Recognizing the Breadth and Biological Significance of a Mechanism Underlying MicroRNA Diversity

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MicroRNAs (miRNAs) are short ribonucleic acid molecules that regulate the expression of a gene after it has been transcribed into messenger RNA (mRNA). This form of post-transcriptional regulation is accomplished when miRNA binds to complementary sequence on its target mRNA. Depending on the miRNA and target, the effect is usually repression of translation or mRNA degradation, yet positive regulation may also be possible. The human genome encodes more than 1,000 miRNAs targeting at least half of all genes, and miRNAs appear to be involved in myriad biological processes. Their abnormal expression has been implicated in a number of diseases, and some miRNA-based cancer therapies are already under investigation. In addition to the large number of canonical miRNAs, addition of one or a few nucleotides to the 3' end of any given miRNA may modify its stability and activity. In humans, such nucleotide additions are thought to be primarily accomplished by just three nucleotidyl transferases (PAPD4, PAPD5 and ZCCHC11). Widespread occurrence of variable 3' nucleotide additions to miRNAs has been observed in high-throughput sequencing datasets. However, the degree to which this sort of miRNA diversity is biologically meaningful, or simply the result of artifacts during transcriptome sequencing, has remained uncertain.

Significant new glimpses into the depth and biological importance of 3' miRNA nucleotide additions have been made in a paper published by co-leading authors Stacia Wyman and Emily Knouf (Human Biology Division), senior author Muneesh Tewari (Human Biology Division) and three other researchers affiliated with Fred Hutchinson Cancer Research Center (Rachael Parkin, Daniel Lin and Brian Fritz). Contributions were also made by collaborators at Seattle's NanoString Technologies. In carefully controlled sequencing experiments, Wyman, Knouf and colleagues demonstrated that 3' nucleotide additions occur physiologically (and often) in normal tissue. The research team then adapted NanoString Technologies' nCounter assay for the high-throughput quantification of 3' miRNA variants. Using their new approach, Wyman *et al*. detected miRNA variants that changed dynamically during the differentiation of human embryonic stem cells,

exemplifying that 3' miRNA variation is biologically regulated. Lastly, the researchers used RNA interference to individually suppress eight candidate nucleotidyl transferases in colon-cancer cell culture, again employing their adapted protocol to monitor 3' miRNA variants. Seven enzymes affected 3' variation, three of which (MTPAP, ZCCHC6 and TUT1) were not known previously to modify miRNAs. This impressive body of work illustrates that 3' nucleotide addition is a much more common and biologically meaningful mechanism of generating miRNA transcriptome complexity than previously recognized. The authors' findings will likely motivate new research into this facet of post-transcriptional gene regulation. Their identification of a greater diversity of relevant nucleotidyl transferases may also contribute to future miRNA-based cancer therapies.

Wyman SK, Knouf EC, Parkin RK, Fritz BR, Lin DW, Dennis LM, Krouse MA, Webster PJ, Tewari M. 2011. Post-transcriptional generation of miRNA variants by multiple nucleotidyl transferases contributes to miRNA transcriptome complexity. *Genome Research* 21:1450-61.

Photo by Dean Forbes

Principal investigator on the study, Dr. Muneesh Tewari of the Hutchinson Center's Human Biology Division.