A Package for the Genome-wide Dissection of miRNA-mRNA Interactions Applied to the Plasma Cell Differentiation Process

Uva P (1), Mentzen W (1), Orfanelli U (2), Cenci S (2)

(1) CRS4 Bioinformatics Laboratory - Parco Scientifico e Tecnologico POLARIS, Pula (CA), Italy
(2) Division of Genetics and Cell Biology, Lab of Age Related Disorders - San Raffaele Scientific Institute & Università Vita-Salute San Raffaele, Milan, Italy

Motivation

MicroRNAs (miRNAs) have emerged as a mechanism for the regulation of a wide range of biological processes, including cell differentiation and proliferation. Although new miRNAs are continually discovered, the biological role of most of them still remains unclear. One major challenge for the comprehension of miRNA functions resides in the prediction of target mRNAs, which is generally based on physical and evolutionary properties of the target sites. However, all the proposed approaches suffer from the presence of high false positive rates. Since experimental evidences in animals support the role for miRNAs in target degradation rather than translation inhibition, the integration of in-silico target predictions with genome-wide expression profiles of both miRNAs and mRNAs has been adopted as a strategy for the detection of functional miRNA-mRNA interactions. Available tools have been developed for the integrative analysis of miRNA-mRNA expression data (MAGIA, MMIA) providing easy-to-use solutions for experimental researchers. Unfortunately such tools are not tailored for the integration in more complex analysis workflows and, most importantly, they are focused only on miRNA predictions in human. Here we describe an R package for the integrative analysis of miRNA and mRNA expression profiles. The software provides a ranked list of relevant miRNAs and an annotated list of potential targets. The package also generates a miRNA-pathway chart that maps miRNAs to the general biological functions shared by their targets and indicates the presence of coordinated miRNA targeting of functionally related genes.

Methods

The analysis workflow starts from the prediction of miRNA target sites. Updated predictions based on miRBase v16 for human (hg18), mouse (mm9) and rat (rn4) genomes obtained with the PITA algorithm are currently available. Predictions have been filtered for target site conservation based on phastCons conservation scores. Predictions generated by additional algorithms can be easily integrated. MiRNAs were ranked by correlation with their predicted targets, and statistical significance of miRNA-target interactions was assessed by comparison to random targets. Results are available in a HTML
report containing the selected miRNAs, annotated lists of targets, plots displaying the miRNA-mRNA expression profiles at different correlation cutoffs, and statistical p-values from random permutations. Finally, we generated a miRNA-pathway map where miRNA functions were inferred from the significant association between target genes and biological annotations (KEGG, GO Terms). For each pathway, we also defined a ‘cotargeting score’ for the presence of multiple miRNAs targeting the same gene, and a ‘cooperative score’ for the evidence of miRNAs targeting functionally related genes within the same pathway. Being developed in R, the package allows for easy integration with additional target predictions and user defined gene set collections for generating more focused miRNA-pathway maps.

Results
We applied our software to the analysis of matched mRNA-miRNA expression profiles obtained from mouse primary in-vitro B to plasma cell differentiation. Upon binding to a specific antigen, B cells activate a complex program of differentiation leading to specialized short-lived antibody-secreting plasma cells. The molecular events causing cell death in short-lived plasma cells are tightly linked to antibody production, with a decrease of the proteasomal activity, onset of apoptosis and sensitization to proteasome inhibitors (PI). PI recently proved powerful against many tumors, with exquisite potency against multiple myeloma (MM), a frequent and still incurable plasma cell malignancy. However, why MM is so sensitive, and how MM becomes resistant are all unresolved issues. By offering a cellular model of progressive proteostenosis linked to accelerated senescence, plasma cells enable the dissection of the molecular mechanisms of how proteostasis, cellular senescence and lifespan control intertwine. MiRNAs may contribute to basal and acquired PI resistance which prompt us to define more accurately the role of miRNAs in B to plasma cell differentiation in order to unveil novel strategies against age-related diseases. Application of the software to the dataset allowed for prioritization of a dozen of miRNAs potentially involved during plasma cell differentiation, with a number of filtered targets ranging from 20 to 200. Interestingly, the miRNA-pathway map highlighted several plasma cell differentiation specific pathways that were coordinately targeted by multiple miRNAs. Experimental validation of proposed miRNA-mRNA interactions is in progress.

Contact email
paolo.uva@crs4.it