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Ago-associated human usRNAs and other similar small RNAs

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We report the characterization of several subclasses of unusually small RNAs (usRNAs) in human and viral genomes. Prior to our work, two popular, yet incorrect presumptions led to all small RNAs less than 20 bases in length (usRNAs) being thought of as transient degradation products of longer RNAs or as RNAs that cannot be reliably mapped to the genome. In this work, we show that while 100% of usRNAs cannot be mapped reliably to the genomes, ~16% of RNAs as small as 15 bases can be reliably mapped to the genome, corresponding to thousands of new small RNAs. We demonstrate that these 5'-phosphate containing usRNAs are accurately, reproducibly and repeatedly, produced across various tissues, and have very unique biological characteristics, thus contesting the hypothesis that these RNAs can be ignored as transient degradation products.

Our observations began with the identification of a Kaposi sarcoma-associated herpesvirus us-K12-1 RNA (17 bases) that is as effective as the K12-1 miRNA in regulating human RAD21, a novel K12-1 target. High-throughput sequencing followed by bioinformatics analysis and molecular profiling reveals a diverse set of human miRNA-derived usRNAs and other non-miRNA-derived usRNAs. Human miRNA-derived usRNAs preferentially match to 5' ends of miRNAs, and are also more likely to associate with the siRNA effector protein Ago2, than Ago1. Many non-miRNA-derived usRNAs associate with Ago proteins and also frequently contain C-rich 3'-specific motifs that are highly position specific, and are overrepresented in comparison to piRNAs and TSSa-RNAs. In summary, our observations suggest that ~ 30% of usRNAs could have evolved to participate in biological processes including gene-silencing.

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