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Preview

The Epithelial-Mesenchymal Transition, as Hacked by a microRNA Combinatorial Code

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A new study coupling bioinformatic and experimental investigations highlights the importance of combinatorial microRNA targeting in human EMT, a phenotypic program underlying normal and pathological processes.

The vast majority of our genome encodes for non-protein-coding molecules, whose precise functions are mostly unknown. Among these, microRNAs (miRNAs), a class of small endogenous regulatory non-coding RNAs, have emerged in the last 20 years as major non-coding RNA species. The current paradigm for miRNA function is that miRNAs, as a whole, exert their function through a labyrinth of combinatorial interactions. However, most studies have focused their attention on single miRNA-target gene interactions, thereby neglecting the intrinsic combinatorial nature of gene regulation in higher eukaryotes. Frequently, these studies also adopted experimental protocols which require molecular concentration levels far from the physiological ones.

In this issue of *Cell Systems*, Joe Cursons, Cameron Bracken, Melissa Davis, and their colleagues represents a departure from this standard approach. Using a combination of bioinformatics, high-throughput sequencing, and functional validation, Cursons and colleagues were able to precisely describe the behavior of a circuit composed by a set of miRNAs acting combinatorially at molecular concentration close to cellular endogenous levels (Cursons et al., 2018). Their focus is the human epithelial-mesenchymal transition (EMT), an important biological switch implicated both in development and diseases.

MiRNAs are a class of tiny ~22 nt RNAs whose primary function is to manage post-transcriptional repression of mRNA targets. Pervasive in the human genome, with more than 2,000 examples currently annotated, their primary action is thought to be RNA silencing and translational inhibition of target protein-coding genes (Bartel, 2018).

Both computational as well as experimental clues support the notion that over half of the human transcriptome is regulated by miRNAs. At the same time, all the miRNA target prediction tools, developed over many years, despite the inherent differences arising from the diversity of the algorithms used, suggest that a typical miRNA can repress possibly hundreds of target genes. Conversely, a single gene can be regulated by several miRNAs. Together, this suggests the presence of a complex combinatorial regulatory code embedded in the human post-transcriptional regulatory network (Krek et al., 2005; Tokar et al., 2018).

In this context, recent studies have focused on the interplay between miRNAs and transcription factors (TFs) and in particular on their mutual involvement in complex circuits with feed-forward or feedback loop topologies. This class of regulatory motifs have an important role in driving differentiation processes and are involved in various aspects of physiology and disease, including cancer (Bracken et al., 2016).

Among these regulatory circuits, one of the most studied is the feedback loop which involves TFs of the Zeb family and miRNAs of the mir-200 family which together control the so called epithelial to mesenchymal transition, a critical step in development and embryogenesis, which has also a major role in tumorigenesis. EMT represents the key process by which differentiated epithelial cells can change their status and gain the ability to invade, to resist apoptosis, and eventually to disseminate leading to metastasis formation (Gregory et al., 2008; Hanahan and Weinberg, 2011).

Given this complexity, how does one test the role of miRNAs in regulating the EMT experimentally? Cursons et al. combined computational and experimental methods to identify co-regulated miRNAs that have the potential to cooperate during EMT and they demonstrate that the combinatorial activity of co-regulated miRNAs is an intrinsic property of post-transcriptional network regulation. Using a human mammary cell model of EMT, Cursons et al. provide evidence that miRNAs can act as a secondary regulatory layer after transcription, amplifying transcriptional effects on relevant EMT-associated processes (such as cell-adhesion and extracellular matrix organization) while simultaneously buffering transcriptional effects on non-EMT genes. They particularly pinpoint that a set of miRNAs, composed by miR-200c-3p, miR-141-3p, miR-182-5p and miR-183-5p, are able to cooperate in a combinatorial manner, through co-regulation and cooperative targeting of functionally related transcripts, even when operating in sub-nanomolar concentrations in the context of EMT.

The main idea of the article (Figure 1) is that co-expressed miRNAs that jointly target multiple genes in a common pathway enhance their common function, a notion the authors addressed also in previous studies (Bracken et al., 2016) and is at the core of the present paper. Strikingly, the authors were able to show that co-transfection with low levels of miR-200 and miR-182/183 had a co-operative effect on epithelial gene expression, and an almost negligible effect on off-target mRNAs. These observations

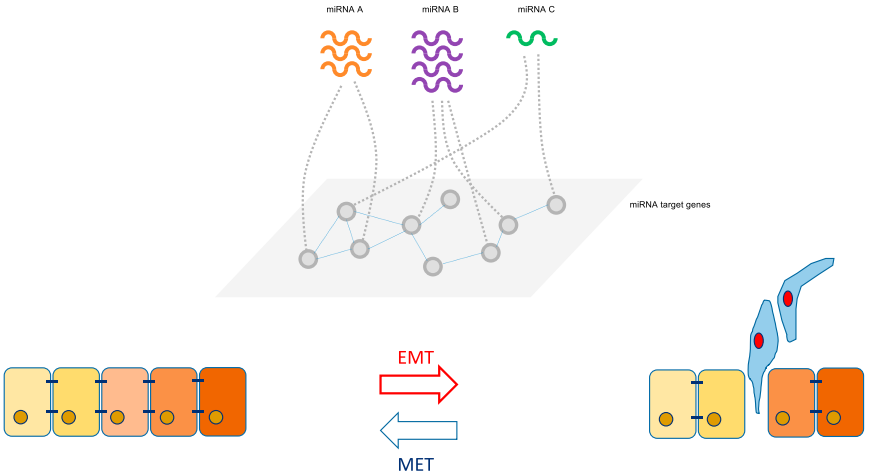

Figure 1. A Set of miRNAs Acting in a Combinatorial Manner on a Network of Target Genes Coordinates Post-Transcriptional Control of EMT

Within a cell, co-regulated miRNAs can act in a combinatorial manner on a set of target genes, usually embedded in a network of interactions, eventually interfering with complex phenotypic patterns. Cursons et al. investigated the cooperative role of a combination of few miRNAs, including the miR-200 and miR-182/183 family members, in the post-transcriptional regulation of EMT (human epithelial-mesenchymal transition), an important biological switch implicated both in development and disease. In particular, they proved that the combinatorial treatment could alter the cellular phenotype with miRNA concentrations closer to physiological levels and with less off-target effects.

support a model in which miRNAs exert their action via the collaborative network of several targets, and at the same time, due to the moderate level of concentration do not influence the expression of unrelated genes. The approach by Cursons and co-workers, goes well beyond the particular EMT pathway to which it has been applied. It points out a new powerful way to investigate the properties of the "real" miRNA post-transcriptional networks (Pinzón et al., 2017) and could be extrapolated, in principle to other model systems.

The work of Cursons et al. could also facilitate the building of fine-tuned, reliable, and quantitative models of miRNA regulation along the line of what was done in the past years for transcriptional regulation (Bintu et al., 2005). Such models would allow one to address a few long-standing questions in miRNA biology like the interplay between the number of miRNA binding sites (and of miRNA species) and their strength or the relevance of the so called "sponge effect" due to the presence of competing endogenous RNA (ceRNA).

One major outstanding question is whether it is more effective for the miRNA to bind the target messenger RNA with several weak binding sites or with a single strong one. A complete answer to this question requires not only careful models of miRNA-mRNA interaction but also reliable experimental data in physiological conditions. Understanding this fundamental question would represent a major achievement in basic science. It could also have potential clinical applications, which now seem within reach, thanks to the results of Cursons and co-workers. We hope that future experimental and computational work will substantiate and broaden this design paradigm.

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