(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 October 2010 (21.10.2010)

PCT

(10) International Publication Number
WO 2010/120686 A2

(51) International Patent Classification:
G01N 33/53 (2006.01)

(21) International Application Number:
PCT/US2010/030736

(22) International Filing Date:
12 April 2010 (12.04.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/168,824 13 April 2009 (13.04.2009) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: METHODS FOR THE DIAGNOSIS AND TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

(57) Abstract: The chemokine MCP-1 is closely associated with the development of the condition benign prostatic hyperplasia (BPH) and determination of the concentration of MCP-1 in prostatic secretions or in urine after prostatic massage, or determination of the expression of the MCP-1 gene by determination of the concentration of MCP-1 -specific mRNA can be used to specifically diagnose BPH and distinguish it from other diseases or conditions affecting the prostate, such as prostatitis and prostatic carcinoma. In addition, MCP-1 can serve as a target for therapy of BPH and agents affecting the expression or activity of MCP-1 can serve as therapeutic agents for BPH.

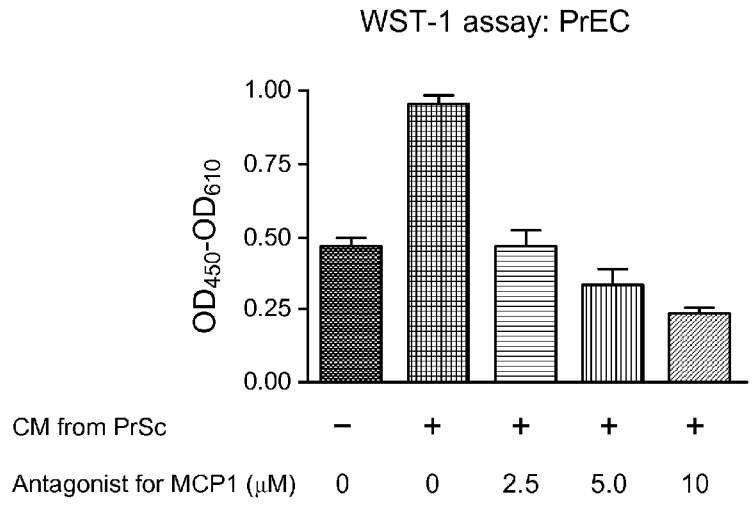

FIGURE 1

WO 2010/120686 A2

METHODS FOR THE DIAGNOSIS AND TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

by

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CROSS-REFERENCES

[0001] This PCT application claims priority from United States Provisional Application Serial No. 61/168,824, by Parsons et al., entitled "Methods for the Diagnosis and Treatment of Benign Prostatic Hyperplasia" and filed on April 13, 2009, the contents of which are hereby incorporated herein in their entirety by this reference.

FIELD OF THE INVENTION

[0002] This invention is directed to methods and devices for the diagnosis of benign prostatic hyperplasia, especially methods and devices for distinguishing benign prostatic hyperplasia from other diseases and conditions affecting the prostate, such as prostatitis and prostatic carcinoma.

BACKGROUND OF THE INVENTION

[0003] As men age, they frequently begin to show various symptoms related to the lower urinary tract. In many cases, such symptoms are associated with enlargement of the prostate gland. Such symptoms include frequency of urination, especially at night, urinary urgency, urinary hesitancy, urinary retention, and increased susceptibility to urinary infections.

[0004] Although there are a number of conditions associated with these symptoms, sometimes described in general as "lower urinary tract symptoms" or "LUTS", three particular conditions are most important. These conditions are benign prostatic hyperplasia (BPH), prostatitis, and prostatic carcinoma.

[0005] BPH is characterized by hyperplasia of prostatic stromal and epithelial cells, resulting in the formation of large, fairly discrete nodules in the periurethral region of the prostate. When sufficiently large, the nodules compress the urethral canal to cause partial, or sometimes virtually complete, obstruction of the urethra which interferes with the normal flow of urine.

[0006] The occurrence of BPH is definitely a function of age. Adenomatous prostate growth is believed to begin at approximately 30 years of age in most men. An estimated 50% of men have histological evidence of BPH by age 50 years and 75% by age 80 years. In 40-50% of these patients, BPH becomes clinically significant.

[0007] Despite the "benign" label, BPH can be a progressive disease, especially if left untreated. Incomplete voiding results in stasis of bacteria in the bladder residue, which can lead to an increased risk of urinary tract infections. Urinary bladder stones can be formed from the crystallization of salts in the residual urine; the formation of such stones can lead to severe pain and other symptoms. Urinary retention, termed acute or chronic, is another form of progression of BPH. Acute urinary retention is the inability to void, while in chronic urinary retention the residual urinary volume gradually increases and the bladder distends. Some patients who suffer from chronic urinary retention may eventually progress to renal failure, a condition termed obstructive uropathy.

[0008] Androgens are considered to play a permissive role in BPH by most experts. This means that androgens have to be present for BPH to occur, but do not necessarily directly cause the condition. This is supported by the fact

that castrated boys do not develop BPH when they age, unlike intact men. Additionally, administering exogenous testosterone is not associated with a significant increase in the risk of BPH symptoms. Dihydrotestosterone (DHT), a metabolite of testosterone, is a critical mediator of prostatic growth. DHT is synthesized in the prostate from circulating testosterone by the action of the enzyme 5α-reductase, type 2. This enzyme is localized principally in the stromal cells; hence, these cells are the main site for the synthesis of DHT. DHT can act in an autocrine fashion on the stromal cells or in paracrine fashion by diffusing into nearby epithelial cells. In both of these cell types, DHT binds to nuclear androgen and signals the transcription of growth factors that are mitogenic to the epithelial and stromal cells. DHT is 10 times more potent than testosterone because it dissociates from the androgen receptor more slowly. The importance of DHT in causing nodular hyperplasia is supported by clinical observations in which an inhibitor of 5α-reductase is given to men with this condition. Therapy with 5α-reductase inhibitor markedly reduces the DHT content of the prostate and in turn reduces prostate volume and, in many cases, BPH symptoms.

[0009] There is growing evidence that estrogens play a role in the etiology of BPH. This conclusion is based on the fact that BPH occurs when men generally have elevated estrogen levels and relatively reduced free testosterone levels, and when prostate tissue becomes more sensitive to estrogens and less responsive to DHT. Cells taken from the prostates of men who have BPH have been shown to grow in response to high estradiol levels with low androgen levels present. Estrogens may render cells more susceptible to the action of DHT.

[0010] One of the most significant issues associated with BPH is the differential diagnosis of BPH, particularly with respect to other diseases and conditions affecting the male lower urinary tract, especially prostatitis and prostatic carcinoma. This is extremely important because of the different treatments that are considered optimal for each of these diseases and conditions. It is particularly important to distinguish BPH from prostatic

carcinoma, as detailed further below. This is because the treatment of these conditions differs widely.

[0011] For BPH, treatment is typically fairly conservative, such as lifestyle changes such as avoidance of caffeine and alcohol, and the use of medications such as alpha-blockers (α_1-adrenergic receptor antagonists) such as doxazosin, terazosin, alfuzosin, and tamsulosin, as well as 5α-reductase inhibitors such as finasteride or dutasteride. Saw palmetto extracts (*Serenoa repens*) and other herbal extracts may also be beneficial.

[0012] For cases in which such conservative treatments do not provide sufficient relief, minimally invasive therapies such as transurethral microwave thermotherapy (TUMT) or transurethral needle ablation (TUNA) can be applied to shrink the prostate. These therapies can be performed on an outpatient basis in a doctor's office.

[0013] Another treatment option is the surgical transurethral resection of the prostate (TURP). This is a surgical procedure involving removal of part of the prostate through the urethra. Like all surgical procedures of the lower urinary tract, such a procedure can cause complications such as bleeding, thrombosis, hyponatremia due to bladder irrigation, and urinary tract complications such as impotence.

[0014] By contrast, prostatic carcinoma (prostatic adenocarcinoma), while typically presenting with no significant symptoms or symptoms comparable to BPH, is a much more serious disease. Prostatic carcinomas, while often slow-growing, can grow more rapidly and metastasize to other organs, such as the bones, lymph nodes, rectum, and bladder. Treatment of prostatic carcinoma is typically far more invasive and aggressive than for BPH, involving surgery, radiation, chemotherapy, and other treatment modalities.

[0015] Prostatitis is an inflammation of the prostate, frequently associated with acute or chronic bacterial infection, although it can result from other causes. Prostatitis can cause symptoms similar to those of BPH, although it can also be associated with other symptoms, such as pelvic pain in men.

[0016] Diagnosis of BPH, however, is not always clearcut. Rectal examination (palpation of the prostate through the rectum) may reveal a markedly enlarged prostate, usually affecting the middle lobe of the prostate. Elevated prostate specific antigen (PSA) levels can occur in BPH, but also occur in prostatic carcinoma. Attempts have been made to reinterpret PSA levels in terms of prostatic volume, leading to a determination of PSA density. Attempts have also been made to distinguish between bound and free PSA. However, these attempted refinements of the determination of PSA levels have been questioned. Ultrasound examination of the testicles, prostate, and kidneys is also frequently performed,

[0017] Because of the severe physical and psychological consequences of a misdiagnosis of BPH as prostatic carcinoma, therefore, there exists a need for an improved diagnostic method for the differential diagnosis of BPH as distinguished from other diseases or conditions that can cause LUTS in men. This is particularly important because current diagnostic methods often cannot clearly distinguish these two conditions (BPH and prostatic carcinoma). A patient with BPH who is misdiagnosed as having prostatic carcinoma may, therefore, be forced to undergo extremely aggressive treatment, with a serious risk of side effects, when such treatment is not actually needed. Additionally, such a patient, who is mistakenly given a diagnosis of cancer, will frequently suffer severe psychological consequences, such as depression, that will interfere with his quality of life and may prevent him from participating actively in decisions concerning the course of his treatment. Preferably, such a diagnostic method would be precise and would avoid false positives (diagnosis of BPH when the patient does not have this condition) and false negatives (diagnosis of no BPH

when, in fact, the patient has the condition). Preferably, such a diagnostic method would avoid subjective determinations based on imprecise impressions such as the size of the prostate as felt in rectal examination, would be non-invasive, and would be rapid and simple to perform.

SUMMARY OF THE INVENTION

[0018] As detailed below, a diagnostic test based on the concentration of the chemokine Monocyte Chemoattractant Protein-1 (MCP-1) meets the needs for an improved method for diagnosing BPH.

[0019] One aspect of the invention is a method of diagnosing BPH comprising the steps of:

(1) obtaining a sample of prostatic fluid or urine from a male patient suspected of having BPH;

(2) performing an assay for MCP-1 on the sample to measure the concentration of MCP-1 in the sample; and

(3) comparing the concentration of MCP-1 in the sample with a reference value or comparing a ratio of the concentration of MCP-1 with a parameter selected from the group consisting of total protein and weight of the prostate gland, such that if the concentration of MCP-1 or the ratio of the concentration of MCP-1 to the parameter is at or above a defined reference value, the male patient has BPH.

[0020] Typically, the assay for MCP-1 is an immunoassay, such as a sandwich immunoassay. The sandwich immunoassay can be an ELISA assay. Other immunoassay techniques, such as the enzyme multiplied immunoassay technique (EMIT), a cloned enzyme donor immunoassay (CEDIA), an immuno-PCR assay, a phosphor immunoassay, a quantum dot immunoassay, a solid phase, light-scattering immunoassay, or a surface effect immunoassay, can be

used. If the immunoassay is a sandwich immunoassay, it can be performed in a lateral flow device.

[0021] Typically, if the sample is prostatic fluid, in one alternative, the reference value for the concentration of MCP-1 in prostatic fluid is about 5000 intensity units as measured in densitometry in a conventional MCP-1 immunoassay. In another alternative, the method comprises determining a ratio of the level of MCP-1 in prostatic fluid to the total weight of the prostate gland, such that a ratio of greater than about 6.0 intensity units/g prostate gland weight is diagnostic of BPH. In still another alternative, the method comprises determining a ratio of the level of MCP-1 in prostatic fluid to the total protein in the prostatic fluid, such that a ratio of greater than about 400 intensity units/μg total protein is diagnostic of the existence of BPH.

[0022] Alternatively, the sample can be urine. If the sample is urine, typically the urine sample is collected after prostatic massage. In one alternative, any detectable MCP-1 concentration is diagnostic of BPH. In another alternative, a MCP-1 concentration of at least 10 pg/ml is diagnostic of BPH.

[0023] Another diagnostic method within the scope of the invention is a method for diagnosing BPH comprising the steps of:

(1) hybridizing mRNA isolated from prostatic cells with a probe that corresponds to a sequence within mRNA encoding MCP-1 protein under conditions that substantially only mRNA encoding MCP-1 protein hybridizes to the probe;

(2) isolating the hybridized mRNA; and

(3) determining the quantity of mRNA encoding the MCP-1 protein to diagnose the existence of BPH.

[0024] Another aspect of the present invention is a method for treating or preventing BPH comprising the step of administering a therapeutically effective

quantity of an agent that inhibits the expression or activity of MCP-1 to a patient who has or is at risk of developing BPH in order to inhibit the expression or activity of MCP-1 to a sufficient degree that BPH is treated or its occurrence prevented.

[0025] When the agent that inhibits the expression or activity of MCP-1 inhibits the expression of MCP-1, in one alternative, the agent is an inhibitory nucleotide selected from the group consisting of short interfering RNA (siRNA), microRNA (miRNA), and synthetic hairpin RNA (shRNA), anti-sense nucleic acids, and complementary DNA (cDNA). In another alternative, the agent that inhibits the expression of MCP-1 is an angiotensin receptor blocker (ARB).

[0026] When the agent that inhibits the expression or activity of MCP-1 inhibits the activity of MCP-1, in one alternative, the agent is an antibody that specifically binds MCP-1. In another alternative, the agent is an antibody that specifically binds CCR2. In still another alternative, the agent is an antagonist of CCR2, such as, but not limited to, 1′-[2-[4-(trifluoromethyl)phenyl]ethyl]-spiro[4H-3,1-benzoxazine-4,4′-piperidin]-2(1H)-one hydrochloride.

[0027] Another aspect of the present invention is a pharmaceutical composition for the treatment or prevention of BPH comprising:

(1) a therapeutically effective dose of an agent that inhibits the expression or activity of MCP-1; and

(2) a pharmaceutically acceptable carrier, diluent or excipient in unit dosage form.

[0028] In this pharmaceutical composition, the agent that inhibits the expression or activity of MCP-1 is as described above.

[0029] Another aspect of the present invention is a method of screening for a compound inhibiting the expression and/or activity of MCP-1 comprising the steps of:

(1) administering a compound potentially inhibiting the expression and/or activity of MCP-1 to a first sample of cells that demonstrate a detectable response to MCP-1;

(2) determining the response to MCP-1 in the first sample of cells; and

(3) comparing the response to MCP-1 in the first sample of cells with the response to MCP-1 in a second sample of cells to which the compound potentially inhibiting the expression and/or activity of MCP-1 to determine whether the compound inhibits the expression and/or activity of MCP-1.

[0030] In one alternative, the cells that demonstrate a detectable response to MCP-1 are cells that endogenously express and secrete MCP-1. In another alternative, the cells that demonstrate a detectable response to MCP-1 are cells that express a receptor for MCP-1. The response to MCP-1 that is measured can be cell proliferation. The compound potentially inhibiting the expression and/or activity of MCP-1 can be selected from the group consisting of a protein, a peptide, a peptidomimetic, a nucleic acid, a steroid, an alkaloid, a prostaglandin or analogue thereof, a prostacyclin or analogue thereof, a receptor agonist, a receptor antagonist, a monosaccharide, a disaccharide, a carbohydrate larger than a disaccharide, or a small molecule that is other than a steroid, an alkaloid, a monosaccharide, a disaccharide, a prostaglandin or analogue thereof, and a prostacyclin or analogue thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

[0032] Figure 1 is a graph showing that conditioned medium from PrSC stimulated the proliferation of PrEC, and that this proliferation was completely inhibited by a specific MCP-1 inhibitor, RS-102895.

[0033] Figure 2 is a scatter plot of 147 cytokines with the average OD of each cytokine in an immunoassay specific for that cytokine from the large prostate group plotted on the y axis and the average OD of each cytokine from the small prostate group plotted on the x axis. The position of MCP-1 on the scatter plot is indicated.

[0034] Figure 3 is a plot of the detailed data for a number of cytokines, with the OD of the cytokine in an immunoassay specific for that cytokine on the y axis and the weight of the prostate gland being measured on the x axis. (A) MCP-1; (B) Activin A; (C) FGF-7; (D) FGF-4; (E) Endoglin; (F) IL-6; (G) IL-7; and (H) IL-1β.

[0035] Figure 4 shows the results of analysis for MCP-1 and IL-1β for prostatic fluid from patients in the category Gleason 6 with minimum disease and no hormonal treatment. In Figure 4, Panel (A) plots the results for concentration of MCP-1 as represented by the ratio of OD in an MCP-1-specific immunoassay to total protein on the y axis and the weight of the prostate gland being measured on the x axis. Panel (B) plots the results for concentration of MCP-1 as represented by the ratio of OD in an MCP-1-specific immunoassay to total protein on the y axis in two groups, separated on the x axis: a first group with prostate gland weights < 50 g and a second group with prostate gland weights >80 g. Panel (C) plots the concentration of MCP-1 as represented by the OD in a MCP-1 specific immunoassay on the x axis versus the I-PSS score on the y

axis. Panel (D) plots the results for concentration of IL-1β as represented by the ratio of OD in an IL-1β-specific immunoassay to total protein on the y axis versus gland weight on the x axis. Panel (E) plots the concentration of IL-1β as represented by the OD in an IL-1β-specific immunoassay on the x axis versus the I-PSS score on the y axis.

[0036] Figure 5 shows the effect of MCP-1 on the proliferation of the human cell lines PrEC (primary prostate basal epithelial cell) and PrSC (primary prostate stromal cell). In Panel (A), the effects of MCP-1 on the proliferation of PrEC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = MCP-1 1 ng/ml; ▼ = MCP-1 10 ng/ml; ♦ = MCP-1 100 ng/ml). In Panel (B), the effects of MCP-1 on the proliferation of PrSC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = MCP-1 1 ng/ml; ▼ = MCP-1 10 ng/ml; ♦ = MCP-1 100 ng/ml). In Panel (C), the effects of RS102895, the specific antagonist of CCR2 (also known as CD192, the receptor for MCP-1) on the proliferation of PrEC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = RS102895 1.25 μM; ▼ = RS102895 2.5 μM; ♦ = RS102895 5.0 μM; ● = RS102895 10 μM). In Panel (D), the effects of RS102895, the specific antagonist of CCR2 (also known as CD192, the receptor for MCP-1) on the proliferation of PrSC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = RS102895 1.25 μM; ▼ = RS102895 2.5 μM; ♦ = RS102895 5.0 μM; ● = RS102895 10 μM).

[0037] Figure 6 shows Western blots for CCR2 in PrEC and PrSC.

[0038] Figure 7 shows the results of immunoassays for the detection of MCP-1 in PrEC and PrSC cells. In the left panel of Figure 7, MCP-1 concentration (determined by immunoassay) is shown in pg/ml. In the right panel of Figure 7, MCP-1 concentration (determined by immunoassay) is shown in pg/ml for PrEC, the LNCap cell line (derived from androgen-sensitive human

prostate adenocarcinoma cells), the C42B cell line (also derived from prostate adenocarcinoma cells), the PC3 cell line (also derived from prostate adenocarcinoma cells), and the hFOB cell line (a human fetal osteoblast progenitor cell line).

[0039] Figure 8 shows that the specific antagonist of CCR2 WS102895 inhibits the effects of MCP-1 on PrEC cells. In Figure 8, proliferation of PrEC cells is represented as OD_{450}-OD_{610} in the proliferation assay (the WST-1 assay described above). Starting from the leftmost bar, the bars represent: (1) no MCP-1 and no WS 102895; (2) 10 nM MCP-1 and no WS102895; (3) 10 nM MCP-1 and 2.5 μM WS102985; (4) 10 nM MCP-1 and 5.0 μM WS102985; and (5) 10 nM MCP-1 and 10 μM WS102985. These results clearly show that WS102895 in inhibits the effects of MCP-1 on PrEC cells, blocking their proliferation.

[0040] Figure 9 shows that the specific antagonist of CCR2 WS102895 also blocks the growth effect of conditioned media from PrSC to PrEC. In Figure 9, proliferation of PrEC cells is represented as OD_{450}-OD_{610} in the proliferation assay. Starting from the leftmost bar, the bars represent: (1) no conditioned media and no WS 102895; (2) conditioned media and no WS102895; (3) conditioned media and 2.5 μM WS102985; (4) conditioned media and 5.0 μM WS102985; and (5) conditioned media and 10 μM WS102985.

[0041] Figure 10 shows that a specific monoclonal antibody binding MCP-1 blocks the effect of PrSC-conditioned media in stimulating proliferation of PrEC. In the top panel of Figure 10, conditioned media was used as in Figure 9 and proliferation of PrEC cells is represented as OD_{450}-OD_{610} in the proliferation assay. Starting from the leftmost bar, the bars represent: (1) no conditioned media and no anti-MCP-1 mAb; (2) conditioned media and no anti-MCP-1 mAb; (3) conditioned media and anti-MCP-1 mAb at 1.0 μg/ml; and (4) conditioned media and anti-MCP-1 mAb at 5.0 μg/ml. However, the anti-MCP-1 mAb has a

very minimal effect on the proliferation of PrEC cells, as shown in the bottom panel of Figure 10. In the bottom panel of Figure 10, the bars represent, starting from the left: (1) no anti-MCP-1 antibody; (2) anti-MCP-1 antibody at 0.25 μg/ml; (3) anti-MCP-1 antibody at 1.0 μg/ml; and (4) anti-MCP-1 antibody at 5.0 μg/ml.

[0042] Figure 11 shows that MCP-1 and IL-1β levels correlate with CD68, a macrophage-specific marker, in prostatic juice. In Figure 11, in Panel (A), the ratio of CD68/β-actin is plotted on the y axis and MCP-1 concentration in pg/ml is plotted on the x axis. In Panel (B), the ratio of CD68/β-actin is plotted on the y axis and IL-1β concentration in pg/ml is plotted on the x axis. In Panel (C), the concentration of IL-1β in pg/ml is plotted on the x axis and the concentration of MCP-1 in pg/ml is plotted on the y axis. In Panel (D), the results are divided into two groups, those with low CD68 concentration and those with high CD68 concentration, and the I-PSS score for each sample within each group is plotted separately, showing a correlation between high CD68 concentration and higher I-PSS score.

[0043] Figure 12 shows that conditioned medium from monocytes/macrophages stimulates the proliferation of PrEC. In Figure 12, the left panel shows results with PrEC and the right panel shows results with PrSC. In each panel, starting from the leftmost bar, the bars represent: (1) no conditioned medium from monocytes/macrophages and no MCP-1; (2) no conditioned medium from monocytes/macrophages and MCP-1; (3) conditioned medium from monocytes/macrophages and no MCP-1; and (4) conditioned medium from monocytes/macrophages and MCP-1. The results show a substantially greater effect with PrEC than with PrSC.

[0044] Figure 13 shows that inflammatory cytokines also enhance the secretion of MCP-1 from PrEC and PrSC. The results in the left panel of Figure 13 are for PrEC; in the right panel, for PrSC. In each panel, starting from the

leftmost bar, the bars represent: (1) control; (2) IL-1β; (3) IFN-γ; and (4) IL-2. For each graph, MCP-1 in pg/ml is shown on the y axis. In particular, IFN-γ has a substantial effect in stimulating MCP-1 secretion in PrEC cells, and all of IL-1β, IFN-γ and IL-2 have a substantial effect on the secretion of MCP-1 in PrSC cells.

[0045] Figure 14 is a schematic showing the role of MCP-1 in BPH, showing the interactions with stromal cells, basal cells, luminal cells, macrophages, and T cells, including interactions mediated by IL-1β and IFN-γ.

[0046] Figure 15 shows the effect of the synthetic androgen methyltrienolone (R1881) on the secretion of MCP-1 by PrSC and the expression of the MCP-1 gene. In the left panel of Figure 15, results for ELISA for MCP-1 in the supernatant of PrSC are shown; MCP-1 in pg/ml is shown on the y axis, and starting with the leftmost bar, R1881 was used as follows: (1) no R1881; (2) 0.1 nM R1881; (3) 1.0 nM R1881; and (4) 1.0 nM R1881. In the right panel of Figure 15, results for real-time PCR are shown on the y axis, representing mRNA specific for MCP-1; again, starting with the leftmost bar, R1881 was used as follows: (1) no R1881; (2) 0.1 nM R1881; (3) 1.0 nM R1881; and (4) 1.0 nM R1881.

[0047] Figure 16 shows the level of MCP-1 protein in patients after massage. In Panel (A) of Figure 16, the data is grouped into two parts: on the left, I-PSS scores are plotted for those patients for which MCP-1 was undetectable in urine after massage, and, on the right, I-PSS scores are plotted for those patients for which MCP-1 was detectable in urine after massage. In Panel (B) of Figure 16, the data is again grouped into two parts: on the left, estimated prostate gland size is plotted for those patients for which MCP-1 was undetectable in urine after massage, and, on the right, estimated prostate gland size is plotted for those patients for which MCP-1 was detectable in urine after massage. In Panel (C) of Figure 16, the data is again grouped into two parts: on the left, the age of the patient is plotted for those patients for which MCP-1 was

undetectable in urine after massage, and, on the right, the age of the patient is plotted for those patients for which MCP-1 was detectable in urine after massage.

[0048] Figure 17 presents additional analysis of the data from the patients of Figure 16. In Panel (A) of Figure 17, MCP-1 on the x axis is plotted versus I-PSS score on the y axis. In Panel (B) of Figure 17, MCP-1 on the x axis is plotted versus gland weight (in g) on the y axis. In Panel (C) of Figure 17, MCP-1/total protein on the x axis is plotted versus I-PSS score on the y axis. In Panel (D) of Figure 17, MCP-1/total protein on the x axis is plotted versus gland weight (in g) on the y axis.

[0049] Figure 18 presents an analysis of the MCP-1 concentration in serum for these patients. In the graph shown in Figure 18, MCP-1 concentration in pg/ml is shown for serum (x2 diluted). The data is grouped into three groups: the leftmost group is patients considered "normal"; the center group is patients for which there has been treatment failure; the rightmost group is patients with an AUA score of >20.

[0050] Figure 19 shows results of a study of expression of the gene for MCP-1 as measured in 25 paired normal-tumor RRP (radical retropublic prostatectomy) tissues and 9 BPH samples. Expression ratios of test sample/BPH reference are color coded, with red indicating higher expression in test sample relative to BPH and green indicating lower expression in the test sample relative to BPH. These results indicate that expression of MCP-1 is characteristic of BPH and not of prostatic carcinoma.

DETAILED DESCRIPTION OF THE INVENTION

[0051] The present invention is based on the discovery that the cytokine Monocyte Chemoattractant Protein-1 (MCP-1) is strongly associated with the development of BPH and can serve as a marker for its diagnosis, as well as a target for its treatment.

[0052] Human MCP-1 is a 76-amino-acid protein with a pI of 9.7. The protein contains two disulfide bonds. The protein has the amino acid sequence: QPDAINAPVTCCYNFTNRKISVQRLASYRRITSSKCPKEAVIFKTIVAKEICADPK QKWVQDSMDHLDKQTQTPKT (SEQ ID NO: 1). Human MCP-1 is cleaved from a 99-amino acid precursor protein with the amino acid sequence: MKVSAALLCLLLIAATFIPQGLAQPDAINAPVTCCYNFTNRKISVQRLASYRRITS SKCPKEAVIFKTIVAKEICADPKQKWVQDSMDHLDKQTQTPKT (SEQ ID NO: 2). The human MCP-1 protein has the SwissProt accession number P13500. Structurally, human MCP-1 has the tertiary structure of a dimmer consisting of two three-stranded Greek keys upon which lie two antiparallel α-helices. The structure of the human MCP-1 gene has three exons separated by introns; the gene is on chromosome 17q11.2-q21. MCP-1 is also known as CCL2.

[0053] MCP-1 is a monocyte/macrophage chemotactic factor and induces monocytes to produce proinflammatory cytokines such as IL-1 and IL-6. MCP-1 also induces the migration of smooth muscle cells *in vitro* and enhances *in vitro* collagen expression by fibroblasts, partly by enhancing TGF-β. MCP-1 also chemoattracts basophils and CD4-positive and CD8-positive T lymphocytes. MCP-1 also plays central roles in the pathogenesis of lung fibrosis and diabetic nephropathy.

[0054] In benign prostatic hyperplasia (BPH), BPH nodules frequently occur concurrently together with chronic inflammatory infiltrates, T cells, and macrophages. BPH is characterized by increased expression of the cytokines IL-2, IL-4, IL-8, IL-15 and IFNγ. BPH is also characterized by downregulation of macrophage inhibitory cytokine-1/prostate derived factor.

[0055] This invention, therefore, is based on the hypothesis that MCP-1 is the key cytokine involved in BPH development. Accordingly, MCP-1 may induce epithelial and stromal proliferation and may also induce extracellular matrix deposition. Therefore, because of this hypothesis, and the specific involvement of MCP-1 in BPH development, MCP-1 can be a specific marker for BPH and can be used to discriminate BPH from prostatic carcinoma. The use of MCP-1 as a specific marker for BPH is described below. Also, MCP-1 can be a novel target for BPH therapy, either alone or in conjunction with other therapies. The use of MCP-1 as a target for BPH therapy is described below.

[0056] Accordingly, one aspect of the present invention is a method of diagnosing BPH comprising the steps of:

(1) obtaining a sample of prostatic fluid or urine from a male patient suspected of having BPH;

(2) performing an assay for MCP-1 on the sample to measure the concentration of MCP-1 in the sample; and

(3) comparing the concentration of MCP-1 in the sample with a reference value or comparing a ratio of the concentration of MCP-1 with a parameter selected from the group consisting of total protein and weight of the prostate gland, such that if the concentration of MCP-1 or the ratio of the concentration of MCP-1 to the parameter is at or above a defined reference value, the male patient has BPH.

[0057] Typically, the reference value for the concentration of MCP-1 in prostatic fluid is about 5000 intensity units as measured in densitometry in a conventional MCP-1 immunoassay.

[0058] Typically, the assay for MCP-1 is an immunoassay.

[0059] When the assay for MCP-1 is an immunoassay, many immunoassay formats are suitable for use in immunoassays in this method of the present invention. Because MCP-1 is a sufficiently large protein antigen, it is capable of binding multiple antibody molecules. This enables the use of a sandwich immunoassay format, in which the MCP-1 protein molecule is bound to both an unlabeled antibody, typically bound to a solid support, and a labeled antibody. The MCP-1 protein molecule is then detected by the label of the labeled antibody.

[0060] One particularly useful sandwich immunoassay format is the enzyme-linked immunosorbent (ELISA) assay. In the ELISA assay, a non-labeled antibody is attached to the surface of a solid phase, such as that of a microtiter well, a magnetic particle, or a plastic bead. This attachment facilitates the separation of bound labeled reactants from free labeled reactants; since only the bound labeled reactants (labeled antibody) bound to the antigen (MCP-1) in the sandwich need to be detected or determined. In one common approach for performing ELISA assays, an aliquot of sample presumed to contain a quantity of MCP-1 is added to and allowed to bind with a solid phase antibody as described above. After washing, enzyme-labeled antibody is added to form the sandwich complex as described above, i.e., solid phase Ab-Ag-Ab (enzyme-labeled). Excess unbound antibody is then washed away, and a suitable enzyme substrate is added, along with appropriate buffers and coenzymes if required. The enzyme then catalytically converts the substrate to a detectable product, the quantity of which is proportional to the quantity of antigen in the sample.

[0061] A number of suitable enzymes are known in the art for use in ELISA assays. These enzymes include, but are not limited to, alkaline phosphatase, horseradish peroxidase, glucose 6-phosphate dehydrogenase, and β-galactosidase. Other enzyme labels are also known in the art. Enzyme-labeled antibodies can be prepared by covalent coupling procedures involving reagents such as, but not limited to, glutaraldehyde, N-succinimidyl 3-[pyridyl]

propionate, carbodiimides, carbonyldiimidazole, and other cross-linking reagents known in the art, such as those described in G.T. Hermanson, "Bioconjugate Techniques" (Academic Press, San Diego, 1996), pp. 630-637, incorporated herein by this reference. As another alternative, recombinant antibody conjugates can be prepared by genetic engineering techniques known in the art, the conjugates being produced by transcription and translation of gene fusions.

[0062] In many cases, the enzyme in the enzyme-labeled antibody in ELISA produces a product that is monitored and/or quantitated photometrically, such as by spectroscopy. However, in some alternatives, the enzyme produces a product that is monitored and/or quantitated by other means, such as detection of fluorescence, bioluminescence, or chemiluminescence. For example, immunoassays that use horseradish peroxidase as the enzyme label can be assayed by the detection of chemiluminescence using a mixture of luminol, peroxide, and an enhancer such as *p*-iodophenol or by using an acridan derivative. Umbelliferone phosphate is a nonfluorescent substrate that is converted to the highly fluorescent umbelliferone by the catalytic activity of the enzyme alkaline phosphatase. Another sensitive assay using an alkaline phosphatase label uses a chemiluminescent adamantyl 1,2-dioxetane aryl phosphate substrate, which is dephosphorylated by the catalytic activity of alkaline phosphate and decomposes with a concomitant long-lived glow of light, such that the detection limit for alkaline phosphatase using this assay can be as low as 1 zeptomole (10^{-21} moles). In another alternative, an enzyme cascade is used, The advantage of the use of an enzyme cascade is that it combines the amplification properties of two enzymes—the alkaline phosphatase present in the antibody label and the second enzyme, alcohol dehydrogenase, in the assay reagent, thereby producing an extremely sensitive assay. For example, the alkaline phosphatase can dephosphorylate NADP to NAD, which then takes part in a reaction catalyzed by alcohol dehydrogenase and the enzyme diaphorase in which ethanol is oxidized to acetaldehyde and *p*-iodonitrotetrazolium violet is reduced to the detectable formazan by the catalytic activity of diaphorase.

[0063] Other assay techniques involving enzyme-labeled antibodies are known in the art. For example, enzyme multiplied immunoassay technique (EMIT) is a homogeneous immunoassay. In this technique, antibody against the analyte (MCP-1) is added together with substrate for an enzyme label to a sample. Binding of the antibody and the MCP-1 occurs. An aliquot of a conjugate of an enzyme that can catalyze a reaction involving the substrate and the MCP-1 is added to the sample. The enzyme-MCP-1 conjugate then binds with any excess antibody to MCP-1, forming an antibody-enzyme-MCP-1 conjugate complex. In the absence of free MCP-1 in the original sample, the enzyme is inactivated, such as by the binding of the enzyme in the enzyme-MCP-1 conjugate to the antibody which physically blocks access of the substrate to the active site of the enzyme or by changing the conformation of the enzyme and thus altering the activity. In this assay, which is a homogeneous assay not requiring separation of bound and free label, the greater the concentration of analyte (MCP-1), the greater is the quantity of enzyme activity.

[0064] Still another antibody immunoassay format known in the art is cloned enzyme donor immunoassay (CEDIA). In CEDIA, inactive fragments (the enzyme donor and acceptor) of the enzyme β-galactosidase are prepared by manipulation of the *Z* gene of the *lac* operon of the bacterium *Escherichia coli*. These two fragments spontaneously reassemble to form active enzyme, even if the enzyme donor is attached to an antigen, such as MCP-1. However, binding of antibody to the enzyme donor inhibits reassembly, thus blocking the formation of active enzyme and preventing enzyme activity. Thus, competition between the antigen (MCP-1) and the enzyme donor antigen conjugate for a fixed quantity of antibody in the presence of the enzyme acceptor modulates the measured enzyme activity so that high concentrations of antigen produce the least inhibition of enzyme activity and the greatest measured enzyme activity, while low concentrations of antigen produce the most inhibition of enzyme activity and the least measured enzyme activity.

[0065] Other immunoassay formats are known using various other types of labeled antibodies, such as are well known in the art. For example, immunoassays can be performed using radiolabeled antibodies, antibodies labeled with fluorescent, chemiluminescent, electrochemiluminescent, or bioluminescent labels, antibodies labeled with colloidal metals such as gold or silver, as well as other labels known in the art, such as, but not limited to, Surface-Enhanced Resonant Raman Spectroscopy (SERRS) labels, or signaling aptamers. For example, for bioluminescent labels, native or recombinant apoaequorin from the bioluminescent jellyfish *Aequorea* can be used as the label; the label is activated by reactions with coelenterazine, and light emission at 469 nm is triggered by reaction with calcium ions, such as calcium chloride. In fluorescence excitation transfer immunoassay, a fluorophore (donor)-labeled antigen competes with an antigen in the sample for binding sites on an antibody labeled with a fluorescent dye (acceptor). The fluorescence of the donor is quenched when it is bound to the acceptor-labeled antibody. Another alternative assay format is immuno-PCR. Immuno-PCR is a heterogeneous immunoassay in which a piece of single- or double-stranded DNA is used as a label for an antibody in a sandwich assay. Bound DNA label is amplified using PCR. The amplified DNA produced is separated by gel electrophoresis and quantitated by densitometric scanning of a gel stained with the DNA-specific stain ethidium bromide. Still another alternative assay format is luminescent oxygen channeling immunoassay (LOCI). LOCI is a homogeneous sandwich immunoassay in which an antigen (MCP-1 in this example) links an antibody-coated sensitizer dye-loaded particle (250-nm diameter) and an antibody-coated particle (250-nm diameter) loaded with a mixture of a precursor of a chemiluminescent compound and a fluorophore. Irradiation produces singlet oxygen at the surface of the sensitizer dye-loaded particle. This diffuses or "channels" to the other particle held in close proximity by the immunochemical reaction between the antigen (MCP-1 in this example) and antibodies on the particles. The singlet oxygen reacts with the chemiluminescent compound precursor in the particle to form a

chemiluminescent dioxane compound, which then decomposes to emit light via a fluorophore-sensitized mechanism. No signal is obtained from precursor fluorophore-loaded particles that are not linked by an immunological reaction with an antigen (MCP-1 in this example). Yet another alternative assay format is phosphor immunoassay. Phosphor immunoassay is a heterogeneous immunoassay in which an upconverting phosphor nanoparticle is used as a label. The nanoparticle (200- to 400-nm diameter) is a crystalline lanthanide oxysulfide. It absorbs two or more photons of infrared light (980 nm) and produces light emission at a shorter wavelength (anti-Stokes shift). The phosphorescence is not influences by reaction conditions such as temperature or buffer composition and there is no upconverted signal from biological components in the sample, resulting in a low background. Still another alternative assay format is quantum dot immunoassay. Quantum dot immunoassay is a heterogeneous immunoassay in which a nanometer-sized (less than 10 nm) semiconductor quantum dot is used as a label. A quantum dot is a highly fluorescent nanocrystal composed of CdSe, ZnSe, InP or InAs or a layer of ZnS or CdS on, for example, an CdSe core. Yet another alternative assay format is solid phase, light-scattering immunoassay, in which indium spheres are coated on glass to measure an antibody binding to an antigen (MCP-1 in this example). Binding of antibody to antigen increases dielectric layer thickness, which produces a greater degree of scatter than in areas where only an antigen is bound; quantitation is achieved by densitometry. Still another alternative assay format is surface effect immunoassay, in which an antibody is immobilized on the surface of a waveguide (a quartz, glass, or plastic slide, or a gold- or silver-coated prism), and binding of an antigen is measured directly by total internal reflection fluorescence, surface plasmon resonance, or attenuated total reflection. Other assay formats are known in the art.

[0066] In one alternative, a sandwich assay can be performed in a lateral flow assay device as generally known in the art. In such devices, the assay platform is typically constructed of a solid support that provides lateral flow of a

sample through the assay platform when a sample is applied to a sampling platform that is in operable contact with the assay platform. The sampling platform and the assay platform are typically constructed of a material such as nitrocellulose, glass fiber, paper, nylon, or a synthetic nanoporous polymer. Suitable materials are well known in the art and are described, for example, in U.S. Patent No. 7,256,053 to Hu, 7,214,417 to Lee et al., 7,238,538 to Freitag et al., 7,238,322 to Wang et al., 7,229,839 to Thayer et al., 7,226,793 to Jerome et al., RE39,664 to Gordon et al., 7,205,159 to Cole et al., 7,189,522 to Esfandiari, 7,186,566 to Qian, 7,166,208 to Zweig, 7,144,742 to Boehringer et al., 7,132,078 to Rawson et al., 7,097,983 to Markovsky et al., 7,090,803 to Gould et al., 7,045,342 to Nazareth et al., 7,030,210 to Cleaver et al., 6,981,522 to O'Connor et al., 6,924,153 to Boehringer et al., 6,849,414 to Guan et al., 6,844,200 to Brock, 6,841,159 to Simonson, 6,767,714 to Nazareth et al., 6,699,722 to Bauer et al., 6,656,744 to Pronovost et al., 6,528,323 to Thayer et al., 6,297,020 to Brock, 6,140,134 to Rittenburg, 6,136,610 to Polito et al., 5,965,458 to Kouvonen et al., 5,712,170 to Kouvanen et al., 4,956,302 to Gordon et al., and 4,943,522 to Eisinger et al., all of which are incorporated herein by this reference. The use of such devices for the performance of sandwich immunoassays is also well known in the art, and is described, for example, in U.S. Patent No. 7,141,436 to Gatto-Menking et al. and 6,017,767 to Chandler, both of which are incorporated herein by this reference.

[0067] As used herein, unless further defined, the term "antibody" encompasses both polyclonal and monoclonal antibodies, as well as genetically engineered antibodies such as chimeric or humanized antibodies of the appropriate binding specificity. As used herein, unless further defined, the term "antibody" also encompasses antibody fragments such as sFv, Fv, Fab, Fab' and $F(ab)'_2$ fragments. Because, in a sandwich immunoassay, it is required that the antigen (MCP-1 protein) bind to multiple antibody molecules (at least one unlabeled antibody molecule and at least one labeled antibody molecule), while there is no requirement for an antibody molecule to bind to more than one

antigen, suitable antibody fragments that can bind only one antigen molecule can be used in assays according to the present invention.

[0068] The results presented herein demonstrate that MCP-1 is a specific marker for the presence of BPH. In order to diagnose BPH, therefore, the level of MCP-1 can be compared against a number of measurements of prostatic gland size or function, as described below.

[0069] As discussed further below, MCP-1 can be measured in either prostatic fluid or urine.

[0070] In one alternative, the level of MCP-1 in prostatic fluid in intensity units as measured in densitometry in a conventional MCP-1 immunoassay per ml of prostatic fluid is determined. A ratio of greater than about 5,000 intensity units/ml prostatic fluid is diagnostic of the existence of BPH.

[0071] In another alternative, the level of MCP-1 in prostatic fluid in intensity units as measured in densitometry in a conventional MCP-1 immunoassay is compared to the total weight of the prostate gland, in grams, to generate a ratio of MCP-1 to prostate gland weight. A ratio of greater than about 6.0 intensity units/g prostate gland weight is diagnostic of the existence of BPH.

[0072] In another alternative, the level of MCP-1 in prostatic fluid in intensity units as measured in densitometry in a conventional MCP-1 immunoassay is compared to total protein in prostatic fluid. A ratio of 400 intensity units/μg total protein is diagnostic of the existence of BPH.

[0073] In still another alternative, the concentration of MCP-1 in urine after prostatic massage is measured; concentration of MCP-1 is expressed in intensity units as measured in densitometry in a conventional MCP-1 immunoassay. In one alternative, any detectable MCP-1 concentration is

diagnostic of BPH. In another alternative, a MCP-1 concentration of at least 10 pg/ml is diagnostic of BPH.

[0074] Because the quantity of MCP-1 protein expression can be correlated with the quantity of mRNA that encodes the MCP-1 protein and can be translated into MCP-1 protein, another aspect of the present invention is a method of determining the quantity of mRNA encoding the MCP-1 protein. As detailed below in the examples, the upregulation of MCP-1 expression is strongly characteristic of the diagnosis of BPH.

[0075] In general, this method comprises:

(1) hybridizing mRNA isolated from prostatic cells with a probe that corresponds to a sequence within mRNA encoding MCP-1 protein under conditions that substantially only mRNA encoding MCP-1 protein hybridizes to the probe;

(2) isolating the hybridized mRNA; and

(3) determining the quantity of mRNA encoding the MCP-1 protein to diagnose the existence of BPH.

[0076] In one alternative, the concentration of MCP-1 can be correlated with the existence and severity of LUTS. This enables one skilled in the art to predict the course of LUTS and to prescribe appropriate treatment for a patient who has LUTS or may experience LUTS.

[0077] Another aspect of the present invention is a method for treating or preventing BPH. In general, this method comprises the step of administering a therapeutically effective quantity of an agent that inhibits the expression or activity of MCP-1 to a patient who has or is at risk of developing BPH in order to inhibit the expression or activity of MCP-1 to a sufficient degree that BPH is treated or its occurrence prevented.

[0078] In one alternative, the agent that inhibits the expression or activity of MCP-1 is an agent that inhibits the expression of MCP-1. Inhibition of the expression of MCP-1 can be carried out at either the level of transcription of the gene for MCP-1 into mRNA or the translation of mRNA for MCP-1 into the corresponding protein.

[0079] In some embodiments, inhibitory nucleotides are used to suppress MCP-1 expression. These include short interfering RNA (siRNA), microRNA (miRNA), and synthetic hairpin RNA (shRNA), anti-sense nucleic acids, or complementary DNA (cDNA). In some preferred embodiments, a siRNA targeting MCP-1 expression is used. Interference with the function and expression of endogenous genes by double-stranded RNA such as siRNA has been shown in various organisms. See, e.g., A. Fire et al., "Potent and Specific Genetic Interference by Double-Stranded RNA in *Caenorhabditis elegans*," Nature 391:806-811 (1998); J.R. Kennerdell & R.W. Carthew, "Use of dsDNA-Mediated Genetic Interference to Demonstrate that *frizzled* and *frizzled 2* Act in the Wingless Pathway," Cell 95:1017-1026 (1998); F. Wianni & M. Zernicka-Goetz, "Specific Interference with Gene Function by Double-Stranded RNA in Early Mouse Development," Nat. Cell Biol. 2:70-75 (2000). siRNAs can include hairpin loops comprising self-complementary sequences or double stranded sequences. siRNAs typically have fewer than 100 base pairs and can be, e.g., about 30 bps or shorter, and can be made by approaches known in the art, including the use of complementary DNA strands or synthetic approaches. Such double-stranded RNA can be synthesized by in vitro transcription of single-stranded RNA read from both directions of a template and in vitro annealing of sense and antisense RNA strands. Double-stranded RNA targeting MCP-1 can also be synthesized from a cDNA vector construct in which a MCP-1 gene (e.g., human MCP-1 gene) is cloned in opposing orientations separated by an inverted repeat. Following cell transfection, the RNA is transcribed and the complementary strands reanneal. Double-stranded RNA targeting the MCP-1 gene can be introduced into a cell (e.g., a prostatic cell) by transfection of an

appropriate construct. Typically, RNA interference mediated by siRNA, miRNA, or shRNA is mediated at the level of translation; in other words, these interfering RNA molecules prevent translation of the corresponding mRNA molecules and lead to their degradation. It is also possible that RNA interference may also operate at the level of transcription, blocking transcription of the regions of the genome corresponding to these interfering RNA molecules. The structure and function of these interfering RNA molecules are well known in the art and are described, for example, in R.F. Gesteland et al., eds, "The RNA World" (3rd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2006), pp. 535-565, incorporated herein by this reference.

[0080] For these approaches, cloning into vectors and transfection methods are also well known in the art and are described, for example, in J. Sambrook & D.R. Russell, "Molecular Cloning: A Laboratory Manual" (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001), incorporated herein by this reference.

[0081] In addition to double stranded RNAs, other nucleic acid agents targeting MCP-1 can also be employed in the methods of the present invention, e.g., antisense nucleic acids. Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific target mRNA molecule. In the cell, the single stranded antisense molecule hybridizes to that mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the translation of mRNA into protein, and, thus, with the expression of a gene that is transcribed into that mRNA. Antisense methods have been used to inhibit the expression of many genes in vitro. See, e.g., C.J. Marcus-Sekura,"Techniques for Using Antisense Oligodeoxyribonucleotides to Study Gene Expression," Anal. Biochem. 172:289-295 (1988); J.E. Hambor et al., "Use of an Epstein-Barr Virus Episomal Replicon for Anti-Sense RNA-Mediated Gene Inhibition in a Human Cytotoxic T-Cell Clone," Proc. Natl. Acad. Sci. U.S.A.

85:4010-4014 (1988); H Arima et al., "Specific Inhibition of Interleukin-10 Production in Murine Macrophage-Like Cells by Phosphorothioate Antisense Oligonucleotides," Antisense Nucl. Acid Drug Dev. 8:319-327 (1998); and W.-F. Hou et al., "Effect of Antisense Oligodeoxynucleotides Directed to Individual Calmodulin Gene Transcripts on the Proliferation and Differentiation of PC12 Cells," Antisense Nucl. Acid Drug Dev. 8:295-308 (1998), all incorporated herein by this reference. Antisense technology is described further in C. Lichtenstein & W. Nellen, eds., "Antisense Technology: A Practical Approach" (IRL Press, Oxford, 1997), incorporated herein by this reference.

[0082] MCP-1 nucleotide sequences, such as those derived from mRNA, are known in the art, including the human MCP-1 gene sequence reported as NM_002982 in GenBank, and described further in G. Pacheco-Rodriguez et al., "Chemokine-Enhanced Chemotaxis of Lymphangioleiomyomatosis Cells with Mutations in the Tumor Suppressor TSC2 Gene," J. Immunol. 182: 1270-1277 (2009), incorporated herein by this reference. Based on the known sequences, inhibitory nucleotides (e.g., siRNA, miRNA, or shRNA) targeting PAR1 can be readily synthesized using methods well known in the art. Exemplary siRNAs according to the invention could have up to 29 bps, 25 bps, 22 bps, 21 bps, 20 bps, 15 bps, 10 bps, 5 bps or any integral number of base pairs between these numbers. Tools for designing optimal inhibitory siRNAs include that available from DNAengine Inc. (Seattle, WA) and Ambion, Inc. (Austin, TX).

[0083] As another alternative, the agent that inhibits the expression or activity of MCP-1 is an agent that inhibits the activity of MCP-1. For example, such an agent can be an antibody that specifically binds either MCP-1 or its receptor, CCR2. The preparation of such antibodies is well known in the art and need not be described further herein. In general, antibodies according to the present invention can be of any class, such as IgG, IgA, IgD, IgE, IgM, or IgY, although IgG antibodies are typically preferred. Antibodies can be of any mammalian or avian origin, including human, murine (mouse or rat), donkey,

sheep, goat, rabbit, camel, horse, or chicken. In some alternatives, the antibodies can be bispecific. The antibodies can be modified by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, or other modifications known in the art. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., "Antibodies: A Laboratory Manual", (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981), or by other standard methods known in the art. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. For example, suitable antibodies can be produced by phage display or other techniques. Additionally, and not by way of limitation, human antibodies can be made by a variety of techniques, including phage display methods using antibody libraries derived from human immunoglobulin sequences and by the use of transgenic mice that are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes can be introduced randomly or by homologous recombination into mouse embryonic stem cells. The antibodies can also be produced by expression of polynucleotides encoding these antibodies. Additionally, antibodies according to the present invention can be fused to marker sequences, such as a peptide tag to facilitate purification; a suitable tag is a hexahistidine tag. The antibodies can also be conjugated to a diagnostic or

therapeutic agent by methods known in the art. Techniques for preparing such conjugates are well known in the art.

[0084] Other methods of preparing these monoclonal antibodies, as well as chimeric antibodies, humanized antibodies, and single-chain antibodies, are known in the art. In general, for administration to human patients, human antibodies, chimeric antibodies, or humanized antibodies are preferred.

[0085] One particular monoclonal antibody that has been shown to inhibit MCP-1 stimulation of PrEC growth (K. Fujita et al., "Monocyte Chemotactic Protein-1 (MCP-1:CCL2) is Associated With Prostatic Growth Dysregulation," Prostate 70: 473-478 (2010), incorporated herein by this reference) is the mouse monoclonal anti-MCP-1 antibody designated MAB279, commercially available from R&D Systems, Minneapolis. MN. This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with *Escherichia coli*-derived, recombinant human CCL2 (rhCCL2). The IgG fraction from ascites fluid was purified by Protein A affinity chromatography.

[0086] Other suitable antibodies with appropriate binding specificity can be used to inhibit the activity of MCP-1. These antibodies include, but are not limited to:

(1) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(2) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; and

(3) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-

determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(4) chimeric antibodies derived from:

(a) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(b) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; or

(c) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279; and

(5) humanized antibodies derived from:

(a) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

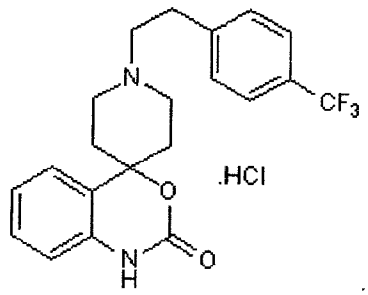
(b) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; or

(c) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes

at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279.

[0087] Methods for the production of chimeric and humanized antibodies are well known in the art.

[0088] In another alternative, the agent that inhibits the activity of MCP-1 is an antagonist of the MCP-1 receptor CCR2, also known as CD192. One suitable antagonist is RS102895, which is a CCR2-selective chemokine receptor antagonist whose systemic chemical name is 1′-[2-[4-(trifluoromethyl)phenyl]ethyl]-spiro[4H-3,1-benzoxazine-4,4′-piperidin]-2(1H)-one hydrochloride. RS102895 has a molecular weight of 426.86 g/mol; it is CAS: 300815-41-2. Its formula is



[0089] Another class of agents that inhibit the expression of MCP-1 is angiotensin receptor blockers (ARBs). ARBs include, but are not limited to, candesartan (cilexetil), irbesartan, losartan, olmesartan (medoxomil), telmisartan, valsartan, eprosartan, and the salts, solvates, analogues, congeners, bioisosteres, and prodrugs thereof. ARBs have been shown to inhibit the expression of MCP-1.

[0090] In another alternative, the agent that inhibits the activity of MCP-1 is an antibody that specifically binds CCR2. Typically, this antibody is a monoclonal antibody; it can alternatively be a chimeric or humanized antibody.

[0091] Other antagonists of CCR2 are also known in the art.

[0092] As used herein, terms such as "treatment," "treating," and similar terminology includes the administration of compounds or agents to a subject to prevent or delay the onset of the symptoms, complications, or biochemical indicia of a disease (e.g., BPH), alleviating the symptoms or arresting or inhibiting further development of the disease, condition, or disorder. Subjects in need of treatment include patients already suffering from the disease or disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. As used herein, the terms "treating," or similar terminology do not imply a cure for BPH or any other disease or condition; rather, this terminology is used to refer to any clinically detectable improvement in the disease or condition being treated or alleviated, including, but not limited to, reduction in LUTS symptoms, reduction of urinary frequency, reduction of pain, reduction of urethral obstruction, reduction of urinary retention, improvement in subjective well-being experienced by the patient, or any other clinically detectable improvement. As used herein, the term "therapeutically effective amount" refers to the amount of a therapy (e.g., a prophylactic or therapeutic agent) which is sufficient to treat BPH as described above.

[0093] The agents described above can be administered directly to subjects in need of treatment. However, therapeutic agents are preferably administered to the subjects in pharmaceutical compositions which comprise the agent that inhibits the expression or activity of MCP-1 and/or other active agents in a therapeutically effective dose along with a pharmaceutically acceptable carrier, diluent or excipient in unit dosage form. Pharmaceutically acceptable carriers are agents which are not biologically or otherwise undesirable, i.e., the

agents can be administered to a subject along with the agent that inhibits the expression or activity of MCP-1 without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the pharmaceutical composition in which it is contained. The compositions can additionally contain other therapeutic agents that are suitable for treating or preventing BPH as described above. Pharmaceutically acceptable carriers enhance or stabilize the composition, or can facilitate preparation of the composition. Pharmaceutically acceptable carriers include solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The pharmaceutically acceptable carrier should be suitable for various routes of administration described herein.

[0094] Therefore, pharmaceutical compositions according to the present invention for the treatment or prevention of BPH in a subject comprise:

(1) a therapeutically effective dose of an agent that inhibits the expression or activity of MCP-1; and

(2) a pharmaceutically acceptable carrier, diluent or excipient in unit dosage form.

[0095] Optionally, the pharmaceutical composition can further comprise an additional therapeutic agent for the treatment or prevention of BPH.

[0096] A pharmaceutical composition containing an agent that inhibits the expression or activity of MCP-1 and/or other therapeutic agents can be administered by a variety of methods known in the art. The routes and/or modes of administration vary depending upon the desired results. Depending on the route of administration, the active therapeutic agent may be coated in a material to protect the compound from the action of acids and other compounds that may inactivate the agent. Conventional pharmaceutical practice can be employed to provide suitable formulations or compositions for the administration of such

antagonists to subjects. Any appropriate route of administration can be employed, for example, but not limited to, intravenous, parenteral, intraperitoneal, intravenous, transcutaneous, subcutaneous, intramuscular, intraurethral, oral administration. Depending on the degree of BPH and other conditions affecting the urinary tract, as well as other conditions affecting of the subject to be treated, either systemic or localized delivery of the therapeutic agent can be used in the course of treatment.

[0097] In some embodiments, local administration of the agent that inhibits the expression or activity of MCP-1 is desired in order to achieve the intended therapeutic effect. Many methods of localized delivery of therapeutic agents can be used in the practice of the invention. For example, such agents can be administered directly to the lower urinary tract by suppository, by direct injection or infusion, or by other means known in the art.

[0098] Pharmaceutical compositions of the invention can be prepared in accordance with methods well known and routinely practiced in the art. See, e.g., Remington: *The Science and Practice of Pharmacy*, Mack Publishing Co., 20th ed., 2000; and *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Pharmaceutical compositions are preferably manufactured under GMP conditions. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymers, lactide/glycolide copolymers, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for molecules of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, e.g.,

polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or can be oily solutions for administration or gels.

[0099] The agent that inhibits the expression or activity of MCP-1 for use in the methods of the invention is typically administered to a subject in an amount that is sufficient to achieve the desired therapeutic effect (e.g., eliminating or ameliorating symptoms associated with BPH) in a subject in need thereof. Typically, a therapeutically effective dose or efficacious dose of the agent that inhibits the expression or activity of MCP-1 is employed in the pharmaceutical compositions of the invention. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular agent that inhibits the expression or activity of MCP-1, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the severity of the condition, other health considerations affecting the subject, and the status of liver and kidney function of the subject. It also depends on the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular agent that inhibits the expression or activity of MCP-1 employed, as well as the age, weight, condition, general health and prior medical history of the subject being treated, and like factors. Methods for determining optimal dosages are described in the art, e.g., Remington: *The Science and Practice of Pharmacy*, Mack Publishing Co., 20th ed., 2000. Typically, a pharmaceutically effective dosage would be between about 0.001 and 100 mg/kg body weight of the subject to be treated.

[0100] The agent that inhibits the expression or activity of MCP-1 and, if desired, other therapeutic regimens described above are usually administered to the subjects on multiple occasions. Intervals between single dosages can be

weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the agent that inhibits the expression or activity of MCP-1 and the other therapeutic agents, if any, used in the subject. In some methods, dosage is adjusted to achieve a plasma compound concentration of 1–1000 μg/ml and in some methods 25–300 μg/ml. Alternatively, the agent that inhibits the expression or activity of MCP-1 can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life in the subject of the agent that inhibits the expression or activity of MCP-1 and the other drugs included in a pharmaceutical composition. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some subjects may continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the subject can be administered a prophylactic regime.

[0101] For the purposes of the present application, treatment can be monitored by observing one or more of the improving symptoms associated with BPH or LUTS, such as decreased urinary frequency, decreased urinary retention, decreased pain, improved urinary flow, or any other symptom associated with improvement in BPH or LUTS.

[0102] Preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions contemplated by the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0103] Pharmaceutical formulations for parenteral administration include aqueous solutions of the agent that inhibits the expression or activity of MCP-1 in water-soluble form. Additionally, suspensions of the agent that inhibits the expression or activity of MCP-1 can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or modulators which increase the solubility of the agent that inhibits the expression or activity of MCP-1 to allow for the preparation of highly concentrated solutions. Pharmaceutical preparations for oral use can be obtained by combining the active modulators with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating modulators may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0104] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different doses of agent that inhibits the expression or activity of MCP-1.

[0105] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the agent that inhibits the expression or activity of MCP-1 may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

[0106] Other ingredients such as stabilizers, for example, antioxidants such as sodium citrate, ascorbyl palmitate, propyl gallate, reducing agents, ascorbic acid, vitamin E, sodium bisulfite, butylated hydroxytoluene, BHA, acetylcysteine, monothioglycerol, phenyl-α-naphthylamine, or lecithin can be used. Also, chelators such as EDTA can be used. Other ingredients that are conventional in the area of pharmaceutical compositions and formulations, such as lubricants in tablets or pills, coloring agents, or flavoring agents, can be used. Also, conventional pharmaceutical excipients or carriers can be used. The pharmaceutical excipients can include, but are not necessarily limited to, calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. Other pharmaceutical excipients are well known in the art. Exemplary pharmaceutically acceptable carriers include, but are not limited to, any and/or all of solvents, including aqueous and non-aqueous solvents, dispersion media, coatings, antibacterial and/or antifungal agents, isotonic and/or absorption delaying agents, and/or the like. The use of such media and/or agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional medium, carrier, or agent is incompatible with the active ingredient or ingredients, its use in a composition according to the present invention is contemplated. Supplementary active ingredients can also be

incorporated into the compositions, particularly as described above. For administration of any of the compounds used in the present invention, preparations should meet sterility, pyrogenicity, general safety, and purity standards as required by the FDA Office of Biologics Standards or by other regulatory organizations regulating drugs.

[0107] Sustained-release formulations or controlled-release formulations are well-known in the art. For example, the sustained-release or controlled-release formulation can be (1) an oral matrix sustained-release or controlled-release formulation; (2) an oral multilayered sustained-release or controlled-release tablet formulation; (3) an oral multiparticulate sustained-release or controlled-release formulation; (4) an oral osmotic sustained-release or controlled-release formulation; (5) an oral chewable sustained-release or controlled-release formulation; or (6) a dermal sustained-release or controlled-release patch formulation.

[0108] The pharmacokinetic principles of controlled drug delivery are described, for example, in B.M. Silber et al., "Pharmacokinetic/Pharmacodynamic Basis of Controlled Drug Delivery" in Controlled Drug Delivery: Fundamentals and Applications (J.R. Robinson & V.H.L. Lee, eds, 2d ed., Marcel Dekker, New York, 1987), ch. 5, pp. 213-251, incorporated herein by this reference.

[0109] One of ordinary skill in the art can readily prepare formulations for controlled release or sustained release comprising an agent that inhibits the expression or activity of MCP-1 by modifying the formulations described above, such as according to principles disclosed in V.H.K. Li et al, "Influence of Drug Properties and Routes of Drug Administration on the Design of Sustained and Controlled Release Systems" in Controlled Drug Delivery: Fundamentals and Applications (J.R. Robinson & V.H.L. Lee, eds, 2d ed., Marcel Dekker, New York, 1987), ch. 1, pp. 3-94, incorporated herein by this reference. This process of preparation typically takes into account physicochemical properties of the agent

that inhibits the expression or activity of MCP-1, such as aqueous solubility, partition coefficient, molecular size, stability of the agent that inhibits the expression or activity of MCP-1, and binding of the agent that inhibits the expression or activity of MCP-1 to proteins and other biological macromolecules. This process of preparation also takes into account biological factors, such as absorption, distribution, metabolism, duration of action, the possible existence of side effects, and margin of safety, for the agent that inhibits the expression or activity of MCP-1. Accordingly, one of ordinary skill in the art could modify the formulations in order to incorporate an agent that inhibits the expression or activity of MCP-1 into a formulation having the desirable properties described above for a particular application.

[0110] Another aspect of the present invention is a method of screening for a compound inhibiting the expression and/or activity of MCP-1 to identify additional agents suitable for treating BPH.

[0111] In general, such a screening method comprises:

(1) administering a compound potentially inhibiting the expression and/or activity of MCP-1 to a first sample of cells that demonstrate a detectable response to MCP-1;

(2) determining the response to MCP-1 in the first sample of cells; and

(3) comparing the response to MCP-1 in the first sample of cells with the response to MCP-1 in a second sample of cells to which the compound potentially inhibiting the expression and/or activity of MCP-1 to determine whether the compound inhibits the expression and/or activity of MCP-1.

[0112] The cells that demonstrate a detectable response to MCP-1 can be cells that endogenously express and secrete MCP-1, such as PrSC. Alternatively, the cells that demonstrate a detectable response to MCP-1 can be cells that express a receptor for MCP-1, i.e., the receptor CCR2; the cells need

not endogenously express and secrete MCP-1. The response to MCP-1 can be cell proliferation, which can be determined using the WST-1 assay as described below.

[0113] The compound potentially inhibiting the expression and/or activity of MCP-1 can be a protein, a peptide, a peptidomimetic, a nucleic acid, a steroid, an alkaloid, a prostaglandin or analogue thereof, a prostacyclin or analogue thereof, a receptor agonist, a receptor antagonist, a monosaccharide, a disaccharide, a carbohydrate larger than a disaccharide, or a small molecule that is other than a steroid, an alkaloid, a monosaccharide, a disaccharide, a prostaglandin or analogue thereof, or a prostacyclin or analogue thereof.

[0114] The invention is illustrated by the following examples. These examples are for illustrative purposes only, and are not intended to limit the invention.

EXAMPLES

Example 1

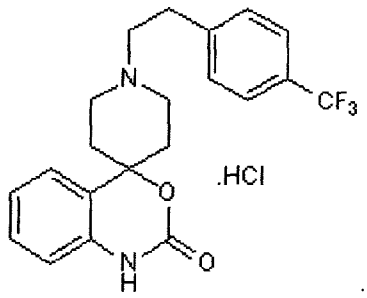
Monocyte Chemotactic Protein-1 (MCP-1; CCL2) Is Associated with Prostatic Growth Dysregulation

[0115] Introduction and Objective. Chronic inflammation is commonly observed in benign prostate hyperplasia (BPH), and prostate tissue often contains increased inflammatory infiltrates, including T cells and macrophages. Cytokines are not only key mediators of inflammation, but may also play important roles in the initiation and progression of BPH. In order to determine what cytokines might be involved in prostatic enlargement, prostatic fluids were analyzed by human cytokine antibody microarray.

[0116] Methods. Prostatic fluid was expressed from fresh radical prostatectomy cases and frozen. For this study, prostatic fluids from 21 specimens harboring only minimal volume prostate cancer were chosen, in hopes of minimizing the contribution of prostate cancer to the cytokine profile. These cases were stratified by prostate weight, and their prostatic fluid cytokines initially analyzed by human cytokine antibody microarray, thence by quantitative ELISA. The human cell lines PrEC (primary prostate basal epithelial cell) and PrSC (primary prostate stromal cell) were used for in vitro ELISA, proliferation, and western blot assays.

[0117] Results. Among the 174 cytokines on the array, Monocyte Chemotactic Protein-1 (MCP-1:CCL2) was one of the most upregulated in cases with large prostate volume. In order to study a possible origin of MCP-1, PrSC were studied in vitro by ELISA and found to secrete MCP-1, and western blotting showed that both PrSC and PrEC express the MCP-1 receptor, CCR2. Proliferation assays showed that MCP-1 stimulates the proliferation of PrEC (but not PrSC), and that a specific MCP-1 antagonist suppresses the proliferation of both PrSC and PrEC. Conditioned medium from PrSC stimulated the proliferation of PrEC as well, an effect that was completely inhibited by the MCP-1 antagonist (Figure 1), and also by a neutralizing monoclonal antibody against MCP-1 (ruling out direct toxic effect of the inhibitor). In Figure 1, proliferation of PrEC cells was stimulated by conditioned medium from PrSC, but this effect was inhibited by the MCP-1 antagonist RS-102895, as shown in the results of Figure 1.

[0118] RS-102895 is a CCR2-selective chemokine receptor antagonist whose systemic chemical name is 1′-[2-[4-(Trifluoromethyl)phenyl]ethyl]-spiro[4H-3,1-benzoxazine-4,4′-piperidin]-2(1H)-one hydrochloride. Its formula is



[0119] Conclusions. The cytokine MCP-1, of prostatic stromal cell origin, may play an important role in prostatic enlargement and BPH, and is both a growth factor for epithelial and stromal cells, and a known macrophage chemoattractant. MCP-1 merits further study as a potential BPH marker and therapeutic target.

Example 2

Prostate Monocyte Chemotactic Protein (MCP-1): A Novel Biomarker for Lower Urinary Tract Symptoms Associated with Benign Prostatic Hyperplasia

[0120] Introduction and Objective. Immunohistochemical and array-based studies indicate that monocyte chemotactic protein-1 (MCP-1), a potent inflammatory mediator, is significantly upregulated in benign prostatic hyperplasia (BPH) tissues. This purpose of this study was to evaluate associations of MCP-1 in prostatic fluid and urine with prostate volume and lower urinary tract symptoms (LUTS).

[0121] Methods. For prostatic fluid analyses, expressed prostate secretions were collected from fresh radical prostatectomy specimens from 46 men with very small volume Gleason 3+3=6 pT2 disease. For urinary analyses, samples were collected after prostate massage from 42 men without a diagnosis of prostate cancer. MCP-1 levels were measured by ELISA. For the ELISA measurements, MCP-1 was measured by a Human CCL2 (MCP-1) ELISA

Ready-SET-GO kit (eBioscience, San Diego, CA). MCP-1 was measured using the manufacturer's recommendations. Associations of MCP-1 expression in prostatic fluid with prostate weight and International Prostate Symptom Score (I-PSS) were examined utilizing multivariable linear and logistic regression modeling adjusted for age, pre-operative serum PSA and (in the models with I-PSS as the outcome) prostate weight. Prostate enlargement was defined as prostate weight >40 g. Linear regression was used to evaluate associations of MCP-1 with I-PSS and prostate volume.

[0122] Results. In prostatic fluid analyses, there were no significant correlations of prostate weight with MCP-1 ($p = 0.19$) or I-PSS ($p = 0.26$). In multivariable regression, prostate weight was associated with increased age ($p = 0.04$) and increased PSA ($p < 0.001$) but not MCP-1 ($p = 0.71$); prostate enlargement was associated with increased PSA ($p = 0.01$) but not age ($p = 0.23$) or MCP-1 ($p = 0.38$). Increased I-PSS was associated with increased PSA ($p = 0.03$) and increased age ($p = 0.07$) but not prostate weight ($p = 0.47$). In multivariable regression, increased I-PSS was significantly associated with higher MCP-1 ($p = 0.05$); moreover, there was a trend toward severe LUTS (I-PSS > 19) with higher MCP-1 ($p = 0.06$). In urine analyses, higher urinary MCP-1 was significantly associated with higher IPSS ($p=0.04$) but not prostate volume ($p=0.44$).

[0123] Conclusions In this cohort, increased expression of MCP-1 in prostatic fluid and urine was significantly associated with increased LUTS. These data suggest that MCP-1 may be a novel biomarker for LUTS associated with BPH independent of prostate volume, age, and PSA. In addition, these data indicate that prostate-generated inflammatory mediators may substantially contribute to LUTS pathogenesis in men with BPH.

Example 3

Analysis of Cytokines Associated with Existence of BPH

[0124] To determine which, if any cytokines can be clearly associated with the existence of BPH, the concentrations of a number of cytokines in prostatic fluid were measured and correlated with the extent of BPH as measured by the I-PSS scoring scale, the weight of the prostate gland, and other variables affecting prostatic health as described below.

[0125] Table 1 shows the upregulation of cytokines in large prostates, with the ratio of the cytokine in large (>60 g) versus small (40 g) prostates indicated for each cytokine.

Table 1

Upregulation of Cytokines in Large Prostates

Cytokine	Large (>60 g)/Small (40 g)
IL-1β	5.07
IL-7	4.63
Activin A	4.47
MCP-1	3.66
IL-6	3.09
FGF-4	2.78
FGF-7	2.75
IGFBP-4	2.74
IGF-I	2.66
Endoglin	2.37
AgRP	1.98
BLC	1.95

[0126] Figure 2 is a scatter plot of 147 cytokines with the average OD of each cytokine in an immunoassay specific for that cytokine from the large prostate group plotted on the y axis and the average OD of each cytokine from the small prostate group plotted on the x axis. The position of MCP-1 on the scatter plot is indicated.

[0127] Figure 3 is a plot of the detailed data for a number of cytokines, with the OD of the cytokine in an immunoassay specific for that cytokine on the y axis and the weight of the prostate gland being measured on the x axis. (A) MCP-1; (B) Activin A; (C) FGF-7; (D) FGF-4; (E) Endoglin; (F) IL-6; (G) IL-7; and (H) IL-1β.

[0128] Accordingly, in view of these results, a new cohort of prostatic fluids was collected from patients in the category Gleason 6 with minimum disease and no hormonal treatment. These were analyzed for MCP-1, Activin A, and IL-1β. MCP-1 in prostatic juices, cell culture supernatants, and urine were measured by a Human CCL2 (MCP-1) ELISA Ready-SET-GO kit (eBioscience, San Diego, CA). Forty five prostatic juices were different cohort from that used in the cytokine array experiment. MCP-1 was measured based on the manufacturer's recommendations. Prostatic juices and cell culture supernatants from PrSC were assayed at a 16- and 4-fold dilution, respectively. The results for MCP-1 and IL-1β were plotted in Figure 4. In Figure 4, Panel (A) plots the results for concentration of MCP-1 as represented by the ratio of OD in an MCP-1-specific immunoassay to total protein on the y axis and the weight of the prostate gland being measured on the x axis. Panel (B) plots the results for concentration of MCP-1 as represented by the ratio of OD in an MCP-1-specific immunoassay to total protein on the y axis in two groups, separated on the x axis: a first group with prostate gland weights < 50 g and a second group with prostate gland weights >80 g. Panel (C) plots the concentration of MCP-1 as represented by the OD in a MCP-1 specific immunoassay on the x axis versus

the I-PSS score on the y axis. Panel (D) plots the results for concentration of IL-1β as represented by the ratio of OD in an IL-1β-specific immunoassay to total protein on the y axis versus gland weight on the x axis. Panel (E) plots the concentration of IL-1β as represented by the OD in an IL-1β-specific immunoassay on the x axis versus the I-PSS score on the y axis.

[0129] The analysis of the results from Figure 4 led to the following conclusions: (1) MCP-1 concentration is not associated with prostatic size. This was apparent in multivariable regression, adjusting for PSA and age. (2) I-PSS score was not associated with prostate size (previously known from other work). (3) Increased MCP-1 concentration is associated with increased I-PSS score, independent of age, PSA, and prostate size.

[0130] The effect of MCP-1 on the proliferation of the human cell lines PrEC (primary prostate basal epithelial cell) and PrSC (primary prostate stromal cell) was determined. The results are shown in Figure 5. In Panel (A), the effects of MCP-1 on the proliferation of PrEC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = MCP-1 1 ng/ml; ▼ = MCP-1 10 ng/ml; ♦ = MCP-1 100 ng/ml). In Panel (B), the effects of MCP-1 on the proliferation of PrSC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = MCP-1 1 ng/ml; ▼ = MCP-1 10 ng/ml; ♦ = MCP-1 100 ng/ml). In Panel (C), the effects of RS102895, the specific antagonist of CCR2 (also known as CD192, the receptor for MCP-1) on the proliferation of PrEC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = RS102895 1.25 μM; ▼ = RS102895 2.5 μM; ♦ = RS102895 5.0 μM; ● = RS102895 10 μM). In Panel (D), the effects of RS102895, the specific antagonist of CCR2 (also known as CD192, the receptor for MCP-1) on the proliferation of PrSC cells as measured by the WST-1 assay are shown (■ = control in DMSO; ▲ = RS102895 1.25 μM; ▼ = RS102895 2.5 μM; ♦ = RS102895 5.0 μM; ● = RS102895 10 μM).

[0131] Both PrEC and PrSC express CCR2, the receptor for MCP-1. This is shown in the results presented in Figure 6, which shows Western blots for CCR2 in PrEC and PrSC. Both cells express a 44-kD protein that binds to antibody for CCR2.

[0132] PrSC cells secrete MCP-1. The results of immunoassays for the detection of MCP-1 in PrEC and PrSC cells are shown in Figure 7 (left panel). In the left panel of Figure 7, MCP-1 concentration (determined by immunoassay) is shown in pg/ml. In the right panel of Figure 7, MCP-1 concentration (determined by immunoassay) is shown in pg/ml for PrEC, the LNCap cell line (derived from androgen-sensitive human prostate adenocarcinoma cells), the C42B cell line (also derived from prostate adenocarcinoma cells), the PC3 cell line (also derived from prostate adenocarcinoma cells), and the hFOB cell line (a human fetal osteoblast progenitor cell line).

[0133] The specific antagonist of CCR2 WS102895 inhibits the effects of MCP-1 on PrEC cells. The results are shown in Figure 8. In Figure 8, proliferation of PrEC cells is represented as OD_{450}-OD_{610} in the proliferation assay (the WST-1 assay described above). Starting from the leftmost bar, the bars represent: (1) no MCP-1 and no WS 102895; (2) 10 nM MCP-1 and no WS102895; (3) 10 nM MCP-1 and 2.5 μM WS102985; (4) 10 nM MCP-1 and 5.0 μM WS102985; and (5) 10 nM MCP-1 and 10 μM WS102985. These results clearly show that WS102895 in inhibits the effects of MCP-1 on PrEC cells, blocking their proliferation.

[0134] The specific antagonist of CCR2 WS102895 also blocks the growth effect of conditioned media from PrSC to PrEC. These results are shown in Figure 9. PrEBM media was conditioned on PrSC for two days. In Figure 9, proliferation of PrEC cells is represented as OD_{450}-OD_{610} in the proliferation assay (the WST-1 assay described above). Starting from the leftmost bar, the

bars represent: (1) no conditioned media and no WS 102895; (2) conditioned media and no WS102895; (3) conditioned media and 2.5 μM WS102985; (4) conditioned media and 5.0 μM WS102985; and (5) conditioned media and 10 μM WS102985. This again shows that WS102895 in inhibits the effects of MCP-1 on PrEC cells, blocking their proliferation. The MCP-1 was secreted by PrSC cells and was present in the conditioned media.

[0135] Utilizing a specific monoclonal antibody binding MCP-1 blocks the effect of PrSC-conditioned media in stimulating proliferation of PrEC. The results are shown in Figure 10. In the top panel of Figure 10, conditioned media was used as in Figure 9 and proliferation of PrEC cells is represented as OD_{450}-OD_{610} in the proliferation assay (the WST-1 assay described above). Starting from the leftmost bar, the bars represent: (1) no conditioned media and no anti-MCP-1 mAb; (2) conditioned media and no anti-MCP-1 mAb; (3) conditioned media and anti-MCP-1 mAb at 1.0 μg/ml; and (4) conditioned media and anti-MCP-1 mAb at 5.0 μg/ml. However, the anti-MCP-1 mAb has a very minimal effect on the proliferation of PrEC cells, as shown in the bottom panel of Figure 10. In the bottom panel of Figure 10, the bars represent, starting from the left: (1) no anti-MCP-1 antibody; (2) anti-MCP-1 antibody at 0.25 μg/ml; (3) anti-MCP-1 antibody at 1.0 μg/ml; and (4) anti-MCP-1 antibody at 5.0 μg/ml.

[0136] MCP-1 and IL-1β levels correlate with CD68, a macrophage-specific marker, in prostatic juice. The results are shown in Figure 11. In Figure 11, in Panel (A), the ratio of CD68/β-actin is plotted on the y axis and MCP-1 concentration in pg/ml is plotted on the x axis. In Panel (B), the ratio of CD68/β-actin is plotted on the y axis and IL-1β concentration in pg/ml is plotted on the x axis. In Panel (C), the concentration of IL-1β in pg/ml is plotted on the x axis and the concentration of MCP-1 in pg/ml is plotted on the y axis. In Panel (D), the results are divided into two groups, those with low CD68 concentration and those with high CD68 concentration, and the I-PSS score for each sample within each

group is plotted separately, showing a correlation between high CD68 concentration and higher I-PSS score. A higher I-PSS score means a greater severity of LUTS symptoms.

[0137] Conditioned medium from monocytes/macrophages stimulates the proliferation of PrEC. The results are shown in Figure 12. In Figure 12, the left panel shows results with PrEC and the right panel shows results with PrSC. In each panel, starting from the leftmost bar, the bars represent: (1) no conditioned medium from monocytes/macrophages and no MCP-1; (2) no conditioned medium from monocytes/macrophages and MCP-1; (3) conditioned medium from monocytes/macrophages and no MCP-1; and (4) conditioned medium from monocytes/macrophages and MCP-1. The results show a substantially greater effect with PrEC than with PrSC.

[0138] IL-1β and interferons are known to synergistically induce the synthesis of MCP-1 (and MCP-2) in fibroblasts and epithelial cells. Also, it is known that IL-1β is secreted from monocytes/macrophages.

[0139] Inflammatory cytokines also enhance the secretion of MCP-1 from PrEC and PrSC. The results are shown in Figure 13. The results in the left panel of Figure 13 are for PrEC; in the right panel, for PrSC. In each panel, starting from the leftmost bar, the bars represent: (1) control; (2) IL-1β; (3) IFN-γ; and (4) IL-2. For each graph, MCP-1 in pg/ml is shown on the y axis. In particular, IFN-γ has a substantial effect in stimulating MCP-1 secretion in PrEC cells, and all of IL-1β, IFN-γ and IL-2 have a substantial effect on the secretion of MCP-1 in PrSC cells. It is known that increased expression of IL-2, IL-4, IL-8, IL-15, and IFN-γ occurs in BPH.

[0140] Figure 14 is a schematic showing the role of MCP-1 in BPH, showing the interactions with stromal cells, basal cells, luminal cells,

macrophages, and T cells, including interactions mediated by IL-1β and IFN-γ. MCP-1 is known to be chemotactic for cells playing a substantial role in inflammation.

[0141] Androgens may also have an effect on MCP-1 secretion from PrSC. The results are shown in Figure 15 with the synthetic androgen methyltrienolone (R1881). In the left panel of Figure 15, results for ELISA for MCP-1 in the supernatant of PrSC are shown; MCP-1 in pg/ml is shown on the y axis, and starting with the leftmost bar, R1881 was used as follows: (1) no R1881; (2) 0.1 nM R1881; (3) 1.0 nM R1881; and (4) 1.0 nM R1881. In the right panel of Figure 15, results for real-time PCR are shown on the y axis, representing mRNA specific for MCP-1; again, starting with the leftmost bar, R1881 was used as follows: (1) no R1881; (2) 0.1 nM R1881; (3) 1.0 nM R1881; and (4) 1.0 nM R1881. The results shown in Figure 15 are for incubation for 4 days; the ELISA may have been skewed by the numbers of PrSC cells, because R1881 stimulates the growth of PrSC cells. With 2 days of incubation, there was no difference in mRNA of MCP-1 and in MCP-1 secretion from PrSC.

[0142] MCP-1 levels were measured in urine of patients after massage. A total of 28 patients were studied. All patients were confirmed as negative for prostatic carcinoma by biopsy. MCP-1 levels in urine were measured by ELISA as described above. I-PSS scores were also evaluated for these patients. Protein levels in the urine were measured by the Bradford method. The volumes of the prostate glands in these patients were also estimated from transurethral ultrasound (TRUS) or digital rectal examination (DRE).

[0143] The results are shown in Figure 16. In Panel (A) of Figure 16, the data is grouped into two parts: on the left, I-PSS scores are plotted for those patients for which MCP-1 was undetectable in urine after massage, and, on the right, I-PSS scores are plotted for those patients for which MCP-1 was detectable in urine after massage. In Panel (B) of Figure 16, the data is again grouped into

two parts: on the left, estimated prostate gland size is plotted for those patients for which MCP-1 was undetectable in urine after massage, and, on the right, estimated prostate gland size is plotted for those patients for which MCP-1 was detectable in urine after massage. In Panel (C) of Figure 16, the data is again grouped into two parts: on the left, the age of the patient is plotted for those patients for which MCP-1 was undetectable in urine after massage, and, on the right, the age of the patient is plotted for those patients for which MCP-1 was detectable in urine after massage.

[0144] Figure 17 presents additional analysis of data from these patients. In Panel (A) of Figure 17, MCP-1 on the x axis is plotted versus I-PSS score on the y axis. In Panel (B) of Figure 17, MCP-1 on the x axis is plotted versus gland weight (in g) on the y axis. In Panel (C) of Figure 17, MCP-1/total protein on the x axis is plotted versus I-PSS score on the y axis. In Panel (D) of Figure 17, MCP-1/total protein on the x axis is plotted versus gland weight (in g) on the y axis.

[0145] Figure 18 presents an analysis of the MCP-1 concentration in serum for these patients. In the graph shown in Figure 18, MCP-1 concentration in pg/ml is shown for serum (x2 diluted). The data is grouped into three groups: the leftmost group is patients considered "normal"; the center group is patients for which there has been treatment failure; the rightmost group is patients with an AUA score of >20.

Example 4

mRNA Analysis of Inflammatory Cytokines in Normal Prostate Epithelial Cells, Benign Prostate Hyperplasia Cells, and Human BPH Tissue Samples

[0146] Introduction: The histologic finding of benign prostatic hyperplasia associated with chronic inflammation occurs in up to 80% of men,

suggesting a role for inflammation in BPH. The expression levels of 84 inflammatory cytokines in a BPH cell line and in BPH patient samples, as compared to those in a normal prostate epithelial cell line, were investigated.

[0147] Methods: Total RNA was isolated from the BPH-1 cell line, the PrEC cell line, and the paraffin-embedded tissue specimens from 6 BPH patients obtained from transurethral resections of the prostate. First strand cDNA synthesis was performed with real-time PCR using the SuperArray inflammatory cytokine gene array. The array contained 84 disease-focused genes and several controls. Data was analyzed according to the $\Delta\Delta$Ct method (Ct=threshold cycle). Expression in PrEC cells was compared with expression in BPH-1 cells and human BPH samples.

[0148] Results: The BPH-1 cells demonstrated 380-fold increased expression of monocyte chemotactic protein (MCP-1), followed by IL-8-RB (95-fold) and IL-10-RA (100-fold), when compared to PrEC cells. Sufficient RNA was obtained from 4 of the human samples, which also revealed an average of 9000-fold increased expression of MCP-1 in 2 cases, when compared to PrEC cells.

[0149] Conclusions: The overexpression of MCP-1 in BPH cells and clinical samples suggests a link between inflammatory cytokine overexpression and BPH cell proliferation. Subsequent investigations will examine the cause of overexpression and the effect of anti-inflammatory agents on cellular proliferation.

Example 5

Comparison of Gene Expression for MCP-1 in BPH and Prostatic Carcinoma

[0150] Expression of the gene for MCP-1 was measured in 25 paired normal-tumor RRP (radical retropublic prostatectomy) tissues and 9 BPH

samples. The results are shown in Figure 19. Expression ratios of test sample/BPH reference are color coded, with red indicating higher expression in test sample relative to BPH and green indicating lower expression in the test sample relative to BPH. These results indicate that expression of MCP-1 is characteristic of BPH and not of prostatic carcinoma.

ADVANTAGES OF THE INVENTION

[0151] Methods of diagnosis of BPH according to the present invention, based on the concentration of MCP-1 or mRNA encoding MCP-1, offer improved diagnosis of this condition while clearly distinguishing it from prostatic carcinoma and prostatitis. The methods of diagnosis according to the present invention therefore enable individuals suspected of having BPH to get appropriate treatment more rapidly without the necessity of their being subjected to unneeded, inappropriate, and invasive therapies, particularly those intended to treat prostatic carcinoma.

[0152] Additionally, treatment methods according to the present invention directed to the activity of MCP-1 are suitable for the treatment of diagnosed BPH and are specific for that condition, without serious side effects.

[0153] Diagnostic methods according to the present invention possess industrial applicability, such as for screening compounds for their effect on the progression of BPH, and can be performed *in vitro*.

[0154] Treatment methods according to the present invention also possess industrial applicability for the preparation of a medicament for the treatment of BPH.

[0155] With respect to ranges of values, the invention encompasses each intervening value between the upper and lower limits of the range to at least a tenth of the lower limit's unit, unless the context clearly indicates otherwise. Moreover, the invention encompasses any other stated intervening values and ranges including either or both of the upper and lower limits of the range, unless specifically excluded from the stated range.

[0156] Unless defined otherwise, the meanings of all technical and scientific terms used herein are those commonly understood by one of ordinary skill in the art to which this invention belongs. One of ordinary skill in the art will also appreciate that any methods and materials similar or equivalent to those described herein can also be used to practice or test this invention.

[0157] The publications and patents discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0158] All the publications cited are incorporated herein by reference in their entireties, including all published patents, patent applications, literature references, as well as those publications that have been incorporated in those published documents. However, to the extent that any publication incorporated herein by reference refers to information to be published, applicants do not admit that any such information published after the filing date of this application to be prior art. Similarly, all GenBank sequences and other sequence information obtainable from publicly accessible databases are herein incorporated by reference as if each was specifically and individually indicated to be incorporated by reference.

[0159] As used in this specification and in the appended claims, the singular forms include the plural forms. For example the terms "a," "an," and "the" include plural references unless the content clearly dictates otherwise. Additionally, the term "at least" preceding a series of elements is to be understood as referring to every element in the series. The inventions illustratively described herein can suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising," "including," "containing," etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the future shown and described or any portion thereof, and it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions herein disclosed can be resorted by those skilled in the art, and that such modifications and variations are considered to be within the scope of the inventions disclosed herein. The inventions have been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the scope of the generic disclosure also form part of these inventions. This includes the generic description of each invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised materials specifically resided therein. In addition, where features or aspects of an invention are described in terms of the Markush group, those schooled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. It is also to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of in the art upon reviewing the above description. The scope of the invention should therefore, be determined not with reference to the above description, but should instead be

determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described. Such equivalents are intended to be encompassed by the following claims.

I claim:

1. A method of diagnosing BPH comprising the steps of:

(a) obtaining a sample of prostatic fluid or urine from a male patient suspected of having BPH;

(b) performing an assay for MCP-1 on the sample to measure the concentration of MCP-1 in the sample; and

(c) comparing the concentration of MCP-1 in the sample with a reference value or comparing a ratio of the concentration of MCP-1 with a parameter selected from the group consisting of total protein and weight of the prostate gland, such that if the concentration of MCP-1 or the ratio of the concentration of MCP-1 to the parameter is at or above a defined reference value, the male patient has BPH.

2. The method of claim 1 wherein the assay for MCP-1 is an immunoassay.

3. The method of claim 2 wherein the immunoassay is a sandwich immunoassay.

4. The method of claim 3 wherein the sandwich immunoassay is an ELISA assay.

5. The method of claim 4 wherein the enzyme label of the ELISA assay is selected from the group consisting of alkaline phosphatase, horseradish peroxidase, glucose 6-phosphate dehydrogenase, and β-galactosidase.

6. The method of claim 4 wherein the enzyme label of the ELISA assay is detected photometrically.

7. The method of claim 4 wherein the enzyme label of the ELISA assay is detected by detection of fluorescence.

8. The method of claim 4 wherein the enzyme label of the ELISA assay is detected by detection of chemiluminescence.

9. The method of claim 4 wherein the enzyme label of the ELISA assay is detected by detection of bioluminescence.

10. The method of claim 2 wherein the immunoassay is performed by the enzyme multiplied immunoassay technique (EMIT).

11. The method of claim 2 wherein the immunoassay is a cloned enzyme donor immunoassay (CEDIA).

12. The method of claim 2 wherein the immunoassay is performed using a labeled antibody selected from the group consisting of a radiolabeled antibody, a fluorescent-labeled antibody, a chemiluminescent-labeled antibody, an electrochemiluminescent-labeled antibody, a bioluminescent-labeled antibody, an antibody labeled with a colloidal metal, an antibody labeled with a Surface-Enhanced Resonant Raman Spectroscopy (SERRS), and an antibody labeled with a signaling aptamer.

13. The method of claim 2 wherein the immunoassay is an immuno-PCR assay.

14. The method of claim 2 wherein the immunoassay is phosphor immunoassay.

15. The method of claim 2 wherein the immunoassay is quantum dot immunoassay.

16. The method of claim 2 wherein the immunoassay is solid phase, light-scattering immunoassay.

17. The method of claim 2 wherein the immunoassay is surface effect immunoassay.

18. The method of claim 3 wherein the sandwich immunoassay is performed in a lateral flow device.

19. The method of claim 1 wherein the sample is prostatic fluid.

20. The method of claim 19 wherein the reference value for the concentration of MCP-1 in prostatic fluid is about 5000 intensity units as measured in densitometry in a conventional MCP-1 immunoassay.

21. The method of claim 19 wherein the method comprises determining a ratio of the level of MCP-1 in prostatic fluid to the total weight of the prostate gland, such that a ratio of greater than about 6.0 intensity units/g prostate gland weight is diagnostic of BPH.

22. The method of claim 19 wherein the method comprises determining a ratio of the level of MCP-1 in prostatic fluid to the total protein in the prostatic fluid, such that a ratio of greater than about 400 intensity units/μg total protein is diagnostic of the existence of BPH.

23. The method of claim 1 wherein the sample is urine.

24. The method of claim 23 wherein the urine sample is collected after prostatic massage.

25. The method of claim 24 wherein a detectable MCP-1 concentration in urine is diagnostic of BPH.

26. The method of claim 24 wherein a concentration of MCP-1 in urine of at least 10 pg/ml is diagnostic of BPH.

27. A method for diagnosing BPH comprising the steps of:

(a) hybridizing mRNA isolated from prostatic cells with a probe that corresponds to a sequence within mRNA encoding MCP-1 protein under conditions that substantially only mRNA encoding MCP-1 protein hybridizes to the probe;

(b) isolating the hybridized mRNA; and

(c) determining the quantity of mRNA encoding the MCP-1 protein to diagnose the existence of BPH.

28. A method for treating or preventing BPH comprising the step of administering a therapeutically effective quantity of an agent that inhibits the expression or activity of MCP-1 to a patient who has or is at risk of developing BPH in order to inhibit the expression or activity of MCP-1 to a sufficient degree that BPH is treated or its occurrence prevented.

29. The method of claim 28 wherein the agent that inhibits the expression or activity of MCP-1 inhibits the expression of MCP-1.

30. The method of claim 29 wherein the agent is an inhibitory nucleotide selected from the group consisting of short interfering RNA (siRNA), microRNA (miRNA), and synthetic hairpin RNA (shRNA), anti-sense nucleic acids, and complementary DNA (cDNA).

31. The method of claim 29 wherein the agent is an angiotensin receptor blocker.

32. The method of claim 31 wherein the angiotensin receptor blocker is selected from the group consisting of candesartan (cilexetil), irbesartan, losartan, olmesartan (medoxomil), telmisartan, valsartan, eprosartan, and the salts, solvates, analogues, congeners, bioisosteres, and prodrugs thereof.

33. The method of claim 28 wherein the agent that inhibits the expression or activity of MCP-1 inhibits the activity of MCP-1.

34. The method of claim 33 wherein the agent that inhibits the activity of MCP-1 is an antibody that specifically binds MCP-1.

35. The method of claim 34 wherein the antibody that binds MCP-1 is a monoclonal antibody.

36. The method of claim 35 wherein the monoclonal antibody is MAB279.

37. The method of claim 35 wherein the monoclonal antibody is selected from the group consisting of:

(1) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(2) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; and

(3) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same

epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(4) chimeric antibodies derived from:

(a) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(b) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; or

(c) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279; and

(5) humanized antibodies derived from:

(a) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(b) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; or

(c) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as

the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279.

38. The method of claim 33 wherein the agent that inhibits the activity of MCP-1 is an antibody that specifically binds CCR2.

39. The method of claim 38 wherein the antibody is a monoclonal antibody.

40. The method of claim 39 wherein the antibody is an antibody selected from the group consisting of a chimeric antibody and a humanized antibody.

41. The method of claim 33 wherein the agent that inhibits the activity of MCP-1 is an antagonist of CCR2.

42. The method of claim 41 wherein the antagonist of CCR2 is 1′-[2-[4-(trifluoromethyl)phenyl]ethyl]-spiro[4H-3,1-benzoxazine-4,4′-piperidin]-2(1H)-one hydrochloride.

43. A pharmaceutical composition for the treatment or prevention of BPH comprising:

(a) a therapeutically effective dose of an agent that inhibits the expression or activity of MCP-1; and

(b) a pharmaceutically acceptable carrier, diluent or excipient in unit dosage form.

44. The pharmaceutical composition of claim 43 wherein the agent that inhibits the expression or activity of MCP-1 inhibits the expression of MCP-1.

45. The pharmaceutical composition of claim 44 wherein the agent is an inhibitory nucleotide selected from the group consisting of short interfering RNA (siRNA), microRNA (miRNA), and synthetic hairpin RNA (shRNA), anti-sense nucleic acids, and complementary DNA (cDNA).

46. The pharmaceutical composition of claim 44 wherein the agent is an angiotensin receptor blocker.

47. The pharmaceutical composition of claim 46 wherein the angiotensin receptor blocker is selected from the group consisting of candesartan (cilexetil), irbesartan, losartan, olmesartan (medoxomil), telmisartan, valsartan, eprosartan, and the salts, solvates, analogues, congeners, bioisosteres, and prodrugs thereof.

48. The pharmaceutical composition of claim 43 wherein the agent that inhibits the expression or activity of MCP-1 inhibits the activity of MCP-1.

49. The pharmaceutical composition of claim 48 wherein the agent that inhibits the activity of MCP-1 is an antibody that specifically binds MCP-1.

50. The pharmaceutical composition of claim 49 wherein the antibody is a monoclonal antibody.

51. The pharmaceutical composition of claim 50 wherein the monoclonal antibody is MAB279.

52. The pharmaceutical composition of claim 50 wherein the monoclonal antibody is selected from the group consisting of:

(1) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(2) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; and

(3) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(4) chimeric antibodies derived from:

(a) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(b) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; or

(c) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279; and

(5) humanized antibodies derived from:

(a) a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279;

(b) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of MAB279 and which competes at least about 80% as effectively on a molar basis with MAB279 as MAB279 itself for the binding of MCP-1; or

(c) a monoclonal antibody having complementarity-determining regions of its heavy and light chains that are identical with the complementarity-determining regions of a monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279 and that competes at least about 80% as effectively on a molar basis for the binding of MCP-1 as the monoclonal antibody specifically binding the same epitope of MCP-1 as is bound by MAB279.

53. The pharmaceutical composition of claim 48 wherein the agent that inhibits the activity of MCP-1 is an antibody that specifically binds CCR2.

54. The pharmaceutical composition of claim 48 wherein the agent that inhibits the activity of MCP-1 is an antagonist of CCR2.

55. The pharmaceutical composition of claim 54 wherein the antagonist of CCR2 is 1′-[2-[4-(trifluoromethyl)phenyl]ethyl]-spiro[4H-3,1-benzoxazine-4,4′-piperidin]-2(1H)-one hydrochloride.

56. A method of screening for a compound inhibiting the expression and/or activity of MCP-1 comprising the steps of:

(a) administering a compound potentially inhibiting the expression and/or activity of MCP-1 to a first sample of cells that demonstrate a detectable response to MCP-1;

(b) determining the response to MCP-1 in the first sample of cells; and

(c) comparing the response to MCP-1 in the first sample of cells with the response to MCP-1 in a second sample of cells to which the compound potentially inhibiting the expression and/or activity of MCP-1 to determine whether the compound inhibits the expression and/or activity of MCP-1.

57. The method of claim 56 wherein the cells that demonstrate a detectable response to MCP-1 are cells that endogenously express and secrete MCP-1.

58. The method of claim 56 wherein the cells that demonstrate a detectable response to MCP-1 are cells that express a receptor for MCP-1.

59. The method of claim 56 wherein the response to MCP-1 is cell proliferation.

60. The method of claim 56 wherein the compound potentially inhibiting the expression and/or activity of MCP-1 is selected from the group consisting of a protein, a peptide, a peptidomimetic, a nucleic acid, a steroid, an alkaloid, a prostaglandin or analogue thereof, a prostacyclin or analogue thereof, a receptor agonist, a receptor antagonist, a monosaccharide, a disaccharide, a carbohydrate larger than a disaccharide, or a small molecule that is other than a steroid, an alkaloid, a monosaccharide, a disaccharide, a prostaglandin or analogue thereof, and a prostacyclin or analogue thereof.

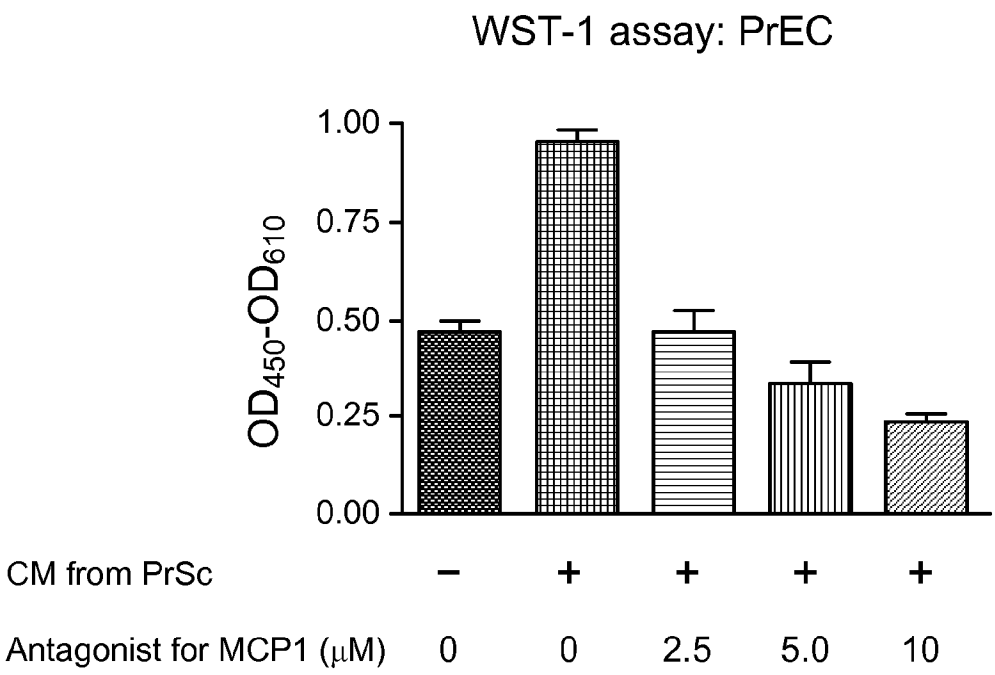

WST-1 assay: PrEC
1.00
0.75
0.50
0.25
0.00
OD450-OD610
CM from PrSc
Antagonist for MCP1 (μM)
−
+
+
+
+
0
0
2.5
5.0
10

FIGURE 1

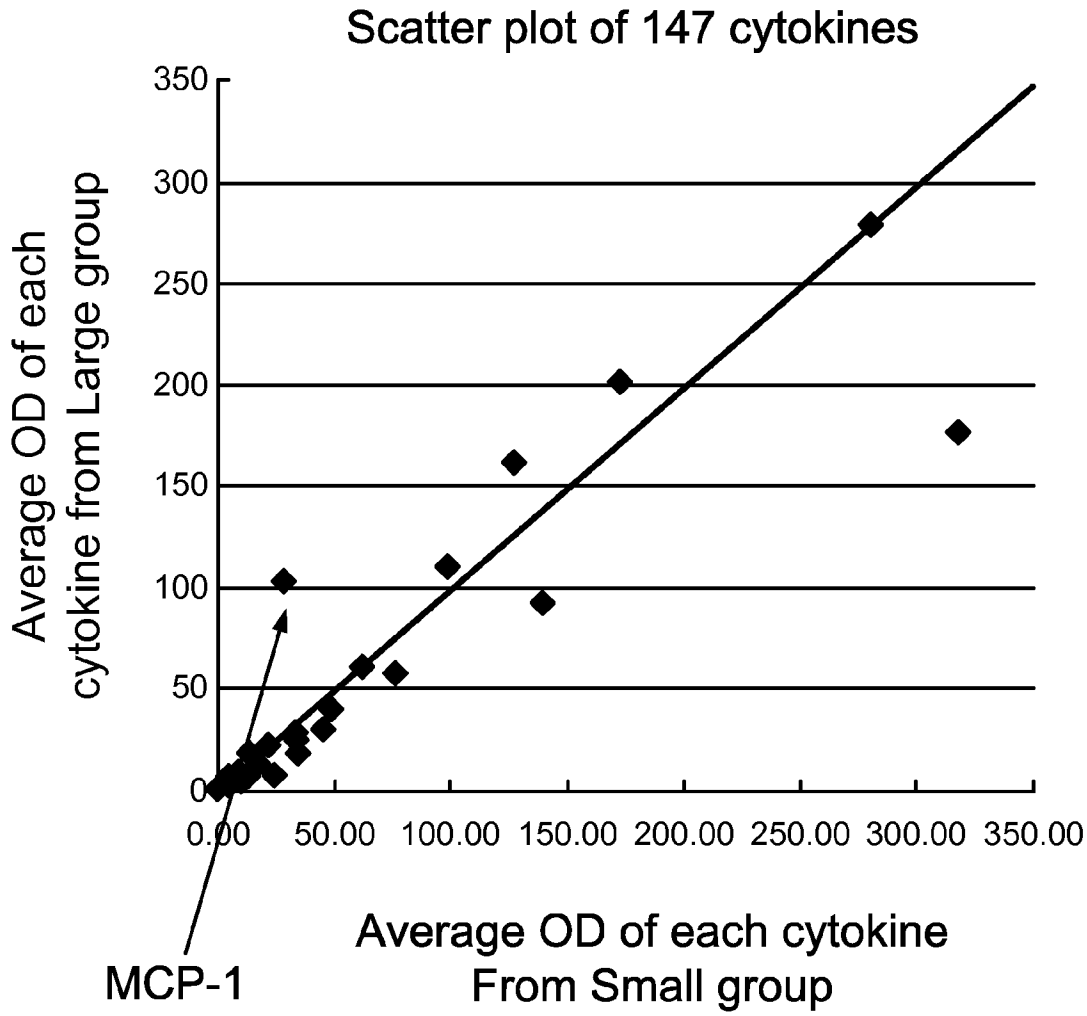
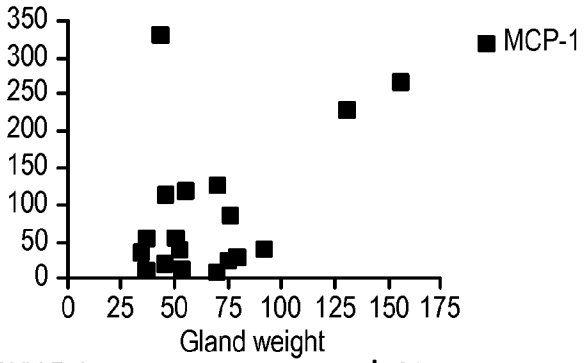
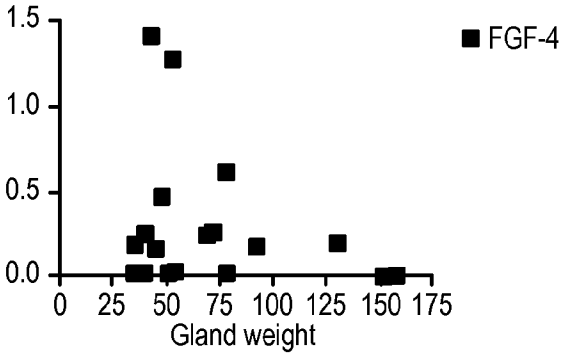

Scatter plot of 147 cytokines
Average OD of each cytokine from Large group
350
300
250
200
150
100
50
0
0.00
50.00
100.00
150.00
200.00
250.00
300.00
350.00
Average OD of each cytokine From Small group
MCP-1

FIGURE 2

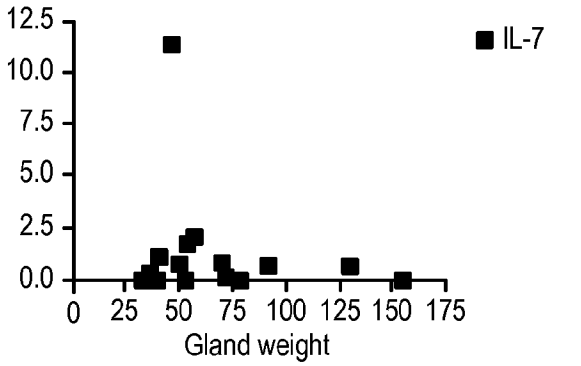
3/21 Cytokine antibody array



Number of XY Pairs	21
Pearson r	0.5103
95% confidence interval	0.1008 to 0.7720
P value (two-tailed)	0.0181
P value summary	*
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.2604



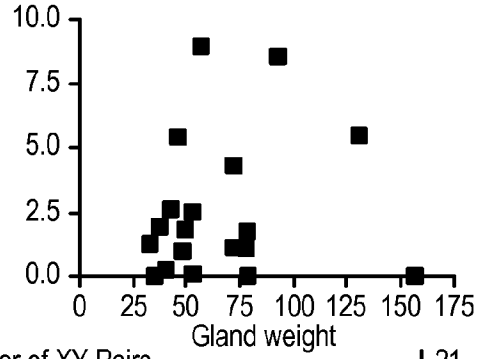
Number of XY Pairs	21
Pearson r	-0.1257
95% confidence interval	-0.5288 to 0.3237
P value (two-tailed)	0.5873
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.01579



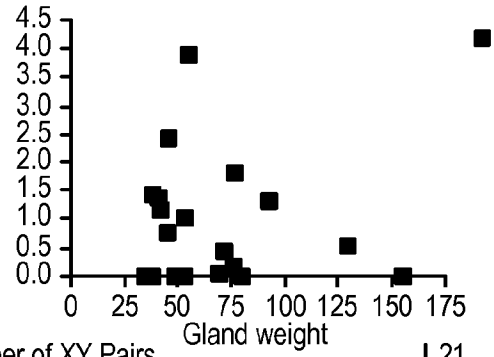
Number of XY Pairs	21
Pearson r	-0.1131
95% confidence interval	-0.5195 to 0.3350
P value (two-tailed)	0.6254
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.01280

FIGURE 3A

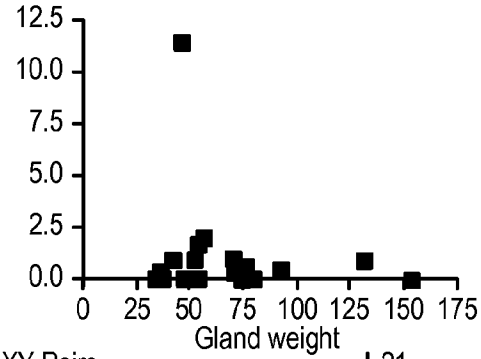
Cytokine antibody array



Number of XY Pairs	21
Pearson r	0.1957
95% confidence interval	-0.2579 to 0.5786
P value (two-tailed)	0.3952
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.03831



Number of XY Pairs	21
Pearson r	-0.06340
95% confidence interval	-0.4820 to 0.3787
P value (two-tailed)	0.7848
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.004020



Number of XY Pairs	21
Pearson r	-0.1131
95% confidence interval	-0.5195 to 0.3350
P value (two-tailed)	0.6254
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.01280

FIGURE 3B

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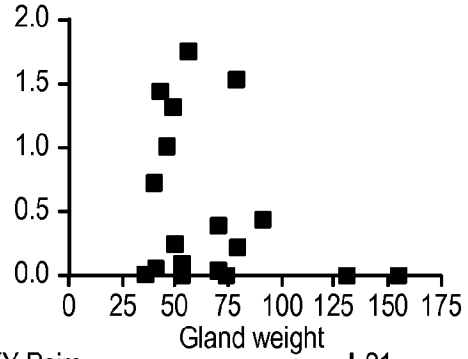
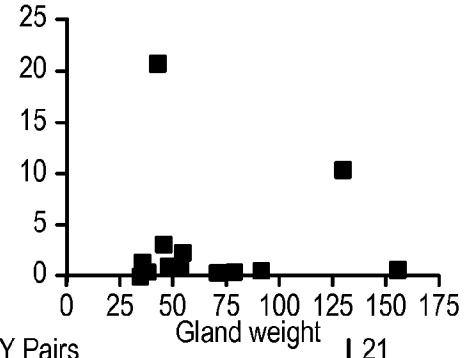
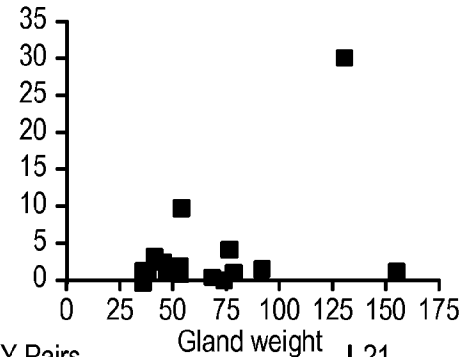
Cytokine antibody array



Number of XY Pairs	21
Pearson r	-0.1610
95% confidence interval	-0.5543 to 0.2910
P value (two-tailed)	0.4856
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.02593



Number of XY Pairs	21
Pearson r	0.05319
95% confidence interval	-0.3875 to 0.4741
P value (two-tailed)	0.8189
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.002830



Number of XY Pairs	21
Pearson r	0.4451
95% confidence interval	0.01647 to 0.7355
P value (two-tailed)	0.0432
P value summary	*
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.1981

FIGURE 3C

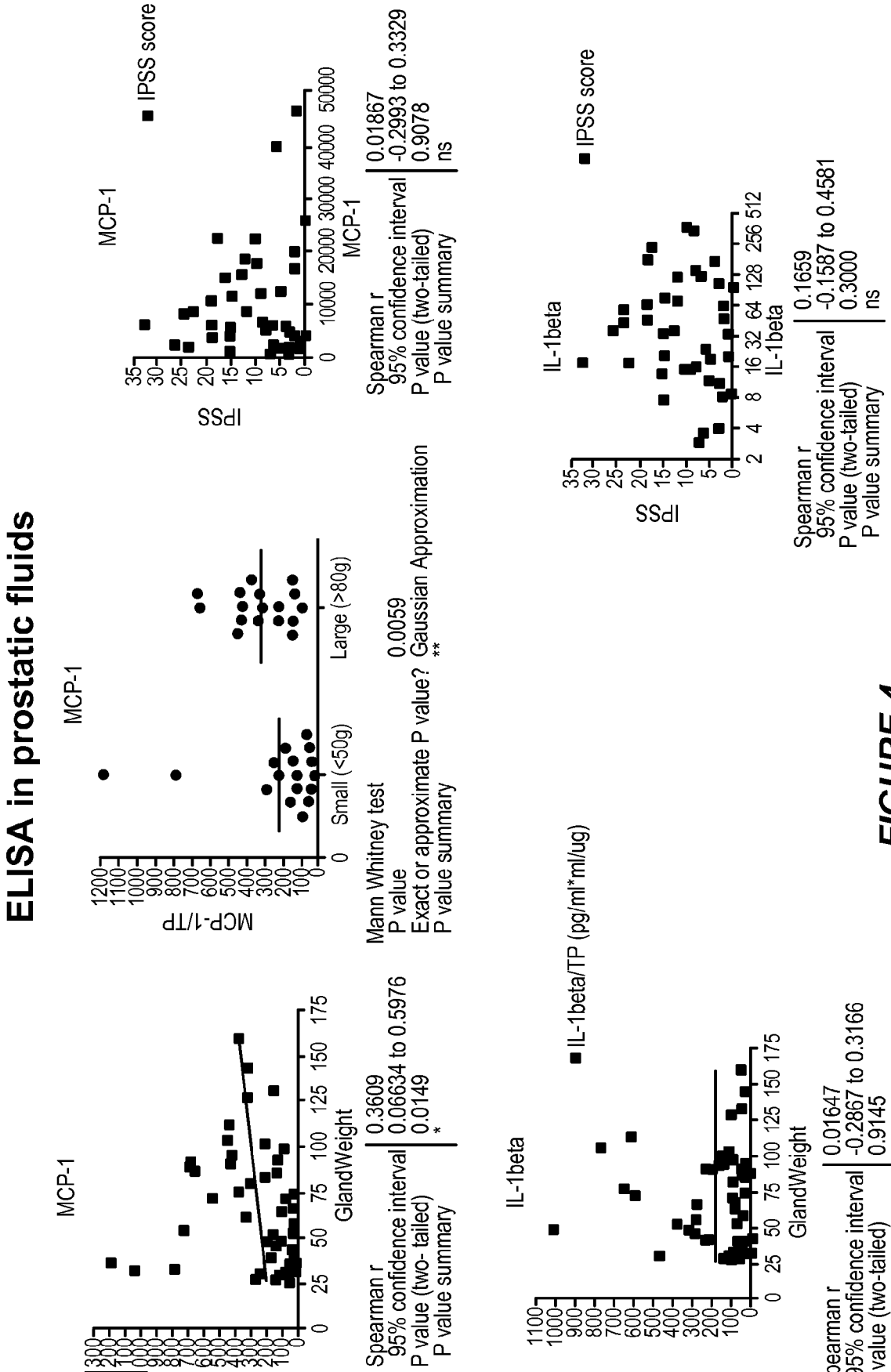

ELISA in prostatic fluids
MCP-1
MCP-1/TP
Small (<50g)
Large (>80g)
Mann Whitney test
P value 0.0059
Exact or approximate P value? Gaussian Approximation
P value summary **
MCP-1
GlandWeight
Spearman r 0.3609
95% confidence interval 0.06634 to 0.5976
P value (two- tailed) 0.0149
P value summary *
MCP-1
IPSS
IPSS score
Spearman r 0.01867
95% confidence interval -0.2993 to 0.3329
P value (two-tailed) 0.9078
P value summary ns
IL-1beta
IL-1beta/TP (pg/ml*ml/ug)
GlandWeight
Spearman r 0.01647
95% confidence interval -0.2867 to 0.3166
P value (two-tailed) 0.9145
P value summary ns
IL-1beta
IPSS
IPSS score
IL-1beta
Spearman r 0.1659
95% confidence interval -0.1587 to 0.4581
P value (two-tailed) 0.3000
P value summary ns

FIGURE 4

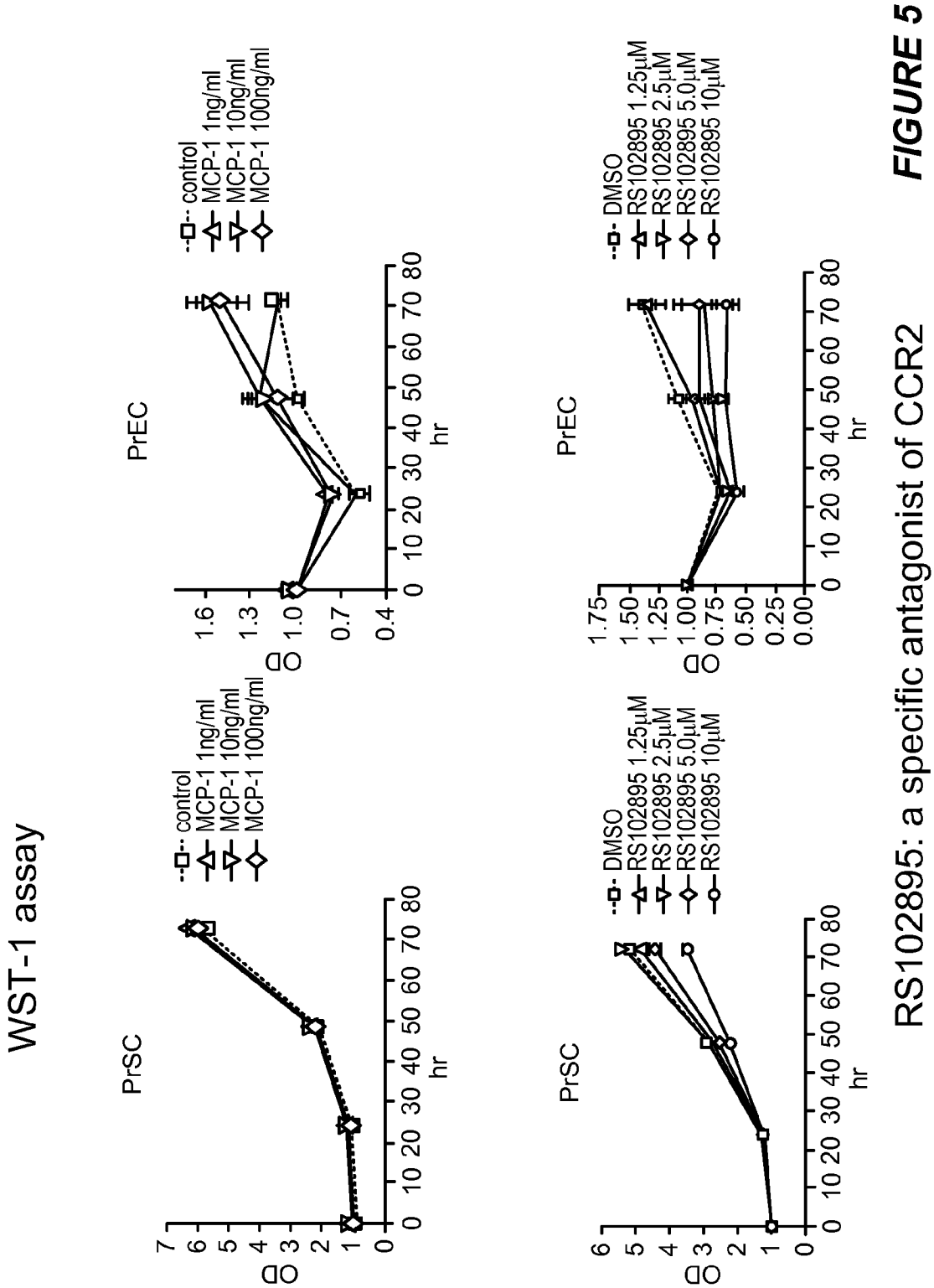

WST-1 assay
PrSC
PrEC
OD
hr
control
MCP-1 1ng/ml
MCP-1 10ng/ml
MCP-1 100ng/ml
DMSO
RS102895 1.25μM
RS102895 2.5μM
RS102895 5.0μM
RS102895 10μM
RS102895: a specific antagonist of CCR2

FIGURE 5

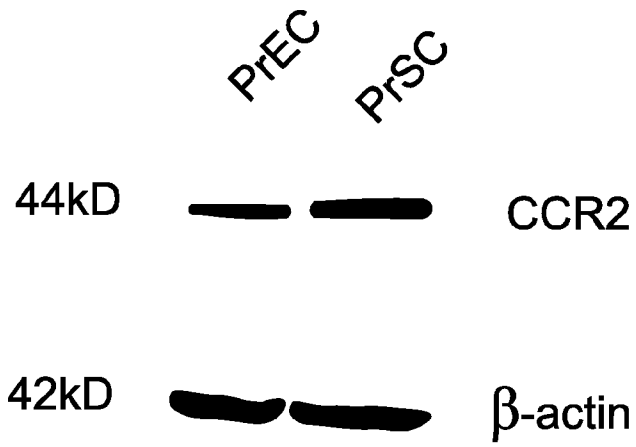


FIGURE 6

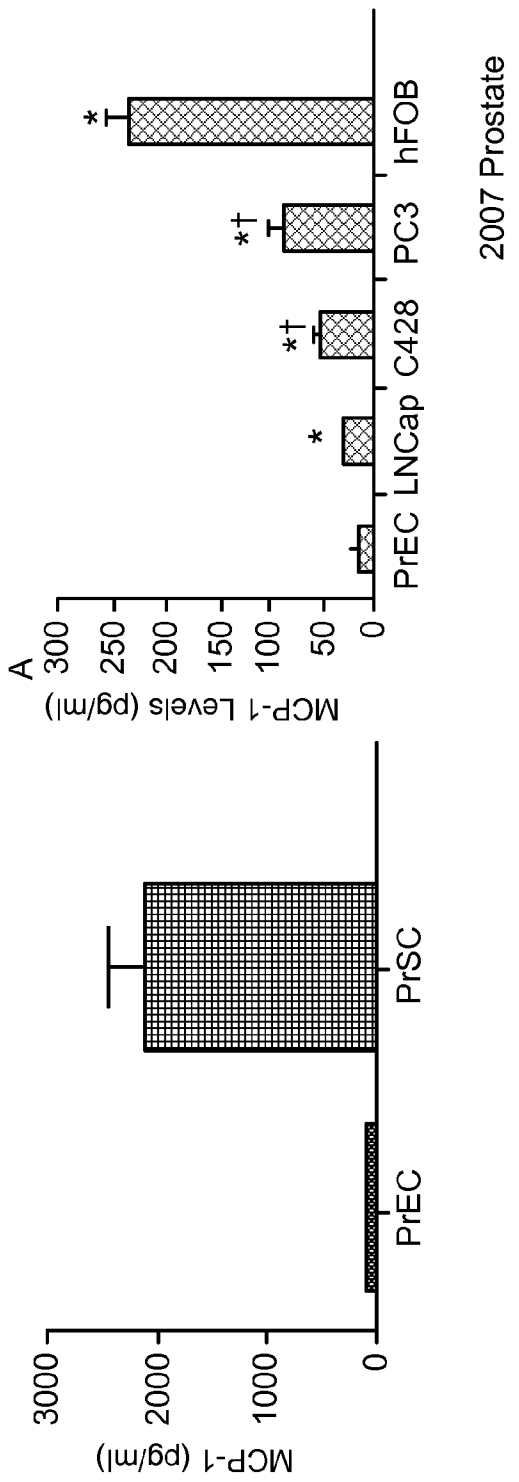

A
MCP-1 Levels (pg/ml)
300
250
200
150
100
50
0
PrEC
LNCap
C428
PC3
hFOB
2007 Prostate
*
*†
*†
*
MCP-1 (pg/ml)
3000
2000
1000
0
PrEC
PrSC

FIGURE 7

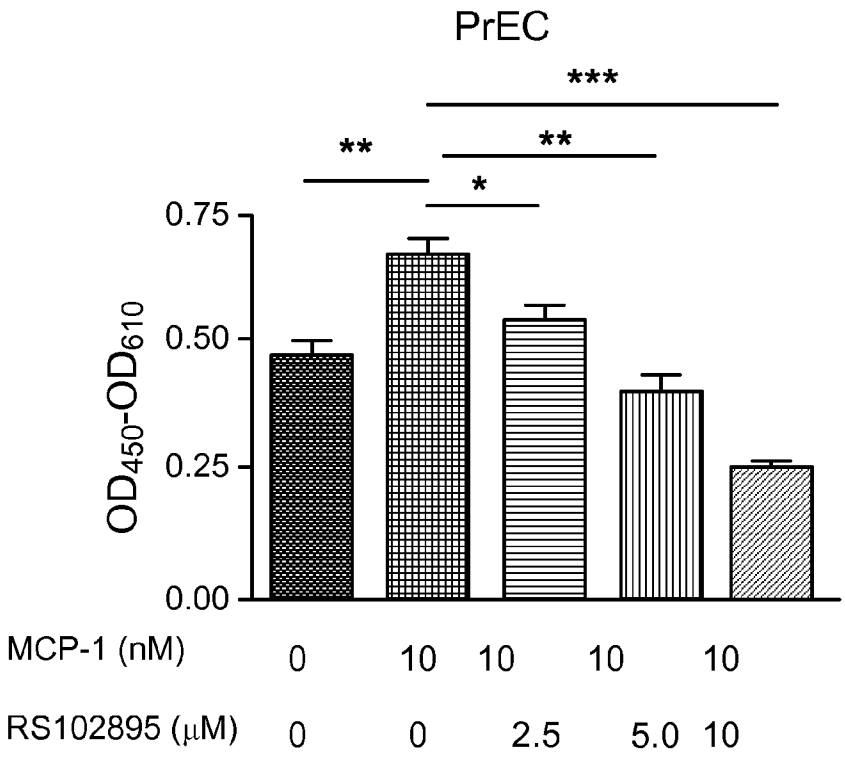
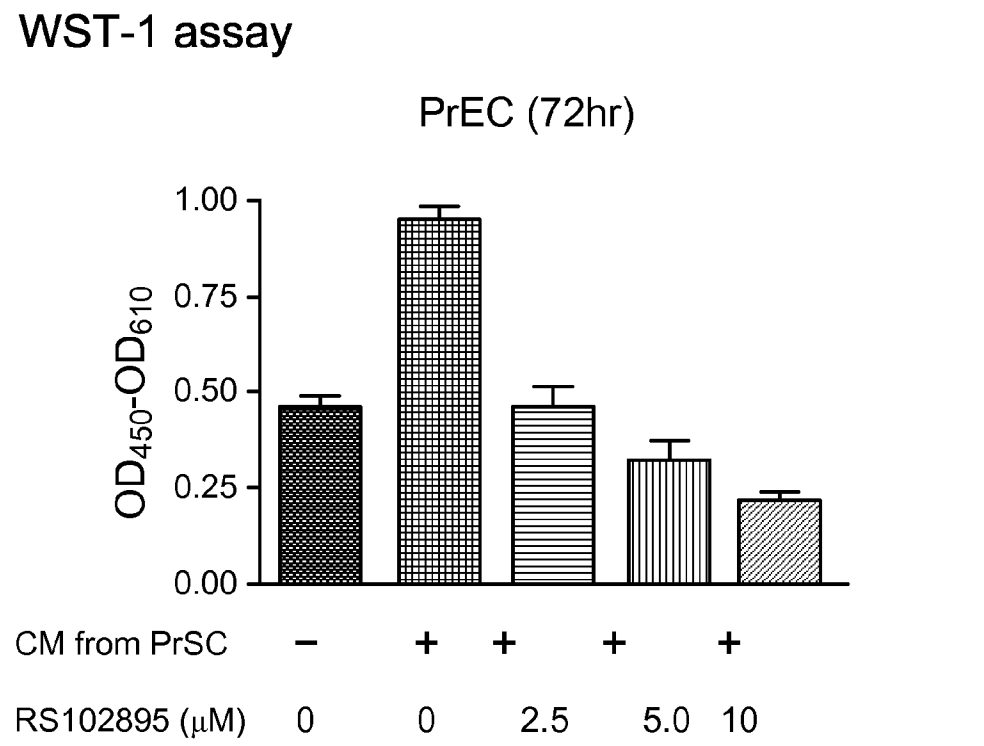

PrEC
***
**
**
*
0.75
0.50
0.25
0.00
OD450-OD610
MCP-1 (nM)
0
10
10
10
10
RS102895 (μM)
0
0
2.5
5.0
10

FIGURE 8



CM from PrSC: PrEBM media conditioned on PrSC for 2 days.

FIGURE 9

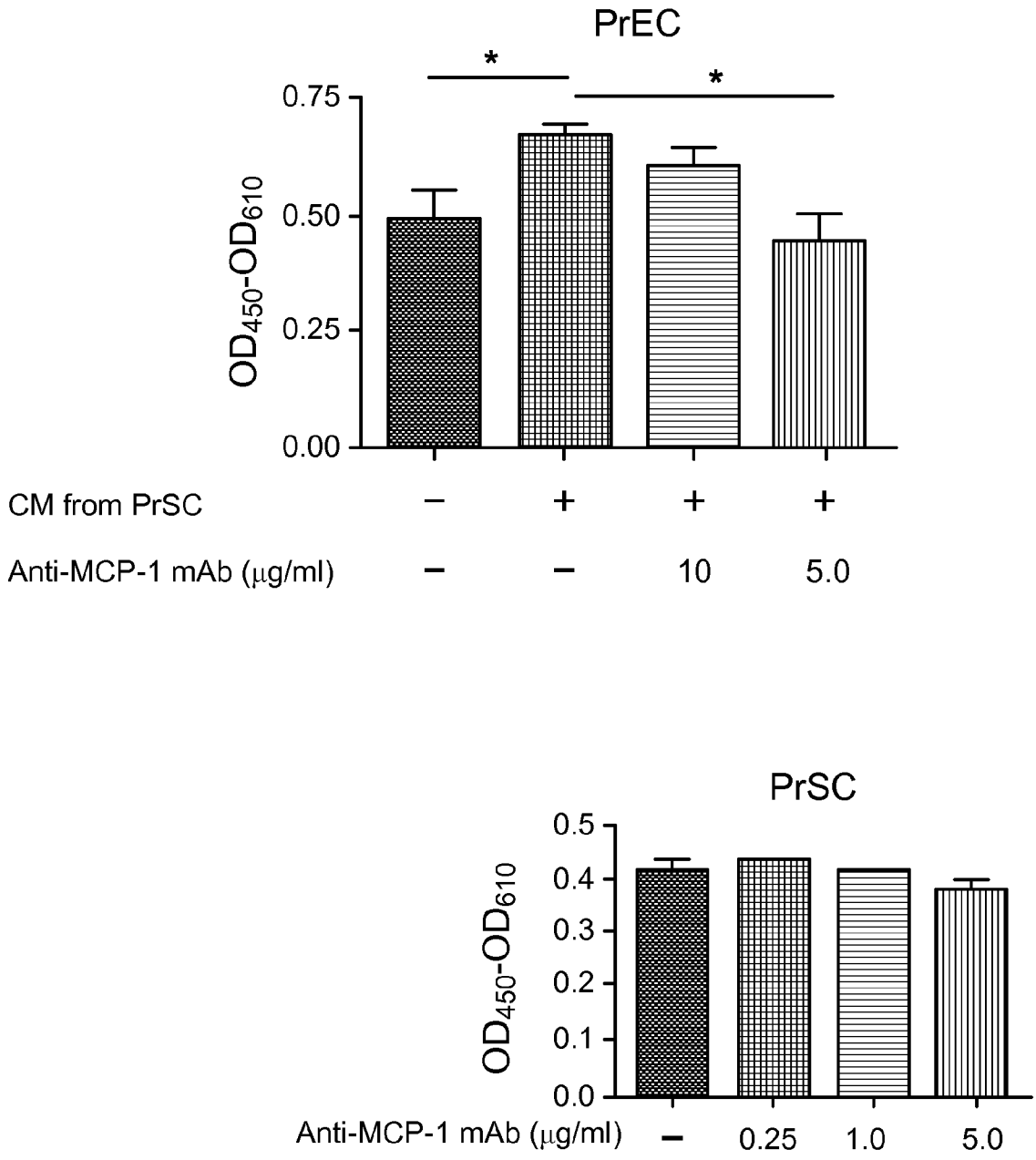

PrEC
*
*
0.75
0.50
0.25
0.00
OD450-OD610
CM from PrSC
– + + +
Anti-MCP-1 mAb (μg/ml)
– – 10 5.0
PrSC
0.5
0.4
0.3
0.2
0.1
0.0
OD450-OD610
Anti-MCP-1 mAb (μg/ml)
– 0.25 1.0 5.0

FIGURE 10

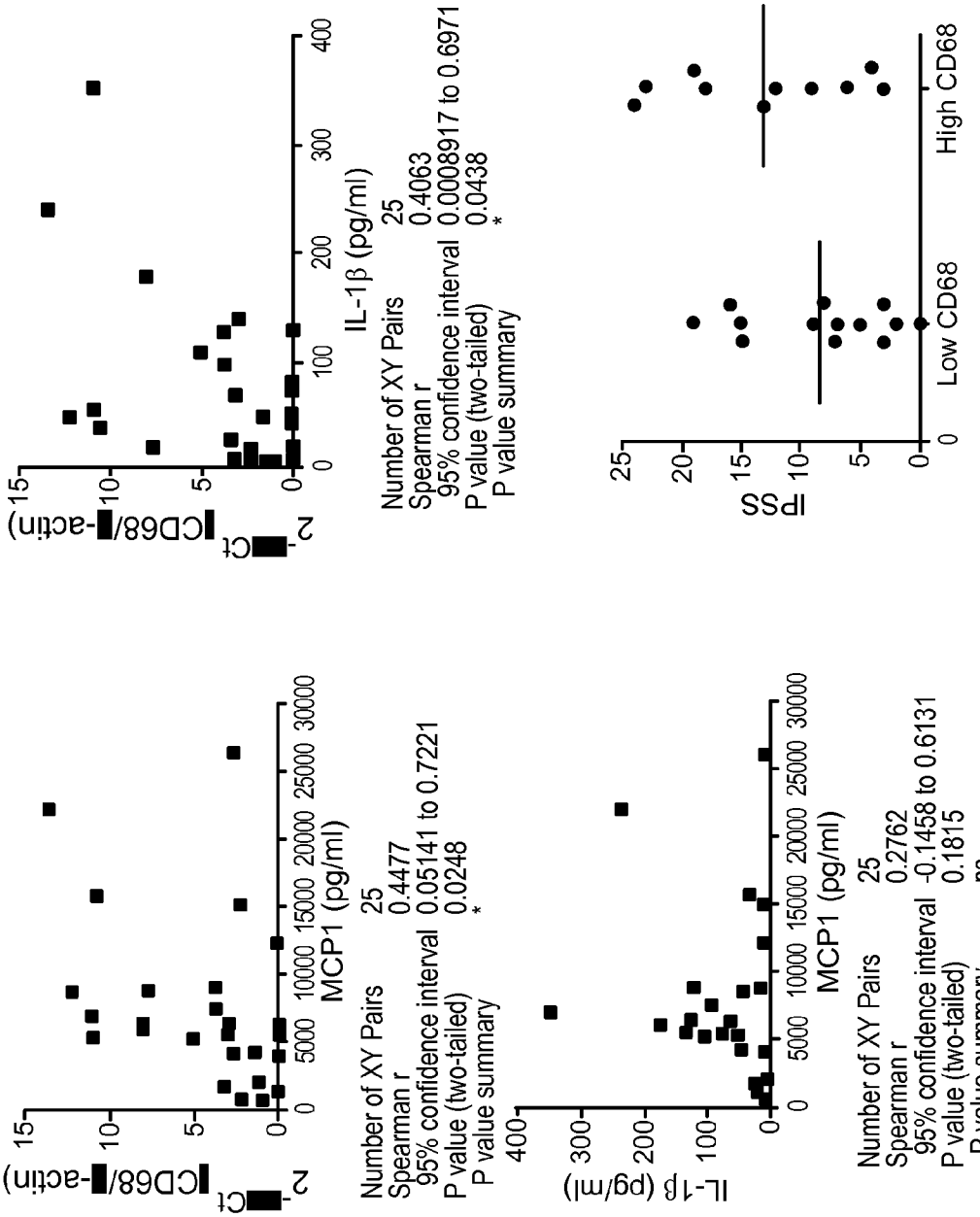

2-ΔCt (CD68/β-actin)
MCP1 (pg/ml)
Number of XY Pairs 25
Spearman r 0.4477
95% confidence interval 0.05141 to 0.7221
P value (two-tailed) 0.0248
P value summary *
IL-1β (pg/ml)
Number of XY Pairs 25
Spearman r 0.4063
95% confidence interval 0.0008917 to 0.6971
P value (two-tailed) 0.0438
P value summary *
IL-1β (pg/ml)
Number of XY Pairs 25
Spearman r 0.2762
95% confidence interval -0.1458 to 0.6131
P value (two-tailed) 0.1815
P value summary ns
IPSS
Low CD68
High CD68

FIGURE 11

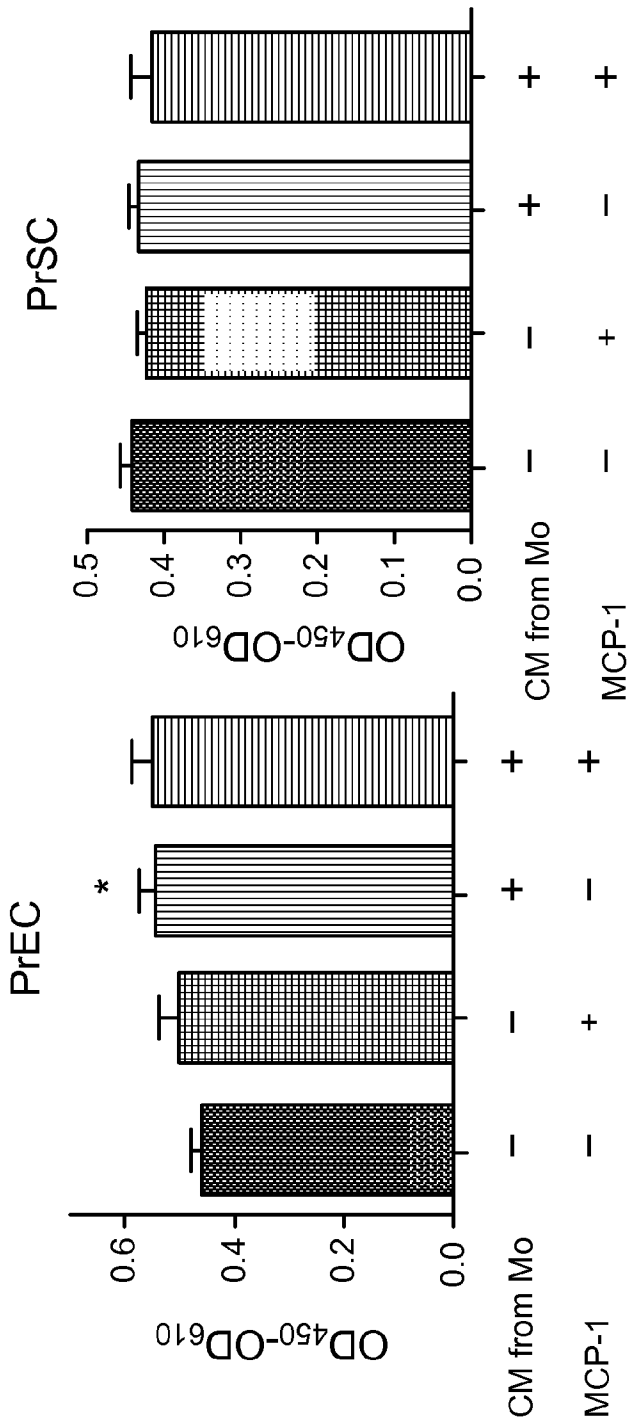
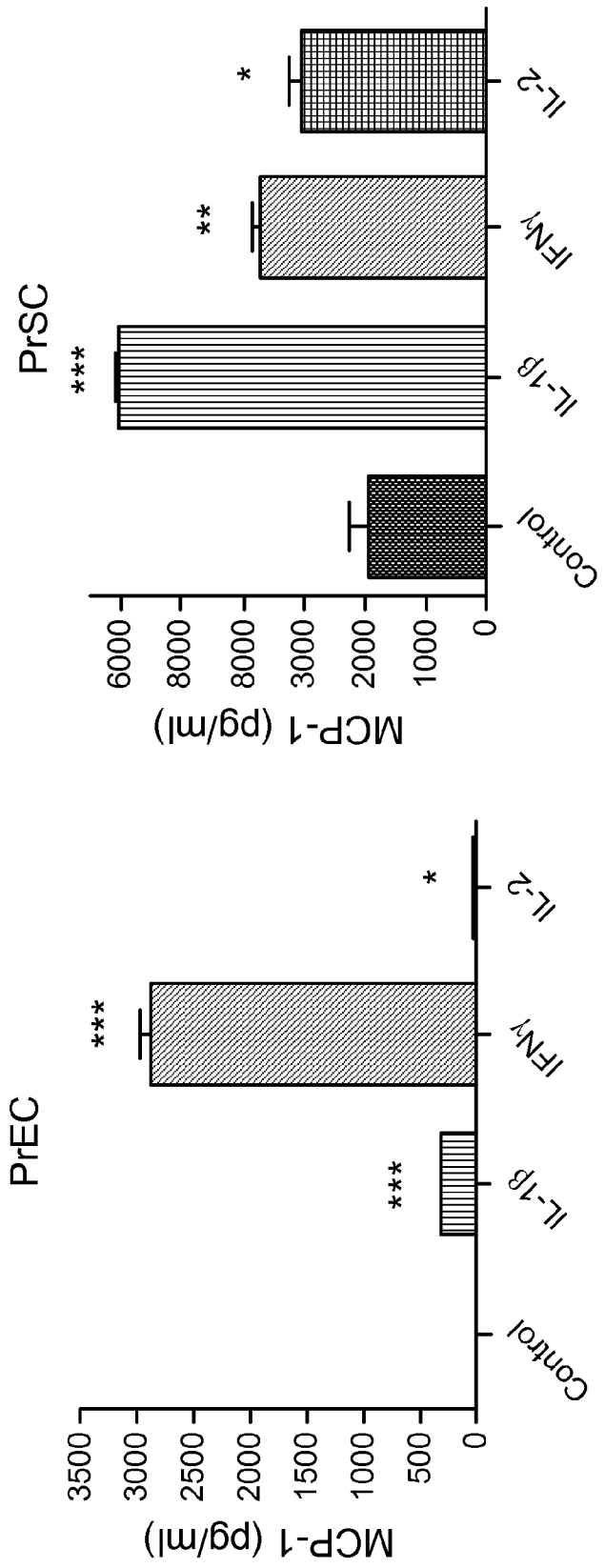


FIGURE 12



○ Increased expression of IL-2, IL-4, IL-8, IL-15 and IFNgamma in BPH tissues.

FIGURE 13

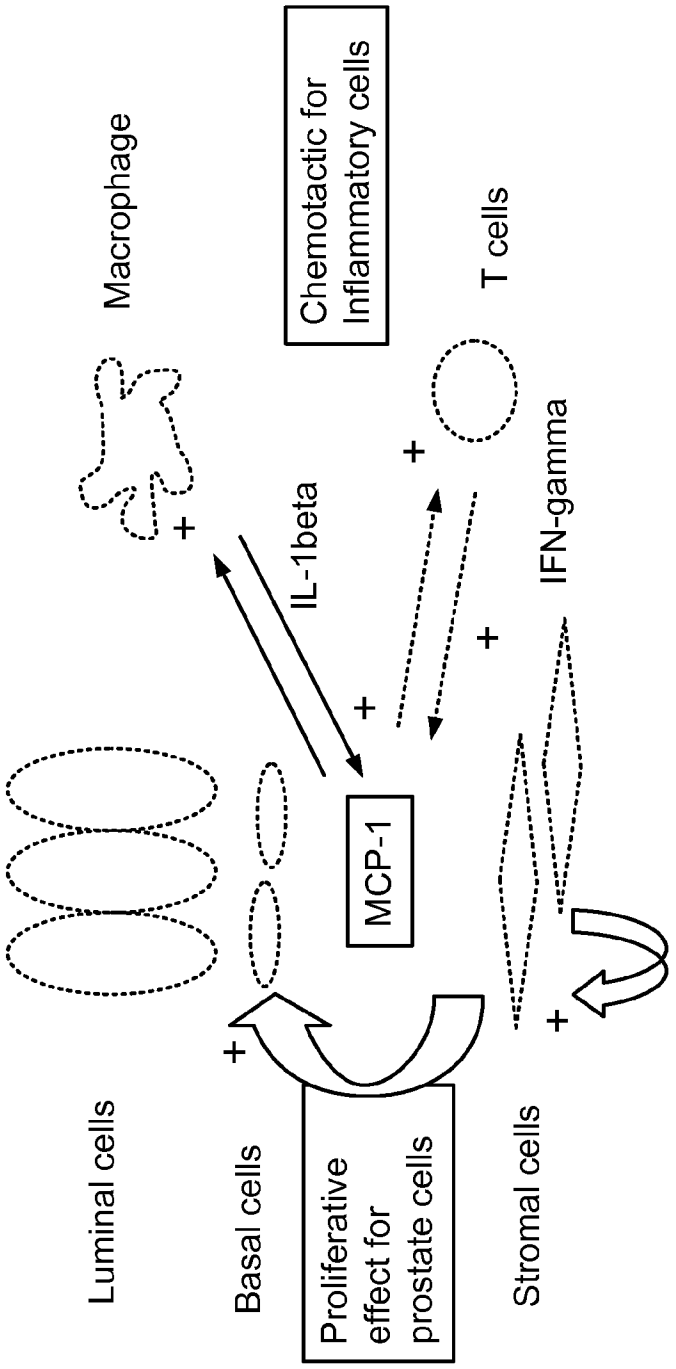

Macrophage
Chemotactic for Inflammatory cells
T cells
IL-1beta
IFN-gamma
MCP-1
Luminal cells
Basal cells
Proliferative effect for prostate cells
Stromal cells
+

FIGURE 14

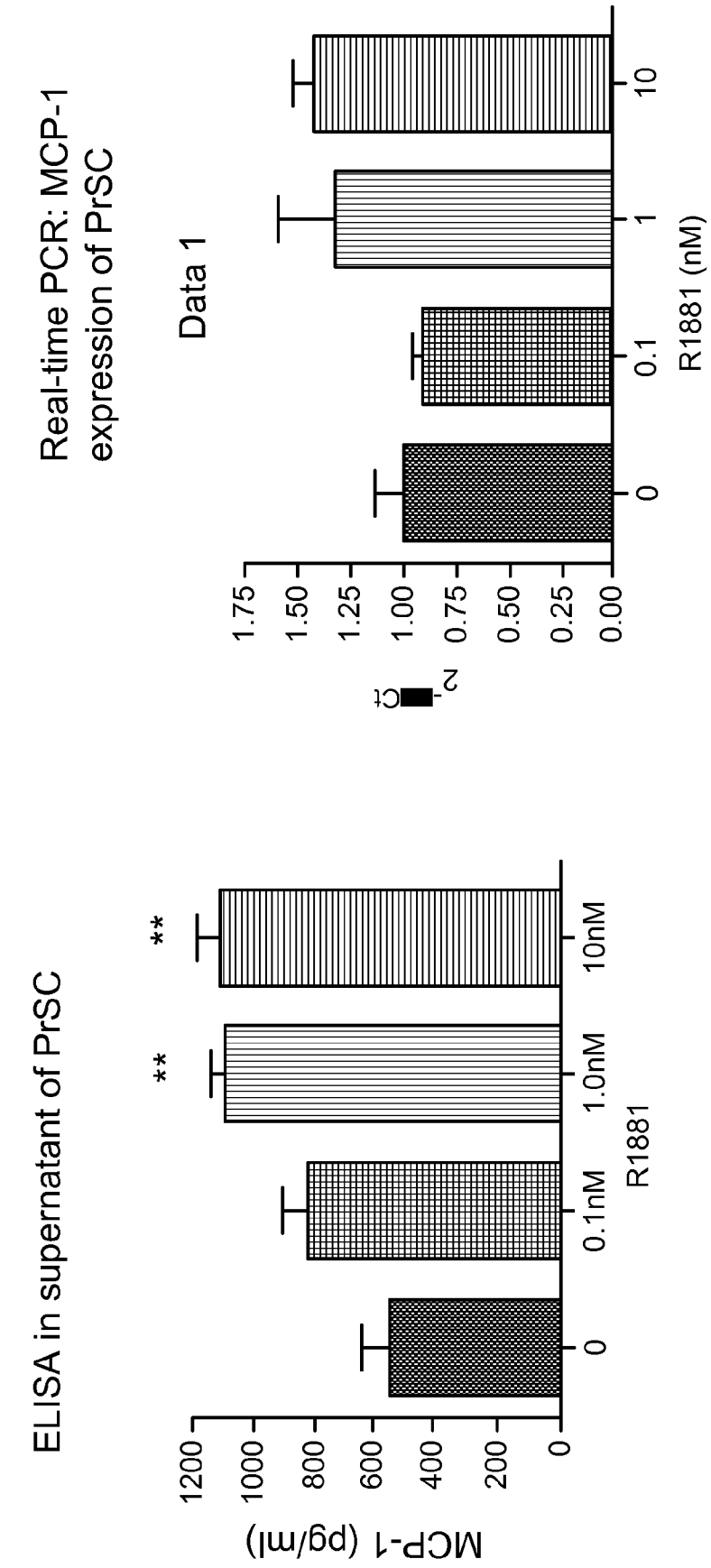

ELISA in supernatant of PrSC
MCP-1 (pg/ml)
1200
1000
800
600
400
200
0
0
0.1nM
1.0nM
10nM
R1881
**
**
4-days of incubation
Real-time PCR: MCP-1 expression of PrSC
Data 1
2-ΔCt
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
0.1
1
10
R1881 (nM)

FIGURE 15

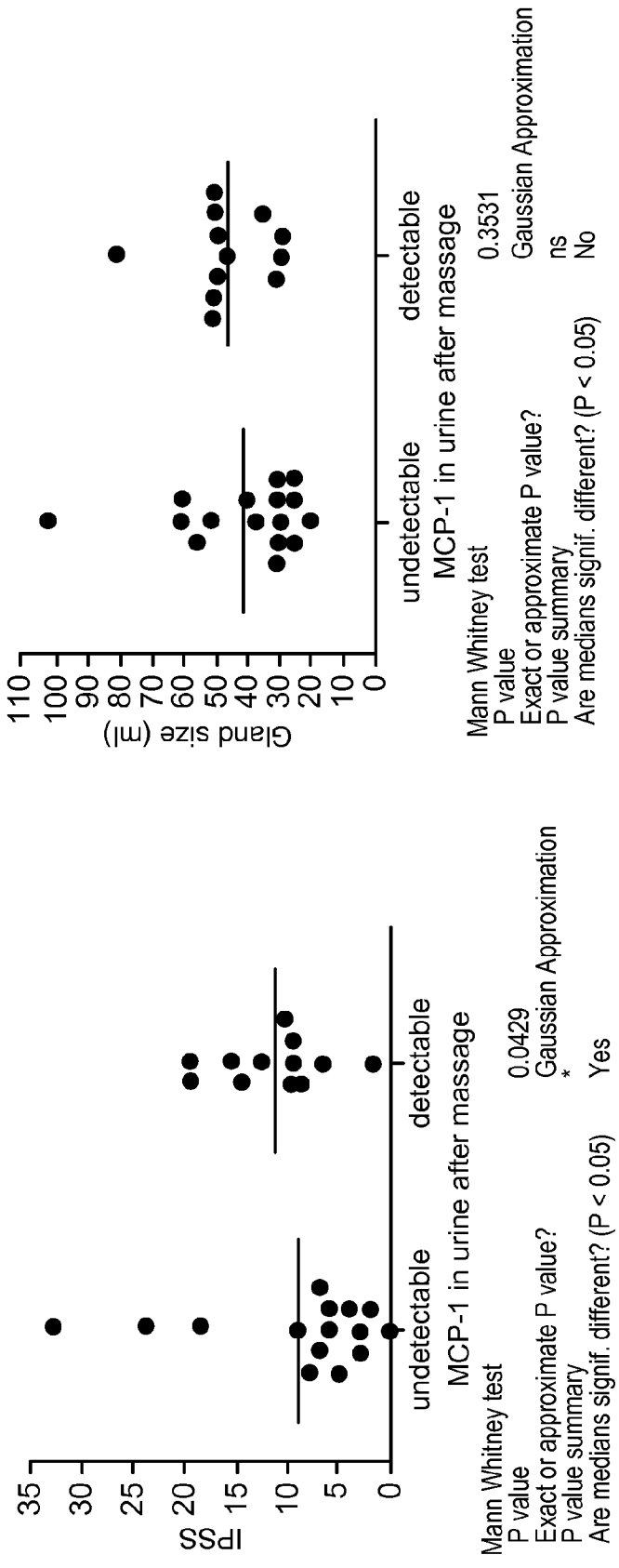

IPSS
35
30
25
20
15
10
5
0
undetectable
detectable
MCP-1 in urine after massage
Mann Whitney test
P value 0.0429
Exact or approximate P value? Gaussian Approximation
P value summary *
Are medians signif. different? (P < 0.05) Yes
Gland size (ml)
110
100
90
80
70
60
50
40
30
20
10
0
undetectable
detectable
MCP-1 in urine after massage
Mann Whitney test
P value 0.3531
Exact or approximate P value? Gaussian Approximation
P value summary ns
Are medians signif. different? (P < 0.05) No
Age (year)
85
80
75
70
65
60
55
50
45
undetectable
detectable
Urinary MCP-1
Mann Whitney test
P value 0.3902

FIGURE 16

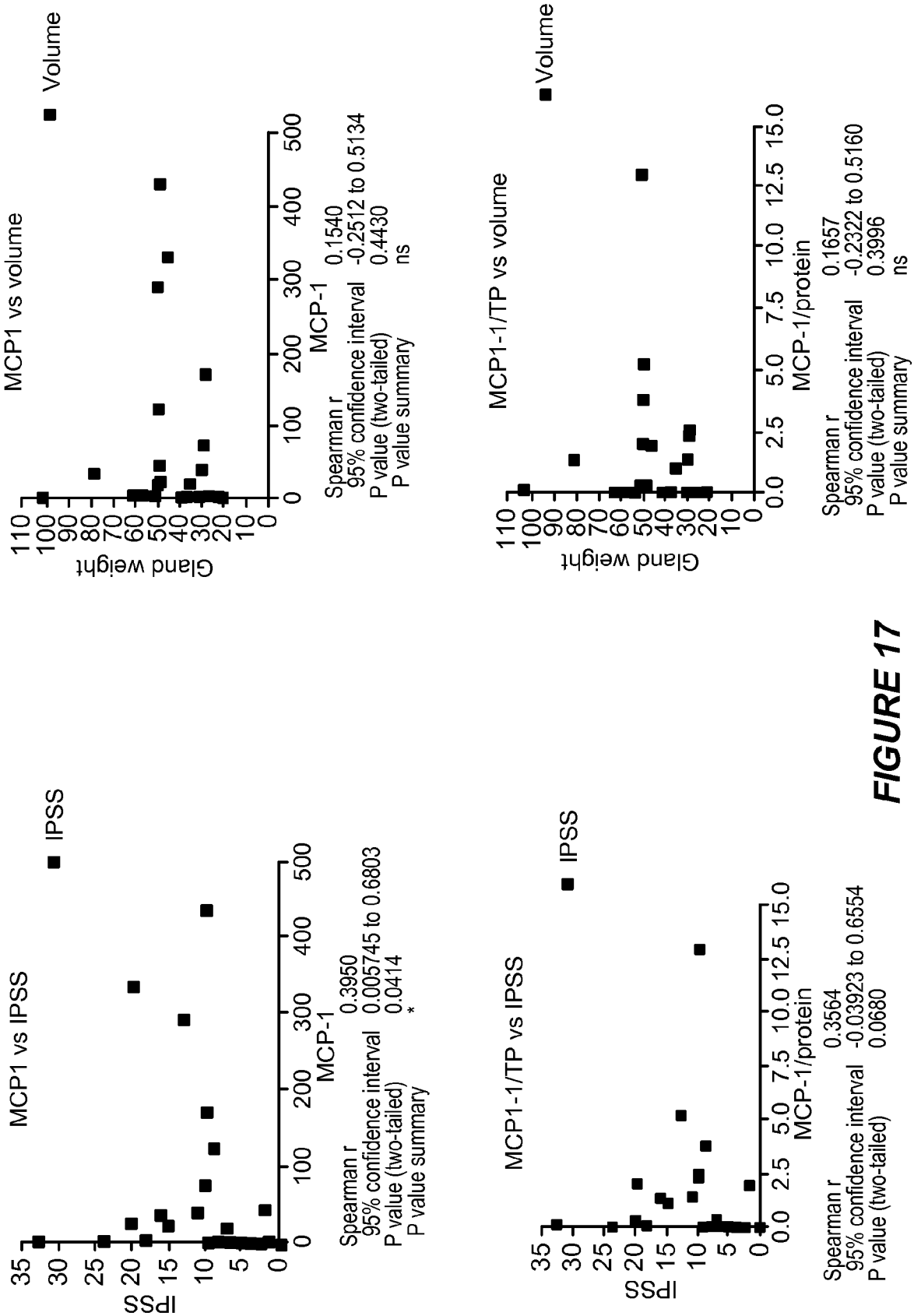

MCP1 vs IPSS
IPSS
MCP-1
Spearman r 0.3950
95% confidence interval 0.005745 to 0.6803
P value (two-tailed) 0.0414
P value summary *
MCP1-1/TP vs IPSS
IPSS
MCP-1/protein
Spearman r 0.3564
95% confidence interval -0.03923 to 0.6554
P value (two-tailed) 0.0680
MCP1 vs volume
Volume
Gland weight
MCP-1
Spearman r 0.1540
95% confidence interval -0.2512 to 0.5134
P value (two-tailed) 0.4430
P value summary ns
MCP1-1/TP vs volume
Volume
Gland weight
MCP-1/protein
Spearman r 0.1657
95% confidence interval -0.2322 to 0.5160
P value (two-tailed) 0.3996
P value summary ns

FIGURE 17

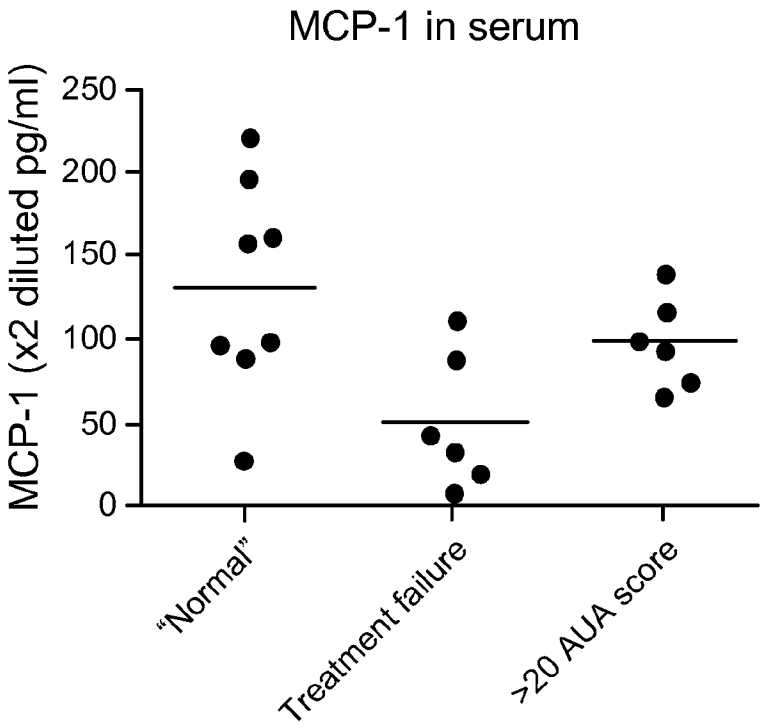

MCP-1 in serum
MCP-1 (x2 diluted pg/ml)
250
200
150
100
50
0
"Normal"
Treatment failure
>20 AUA score

FIGURE 18

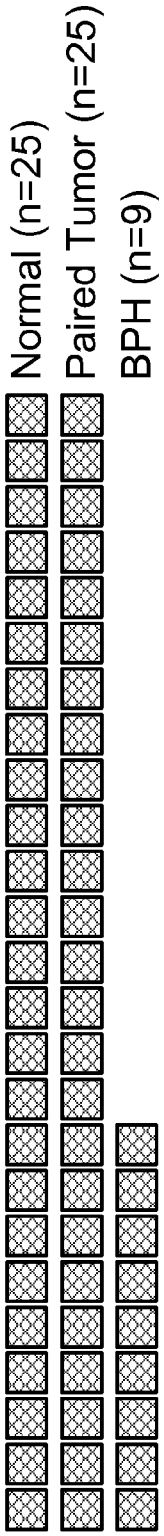


FIGURE 19