Human Pathology (2017) **61**, 26–32

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

In this issue

Clinical significance of epithelial-mesenchymal transition markers in prostate cancer☆,☆☆

Sandy Figiel MSC[a], Caroline Vasseur MD[b], Franck Bruyere MD, PhD[c], Francois Rozet MD[d], Karine Maheo PhD[a], Gaelle Fromont MD, PhD[a,b,*]

[a]*Inserm U1069, 37000 Tours, France*
[b]*Department of Pathology, CHU-Universite de Tours, 37000 Tours, France*
[c]*Department of Urology, CHU-Universite de Tours, Tours, France*
[d]*Department of Urology, Institut Mutualiste Montsouris, 75014 Paris, France*

Received 26 August 2016; revised 4 October 2016; accepted 14 October 2016

Keywords:
Prostate cancer;
Epithelial-to-mesenchymal transition;
Prognosis;
Zeb1;
Metastases

Summary The process of epithelial-to-mesenchymal transition (EMT) contributes to cancer progression, with activation of transcription factors leading to loss of epithelial characteristics and acquirement of mesenchymal properties. We analyzed in human prostate cancer (PCa) the expression of EMT markers at the different stages of PCa natural history, and evaluated its clinical significance. The expression of the key EMT transcription factor Zeb1, together with E-cadherin, vimentin, and N-cadherin, was evaluated by immunohistochemistry on tissue microarrays containing samples of normal prostate (n = 58), clinically localized cancer (CLC) (n = 242), castration-resistant PCa (CRPC) (n = 48), and metastases (n = 43). Zeb 1 expression was not found in normal tissues, and significantly increased with disease progression from pT2 (20% of cases) to pT3 tumors (34%), and then from CLC to metastases and CRPC (62% and 92%). The expression of EMT target genes was more fluctuant according to disease stages, although in CLC N-cadherin was closely associated with Zeb1 staining. In CLC, after adjusting for classical prognostic markers, only vimentin expression was significantly predictive of shorter recurrence-free survival. In CRPC, preserved E-cadherin staining was associated with longer overall survival, and Zeb1 expression in metastases was predictive of decreased survival. Although Zeb1 expression increased according to the different steps of PCa progression, the expression of its target genes does not seem to follow the same kinetics. However, the potential clinical interest of these EMT markers at several stages of the disease is strongly suggested by their predictive value on both recurrence-free and overall survival.

1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed non-cutaneous cancer in men, and a leading cause of cancer-related death in industrialized countries [1]. Locally confined (pT2) or even locally advanced (pT3) PCa can be treated by surgery, with most often a favorable oncologic outcome. For metastatic PCa, either at diagnosis or after recurrence, androgen-deprivation therapy (ADT) is the standard of care. In these patients, despite an initial response to hormonal treatment, the tumors eventually progress to a castration-resistant stage, leading to poor prognosis. At each step of the disease, biomarkers are needed to better predict recurrence after treatment and survival, in order to improve clinical management.

☆ Competing interests: No conflict of interest.
☆☆ Funding/Support: Region Centre.
* Corresponding author at: Service d'Anatomie Pathologique, Hopital Bretonneau, CHRU Tours, Blvd Tonnelle, 37000 Tours, France.
E-mail address: gaelle.fromont-hankard@univ-tours.fr (G. Fromont).

http://dx.doi.org/10.1016/j.humpath.2016.10.013
0046-8177/

Cancer progression is dependent upon the epithelial-to-mesenchymal transition (EMT) process, which allows epithelial cancer cells to acquire migratory and invasive characteristics, properties essential for dissemination and distant metastasis. EMT is mediated by key transcription factors, which induce the repression of epithelial genes like E-cadherin and the induction of mesenchymal genes like vimentin and N-cadherin. Among the transcription factors involved in EMT, Zeb 1 (zinc finger enhancer binding protein) is a central actor that can be induced not only by microenvironmental factors, by also by other EMT transcription factors, like SNAIL [2]. It has been shown in vitro that Zeb1 can induce EMT in PCa cells, repress E-cadherin, increase mesenchymal markers, and promote cancer cell migration and invasion [3]. Zeb1 expression in human cancers has been evidenced in colorectal carcinoma, uterine cancer, and clinically localized PCa [3,4]. EMT target genes have also been studied in vivo in human PCa, mainly E-cadherin which repression has been associated with increased Gleason score in clinically localized cancer [5-8].

The joint analysis of both Zeb1 and target genes has not been performed at the different stages of PCa natural history, and their clinical significance remains unknown.

2. Materials and methods

2.1. Patients and samples

Normal prostate tissues (n = 58) were obtained from patients treated by cystoprostatectomy for bladder carcinoma, without incidental prostate cancer. Hormone naïve clinically localized cancer samples (CLC) (n = 242), were obtained from patients treated by radical prostatectomy (without previous hormonal deprivation therapy) for localized PCa at Tours University Hospital and Institut Mutualiste Montsouris.

CLC cases (n = 242) were composed of 121 tumors with negative surgical margins and biochemical relapse (defined as 2 consecutive increases in serum PSA 0.2 ng/mL or greater), matched with 121 tumors without recurrence. Each patient with biochemical relapse was matched with 1 patient with identical age group, preoperative PSA, Gleason score and pathological stage, but who was free of recurrence after at least the same follow-up. This matching allows to have already taken into account the traditional predictive markers, in order to analyze the prognostic value of candidate markers. The median time to recurrence was 19 months (range, 2–90), and the median follow-up in the group of patients without recurrence was 55 months (range, 25–95).

Forty-eight cases of castration-resistant prostate cancers (CRPC) were selected from patients treated with exclusive ADT. Patients were selected when they initially responded to exclusive ADT and had post hormonal relapse tissue sample suitable for analysis. Hormonal relapse was defined as 2 consecutive rises in PSA, with serum testosterone under castration level (50 ng/dL). Tissues were collected by transurethral resection, performed because of lower urinary tract symptoms associated with local tumor progression. The median follow-up after tissue collection was 9 months (range, 0.3–86.5). The median overall survival from tissue collection to death was 7.6 months (0.3–37.6 months).

Forty-three cases of metastatic prostate cancer were selected from patients with tissues available for analysis, either lymph nodes (n = 28) or bone (n = 15). Among these patients, 11 had been previously treated by hormone deprivation (all with bone metastasis) and were castration resistant. The median follow-up after tissue collection was 18 months (range 1–139). The median overall survival from tissue collection to death was 14 months (1–120 months).

The characteristics of patients and tissues are summarized in Table 1. Written informed consents were obtained from patients in accordance with the requirements of the medical ethics committee of our institutes.

2.2. Immunohistochemistry on tissue microarray

2.2.1. TMA construction

TMAs were constructed using formalin-fixed paraffin-embedded tissue samples. Original slides stained with hematoxylin-eosin were reviewed using the 2009 TNM classification and the 2014 modified "Gleason" system. Areas of normal tissue, tumoral tissue or PIN (prostate intraepithelial neoplasia) were selected. For each case, a minimum of 4 cores (0.6-1 mm diameter) were transferred from the selected areas to the recipient block, using a TMA workstation (Manual Tissue Arrayer MTA Booster, Alphelys, France). Serial 3 μm sections of the TMA blocks were used for immunohistochemistry. One section in 10 was stained with hematoxylin-eosin to check that the cores adequately represented diagnostic areas.

Table 1 Patients and tissues characteristics

Groups	NL (n = 58)	CLC (n = 242)	CRPC (n = 48)	Metastases (n = 43)
Age y, median (range)	64 (48-80)	63 (46-74)	72 (56-86)	66 (51-77)
PSA (ng/mL)	–	9.4 (1.5-23)	12.5 (0.2-285)	12.4 (3.1-4894)
pTNM				
pT2	NA	152	NA	NA
pT3		90		
Gleason score				
6	NA	58	NA	NA
7 (3 + 4)		66		
7 (4 + 3)		98		
8 and more		20		

Abbreviations: NL, normal prostate tissue; CLC, hormone-naïve clinically localized cancer; CRPC, castration-resistant prostate cancer; NA, not applicable.