MiR-202 regulates female fecundity by controlling early follicle development in medaka ovary

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POSTER 60

MIR-202 REGULATES FEMALE FECUNDITY BY CONTROLLING EARLY STEPS OF OOGENESIS IN THE MEDAKA OVARY

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Introduction

In fish, the female fecundity is closely linked to the proper completion of oogenesis that takes place in the ovary. An increasing number of studies in mammals have recently provided evidence that small non-coding microRNAs (miRNAs) play an important role in the regulation of this cellular process. However, data related to the role of miRNAs in the fish ovary remain rather scarce and *in vivo* functional evidence of the role specific miRNAs in the fish ovary has never been provided to date. In the present work, we aimed at studying the role(s) of *miR-202*, a miRNA specifically expressed in the vertebrate gonads, in the medaka ovary.

Methods

We first used fluorescent *in situ* hybridization to determine the precise cellular expression of *miR-202* in the medaka ovary. We then generated a mutant fish line (CRISPR/Cas9) to analyze its role in female fecundity and oogenesis. Phenotype of mutant females was analyzed at both cellular and molecular levels. Fluorescent imaging (2D and 3D) and image processing strategies were developed to analyze the cellular modifications. A genome-wide transcriptomic analysis (microarrays) was performed on mutant ovaries to analyze gene expression modifications.

Results and Discussion

Our results showed that *miR-202* is highly and specifically expressed in granulosa cells of ovarian follicles at all stages. Analysis of mutant females revealed that the inactivation of *miR-202* drastically reduced the fish fecundity (reduced number of spawned eggs) and impaired the egg quality. Further image analyses of mutant ovaries showed that early steps of follicular growth are affected, leading to an accumulation of small follicles and to a lower number of larger follicles. While data obtained by transcriptomic analysis were consistente with this cellular phenotype (*i.e.* down-regulation of genes encoding key steroidogenic enzymes such as *cyp19a1a* and *cyp17*), it also revealed the down-regulation of key genes expressed in early meiotic oocytes within the germinal cradle, including *foxl3* and *sycp3*. Finally, results shed light on novel molecular players that are dysregulated in mutant females, such as *setd4*, *npr1b* and *srgap3*.

Conclusion

Here, we provide evidence that *mir-202* is a key mircoRNA necessary for female reproductive success in medaka. Our results indicate that *mir-202* is necessary for the early steps of oogenesis. Thus, this study largely contributes to elucidate the regulatory mechanisms that control the early steps of oogenesis, in particular the follicle recruitment that remains poorly understood to date.

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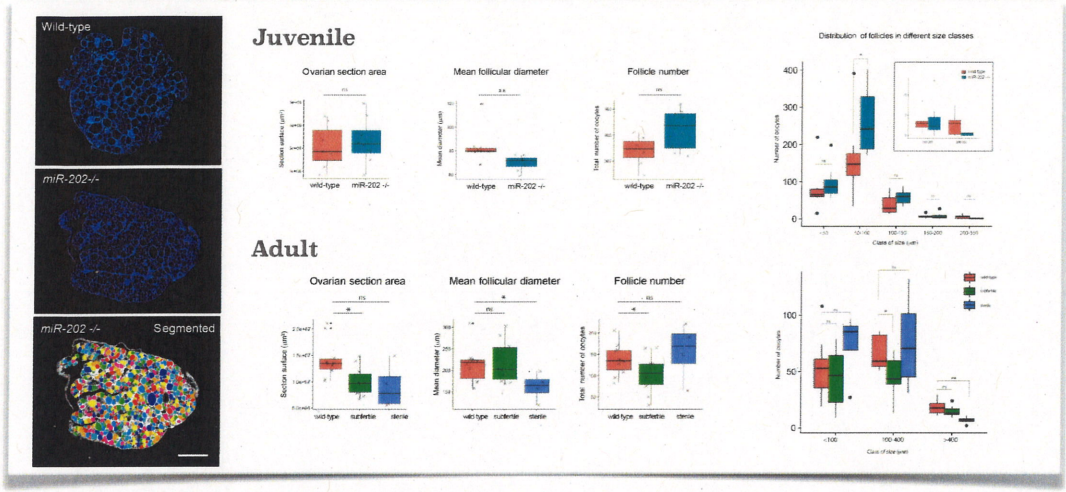
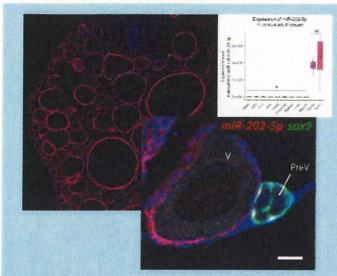


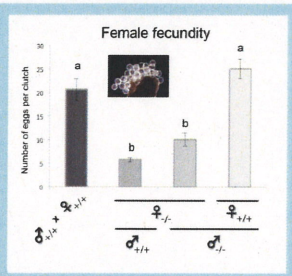
KO of mir-202 drastically reduces the number and quality of eggs laid

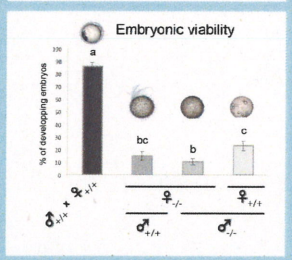
Follicle recruitment and growth are impaired. Many key ovarian genes are dysregulated.

The role of microRNAs in the regulation of animal reproduction is still poorly understood, despite a growing interest for their role in a wide variety of biological processes through post-transcriptional gene regulations. Here, we showed that miR-202-5p, a gonad-specific miRNA, is highly expressed in somatic follicular cells. We generated a mutant fish line (using CRISPR/Cas9), which provides the first functional evidence that a microRNA is necessary for fish reproduction, in particular for female fecundity. We further analyzed this phenotype by histological and molecular quantitative analyses.

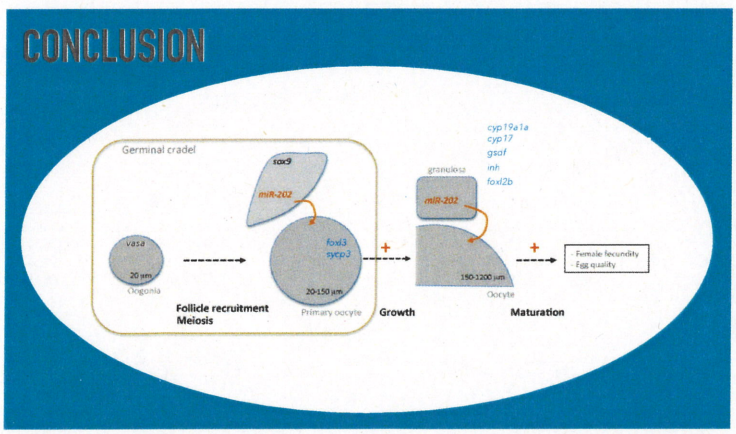


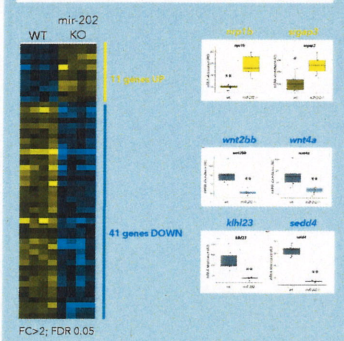
MiR-202-5p, the predominant mature form
Is detected in somatic supporting cells





Mir-202 KO reduces fertility of adult females





Dysregulation of key ovarian genes in juveniles
Microarray analysis

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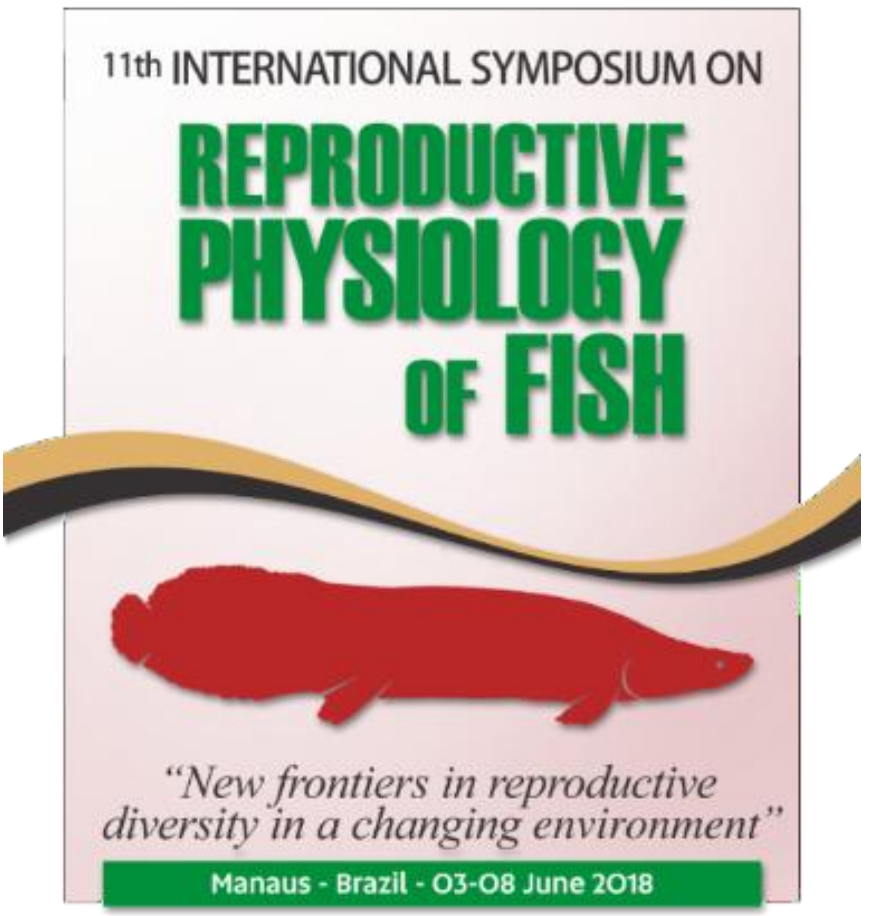

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