Investigating the Profile of miRNAs in the Mammalian Male Reproductive Tract

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Abstract

Approximately 20 % of the human population is affected by infertility, leading to an increasing concern regarding the reproductive health of our species. In around half these cases, a male factor is implicated and as a result, many research groups are actively exploring the causes of male infertility and the development of therapeutic interventions to alleviate this infertility. After leaving the testis, nascent sperm gain their potential for functional competence as they progressively transit the epididymis, a long and convoluted tubule that connects the testes to the vas deferens. This accessory organ of the male reproductive tract is characterised by segment specific microenvironments that result from differential protein secretion by the epithelium of the tubule. Recently, it has been shown that an additional tier of regulation involving non-protein-coding RNAs (ncRNAs), such as the microRNA (miRNA) small RNAs, is also highly influential in creating the dynamic intraluminal environment of the epididymis. There is also emerging interest in the contribution that these species of epididymal small RNA (sRNA) have in transgenerational inheritance owing to their potential to be transferred to maturing spermatozoa within the lumen of the duct. Thus, in recognition of the potential importance of epididymal sRNA, the aims of this project were to investigate the profile of miRNAs differentially expressed throughout the mouse epididymis, with a particular focus on identifying novel and miRNAs generated within this organ. The results of this study revealed that mouse epididymal epithelial cells are characterised by a cohort of 218 miRNAs. Interestingly, these populations were relatively stable, with only a small portion of these molecules (15 %) undergoing the significant changes expected of candidates involved in regulating differential gene expression along the length of the tubule. A number of these miRNAs were identified as playing regulatory roles in pathways well documented to influence epididymal physiology, including 12 and 10 miRNAs mapping to androgen regulation and endocytotic pathways, respectively. An impressive 295 miRNA species were identified within the spermatozoa sourced from differing epididymal segments. In marked contrast to epithelial cells, the miRNA population harboured by epididymal spermatozoa was found to be far more variable, with pronounced changed in both the number and abundance of

miRNAs in sperm being observed as these cells progress through the epididymis. Among the miRNAs enriched in caudal sperm are a cohort of 28 molecules that have been experimentally confirmed to target the genes encoding several members of the TGFβ signalling pathway, which has been documented in the modulation of the female reproductive tract prior to fertilization. Further studies revealed that epididymosomes, small exosome-like vesicles produced by the epididymal epithelium, are replete with 358 miRNAs, ~48 % of which were characterised by significant changes in accumulation between proximal and distal ends of the tract. Additionally, the first empirical evidence to suggest that epididymosomes may transfer their payload to sperm after co-incubation *in vitro* has been provided. Analysis of the presence of novel miRNAs (*Nov-miR*s) in the mouse epididymis resulted in the identification of 22 putative candidates, mapping to > 6,200 reads. Of these, five were selected for further validation and target identification, resulting in the documentation of 19 key biological processes potentially regulated by these molecules. Three of the five *Nov-miR*s chosen for validation were confirmed to be present in sperm via RT-qPCR. The ongoing characterisation of these *Nov-miR*s and the role they play in regulation of epididymal physiology will form the basis of future work in the Nixon laboratory.

Declaration

“I hereby certify that the work embodied in this thesis is the result of original research that has not been submitted for a higher degree to any other University or Institution”

Signed: _____

Jackson Reilly

Author Contributions

Publication Title

Next Generation Sequencing Analysis Reveals Segmental Patterns of microRNA Expression in Mouse Epididymal Epithelial Cells

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Brett Nixon, Simone J. Stanger, Bettina P. Mihalas, Jackson N. Reilly, Amanda L. Anderson, Mathew D. Dun, Sonika Tyagi, Janet E. Holt, Eileen McLaughlin

Citation

Nixon, B., et al., Next Generation Sequencing Analysis Reveals Segmental Patterns of microRNA Expression in Mouse Epididymal Epithelial Cells. PLoS One, 2015. 10(8): p. e0135605.

Statement of Contribution

Conceived and designed the experiments: B.N, J.E.H and E.A.M. Performed the experiments: S.J.S, B.P.M, J.N.R, A.L.A, and M.D.D. Analyzed the data: B.N, E.A.M, J.E.H, S.J.S, B.P.M and S.T. Contributed reagents/materials/analysis tools: B.N and S.T. Wrote the paper: B.N, S.J.S, B.P.M, J.N.R, A.L.A, M.D.D, S.T, J.E.H and E.A.M.

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Publication Title

The microRNA signature of mouse spermatozoa is substantially modified during epididymal maturation

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Brett Nixon, Simone J. Stanger, Bettina P. Mihalas, Jackson N. Reilly, Amanda L. Anderson, Sonika Tyagi, Janet E. Holt, Eileen A. McLaughlin

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Publication Title

Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome

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Statement of Contribution

J.N.R., S.J.S., A.L.A., K.H., K.C. and B.P.M. carried out the laboratory work and participated in data analysis and drafting of the manuscript; S.T. performed next generation sequence analyses and bioinformatics. E.A.M., J.E.H. and A.L.E. participated in the design of the study, data analysis, and drafting of the manuscript; B.N. conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

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Abbreviations

AGO2	Argonaute 2
AGRF	Australian Genome Research Facility
BLVRA	Bilverdin Reductase A
Ces7	Carboxylesterase 7
CPM	Counts Per Million
DCR1	DICER1
DGCR8	DiGeorge Syndrome Critical Region 8
ESCRT	Endosomal Sorting Complex
Exportin 5	Exp5
ICSI	Intracytoplasmic Sperm Injection
lncRNA	Long-non-coding RNA
MDS	Multi-Dimensional Scaling
miRNA	microRNA
ncRNA	Non-protein-coding RNA
NFW	Nuclease Free Water
NGS	Next Generation Sequencing
PACT	Protein Kinase R-activating
PAZ	Piwi/Argonaute/Zwille
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
piRNA	Piwi-Interacting RNA
pre-miRNA	Precursor miRNA
pri-miRNA	Primary miRNA

qPCR	Quantitative PCR
RISC	RNA Induced Silencing Complex
RNAi	RNA Interference
ROS	Reactive Oxygen Species
RT-qPCR	Reverse Transcription qPCR
sRNA	Small ncRNA
sRNA WB	Small RNA Workbench
TGFβ	Transforming Growth Factor Beta
TRBP	*trans*-Activation Response RNA Binding Protein
tRF	Transfer RNA Fragment
UTR	Untranslated Region