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Comparative profiling of genes and miRNAs expressed in the newborn, young adult, and aged human epididymides

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To understand roles of transcriptional factors and miRNAs in regulating gene expression in the epididymis from postnatal development through aging, systematic profiling of genes and miRNAs expressed in the newborn, young adult, and aged human epididymides was performed by cDNA array and miRNA array analysis, respectively. The newborn human epididymis expressed the fewest mRNAs but the largest number of miRNAs, whereas the adult and aged epididymides expressed the most mRNAs but the fewest miRNAs, a negative correlation between mRNAs and miRNA during aging. By integrative analysis, a set of miRNA targets were predicted based on the miRNA and cDNA arrays. In the newborn epididymis, 127 miRNAs were exclusively or preferentially expressed but only 3 and 2 miRNAs showed an age-enriched expression pattern in the adult and aged epididymides, respectively. This study provides a basic database as well as new insights and foundations for further studies on the complex regulation of gene expression in the epididymis.

Keywords epididymis; microarray; miRNA; transcriptome; transcriptional factor

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Introduction

The epididymis plays an important role in sperm maturation and sperm storage [1,2]. From birth to adulthood, to achieve full sperm fertility, the epididymis undergoes major changes in the epithelial cells with the differentiation of principal, narrow, basal, and halo cells [3,4]. In humans, from the newborn period to adulthood, the epididymal tubular diameter, luminal diameter, and height of the epithelium increase dramatically [5]. On the other hand, aging in the mammalian epididymis is associated with a general decline in physiological functions, particularly fertility. Remarkable cellular and histological changes take place during aging along with a decrease in semen volume and epididymally acquired sperm motility. At the same time, the proportion of abnormal spermatozoa increases [6]. The luminal diameter of the epididymis is significantly reduced, whereas the epithelial cell height increases [7–9]. In addition, there is a dramatic increase in halo cells that were postulated to be either lymphocytes or monocytes [7,10], suggesting an increase in innate immune activity.

The molecular bases of age-dependent fertility development and decline are largely unknown and the accompanying cellular and morphological changes in the epididymis from birth through adolescence to senescence are also little understood. However, it is well established that a functional epididymis is dependent upon androgen produced by the testis and androgen levels change dramatically during the lifespan. During human male development, the greatest testosterone secretion can be observed in the first half of gestation, gradually decreasing throughout the first year of life [11,12]. The testosterone content of the epididymis reaches a plateau at puberty and remains at a relatively high level until adulthood. Many genes show androgen-dependent expression patterns as revealed by individual studies and the comprehensive profiling of gene expression changes before and after orchidectomy in the rat [13–16]. In the mouse, by castration and dihydrotestosterone (DHT) replacement, the androgen-responsive genes including up- or down-regulated by DHT were identified [17]. Previous

studies reported changes in gene expression during epididymal development and aging including 5α-reductases, E-cadherin, occludin, and ZO-1 which decline during aging [18]. By using cDNA arrays, changes in gene expression in the young and old rat epididymides were identified and an overwhelming decrease in gene expression during aging was found [19,20].

However, up to now, there is no gene expression profiling available defining epididymal gene expression changes throughout the entire lifespan of any mammal, including humans. Toward filling this gap in our knowledge, epididymal gene expression patterns of the newborn, young adult, and aged were profiled by employing whole genome microarray systems. To further understand how these ever-changing gene expression patterns were achieved, all known transcription factors (TFs) were profiled within the epididymides of at these three ages. However, the regulation of TFs alone may not be enough to account for all the changes seen in the network of gene expression pathways. In fact, previous studies showed that in addition to TFs, during many biological processes, miRNAs also regulated gene expression [21]. Given that gene expression is highly regulated and there are no studies addressing miRNAs in the epididymis, a comprehensive survey of the miRNA expression in the epididymis was conducted by using miRNA arrays. An integrative analysis was performed by combining the gene and miRNA expression profiles. This report provides a database for further research on miRNAs in the epididymis. All array data were deposited for public use on our website: http://www.scbit.org/human_epididymis_transcriptomes.

Materials and Methods

Sample collection and RNA extraction

Human epididymis samples used in this study were obtained from Yuhuangding Hospital (Yantai, China) and Renji Hospital (Shanghai, China). The newborn and adult epididymides were obtained from accident victims after family informed consent. The adult was 25 years old and both newborn and adult were healthy. The aged epididymis was obtained after informed consent from a 75-year-old man who suffered from prostate cancer and did not receive any hormone intervention before castration. The Ethics Committee of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, approved all the above procedures. Tissues were frozen in liquid nitrogen and total RNAs were prepared using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instruction. Total RNA integrity was judged by denaturing agarose gel electrophoresis. The RNA purity was indicated by the 260–280 nm absorbance ratio of 1.9–2.0.

Microarray and hybridization

Human Genome U133 plus 2.0 microarray platforms (Affymetrix, Shanghai, China) used in this study contain 54,000 probe sets, representing ~38,500 well-known human transcripts (estimated by UniGene coverage). Gene Company Ltd (Shanghai, China) performed microarray target preparations including the synthesis of double-strand DNA, biotin labeling of cRNA, and hybridization. Briefly, after preparation of the hybridization cocktail of the fragmented-biotinylated cRNA, each sample was checked for cRNA integrity by hybridizing to the test-chips and the ratio $3'-5'$ of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and *actin* should be less than 3.0. After that, the Human Genome U133 plus 2.0 microarray was directly loaded with hybridization solution. The steps of washing and scanning were performed as before [22]. For miRNA profiling, the miRCURY array microarray (Exiqon, Vedbaek, Denmark) used in this study was referenced from the Sanger miRNA database (http://microrna.sanger.ac.uk). Small RNAs (<200 nt) were extracted with mirVana miRNA isolation kit (Ambion, Austin, USA). miRNA labeling was performed with miRCURY array labeling kit (Exiqon) and hybridization was conducted by using miRCURY array microarray kit at 60°C. Finally, the image acquisition and quantification were performed using a Genepix 4000B microarray scanner (Molecular Devices, Sunnyvale, USA).

Statistical and comparative analysis

For gene expression profiling, the raw data of all probe sets for each array produced by scanning were normalized according to 100 housekeeping genes in the mask file of U133 plus 2.0 to a mean intensity of 2000 before further statistical analysis. Using the GCOS (GeneChip Operation System; Affymetrix), data were analyzed to determine whether the transcripts on the array were 'Present', 'Marginal' or 'Absent' and to calculate the raw signal representing the abundance of the tested transcripts in each sample. Initially, a transcript was considered expressed in a sample if its detection *P*-value was <0.04 and its relative signal was no less than 50 and was designated 'Present' by the statistical analysis.

For the comparative analysis, data from each age were compared with the other two ages. To qualify as age-specific, a transcript should meet the following conditions: (i) the detection call in a given age epididymis by GCOS analysis is assigned 'Present' and it is 'Absent' in the epididymis of other two ages, and (ii) the scaling intensity in the epididymis of a given age is no less than 50.

We assembled a database of 1494 TFs of known transcriptional activity derived from GO (Gene Ontology: www.geneontology.org) annotation or validated in the published literature. The full list of TFs is also available on

our website: http://www.scbit.org/human_epididymis_transcriptomes. Our TF database was integrated into the microarray data for further analysis. TF expression was judged 'Present' by a signal no less than 20 instead of signal no less than 50 because TF expression level is relatively low *in vivo*. The software used in these analyses includes Microsoft Excel and Cluster 3.0.

For miRNA arrays, the signal for each miRNA was normalized to snRNA U6 in each array to allow comparative analysis among the three samples. The expressed miRNA should have a normalized value of no less than 1.5 folds of the highest empty control in each array. Age-specific or preferentially expressed miRNAs are defined as those for which the normalized data qualifies as 'Present' in one age, but 'Absent' in the other two developmental ages.

Integration analysis of correlated miRNA–mRNA pairs based on two level profiles

Generally, it is reported that miRNAs lead to the repression of gene expression or mRNA degradation [23]. In other words, there is a negative correlation between miRNA and its target genes. The correlation analysis between miRNAs and mRNAs was performed as described previously [24]. Briefly, 174 miRNAs differentially expressed in any two samples with more than 2-fold up- or down-regulation were selected. The 18,349 transcripts expressed ubiquitously within three ages were selected. Correlation analysis was performed on expression of 174 miRNAs and 18,349 transcripts within the newborn, adult, and aged samples. An interaction network between miRNAs and transcripts was obtained when the P-value was <0.01 [24]. Moreover, the pairs with P-values $<0.1/18{,}349$ were selected [24]. The miRNA target prediction was performed by employing TargetScan 4.2 (http://www.targetscan.org) and miRanda (http://www.microrna.org) and their intersections were adopted.

Quantitative real-time RT–PCR

The cDNA samples for quantitative PCR analysis were synthesized by oligo(dT)20 priming in a final volume of 25 μl using ReverTra Ace first-strand cDNA synthesis kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The cDNA samples from the newborn, adult, and aged epididymides were diluted to a final volume of 200 μl to produce 20× cDNA stocks. All primers for genes of interests were designed using Primer Premier 5.0 software and synthesized at Sangon Company Ltd (Shanghai, China). The sequences of primers chosen for each gene and the product length as well as the accession number for each transcript are indicated in **Supplementary Table S1**. Quantitative real-time PCR was conducted using QuantiTect SYBR Green PCR reagent (Qiagen, Valencia, USA). The PCRs were performed on DNA Engine Opitcon 2.0 (Bio-Rad, Hercules, USA) in a 96-well format. Melting curve analysis was conducted from 72 to 95°C, read every 0.5°C to check the specificity of proliferation products. Each reaction was conducted in triplicate and each experiment was performed three independent times. Relative quantification analyses were undertaken based on Relative Standard Curve Method as described at http://www.uic.edu/depts/rrc/cgf/realtime/stdcurve.html.

Results

Increasing number of expressed genes in the epididymis between birth and adulthood

The number of genes expressed in the epididymis increased from the newborn to the young adult with a slight difference between the young and old epididymides. Totally, 25,577 transcripts were detected as 'Present' in the human epididymis, almost half of the total probes on the array. In detail, according to the age-expressed qualifier criteria, 12,380, 16,002, and 16,017 transcripts were detected in the newborn, young adult, and aged human epididymides, respectively, accounting for about 23%, 29%, and 29% of the total probes on the array (**Fig. 1**).

On the basis of the criteria for judging age-specific qualifiers, 260, 165, and 166 transcripts can be considered exclusively or preferentially expressed in the newborn, adult, and aged epididymides, respectively. Thus, the newborn epididymis expressed the greatest number of age-specific qualifiers, whereas the aged epididymis expressed the fewest (**Fig. 1**). **Supplementary Tables S2–S4** selectively show the age-specific or preferentially expressed genes with relatively higher intensity. Interestingly, it should be noted that the catalogue of adult-specific transcripts is mostly composed of motor proteins, including tropomyosin 2, tropomyosin 3, myosin, titin, myosin-binding protein C, troponin 1, and troponin C1

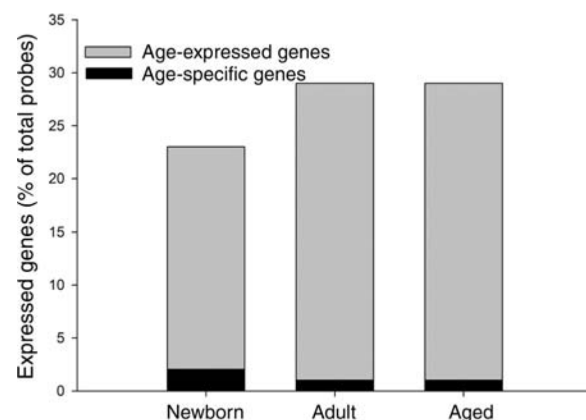

Figure 1 Gene expression profile in the newborn, adult, and aged human epididymides Gray bars represent the expressed genes (% of total probes on the array) for each age, whereas the black bars show the age-specific genes (% of total).

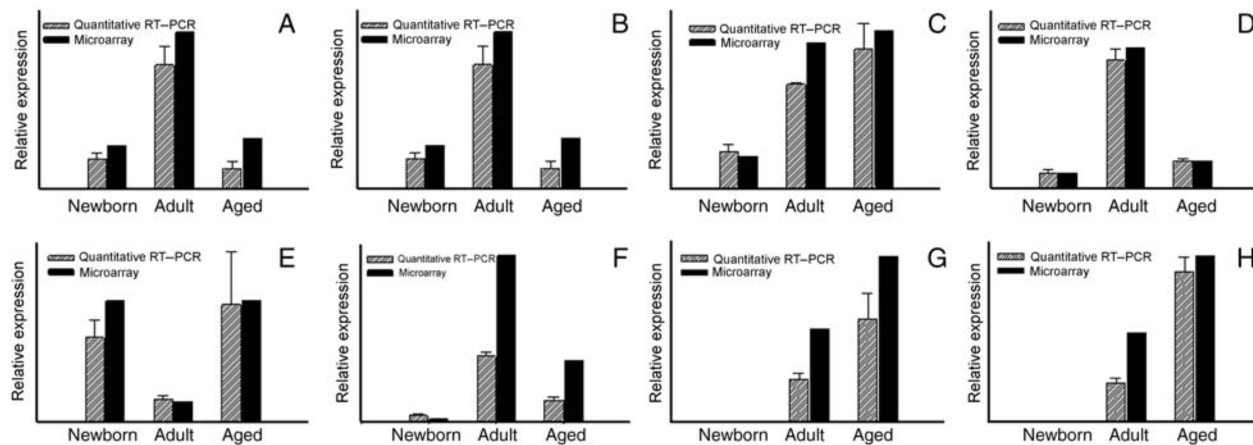

Figure 2 Confirmation of microarray results via quantitative real-time RT−PCR (A) Caveolin 1, (B) *CKM*, (C) guanidinoacetate *N*-methyltransferase (*GAMT*), (D) *SPAG11B* variant *H* (also known as HE2 gamma 1), (E) beta-defensin 1 (*DEFB1*), (F) beta-defensin 107A (*DEFB107A*), (G) *DEFB119*, and (H) *DEFB126*.

(**Supplementary Table S3**). On the other hand, several members of the beta-defensin family showed differently regulated patterns during epididymal postnatal development, such as *DEFB32* (beta-defensin 32), *DEFB105*, *DEFB119*, *DEFB121*, *DEFB126*, and *DEFB129*. Their expression increased from birth to adult and reached the highest amount in the aged epididymis. To evaluate the authenticity of microarray results, we performed real-time quantitative RT−PCR on eight genes randomly selected. As shown in **Fig. 2**, the PCR assays are well consistent with the array results.

Moreover, we conducted a comparative analysis of gene expression in the young adult epididymis compared with the newborn and identified 2758 genes that were differentially regulated. Among them, 2233 transcripts were up-regulated in the young adult but only 525 were down-regulated compared with the newborn. However, compared with the adult epididymis, 392 genes were up-regulated and 427 genes were down-regulated in the aged epididymis, indicating a relatively small change between these two ages (**Fig. 3**).

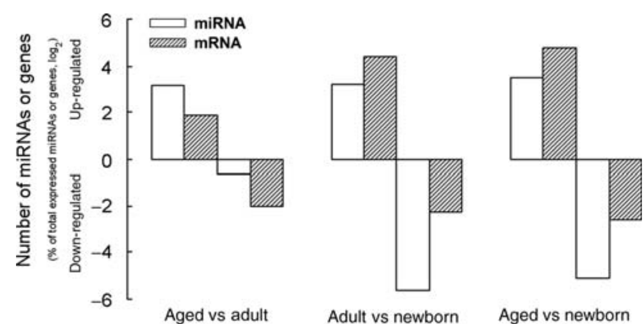

Figure 3 Comparative analysis showing changes for miRNAs and genes expressions between any two age epididymides The vertical scale represents the ratio of miRNAs or genes that show at least 2-fold changes in either direction to the total number of expressed miRNAs or genes. The ratio on the scale is demonstrated by logarithm using 2 as base. The bars above the *X*-axis indicate up-regulated and below indicate down-regulated expression for both miRNAs and genes.

TF expression was temporally regulated from birth until aging in the epididymis

Transcriptional factors perform vital roles in regulating gene expression. We listed human 1494 TFs by collecting known TFs with TF activity annotation or identified in the previously published literature and their expression is available according to the array analysis. As a whole, the newborn, young, and old epididymides, respectively, expressed 755, 937, and 968 TFs, in possession of 50%, 63%, and 65% of the total 1494 known TFs [**Fig. 4(A)**]. Similarly, 229 age-specific TFs were identified. Individually, 42, 88, and 99 TFs were restrictively expressed in the newborn, young, and old human epididymides [**Fig. 4(A)**] and the most abundant are listed in **Supplementary Table S5**. On the other hand, from the newborn to adult and aged epididymides, 281 TFs increased in expression and 81 declined [**Fig. 4(B)**]. For example, *AR* (androgen receptor) and *TFE3* [transcription factor binding to *IGHM* (immunoglobulin heavy constant mu) enhancer 3] were expressed at a low level in the newborn but at a high level in the adult man. *ER1* (estrogen receptor 1) showed the highest intensity in the adult, but *TCF3* (transcription factor 3) and *HSF2* (heat shock factor 2) were more highly expressed within the aged epididymis compared with that of the newborn and adult. Moreover, 375 TFs demonstrated the highest expression and 162 TFs showed the lowest expression in the young adult human epididymis in contrast to the other two ages.

The largest number of miRNAs was expressed in the newborn epididymis

At the miRNA level, the arrays demonstrated that the newborn epididymis expressed the largest number of

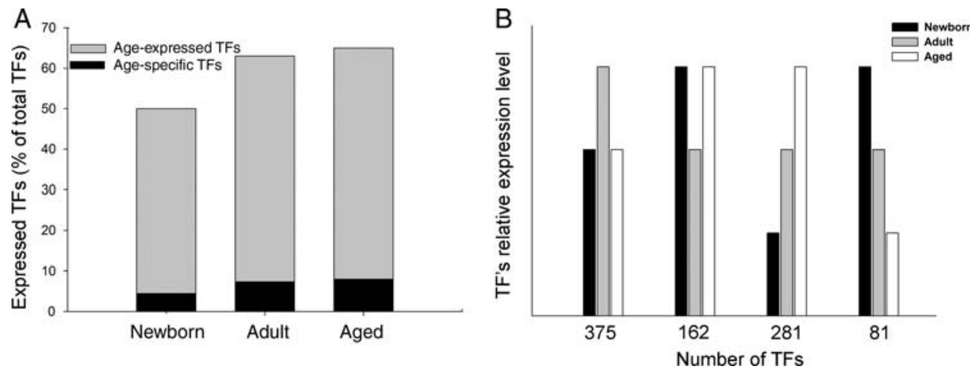

Figure 4 TF expression profiles in three ages of the human epididymis (A) TF expression in the newborn, adult, and aged human epididymides. Gray bars represent the expressed TFs (% of total TFs from list) for each age, whereas the black bars show the age-specific TFs (% of total TFs). (B) Different patterns of TF expression from the newborn, to the adult and aged human epididymides. *Y*-axis represents the relative level of TF expression, whereas *X*-axis indicates the number of TFs displaying each pattern.

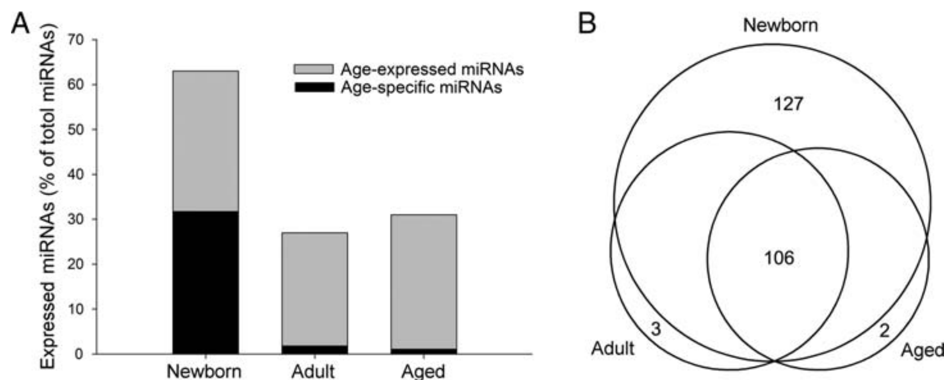

Figure 5 miRNAs expressed within the epididymis of newborn, young adult, and aged human (A) Age-expressed or age-specific miRNAs number in each age. Gray bars represent the expressed miRNAs (% of total miRNAs on the array) for each age, whereas the black bars show the age-specific genes (% of total miRNAs). (B) Venn diagram of miRNA expression in three age epididymides.

miRNAs, almost equal to the sum of those detected in the young adult and aged epididymides. In detail, the newborn expressed 251 miRNAs, whereas only 109 and 125 miRNAs probes were detected as positive on the arrays in the adult and aged human epididymides, accounting for 63%, 27%, and 31% of 402 known miRNAs, respectively [**Fig. 5(A)**]. Thus, the neonatal epididymis expressed the fewest genes but the greatest number of miRNAs compared with the other two ages. Thus, quantitatively, expressions of both genes and miRNAs were similar in the young adult and aged epididymides [**Figs. 1** and **5(A)**]. Clustering analysis of miRNA and gene expression exhibited similar results (data not shown).

The 106 miRNAs expressed ubiquitously in all three ages represent 26% of the total miRNA probes on the array [**Fig. 5(B)**]. These miRNAs showed widespread distribution with high or low level throughout the whole life-span, including *let-7a*, *b*, *c*, *d*, *f*, *miR-125a*, *miR-125b*, *miR-143*, *miR-23b*, *miR-26a*, and *rno-miR-347* (**Table 1**), implying their crucial roles in the maintenance of basic epididymal functions. Surprisingly, we identified 127 miRNAs specific for the newborn epididymis, corresponding to 31% of total known miRNAs on the array. For example, *miR-375*, *miR-208*, *miR-494*, *miR-452*, and *miR-198* showed abundant expression at birth compared with the other two ages (**Table 1**). This is in sharp contrast to only three and two unique miRNAs showing age-preferential expression in the adult and aged epididymides, respectively (**Fig. 5**).

miRNAs likely lead to clearance of mRNAs in the epididymis

Given that miRNAs regulate gene expression not only by preventing protein translation but also by degrading mRNAs as proved by many previous studies [25−27], we hypothesized that within the epididymis, miRNAs also regulate gene expression and lead to mRNA degradation. To this end, we integrated profiles of miRNA and mRNA expressions from the newborn to adult and aged epididymides for further analysis. As expected, the mRNA and miRNA profiles show negative correlation in the following aspects. First, as shown in **Figs. 1** and **4**, the newborn

Table 1 Representative miRNAs expressed in the infant, adult, and aged human epididymides

Description	Representative miRNAs
Ubiquitously expressed	hsa-let-7a, hsa-let-7b, hsa-let-7c, hsa-let-7d, hsa-let-7f, hsa-let-7g, hsa-miR-125a, hsa-miR-125b, hsa-miR-26a, mmu-miR-290, rno-miR-347, hsa-miR-143, has-miR-373, has-miR-23b, has-miR-100
Newborn-abundant or -specific	mmu-miR-433-5p, hsa-miR-375, hsa-miR-382, hsa-miR-208, hsa-miR-494, hsa-miR-513, mmu-miR-298, hsa-miR-503, hsa-miR-498, hsa-miR-198, hsa-miR-452, has-miR-492
Adult-abundant or -specific	hsa-miR-143, hsa-miR-7a, hsa-miR-29a, hsa-miR-24, hsa-miR-27b, hsa-miR-27a, has-miR-21, hsa-miR-23a
Aged-abundant or -specific	hsa-miR-222, hsa-miR-221, has-miR-29c, hsa-miR-193b, hsa-miR-374

Table 2 miRNA–mRNA high correlation pairs

miRNA (hsa-)	mRNA	*r*	Binding sites	miRNA (hsa-)	mRNA	*r*	Binding sites
miR-29b	KIAA0355	−1.00000	1	miR-34c	SATB1	−1.00000	1
miR-214	C2orf17	−0.99999	1	miR-185	CDC42	−1.00000	2
miR-130b	BLCAP	−0.99993	1	miR-141	JAG1	−1.00000	1
miR-130b	MECP2	−0.99995	4	let-7f	APPBP2	−0.99997	1
miR-130b	FBXL11	−0.99990	3	miR-129	UNC84B	−0.99992	1
miR-106b	BCL2L11	−0.99994	2	miR-145	CCNL1	−1.00000	1
miR-106b	STAT3	−0.99999	3	miR-206	JUND	−1.00000	1
miR-106b	RABEP1	−1.00000	2	miR-29c	PLP1	−0.99989	1
miR-181b	SIRT1	−0.99992	1	miR-211	UBE2R2	−0.99988	1
miR-181b	NCOA2	−0.99998	1	miR-215	FNDC3B	−0.99998	1
miR-122a	MBNL1	−0.99989	2	miR-27b	PSMA1	−1.00000	1

epididymis expressed the minimum number of mRNAs but maximum miRNAs, whereas the reverse was true in the adult and aged epididymides. Second, we compared the up- and down-regulated mRNAs and miRNAs between random pairs from newborn, adult, and aged epididymides. As indicated in **Fig. 3**, the difference in the number of expressed genes between the aged and the adult epididymides is the least notable, whereas the change in gene expression from the newborn to the aged epididymides is the most significant. Similarly, the difference between numbers of miRNAs showing more than 2-fold up- or down-regulation in the newborn compared with the adult human epididymis was the greatest, whereas the aged and adult epididymides were similar. Compared with the newborn epididymis, 2233 and 525 transcripts were up- and down-regulated in the adult human, but in contrast, 24 and 129 miRNAs were up- and down-regulated, respectively. The numbers of up- or down-regulated genes were in inverse proportion to those of miRNAs. Similarly, this is the same for the comparisons of mRNAs and miRNAs between the newborn and the aged epididymides. The difference between adult and aged samples also shows similar regulation, though not so remarkable as the other two pairs.

Aided by computational analysis, miRNA potential targets can be predicted according to several algorithms as reported before, but false positives always dampen the prediction accuracy. However, by virtue of available mRNA and miRNA expression profiles, miRNA target prediction will become more accurate than before. For example, by employing miRNA target prediction algorithms and based on the negative correlation between *miR-130b* and gene expression profiles, it is likely that *miR-130b* targets *blcap*, *mecp2*, and *fbxl11* in the epididymis. By this means, we predicted 22 possible target genes for 17 miRNAs (**Table 2**).

Discussion

In summary, by using genome-wide microarray analysis, we profiled gene expression in the human epididymis from three distinct ages simultaneously and identified a far larger number of genes with temporally regulated patterns than previously reported [19,20]. In spite of the well-known importance of miRNAs as regulatory factors of gene expression in many biological processes, so far, no studies have analyzed miRNAs in the epididymis. This is not only the first time to report a genome-wide analysis of gene

expression in the human epididymis from three distinct ages but also the first time to study miRNA in a mammalian epididymis. Our data shed new light on the well-orchestrated gene expression and regulation in the epididymis and provide a broad foundation for further studies.

Androgen regulates the structure and function of the epididymis by altering the expression of a large number of genes [28]. Specific genes within the different regions of the epididymis are known to be under the influence of androgens based on genome-wide profiling [15,17,29,30]. Androgen likely plays vital roles in gene expression during all three life ages. We identified genes showing major changes during the lifespan related to the maturation and aging processes. Interestingly, creatine kinase muscle (*CKM*) and a large number of motor proteins, such as myosin, tropomyosin, and titin also known as connectin, are predominantly expressed in the young man, but they dramatically declined in the elder human epididymis (**Supplementary Tables S3** and **S4**). *CKM*, also known as phosphocreatine kinase catalyses the conversion of creatine to phosphocreatine, serves as an energy reservoir for the rapid generation of ATP. The abundant expression of *CKM* and these motor genes reflects the robust muscle movement within the epididymis in the young man characteristic of high fertility. However, glutathione peroxidase 1 (*GPX-1*), known as a testosterone-repressed gene in the rat epididymis [15], was expressed at a lower level in the young adult compared with the epididymis of the other two ages. Androgen administration and efferent duct ligation studies revealed that androgen differentially influences gene expression of the four parts of the rat epididymis: initial segment, caput, corpus, and cauda [28,31–33]. Thus, more information will be obtained in future studies in which the samples will be dissected into several regions for microarray assays.

It is noteworthy, in the aged human epididymis, almost all beta-defensins showed enhanced expression in contrast to the newborn and adult epididymides (data not shown). Our previous work has revealed that Bin1b, an antimicrobial peptide member of beta-defensin family, is specifically expressed in the rat epididymis and is important for the initiation of sperm maturation [34,35]. Substantial evidence accumulated in recent years indicates that mammalian defensins are multifunctional and, by interacting with host cell receptor(s), participate in both the innate and adaptive antimicrobial immunity of the host [36]. In the aged rat, spermatozoa from the cauda epididymidis exhibited a different susceptibility to the combined effects of *in vivo* and *in vitro* oxidative challenges compared with the spermatozoa from young rats [37,38]. The augmented expression of these beta-defensins in the aged human is likely to be activated by the increasing pathogenic invaders in the epididymis to protect the aged host from infections. Given that the number of halo cells intensively increased in the aged epididymis, it will be intriguing to investigate the relation between the halo cell and beta-defensins in further studies.

Among the list of newborn-specific genes, the gamma globin genes (*Hbg1* and *Hbg2*) demonstrated the highest expression, which is well consistent with previous reports showing that *Hbg1* and *Hbg2* are normally expressed in the fetal liver, spleen, and bone marrow and these two gamma chains together with two alpha chains constitute fetal hemoglobin (*HbF*) which comprises 50–95% of the hemoglobin in newborns [39,40]. However, the roles of these highly expressed *Hbg* genes in the newborn epididymis remain to be elucidated.

miRNAs likely lead to age-specific gene expression through mRNA cleavage [21]. The adult epididymis expressed the most genes; however, the newborn epididymis expressed the highest number of miRNAs. On the other hand, the number of newborn-specific miRNAs and mRNAs were far more than in the adult and aged epididymides. Previous studies showed that miRNA expression was regulated by androgen [41,42]. The expressions of *miR-92* and *miR-106a* are shown to be down-regulated and *miR-125b*, *miR-16*, *miR-21*, *miR-30c*, and *miR-100* are up-regulated by androgen, respectively [42]. The androgen level in the newborn is relatively low compared with the fetus and adult epididymis. Therefore, it would be interesting to study the relationship between the low androgen level and the highly expressed miRNAs and identify all the androgen-responsive miRNAs within the epididymis by using a castrated mouse model. On the other hand, as widely proved, a miRNA can repress hundreds of target mRNAs, but the physiological roles of specific miRNA–mRNA interactions remain largely elusive. Actually, Giraldez *et al*. found that *miR-430* accelerates the deadenylation of target mRNAs and clearance of maternal mRNAs during early embryogenesis by using a microarray approach and *in vivo* target validation [43–45]. Also, it is reported that the early zygotic onset of fly *miR-309* cluster miRNA expression acts to promote the turnover of many maternally deposited mRNAs in the fly [43]. Therefore, further studies are necessary to confirm the hypothesis that the newborn epididymis expressed so many miRNAs to repress or eliminate the deposited fetal mRNAs, which are not needed after birth.

TFs and miRNAs both exhibited highly age-specific expression patterns in the newborns, young, and aged human epididymis. Although differing from each other in many aspects, miRNAs and TFs also share some common features such as pleiotropy, cooperative activity as reviewed by Hobert [21]. In our study, about two-thirds of known TFs were expressed in the adult epididymis; however, most of the known miRNAs were detected within the newborn epididymis, implying sharp differences in gene regulation networks in newborn compared with adult human. The

possible explanation is that miRNAs play major roles in regulating gene expression in the newborn epididymis, when TF pathways are not well established. But after adulthood, more TFs participate in the regulation processes.

This study surveyed the gene and miRNA expressions in representative epididymides encompassing the whole human lifespan. These simultaneous analyses allow comparisons that provide novel insights into the temporal and androgen-dependent gene/miRNA expression involved in the development and aging processes in the human epididymis. However, it is very difficult to obtain normal human epididymal samples, especially for such samples of three distinct ages, so a pity for this study is that only one sample was conducted for each age without more replications. In the future, as they become available, epididymides from different genetic backgrounds will be utilized in expanded studies that will allow more in-depth analysis.

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Supplementary Data

Supplementary data are available at *ABBS* online.

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