OF MIRTRONS AND 3’ MIRNA ISOFORMS:MICRORNAS FORMED BY ALTERNATIVE PATHWAYS

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Introduction:microRNAs (miRNAs) are non-coding RNA molecules of 20-30 nucleotides in length. They form an extensive regulatory network similar to that of transcription factors.In animal cells, most miRNAs are believed to use the “canonical” pathway involving the Drosha/DGCR8 complex and Dicer. However, recent investigations revealed several alternative maturation routes that bypass either of the two cleavage steps of the canonical pathway. The most prominent Drosha-independent pathway is the mirtron pathway which was first described in invertebratesand relies on the splicing machinery. However, due to the long average intron length, it was not obvious whether this pathway existsin higher organisms. In addition to alternative maturation mechanisms, the alternative usage of miRNA arms and the diversity of the 5’/3’ sequence of miRNAs can also increase the complexity of miRNA regulation. We are investigating the existence and the role of the mammalian mirtron pathway, as well as the 3’ isomir diversity of human miRNAs.

Methods: By expressing natural and artificial miRNA constructs in mammalian cells, we measure the level of miRNAs by Northern blot and qRT-PCR. We use 3’ isoform specific assays to detect different miRNA species from the same locus; we also apply luciferase assays to test the function of the mature miRNAs.

Results: We could prove that the mirtron pathway indeed exists in higher vertebrates, including humans. We showed that predicted mirtronic miRNAs are formed independently of the Drosha/DGCR8 complex, using the splicing apparatus of the cells. Moreover, the flanking exons do not influence functional mirtrons, provided that the sequences are splicing-competent. In addition, we provided evidence for the first time that functional miRNAs can be formed simultaneously from both arms of the hsa-miR-877 mirtron locus. Finally, we revealed that several miRNA species exist in various 3’ isoforms which can severely influence detection accuracy by qRT-PCR and may represent different regulatory functions.

Conclusions: Our results indicate that the miRNA repertoire and variability in the cells are far more complex thanpreviously anticipated. Nevertheless, it has to be emphasized that although bioinformatic predictions are useful as investigative pre-screens, they must always be followed by experimental verifications.

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