Molecular profiling of human prostate tissues: insights into gene expression patterns of prostate development during puberty

Saravana Mohan Dhanasekaran,*[,1] Atreya Dash,[†,1] Jianjun Yu,*[,1] Ira P. Maine,* Bharathi Laxman,* Scott A. Tomlins,* Chad J. Creighton,[‡] Anjana Menon,* Mark A. Rubin,[§] and Arul M. Chinnaiyan*[,†,‡,2]

*Department of Pathology, [†]Michigan Urology Center, [‡]Bioinformatics Program, University of Michigan Medical School, Ann Arbor, Michigan, USA; and [§]Department of Pathology, Brigham and Women's Hospital, Dana Farber Cancer Institute, and Harvard Medical School, Boston, Massachusetts, USA

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SPECIFIC AIMS

Our aim was to identify gene expression changes occurring in the human prostate gland at puberty, a crucial juncture in prostate development that is androgen dependent, and to thereby identify novel in vivo targets of androgen action.

PRINCIPAL FINDINGS

Prostate tissues obtained from cadaveric organ donors were grouped into two categories: the pubertal group comprised individuals from 9 to 13 years of age and the adult group of donors between 19 and 57 years. The total RNA from these samples was analyzed on a 20,000 element cDNA microarray chip against a reference of normal pooled adult prostate RNA from a commercial source. Significance analysis of microarray identified 375 genes to be dysregulated in the pubertal group.

1. Pubertal prostates exhibit a distinct gene expression signature

Based on statistical analysis, we identified 375 genes as significantly dysregulated in pubertal prostates compared with adult tissue. Of the 375 genes, we found 131 to be overexpressed in the pubertal group and 244 were underexpressed. The pubertal prostate signature was comprised of the expression pattern of these 375 genes.

2. Pubertal prostate signature contains several androgen-regulated genes

We found several known androgen-regulated genes (ARGs) to be present in the pubertal signature. We searched the promoters of these 360 reference sequences that represent the pubertal prostate signature and found 52 to contain one or more putative androgen response elements (AREs). Along with known androgen-regulated genes, this analysis identified several potential in vivo targets of androgen action.

3. TIARP, a novel target of androgen action

TNF α−induced adipocyte-related protein (TIARP) was found to be underexpressed in the pubertal signature. Promoter search identified a putative ARE and RT-PCR analysis on androgen-treated LNCaP cells demonstrated that TIARP mRNA was induced by androgen in this prostate cancer cell line.

4. The pubertal signature shows similarity to the expression pattern of benign prostatic hyperplasia

Comparison of pubertal signature genes with expression data obtained from other prostatic disease conditions revealed an overlap in a subset of genes between benign prostatic hyperplasia (BPH) and the pubertal group.

CONCLUSIONS AND SIGNIFICANCE

We compared the gene expression patterns of pubertal human prostate and adult tissues using cDNA microarrays. These rare samples were obtained with approval from the institutional review board and the Michigan Transplantation Society. Tissue was sectioned in order to perform H&E stain for morphological observation

[1] These authors contributed equally to this work.

[2] Correspondence: Department of Pathology and Urology, University of Michigan Medical School, 1301 Catherine Rd., MSI Rm. 4237, Ann Arbor, MI 48109-0602, USA. E-mail: arul@umich.edu

and for immunohistochemistry with androgen receptor antibody. The androgen receptor staining was more nuclear in adult tissues when compared with the pubertal samples. As expected, serum testosterone levels were low in pubertal group vs. the adults. Serum PSA levels indicated that this androgen-responsive protein's level (correlated with testosterone values) had higher PSA levels in adults and lower or undetectable amount in pubertal prostates.

Extensive microarray analyses have been reported for various pathological conditions of prostate such as localized prostate cancer (PCA), metastatic prostate cancer (HR-MET), and BPH. Several correlations and expression patterns have been identified from these studies. Several other prostate cancer cell lines (such as DU145, LNCaP, PC3, and prostate fibroblasts) have been profiled. Three independent studies have documented transcriptional program activated by androgen in LNCaP cells (**Fig. 1**). A large microarray data set that documents gene expression patterns of hundreds of prostate tissues has been generated by us and other independent groups. Our pubertal data set will add to this collection and will facilitate cross-indexing among various data sets to examine the expression pattern of a given gene across various normal and diseased conditions. The study reported here is the first microarray analysis to look at the gene expression patterns of human pubertal tissue, a developmentally important stage in prostate. Statistical analysis identified 375 genes to be differentially expressed in pubertal samples and this expression pattern comprised the pubertal prostate signature. These genes belonged to several functional groups. The signature reflected the cellular composition of prostate at this stage. Contributions to gene expression from both epithelial and stromal components were readily observed. Comparisons revealed that genes such as laminin A4, T-box5,3, fibronectin, nidogen, aldehyde dehydrogenase, TIMP1, tenascin (TNXB), fibulin, PDGFRA, and SPARC were highly expressed in pubertal tissues and the previously reported expression pattern for genital foreskin and prostate fibroblasts. High expression of these genes observed in pubertal tissues may reflect the stromal contribution to the expression profile. Genes such as EPS8, S100P, LPL, mal, crystallin gamma, ITM2A, and A2M were found to be underexpressed in fibroblasts but highly expressed in the pubertal prostate, implying that the presence of these genes within the pubertal prostates may be contributed by the epithelial compartment.

Analysis of the pubertal signature also revealed the presence of several known ARGs. Several studies have documented the transcriptional regulation of various genes by androgen using DNA microarrays, including our own unpublished data. Genes such as TMEPAI, NKx3.1, fatty acid synthase (FASN), and ankylosis homologue (ANKH), which have been confirmed as ARGs by independent studies using different techniques, are under expressed in pubertal prostate as expected. To identify novel androgen-responsive genes that are important in vivo, we searched the promoters of 360

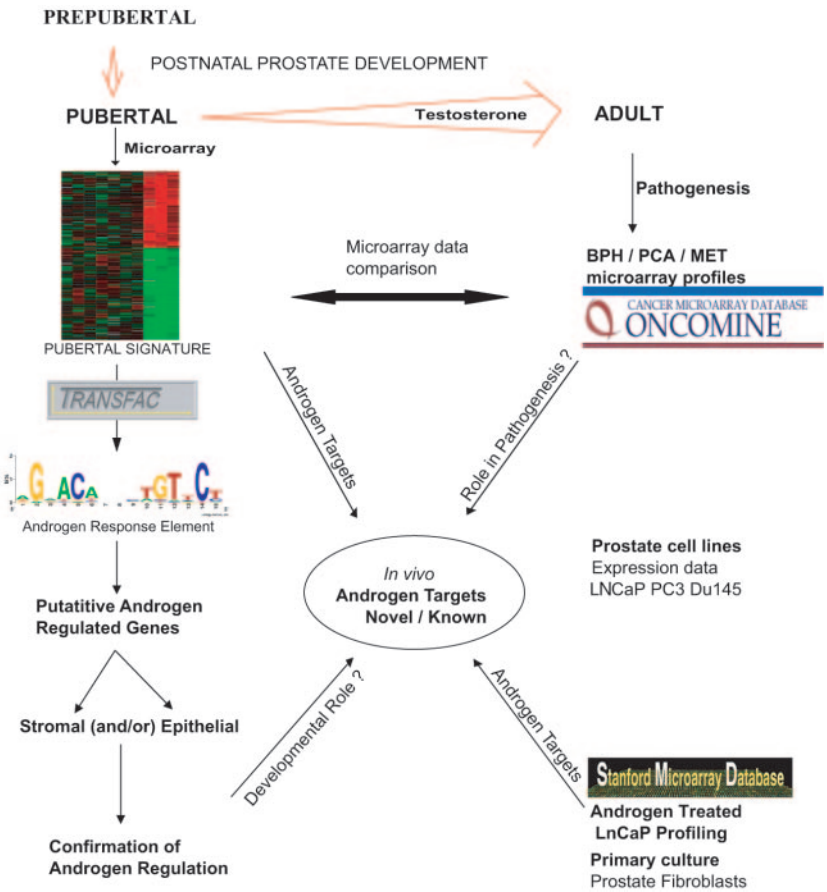

Figure 1. Schematic diagram of pubertal prostate profiling study. Testosterone is essential for pubertal prostate development and maintenance of adult tissue structure and function. The pubertal gene signature was obtained using cDNA microarrays. The pubertal signature was compared with the adult pathological states of prostate (i.e., PCA, BPH, and MET). Promoter searches with TRANSFAC identified genes with putative androgen targets. Putative androgen targets may be from either the epithelial and/or stromal compartments. Validating the androgen regulation identifies novel in vivo androgen targets. Databases hosting the data sets used in the study are indicated.

reference sequences that represented the pubertal prostate signature. This analysis identified 52 genes having one or more potential androgen response elements. We confirmed two genes from this list, TMEPAI, a gene known to be induced by androgen, and TIARP, a gene previously unknown as an androgen target. TIARP was reported to be highly expressed in adipocytes. In androgen receptor knock out mice, a decrease in adipocyte number and size was observed. If androgen induction of TIARP could be reproduced in an adipocyte model, it may rekindle efforts to explain the adipocyte phenotype observed in androgen receptor knockout mice. This will identify possible relationships between androgen induction of TIARP and adipocyte morphology and proliferation. The pubertal signature showed an overlap in gene expression pattern with BPH samples when it was cross-indexed with the expression values obtained for BPH, PCA, and HR-MET samples. Overlap was also observed when comparisons were made with an independent BPH data set. The stromal-epithelial ratio remains constant from birth to age 40 in nonhyperplastic glands and is similar to ratios in asymptomatic and symptomatic BPH tissues. However, there is a difference between pubertal stage and adult prostates in the percentage of smooth muscle and connective tissue that make up the stromal compartment. Based on the observation that ontogenetic processes are recapitulated in development of stromal nodules in BPH, earlier findings suggest a "reawakening" of fetal processes in BPH. The significance of genes that contribute the shared signature between BPH and pubertal tissues needs further study. In summary, we report here the gene expression signature of human pubertal prostate as identified using cDNA microarray technology as well as several potential in vivo ARGs identified from the data set. TIARP was identified and confirmed as a novel androgen-regulated gene in a prostate cell line. FJ