Published in final edited form as:

Cytokine. 2008 August ; 43(2): 194–199. doi:10.1016/j.cyto.2008.05.012.

The Inflammatory Microenvironment of the Aging Prostate Facilitates Cellular Proliferation and Hypertrophy

L.A. Begley, S. Kasina, J. MacDonald, and J.A. Macoska*

Department of Urology and the Comprehensive Cancer Center, The University of Michigan, Ann Arbor, Michigan, The United States of America

Abstract

Benign Prostatic Hypertrophy (BPH, also known as benign prostatic hyperplasia or benign prostatic enlargement), is one of the most common benign proliferative conditions associated with aging in men and is pathologically characterized by the proliferation of fibroblast/myofibroblast and epithelial cell types in the prostate. Previous studies from our laboratory have shown that the CXC-type chemokines, CXCL5 and CXCL12, are secreted by aging prostate stroma and promotes both proliferative and transcriptional responses from prostate epithelial cells. Using array-based gene expression profiling and quantitative reverse-transcriptase polymerase chain reaction, we now show that the transcriptome of the aging prostate stroma is characterized by the up-regulation of several genes that encode secreted inflammatory mediators, including secreted CXC-type chemokines (CXCL1, CXCL2, CXCL5, CXCL6, CXCL12), interleukins (IL11, IL33), and transcripts with cytokine homology (CYTL1). At the protein level, ELISA experiments demonstrated that CXCL1, CXCL5, and CXCL6 were secreted by primary prostate stromal fibroblasts explanted from aging prostate stroma. Dose-response assays confirmed that, like CXCL5 and CXCL12, CXCL1 and CXCL6 promote low-level proliferative responses from both prostate stromal fibroblasts and epithelial cells. Taken together, these data suggest that inflammatory mediators are secreted by prostatic stroma consequent to aging, that the levels of these mediators are sufficient to promote low-level increases in the proliferative rate of both epithelial and stromal fibroblast cell types. Moreover, these processes may account for the low-level, but cumulative, proliferation of both epithelial and fibroblastic/myofibroblastic cell types that characterizes the aging-associated development of benign prostatic hypertrophy.

Keywords

chemokines; prostate; hypertrophy; proliferation; aging

INTRODUCTION

Benign Prostatic Hypertrophy (BPH) is one of the most common benign proliferative conditions associated with aging in men and is pathologically characterized by the proliferation of fibroblast/myofibroblast and epithelial cell types in the prostate [1]. In a survey of 1709 men without cancer recently reported by the Massachusetts Male Aging Study, the frequency of

* Address Correspondence To: Jill A. Macoska, Ph.D. Department of Urology The University of Michigan 6217 CCGC 1500 East Medical Center Drive Ann Arbor, MI 48109-0944 (734) 647-8121 TEL (734) 647-9271 FAX jcoska@umich.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

clinical BPH (defined in terms of frequency/difficulty with urinating and evidence of an enlarged/swollen prostate) rose from 8.4% in men 38-49 years of age to 33.5% in men aged 60-70 years ($p < 0.001$) [2]. Using lower urinary tract symptoms (LUTS) as a surrogate measure for BPH, the Triumph project in the Netherlands reported a 2.7% prevalence rate for BPH in men 45-49 years of age, which increased to 24% in men 80 years of age [3]. For the year 2007, the American Cancer Society estimates that the probability of developing an invasive prostate cancer (PCa) rises from .01% between birth and the fourth decade of life to 2.59% in the fifth decade, 7.03% in the sixth decade, 13.83% in the seventh decade and 17.12% in the eighth decade of life for American men [4]. Clearly, age is a major risk factor for the development of both BPH and PCa.

BPH is pathologically characterized by the proliferation of fibroblast/myofibroblast and epithelial cell types within the periurethral, or transitional zone, region of the prostate gland [1-3,4]. Previous studies have shown that BPH develops consequent to a gradual increase in prostatic volume that occurs over decades of life through a process of low-level, but cumulative, cellular proliferation that increases post-pubertal prostatic volume by approximately 0.8-1.6%, equivalent to only 0.2-0.4 ml, per year [5,6]. Recent studies from our laboratory demonstrate that an inflammatory mediator, the CXC-type chemokine, CXCL12, is secreted at sub-nanomolar levels by aging prostate stromal fibroblasts and stimulates a significant, low-level proliferative response, as well as a robust and complex pro-proliferative transcriptional response, in prostate epithelial cells [7,8]. Immunohistochemical studies examining the histopathology of BPH have reported the presence of pervasive inflammatory infiltrate comprising leukocytes associated with acute inflammation, chronic inflammation, or both [9-11]. These studies suggest the intriguing possibility that the aging prostate provides an inflammatory microenvironment that is both conducive is not only chemotactic for several types of inflammatory cells but also conducive to the proliferation of cell types associated with the development of BPH. The studies presented here were intended to begin to test this hypothesis by investigating whether inflammatory mediators are secreted by aging stroma, and whether these mediators promote the proliferation of both stromal fibroblasts/myofibroblasts and epithelial prostate cells.

METHODS

Cell Cultures

N15C6 and N1 cells were produced through the immortalization of primary human prostate epithelial or stromal fibroblast cultures, respectively, by transduction with a recombinant retrovirus encoding the E6 and E7 genes of HPV-16, as previously described [12]. Both N15C6 and N1 cells are non-transformed and grow continuously in culture but do not form colonies in soft agar or tumors in immuno-compromised mice [13,14]. N15C6 and N1 cells were maintained in 5% HIE media [Ham's F12 (Mediatech Inc. Herndon, Virginia) with 5% FBS (Life Technologies, Inc.), 5 ug/ml insulin, 10 ng/ml EGF, 1ug/ml hydrocortisone (Sigma Chemical Co., St. Louis, MO) or in defined serum-free HIE (SF) media supplemented to 5mM ethanolamine (Sigma Aldrich), 10mM HEPES (Sigma Aldrich), 5 ug/ul transferrin (Sigma Aldrich), 10 uM 3,3',5-triiodo-L-thyronine (Sigma Alrdich), 50 uM sodium selenite (Sigma Aldrich), 0.1% BSA (JRH Biosciences Lenexa, Kansas), 0.05 mg/ml gentamycin (Gibco), and 0.5 ug/ml fungizone (Cambrex Bioscience, Walkersville, Maryland). BPH-1 cells are SV40 Large T-immortalized, non-transformed prostate epithelial cells and were maintained in 5% HIE media [15]. LNCaP cells, a widely-used transformed prostate epithelial cell line originating from a prostate cancer lymph node metastasis, were acquired from the American Type Culture Collection (ATCC# CRL-1740), were maintained in 10% RPMI media and 0.5 ug/ml fungizone, and were used at passages 25-35 [16]. PC3 cells, a widely-used transformed prostate epithelial cell line originating from a prostate cancer dural metastasis, were acquired

from the American Type Culture Collection (ATCC# CRL-1435), were maintained in 10% RPMI media and 0.5 ug/ml fungizone [17]. Primary stromal fibroblasts from the periurethral area of the human prostate were isolated, explanted, and cultured as described previously [7]. For the studies described here, a total of 6 primary prostate stromal fibroblast cell populations were cultured from the prostates of younger patients (defined as <55 years of age) and 7 from older patients (defined as > 65 years of age).

Affymetrix Human Genome U133A Array Data Acquisition

As previously described, RNA was purified from trypsinized cultured cells by homogenization in Trizol (Invitrogen, Carlsbad, CA) and additional processing using the RNeasy (Qiagen, Valencia, CA) cleanup procedure. Ten ug of RNA was used to obtain labeled cRNA and hybridized to U133A GeneChips following the Affymetrix Standard protocol. Expression intensity values for each gene were estimated using a method called Robust Multi-array Average (RMA) using tools available through Bioconductor (www.bioconductor.org). GeneChip gene expression values were normalized using a quantile normalization procedure [7,8]

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

All quantitative real-time assays were conducted as previously described with an Applied Biosystems 7900HT instrument and reagents [14]. Cells were grown to 70% confluence in 60mM dishes prior to RNA purification using the Trizol reagent (Invitrogen Life Technologies). The patient samples used in these studies included primary prostate stromal fibroblasts explanted and cultured from the peri-urethral area of prostates from 5 patients aged < 55 ('younger' group), and 4 aged > 65 ('older' group). For all experiments, one microgram of RNA was reverse transcribed by use of Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA). The resulting cDNA was diluted 1:100. Real-time PCR was performed by use of Assays on Demand (Applied BioSystems, Foster City, CA) according to the manufacturer's instructions. All reactions were performed in duplicate including no-template controls and an endogenous control probe, RPLPO (ribosomal protein, large, PO), to assess template concentration. Cycle numbers to threshold were calculated by subtracting control from experimental values and normalized to those obtained for RPLPO expression in the same samples [8]. These values were averaged within groups and standard deviations determined to permit statistical analyses. FAM conjugated, gene specific assays were Hs00236937_m1 for CXCL1, Hs00171085_m1 for CXCL5, Hs00237017_m1 for CXCL6, Hs00184064_m1 for CYTL1, Hs00174148_m1 for IL-11, and Hs99999902_m1 for the control, RPLPO.

ELISA Assays

The patient samples used in these studies included primary prostate stromal fibroblasts explanted and cultured from the peri-urethral area of prostates from 4 patients aged < 55 ('younger' group), and 5 aged > 65 ('older' group). For these experiments, semi-confluent cells were grown in defined serum-free media over a 48 hour period. The resulting conditioned media was collected, serially concentrated using Centriplus centrifugal filters (Amicon) with a 3 kD molecular weight cutoff, and assessed using the R&D Systems DuoSet kits for Human CXCL5/ENA-78 (DY254), CXCL1/GRO alpha (DY275), or CXCL6/GCP-2 (DY 333). All ELISA assays were performed in duplicate three separate times, the values averaged and standard deviations calculated. [7].

Proliferation Assays

For each proliferation assay, cells were plated at 50,000 cell/well in triplicate per time point and media composition in six-well plates and counted after 24 and 96 hours of incubation as described previously [13]. To assess the effects of exogenous chemokines on cellular

proliferation, recombinant human CXCL1 (R&D systems 275-GR), CXCL5 (R&D Systems, 254-X) or CXCL6 (R&D systems 333-GC) was added at the desired concentration in 1 ml SF HIE to each well, with parallel controls grown in SF HIE alone or complete media with serum. The cells were re-fed at 48 and 72 hours growth and counted at 24 and 96 hours growth. Cell counts from the triplicate measures were averaged and normalized to 50,000 cells at 24 hours to account for any plating discrepancies. Each proliferation assay was repeated 3 times, and cell number averages and standard deviations were calculated for each time point under each media composition.

Statistical Analysis

Normalized array-acquired transcript expression values were analyzed using a t-statistic test and by calculation of fold change between data sets. Genes that exhibited both a large t-statistic (> 10.0) and a large fold change (> 2.0) were considered to be differentially expressed. qRT-PCR data assessing transcript levels and ELISA data assessing protein levels in patient samples was tested using the non-parametric Mann-Whitney rank sum test. Differences in cellular proliferation were assessed using t-tests. In all tests, $p < .05$ was considered statistically significant.

RESULTS

As we have reported previously, the transcriptome of primary stromal fibroblasts explanted from the prostates of older (> 64 years) patients is substantially different from that of fibroblasts explanted from the prostates of younger (aged < 52 years) patients [7]. As seen in Table I, the transcriptome of primary prostate stromal fibroblasts explanted from older patients includes significantly higher levels of 26 transcripts encoding secreted proteins. The majority of these transcripts (18/26, 70%) encode secreted inflammatory mediators, and 10 of these map to 8 genes that encode secreted CXC-type chemokines (CXCL1, CXCL2, CXCL5, CXCL6, CXCL12), interleukins (IL11, IL33), or transcripts with cytokine homology (CYTL1).

The transcript levels for six of these chemokines and cytokines – CYTL1, CXCL1, CXCL5, CXCL6, and IL11 – were also examined using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). As seen in Figure 1A, the transcript levels for these genes were predominantly higher for RNA purified from primary prostate stromal fibroblasts from 4 older compared to 5 younger patients. Variation in transcript levels was more evident in RNA purified from primary prostate stromal fibroblasts cultured from younger than older patients. This finding was consistent with that observed for CXCL12, which we have previously reported [7]. When averaged together within groups, transcript levels for CXCL6 were found to be ~10-fold higher for primary prostate stromal fibroblasts cultured from older compared to younger patients ($p < .05$) (Figure 1B).

To determine whether the observed differences in transcript levels were paralleled by similar differences in secreted protein levels, conditioned media from primary prostate stromal fibroblasts explanted and cultured from 5 older and 4 younger patients was prepared and evaluated by ELISA. Among these, 2 fibroblast populations cultured from the prostates of older patients and 3 from younger patients were the same as those evaluated for transcript levels. Although limiting quantities of conditioned media abrogated the ability to test for the presence of all of the proteins encoded by all of the transcripts validated by qRT-PCR, there was sufficient media to examine the levels of CXCL1, CXCL5, and CXCL6. We have previously reported that older prostate fibroblasts secreted significantly higher levels of CXCL12 than younger prostate fibroblasts [7]. Similarly, these studies showed that significantly higher ($p < .05$) levels of CXCL1, CXCL5, and CXCL6 were present in the media conditioned by primary prostate stromal fibroblasts from older compared to younger patients (Figure 1C). All three chemokines were present at levels of ~1.0 ng/ml in media conditioned by primary prostate

stromal fibroblasts from older patients, but only at levels of ~0.01 – 0.1 ng/ml in media conditioned by primary prostate stromal fibroblasts from younger patients.

For comparison, five prostate cell lines – N1 prostate stromal fibroblast cells and N15C6, BPH-1, LNCaP, and PC3 prostate epithelial cells – were grown in serum-free media, the conditioned media collected, and assessed by ELISA. These experiments showed that CXCL1, CXCL5 and CXCL6 secretion by N1 prostate stromal fibroblast cells closely mimicked that observed for prostate stromal fibroblasts cultured from older patients (Figure 1D). The four prostate epithelial cell lines all exhibited lower levels of chemokine secretion than N1 cells, though secretion levels varied considerably between cell lines for each chemokine tested (Figure 1D). These results parallel those observed for CXCL5, which was found to be secreted at levels approximately 10-100 fold higher by N15C6, BPH-1 and PC3 compared to LNCaP cells [18].

Benign prostatic hypertrophy is characterized by the proliferation of both stromal fibroblasts/myofibroblasts and glandular epithelial cells. Therefore, it was important to determine whether the secretion of subnanomolar levels of chemokines by prostate stromal fibroblasts explanted from older patients was sufficient to induce the proliferation of both stromal fibroblast and glandular epithelial cell types, thus, mimic prostatic hypertrophy in vitro. To accomplish this, the difference in cell numbers for N15C6, LNCaP or PC3 prostate epithelial cells, or N1 prostate stromal fibroblasts, after 96 hours growth in serum-free media alone or supplemented with escalating levels of each of the three chemokines was determined. As seen in Figure 2A, N1 fibroblasts responded proliferatively to sub-nanomolar levels of CXCL1 and CXCL5, but not CXCL6. N15C6 cells proliferated in response to sub-nanomolar levels of CXCL1 and CXCL6 (Figure 2B), in parallel with our previous findings these cells proliferated in response to sub-nanomolar levels of CXCL5 [18]. Both LNCaP and PC3 cells proliferated in response to CXCL1, but not CXCL6 (Figure 2C,D). In parallel with our previous findings, PC3 cells demonstrated the least robust proliferative response of all epithelial cell lines examined towards all three chemokines tested (Figure 2C, 2D) [18].

Taken together, these data show that subnanomolar levels of CXCL1, CXCL5 and CXCL6 promote proliferative responses even in cells that endogenously secrete these chemokines, though this response was clearly weakest for the most aggressive prostate cancer cell line tested, PC3 cells.

DISCUSSION

The data presented above suggest that inflammatory mediators are secreted by prostatic stroma consequent to aging, and that the levels of these mediators are sufficient to promote low-level increases in the proliferative rate of both epithelial and stromal fibroblast cell types. Though preliminary and requiring further examination and validation, these findings are consistent with what is known regarding the etiology of BPH in vivo, which develops over decades of life through a process of low-level, but cumulative, proliferation of both epithelial and fibroblastic/myofibroblastic cell types [5,6].

An intriguing aspect of the current study is the observation that the transcriptome of aging prostate stroma is characterized by the up-regulation of genes encoding secreted proteins, and that the majority of these genes actually encode cytokine- and chemokine-type inflammatory mediators. In particular, three CXC-type chemokines, CXCL1, CXCL5 and CXCL6, which are secreted as part of the aging prostate stromal proteome are all pro-angiogenic, neutrophilic chemotactic factors. Neutrophils are the primary type of leukocyte involved in acute inflammatory responses, which are the initial responses of the body to harmful stimuli. A cascade of biochemical events involving the local vascular system, the immune system, and

various cells within the injured tissue, then propagates and matures these inflammatory responses from acute to chronic. Chronic inflammation leads to a progressive shift in the type of cells which are present at the site of inflammation towards macrophages, lymphocytes, and plasma cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [19,20].

The presence of inflammatory infiltrate consistent with acute or chronic inflammation coincident with PCA and/or BPH has been reported in several studies examining the histology of the aging prostate. De Marzo et al. have identified a type of hyperproliferative lesion in the prostate, termed proliferative inflammatory atrophy, or PIA, that is associated with inflammation and is often observed in proximity to high-grade prostatic intraepithelial neoplasia (PIN), consistent with a model in which the proliferative epithelium in PIA may progress to PIN and/or adenocarcinoma [21-23]. There is also some evidence that inflammation may play a role in the etiology of benign proliferative disease in the prostate, e.g., BPH. For example, Nickel et al. described studies that examined sectioned transurethral resection of the prostate (TURP) specimens from 80 consecutive patients with a diagnosis of BPH but no history or symptoms of prostatic inflammation (prostatitis). Inflammatory cells were detected in 90% of specimens examined, suggesting that prostatic inflammation is an extremely common histological finding in patients with BPH who have no symptoms of prostatitis, though the clinical significance of asymptomatic chronic prostatitis associated with BPH had yet to be determined. Neutrophilic infiltrates were identified in mild forms of glandular inflammation, whereas lymphocytic infiltrates characterized the more commonly observed periglandular and stromal inflammation [9]. In another study, Gerstenbluth et al. identified pervasive chronic lymphocytic prostatitis in whole mount radical prostatectomy specimens from a series of 40 consecutive patients with clinically localized prostate cancer. Although inflammation was associated with both BPH and cancer, it was observed more frequently associated with BPH, findings which indirectly supported a potential role for inflammation in the pathogenesis of BPH [10]. Data recently reported by Roehrborn obtained from examining baseline prostate biopsies in a subgroup of 1197 randomly selected patients in the Medical Therapy of Prostatic Symptoms (MTOPS) study demonstrated chronic inflammatory infiltrate in 30–60% of men with BPH. Patients with chronic inflammatory infiltrate had larger prostate volumes and demonstrated significantly more clinical progression and acute urinary retention than those who had no inflammation [11]. Theyer et al. reported that the majority of BPH tissues examined demonstrated infiltration of various T-cell lymphocyte populations typically associated with chronic inflammation [24]. Finally, a recent histological study of sextant needle biopsies from 93 patients diagnosed as biopsy-negative for cancer found that all 93 patients demonstrated high levels (G2 or G3) of polymorphonuclear leukocytic infiltrate, but that only 7 demonstrated similar levels of mononuclear leukocytic infiltrate [25].

The studies just described that associate acute and chronic inflammation with BPH, and our finding that aging prostate stroma secretes chemotactic factors associated with acute inflammation, provides the basis for suggesting a model for inflammation in the etiology of BPH. In this model, low-level secretion of specific CXC-type chemokines, e.g., CXCL1, CXCL5, and CXCL6, by aging prostate stroma may produce a persistent, but weak, neutrophilic chemotactic gradient. Activated neutrophils within prostatic tissues may then release chemokines and cytokines, e.g., TNF-alpha and IL-1, which are chemotactic for activated macrophages [19,20]. Both activated neutrophils and macrophages may then secrete cytokines, such as IL-1, IL-6, and IL-8 that are chemotactic for various types of lymphocytes involved in the chronic inflammatory response [19,26]. Activated macrophages also secrete TGF-beta, which is chemotactic for various types of lymphocytes, is pro-angiogenic like CXCL1, CXCL5, CXCL6, and CXCL12, and has been shown to promote both the development of reactive stroma and the proliferation of prostate epithelial cells [19,27]. Moreover, all of these different types of activated leukocytes secrete cytokines that are growth-stimulatory for

diverse cell types. For example, T-lymphocytes isolated from BPH tissues have been shown to express high amounts of IFN-gamma and IL-2 that promoted the proliferation of prostate stromal cells [28]. Taken together, these studies suggest the possibility that many of these immune-mediated responses could act synergistically with aging prostate stroma to promote the low-level, but cumulative, proliferation of stromal fibroblasts/myofibroblasts and epithelial cells associated with the development and progression of BPH. It is also possible that these same processes may promote the proliferation of malignant prostate epithelial cells in the development and progression of PCa. Though intriguing, further studies employing the use of experimental models to test this hypothesis are clearly required to fully elucidate the potential role of interactions between the aging, inflammatory prostatic microenvironment and components of the immune system in the etiology of benign and malignant proliferative diseases in the prostate.

ACKNOWLEDGEMENTS

This work was supported by awards from the National Institutes of Health NIDDK George M. O'Brien Center for Urologic Research at the University of Michigan 1 P50 DK065313 (J.A.M.) and the University of Michigan's Cancer Center Support Grant (5 P30 CA46592).

LITERATURE CITED

1. Bierhoff E, Vogel J, Benz M, Giefer T, Wernert N, Pfeifer U. Stromal nodules in benign prostatic hyperplasia. *Eur. Urol* 1996;29:345–54. [PubMed: 8740022]
2. Meigs JB, Mohr B, Barry MJ, Collins MM, McKinlay JB. Risk factors for clinical benign prostatic enlargement in a community-based population of healthy aging men. *J Clin Epidemiol* 2001;54:935–44. [PubMed: 11520654]
3. Verhamme K, Dieleman J, Bleumink G, van der Lei J, Sturkenboom M. Incidence and prevalence of lower urinary tract symptoms suggestive of benign prostatic enlargement in primary care-the Triumph project. *Eur Urol* 2002;42:323–328. [PubMed: 12361895]
4. Jemal, A.; Siegel, R.; Ward, EM.; Thun, MJ. *Cancer Facts and Figures 2007*. American Cancer Society; Atlanta: 2007.
5. Jacobsen SJ, Girman CJ, Lieber MM. Natural history of benign prostatic hyperplasia. *Urology* Dec; 2001 58(6 Suppl 1):5–16. [PubMed: 11750242]discussion 16
6. Jakobsen H, Torp-Pedersen S, Juul N. Ultrasonic evaluation of age-related human prostatic growth and development of benign prostatic hyperplasia. *Scand J Urol Nephrol Suppl* 1988;107:26–31. [PubMed: 2453919]
7. Begley L, Monteleon C, Shah RB, Macdonald JW, Macoska JA. CXCL12 overexpression and secretion by aging fibroblasts enhance human prostate epithelial proliferation in vitro. *Aging Cell* 2005;4:291–8. [PubMed: 16300481]
8. Begley LA, MacDonald JW, Day ML, Macoska JA. CXCL12 Activates A Robust Transcriptional Response In Human Prostate Epithelial Cells. *J Biol Chem* Sep 14;2007 282(37):26767–74. [PubMed: 17631494]Epub 2007 Jul 12
9. Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic enlargement. *BJU Int* 1999;84:976–81. [PubMed: 10571623]
10. Gerstenbluth RE, Seftel AD, MacLennan GT, Rao RN, Corty EW, Ferguson K, Resnick MI. Distribution of chronic prostatitis in radical prostatectomy specimens with up-regulation of bcl-2 in areas of inflammation. *J Urol* 2002;167:2267–70. [PubMed: 11956490]
11. Roehrborn, CG.; Kaplan, SA.; Noble, WD.; Lucia, MS.; Slawin, KM.; McVary, KT.; Kusek, JW.; Nyberg, LM. The impact of acute or chronic inflammation in baseline biopsy on the risk of clinical progression of BPE: Results from the MTOPS study; AUA Meeting 2005; Abstract #1277
12. Schwab TS, Stewart T, Lehr J, Pienta KJ, Rhim JS, Macoska JA. Phenotypic characterization of immortalized normal and primary tumor-derived human prostate epithelial cell cultures. *Prostate* Jul 1;2000 44(2):164–71. [PubMed: 10881026]

13. Macoska JA, Paris P, Collins C, Andaya A, Beheshti B, Chaib H, Kant R, Begley L, MacDonald JW, Squire JA. Evolution of 8p loss in transformed human prostate epithelial cells. *Cancer Genet. Cytogenet* 2004;154:36–43. [PubMed: 15381370]
14. Begley L, Keeney D, Beheshti B, Squire JA, Kant R, Chaib H, Macdonald JW, Rhim J, Macoska JA. Concordant Copy Number And Transcriptional Activity Of Genes Mapping To Derivative Chromosomes 8 During Cellular Immortalization In Vitro. *Genes Chromosomes Cancer* Feb;2006 45(2):136–46. [PubMed: 16235240]Epub Oct 18, 2005
15. Hayward SW, Dahiya R, Cunha GR, Bartek J, Deshpande N, Narayan P. Establishment and characterization of an immortalized but non-transformed human prostate epithelial cell line: BPH-1. *In Vitro Cell Dev Biol Anim* Jan;1995 31(1):14–24. [PubMed: 7535634]
16. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP. LNCaP model of human prostatic carcinoma. *Cancer Res* 1983;43:1809–1818. [PubMed: 6831420]
17. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* Jul;1979 17(1):16–23. [PubMed: 447482]
18. Begley LA, Kasina S, Mehra R, Adsule S, Admon AJ, Lonigro RJ, Chinnaiyan AM, Macoska JA. CXCL5 promotes prostate cancer progression. *Neoplasia* Mar;2008 10(3):244–54. [PubMed: 18320069]
19. Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. *Front Biosci* Jan 1;1997 2:d12–26. [PubMed: 9159205]
20. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. *J Leukoc Biol* Apr;2001 69(4): 513–21. [PubMed: 11310836]
21. De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* 1999;155:1985–92. [PubMed: 10595928]
22. Putzi MJ, De Marzo AM. Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. *Urology* 2000;56:828–32. [PubMed: 11068311]
23. Palapattu GS, Sutcliffe S, Bastian PJ, Platz EA, De Marzo AM, Isaacs WB, Nelson WG. Prostate carcinogenesis and inflammation: emerging insights. *Carcinogenesis* 2004;26(7):1170–1181. [PubMed: 15498784]
24. Theyer G, Kramer G, Assmann I, Sherwood E, Preinfalk W, Marberger M, Zechner O, Steiner GE. Phenotypic characterization of infiltrating leukocytes in benign prostatic hyperplasia. *Lab Invest* Jan; 1992 66(1):96–107. [PubMed: 1370561]
25. Okada K, Kojima M, Naya Y, Kamoi K, Yokoyama K, Takamatsu T, Miki T. Correlation of histological inflammation in needle biopsy specimens with serum prostate- specific antigen levels in men with negative biopsy for prostate cancer. *Urology* Jun;2000 55(6):892–8. [PubMed: 10840104]
26. Takata H, Tomiyama H, Fujiwara M, Kobayashi N, Takiguchi M. Cutting edge: expression of chemokine receptor CXCR1 on human effector CD8+ T cells. *J Immunol* Aug 15;2004 173(4):2231–5. [PubMed: 15294933]
27. Singh H, Dang TD, Ayala GE, Rowley DR. Transforming growth factor-beta1 induced myofibroblasts regulate LNCaP cell death. *J Urol* Dec;2004 172(6 Pt 1):2421–5. [PubMed: 15538284]
28. Kramer G, Steiner GE, Handisurya A, Stix U, Haitel A, Knerer B, Gessl A, Lee C, Marberger M. Increased expression of lymphocyte-derived cytokines in benign hyperplastic prostate tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation. *Prostate* Jun 1;2002 52(1):43–58. [PubMed: 11992619]

Figure 1. Inflammatory Mediators Are Expressed by Aging Prostate Stroma

A. RNA purified from primary prostate stromal fibroblasts explanted and cultured from the prostates of 5 younger (white triangles) or 4 older (black triangles) patients was subjected to quantitative reverse transcription polymerase chain reaction analysis for transcripts encoding cytokine- and chemokine-type inflammatory mediators identified through gene expression profiling experiments. The graph shows the transcript expression values after normalization to the constitutively expressed control transcript, RPLPO, plotted on a logarithmic scale (y-axis).

B. Fold transcript levels for CYTL1, CXCL1, CXCL5, CXCL6 and IL11 for primary prostate stromal fibroblasts explanted and cultured from the prostates of 4 older compared to 5 younger patients. Transcript levels for CXCL6 were significantly higher (asterisk, $p < .05$) for prostate stromal fibroblasts explanted and cultured from older compared to younger patients.

C. Protein levels (pg/ml) for the CXC-type chemokines CXCL1, CXCL5 and CXCL6 secreted into media conditioned by primary prostate stromal fibroblasts were significantly higher (*, $p < .05$) for cells explanted and cultured from the prostates of 5 older (black bars) compared to 4 younger (gray bars) patients. The graph shows the quantity of protein secreted in pg/ml plotted on a logarithmic scale (x-axis).

D. Protein levels (pg/ml) for the CXC-type chemokines CXCL1, CXCL5 and CXCL6 secreted into media conditioned by N1 prostate stromal fibroblasts (black bars) or CXCL1 and CXCL6 by LNCaP (dark gray bars), PC3 (medium gray bars), N15C6 (light gray bars) or BPH-1 (white bars) prostate epithelial cells were determined by ELISA. The graph shows the quantity of protein secreted in pg/ml plotted on a logarithmic scale (x-axis).

Figure 2. Inflammatory Mediators Expressed by Aging Prostate Stroma Promote Fibroblast and Epithelial Cell Proliferation

A. The fold proliferation of N1 prostate stromal fibroblast cells in serum-free media supplemented with CXCL5 (light gray bars), CXCL1 (dark gray bars) or CXCL6 (black bars) at the concentrations indicated relative to proliferation in un-supplemented serum-free media (set at 1-fold) is shown. The histograms indicate average fold proliferation and standard errors obtained over replicate experiments. An asterisk indicates significant levels of proliferation ($p < .05$) in chemokine-supplemented serum-free media compared to proliferation in un-supplemented serum-free media.

B., C. and D. The fold proliferation of N15C6 (B), LNCaP (C) or PC3 (D) prostate epithelial prostate cells in serum-free media supplemented with CXCL1 (dark gray bars) or CXCL6 (black bars) at the concentrations indicated relative to proliferation in un-supplemented serum-free media (set at 1-fold) is shown. The histograms indicate average fold proliferation and standard errors obtained over replicate experiments. An asterisk indicates significant levels of proliferation ($p < .05$) in chemokine-supplemented serum-free media compared to proliferation in un-supplemented serum-free media.

TABLE I
DIFFERENTIAL EXPRESSION OF GENES BY AGING FIBROBLASTS THAT ENCODE SECRETED PROTEINS

U133 A PROBE SET	GENE	FOLD CHANGE	TREND IN OLDER FIBROBLASTS	SECRETED	INFLAMMATORY MEDIATOR/INFLAMMATORY RESPONSE	FUNCTION
205239_at	AREG	3.12	UP	YES	YES	cytokine activity; growth factor activity; signaling
217767_at	C3	2.35	UP	YES	YES	complement activation
201925_s_at	CD55	2.18	UP	YES	YES	complement activation
201926_s_at	CD55	2.04	UP	YES	YES	chemokine activity, growth factor activity; signaling
204470_at	CXCL1	2.41	UP	YES	YES	chemokine activity, growth factor activity; signaling
203666_at	CXCL12	2.62	UP	YES	YES	chemokine activity, growth factor activity; signaling
209687_at	CXCL12	4.20	UP	YES	YES	chemokine (C-X-C motif) ligand 2
209774_x_at	CXCL2	2.19	UP	YES	YES	chemokine activity, growth factor activity; signaling
214974_x_at	CXCL5	2.25	UP	YES	YES	chemokine activity, growth factor activity; signaling
215101_s_at	CXCL5	2.97	UP	YES	YES	chemokine activity, growth factor activity; signaling
206336_at	CXCL6	2.97	UP	YES	YES	chemokine activity, growth factor activity; signaling
219837_s_at	CYTL1	2.30	UP	YES	YES	cytokine activity; growth factor activity; signaling
206924_at	IL11	3.06	UP	YES	YES	cytokine activity; growth factor activity; signaling
209821_at	IL33	2.25	UP	YES	YES	cytokine activity; protein binding
206025_s_at	TNFAIP6	2.86	UP	YES	YES	cell adhesion, signaling
206026_s_at	TNFAIP6	2.66	UP	YES	YES	cell adhesion, signaling
220092_s_at	ANTXR1	2.06	UP	YES (inferred)	YES (inferred)	signaling
211312_s_at	WISP1	2.07	UP	YES	NO	cell growth, cell adhesion, signaling (Wnt pathway)
219049_at	ChGn	2.10	UP	YES	NO	development (nervous system), biosynthesis, signaling
210587_at	INHBE	2.21	UP	YES	NO	hormone/growth factor activity
204491_at	PDE4D	2.25	UP	YES	NO	signaling
212353_at	SULF1	2.09	UP	YES	NO	apoptosis, metabolism
206796_at	WISP1	3.21	UP	YES	NO	cell adhesion; signaling (Wnt pathway); regulation of cell growth
204830_x_at	PSG5	-2.21	DOWN	YES	YES (inferred)	carcinoembryonic antigen
205200_at	CLEC3B	-2.51	DOWN	YES	NO	development (skeletal)
221029_s_at	WNT5B	-2.34	DOWN	YES	NO	signaling (Wnt pathway)