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Original article

Genetic profile identification in clinically localized prostate carcinoma☆

Michele Gallucci, M.D.[a], Roberta Merola, Ph.D.[b], Costantino Leonardo, M.D.[a], Piero De Carli, M.D.[a], Antonella Farsetti, M.D.[c], Steno Sentinelli, M.D.[d], Isabella Sperduti, Ph.D.[e], Marcella Mottolese, Ph.D.[d], Paolo Carlini, M.D.[f], Erika Vico, Ph.D.[b], Giuseppe Simone, M.D.[a], AnnaMaria Cianciulli, Ph.D.[b,*]

[a] *Department of Urology, Regina Elena Cancer Institute, Rome, Italy*
[b] *Department of Clinical Pathology (Cytogenetic Unit), Regina Elena Cancer Institute, Rome, Italy*
[c] *Institute of Neurobiology and Molecular Medicine, National Research Council, Rome, Italy*
[d] *Department of Pathology, Regina Elena Cancer Institute, Rome, Italy*
[e] *Biostatistic Unit, Regina Elena Cancer Institute, Rome, Italy*
[f] *Department of Oncology, Regina Elena Cancer Institute, Rome, Italy*

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Abstract

Purpose: To confirm our previously obtained results, we genetically characterized prostate cancer from patients undergo radical prostatectomy in a retrospective study.

Materials and methods: Histological sections were evaluated for 106 patients treated with surgery from 1991 to 2004. With fluorescence in situ hybridization (FISH) method, the status of LPL (8p22), c-MYC (8q24) genes and 7, 8, X chromosomes was evaluated.

Results: Chromosomes 7, 8, X aneusomy was demonstrated in 91.5%, 78.3%, and 51.9% of the samples, respectively, whereas LPL deletion and MYC amplification were found in 76.0% and 1.6%. A genetic profile was considered as unfavorable when at least two aneusomic chromosomes and one altered gene were present. Tumors with an adverse genetic profile were more frequently present in patients with higher stages ($P = 0.02$), biochemical/clinical progression ($P = 0.03$), and Gleason grade 4 + 3 ($P = 0.02$). Multiple correspondence analysis identified one tumor group characterized by chromosome 8 aneusomy, X polysomy, LPL gene deletion, Gleason > 7 and 4 + 3 associated with progression.

Conclusions: In this study, we recognized the predictive power of previously identified cytogenetic profiles. Assessment of genetic set may characterize each patient and have influence on postoperative therapeutic strategies.

Keywords: Prostate cancer; Cancer genetics; Chromosomal abnormalities; Prognostic studies

1. Introduction

The prognosis and choice of therapy for prostate cancer (PC) are based primarily on 3 parameters obtained at the time of diagnosis: clinical stage, serum prostate-specific antigen (PSA), and degree of tumor differentiation (Gleason score), however, these do not provide enough predictive information regarding the clinical outcome [1]. The identification of patients who have an increased risk of progression and/or postoperative recurrence is an important goal for PC research, as such patients could be candidates for newer treatments and follow-up strategies.

The goal is to recognize, through emerging technologies, the genetic profiles driving the aggressiveness of PC. With the rapid development of molecular cytogenetics, a series of genetic alterations on multiple chromosomes has been detected in PC [2,3]. Deletion in sporadic PC most commonly occurs at chromosomal locus 8p21–22 [4,5,6]; 8p22 loss, concurrent with the gain of copy number of chromosome 8 (aneusomy), may successfully predict disease recurrence [7]. The commonly deleted region of 8p22 includes the LPL (lipoprotein lipase) gene that is suggested to be responsible for the initiation or early event in prostate tumorigenesis. Another important genetic alteration in PC is 8q24 overrepresentation, which is commonly found in advanced, meta-

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* Corresponding author. Tel./fax: +39-6-52665966.
E-mail address: cianciulli@ifo.it (A.M. Cianciulli).

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static, and androgen-independent PC [8]. The region contains an oncogene MYC, which regulates cell proliferation and apoptosis [9]. Aneusomies of chromosomes 7 have also been observed frequently with the gain of chromosome 7 and loss of 7q31 [10]. Chromosome 7 alterations were known to be associated with higher tumor grade, advanced pathological stage and poor prognosis [11]. The androgen receptor gene located on chromosome Xq11–13 encodes the androgen receptor protein through which androgens exert their intracellular regulation of prostate growth and cellular differentiation [12]. Additional androgen receptor gene copies are present in patients with PC due to polysomy of the chromosome X [13].

Our previous contribution to this area of investigation has demonstrated that the combined 7, 8, X chromosomes and MYC (8q24), LPL (8p22) gene anomaly patterns identify cytogenetic profiles as additional markers to pathological features in clinically localized prostate carcinoma [14].

In order to develop a more detailed understanding of the involvement of chromosomes 7, 8, X and MYC (8q24), LPL (8p22) gene anomalies in human PC and to define the potentially predictive biological profiles for a bad or good prognosis, in this study we confirmed our previously obtained results by analyzing independent set samples from patients with adequate follow-up in a retrospective study. The criteria for patient selection were absence of hormonal neoadjuvant treatment before surgery and diagnosis of clinically localized disease.

These results allowed us to identify a poor genetic profile (at least 2 aneusomic chromosomes and 1 altered gene present), that can be integrated with the grade and clinical information. The aim would be to clearly divide cancer patients into 2 groups, with indolent or aggressive prostate tumors. Moreover, the association of specific genetic lesions (chromosome X polysomy, chromosome 8 aneusomy, and LPL gene deletion) with progression may help to improve therapeutic options after surgery.

2. Materials and methods

2.1. Patients

The samples included in the previous investigation were excluded in this validation study, which was done on an independent set. Histological sections were evaluated for 106 patients treated with radical prostatectomy for clinically circumscribed PC from 1991 to 2004. Patients who received neoadjuvant treatment were excluded from the study. Complete information on pretreatment PSA-values was available in all patients. Postoperative serial PSA measurements were done semiannually within the first 2 years and annually thereafter. A postoperative PSA-level of 0.1 ng/ml and rinsing concentrations were considered as biochemical evidence of tumor recurrence. All samples were reviewed by a pathologist with experience in uropathology. The largest tumor focus and/or the focus with the worst Gleason grade were marked on the slides. The pathologic stage for each case was assigned according to the Union Internationale Contre le Cancer (UICC, 2002) TNM system [15]. In 23 tumors with bilateral involvement, the slides obtained from both lobes were selected for genetic evaluation, which was consequently performed on 129 foci. The Gleason grade (G) was determined for each prostate carcinoma focus. All clinicopathological characteristics are illustrated in Table 1. Follow-up data were obtained from hospital charts and correspondence with the referring physicians. In these patients, with a median follow-up of 48 months, 34 relapses (18 biochemical and 16 clinical progression), and 10 prostate cancer-related deaths were recorded. As a negative control population, normal prostatic tissue samples from patients undergoing cystectomy were used. In addition, as positive control we used prostatic cell lines (LNCaP).

Table 1
Clinicopathologic characteristics

Characteristics	*n* Cases	%
No patients	106	
PSA		
0–3.9	8	7.5
4–9.9	25	23.6
10–19.9	31	29.2
>20	18	17.0
Histologic stage		
$T_{2a–2b}$	15	14.1
T_{2c}	42	39.6
$T_{3a–3b}$	47	44.4
T_4	1	0.9
Gleason grade		
<7	30	23.3
3 + 4	50	38.8
4 + 3	22	17.1
>7	27	20.9
Lymph node		
Negative	99	76.7
Positive	2	1.6
Follow-up (months)		
Median	48.00	
Range	6–178	

2.2. Fluorescence in situ hybridization (FISH) analysis

The Vysis ProVysion Multi-color mixture (Vysis, Inc., Downers Grove, IL) was used for detection and quantification of 8 chromosome labeled with SpectrumAqua, LPL (8p22) gene labeled with SpectrumOrange, and c-MYC gene (8q24) labeled with SpectrumGreen. We also employed chromosome enumeration probes (CEP) specific for X and 7 chromosomes (Vysis, Inc.). In brief, after deparaffinization, specimens were incubated in pretreatment solution (80°C, 20 min) and then digested with protease (37°C, 25 min) (Paraffin Pretreatment Kit II; Vysis, Inc. Downers Grove, IL). The probes were applied, a coverslip sealed to the slide, and the specimens denatured (75°C, 5 min) and hybridized overnight (37°C) in a humidified chamber (Hybrite