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# Expression of the chemokine receptor CCR7 in prostate cancer presenting with generalized lymphadenopathy: Report of a case, review of the literature, and analysis of chemokine receptor expression

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**Abstract**

**Purpose:** Generalized lymphadenopathy is a rare presentation of prostate cancer. We report a case and review reported cases in the literature. Because of the association of chemokine receptor expression with specific metastatic patterns, we tested for expression of chemokine receptors known to mediate migration to lymph nodes.

**Methods:** We performed a MEDLINE (National Library of Medicine, Bethesda, MD) database search for case reports during the last 32 years using "prostate cancer," "lymphadenopathy," "metastatic to lymph nodes," and "mimicking lymphoma" as keywords. Expression of the CXCR4 and CCR7 chemokine receptors was assessed by immunohistochemistry. Laser capture microdissection and reverse transcription polymerase chain reaction for CXCR4 were used to exclude nonspecific binding.

**Results:** Of 153 patients with prostate cancer presenting with lymphadenopathy (LAD) described in the literature, 67 (44%) presented with supraclavicular adenopathy, 29 (19%) retroperitoneal, 22 (14%) mediastinal, 15 (10%) cervical, 9 (6%) inguinal, and 2 (1%) axillary LAD. Only 9 patients presenting with generalized LAD have been previously reported. Monoclonal antibodies to CCR7 showed intense staining in the patient's tumor epithelium. Little or no staining was observed for CXCR4. Reverse transcription polymerase chain reaction for chemokine receptors on ribonucleic acid (RNA) recovered from the patient's sample failed to express messenger RNA for CXCR4 but did express messenger RNA for CCR1, CCR4, and CCR5.

**Conclusions:** Prostate cancer may present on rare occasions with generalized adenopathy. Variable expression of chemokine receptors may be associated with organ specific patterns of metastasis. In this case, expression of CCR7 may have accounted for the unusual predilection of this patient's prostate cancer for lymph nodes. 

*Keywords:* Prostate cancer; Lymphadenopathy; Chemokine receptors

## 1. Introduction

Prostate cancer is usually asymptomatic or presents with local symptoms such as urinary urgency, nocturia, frequency, and hesitancy [1]. When metastases are present, the axial skeleton and the pelvic lymph nodes are the most prevalent sites [2]. Generalized lymphadenopathy (LAD) as the initial presentation of prostate cancer is extremely rare [2]. We present a case of prostate cancer presenting with generalized LAD, a review the literature on this rare metastatic pattern, and an analysis of chemokine receptor expression in the tumor.

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## 2. Case report

A 59-year-old white man presented with a 2-day history of right upper extremity edema. He had a history of left axillary subclavian vein thrombosis one-year before associated with thoracic outlet syndrome. The patient denied any urinary symptoms or bone pain. He did not smoke and consumed 6 beers per day. His father died at age 70 with prostate cancer, and he had a 65-year-old brother also diagnosed with prostate cancer.

Vital signs were unremarkable. Physical examination revealed a well developed man with mild right-upper extremity edema and large, bilateral, nontender, rubbery, cervical, supraclavicular, axillary and inguinal LAD. Abdominal examination was within normal limits. Digital rectal examination showed an enlarged hardened right prostatic lobe without distinct nodularity. The remainder of the physical examination was unremarkable.

Laboratory studies on admission included a normal complete blood count, complete metabolic profile, prothrombin time, activated partial thromboplastin time, calcium, alkaline phosphatase, and lactate dehydrogenase. Prostate specific antigen (PSA) level was increased at 260 ng/mL (range, 0–4). A chest x-ray showed a right cervical rib with clear lung fields. Duplex ultrasound of the right-upper extremity showed a partial filling defect consistent with a thrombus at the junction of the right internal jugular and subclavian vein. A hypercoagulability evaluation, including functional assays for activated protein C resistance, protein C and S levels, antithrombin III levels, and homocysteine, was negative. Computerized tomography of the neck showed bilateral cervical LAD (maximal size 2 cm). A chest CT revealed bilateral axillary LAD (maximal size 1.9 × 2.5 cm) and enlarged mediastinal lymph nodes (maximal size 1.8 × 2.5 cm). An abdominal CT revealed normal liver and spleen, and enlarged peri-celiac and periaortic lymph nodes. CT of the pelvis was positive for a 2-cm left external iliac lymph node. A fine-needle aspiration biopsy of a cervical node was performed and displayed cellular evidence of adenocarcinoma. The patient underwent a transrectal, ultrasound guided prostate biopsy revealing prostatic adenocarcinoma (Gleason Grade 4 + 3 = 7).

A whole body bone scan showed increased tracer activity in the third and fourth cervical vertebrae, seventh thoracic vertebra, and left ninth rib, suggestive of metastases. Immunoperoxidase staining of the cervical lymph node tissue was positive for PSA. The patient was treated with full anticoagulation, and was started on oral flutamide and the GnRH agonist goserelin.

## 3. Methods

### *3.1. Immunohistochemical methods*

Formalin-fixed, paraffin-embedded prostate cancer tissue samples from the patient obtained during initial evaluation were de-waxed and placed in a pressure cooker containing 0.01 M buffered sodium citrate solution (pH 6.0), boiled, and chilled to room temperature for antigen retrieval. The slides were then incubated overnight at room temperature with anti-human CXCR4 antibody MBA171 IgG2a (Clone 44708.111, R&D Systems, Minneapolis, MN) diluted 1:100 (10 mg/ml) or with anti-human CCR7 antibody (Clone 150503, R&D Systems) diluted at 25 μg/ml. A streptavidin/biotin detection method with 3,3′-diaminobenzidine tetrahydrochloride was used for signal detection, and Harris hematoxylin was used as a counterstain. Sections of lymph nodes were used as positive controls, and IgG2a was used as a negative control.

### *3.2. Laser capture microdissection (LCM) and reverse transcription polymerase chain reaction (RT-PCR)*

Microdissection of the prostate tissue sample was performed using a PixCell II laser capture microscope equipped with an infrared diode laser (Arcturus Engineering, Mountain View, CA) on deparaffinized slides. The laser power was 70 mV, the pulse width was 2 milliseconds, the spot size was 7.5 μm, and 500–600 laser shots were needed to collect the ribonucleic acid (RNA) from 2 slides. Two strategies were followed. Either the selected areas were microdissected from the tissue, or, where the area of interest made up the majority of the section, it was found most efficient to dissect and discard areas that were not of interest. RNA was recovered from LCM Caps according to the manufacturer instructions (Arcturus Engineering). Complementary deoxyribonucleic acid synthesis was performed for 30 minutes at 45°C with SuperScript RT (Invitrogen Corp., Carlsbad, CA) and platinum Taq for an initial 2 minutes at 94°C, followed by 40 cycles of 30 seconds at 94°C, 45 seconds at 55°C, and 1 minute at 72°C, with a final 10-minute extension at 72°C. PCR primers that were used included: CCR1 5′-gcgaattccatggaaactccaaacaccaca, [BC051306, 60–80] 3′-gcggatccctaggccccaaaggccctctcgtt, [BC051306, 160–141] CCR4 5′-gcgaattccatgaaccccacggatatagca,) [X85740.1, 183–203] 3′-cggatccctactccc caaatgccttgatgcc, [X85740.1, 299–279] CCR5 5′-gcgaattccatggattatcaagtgtcaagt[NM_000579.1, 358–378], 3′-gcggatccctagcgggct gcgatttgcttcac[NM_000579.1, 450–430], and 2 sets of primers for CXCR4 5′-ggcagcaggtagcaaagtga, [NM_003467, 14–33] 3′)tgagacaaagaggaggtcgg, [NM_003467, 264–248] and 5′-ggaagcttcatggaggggatcagtatatac [NM_003467, 88–109],3′-ggtctagattagctggagtgaaaacttg. [NM_003467, 1147–1128]. RNA quality was assessed by RT-PCR using primers for glyceraldehyde-3-phosphate dehydrogenase. RT-negative controls, from which the RT was omitted, were included in all reactions. Positive controls included RNA isolated from PC-3 prostate cancer cells originally isolated from a vertebral metastasis of a human patient with prostate cancer; these were obtained from American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin.