Understanding the 'sperm RNA code' for epigenetic inheritance with emerging tools

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Summary

Recent discoveries have unraveled a novel layer of inheritance, revealing that mammalian sperm convey not just genetic information via DNA but also carry diverse RNA molecules that can mediate the intergenerational transmission of environmental experiences (e.g. diet, mental stress, endocrine disruptors, and toxins) beyond DNA sequence. Our understanding of the categories of sperm RNAs (e.g., miRNAs, tsRNAs, rsRNAs, lnRNAs), their associated RNA modifications, and their spatial compartmentalization is now expanding and has begun to reveal the functional diversity and information capacity of these molecules, which support the concept of 'sperm RNA code' in programming specific offspring phenotypes during embryonic development. Here I discuss the challenges and opportunities in solidifying the field of mammalian sperm RNA-mediated epigenetic inheritance, including the identification of the key sperm RNAs that are responsible for the paternal phenotype transmission, and the cellular and molecular events that are triggered by sperm RNAs during embryo development. Complete deciphering of the 'sperm RNA code' with regard to various paternal environmental exposures could move the field towards translational applications and precision medicine.

During the exploration of sperm RNA code, we realized that both tsRNAs and rsRNAs are highly modified. Some of the RNA modifications they carry will prevent their detection by using traditional RNA-seq, thus generating substantial bias in their discovery. To conquer this problem, we have developed a new method *PANDORA-seq,* to overcome RNA modifications that prevent sncRNA detection in traditional RNA-seq, showing that the previously undetectable, highly modified tsRNAs/rsRNAs are even more dominant in sperm than we previously thought when using traditional sncRNA-seq. Importantly, using PANDORA-seq we have further found that the signature of mammalian sperm sncRNAs is spatially organized, with unique tsRNA & rsRNA composition in the sperm heads compared to that of whole sperm, which provides important clue regarding undiscovered tsRNA/rsRNA function in the nuclear compartment.

The abundant existence of highly modified tsRNAs/rsRNAs has raises new questions about the functional impact of RNA moderations, which has made the deciphering of site-specific modifications on tsRNA/rsRNA an imminent task, as these modifications would hold key to their functionality such as in the case of sperm RNA-mediated epigenetic inheritance. We are currently using a novel sequencing method *MLC-Seq,* a novel mass spectrometry-based direct sequencing methods enabling the simultaneous identification of both RNA sequence & RNA modifications of small RNAs (e.g., tsRNAs and rsRNAs) with stoichiometric precision, which would be valuable for further in-depth exploration of sperm tsRNA/rsRNA functionality.

The emerging concept of sperm RNA code and the new tools developed here present a transformative opportunity not only in the study of sperm mediated epigenetic inheritance of paternal traits, but also in exploring the roles of highly modified small RNAs in a wide range of physiological and disease conditions.

Related reference:

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