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·*Review*·

Identification of epididymis-specific transcripts in the mouse and rat by transcriptional profiling

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Abstract

As part of our efforts to identify novel contraceptive targets in the epididymis we performed transcriptional profiling on each of the 10 and 19 segments of the mouse and rat epididymidis, respectively, using Affymetrix whole genome microarrays. A total of 17 096 and 16 360 probe sets representing transcripts were identified as being expressed in the segmented mouse and rat epididymal transcriptomes, respectively. Comparison of the expressed murine transcripts against a mouse transcriptional profiling database derived from 22 other mouse tissues identified 77 transcripts that were expressed uniquely in the epididymis. The expression of these genes was further evaluated by reverse transcription polymerase chain reaction (RT-PCR) analysis of RNA from 21 mouse tissues. RT-PCR analysis confirmed epididymis-specific expression of Defensin Beta 13 and identified two additional genes with expression restricted only to the epididymis and testis. Comparison of the 16 360 expressed transcripts in the rat epididymis with data of 21 other tissues from a rat transcriptional profiling database identified 110 transcripts specific for the epididymis. Sixty-two of these transcripts were further investigated by qPCR analysis. Only Defensin 22 (E3 epididymal protein) was shown to be completely specific for the epididymis. In addition, 14 transcripts showed more than 100-fold selective expression in the epididymis. The products of these genes might play important roles in epididymal and/or sperm function and further investigation and validation as contraceptive targets are warranted. The results of the studies described in this report are available at the Mammalian Reproductive Genetics (MRG) Database (http://mrg.genetics.washington.edu/). *(Asian J Androl 2007 July; 9: 522–527)*

Keywords: mouse; rat; epididymis; transcriptional profiling; contraception; microarray; epididymis-specific; epididymis-selective

1 The epididymis as a target for contraception

Non-hormonal approaches to achieving contraception represent an innovative strategy for the development of novel contraceptive therapeutics. The strategy is designed to identify specific gene products associated with the reproductive tissues that mediate physiological processes required for the production of a mature gamete. In men, the goal is to inhibit successful sperm production or maturation. The concept of post-testicular maturation of spermatozoa as a target for male contraception is well established [1–3]. Sperm acquire progressive motility and the ability to undergo capacitation during transit through the epididymis. These changes are a result of, in part, changes in the composition of the epididymal luminal fluid microenvironment. Inhibition of these post-testicular maturational events in sperm by modulating the function of specific proteins associated with the epididymis represents an attractive option of contraception intervention [4, 5]. An advantage of targeting the epididymis

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through a non-hormonal approach is that normal male endocrine function is unlikely to be affected. This would preclude targeting the process of spermatogenesis, thus avoiding potential issues with aneuploidy. Moreover, reversibility of the contraceptive effect would be rapid owing to the lack of a latency period seen with strategies targeting the processes of spermatogenesis.

As contraceptives are frequently given to healthy people for long periods of time, it is also essential that contraceptive drugs do not have significant side-effects. One way to minimize any potential off-target, drug profile is to focus on targets that are either unique to, or highly selective for, the reproductive tract. To this end, a large-scale segmental transcriptional profiling of both mouse and rat epididymides using commercial whole genome microarrays was performed. The data were compared to those from a large in-house database of other tissues derived from the same platform and generated with exactly the same protocol. Those putative epididymis-specific transcripts were investigated further using more sensitive amplification-based technologies.

2 Segmental profiling of the rat and murine epididymal transcriptomes

To date, numerous strategies for identifying novel epididymis-specific transcripts or proteins have been used, including micro-arrays [6–8], proteomics [9] and signal sequence traps (Johnston, unpublished data). As part of our effort to identify novel epididymis-based contraceptive targets, we undertook a strategy to catalogue the mouse and rat epididymis transcriptomes at the highest possible resolution in a manner that would allow us to rapidly compare the expressed transcripts with large multi-tissue databases in order to identify novel epididymis-specific transcripts.

Most of the published reports on transcriptional profiling of the epididymis have used epididymal tissue dissected into conventional caput, corpus, cauda regions [8] or slightly more refined regions, such as the initial segment (in the rat) [6]. In describing the localization of epididymal gene expression or gene products, these regions have also been described in greater detail (e.g. proximal-, mid- or distal-caput). At the time we were initiating our studies, one of us (Terry T. Turner), had begun investigating the biological role of epididymal segmentation in the mouse [10] and had demonstrated the ability to micro-dissect the caput region reproducibly. As it had become increasingly apparent from the literature that many gene expression and protein localizations within these regions are restricted to one or more segments, we hypothesized that it would be possible and beneficial to micro-dissect out all of the segments of the mouse epididymis and subject each of these segments to transcriptional profiling using genome-wide arrays. It was felt that this strategy would provide a high resolution cataloging of the mouse epididymal transcriptome and assist in the identification and characterization of putative targets. Complete segmentation of C57BL/6 mouse epididymidis reproducibly identified 10 distinct epididymal segments (Figure 1A) [11]. The expression profiles and several analyses of these data have been reported [11].

After completing the study in the mouse, several factors prompted us to use this strategy to investigate the segmented transcriptome of the Sprague-Dawley rat epididymis. First, this approach would help to identify additional contraceptive targets. Second, the rat is the most commonly used animal model for studies of the epididymis. Third, this species is a frequently used model within the pharmaceutical industry for efficacy and toxicology studies and we recognized that data from these studies would be important for planning and interpreting such studies. Fourth, we had es-

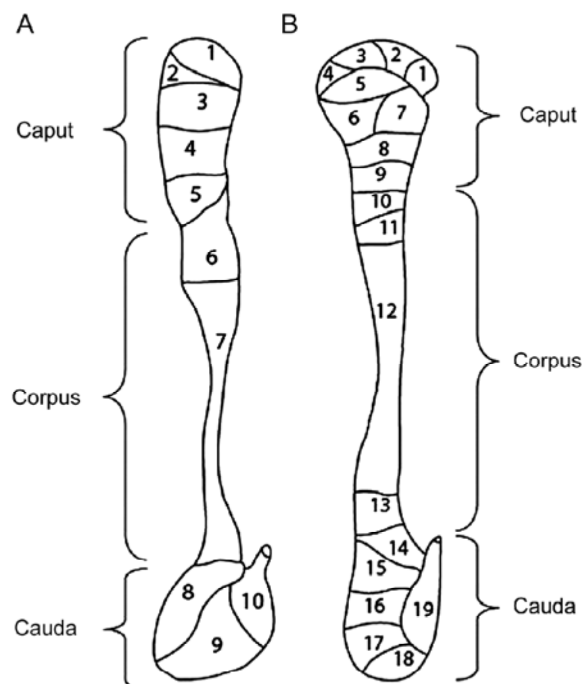

Figure 1. Segmental structure of the murine (A) and rat (B) epididymis. A schematic pattern of the epididymal segmentation across each species is shown. Each individual segment was microdissected and the gene expression pattern identified. The murine and rat epididymides are not drawn to scale. The average rat epididymis is approximately two and a half times the length of the murine epididymis and forty times the total mass (data not shown). Modified with permission from Jelinsky *et al.* [12].

tablished a large-scale transcriptional profiling effort of the entire male rat reproductive tract and we were confident that having the same high-resolution view of the rat epididymal transcriptome would ultimately be beneficial to our future efforts. Complete microdissection of Sprague-Dawley epididymides reproducibly identified 19 distinct epididymal segments (Figure 1B). Analysis of these data have been reported [12].

Importantly, the two profiling efforts described above have considerable importance beyond the identification of novel targets. The data from these studies provide a high resolution, physiologically relevant index of gene expression along the full length of mouse and rat epididymides, elucidate the epididymal expression of individual genes [11, 12], gene families [11, 12] and genes that comprise biological pathways, and allow comparison of gene expression between the two species [12]. Interested readers are encouraged to view these primary references and to utilize the data available on the public database (Mammalian Reproductive Genetics, MRG). The present report will provide novel data about epididymis-specific genes identified from our profiling efforts and will characterize the expression profiles of those transcripts.

3 Identification of epididymis-specific transcripts in the mouse

The 17 096 transcripts expressed in the mouse epididymis were compared to an internal database of 22 mouse transcriptional profiling data to identify transcripts that were detected only in the samples of RNA from the mouse epididymis. The criteria to be considered "epididymis specific" are described in Johnston *et al.* [11]. Briefly, the transcript had to be called "present" in at least 67% of the samples in at least one epididymal segment with a mean expression of > 50 signal units and the transcript had never to be called "present" in any of the samples from the other 22 tissues. The data for each tissue were comprised of between 3 and 12 replicates. RNA from the following tissues were used for comparison: adrenal, bladder, brain, colon, embryo, eye, heart, kidney, liver, lung, lymph node, ovary, pituitary, prostate, salivary gland, small intestine, spleen, stomach, testis, thymus, uterus and vagina. Seventy-seven transcripts met the criteria for epididymal specificity, including a number of genes historically studied in the epididymis, such as lipocalin 5, glutathione peroxidase 5 and Adam 7. Interestingly, a large number of transcripts corresponding to β-defensin genes were also identified, including β-defensins 2, 9, 11, 13 and 15. The analysis also identified transcripts of several uncharacterized or hypothetical genes, including Hypothetical Protein A230091H23, RIKEN cDNA 9230116B18 Gene and cDNA Sequence AJ554213. A list of 49 epididymis-specific probe sets is provided in Johnston *et al.* [11]. The identity of the remaining 28 probe sets, so far as they were known, is provided in Table 1. The identity of all 77 probe sets can be found on the MRG database by selecting "Download Database" and selecting the link for "Datasets from Wyeth Research".

We wished to confirm and characterize further the expression of transcripts identified as being epididymis-specific from the above analysis. In addition, we decided to increase the number of probe sets under evaluation by investigating both epididymis-specific probe sets (described above) and epididymis-selective probe sets. The criteria for epididymal-selective probe sets is described in Johnston *et al.* [11]. Briefly, for a transcript to be epididymal-selective, it had to present in 66% of the samples in at least one segment with a mean expression of > 50 signal units and it had to have a signal value > 3-fold higher than all of the other 21 normal tissues. As these criteria were less stringent, all of the 77 epididymis-specific transcripts met the criteria for being epididymis-selective. This resulted in the identification of 307 murine epididymis-selective transcripts. The identity of the epididymis-selective transcripts is available on the MRG database by selecting "Download Database" and selecting the link for "Datasets from Wyeth Research". The tissue expression profile of each of the 307 transcripts was visually inspected to identify those transcripts that had high signal values in other tissues and these probe sets were excluded. Approximately 130 appeared to show high selectivity in the epididymis and were selected for further analysis.

The tissue distribution of these transcripts was analyzed by RT-PCR analysis of RNA from a total of 21 normal tissues, including the epididymis. The tissues used in these analyses were ovary, epididymis, testis, prostate, embryo, muscle, kidney, liver, placenta, heart, intestine, lung, brain, pancreas, stomach, hypothalamus, spleen, thymus, uterus, pituitary and colon. This analysis showed that only one transcript corresponding to Defensin β13 was expressed solely in the epididymis (Figure 2). Two additional transcripts corresponding to Eppin and RIKEN cDNA 9230105I15 gene each had strong expression in the epididymal samples and weak expression in the testis (data not shown).

4 Identification of epididymal-specific transcripts in the rat

After completing the analysis of epididymis-specific transcripts in the mouse, a similar strategy was used to

Table 1. Unreported epididymis-specific probe sets.

Probe set	Gene symbol	Gene description
1427975_at	*2210403B10Rik*	RIKEN cDNA 2210403B10 gene
1428022_at	*BC027556*	cDNA sequence BC027556
1429140_at	*9830002I17Rik*	RIKEN cDNA 9830002I17 gene
1429935_at	*9230102M18Rik*	RIKEN cDNA 9230102M18 gene
1430107_at	*9230116B18Rik*	RIKEN cDNA 9230116B18 gene
1430331_at		
1431444_at	*9230105I15Rik*	RIKEN cDNA 9230105I15 gene
1431468_at		
1432406_at		
1432468_at	*9230102I19Rik*	RIKEN cDNA 9230102I19 gene
1434409_at	*na*	similar to hypothetical protein FLJ20811
1438444_at	*A230091H23*	hypothetical protein A230091H23
1440344_at		
1440419_at	*na*	LOC381667
1441094_at	*na*	similar to Alcohol dehydrogenase 6
1442012_at		
1443122_at		
1444427_at	*D930038D03Rik*	RIKEN cDNA D930038D03 gene
1444540_at		
1444658_at	*AJ554213*	cDNA sequence AJ554213
1445804_at		
1447395_at	*na*	similar to epididymis specific clone 42; chromosome 20 open reading frame 63; defensin beta 18
1455120_at	*BC034099*	cDNA sequence BC034099
1455423_at	*(AY238603, na)*	(cDNA sequence AY238603, similar to Nur77 downstream protein 1)
1456543_at	*Gpr73*	G protein-coupled receptor 73
1456804_at		
1457869_at		
1459448_at	*D630003K02Rik*	RIKEN cDNA D630003K02 gene

identify additional epididymis-specific transcripts in the rat. To this end, the 16 360 transcripts expressed in the rat epididymis were compared with a Wyeth Research database of rat profiling data from 22 distinct normal tissues. The tissues used for comparison were similar to those used for the mouse and are listed in the Materials and Methods of Jelinsky *et al.* [12]. The criterion for epididymal specificity was effectively identical to that used for the mouse and 110 transcripts met the criteria for epididymal specificity. Among the 110 transcripts were known "epididymis specific" genes, such as *Cystatin 11*, *Adam7* and *LCN5*. Many of the identified transcripts have not been reported to be epididymis-specific, including *Adora1*, *Gdf15* and *Kcng3*, and their function in the epididymis is not known. The complete list of probe sets has been provided [12] and has been placed on the MRG.

We characterized the expression of the 110 epididymis-selective genes in 11 additional types of normal rat tissue, including bone, bone marrow, cerebellum, hippocampus, hypothalamus, cartilage, esophagus, white fat, meniscus, muscle and pancreas. We found that 48 transcripts were also expressed in at least one of these additional 11 tissues. The tissue expression of the remaining 62 transcripts was characterized by qPCR. Using PrimerExpress (Applied Biosystems, Foster City, CA, USA) transcript-specific primer/probe sets were identified and cross-reactivity of each primer probe set sequence was evaluated using the BLAST algorithm and determined to be specific for each qualifier. Twelve point standard curves were generated for caput, corpus, and cauda epididymidal total RNA and used to validate all 62 probe sets. One primer probe set failed to be validated

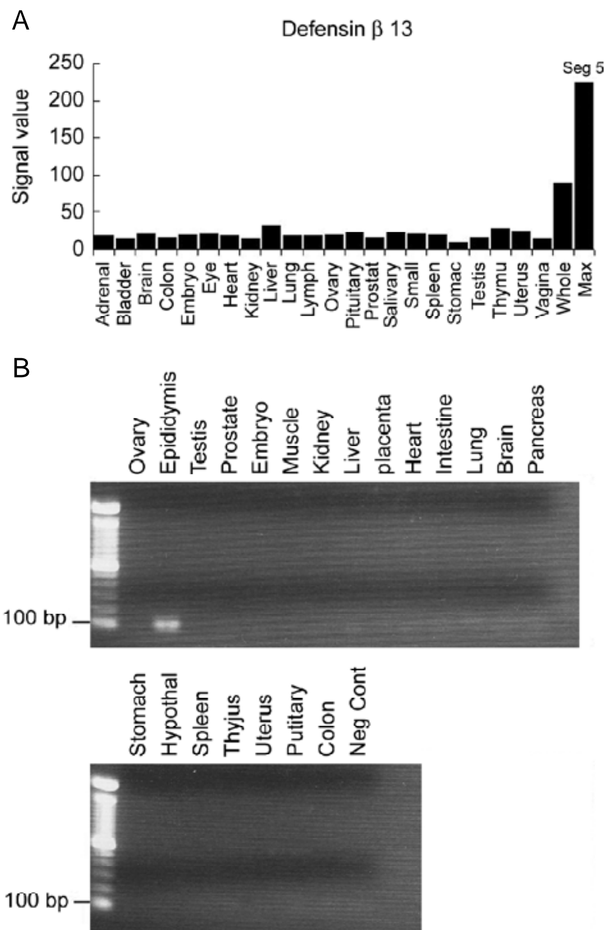

Figure 2. Tissue expression profiles of mouse Defensin β13. (A): Electronic tissue distribution of mRNA levels for Defensin β13 in 22 normal murine tissues as described in [11]. "Whole" refers to the entire epididymis and "Max" refers to the signal value obtained from the epididymal segment with the highest expression (Seg 5, segment 5). (B): Reverse transcription polymerase chain reaction analysis of tissue expression of Defensin β 13 in 21 murine tissues. A negative control was performed by substituting water in the cDNA reaction and using an equivalent volume of this mixture in PCR.

and was therefore eliminated from further analysis.

A panel of 28 tissues was evaluated, including 19 non-reproductive tissues and 9 male reproductive tissues. The 19 non-reproductive tissues were adipose tissue, adrenal gland, bone, cerebellum, cerebrum, colon, heart, small intestine, kidney, liver, lung, meniscus, pancreas, salivary gland, skeletal muscle, spleen, stomach, synovium and thymus. The nine male reproductive tissues were bulbourethral gland, prostate, seminal vesicle, testis, vas deferens, caput epididymidis, corpus epididymidis, cauda epididymidis and whole epididymis. All tissues were dissected in triplicate from littermates and frozen in liquid nitrogen. Total RNA was isolated using CsCl2 centrifugation followed by DNAase treatment on QIAgen RNAeasy columns and expression of each of the 61 transcripts in the 28 tissues was determined as previously described [12].

In total, 14 transcripts were found to have >100-fold higher expression in epididymal tissue than any other non-reproductive tissue and 5 were > 10 000-fold higher in the epididymis tissue than any other non-reproductive tissue. These 5 transcripts included ERABP (> 30 000), Defβ22 (> 28 000), Spag 11 (> 20 000), Transmembrane Epididymal Protein 1 (> 16 000) and cystatin 8 (> 11 000). These genes that are so highly enriched may be critical for epididymal function. Davies *et al.* [13] reported that disruption of mouse HE6 (Transmembrane Epididymal Protein 1) downregulates genes specific to the caput epididymidis and results in male infertility, indicating that this approach is valid. Other epididymis-specific genes, such as beta-galactosidase-like protein, ribonuclease 12 and indoleamine-2,3-dioxygenase have not been implicated in male reproduction, but might provide novel putative targets for male contraception. A representative electronic distribution and qPCR analysis for transmembrane epididymal protein 1 is shown in Figure 3. The identities of all 14 genes and the qPCR data have been placed on the MRG database and can be found as described above.

5 Conclusion

The epididymis-selective genes described in this manuscript were identified using sensitive technologies that are amenable to high volume screening. These data should be viewed in the context of the sensitivity of the microarray technology and amplification procedures used to determine their expression and the panel of tissues used for analysis. Evaluation of these transcripts using additional tissues and/or more sensitive assays could alter their designation as epididymis selective/specific. This analysis provides an important dataset of genes and gene products to be further evaluated in order to understand the role they might play in epididymal function. It is the authors' hope that this resource will assist interested investigators to develop novel hypothesis about the cellular and molecular biology of the epididymis and, ultimately, lead to new insights into epididymal function.

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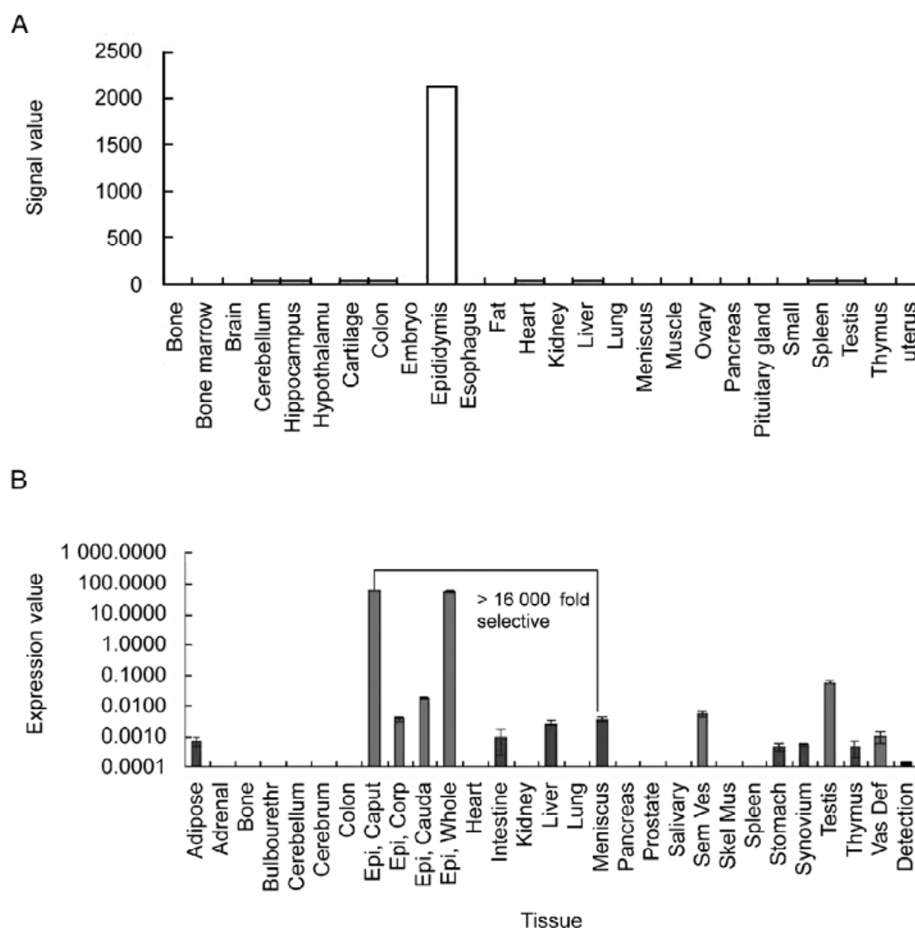

Figure 3. Tissue expression profiles of rat transmembrane epididymal protein 1. (A): Tissue expression profiles of rat transmembrane epididymal protein 1 from a profiling database of rat microarray data. Fifty-nine tissues were evaluated. (B): qPCR analysis of transmembrane epididymal protein 1. Epi, epididymidis.

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