Molecular Systems Biology 8; Article number 569; doi:10.1038/msb.2012.2
Citation: ***Molecular Systems Biology*** **8:569**

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molecular systems biology

NEWS AND VIEWS

miRNAs versus oncogenes: the power of social networking

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Molecular Systems Biology **8**: 569; published online 14 February 2012; doi:10.1038/msb.2012.2

microRNAs (miRNAs) are small, non-coding RNAs that regulate the expression of proteins through specific target sites in the corresponding transcripts. These small RNAs may function as oncogenes or tumor suppressors by modulating the levels of critical proteins, and their relevance in human disease and therapy is now under intense investigation. The human genome encodes about 1500 miRNAs that are thought to regulate more than 30% of protein-coding genes. Individual miRNAs can target multiple genes and each protein-coding gene can be regulated by several miRNAs, making the analysis of these networks difficult to explore. In a recent article published in *Molecular Systems Biology,* Uhlmann *et al* (2012) report a combined strategy to analyze the multiple miRNA–protein interactions that regulate cell proliferation in response to epidermal growth factor receptor (EGFR), an oncogenic pathway highly relevant in breast cancer. This analysis provides an unprecedented view of the combinatorial effort of miRNAs to control a signaling pathway at different levels. As oncogenic pathways are often resistant to the inhibition of individual regulators, this analysis also provides the molecular basis for selecting individual miRNAs, or a set of a few miRNAs, whose combined activity may be strong enough to treat breast tumors.

Activation of the EGFR drives a signaling network that can lead to cell proliferation, survival, angiogenesis, invasion and metastasis. Aberrant expression or activity of EGFR has been strongly linked to the etiology of several human epithelial cancers, including lung, colorectal and breast cancer, among others (Wheeler *et al*, 2010). Not surprisingly, the EGFR pathway is probably the most explored next-generation target for breast cancer therapy, and several downstream signal transducers, such as RAS, AKT or CDKs, are also currently considered as cancer targets (Weickhardt *et al*, 2010; Wheeler *et al*, 2010). The EGFR pathway controls the expression of several miRNAs (Avraham *et al*, 2010; Garofalo *et al*, 2011), and some transcription factors and cell cycle regulators downstream of EGFR are known to be regulated by miRNAs at different levels (Avraham *et al*, 2010; Bueno and Malumbres, 2011). However, current information is scarce and typically describes single interactions between a miRNA and a relevant target transcript. Uhlmann *et al* (2012) used a genome-wide library of miRNA mimics to test their effect on the expression of 26 proteins in the EGFR-driven cell cycle pathway using reverse-phase protein arrays. Their findings validated some of the miRNA–protein interactions already known, such as the control of KRAS by miR-143 or the regulation of EGFR by miR-7 (Chen *et al*, 2009; Webster *et al*, 2009). In addition, other phenotypic interactions were found between 241 miRNAs and 25 EGFR network transcripts, revealing a total of 355 edges connecting them (Uhlmann *et al*, 2012).

The high density of connections led the authors to investigate possible associations among the identified inter-

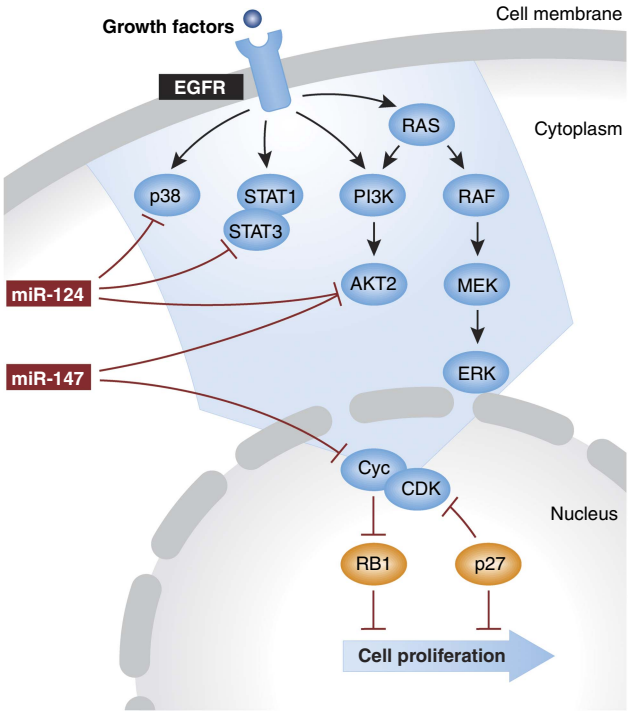

Figure 1 Control of the EGFR pathway and cell cycle entry by miRNAs. The EGF receptor uses several downstream pathways to drive cell cycle progression. Similarly, miRNAs simultaneously modulate several of these downstream pathways to effect cell cycle progression. For instance, miR-124 co-down-regulates AKT2, p38MAPK and STAT3 to inhibit the EGFR pathway leading to CDK downregulation, $p27^{Kip1}$ upregulation and cell cycle inhibition. miR-147 also participates in AKT2 downregulation and simultaneously represses Cyclins (Cyc). These two examples show how a single miRNA can target several components of a signaling pathway and how two miRNAs can co-regulate the same target. These data also suggest the therapeutic value of miR-124 and miR-147 in treating EGFR-dependent tumors. Only a few representative molecules are shown for clarity.

actions. Using a novel framework for complex network analysis, they found that proteins controlling the EGFR-driven G1/S transition are frequently co-regulated by several miRNAs (Figure 1). For instance, independent expression of 19 miRNAs led to the concomitant downregulation of the cyclin-dependent kinase (CDK) 4 and upregulation of $p27^{Kip1}$, a known CDK inhibitor, and both events likely contributed to cell cycle inhibition by these miRNAs (Uhlmann *et al*, 2012). The fact that the retinoblastoma protein was also concomitantly downregulated may seem surprising given the antiproliferative function of this molecule. The use of reporter assays on specific 3′-UTRs indicated that these changes were indirect and associated with direct co-repression of several EGFR core proteins. Thus, miR-124 was able to directly downregulate AKT2, p38MAPK and STAT3, probably resulting in the observed changes in cell cycle regulators (Figure 1). miR-124, miR-147 and miR-193a-3p showed the highest score in the network analysis, and their expression has a significant effect in the EGFR pathway by simultaneously targeting several of its components in several different cell lines (Uhlmann *et al*, 2012). As the expression of these miRNAs ultimately results in cell cycle inhibition, these three miRNAs may be relevant tumor suppressors by co-targeting several EGFR network proteins.

The fact that individual miRNAs may target several components of a single signaling pathway seems reasonably obvious. However, clear examples of this type of multi-level regulation are rare in the literature, probably due to current technical challenges, including difficulties in detecting miRNA–protein interactions with moderate effects. The study by Uhlmann *et al* (2012) now provides an experimental framework to detect multiple interactions in a single pathway that may be extended to a higher number of proteins or to other pathways. The analysis of endogenous miRNAs, rather than overexpressing exogenous sequences, and the separation of direct from indirect interactions remain as major challenges in these massive studies.

A further level of complexity that needs to be clarified is how the combinational effect of several miRNAs can regulate the levels of a single protein. Although both miR-124 and miR-147 target AKT2 (Uhlmann *et al*, 2012; Figure 1), the relevance of this co-regulation deserves further analysis. Is it additive or simply redundant? Furthermore, the $p27^{Kip1}$ family member $p21^{Cip1}$ may be regulated by 28 miRNAs (Wu *et al*, 2010), and MYC, a major oncogene in several pathologies, can be targeted by nearly a dozen miRNAs with additive effects (Bueno *et al*, 2011). The fact that miRNA-target sites in many proteins are not evolutionarily conserved (Bueno *et al*, 2011; Uhlmann *et al*, 2012) suggests that the combinatorial effect of several miRNAs, rather than a single miRNA, may be the critical factor for modulating the levels of specific oncogenes.

In general, it seems that individual miRNAs affect the expression of many proteins, even in the same pathway (Uhlmann *et al*, 2012), but in a rather mild manner. In addition, one gene can be regulated by several miRNAs (Wu *et al*, 2010; Bueno *et al*, 2011). Researchers need to use new technical approaches, such as the one reported by Uhlmann *et al* (2012), to characterize multiplicity, redundancy and the additive effect of miRNAs in these complex networks. These studies could identify miRNAs that may be efficient in the inhibition of a key pathway or even in the simultaneous inhibition of several pathways regulating cell survival and proliferation. The fact that AKT1 and ERK2, two major kinases in the PI3K and RAS oncogenic pathways, may be co-downregulated by 30 miRNAs (Uhlmann *et al*, 2012) is certainly a promising starting point that highlights the therapeutic potential of this approach. miRNAs seem to function like short, additive or redundant tweets with multiple targets, and it is now obvious that 'social' networking is essential for the effect of these sequences in cell signaling. We now need to identify the relevant networks and validate their social utility.

Conflict of interest

The author declares that he has no conflict of interest.

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