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Systems biology

DIANA-mirPath: Integrating human and mouse microRNAs in pathways

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ABSTRACT

Summary: DIANA-mirPath is a web-based computational tool developed to identify molecular pathways potentially altered by the expression of single or multiple microRNAs. The software performs an enrichment analysis of multiple microRNA target genes comparing each set of microRNA targets to all known KEGG pathways. The combinatorial effect of co-expressed microRNAs in the modulation of a given pathway is taken into account by the simultaneous analysis of multiple microRNAs. The graphical output of the program provides an overview of the parts of the pathway modulated by microRNAs, facilitating the interpretation and presentation of the analysis results.

Availability: The software is available at http://microrna.gr/mirpath and is free for all users with no login or download requirement.

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1 INTRODUCTION

Post-transcriptional regulation of protein coding genes is emerging as one of the new frontiers in modern cellular biology. MicroRNAs (miRNAs) are ~22-nt long non-coding RNAs that play an important role as fine regulators of cellular processes through specific post-transcriptional repression of protein coding genes (Filipowicz *et al*., 2008). MiRNAs have been shown to factor into several physiological and pathological human conditions such as stem cell differentiation (Li and Gregory, 2008), immune response (Bi *et al*., 2009), blood lineage and transformation (Garzon and Croce, 2008), tumor development (Esquela-Kerscher and Slack, 2006) and metastasis (Lujambio *et al*., 2008).

MiRNAs are functionally related with both signaling (Cui *et al*., 2006) and metabolic (Tibiche and Wang, 2008) networks and also extensively interact with transcription factors (Yu *et al*., 2008) through distinct topological patterns, integrating transcriptional and post-transcriptional mechanisms in biological regulatory networks. Despite the growing evidence for miRNA involvement in central biological processes (Zhang and Su, 2009), the systematic integration of miRNAs in biological pathways remains rather incomplete. Currently there are only two miRNA-specific functional analysis tools available. MiRGator (Nam *et al*., 2008) performs a miRNA functional analysis by mapping the predicted targets of a single miRNA in pathways. The source of miRNA target genes used in the analysis may be any of three target prediction programs [TargetScanS (Lewis *et al*., 2005), PicTar (Krek *et al*., 2005) and miRanda (John *et al*., 2004)]. Results are presented in a tabular format sorted by the enrichment *P*-value of each pathway. MiRDB (Wang, 2008) is a miRNA target prediction program which additionally offers precompiled information regarding miRNAs enrichment in a single pathway.

Here we introduce DIANA-mirPath, a web-based application that performs an enrichment analysis of predicted target genes of one or more miRNAs in biological pathways. It is known that miRNAs have multiple target genes and there is strong evidence that some miRNAs can act in concert with each other in order to modulate a molecular pathway (Ivanovska and Cleary, 2008). The combinatorial effect of co-expressed miRNAs in the modulation of a given pathway is addressed by our tool through the simultaneous analysis of multiple miRNAs. MiRNA target genes implicated in a given pathway are graphically annotated on the pathway map providing a direct overview of the miRNA modulated parts, facilitating the interpretation and presentation of miRNA-dependent regulation of biological pathways.

2 METHODS

The input of DIANA-mirPath is a list of miRNA target genes, defined in a user-friendly web interface by simply selecting the miRNA name and the target prediction software of preference. Retrieval of miRNA target genes is automated for the three miRNA target prediction programs that achieved precision levels higher than 60% in a recent comparison (Selbach *et al*., 2008): DIANA-microT (Maragkakis *et al*., 2009), PicTar (Krek *et al*., 2005) and TargetScan (Lewis *et al*., 2005). Alternatively any list of human or mouse miRNA target genes compiled by the user can be used as input by the application. DIANA-mirPath performs an enrichment analysis of the input datasets by comparing each set of genes to all available biological pathways provided by the *Kyoto Encyclopedia of Genes and Genomes* (KEGG) (Kanehisa and Goto, 2000). KEGG is a database resource that provides knowledge about several genomes as well as their relationships to biological systems and has been utilized as a systematic knowledge base for molecular and network

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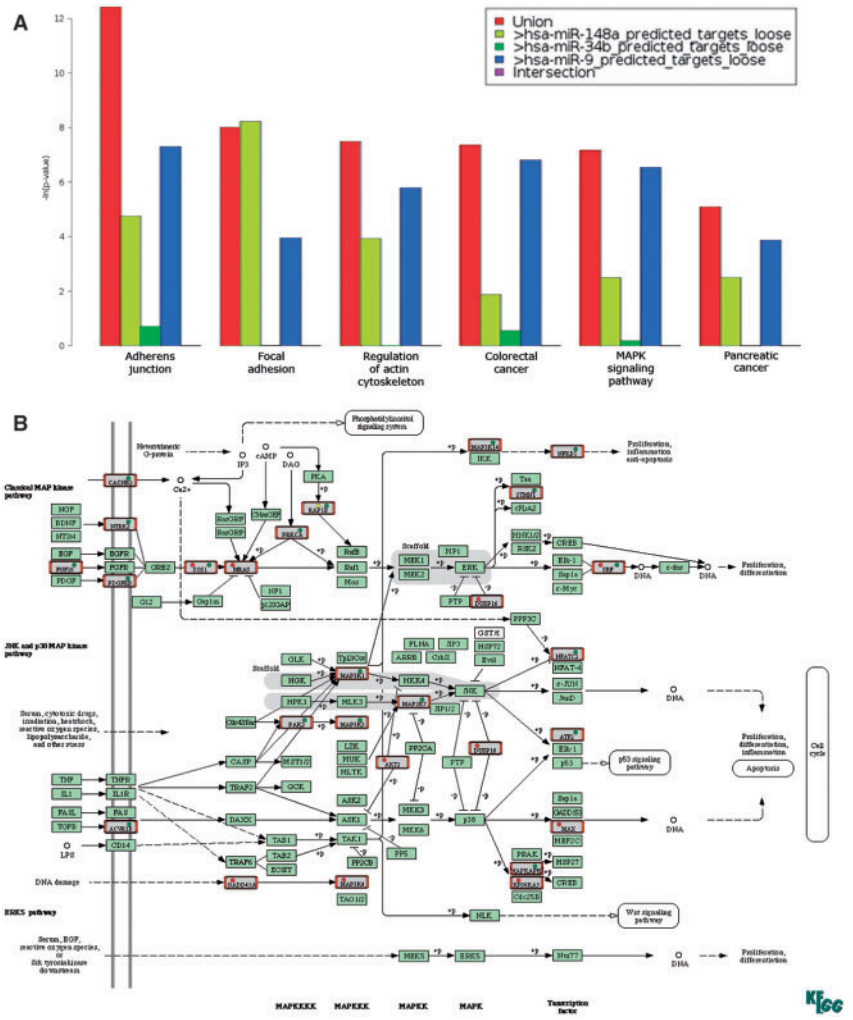

Fig. 1. DIANA-mirPath analysis, based on DIANA-microT 3.0 predictions, applied to explore altered biological processes by the epigenetically mediated silencing of miR-148a, miR-34b and miR-9, associated with human cancer metastasis. (**A**) The combinatorial effect of the miRNA signature is visible in the bar plot graph of the –ln *P* values. The Union dataset –ln *P*s (red bars) are higher than the –ln *P* values obtained for each single miRNA (yellow, green and blue bars) in most of the top targeted pathways. (**B**) The graphical annotation of the MAPK pathway produced by DIANA-mirPath. Targets of different miRNAs are differentiated by a coloured dot in the top of the highlight rectangle for a maximum of three miRNAs, in case of larger input datasets a mouse over option displays the names of miRNAs targeting the selected gene.

biology. Particularly the KEGG PATHWAY Database provides wiring diagrams of interaction and reaction networks between genes. The input dataset enrichment analysis is performed by a Pearson's chi-squared test $\{\chi^2 = \Sigma[(O - E)^2/E]\}$, where O (Observed) is the number of genes in the input dataset found to participate in a given pathway and E (Expected) is the number of genes expected by chance, given the pathway and input list size, to be member of that pathway. The input dataset enrichment in each KEGG Pathway is represented by the negative natural logarithm of the *P*-value (–ln *P*). The algorithm also performs an enrichment analysis of the Union and Intersection sets.

The enrichment *P*-value of the Union dataset in a specific pathway will reflect the coordinated downregulation of the pathway by all co-expressed miRNAs whereas the Intersection dataset gives an overview of the cooperative downregulation of single genes by all of the expressed miRNAs. A bar plot graph of the enrichment –ln *P* values is produced to facilitate the comparison of each pathway enrichment in different datasets (Fig. 1A). In the DIANA-mirPath output page all pathways are sorted according to a descending enrichment statistical score (–ln *P*) along with the number and names of each miRNAs target genes involved in each KEGG Pathway. MiRNA target genes found to be implicated in a given pathway are graphically annotated as an overlay of the pathway wiring diagram provided by the KEGG database and single genes or datasets can be independently highlighted by the user to facilitate the identification of genes or datasets of interest directly on the pathway map.

3 CONCLUSION

DIANA-mirPath is developed in order to estimate the impact of co-expressed miRNAs in biological pathways. As a representative scenario we apply DIANA-mirPath in the functional analysis of

miRNAs associated with human metastatic cancer cells. In Lujambio *et al*. (2008), a DNA methylation-associated silencing of tumor suppressor miRNAs (miR-148a, miR-34b/c and miR-9) was found to contribute to the development of human cancer metastasis. In the same study, transfection of these miRNAs into the metastatic cell lines resulted in a lower capability of migration and less tumor growth. A functional analysis of this miRNA signature performed with DIANA-mirPath identifies both mitogenic and motility pathways to be extensively downregulated by the combined action of these three miRNAs. Top rated pathways involved cell–matrix and cell–cell adhesions, are known to play essential roles in cell motility, invasion and proliferation. Furthermore, the MAPK cascade (Fig. 1B), a highly conserved module that is involved in cell proliferation, differentiation and migration is also found to be significantly modulated by the presence or absence of these miRNAs. In the aforementioned case DIANA-mirPath is able to give a systemic explanation of the two observed phenotypes. In accordance with the particular emphasis given to the analysis of the coordinated modulation of a biological process by co-regulated microRNAs in the development of this tool, the example indicates that the global effect of the downregulated miRNAs might not only depend on single central target genes (i.e. well characterized oncogenes or tumor suppressor genes) but also through modulation of multiple components of proliferative and motility related pathways resulting on a more extended and coordinated downregulation. Given the lack of systematic integration of miRNAs in biological pathways we believe that the development of a tool like DIANA-mirPath can be a substantial aid in the planning and the interpretation of wet lab experiments aiming to infer systemic functions in miRNA expression signatures.

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