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Original article

The role of lymph vessel density and lymphangiogenesis in metastatic tumor spread of nonseminomatous testicular germ cell tumors☆

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Abstract

Objectives: To evaluate the role of lymph vessel density (LVD) and lymphangiogenesis in nonseminomatous testicular germ cell tumors (NSGCT) using the specific lymphatic endothelial cell (LEC) marker LYVE-1.

Materials and methods: NSGCT specimens of 77 patients (32 with and 45 without metastases) were stained immunohistochemically using a LYVE-1 antibody. LVD was measured in different representative areas by the standardized "hot spot" method. Fluorescence double stainings for LYVE-1 and Ki-67 were performed. The median follow-up period was 46 (range 3–170) months.

Results: The mean peritumoral (2.16 ± 2.17) and nontumoral LVD (3.17 ± 3.24) were significantly higher than intratumoral LVD (0.16 ± 0.73) (both: $P = < 0.001$). In 5 patients proliferating LECs were observed. The peritumoral LVD was 2.66 (±2.31) and 1.80 (±2.02) in metastatic and nonmetastatic NSGCT, respectively. A higher peritumoral LVD was associated with the presence of metastases at the time of diagnosis ($P = 0.087$). The mean peritumoral LVD in tumors with and without lymphovascular invasion (LVI) was 3.33 (±2.20) and 1.62 (±1.95), respectively ($P < 0.001$). The presence of LVI detected by LYVE-1 (LVI-LYVE-1) was independently associated with metastatic disease (logistic regression; $P = 0.045$).

Conclusions: The presence of a high peritumoral LVD and LVI-LYVE-1 are both associated with metastatic disease in NSGCT. LVI-LYVE-1 was independently associated with the presence of metastases at the time of diagnosis. Proliferating LECs are present, suggesting that lymphangiogenesis may promote metastatic dissemination of tumor cells in NSGCT.

Keywords: Lymphangiogenesis; Lymphovascular invasion; NSGCT; Testicular cancer; Metastases; Lymph vessel density (LVD)

1. Introduction

Testicular cancer (TC) is the most frequent malignant disease in young men, with nonseminomatous testicular germ cell tumors (NSGCT) affecting around 40% of the patients. Lymphatic spread to regional lymph nodes is frequently found in NSGCT, however, little is known about the mechanisms of tumor cell dissemination and even less on the possible role of lymphangiogenesis during this process [1]. Lymph vessel density (LVD) has been identified as a surrogate marker for lymphangiogenesis [2]. The introduction of new lymphatic endothelial cell (LEC)-specific antibodies, such as LYVE-1, enabled morphologic studies with clear distinction between blood and lymphatic vessels [3–5].

Computer tomography (CT) is the method of choice for the staging of NSGCT. However, up to 46% of stage I patients present with retroperitoneal metastatic disease at diagnosis [6]. The presence of lymphovascular invasion (LVI), defined as the presence of blood vessel invasion (VI) and/or lymph vessel invasion, represents the most important

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predictive marker in NSGCT. It is associated with an odds ratio of 3.5 for the presence of occult retroperitoneal metastases at diagnosis and a relative risk of relapse of 30%–50% [6–8]. Based on the presence or absence of LVI, patients with stage I NSGCT will be observed or treated by chemotherapy or retroperitoneal lymph node dissection (RPLND) following radical orchiectomy. However, a large proportion of patients may be overtreated by chemotherapy or RPLND [7]. Therefore, new independent predictive markers are required for clinical decision making in patients with NSGCT.

We tested whether there is an association between LVD values and the presence of metastases at diagnosis in patients with NSGCT using the LEC-specific marker LYVE-1.

2. Materials and methods

2.1. Patient population

Paraffin-embedded tumor samples from 77 NSGCT patients who underwent radical orchiectomy between July 1996 and September 2010 were evaluated. Clinical staging (CS) and histopathologic evaluation were performed according to the TNM classification [9–11]. LVI was defined as tumor cell invasion into blood and/or lymph vessels. The median follow-up period was 47 (range 6–176) months. Clinical and pathologic data are presented in Table 1.

2.2. Tissue sample preparation

All hematoxylin and eosin (HE) stained sections were examined by 1 reference pathologist (K.H.) who identified the most representative tissue sections (index tumor). Tissue sections, including intratumoral and peritumoral tissue, were available from all patients. Areas of nontumoral tissue within the tumor section were evaluable in 69 (90%) patients. Paraffin embedded tissue samples were cut in 3-μm sections, mounted, and dried at 37°C overnight (ON).

2.3. Primary antibodies and immunohistochemical procedures

All sections for immunostaining were deparaffinized in Neoclear, rehydrated, and washed in distilled water. For LYVE-1 and LYVE-1/CD-30 double immunostaining blocking of the endogenous peroxidase activity was performed for 30 minutes with 3% H_2O_2 in methanol. After target retrieval, sections were cooled down in iced water. In between washing with Tris-buffered saline (TBS) was performed. As primary antibodies LYVE-1 (DCS-Immunoline, Hamburg, Germany; 1:60, 4°C, ON), CD-30 (DAKO, Glostrup, Denmark; 1:200, 4°C, ON) and Ki-67 (DAKO; clone MIB-1, 1:75, room temperature (RT), one h) were used.

Table 1
Clinicopathologic patient data

Characteristics	Number of Patients	
Total number of patients	77	
Mean patients' age, years ± SD	77	32.0 ± 8.8
Mean tumor size, cm ± SD	72	3.7 ± 2.0
Clinical stage, *n* (%)	77	
I		45 (58)
>I		32 (42)
IGCCCG classification	32	
Good		19 (59)
Intermediate		9 (28)
Poor		3 (9)
Unknown, due to lack of tumor markers		1 (3)
pT stage, *n* (%)	74	
pT1		36 (49)
pT2		32 (43)
pT3		6 (8)
pT4		0 (0)
Histologic condition, *n* (%)		
Seminomatous elements	76	29 (38)
Embryonal carcinoma elements	77	55 (71)
Yolk-sac elements	77	32 (42)
Teratomatous elements	77	40 (52)
Choriocarcinoma elements	77	14 (18)
LVI, *n* (%)	64	
Yes		26 (41)
No		38 (59)
LVI-LYVE-1, *n* (%)	77	
Yes		24 (31)
No		53 (69)
Tumor markers		
Median AFP, ng/ml (range)	72	65 (2–37,759)
Median β-HCG, U/mL (range)	75	15 (2–450,370)
Median LDH, U/l (range)	69	246 (158–4277)
S stage, *n* (%)	75	
S0		14 (19)
S1		44 (59)
S2		15 (20)
S3		2 (3)
Adjuvant treatment, *n* (%)		
CS I		
Surveillance		9 (20)
Chemotherapy		26 (58)
RPLND		6 (13)
Unknown		4 (9)
CS > I		
Chemotherapy		28 (88)
RPLND		1 (3)
Unknown		3 (9)
Median follow-up, months (range)		47 (6–176)
Median time to relapse, months (range)	67	14 (3–19)
Median time to death, months (range)	70	8 (6–43)
Relapse, *n* (%)		4 (6)
Death, *n* (%)		4 (6)
Patients alive, *n* (%)		66 (94)

SD = standard deviation; LVI = lymphovascular invasion defined as the invasion of tumor cells into lymphatics and/or blood vessels; LVI-LYVE-1 = lymph vessel invasion, defined as the invasion of tumor cells into LYVE-1 stained lymphatics; *n* = number of patients; AFP = α-fetoprotein; β-HCG = β-human chorionic gonadotropin; LDH = lactate dehydrogenase; IGCCCG = International Germ Cell Cancer Consensus Group; RPLND = retroperitoneal lymph node dissection.