrating genetic context into nonlinear network models may shed light on functionally important regulatory interactions.

In this issue of *Cancer Discovery*, Genovese and colleagues (9) apply a Context Likelihood of Relatedness (CLR) network modeling algorithm to identify potentially significant miR–mRNA pairs using data from 290 glioblastoma samples from TCGA. Integrative analyses using this method identified a number of potentially significant miR and mRNA interactions worthy of future study, a significant proportion of which may be indirect. However, to further leverage the preexisting knowledge derived from TCGA and to assess the effect of genetic context on these regulatory networks, Genovese and colleagues (9) stratified CLR-inferred global networks by glioblastoma molecular subtype, revealing that nearly 70% of the network edges involved miR and mRNA nodes differentially expressed between proneural and mesenchymal subtypes. This analysis yields a strong prediction that miR–mRNA regulation is likely an important determinant of the biologic phenotype of the proneural and mesenchymal glioblastoma subtypes.

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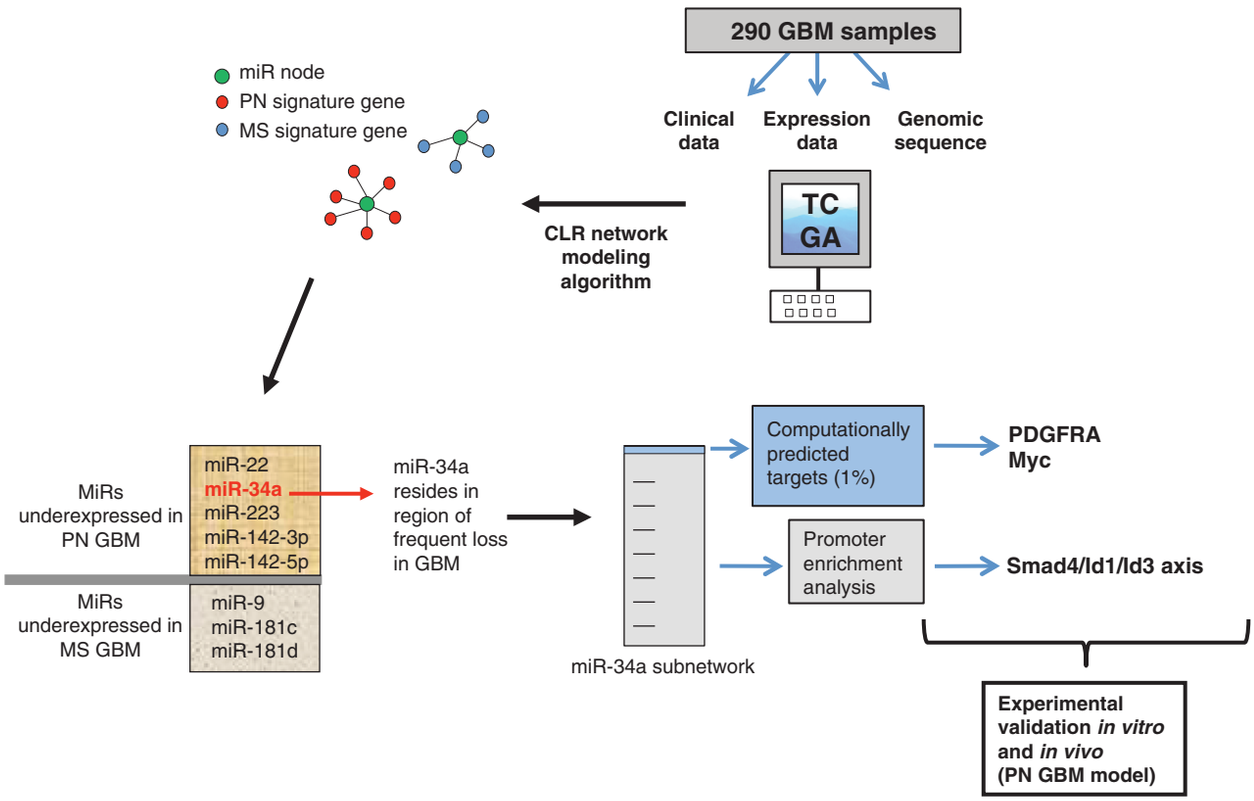

Figure 1. Overview of network modeling derived from complex multidimensional genomic data to identify novel miR–mRNA regulatory networks relevant to glioblastoma (GBM). Leveraging the multidimensional data of TCGA from 290 glioblastoma samples identified putative miR–mRNA regulatory networks by the CLR modeling algorithm. Interrogation of the network within defined proneural (PN) and mesenchymal (MS) subtypes identified 8 underexpressed miRs. *In silico* analyses and experimental validation identified miR-34a as a tumor suppressor in proneural glioblastoma through effects on PDGFRA and the Smad4/Id1/Id3 axis.

Deeper analysis of predicted regulatory networks, focusing primarily on the proneural subtype, showed a potentially important role for miR-34a, which resides in a chromosomal region previously identified by TCGA to be frequently lost in glioblastoma. To validate its potential biologic significance, Genovese and colleagues (9) used a mouse genetic model of proneural glioblastoma harboring a *p53* and *Pten* deletion in neural stem cells and neural progenitors (10). Transcriptomic analysis was conducted to validate the proneural model by presence of a proneural signature in both premalignant embryonic neural stem cells and in malignant tumor spheroids. Reintroduction of miR-34a or miR-142 in the *p53*/*Pten*$^{-/-}$ neural stem cells significantly inhibited tumorigenesis *in vivo*. In addition, knockdown of miR-34a with a 3′UTR stable decoy promoted tumorigenesis in human E6/E7T astrocytes and mouse *Ink4a*/*Arf*/*Pten*$^{-/-}$ as well as *Ink4a*/*Arf*$^{-/-}$;*Pten*$^{-/-}$;*EGFR*$^{VIII/+}$ astrocytes. Thus, miR-34a is effective as a tumor suppressor in the proneural glioblastoma subtype. Initially, network analysis revealed a miR-34a–PDGFRA (platelet-derived growth factor receptor A) node in proneural glioblastoma, and *in vitro* experimentation validated PDGFRA as a direct target of miR-34a.

Interestingly, less than 1% of the miR-34a subnetwork was predicted to be direct, suggesting that the majority of the nodes within the miR-34a network were likely indirect resulting from modulation of transcriptional regulators. An unbiased screen for transcription factor–binding site enrichment at the promoters of CLR-inferred mRNA nodes identified 3 transcription factors: PBX1, Smad4, and Myc. Because PBX1 was not expressed in the human or mouse glioblastoma model systems and because Myc has been described as a valid target of miR-34a, the authors experimentally examined the connection between miR-34a and Smad4. Providing critical functional relevance, Genovese and colleagues (9) show that miR-34a can regulate Smad4 expression and its downstream targets Id1/Id3 and they show that Smad4 expression is sufficient to overcome the tumor-suppressive ability of miR-34a in the proneural glioblastoma model system. Thus, Genovese and colleagues (9) show the potential importance of miR-34a in the proneural subtype of glioblastoma through regulation of PDGFRA and Smad4/Id1/Id3.

The study raises a number of intriguing challenges for researchers aiming to extract biologically meaningful information about regulatory networks from multidimensional cancer genomic datasets. The study reveals the importance of context in mediating the biologic and clinical effects of a regulatory network. In this glioblastoma cohort, increased miR-34a expression correlated with shorter overall survival. This is in contrast to other studies reported for pancreatic and breast cancers, which showed a significant association

between increased expression of miR-34a and favorable prognosis. One conclusion that can be drawn from these disparate results is the critical nature of context in mediating the clinical effects of genetic and epigenetic changes in cancer—a theme likely to resonate in future studies. It is widely recognized that cell context–dependent effects may be important mediators of the impact of changes in miRs, as 3′UTRs can be modified through alternative splicing, thus altering miR targets (11). Well-annotated 3′UTRs may potentially provide valuable additional information toward understanding the biologic impact of changes in miR expression and for defining the prognostic value of miR expression in molecularly heterogeneous cancer subtypes. By showing that the majority of mRNA–miR interactions within glioblastoma are predominantly mediated through indirect mechanisms, including through modulation of transcriptional regulators targeted by a miR, this article raises an important challenge for application of sequence-based prediction algorithms and provides a complementary approach that may be of significant value for regulatory network and novel target discovery.

In summary, Genovese and colleagues (9) provide an important proof-of-concept showing the power of context-based algorithms for *in silico* analysis of complex genomic datasets and highlight the importance of coupling such computational approaches with functional genetic screens and experimental validation to detect novel cancer regulatory networks and new therapeutic targets.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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CANCER DISCOVERY

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