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Region-specific microRNA signatures in the human epididymis

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The epithelium of the human epididymis maintains an appropriate luminal environment for sperm maturation that is essential for male fertility. Regional expression of small noncoding RNAs such as microRNAs contributes to segment-specific gene expression and differentiated functions. MicroRNA profiles were reported in human epididymal tissues but not specifically in the epithelial cells derived from those regions. Here, we reveal miRNA signatures of primary cultures of caput, corpus, and cauda epididymis epithelial cells and of the tissues from which they were derived. We identify 324 epithelial cell-derived microRNAs and 259 tissue-derived microRNAs in the epididymis, some of which displayed regionalized expression patterns in cells and/or tissues. Caput cell-enriched miRNAs included miR-573 and miR-155. Cauda cell-enriched miRNAs included miR-1204 and miR-770. Next, we determined the gene ontology pathways associated with *in silico* predicted target genes of the differentially expressed miRNAs. The effect of androgen receptor stimulation on miRNA expression was also investigated. These data show novel epithelial cell-derived miRNAs that may regulate the expression of important gene networks that are responsible for the regionalized gene expression and function of the epididymis.

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INTRODUCTION

The epididymis epithelium supports a luminal environment that promotes sperm maturation, and each region of the duct (caput, corpus, and cauda) has a unique role in the process. These region-specific functions are maintained by distinct gene expression signatures,^{1–3} which are coordinated by a network of transcription factors and also by noncoding RNAs. MicroRNAs (miRNAs) are one family of small noncoding RNAs that regulate gene expression posttranscriptionally, generally by binding to specific motifs in the 3'-untranslated regions (3'UTRs) of target genes (reviewed by Bartel⁴). miRNAs coordinate diverse biological processes including stem cell maintenance,⁵ development, metabolism,⁶ proliferation,⁷ differentiation,⁸ and apoptosis.⁹ In the epididymis, miRNAs may have tissue-specific roles and also be released from the epididymis epithelium in exosomes, which can be taken up by transiting sperm.^{10,11} Conditional deletion of Dicer, a critical component of the mature miRNA processing pathway, from epididymal principal cells of the mouse has a dramatic effect on the epithelium and impairs male fertility.^{12,13} Androgens are required for normal epididymal structure and function (reviewed by Robaire and Hamzeh¹⁴) and have been shown to regulate miRNA expression in rodents.^{15,16} Region-specific miRNA profiles were characterized in rodents^{17,18} and in whole human epididymis tissue by microarray analysis¹⁹ and RNA-seq.²⁰ We recently described the transcriptome of human caput, corpus, and cauda epididymis tissues and primary epithelial cell cultures derived

from each region,³ though miRNAs were not discussed. Here, we describe the region-specific expression of microRNAs in the caput, corpus, and cauda epididymal cells and tissues. Further, we use *in silico* prediction methods to identify candidate targets of several abundant miRNAs, which may directly impact regional functions of the epididymal epithelium.

PATIENTS AND METHODS

Preparation of primary cultures

Human epididymis tissue was obtained with the institutional review board permission from institutions listed in the author affiliations, and with informed consent from patients undergoing inguinal radical orchiectomy for a clinical diagnosis of testicular cancer. None of the epididymides had extension of the testicular cancer, and no donors were receiving hormone or drug treatments before surgery. Efferent ducts were removed and the three anatomical regions of the epididymis: caput, corpus, and cauda, were separated and segments of each were either snap frozen in liquid nitrogen or epithelial cells were isolated and established in culture as described previously.²¹ For the experiments to test androgen receptor (AR) function, cells were cultured in phenol-red-free CMRL-1066 medium containing 10% fetal bovine serum (FBS), hormone depleted with dextran coated-charcoal (C6241; Sigma, St. Louis, MO, USA), for 72 h before stimulation with vehicle or the synthetic androgen R1881 (1 nmol l⁻¹, methyltrienolone, NLP005005MG; PerkinElmer, Waltham, MA, USA) for a further 16 h.

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RNA sequencing

RNA was extracted using TRIzol (Life Technologies, Carlsbad, CA, USA) as per the manufacturer's protocol. RNA quality was confirmed by NanoDrop (NanoDrop™ One, Thermo Fisher Scientific, Waltham, MA, USA) measurement of OD 260/280 and 260/230 ratios, and the RNA was stored at -80°C under ethanol. RNA integrity was verified by the Bioanalyzer (Agilent, Santa Clara, CA, USA), and RNA-seq libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 as per the manufacturer's Low-throughput protocol (Illumina, San Diego, CA, USA). The libraries were sequenced on Illumina HiSeq2500 machines and generated $1.9 \times 10^7 - 3.9 \times 10^7$ reads per library from the cultured cells (95%–99% mapping to the genome) and $1.4 \times 10^7 - 3.9 \times 10^7$ reads per library from tissues (84%–99% mapping to the genome). Data were analyzed using TopHat and Cufflinks.²² All data are deposited at GEO (<http://www.ncbi.nlm.nih.gov/geo/GSE72986>).

In silico analysis of miRNAs and target prediction

Putative mRNA targets of differentially expressed miRNAs were predicted using TargetScan 7.0 (<http://www.targetscan.org/>)^{23,24} and miRecords.²⁵ Only data generated with TargetScan are presented here, as the miRecord database was incomplete with respect to miRNAs of interest. Gene ontology process enrichment analysis^{26,27} was performed to identify statistically significant biological processes associated with the miRNA targets (as shown by both *P* value and false discovery rate [FDR]).

RESULTS

Regional miRNA expression in the human epididymis

To identify the regional microRNA signature of the epididymis epithelium, we examined RNA-seq data from both cultured human epididymal epithelial (HEE) cells and tissues from the caput, corpus, and cauda segments.³ The majority of the 324 miRNAs identified in HEE cells (**Supplementary Table 1**) were expressed in more than one region, as were most of the 259 tissue miRNAs (**Supplementary Table 2**). Fifty-seven percent (185/324) of the HEE cell miRNAs were also present in the tissues they were derived from. Regionally restricted miRNAs were defined by differential expression of at least 2.5-fold change between caput, corpus, and cauda HEE cells and minimum gene expression levels of ≥ 0.3 fragments per kilobase of transcript per million mapped reads (FPKM; **Table 1**). The same parameters were used in a comparison of differential gene expression for the miRNAs of the caput, corpus, and cauda tissues (**Table 2**).

Caput-enriched miRNAs of the cells and tissues

Two microRNAs (miR-573 and miR-155) were significantly more abundant in caput cells than in corpus or cauda HEE cells (**A and B in Table 1**). miR-30c2 was also enriched in the caput compared to corpus cells, and miR-196A1 was enhanced in the caput over the cauda cells. The most significant differentially expressed miRNAs observed in intact tissues were distinct from those seen in HEE cells, with miR-1247 more abundant in the caput tissue compared to both the corpus and cauda (**A and B in Table 2**). Likewise, miR-4461 was enriched in the caput in comparison to the corpus and cauda tissues though it was abundant in all tissue regions (**A and B in Table 2**). Sixteen other miRNAs were more highly expressed in the caput than the cauda tissue (**B in Table 2**).

Corpus-enriched miRNAs of the cells and tissues

Although our analysis did not identify any corpus-specific cell-derived miRNAs, four miRNAs (miR-4730, miR-196a1, miR-let7d, and miR-3916) were enriched in the corpus over the cauda cells (**C in Table 1**). In the tissues, miR-662 and miR-3936 were more abundant in the corpus than either the caput or cauda.

Table 1: Differentially expressed miRNAs comparing the caput, corpus and cauda epididymis cells

miRNA	FPKM comparison		Log2 fold change	Actual fold change	Differential
	Caput	Corpus			
A.					
miR-573	10.28	0.40	-4.68	25.7	Cap>Corp
miR-155	3.11	0.61	-2.36	5.1	Cap>Corp
miR-30c2	3.92	1.10	-1.83	3.6	Cap>Corp
B.					
	Caput	Cauda			
miR-196a1	15.03	0.44	-5.10	34.4	Cap>Cau
miR-573	10.28	0.58	-4.15	17.8	Cap>Cau
miR-155	3.11	0.45	-2.77	6.8	Cap>Cau
miR-let7i	0.72	8.67	3.59	12.0	Cau>Cap
miR-770	2.12	24.47	3.53	11.5	Cau>Cap
miR-1204	8.54	89.13	3.38	10.4	Cau>Cap
C.					
	Corpus	Cauda			
miR-4730	21.14	0.00	infinity	infinity	Corp>Cau
miR-196a1	72.23	0.44	-7.37	165.2	Corp>Cau
miR-let7d	12.80	1.64	-2.97	7.8	Corp>Cau
miR-3916	34.98	9.98	-1.81	3.5	Corp>Cau
miR-1204	7.84	89.13	3.51	11.4	Cau>Corp
miR-675	0.42	2.90	2.79	6.9	Cau>Corp

A: caput vs corpus; B: caput vs cauda; C: corpus vs cauda. Cap: caput; Corp: corpus; Cau: cauda; FPKM: fragments per kilobase of transcript per million mapped reads

Three other miRNAs (miR-33b, miR-135b, and miR-3074) were enriched in the corpus over the caput tissues (**A in Table 2**). Multiple other tissue-derived miRNAs were enriched in the corpus over the cauda tissues, including the highly expressed miR-205, miR-141, and miR-3648-miR-3687 (**C in Table 2**).

Cauda-enriched miRNAs of the cells and tissues

In HEE cells, miR-1204 showed enhanced expression in the cauda compared to both the caput and corpus cells (**Table 1**). H19-miR-675 was also enriched in the cauda over the corpus cells. miR-770 and miR-let7i were higher in the cauda than the caput cells (**B in Table 1**). In the tissues, miR-146a was more abundant in the cauda compared to the caput and corpus (**B in Table 2**).

Also of note, but solely in the tissues, miR-135b and miR-3074 were more abundant in both the corpus and the cauda (**A in Table 2**) than in the caput (**B in Table 2**).

In silico analysis of microRNA-regulated processes of human epididymal epithelial cells

Putative target genes of the differentially expressed miRNAs were predicted using TargetScan 7.0 (<http://www.targetscan.org/>).^{23,24} We chose this *in silico* prediction tool since it was one of the few such resources that were recently updated and included all the differentially expressed miRNAs in the HEE cells. Moreover, it was a robust predictive tool in our recent work on other miRNAs.^{28–30} Target genes (< -0.2 total context score) were then subjected to gene ontology process enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID).^{26,27} The top 10 predicted targets and their associated processes are shown in **Table 3** and **4**.

MicroRNA-regulated processes of the caput

Processes enriched among the predicted targets of the caput cell-enriched miRNAs (miR-155 and miR-573) were identified first. For miR-155-3p and miR-155-5p, these included predominantly aspects of transcriptional regulation and membrane-enclosed lumens of both nuclei and intracellular organelles (**A and B in Table 3**). Processes associated with miR-573 included

Table 2: Differentially expressed miRNAs comparing the caput, corpus and cauda epididymis tissues

miRNA	FPKM comparison		Log2 fold change	Actual fold change	Differential change
	Caput	Corpus			
A.					
miR-1247	67.97	0.00	infinity	infinity	Cap>Corp
miR-4461	3.58E+6	5.26E+5	-2.77	6.81	Cap>Corp
miR-33b	0.00	10.82	infinity	infinity	Corp>Cap
miR-3074	2.08	44.72	4.43	21.54	Corp>Cap
miR-135b	0.15	2.59	4.14	17.61	Corp>Cap
miR-3936	39.97	263.65	2.72	6.60	Corp>Cap
miR-662	4.84	16.07	1.73	3.32	Corp>Cap
B.					
	Caput	Cauda			
miR-1247	67.97	0.00	infinity	infinity	Cap>Cau
miR-1324	5.20	0.00	infinity	infinity	Cap>Cau
miR-770	45.74	0.27	-7.42	171.75	Cap>Cau
miR-205	466.77	5.06	-6.53	92.31	Cap>Cau
miR-135a2	201.75	2.41	-6.39	83.88	Cap>Cau
miR-4674	62.96	0.80	-6.30	79.03	Cap>Cau
miR-141	337.14	6.92	-5.61	48.73	Cap>Cau
miR-3675	16.77	0.41	-5.35	40.91	Cap>Cau
miR-424	61.67	3.05	-4.34	20.20	Cap>Cau
miR-374c	19.10	1.22	-3.96	15.61	Cap>Cau
miR-100	44.27	3.18	-3.80	13.93	Cap>Cau
miR-4461	3.58E+6	4.13E+5	-3.12	8.68	Cap>Cau
miR-662	4.84	0.62	-2.97	7.84	Cap>Cau
miR-let7d	17.86	2.37	-2.92	7.55	Cap>Cau
miR-3648-miR-3687	12 117.00	1614.22	-2.91	7.51	Cap>Cau
miR-4458	7.05	1.63	-2.12	4.34	Cap>Cau
miR-3916	0.81	28.96	5.15	35.57	Cau>Cap
miR-135b	0.15	4.85	5.04	32.94	Cau>Cap
miR-3074	2.08	54.46	4.71	26.23	Cau>Cap
miR-24-2	5.55	60.77	3.45	10.95	Cau>Cap
miR-4435-1	0.28	3.07	3.44	10.87	Cau>Cap
miR-146a	0.82	6.83	3.07	8.37	Cau>Cap
C.					
	Corpus	Cauda			
miR-1324	3.32	0.00	infinity	infinity	Corp>Cau
miR-4491	6.22	0.00	infinity	infinity	Corp>Cau
miR-205	1770.51	5.06	-8.45	350.16	Corp>Cau
miR-770	92.56	0.27	-8.44	347.54	Corp>Cau
miR-135a2	198.86	2.41	-6.37	82.68	Corp>Cau
miR-141	487.46	6.92	-6.14	70.46	Corp>Cau
miR-4674	48.73	0.80	-5.93	61.17	Corp>Cau
miR-3675	11.42	0.41	-4.80	27.87	Corp>Cau
miR-662	16.07	0.62	-4.70	26.01	Corp>Cau
miR-424	49.11	3.05	-4.01	16.08	Corp>Cau
miR-3936	263.65	18.89	-3.80	13.96	Corp>Cau
miR-3648-miR-3687	17 315.50	1614.22	-3.42	10.73	Corp>Cau
miR-374c	12.58	1.22	-3.36	10.28	Corp>Cau
miR-100	27.17	3.18	-3.10	8.55	Corp>Cau
miR-let7d	15.18	2.37	-2.68	6.41	Corp>Cau
miR-143-miR-145	2.72	0.48	-2.50	5.67	Corp>Cau
miR-4458	5.02	1.63	-1.63	3.09	Corp>Cau
miR-24-2	7.16	60.77	3.09	8.49	Cau>Corp
miR-146a	1.00	6.83	2.77	6.84	Cau>Corp

A: caput vs corpus; B: caput vs cauda; C: corpus vs cauda. Cap: caput; Corp: corpus; Cau: cauda; FPKM: fragments per kilobase of transcript per million mapped reads

some relevant to modification-dependent protein catabolic processes and as for miR-155, to the lumens of nuclei and intracellular organelles including the mitochondrion (C in Table 3).

MicroRNA-regulated processes in the corpus and cauda

Although no corpus-specific HEE cell miRNAs were identified here, processes associated with the predicted targets of cauda cell-enriched miRNAs (miR-1204, miR-770, and miR-let7i) are shown in Table 4. miR-1204-associated processes include “small GTPase-mediated intracellular signaling” and “regulation of cell motion” (A in Table 4). The diverse processes associated with miR-770 included those relevant to metal ion binding (including zinc), transcription, and DNA (B in Table 4). Transcriptional regulation was also enriched in the miR-let7i-associated processes, together with multiple processes of less obvious relevance to HEE cell function including “regulation of neuron differentiation” and “blood vessel development” (C in Table 4).

Androgen-regulated miRNAs of the epididymis

To investigate whether any miRNAs were regulated by androgens in the human epididymis epithelium, caput HEE cells were treated with vehicle or R1881 (1 nmol l⁻¹) for 18 h and gene expression was analyzed by RNA-seq (Yang *et al.*, manuscript in review). Eight miRNAs were differentially expressed following R1881 treatment. Of these, four miRNAs were downregulated (miR-137, miR-3074, miR-3190, and miR-3916) and four were upregulated (miR-4740, miR-506, miR-573, and miR-let7d; $P = 5 \times 10^{-5}$, $q = 0.007$, Table 5).

DISCUSSION

Mechanisms that control gene expression along the human epididymis epithelium are pivotal to coordinating its role in sperm maturation and male fertility. One aspect of this coordination likely involves noncoding RNAs including microRNAs. miRNAs were profiled previously in tissue samples from the human epididymis that contained many cell types.^{19,20,31} In order to focus on the specific functions of cells within the epithelial layer lining the epididymis, we established the HEE cell culture model.²¹ Here, we use RNA-seq analysis to reveal the miRNA signatures of caput, corpus, and cauda HEE cells and the tissues from which they were derived.

Tissue-derived miRNAs

Among the epididymis tissue-derived miRNAs identified in this study, we detected approximately 22.0% (116/527)²⁰ and approximately 24.1% (81/336)¹⁹, respectively of the tissue-derived miRNAs previously reported by others. As previously noted by Belleannee *et al.*¹⁹ most of the tissue miRNAs were present in all three regions, with similar observations of HEE cell-derived miRNAs. Furthermore, most of the HEE cell-derived miRNAs were also evident in the tissues.

MicroRNA-regulated processes of the caput cells

miR-573 and miR-155, which are differentially expressed in caput HEE cells compared to corpus or cauda cells, were investigated previously in other contexts. miR-573 is downregulated by the inflammatory cytokine tumor necrosis factor (TNF)-alpha in primary human airway epithelial cells.³² More relevant to the male reproductive tract, miR-573 is downregulated in testicular tissue from patients with nonobstructive azoospermia.³³ It is perhaps of relevance that we observed miR-573 among miRNAs that were upregulated in androgen-stimulated caput HEE cells. Gene ontology process enrichment analysis on the mRNA targets of miR-573 identified processes related to protein modification by small protein conjugation or removal and modification-dependent protein catabolic process, both of which might be relevant to maintenance of optimal luminal environment in the epididymis. The role of miR-155 in the male reproductive tract has yet to be explored, but it is well characterized as a pleiotropic regulator of both immunity and cancer.^{34–36}

Table 3: Predicted target genes of caput-enriched microRNAs (left) and their enriched processes (right)

<i>Top 10 target genes</i>	<i>TCS</i>	<i>Top 10 enriched GO terms</i>	<i>P</i>	<i>FDR</i>
A. miR-155-3p				
1. Zinc finger protein 140 (<i>ZNF140</i>)	-1.7	1. GO:0031974~membrane-enclosed lumen	2.4E-09	3.4E-06
2. Zinc finger protein 334 (<i>ZNF334</i>)	-1.3	2. GO:0043233~organelle lumen	2.5E-09	3.6E-06
3. ELL associated factor 1 (<i>EAF1</i>)	-1.3	3. GO:0006350~transcription	1.1E-08	1.9E-05
4. NADH dehydrogenase ubiquinone flavoprotein 3, 10kDa (<i>NDUFV3</i>)	-1.0	4. GO:0070013~intracellular organelle lumen	1.1E-08	1.6E-05
5. ZFP14 zinc finger protein (<i>ZFP14</i>)	-1.0	5. GO:0031981~nuclear lumen	1.2E-08	1.7E-05
6. Mitochondrial ribosomal protein L19 (<i>MRPL19</i>)	-0.9	6. GO:0051252~regulation of RNA metabolic process	2.6E-07	4.8E-04
7. Argonaute RISC catalytic component 2 (<i>AGO2</i>)	-0.9	7. GO:0006355~regulation of transcription, DNA-dependent	3.6E-07	6.5E-04
8. Chromosome 12 open reading frame 49 (<i>C12orf49</i>)	-0.8	8. GO:0045449~regulation of transcription	5.9E-07	1.1E-03
9. Tropomyosin 1 alpha (<i>TPM1</i>)	-0.8	9. GO:0005654~nucleoplasm	1.3E-06	1.9E-03
10. Molybdenum cofactor synthesis 2 (<i>MOCS2</i>)	-0.8	10. GO:0005730~nucleolus	8.7E-06	1.2E-02
B. miR-155-5p				
1. Zinc finger protein 385D (<i>ZNF385D</i>)	-0.8	1. GO:0045449~regulation of transcription	1.5E-11	2.6E-08
2. ADP-ribosylation factor-like 5B (<i>ARL5B</i>)	-0.8	2. GO:0030528~transcription regulator activity	2.2E-10	3.0E-07
3. Zinc finger protein 652 (<i>ZNF652</i>)	-0.7	3. GO:0003700~transcription factor activity	4.4E-08	6.1E-05
4. Casein kinase 1, alpha 1 (<i>CSNK1A1</i>)	-0.7	4. GO:0006350~transcription	6.5E-08	1.1E-04
5. Nedd4 family interacting protein 1 (<i>NDFIP1</i>)	-0.7	5. GO:0006357~regulation of transcription from RNA polymerase II promoter	1.2E-07	2.1E-04
6. Sphingosine-1-phosphate receptor 1 (<i>S1PR1</i>)	-0.7	6. GO:0031981~nuclear lumen	1.3E-07	1.7E-04
7. Cell division cycle 73 (<i>CDC73</i>)	-0.6	7. GO:0045893~positive regulation of transcription, DNA-dependent	1.7E-07	2.8E-04
8. Vav 3 guanine nucleotide exchange factor (<i>VAV3</i>)	-0.6	8. GO:0051252~regulation of RNA metabolic process	1.8E-07	3.0E-04
9. Teashirt zinc finger homeobox 3 (<i>TSHZ3</i>)	-0.6	9. GO:0006355~regulation of transcription, DNA-dependent	1.8E-07	3.1E-04
10. V-ets avian erythroblastosis virus E26 oncogene homolog 1 (<i>ETS1</i>)	-0.6	10. GO:0051254~positive regulation of RNA metabolic process	2.0E-07	3.4E-04
C. miR-573				
1. Dynactin associated protein (<i>DYNAP</i>)	-2.4	1. GO:0070647~protein modification by small protein conjugation or removal	2.4E-09	3.4E-06
2. Argonaute RISC catalytic component 2 (<i>AGO2</i>)	-1.5	2. GO:0032446~protein modification by small protein conjugation	2.5E-09	3.6E-06
3. Solute carrier family 25, member 26 (<i>SLC25A26</i>)	-1.0	3. GO:0031974~membrane-enclosed lumen	1.1E-08	1.9E-05
4. RNA binding motif protein 23 (<i>RBM23</i>)	-0.9	4. GO:0043233~organelle lumen	1.1E-08	1.6E-05
5. Fanconi anemia, complementation group M (<i>FANCM</i>)	-0.9	5. GO:0019941~modification-dependent protein catabolic process	1.2E-08	1.7E-05
6. Melan-A (<i>MLANA</i>)	-0.8	6. GO:0043632~modification-dependent macromolecule catabolic process	2.6E-07	4.8E-04
7. Enhancer of yellow 2 homolog Drosophila (<i>ENY2</i>)	-0.8	7. GO:0019001~guanyl nucleotide binding	3.6E-07	6.5E-04
8. Family with sequence similarity 104, member B (<i>FAM104B</i>)	-0.8	8. GO:0032561~guanyl ribonucleotide binding	5.9E-07	1.1E-03
9. BRX1, biogenesis of ribosomes, homolog (S. cerevisiae) (<i>BRX1</i>)	-0.7	9. GO:0051603~proteolysis involved in cellular protein catabolic process	1.3E-06	1.9E-03
10. Killer cell lectin-like receptor subfamily F, member 1 (<i>KLRF1</i>)	-0.7	10. GO:0005525~GTP binding	8.7E-06	1.2E-02

TCS: total context score; FDR: false discovery rate; GO: gene ontology

MicroRNA-regulated processes of the corpus/cauda cells

The role of miR-1204 was investigated in human carcinoma cell lines,^{37,38} though to date there is no information on its involvement in processes relevant to the male reproductive tract. miR-770 is downregulated in testicular tissue from patients with nonobstructive azoospermia.³³ miR-let7i negatively regulates cardiac inflammation and fibrosis³⁹ and is downregulated in the serum from ovarian cancer patients.⁴⁰ However, deciphering its role in the epididymis will require further study.

Androgen-regulated miRNAs in the caput epididymis

Androgens are important for epididymal epithelial structure and function (reviewed by Robaire and Hamzeh¹⁴) and regulate their effects via the AR. We previously showed enrichment of AR protein in caput HEE cells and its nuclear accumulation in these cells in response to R1881.^{3,21} Hence, here, we also examined the effect of R1881 on miRNA expression in caput HEE cells. Among the

miRNAs that were differentially regulated in response to R1881, miR-137 is of interest since this miRNA is differentially expressed after androgen treatment in the prostate adenocarcinoma cell line, LnCaP.⁴¹ Of note, in LnCaP cells, androgen treatment increases miR-137 in contrast to the repression observed here in HEE cells. However, the overamplification of AR in LnCaP cells compared to normal prostate epithelium and the strong context-dependence of AR cofactors could account for these differences. Another androgen-regulated miRNA in the caput epididymis is miR-506, which plays a complex role in cancer. It is oncogenic in melanomas,⁴² a tumor suppressor in ovarian cancer,⁴³ and confers chemoresistance in colon cancer.⁴⁴

In conclusion, our data suggest that further investigation of regionalized miRNA expression along the epididymis may contribute to the understanding of mechanisms controlling segment-specific epididymal epithelial function.

Table 4: Predicted target genes of cauda-enriched microRNAs (left) and their enriched processes (right)

Top 10 target genes	TCS	Top 10 enriched GO terms	P	FDR
A. miR-1204				
1. Von Willebrand factor A domain containing 1 (<i>VWA1</i>)	-1.4	1. GO:0007242--intracellular signaling cascade	3.2E-04	5.7E-01
2. N-acetyltransferase 8-like (GCN5-related, putative) (<i>NAT8L</i>)	-1.2	2. GO:0051270--regulation of cell motion	1.2E-03	2.1E+00
3. Solute carrier family 25 (mitochondrial carrier; dicarboxylate transporter), member 10 (<i>SLC25A10</i>)	-1.2	3. GO:0030036--actin cytoskeleton organization	1.2E-03	2.2E+00
4. Mitochondrial dicarboxylate carrier; Uncharacterized protein; cDNA FLJ60124 (<i>ENSG00000262660</i>)	-1.1	4. GO:0051056--regulation of small GTPase signal transduction	2.0E-03	3.5E+00
5. Leucine-rich repeat containing 56 (<i>LRRCS6</i>)	-1.1	5. GO:0030695--GTPase regulator activity	2.0E-03	3.0E+00
6. Zinc finger, AN1-type domain 3 (<i>ZFAND3</i>)	-1.0	6. GO:0051129--negative regulation of cellular component organization	2.4E-03	4.1E+00
7. Coiled-coil domain containing 127 (<i>CCDC127</i>)	-1.0	7. GO:0000271--polysaccharide biosynthetic process	2.4E-03	4.1E+00
8. Matrix metalloproteinase 11 (stromelysin 3) (<i>MMP11</i>)	-0.9	8. GO:0030029--actin filament-based process	2.6E-03	4.6E+00
9. Protein ITFG3; Uncharacterized protein; cDNA FLJ60496 (<i>ITFG3</i>)	-0.9	9. GO:0060589--nucleoside-triphosphatase regulator activity	2.8E-03	4.1E+00
10. Transmembrane protein 141 (<i>TMEM141</i>)	-0.8	10. GO:0005089--Rho guanyl-nucleotide exchange factor activity	2.9E-03	4.3E+00
B. miR-770-5p				
1. zinc finger, DHHC-type containing 11 (<i>ZDHH11</i>)	-2.9	1. GO:0008270--zinc ion binding	3.62E-09	5.6E-06
2. zinc finger protein 138 (<i>ZNF138</i>)	-1.5	2. GO:0046914--transition metal ion binding	2.23E-08	3.4E-05
3. zinc finger protein 83 (<i>ZNF83</i>)	-1.4	3. GO:0006355--regulation of transcription, DNA-dependent	6.03E-07	1.1E-03
4. glia maturation factor, beta (<i>GMFB</i>)	-1.1	4. GO:0006350--transcription	6.15E-07	1.1E-03
5. zinc finger protein 480 (<i>ZNF480</i>)	-1.0	5. GO:0003677--DNA binding	1.24E-06	1.9E-03
6. zinc finger protein 140 (<i>ZNF140</i>)	-0.9	6. GO:0051252--regulation of RNA metabolic process	2.00E-06	3.5E-03
7. zinc finger protein 107 (<i>ZNF107</i>)	-0.9	7. GO:0045449--regulation of transcription	3.02E-06	5.4E-03
8. motor neuron and pancreas homeobox 1 (<i>MX1</i>)	-0.8	8. GO:0005794--Golgi apparatus	8.52E-05	1.2E-01
9. zinc finger protein 616 (<i>ZNF616</i>)	-0.8	9. GO:0070013--intracellular organelle lumen	2.46E-04	3.5E-01
10. zinc finger protein 676 (<i>ZNF676</i>)	-0.8	10. GO:0043233--organelle lumen	2.49E-04	3.5E-01
C. miR-let7i-3p				
1. Histone cluster 3, H3 (<i>HIST3H3</i>)	-0.9	1. GO:0001568--blood vessel development	4.7E-04	7.8E-01
2. Calcium/calmodulin-dependent protein kinase II inhibitor 1 (<i>CAMK2N1</i>)	-0.9	2. GO:0048514--blood vessel morphogenesis	4.8E-04	8.0E-01
3. Peptidylprolyl isomerase (cyclophilin)-like 4 (<i>PP1L4</i>)	-0.8	3. GO:0001944--vasculature development	5.9E-04	9.8E-01
4. IQ motif containing B1 (<i>IQCB1</i>)	-0.8	4. GO:0045893--positive regulation of transcription, DNA-dependent	1.3E-03	2.2E+00
5. Coiled-coil domain containing 151 (<i>CCDC151</i>)	-0.8	5. GO:0007411--axon guidance	1.4E-03	2.3E+00
6. Ribonuclease, RNase A family, 7 (<i>RNASE7</i>)	-0.8	6. GO:0051254--positive regulation of RNA metabolic process	1.5E-03	2.4E+00
7. RAB, member of RAS oncogene family-like 3 (<i>RABL3</i>)	-0.7	7. GO:0045446--endothelial cell differentiation	1.8E-03	3.0E+00
8. Paraspeckle component 1 (<i>PSPC1</i>)	-0.7	8. GO:0030030--cell projection organization	1.9E-03	3.2E+00
9. X-prolyl aminopeptidase (aminopeptidase P) 3, putative (<i>XPNPEP3</i>)	-0.7	9. GO:0031175--neuron projection development	2.3E-03	3.8E+00
10. Fatty acyl CoA reductase 1 (<i>FAR1</i>)	-0.7	10. GO:0043065--positive regulation of apoptosis	3.1E-03	5.0E+00

TCS: total context score; FDR: false discovery rate; GO: gene ontology

Table 5: Differentially expressed microRNAs in caput human epididymal epithelial cells after R1881 treatment compared to vehicle

miRNAs	Vehicle	R1881	Log2 fold change	Actual fold change
miR-137	4.55	0.06	-6.17	72.2
miR-4740	24.04	1.55	-3.95	15.5
miR-let7d	22.58	1.96	-3.53	11.5
miR-125a	3.85	23.98	2.64	6.2
miR-573	0.76	7.81	3.36	10.3
miR-3177	1.52	18.16	3.58	11.9

Average of 4 RNA-seq replicas. HEE: human epididymal epithelial; miRNAs: microRNAs

AUTHOR CONTRIBUTIONS

JAB, SHL, SEE, and AH acquired, analyzed, and interpreted data. JAB

and AH wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: List of microRNAs in primary human epididymal epithelial cells (fragments per kilobase of transcript per million mapped reads [FPKM] >0.1)

<i>miRNAs</i>	<i>Caput cell</i>	<i>Corpus cell</i>	<i>Cauda cell</i>
miR-4461	1.7E+05	1.8E+05	1.6E+05
miR-548AJ2	1966.26	0.00	0.00
miR-3648, miR-3687	1671.15	957.40	1148.85
miR-3661	1102.61	1940.27	1845.95
miR-1281	1101.70	387.63	1734.55
miR-1282	1014.31	924.35	847.09
miR-4426, RPS27A	777.37	652.50	691.22
miR-1244-3, PTMA	711.12	298.98	326.28
miR-1279	573.42	272.52	146.82
miR-1304, SNORA1, SNORA18, SNORA25, SNORA32, SNORA40, SNORA8, SNORD5, SNORD6, TAF1D	526.49	447.01	460.93
miR-3918	444.70	89.37	33.48
miR-612, NEAT1	395.09	323.26	298.88
miR-762	281.14	261.52	474.86
miR-611, TMEM258	275.76	161.24	219.19
miR-3658, UCK2	274.04	11.72	11.40
miR-663A	261.77	474.04	606.03
miR-4750	247.77	0.00	0.00
miR-1307, USMG5	237.72	299.19	290.34
miR-4722	201.60	250.98	0.00
miR-3650	201.11	0.00	0.00
miR-4489	182.15	0.00	0.00
miR-4635	168.09	0.00	68.57
miR-4728	164.83	106.89	0.00
miR-497HG	164.77	86.98	106.67
miR-1914, miR-647, UCKL1	163.52	76.81	99.04
miR-4709, NPC2	147.87	96.90	106.94
miR-4273	144.19	125.18	42.10
miR-1229	134.65	0.00	131.72
miR-4517, NFATC2IP	128.76	106.77	15.63
miR-3605, PHC2	124.66	91.17	99.54
miR-4746	124.34	31.59	32.04
miR-4721, TUFM	115.52	92.37	89.91
miR-1292, NOP56, SNORD86	112.86	179.88	75.91
miR-4449	111.43	51.91	52.65
miR-4784	108.83	38.87	57.11
miR-25	107.56	12.16	54.35
miR-4700, UNC119B	105.79	20.00	41.59
miR-4263	101.36	155.52	60.15
miR-4292	100.02	121.06	336.41
miR-4516	99.53	108.04	150.05
miR-4785	94.02	170.49	153.80
miR-637	93.21	36.40	72.98
miR-103B2	91.08	0.00	128.09
miR-639, TECR	90.46	78.54	85.37
miR-631	88.55	0.00	0.00
miR-21, VMP1	86.68	60.45	63.40
miR-3684	86.61	0.00	0.00
miR-1260B	86.36	29.21	62.99
miR-636, SRSF2	82.81	63.90	89.84
miR-632, ZNF207	82.38	122.83	112.16
miR-4492	82.05	53.39	98.15
miR-1226	80.00	0.00	22.94
miR-3917, STMN1	77.23	42.78	46.43
miR-196B	73.74	76.55	32.98
miR-4440	67.99	5.78	10.01

Contd...

Supplementary Table 1: Contd...

<i>miRNAs</i>	<i>Caput cell</i>	<i>Corpus cell</i>	<i>Cauda cell</i>
miR-135A1	64.30	0.00	23.67
miR-1248, SNORA81	62.48	33.60	16.85
miR-210	61.67	13.90	17.62
miR-1251	61.23	0.00	0.00
miR-4680, PDCD4	61.21	14.30	14.34
miR-641	60.96	17.72	16.81
miR-3615, SLC9A3R1	57.49	21.56	21.93
miR-4441	54.35	33.48	5.35
miR-3607	54.25	0.00	17.09
miR-4647, SLC35B2	52.83	40.44	40.56
miR-661	51.10	0.00	0.00
miR-3665	50.96	0.00	0.00
miR-937, SCRIB	50.60	52.30	18.96
miR-3191	50.36	57.65	70.64
miR-24-1	49.35	42.02	0.00
miR-553	45.10	42.02	42.62
miR-4253	45.10	0.00	0.00
miR-200C	45.10	0.00	0.00
miR-4312	44.95	20.94	53.39
miR-943, NELFA	44.12	19.36	20.06
miR-4678	43.31	0.00	0.00
miR-324	42.63	29.48	56.10
miR-4741, RBBP8	42.26	42.51	36.76
miR-4754	41.99	0.00	63.03
miR-1180	40.85	84.02	0.00
miR-302A	40.85	0.00	0.00
miR-621, SLC25A15	40.14	28.67	29.08
miR-564, TMEM42	39.32	68.50	12.89
miR-3074	38.91	1.36	1.66
miR-29C	36.62	40.50	47.39
miR-3610, RAD21	36.50	31.86	31.41
miR-93	35.95	0.00	15.95
miR-761, NRD1	33.99	43.41	43.15
miR-4742	33.24	36.73	0.00
miR-663B	30.71	20.74	37.43
miR-3177	29.60	0.00	13.99
miR-3189	28.53	164.97	118.92
miR-5690	28.53	0.00	0.00
miR-4688	28.52	12.94	22.39
miR-604	28.42	0.00	0.00
miR-568	28.32	6.93	15.56
miR-3614, TRIM25	28.24	33.82	31.11
miR-LET7G	27.88	0.00	0.00
miR-4668, UGCG	27.78	28.78	28.47
miR-1227	27.38	9.63	23.97
miR-4687, STIM1	27.24	298.95	247.37
miR-3916	26.91	34.98	9.98
miR-1915	26.78	0.00	0.00
miR-29B2	26.03	0.00	14.93
miR-1278	26.03	0.00	0.00
miR-2467	26.03	0.00	0.00
miR-4497	25.88	0.00	0.00
miR-938	24.70	0.00	0.00
miR-320A	24.39	0.00	0.00
miR-4800, MXD4	24.32	26.93	33.59
miR-27B	23.89	14.56	10.49

Contd...

Supplementary Table 1: Contd...

<i>miRNAs</i>	<i>Caput cell</i>	<i>Corpus cell</i>	<i>Cauda cell</i>
miR-4258	23.30	8.18	16.59
miR-186	23.17	10.79	18.67
miR-4442, TOP2B	22.66	65.67	99.63
miR-4730	22.66	21.14	0.00
miR-26A2	22.19	0.00	28.25
miR-4691	22.02	32.76	13.94
miR-3192	20.86	42.90	0.00
miR-940	20.74	15.48	21.34
miR-1203	20.25	0.00	0.00
miR-5193, UBA7	19.14	28.36	26.92
miR-2117	19.14	23.51	27.37
miR-140	18.66	0.00	0.00
miR-106B	18.54	0.00	27.97
miR-4640	18.52	27.58	56.09
miR-22, miR-22HG	18.24	21.85	24.39
miR-3134, PTBP3	18.13	18.67	17.58
miR-5685	18.11	0.00	0.00
miR-3620	18.08	16.85	29.15
miR-635	17.89	56.60	25.88
miR-4420	17.66	0.00	0.00
miR-193A	17.03	21.25	0.00
miR-298	17.03	0.00	0.00
miR-130A	16.11	0.00	0.00
miR-181B2	16.11	0.00	0.00
miR-192	16.02	0.00	0.00
miR-3120	15.80	0.00	0.00
miR-4308	15.79	0.00	14.93
miR-4723	15.79	0.00	0.00
miR-941-2, miR-941-3, miR-941-4	15.50	19.85	10.06
miR-3909	15.46	8.00	0.00
miR-181B1	15.35	0.00	0.00
miR-153-1	15.26	0.00	0.00
miR-100HG	15.15	25.61	51.07
miR-196A1	15.03	72.23	0.44
miR-638	15.00	5.28	0.00
miR-7-1	14.92	10.42	21.14
miR-548A1	14.82	0.00	0.00
miR-4748	14.80	0.00	0.00
miR-4999	14.47	0.00	0.00
miR-662	14.33	119.82	27.08
miR-4484	13.89	0.00	0.00
miR-1236	13.75	40.74	43.23
miR-30E	13.74	0.00	0.00
miR-378D2	12.42	5.78	10.01
miR-339	12.40	0.00	0.00
miR-135B	12.35	0.00	0.00
miR-3190	12.29	12.37	25.38
miR-1909, REXO1	11.84	58.58	81.37
miR-559	11.25	0.00	0.00
miR-219-1	11.19	12.78	0.00
miR-5006, VWA8	11.16	9.64	9.06
miR-3176	10.93	1.59	1.23
miR-3613	10.93	0.00	0.00
miR-598	10.72	0.00	0.00
miR-4523, TAOK1	10.42	13.25	11.83
miR-708	10.33	0.00	0.00

Contd...

Supplementary Table 1: Contd...

<i>miRNAs</i>	<i>Caput cell</i>	<i>Corpus cell</i>	<i>Cauda cell</i>
miR-4296	10.33	0.00	0.00
miR-573	10.28	0.40	0.58
miR-744	10.23	0.00	0.00
miR-148B	9.80	0.00	0.00
miR-10A	9.71	2.33	9.67
miR-146A	9.40	1.11	1.10
miR-4315-2, PLEKHM1	9.39	11.30	10.37
miR-1225	9.26	0.00	0.00
miR-205, miR-205HG	8.99	104.08	149.66
miR-1204, PVT1	8.54	7.84	89.13
miR-210HG	8.15	4.65	4.91
miR-3911	7.76	15.65	8.27
miR-941-1, miR-941-3, miR-941-4	7.61	15.41	28.02
miR-3939	7.21	0.00	4.14
miR-200A, miR-200B, miR-429	7.19	24.14	8.31
miR-4469, RNF170	6.85	10.56	26.39
miR-3653	6.83	73.59	39.59
miR-1287, PYROXD2	6.72	4.46	7.00
miR-23B	6.51	12.12	0.00
miR-590	6.51	46.23	30.39
miR-499A	6.40	0.00	7.88
miR-484, NDE1	6.26	7.56	8.98
miR-3651, SNORA84	6.22	20.52	18.15
miR-567	6.21	0.00	6.91
miR-345	6.21	0.00	0.00
miR-4763, miR-LET7A3, miR-LET7B, miR-LET7BHG	5.85	39.49	41.06
miR-141	5.47	16.11	11.75
miR-570, SDHAP2	5.14	6.57	11.76
miR-3941	4.97	4.63	4.69
miR-221, miR-222	4.74	5.23	5.91
miR-4767, STS	4.64	13.79	11.98
miR-769	4.57	0.00	0.00
miR-31HG	4.54	3.76	4.15
miR-5187, TOMM40L	4.07	5.32	22.80
miR-30C2	3.92	1.10	0.98
miR-5004, SYNGAP1	3.86	11.00	6.16
miR-5587	3.75	1.79	1.25
miR-34A	3.63	2.73	2.76
miR-135A2	3.54	0.95	1.09
miR-LET7DHG	3.21	2.68	2.83
miR-765	3.20	0.00	0.00
miR-155, miR-155HG	3.11	0.61	0.45
miR-508	3.09	0.00	0.00
miR-4724, RAB11FIP4	2.75	3.95	25.66
miR-5194	2.59	0.00	0.00
miR-218-1	2.43	0.00	0.00
miR-5001, TIGD1	2.00	2.30	2.36
miR-LET7D	1.96	12.80	1.64
miR-331, miR-3685	1.77	1.74	1.91
miR-181D	1.72	1.83	0.00
miR-3714, PLCL2	1.66	0.17	0.23
miR-3180-4	1.62	0.00	0.00
miR-1256, SLC25A53	1.58	1.26	1.36
miR-1291, SNORA34	1.42	5.94	3.50
miR-181A2HG	1.29	0.63	0.88
miR-17, miR-17HG, miR-18A, miR-19A, miR-19B1, miR-20A, miR-92A1	1.10	0.73	0.84

Contd...

Supplementary Table 1: Contd...

<i>miRNAs</i>	<i>Caput cell</i>	<i>Corpus cell</i>	<i>Cauda cell</i>
miR-548I2	1.09	0.00	0.00
miR-4720	0.97	1.25	33.19
miR-548AA1, PALMD	0.91	0.77	0.69
miR-600HG	0.81	0.45	0.39
miR-3619	0.76	0.63	22.01
miR-3677	0.73	0.84	0.40
miR-LET7I	0.72	10.14	8.67
miR-4632, TNFRSF1B	0.72	0.22	0.25
miR-320B2	0.70	0.00	1.39
miR-4324, SLC6A16	0.64	0.12	0.10
miR-548N, OSBPL6	0.50	2.68	2.77
miR-374C	0.48	0.68	3.19
miR-374B, miR-421	0.43	3.57	8.80
miR-143, miR-143HG, miR-145	0.40	0.64	9.23
miR-137, miR-137HG	0.07	0.16	0.15
miR-4690	0.00	251.25	412.35
miR-4749	0.00	97.76	0.00
miR-4669	0.00	84.86	0.00
miR-199A1	0.00	83.60	0.00
miR-149	0.00	67.76	35.79
miR-4665	0.00	67.39	63.32
miR-4651	0.00	58.67	0.00
miR-3136	0.00	39.90	31.28
miR-1972-2	0.00	38.87	0.00
miR-4685	0.00	38.06	0.00
miR-4782	0.00	37.19	29.15
miR-3125	0.00	36.15	0.00
miR-2116	0.00	34.73	0.00
miR-5087	0.00	34.62	21.24
miR-548D2	0.00	28.57	0.00
miR-933	0.00	27.47	0.00
miR-1266	0.00	26.91	0.00
miR-3682	0.00	26.85	0.00
miR-593	0.00	26.73	0.00
miR-4505	0.00	26.58	0.00
miR-4656	0.00	22.65	62.11
miR-3184	0.00	22.61	0.00
miR-622	0.00	22.17	22.00
miR-3132	0.00	21.19	0.00
miR-LET7F2	0.00	19.84	0.00
miR-3138	0.00	19.48	0.00
miR-4653	0.00	16.30	54.72
miR-4786	0.00	15.73	15.95
miR-5192	0.00	15.53	0.00
miR-572	0.00	14.73	27.08
miR-3153	0.00	13.79	13.99
miR-624	0.00	13.38	0.00
miR-5088	0.00	12.18	0.00
miR-4787	0.00	12.16	0.00
miR-877	0.00	10.79	0.00
miR-142	0.00	10.19	0.00
miR-1207	0.00	10.19	0.00
miR-152	0.00	10.19	0.00
miR-3944	0.00	8.30	10.32
miR-5188	0.00	8.00	0.00
miR-4314	0.00	7.76	0.00

Contd...

Supplementary Table 1: Contd...

<i>miRNAs</i>	<i>Caput cell</i>	<i>Corpus cell</i>	<i>Cauda cell</i>
miR-5008	0.00	7.01	0.00
miR-645	0.00	7.01	0.00
miR-3662	0.00	6.69	0.00
miR-615	0.00	6.54	0.00
miR-1324	0.00	6.36	0.00
miR-597	0.00	6.09	0.00
miR-218-2	0.00	4.87	3.52
miR-3973	0.00	3.91	0.00
miR-548F4	0.00	3.90	0.00
miR-98	0.00	3.83	0.00
miR-181A2	0.00	3.47	0.00
miR-365B	0.00	3.34	0.00
miR-548K	0.00	2.78	2.82
miR-181C	0.00	2.55	0.00
miR-3180-5	0.00	2.21	2.52
miR-4519	0.00	0.00	851.22
miR-1249	0.00	0.00	180.16
miR-4701	0.00	0.00	128.52
miR-5581	0.00	0.00	115.31
miR-4520A	0.00	0.00	59.89
miR-4271	0.00	0.00	47.26
miR-302C	0.00	0.00	42.62
miR-2909	0.00	0.00	38.60
miR-4524A	0.00	0.00	38.60
miR-4479	0.00	0.00	32.12
miR-4284	0.00	0.00	29.85
miR-3157	0.00	0.00	28.94
miR-328	0.00	0.00	22.94
miR-363	0.00	0.00	22.94
miR-1-2	0.00	0.00	17.04
miR-LET7A1	0.00	0.00	15.95
miR-3162	0.00	0.00	13.99
miR-92B	0.00	0.00	13.90
miR-554	0.00	0.00	12.90
miR-4644	0.00	0.00	12.34
miR-659	0.00	0.00	12.30
miR-330	0.00	0.00	12.14
miR-5094	0.00	0.00	11.61
miR-644A	0.00	0.00	9.40
miR-4306	0.00	0.00	8.29
miR-648	0.00	0.00	7.12
miR-4313	0.00	0.00	5.12
miR-1250	0.00	0.00	3.15
miR-664, SNORA36B	0.00	0.00	2.94
miR-3689B	0.00	0.00	1.06

miRNAs: microRNAs

Supplementary Table 2: List of microRNAs in primary human epididymal tissues (fragments per kilobase of transcript per million mapped reads [FPKM] >0.1)

<i>miRNAs</i>	<i>Caput tissue</i>	<i>Corpus tissue</i>	<i>Cauda tissue</i>
miR-4461	3.6E+06	5.3E+05	4.1E+05
miR-4709, NPC2	41160.70	121350.00	188.55
miR-3648, miR-3687	12117.00	17315.50	1614.22
miR-1282	1565.40	1400.44	1605.93
miR-663A	1438.89	1222.82	47.05
miR-1244-3, PTMA	862.17	797.60	799.30
miR-3650	833.36	4.67	0.23
miR-4426, RPS27A	733.05	390.24	954.78
miR-611, TMEM258	514.21	652.57	248.28
miR-29C	501.88	1105.79	18.75
miR-4647, SLC35B2	498.77	732.85	77.57
miR-205, miR-205HG	466.77	1770.51	5.06
miR-1279	465.71	144.46	0.00
miR-1538	455.79	7.06	0.00
miR-1307, USMG5	426.02	512.42	389.86
miR-3661	413.86	78.74	271.26
miR-3918	366.64	1374.74	255.72
miR-762	347.98	705.28	50.41
miR-141	337.14	487.46	6.92
miR-4668	326.47	0.00	129.06
miR-4690	312.12	0.00	0.00
miR-1304, SNORA1, SNORA18, SNORA25, SNORA32, SNORA40, SNORA8, SNORD6, TAF1D	305.77	271.49	204.53
miR-155, miR-155HG	298.20	8.98	1.85
miR-4728	255.72	79.32	147.62
miR-5193, UBA7	203.28	251.23	14.82
miR-135A2, RMST	201.75	198.86	2.41
miR-639, TECR	161.93	332.88	121.02
miR-596	159.87	66.17	0.00
miR-663B	151.60	73.48	0.00
miR-4667	142.45	0.00	0.00
miR-1226	124.20	429.02	0.00
miR-3120	121.15	241.20	0.00
miR-636, SRSF2	120.24	116.10	125.52
miR-5691	115.46	0.00	0.00
miR-1909, REXO1	104.45	195.44	81.03
miR-99B	94.99	0.00	0.00
miR-3607	92.46	57.36	0.00
miR-18B	86.70	0.00	0.00
miR-29B2	80.76	75.16	0.00
miR-662	76.83	53.55	0.00
miR-4785	72.93	0.00	0.00
miR-5093	72.36	53.92	0.00
miR-548D2	71.03	0.00	0.00
miR-1247	67.97	0.00	0.00
miR-3682	66.76	174.39	18.63
miR-4674	62.96	48.73	0.80
miR-4691	62.84	19.49	132.11
miR-363	62.06	38.50	0.00
miR-92A2	62.06	0.00	0.00
miR-4516	59.23	92.82	16.63
miR-34C	53.33	0.00	0.00
miR-3912	52.42	0.00	0.00
miR-21, VMP1	49.30	52.76	146.28
miR-1225	47.34	14.68	0.00
miR-1248, SNORA81	46.51	29.13	68.95
miR-3620	46.23	0.00	98.43

Contd...

Supplementary Table 2: Contd...

<i>miRNAs</i>	<i>Caput tissue</i>	<i>Corpus tissue</i>	<i>Cauda tissue</i>
miR-1914, miR-647, UCKL1	45.88	32.86	53.33
miR-100HG	44.27	27.17	3.18
miR-4492	43.19	0.00	171.29
miR-760	43.17	0.00	0.00
miR-2117	43.17	0.00	0.00
miR-4672	40.38	0.00	0.00
miR-761, NRD1	39.38	41.00	38.81
miR-564, TMEM42	36.73	102.04	14.21
miR-4721, TUFM	35.57	40.49	101.44
miR-1238	35.51	44.06	25.20
miR-4750, TBC1D17	33.73	982.62	15.97
miR-505	33.38	0.00	0.00
miR-568	32.26	31.31	15.49
miR-378D2	31.75	19.70	0.00
miR-632, ZNF207	31.60	34.27	99.60
miR-3621	31.42	0.00	0.00
miR-3610, RAD21	31.30	34.26	49.89
miR-3615, SLC9A3R1	30.40	50.69	76.26
miR-22, miR-22HG	30.06	36.51	29.73
miR-3942	29.75	18.46	7.04
miR-3180-4	29.53	16.72	1.39
miR-3917, STMN1	28.96	36.46	168.86
miR-221, miR-222	28.16	28.33	8.73
miR-3656, TRAPPC4	27.47	30.43	113.30
miR-3671	26.41	49.16	0.00
miR-1287, PYROXD2	25.93	28.19	13.40
miR-4687, STIM1	25.58	7.82	239.96
miR-5006, VWA8	23.76	8.57	5.45
miR-135A1	23.67	14.68	31.42
miR-1292, NOP56, SNORD86	22.34	19.47	89.01
miR-3605, PHC2	22.13	26.77	140.39
miR-191	21.30	0.00	0.00
miR-4800, MXD4	20.46	21.64	119.53
miR-645	19.24	0.00	13.66
miR-374C	19.10	12.58	1.22
miR-4680, PDCD4	18.54	21.47	551.67
miR-LET7D	17.86	15.18	2.37
miR-943, NELFA	17.65	22.35	26.01
miR-4741, RBBP8	17.23	11.70	26.43
miR-635	15.87	153.56	0.00
miR-200A, miR-200B, miR-429	14.24	9.23	12.86
miR-210, miR-210HG	13.41	55.42	39.68
miR-3134, PTBP3	13.08	11.02	40.31
miR-497HG	10.12	16.11	7.07
miR-937, SCRIB	9.86	14.53	98.86
miR-4724, RAB11FIP4	9.58	11.29	22.94
miR-3677, miR-940	9.06	35.43	0.64
miR-4517, NFATC2IP	8.92	139.88	18.73
miR-570, SDHAP2	8.24	11.09	7.02
miR-659	8.13	2.47	0.57
miR-4700, UNC119B	8.00	4.02	222.21
miR-4469, RNF170	7.38	9.17	9.75
miR-LET7DHG	7.33	4.88	5.47
miR-3176, SOLH	7.00	7.68	15.60
miR-4763, miR-LET7A3, miR-LET7B, miR-LET7BHG	6.58	17.98	6.43
miR-181A2HG	5.58	3.90	1.34

Contd...

Supplementary Table 2: Contd...

<i>miRNAs</i>	<i>Caput tissue</i>	<i>Corpus tissue</i>	<i>Cauda tissue</i>
miR-3685	5.06	5.17	5.23
miR-664, SNORA36B	4.63	2.87	0.00
miR-3614, TRIM25	4.62	4.94	25.36
miR-5001, TIGD1	4.58	4.34	3.49
miR-132	4.51	9.33	0.12
miR-5004, SYNGAP1	4.26	2.49	2.63
miR-3911	4.04	10.32	8.18
miR-1291, SNORA34	3.87	2.41	7.39
miR-612	3.54	38.31	7.24
miR-3658, UCK2	3.24	1016.70	7.48
miR-3714, PLCL2	3.18	2.84	2.26
miR-31HG	3.06	4.16	6.69
miR-1256, SLC25A53	2.89	3.00	2.67
miR-4315-2, PLEKHM1	2.77	2.80	9.67
miR-548AA1, PALMD	2.72	2.85	0.28
miR-621, SLC25A15	2.53	1.98	21.57
miR-143, miR-143HG, miR-145	2.39	2.72	0.48
miR-4740	2.25	1.55	0.88
miR-31	2.16	3.20	6.27
miR-3074	2.08	44.72	54.46
miR-17, miR-17HG, miR-18A, miR-19A, miR-19B1, miR-20A, miR-92A1	2.01	1.81	1.29
miR-320E	1.71	1.61	0.60
miR-5187, TOMM40L	1.55	1.82	5.64
miR-146B	1.46	2.19	0.01
miR-600HG	1.46	1.17	1.50
miR-548N, OSBPL6	1.27	1.24	0.27
miR-LET7I	1.14	1.41	1.83
miR-4632, TNFRSF1B	1.14	1.49	0.40
miR-1972-2	1.13	0.94	0.09
miR-484, NDE1	0.90	1.32	4.41
miR-146A	0.82	1.00	6.83
miR-3916	0.81	1.21	28.96
miR-4712	0.71	1.06	1.92
miR-3688-2	0.65	0.87	11.27
miR-4720	0.51	0.78	1.70
miR-30C2	0.34	0.11	10.52
miR-4491	0.28	6.22	0.00
miR-7-3HG	0.26	0.22	0.00
miR-573	0.25	0.09	47.53
miR-196A1	0.17	0.15	139.54
miR-135B	0.15	2.59	4.85
miR-5587	0.06	0.28	2.58
miR-631	0.00	230.99	0.00
miR-4489	0.00	144.46	165.26
miR-4714	0.00	137.92	0.00
miR-4467	0.00	126.45	0.00
miR-4256	0.00	111.51	0.00
miR-4449	0.00	88.37	0.00
miR-4292	0.00	79.32	271.37
miR-328	0.00	77.00	0.00
miR-200C	0.00	71.54	0.00
miR-20B	0.00	64.80	0.00
miR-4306	0.00	58.24	0.00
miR-4746	0.00	53.78	61.53
miR-4725	0.00	52.49	0.00
miR-330	0.00	49.30	0.00

Contd...

Supplementary Table 2: Contd...

<i>miRNAs</i>	<i>Caput tissue</i>	<i>Corpus tissue</i>	<i>Cauda tissue</i>
miR-572	0.00	48.06	0.00
miR-339	0.00	47.46	0.00
miR-4804	0.00	45.24	0.00
miR-5690	0.00	45.24	0.00
miR-324	0.00	44.08	176.20
miR-4768	0.00	41.68	0.00
miR-641	0.00	39.91	79.97
miR-4784	0.00	33.09	168.92
miR-3125	0.00	30.78	0.00
miR-LET7A1	0.00	26.78	0.00
miR-1236	0.00	23.96	21.25
miR-4642	0.00	23.47	31.43
miR-5188	0.00	20.90	3.96
miR-196B	0.00	20.74	71.06
miR-4513	0.00	18.37	0.00
miR-1227	0.00	16.39	52.50
miR-133A1	0.00	16.39	0.00
miR-604	0.00	11.94	28.32
miR-331	0.00	11.94	0.00
miR-33B	0.00	10.82	16.51
miR-1324	0.00	10.82	0.00
miR-5002	0.00	10.32	0.00
miR-561	0.00	10.32	0.00
miR-27B	0.00	10.32	0.00
miR-4440	0.00	9.85	25.01
miR-345	0.00	9.85	0.00
miR-4441	0.00	8.99	25.83
miR-593	0.00	8.99	10.28
miR-5000	0.00	7.88	0.00
miR-765	0.00	5.10	0.00
miR-941-1, miR-941-3, miR-941-4	0.00	4.56	15.64
miR-3651, SNORA84	0.00	2.71	0.00
miR-320B2	0.00	2.34	3.73
miR-548I1	0.00	1.73	0.00
miR-548I2	0.00	1.73	0.00
miR-3907	0.00	1.64	0.00
miR-137, miR-137HG	0.00	0.07	0.14
miR-1260B	0.00	0.00	450.62
miR-4701	0.00	0.00	261.36
miR-4273	0.00	0.00	199.26
miR-553	0.00	0.00	163.23
miR-25	0.00	0.00	145.65
miR-3189	0.00	0.00	96.63
miR-4253	0.00	0.00	93.04
miR-302C	0.00	0.00	81.84
miR-4284	0.00	0.00	74.31
miR-4685	0.00	0.00	74.13
miR-1229	0.00	0.00	74.13
miR-4482-1	0.00	0.00	67.41
miR-27A	0.00	0.00	65.58
miR-590	0.00	0.00	52.91
miR-23A	0.00	0.00	48.97
miR-4640	0.00	0.00	47.57
miR-374B	0.00	0.00	42.76
miR-4766	0.00	0.00	40.78
miR-3191	0.00	0.00	39.63

Contd...

Supplementary Table 2: Contd...

<i>miRNAs</i>	<i>Caput tissue</i>	<i>Corpus tissue</i>	<i>Cauda tissue</i>
miR-5687	0.00	0.00	37.85
miR-3117	0.00	0.00	35.21
miR-4742	0.00	0.00	33.32
miR-3153	0.00	0.00	29.95
miR-5087	0.00	0.00	26.92
miR-3138	0.00	0.00	26.86
miR-3662	0.00	0.00	26.00
miR-4263	0.00	0.00	25.28
miR-4653	0.00	0.00	25.20
miR-938	0.00	0.00	24.53
miR-3188	0.00	0.00	22.30
miR-186	0.00	0.00	21.02
miR-149	0.00	0.00	19.32
miR-193A	0.00	0.00	18.75
miR-661	0.00	0.00	18.39
miR-1231	0.00	0.00	16.16
miR-LET7G	0.00	0.00	14.62
miR-421	0.00	0.00	14.11
miR-507	0.00	0.00	13.66
miR-219-1	0.00	0.00	13.53
miR-941-2, miR-941-3, miR-941-4	0.00	0.00	13.16
miR-199B	0.00	0.00	12.15
miR-634	0.00	0.00	11.81
miR-455	0.00	0.00	11.27
miR-7-1	0.00	0.00	11.17
miR-423	0.00	0.00	11.14
miR-622	0.00	0.00	10.70
miR-140	0.00	0.00	10.28
miR-3622B	0.00	0.00	9.14
miR-4311	0.00	0.00	8.35
miR-181A2	0.00	0.00	5.53
miR-769	0.00	0.00	5.03
miR-3909	0.00	0.00	4.86
miR-499A	0.00	0.00	4.47
miR-4524B	0.00	0.00	4.40

miRNAs: microRNAs