Human Pathology (2013) **44**, 1617–1623

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

8q24 amplification is associated with Myc expression and prostate cancer progression and is an independent predictor of recurrence after radical prostatectomy ☆

Gaelle Fromont MD, PhD [a,b,*], Julie Godet HT [a], Antoine Peyret MHS [a], Jacques Irani MD, PhD [c], Olivier Celhay MD [c], François Rozet MD [d], Xavier Cathelineau MD [d], Olivier Cussenot MD, PhD [b,e]

[a] *Department of Pathology, CHU/Universite de Poitiers, 86000 Poitiers, France*
[b] *Centre d'Etude et de Recherche sur les Pathologies Prostatiques, 75020 Paris, France*
[c] *Department of Urology, CHU/Universite de Poitiers, 86000 Poitiers, France*
[d] *Department of Urology, Institut Mutualiste Montsouris, 75014 Paris, France*
[e] *ER2-UPMC, Hôpital Tenon, APHP, 75020 Paris, France*

Received 9 November 2012; revised 14 January 2013; accepted 15 January 2013

Keywords:
Prostatic neoplasms;
Prostatectomy;
Metastasis;
Prognosis;
Immunohistochemistry;
Fluorescence in situ hybridization;
Tissue microarrays;
Myc;
Gene amplification

Summary Genomic alterations affecting the 8q24 region are frequent in prostate cancer. Together with the oncogene *MYC*, other genes located in the surrounding of the amplified region could also be candidate targets. Tissue microarrays were constructed with prostate cancer tissues from (1) a case-control population of patients treated by radical prostatectomy (n = 242; 121 cases with biochemical relapse matched with 121 cancers with identical clinicopathologic features but without relapse), (2) castration-resistant disease (n = 55), and (3) metastatic cancers (n = 28). Fluorescence in situ hybridization and immunohistochemistry were used on tissue microarrays and slides to analyze, respectively, the amplification status of 8q24 and protein expression of genes located at 8q24. Amplification at the *MYC* locus was observed in 29% of cases and was closely associated with both disease progression (from 15% in pT2 tumors to 53% in metastasis; $P = .001$), and Gleason score (from <3% in Gleason 6 tumors to 66% in Gleason 8 and more tumors; $P < .0001$). The expression of genes located at 8q24 did not correlated with the amplification status, except for the Myc protein ($P = .002$). *MYC* amplification status but not Myc protein expression was significantly predictive of biochemical recurrence after prostatectomy, together with the proliferation marker Ki-67 and independently from known prognostic factors, including TNM stage and Gleason score. The *MYC* amplification status could constitute a useful prognostic tool for patients treated by radical prostatectomy, particularly for those with d'Amico intermediate risk, whose clinical behavior is currently difficult to predict.

1. Introduction

Among the genomic alterations associated with both the development and the progression of prostate cancer, those affecting the 8q24 region are of particular interest. Overrepresentation of 8q24 determined by either comparative

☆ Conflict of interest: We have no conflict of interest to declare.

* Corresponding author. Service d'Anatomie Pathologique, CHU Jean Bernard, Rue de la Miletrie, 86000 Poitiers, France.
E-mail address: g.fromont@chu-poitiers.fr (G. Fromont).

0046-8177/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.01.012

genomic hybridization or fluorescence in situ hybridization (FISH) is frequent in prostate cancer cells and has been associated with cancer progression and metastasis [1–5]. Together with 8q24, several regions have been shown to be coamplified at 8q in prostate cancer tissues [6]. In addition, the gained region at 8q24 is known to harbor a number of risk variants that are associated with inherited susceptibility for prostate cancer, with some variants being linked to a more aggressive phenotype [7–10]. The amplified region contains the oncogene *MYC* that has been shown to be overexpressed at both messenger RNA and protein levels in prostate cancer [2,5,11]. However, no association between 8q24 amplification at the *MYC* locus and Myc protein expression has been evidenced to date in prostate tissues. Although it has been suggested that 8q gain on prostate cancer biopsies could be associated with worse survival [3,4], the independent prognostic value of both 8q24 genomic alterations and Myc protein expression in clinically localized prostate cancer remains to be assessed. Moreover, besides *MYC*, other genes located at 8q24, in the surrounding of the amplified region, could also be candidate targets. Among them, the protein thyrosine phosphatase 4A3 (PRL3), whose gene is located at 8q24.3, has been linked to metastatic risk in several types of cancers [12]. The focal adhesion kinase (FAK), at 8q24-qter, regulates several cellular processes including proliferation and migration, and its expression has been shown to be increased in prostate cancer compared with normal tissues [13]. The squalene epoxidase (SQLE), located at 8q24.1, is involved in the synthesis of cholesterol and precursors of the steroid hormones. In breast cancer, SQLE expression has been recently associated with both 8q amplification and poor prognosis [14].

In the present study, we aimed to analyze in a large cohort of prostate cancer tissues the amplification status of 8q24 at the *MYC* locus, together with the protein expression of Myc and other candidate genes located at 8q24, to correlate genomic amplification and protein expression status with disease stage, aggressiveness, and recurrence after treatment.

2. Patients and methods

2.1. Patients

2.1.1. Clinically localized prostate cancer

Among patients treated at the Montsouris Institute between 2000 and 2005 by radical prostatectomy for clinically localized prostate cancer (CLPC), we selected 1200 cases with negative margins and at least 4-year follow-up. Among these, 192 patients experienced *biochemical recurrence*, defined as 2 consecutive increases in serum PSA 0.2 ng/mL or greater. In the cohort, each of these 192 patients was matched with 1 or 2 (when available) patients who presented identical age group (<50, 50-60, 60-70, and >70 years), preoperative prostate specific antigen (PSA) rate group (<10, 10-15, and 15-20 ng/mL), Gleason score, and pathologic stage but were free of recurrence after at least the same follow-up. Eventually, we obtained a total of 544 patients. The centralized review of the initial slides led to pathologic reassessment in 85 cases [15]. When 2 patients without recurrence remained matched with 1 patient with recurrence, the patient with the longer follow-up was chosen. At the end, 121 prostate cancers with biochemical relapse remained matched with 121 tumors without recurrence. The characteristics of patients are summarized in Table 1.

2.1.2. Castration-resistant prostate cancer

Fifty-five cases of castration-resistant prostate cancer (CRPC) were selected from 323 patients treated in the University Hospital of Poitiers with exclusive androgen deprivation therapy, between 1988 and 2008. Patients were selected if they initially responded to exclusive androgen deprivation therapy (decrease in PSA level without clinical or radiologic progression) and had posthormonal relapse tissue sample suitable for analysis. Hormonal therapies were Luteinizing hormone releasing hormone (LHRH) agonist, steroidal or nonsteroidal antiandrogen, or complete androgen blockage. No patient received chemotherapy or radiation therapy. *Hormonal relapse* was defined as 2 consecutive increases in PSA with serum testosterone level under castration level (50 ng/dL). Tissues were collected by transurethral resection, performed in all cases because of lower urinary tract symptoms associated with local progression.

2.1.3. Metastases

Twenty-eight cases of metastases from prostate cancer were obtained from patients treated in the University Hospital of Poitiers. These patients had been previously (in case of bone metastases) or subsequently (lymph node metastases) treated for prostate cancer by either radical prostatectomy or

Table 1 Patient characteristics for the 242 matched prostate cancer cases treated by radical prostatectomy

	Group 1 (R+)	Group 2 (R−)
Age (y), median (range)	63 (46-74)	63 (47-74)
Preoperative PSA (ng/mL), median (range)	9 (1.5-20)	9 (2-20)
Gleason score		
6	30	30
7 (3 + 4)	37	37
7 (4 + 3)	44	44
≥8	10	10
Pathologic stage		
pT2	80	80
pT3	41	41
Follow-up, time to recurrence (mo), median (range)	20 (3-90)	86 (48-128)

Abbreviations: R+, biochemical recurrence after radical prostatectomy; R−, no recurrence.