Genomics Data 6 (2015) 208–211

Contents lists available at ScienceDirect

Genomics Data

journal homepage: http://www.journals.elsevier.com/genomics-data/

Data in Brief

Assessment of microRNA expression in mouse epididymal epithelial cells and spermatozoa by next generation sequencing

Amanda L. Anderson [a], Simone J. Stanger [a], Bettina P. Mihalas [a], Sonika Tyagi [b], Janet E. Holt [c], Eileen A. McLaughlin [a], Brett Nixon [a,*]

[a] Reproductive Science Group, School of Environmental and Life Sciences, Faculty of Science and IT, University of Newcastle, Callaghan, New South Wales, Australia
[b] Australian Genome Research Facility Ltd, The Walter and Eliza Hall Institute, Parkville, Victoria, Australia
[c] School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, New South Wales, Australia

ARTICLE INFO

Article history:
Received 10 September 2015
Accepted 11 September 2015
Available online 18 September 2015

ABSTRACT

The mammalian epididymis is a highly specialized region of the male reproductive tract that is lined with a continuous layer of epithelial cells that display a remarkable level of regionalized secretory and absorptive activity. The luminal environment created by this combined secretory and absorptive activity is directly responsible for promoting the functional maturation of spermatozoa and their maintenance in a quiescent and viable state prior to ejaculation. This study was designed to identify the complement of microRNAs (miRNAs) that are expressed within the mouse epididymal epithelial cells and the maturing populations of spermatozoa. Through the use of Next Generation Sequencing technology we have demonstrated that both epididymal epithelial cells and spermatozoa harbour a complex repertoire of miRNAs that have substantially different expression profiles along the length of the tract. These data, deposited in the Gene Expression Omnibus (GEO) with the accession numbers GSE70197 and GSE70198, afford valuable insight into the post-transcriptional control of gene expression within the epididymis and provide the first evidence for the dynamic transformation of the miRNA content of maturing sperm cells. Ultimately such information promises to inform our understanding of the aetiology of male infertility. Herein we provide a detailed description of the methodology used to generate these important data.

Specifications	
Organism/cell line/tissue	*Mus musculus*/epididymal epithelial cells and spermatozoa
Sex	Male
Sequencer or array type	Illumina HiSeq Next-Generation Sequencing, Small RNA Library Prep
Data format	Raw and processed
Experimental factors	Epididymal tissue, isolated epididymal epithelial cells and spermatozoa
Experimental features	The epididymis was separated into the major anatomical regions; caput, corpus and cauda. From each region, epididymal epithelial cells were isolated, spermatozoa were purified, or the tissue was kept intact.
Consent	Animal ethics approval obtained in accordance with relevant national and international guidelines.
Sample source location	Newcastle, NSW, Australia

* Corresponding author at: School of Environmental and Life Sciences, Faculty of Science and IT, University of Newcastle, University Drive, Callaghan, NSW 2308, Australia.
E-mail address: Brett.Nixon@newcastle.edu.au (B. Nixon).

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70197 — epididymis tissue and epithelial cells.
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70198 — epididymal spermatozoa.

2. Experimental design, materials and methods

2.1. Experimental design

At autopsy, epididymal tissue was dissected from adult Swiss male mice and immediately separated into three major anatomical regions corresponding to the caput, corpus and cauda epididymis. The tissue was either left intact or alternatively, the epithelial cells and spermatozoa were isolated from each region as described below. Total RNA was extracted from each sample and processed for Illumina HiSeq Next-Generation Sequencing for small non-coding miRNAs. The expression profile of selected miRNAs was then validated using TaqMan Real-Time PCR (Fig. 1).

http://dx.doi.org/10.1016/j.gdata.2015.09.012

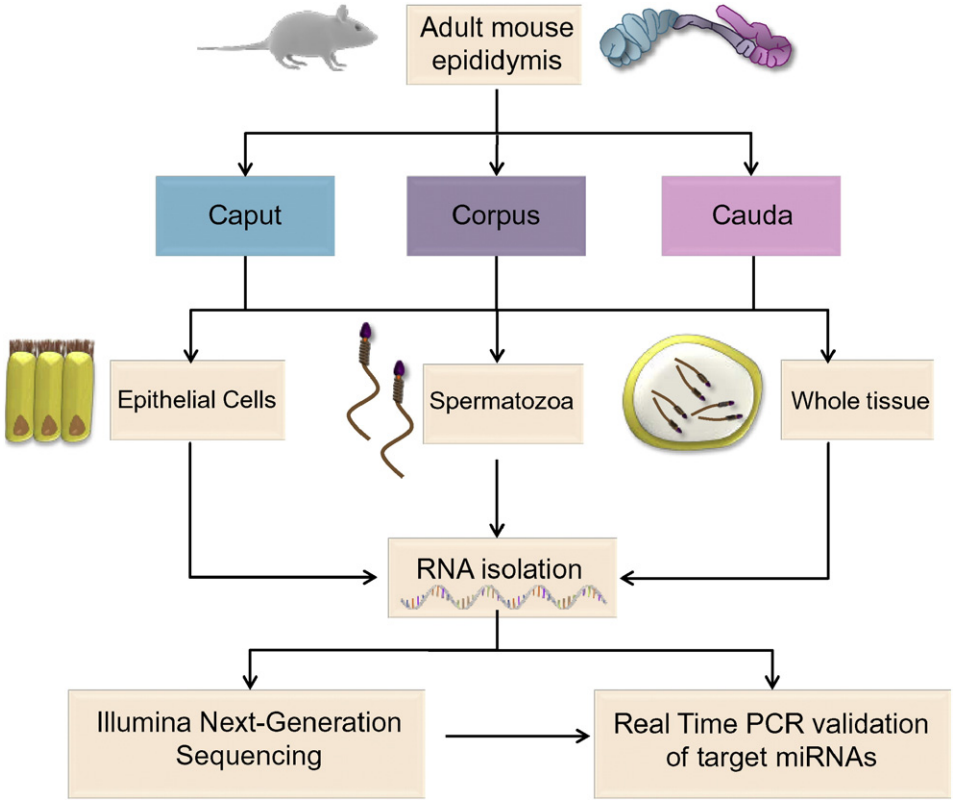

Fig. 1. Experimental design. Adult mouse epididymal tissue was dissected into regions corresponding to the caput, corpus and cauda. From each region, the epididymal epithelial cells were isolated, spermatozoa purified, or the tissue left intact. Total RNA was extracted from each cell (or tissue) sample. Subsequently, the RNA was processed for Illumina HiSeq Next-Generation sequencing, or stored and used for validation of the expression profiles of selected miRNAs.

2.2. Materials and methods

2.2.1. Animal ethics and housing

All experimental procedures were conducted with the approval of the University of Newcastle's Animal Care and Ethics Committee (ACEC) (approval number A-2013–322). Inbred Swiss mice were obtained from a breeding colony held at the institutes' central animal house and maintained according to the recommendations prescribed by the ACEC. Mice were housed under a controlled lighting regime (16L:8D) at 21–22 °C and supplied with food and water ad libitum. Prior to dissection, animals were euthanized via CO_2 inhalation.

2.2.2. Epididymal tissue and cell isolation

Immediately after adult male mice (8 weeks old) were euthanized, their vasculature was perfused with pre-warmed phosphate buffered saline (PBS) to minimize the possibility of blood contamination. The epididymides were then removed, separated from fat and overlying connective tissue and carefully dissected into three anatomical regions corresponding to the caput, corpus and cauda [1]. This material was then pooled ($n = 3$ mice/biological replicate) and subjected directly to RNA extraction and miRNA next-generation sequencing, as described below, to document the 'whole epididymal tissue' miRNA signature.

Alternatively, the perfused caput, corpus and caudal epididymal tissue were prepared for the isolation of epididymal epithelial cells ($n =$ 9–12 mice/biological replicate). To remove contaminating fluids and spermatozoa, the caput and corpus tissue were each placed in a 500 μl droplet of modified Biggers, Whitten, and Whittingham media (BWW; [2]) composed of 91.5 mM NaCl, 4.6 mM KCl, 1.7 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 25 mM $NaHCO_3$, 5.6 mM D-glucose, 0.27 mM sodium pyruvate, 44 mM sodium lactate, 5 U/ml penicillin, 5 μg/ml streptomycin, 20 mM Hepes buffer, and 3 mg/ml BSA. Multiple incisions were made in the tissue with a razor blade, and the luminal contents (fluid and spermatozoa) were allowed to disperse for 30 min at 37 °C. Alternatively, spermatozoa and fluids were cleared from the lumen of the caudal region by retrograde perfusion via the vas deferens [3,4]. All epididymal tissue samples were minced and vigorously washed in sterile PBS multiple times, followed by isolation of epithelial cells via a method adapted from Zuo et al. [5]. Briefly, tissue was digested in 100 μg/ml trypsin (Promega, Madison, WI, USA) in PBS at 37 °C for 30 min with vigorous shaking in a thermomixer (Thermomixer Compact, Eppendorf, Hamburg, Germany) at 1000 rpm. Tissue was collected by centrifugation (800 $\times g$ for 5 min) and further digested with 1 mg/ml collagenase type II (Sigma Aldrich, St. Louis, MO, USA) in PBS for at 37 °C for 30 min with vigorous shaking. The cells were pelleted by centrifugation (800 $\times g$ for 5 min), supernatant discarded, and the cell pellet resuspended in Dulbecco's Modified Eagle Medium (DMEM) containing sodium pyruvate (1 mM), 10% (v/v) foetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) (Gibco, ThermoFisher, Walton, MA, USA). Cells were filtered through a 70 μm cell strainer and incubated in 6-well plates at 32 °C. Each experiment incorporated the use of epididymides from three mice, and each sample was resuspended in 4 ml DMEM and 2 ml of this suspension was plated out onto each well of a 6-well plate. After 4 h of incubation, all non-epithelial cells, such as fibroblasts and muscle cells were attached to the plate, whereas the epithelial cells remained in suspension. A subset of these epithelial cell suspensions was set aside for assessment of purity, while the remainder were frozen at −80 °C prior to total RNA isolation.

Similarly, epididymal spermatozoa (9–12 mice/biological replicate) were collected at autopsy from adult male mice. As with tissue isolation, the mice were first perfused with PBS and the epididymis was then carefully dissected into regions corresponding to the caput, corpus and cauda. Spermatozoa from the proximal regions (caput and corpus) were recovered by placing the tissue in a 1 ml droplet of BWW and making multiple incisions with a razor blade. The spermatozoa were then

gently washed into the medium for 10 min with mild agitation at 37 °C. Purified populations of spermatozoa were recovered from this medium by density gradient centrifugation in which 1 ml of the sperm cell suspension was layered above 3 ml of 27% Percoll/BWW (GE Healthcare, Rydalmere, NSW, Australia). The gradient was centrifuged at 725 ×*g* for 15 min at 37 °C and the spermatozoa collected from the pellet. With regard to cauda epididymal spermatozoa, these cells were isolated free of contamination via retrograde perfusion of the vas deferens [4]. Following enrichment, each sperm sample was gently resuspended in BWW and a subset of the cells were set aside to assess the purity. The remainder of the sample was pelleted by gentle centrifugation (400 ×*g*, 5 min) and frozen at −80 °C prior to processing for total RNA isolation.

To assess the purity of isolated epididymal epithelial cells and spermatozoa, a sub-population of each cell preparation was settled onto microscope slides and labelled with Kwik-Diff (Thermofisher) according to manufacturer's instructions. The percentage of spermatozoa and/or epithelial cells in the preparation was assessed by microscopy (counting a minimum of 200 cells/sample), and only populations enriched >95% for the target cells were used for subsequent RNA isolation. These analyses of cell purity were also complemented with immunoblotting and immunofluorescent labelling strategies incorporating antibodies against well-characterized markers that are either unique to epithelial cells (androgen receptor, keratin 8) or spermatozoa (IZUMO1) [3,4].

2.2.3. Total RNA extraction

Total RNA was extracted from whole epididymal tissue, purified epididymal epithelial cells, and purified epididymal spermatozoa. A Direct-zol RNA MiniPrep Kit (Zymo Research Corporation, Irvine, CA, USA) was used according to manufacturer's instructions. Briefly, whole tissue or isolated cells were lysed directly in 1 ml Tri-Reagent (debris removed by centrifugation at 12,000 ×*g* 1 min) before diluting with 1 ml absolute ethanol and binding to a Zymo-Spin IIC column. The RNA was further purified via a number of stringent washes prior to being eluted from the column into DNase/RNase-free water. Genomic DNA contamination was then eliminated by incubation in 1% DNase (Promega). The purity of all samples was then assessed via determination of their A280/260 ratio and only those samples with values >1.8 were used for sequencing analysis. RNA integrity was also investigated by resolution of a portion of each sample on a denaturing agarose gel and assessment of the intensity of 28S and 18S rRNA bands.

2.2.4. miRNA Next-Generation Sequencing.

Total RNA from each epididymal region was pooled from a minimum of three (whole tissue) to nine (isolated epithelial cells and isolated spermatozoa) animals to generate a single biological replicate. One microgram of two such replicates was subjected to Illumina TruSeq small RNA sample preparation protocol as per the manufacturer's instructions (Illumina Inc. San Diego, CA, USA) at the Australian Genome Research Facility (AGRF, Brisbane, QLD, Australia). This protocol targets mature small RNA product that have a 3'OH group as a result cleavage by RNA processing enzymes. After the adaptors were ligated at the 3′ and 5′ ends, the RNA was reverse transcribed to a library of cDNA fragments, followed by PCR amplification and gel purification of the cDNA. Size selection of cDNA to capture the small RNA fragments (22–30 nucleotides) occurred by excision from the 6% PAGE gel (145–160 bp) and concentrated by ethanol precipitation. The libraries generated from the two biological replicates for each tissue/cell type for each region of the epididymis, were sequenced in triplicate using an Illumina Hiseq-2000 platform as 50 bp single end chemistry at AGRF. The base calls were processed using the Illumina CASAVA 1.8.2 pipeline. The sequence reads were analysed for quality control, all samples contained >94% bases above Q30 and no quality trimming at the ends of reads were utilised. The reads were then screened for the presence of contaminants and trimmed based on their matches to; PhiX, Illumina small RNA sequencing adaptors, ChrM or Mouse rRNA database using flicker script from Illumina. The N bases at the ends were trimmed further and all the reads more than 15 bp were retained for downstream analysis. For read mapping, cleaned sequence reads were then aligned against two different databases: (i) *Mus musculus* genome (Build version mm10), and (ii) microRNA database (miRBASE release 20 at http://www.mirbase.org/). Bowtie aligner version1.1.0 [6] was used for alignment with no mismatches in first 15 bases and with settings optimised for sensitive local alignment (options: -a –m 3 –best –strata). Alignment against the mature miRNA sequences for mouse miRNAs were summarised and counts were recorded for known miRNAs using a quantification function from the mirdeep2 [7] quantifier script.

Differential miRNA expression analysis was undertaken using R script based on, limma and voom libraries (http://www.bioconductor.org/packages/release/bioc/vignettes/limma/inst/doc/usersguide.pdf). A count value of >10 was used as the cutoff for presence of miRNA and the formation of databases (note: a pseudocount of 0.005 was added to all the counts to avoid zero values while computing the fold change). Then expression profiling comparisons were performed for mature miRNAs between the individual epididymal regions with a data filter set to ≥2 fold difference and false discovery rate (FDR) of 0.05. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus [8] and are accessible through GEO Series accession number GSE70197 (epididymal tissue and epithelial cells miRNAs) and GSE70198 (epididymal spermatozoa miRNAs) (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70197; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE70198).

2.2.5. Validation of next-generation sequencing data

In silico analysis of miRNA profiles was undertaken using a suite of techniques [3,4]. Briefly, miRNAs were clustered (Cluster3, Stanford University, Palo Alto, CA, USA) and examined using heatmaps (Java Treeview, Stanford University) to ensure consistency among biological replicates, and via volcano plots to visualize trends associated with differential miRNA expression in each epididymal region. A subset of those miRNAs displaying statistically significant patterns of differential expression (fold-change ≥2 and FDR ≤ 0.05) along the length of the epididymis were selected for further validation. Quantitative real-time PCR with TaqMan miRNA assay reagents (Life Technologies, Carlsbad, CA, USA) was used for validation of miRNA targets, and detailed results and analyses are presented in recent publications by Nixon et al. [3,4].

3. Discussion

Herein we have described the detailed methodology employed for the generation of the first comprehensive libraries of miRNA expression in the epididymis of the adult male mouse. A distinguishing feature of this analysis was our focused on three subsets of epididymal material, namely: entire tissue samples, and purified populations of epithelial cells and spermatozoa. This strategy has enabled us to distinguish the relative contribution of both luminal spermatozoa and the surrounding epithelial cells to the overall epididymal miRNA signature. In the case of epididymal epithelial cells, we were able to establish highly regionalized patterns of miRNA expression that are consistent with the marked division of labour that characterizes epididymal function. Such findings identify miRNAs as an important tier of post-transcriptional gene regulation in the male reproductive tract and suggest that they are likely to be a key element in promoting sperm maturation within this organ. These findings accord with the demonstration that targeted ablation of the miRNA processing machinery, DICER1, leads to male infertility [9,10]. Moreover we have provided the first evidence that the profile of sperm miRNAs is dynamically modified during their passage through the epididymis under normal physiological conditions. Since spermatozoa lack the capacity for de novo transcription these findings identify the epididymis as an important site in establishing the sperm epigenome with the potential to influence the peri-conceptual environment of the female reproductive tract, contribute to the inheritance of

acquired characteristics, and/or alter the developmental trajectory of the resulting offspring. These data and our associated hypotheses are reported in our recent manuscripts [3,4].

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors gratefully acknowledge funding from the National Health and Medical Research Council of Australia (APP1062371) to BN, JEH and EAM. The authors also acknowledge the Australian Genome Research Facility (Melbourne) for sequencing and initial bioinformatic analyses.

References

[1] M.D. Dun, A.L. Anderson, E.G. Bromfield, K.L. Asquith, B. Emmett, E.A. McLaughlin, R.J. Aitken, B. Nixon, Investigation of the expression and functional significance of the novel mouse sperm protein, a disintegrin and metalloprotease with thrombospondin type 1 motifs number 10 (ADAMTS10). Int. J. Androl. 35 (2012) 572–589.

[2] J.D. Biggers, W.K. Whitten, D.G. Whittingham, The culture of mouse embryos in vitro. in: J.C. Daniel (Ed.), Methods in Mammalian Embryology, Freeman Press, San Francisco, CA 1971, pp. 86–116.

[3] B. Nixon, S.J. Stanger, B.P. Mihalas, J. Reilly, A.L. Anderson, M.D. Dun, S. Tyagi, J.E. Holt, E.A. McLaughlin, Next generation sequencing analysis reveals segmental patterns of microRNA expression in mouse epididymal epithelial cells. PLoS One 10 (2015), e0135605.

[4] B. Nixon, S.J. Stanger, B.P. Mihalas, J.N. Reilly, A.L. Anderson, S. Tyagi, J.E. Holt, E.A. McLaughlin, The microRNA signature of mouse spermatozoa is substantially modified during epididymal maturation. Biol. Reprod. (2015), http://dx.doi.org/10.1095/biolreprod.115.132209.

[5] W.L. Zuo, S. Li, J.H. Huang, D.L. Yang, G. Zhang, S.L. Chen, Y.C. Ruan, K.N. Ye, C.H. Cheng, W.L. Zhou, Sodium coupled bicarbonate influx regulates intracellular and apical pH in cultured rat caput epididymal epithelium. PLoS One 6 (2011), e22283.

[6] B. Langmead, C. Trapnell, M. Pop, S.L. Salzberg, Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10 (2009) R25.

[7] M.R. Friedlander, W. Chen, C. Adamidi, J. Maaskola, R. Einspanier, S. Knespel, N. Rajewsky, Discovering microRNAs from deep sequencing data using miRDeep. Nat. Biotechnol. 26 (2008) 407–415.

[8] R. Edgar, M. Domrachev, A.E. Lash, Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30 (2002) 207–210.

[9] I. Bjorkgren, H. Gylling, H. Turunen, I. Huhtaniemi, L. Strauss, M. Poutanen, P. Sipila, Imbalanced lipid homeostasis in the conditional Dicer1 knockout mouse epididymis causes instability of the sperm membrane. FASEB J. 29 (2015) 433–442.

[10] I. Bjorkgren, L. Saastamoinen, A. Krutskikh, I. Huhtaniemi, M. Poutanen, P. Sipila, Dicer1 ablation in the mouse epididymis causes dedifferentiation of the epithelium and imbalance in sex steroid signaling. PLoS One 7 (2012), e38457.