Available online at www.sciencedirect.com
ScienceDirect
Pathology – Research and Practice 205 (2009) 249–254
PATHOLOGY RESEARCH AND PRACTICE
www.elsevier.de/prp

ORIGINAL ARTICLE

Lymphatic vessel densities of lymph node-negative prostate adenocarcinoma in Korea

Hyun-Soo Kim[a], Wooseok Sung[b], Sun Lee[a], Sung-Goo Chang[b], Yong-Koo Park[a,*]

[a]*Department of Pathology, Kyung Hee Medical Center, School of Medicine, Kyung Hee University, #1 Hoegi Dong, Dongdaemoon-gu, Seoul 130-702, Republic of Korea*
[b]*Department of Urology, Kyung Hee Medical Center, School of Medicine, Kyung Hee University, Seoul, Republic of Korea*

Received 23 May 2008; received in revised form 6 September 2008; accepted 20 October 2008

Abstract

Although lymphatic vessel density (LVD) is associated with regional lymph node (LN) metastasis in prostate adenocarcinoma, no study is available that examines whether the LVD is correlated with prognostic factors other than LN metastasis in LN-negative prostate adenocarcinoma. The aim of our study was to analyze intratumoral (IT), peritumoral (PT), and nontumoral (NT) LVDs, and to determine if there is a correlation between the LVD and the clinicopathological parameters in the Korean LN-negative prostate adeocarcinoma patients. Lymphatics were detected by immunohistochemical staining using D2-40 antibody on 39 radical prostatectomy specimens. Mean LVDs of IT, PT, and NT compartments were 5.39 ± 4.22, 10.71 ± 4.61, and 2.04 ± 1.34 per $200\times$ field, respectively. The difference in LVD among the compartments was significant ($P < 0.001$). The IT-LVD was significantly lower in patients with larger tumor volume ($P = 0.029$) and higher preoperative prostate-specific antigen level ($P = 0.008$). The PT-LVD showed no significant correlation with the clinicopathological parameters. Our results suggest that IT- and PT-LVDs may increase in LN-negative prostate adenocarcinoma as a result of lymphangiogenesis, but IT lymphatics may decrease due to mechanical compression and destruction caused by proliferating tumor cells. In addition, IT-LVD may be used as a prognostic factor in LN-negative prostate adenocarcinoma.

Keywords: Lymphatic vessel density; Prostate adenocarcinoma; Lymphangiogenesis

Introduction

The lymphatic system constitutes one of the most important pathways for tumor cell dissemination. Lymph node metastasis (LNM) was believed to be a passive process involving tumor spread via pre-existing peritumoral (PT) lymphatics that follows natural routes of lymph drainage, especially wide open intercellular junction of lymphatic endothelial cells [8]. In the past few years, however, it has become apparent that intratumoral (IT) lymphangiogenesis, the formation of new lymphatics, controlled by a complex network of growth factors, cytokines and chemokines, can contribute actively to tumor metastasis [8]. The significance of PT and IT lymphatics as conduits for cancer cell metastasis has been shown in many studies, but is still controversial. With regard to lymphatic vessel density (LVD), it has been suggested that a positive correlation exists between PT-LVD and LNM in head and neck squamous cell carcinoma [5], cervical carcinoma [19] gastric carcinoma [9], and prostate adenocarcinoma

*Corresponding author. Tel.: +82 2 958 8742; fax: +82 2 957 0489.
E-mail address: ykpark@khmc.or.kr (Y.-K. Park).

0344-0338/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2008.10.005

(PCa) [22,17]. The presence of IT lymphatics has also been reported in cancers affecting many different organs [21,7], and IT-LVD was suggested to be used as a criterion to separate patients at higher risk of an adverse clinical outcome [10]. In contrast, another study reported that PT-LVD offered a better survival capacity for the patients with head and neck squamous cell carcinoma [12]. Others describe that IT lymphatics are non-functional and play a minor role in primary tumor dissemination [15]. Moreover, a recent study reported that PT- and IT-LVDs do not appear to have any prognostic significance in PCa [2].

Some studies have investigated the significance of lymphangiogenesis in LN-negative human cancers, and the results are also controversial. IT lymphangiogenesis was suggested to be a useful discriminator in predicting the outcome of patients with LN-negative oral squamous cell carcinoma [13]. In contrast, another study reported that IT lymphangiogenesis plays no role in LN-negative colorectal carcinoma [18]. Although there is no available study examining lymphatics in LN-negative PCa, we hypothesized that (1) IT lymphangiogenesis may be observed in LN-negative PCa, and (2) it may be correlated with known prognostic factors of PCa. Previous studies assessed the correlation between LVD and LNM in PCa [22,17]. We focused on the difference in LVD according to the prognostic factors other than LNM, including the status of tumor volume, preoperative prostate-specific antigen (PSA) level, Gleason score, and extraprostatic extension. The aim of our study was to evaluate IT-, PT- and nontumoral (NT) LVDs, and to determine their significance using D2-40 antibody in Korean LN-negative PCa patients.

Materials and methods

Patients and tumor specimens

This study was approved by the Institutional Review Board of Kyung Hee University. Thirty-nine radical prostatectomy specimens performed for PCa were selected from the archival cases of the Department of Pathology, Kyung Hee Medical Center, Seoul, Korea. The clinicopathological data, including age group, status of tumor volume, preoperative PSA level, Gleason score, and extraprostatic extension, are summarized in Table 1. Patient age ranged from 49 to 75 years (mean, 63.9-year-old). None of them had received preoperative radiation or chemotherapy. All patients underwent bilateral obturator LN biopsy for intraoperative staging prior to radical prostatectomy, and none of them was LN-positive. Original pathologic diagnoses were confirmed on hematoxylin and eosin (H&E)-stained sections by two pathologists (H.S.K. and Y.K.P.).

Table 1. The clinicopathological data of 39 prostate adenocarcinoma patients.

Parameters	No. of patients (%)
Age (year-old)	
<65	20 (51.3)
$\geqslant 65$	19 (48.7)
Tumor volume (%)	
<20	27 (69.2)
$\geqslant 20$	10 (25.6)
PSA level (ng/mL)	
<4	10 (25.6)
$\geqslant 4$	29 (74.4)
Gleason score	
<7	25 (64.1)
$\geqslant 7$	14 (35.9)
Extraprostatic extension	
Absent	29 (74.4)
Present	9 (23.1)

To determine the tumor volume, we used a modification of the Stanford technique [1,14]. Briefly, the prostate gland was transversely sectioned at 0.3-cm interval from the apex to the base in each case. Each block of the prostatic parenchyma was designated by four quadrants (right anterior, right posterior, left anterior and left posterior). On average, 20 blocks of prostatic parenchyma were obtained from each case. The percent of tumor area was estimated by the area of the tumor and whole parenchyma in each section, calculated using a 1-mm grid. Tumor volume was expressed as the average of all sections. A single paraffin block containing PCa representative of the entire case (same Gleason score as the overall score of the case) was chosen for immunohistochemical detection of lymphatics.

Immunohistochemical staining of D2-40 in lymphatic endothelial cells

Immunohistochemical staining for lymphatics was performed using a D2-40. Briefly, 4-μm sections of formalin-fixed, paraffin-embedded tissues were deparaffinized by Bond Dewax Solution (Vision BioSystems, Mount Waverley,Victoria, Australia), and an antigen retrieval procedure was done using Bond ER Solution (Vision BioSystems) for 30 mins at 100 °C. The endogenous peroxidase was quenched by incubation with hydrogen peroxide for 5 mins. Sections were incubated for 15 min at ambient temperate with mouse monoclonal antibody D2-40 (1:200, Signet Laboratories, Dedham, MA), which reacts with the oncofetal mem-