Reprogramming of miRNA networks in cancer and leukemia

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Motivation

miRNAs are global regulators of protein output. Much of the current effort in miRNA studies is focused on the elucidation of their function. Typically miRNAs have been studied by using the gene profiling approach. Each miRNA has been studied for its single contribution to differential expression or to a compact predictive signature. However the effect of microRNAs on cell pathology and physiology is likely to be complex for two reasons: 1)their activity is exerted in a one-to-many fashion, such that each miRNA can control translation of tens or even hundreds of different coding messengers and 2)a single messenger can be controlled by more than one microRNA. Thus, we propose a paradigm shift to the study of miRNAs in cancer by applying a systems biology approach. For this purpose we built miRNA gene networks by using our very large expression miRNA database. We studied miRNA profiles in 4419 human samples (3312 neoplastic, 1107 non-malignant), corresponding to 50 normal tissues and 51 cancer types. We studied tissue specificity and cancer type specificity. The complexity of our expression database enabled us to perform a detailed analysis of coordinated miRNA activities. We inferred genetic networks directly from miRNA expression data in normal tissues and cancer. We also built, for the first time, specialized miRNA networks for different solid tumors and leukemias.

Methods

Data analysis and Network generation. An SQL miRNA internal database was built with the data retrieved from a large number of different experiments performed in our laboratory. All the results were log2-transformed. The Normalization was performed by using the quantiles normalization, as implemented in Bioconductor "affy"" package. BRB Arraytools was used to perform t-test over 2-classes experiments of F-tests over multiple classes. Target genes selection was performed by DIANA-miRpath, microT-V4.0. The union of the target mRNAs with a score above 3 was used as an input to ClueGO. ClueGO was used to relate differential expression in cancer to functional pathways (KEGG).The network integrates only the positive kappa score term associations and is automatically laid out using Organic layout algorithm supported by Cytoscape. Banjo was used to infer the Bayesian network for the different tissues and diseases. For each tissue or disease all the mature expressed and varying miRNAs were used as input to

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Banjo. The expression values were preprocessed with Gene Pattern to only filter out non varying miRNAs. The static Bayesian network inference algorithm was run on the miRNA expression matrix by using standard parameters. Consensus graphs, based on top 100 networks, were obtained from at least 8 x109 searched networks. We applied the MCL graph-based clustering algorithm to extraction of clusters from miRNA networks. MCL (Neat) has been shown to enable good performances in extracting co-regulated genes from transcriptome networks. yEd graph editor was employed for graphs visualization. Microarray analysis was performed as described by Liu et al, Nature Protocol, 2008. aCGH. 744 comparative genomic hybridization arrays were studied. All platforms were 2-channel based, data were downloaded as normalized values, and genes were annotated according to the gene symbol.

Results

By combining differential expression, genetic networks and DNA copy number alterations, we confirmed, or discovered, a host of miRNAs with comprehensive roles in cancer. We have presented a thorough analysis of miRNA tissue specificity in 50 different normal tissues grouped by 17 systems, corresponding to 1107 human samples. A small set of miRNAs were tissue-specific while many others were broadly expressed. We also studied 51 oncologic or hemato-oncologic disorders and identified cancer type-specific miRNAs. Then we inferred genetic networks for miRNAs in normal tissues and in their pathological counterparts. Normal tissues were represented by single complete miRNA networks. Cancers instead were portrayed by separate and unlinked miRNA sub-nets. Intriguingly, miRNAs independent from the general transcriptional program were often known as cancer-related. This “egocentric” behavior of cancer miRNAs could be the result of positive selection during cancer establishment and progression, as supported by aCGH. Finally, we experimentally validated the miRNA network with ALL originated in Mir155 transgenic mice. Most of miRNAs deregulated in these transgenic mice were located close to hsa-miR-155 in the cancer network. The dissimilar behavior of solid cancers and leukemia might be due to the diverging pathogenetic mechanisms that include differing oncogenic miRNA networks. In the former complex chromosomal aberrations are frequent, whereas in the latter translocations often represent the major driving force. Overall, miRNA networks in cancer cells defined independently regulated miRNAs. The target genes of these uncoordinated miRNA were involved in specific cancer-related pathways.

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