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Original Article

The expression of p-mTOR and COUP-TFII correlates with increased lymphangiogenesis and lymph node metastasis in prostate adenocarcinoma

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Abstract

Background: Mammalian target of rapamycin (mTOR) is a central regulator of major cellular processes such as growth and proliferation. Deregulated mTOR signaling is implicated in a wide spectrum of human malignancies including prostate cancer. The aim of this study is to address the role of phosphorylated mTOR (p-mTOR) in prostate adenocarcinoma-induced lymphangiogenesis and lymph node metastasis as well as to investigate its relationship with chicken ovalbumin upstream promoter transcriptional factor 2 (COUP-TFII) and the vascular endothelial growth factors A/C (VEGF A/C).

Methods: We analyzed 92 paraffin embedded specimens from patients with prostate cancer who underwent radical prostatectomy with pelvic lymph node (LN) dissection. Twenty-four of these men were pathologically assessed to have regional LN metastasis (pN1 group) and 68 with negative lymph nodes (pN0 group). Lymph vessel density was measured using anti-D2-40 and anti-LYVE-1 antibodies. The expression of p-mTOR, COUP-TFII, and VEGF A/C was also evaluated by immunohistochemistry.

Results: Specimens from pN1 group exhibited higher cytoplasmic p-mTOR expression compared to pN0 specimens. Mean vessel densities assessed by COUP-TFII and D2-40 were increased in pN1 tumors and positively associated with higher p-mTOR expression. Interestingly, increased expression of p-mTOR was positively associated with COUP-TFII expression in cancer cells and elevated immunoreactivity for both VEGF A and C, which in turn exhibited higher expression in pN1 group.

Conclusions: Our findings suggest that increased p-mTOR and COUP-TFII expression are implicated in human prostate adenocarcinoma-induced lymphangiogenesis and LN metastasis.

Keywords: Prostate; Adenocarcinoma; mTOR; NR2F2; Metastasis

1. Introduction

The conserved mammalian target of rapamycin (mTOR) is a serine/threonine kinase of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway and a central regulator of fundamental cellular processes such as protein and lipid biosynthesis, lysosome biogenesis, energy metabolism, and cytoskeletal organization. Considering that mTOR plays a key role in cellular physiology, it is predictable that deregulations of the mTOR signaling pathway are implicated in a wide spectrum of human diseases, including cancer. Indeed, abnormal signaling from mutated oncogenes and tumor suppressors constitutively activates the mTOR pathway in a plethora of human malignancies [1]. Oncogenic activation of mTOR consequently induces processes required for cancer cell growth, survival, and proliferation.

Concerning the prostate gland, several observations support the importance of mTOR pathway in cancer pathogenesis. Numerous studies using human tissues or knockout and transgenic mouse models, demonstrate the involvement of mTOR in prostate cancer (PCa) initiation

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and progression [2,3]. Moreover it seems that the mTOR kinase fosters the dissemination and resistance of established, advanced disease in part via acquisition of both epithelial–mesenchymal transition and cancer stem cell phenotypes [4,5].

For numerous types of solid tumors, including prostate adenocarcinoma, the lymphatic system is the primary conduit for initial metastasis. Moreover, high grade (anaplastic) PCa has been shown to correlate with increased lymph node metastatic potential [6]. Chicken ovalbumin upstream promoter transcriptional factor 2 (COUP-TFII) is a member of the steroid/thyroid hormone receptor superfamily that seems to modulate normal as well as pathological lymphangiogenesis and correlates with lymph node metastasis in breast cancer patients [7]. However, little is known about the relationship between the transcription factor COUP-TFII, mTOR, and lymph node metastasis in prostate adenocarcinoma. Based on the current understanding, VEGF-C\VEGFR-3 and other pathways might be involved in the modulation of COUP-TFII in tumor lymphangiogenesis [7]. It is also generally accepted that VEGF-C is a key regulator in lymphangiogenesis and is associated with increased tumoral lymph vessel density (LVD) and metastasis to the regional lymph nodes in a wide spectrum of carcinomas [8,9]. Although many studies suggest a role of m-TOR in angiogenesis, via the upregulation of VEGF-A [10,11], the participation of mTOR in lymphangiogenesis is more complicated. It seems that the kinase plays an important role in the lymphangiogenesis induced by VEGF-C but not VEGF-A [10,12]. Furthermore, it is unclear whether the expression of m-TOR correlates with the presence of lymph node metastasis or not [12–14].

The aim of the present study was to determine the expression status of p-mTOR and compare it with lymphatic parameters (LVD, lymphatic invasion [LVI], etc.) in patients with prostate adenocarcinoma and positive or negative lymph node status (LN+ and LN−). The lymphatics were determined with the lymphatic-specific markers D2-40 and LYVE-1. Moreover, we evaluated the expression of the transcription factor COUP-TFII, the vascular endothelial growth factors A and C (VEGF-A and VEGF-C) in relation to each other, p-mTOR expression, mean LVD, LVI, and lymph node status.

2. Materials and methods

2.1. Patients

Ninety-two archival formalin-fixed, and paraffin embedded tissue samples from patients with prostate adenocarcinoma who underwent radical prostatectomy with pelvic lymph node dissection for clinically localized disease at the General University Hospital of Patras, between 2003 and 2009, were retrospectively collected for this study. Patients, aged from 51 to 75 years, were selected based on the availability of pathologic and clinical data extracted from the primary pathology reports and patients' files, including age, histologic type, grade, and detailed pathologic stage. The material comprised of 24 cases with regional lymph node (LN), metastasis (LN +), and 68 cases with negative lymph node status (LN−). Tumors were graded according to the Gleason system and staged according to the TNM (AJCC 2009) staging system for radical prostatectomy. Cases were further divided in 3 groups, according to Gleason score: grade <7, grade $= 7$, and grade ≥ 8. The study's observational protocol was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of the University Hospital of Patras.

2.2. Immunohistochemistry

Paraffin blocks were cut into 4 μm-thick sections, which were deparaffinised by incubation in xylene at 60°C and rehydrated in serial graded ethanol dilutions, followed by washing in tris-buffered saline (pH 7.6). Antigen retrieval was carried out by microwave treatment the sections in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 0,3% H_2O_2 in distilled water for 20 minutes. To avoid nonspecific binding, slides were treated for 12 minutes with 2% bovine serum albumin in tris-buffered saline. The commercially available primary antibodies for podoplanin (D2-40), LYVE-1, VEGF-A, VEGF-C, COUP-TFII, and p-mTOR were used as described in Table 1. Detection of primary antibodies was performed using a horse radish peroxidase-conjugated polymer according to the manufacturer's instructions (EnVision; Dako, Glostrup,

Table 1
Antibody characteristics

Antibody	Type	Source/cat. no.	Dilution	Incubation (min)
p-mTOR	Polyclonal	Acris/AP02480PU-N	1:120	30, RT
VEGF-A	Polyclonal	Acris/AP15854PU-N	1:100	10, RT
VEGF-C	Polyclonal	Zymed-Invitrogen/ 18–2255	1:300	Overnight, 4°C
COUP-TFII	Monoclonal	Abcam/ab41859	1:200	Overnight, 4°C
D2–40	Monoclonal	Dako/M3619	1:80	30, RT
LYVE-1	Polyclonal	ReliaTech GmbH/102-PA50AG	1:60	Overnight, 4°C