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Original contribution

Androgen deprivation modulates gene expression profile along prostate cancer progression☆,☆☆

Marco Volante MD[a,*], Daniele Tota MD[a], Jessica Giorcelli[a], Enrico Bollito MD[b], Francesca Napoli BSc[a], Simona Vatrano BSc[a], Consuelo Buttigliero MD[a], Luca Molinaro MD[c], Paolo Gontero MD[d], Francesco Porpiglia MD[a], Marcello Tucci MD[e], Mauro Papotti MD[a], Alfredo Berruti MD[f], Ida Rapa PhD[a]

[a]*Department of Oncology at San Luigi Hospital, University of Turin, Orbassano, Turin 10043, Italy*
[b]*Division of Pathology at San Luigi Hospital, Orbassano, Orbassano, Turin 10043, Italy*
[c]*Division of Pathology, Città della Salute Hospital, Turin 10126, Italy*
[d]*Division of Urology, Città della Salute Hospital, Turin 10126, Italy*
[e]*Division of Medical Oncology at San Luigi Hospital, Orbassano, Turin 10043, Italy*
[f]*Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Brescia 25121, Italy*

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Summary Androgen deprivation therapy (ADT) is the standard of care for metastatic prostate cancer and initially induces tumor regression, but invariably results in castration-resistant prostate cancer through various mechanisms, incompletely discovered. Our aim was to analyze the dynamic modulation, determined by ADT, of the expression of selected genes involved in the pathogenesis and progression of prostate cancer (*TMPRSS2:ERG*, *WNT11*, *SPINK1*, *CHGA*, *AR*, and *SPDEF*) using real-time polymerase chain reaction in a series of 59 surgical samples of prostate carcinomas, including 37 cases preoperatively treated with ADT and 22 untreated cases, and in 43 corresponding biopsies. The same genes were analyzed in androgen-deprived and control LNCaP cells. Three genes were significantly up-modulated (*WNT11* and *AR*) or down-modulated (*SPDEF*) in patients treated with ADT versus untreated cases, as well as in androgen-deprived LNCaP cells. The effect of ADT on *CHGA* gene up-modulation was almost exclusively detected in cases positive for the *TMPRSS2:ERG* fusion. The correlation between biopsy and surgical samples was poor for most of the tested genes. Gene expression analysis of separate tumor areas from the same patient showed an extremely heterogeneous profile in the 6 tested cases (all untreated). In conclusion, our results strengthened the implication of ADT in promoting a prostate cancer aggressive phenotype and identified potential biomarkers, with special reference to the *TMPRSS2:ERG* fusion, which might favor the development of neuroendocrine differentiation in hormone-treated patients. However, intratumoral heterogeneity limits the use of gene expression analysis as a potential prognostic or predictive biomarker in patients treated with ADT.

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* Corresponding author at: Department of Oncology, University of Turin, San Luigi Hospital, Regione Gonzole 10, 10043 Orbassano, Orbassano, Turin 10043, Italy.
E-mail addresses: marco.volante@unito.it (M. Volante), tota.daniele@gmail.com (D. Tota), gio.jessica@gmail.com (J. Giorcelli), e.bollito@libero.it (E. Bollito), francesca.napoli2386@gmail.com (F. Napoli), simona.vatrano@unito.it (S. Vatrano), consuelo.buttigliero@gmail.com (C. Buttigliero), luca.molinaro@unito.it (L. Molinaro), paolo.gontero@unito.it (P. Gontero), francesco.porpiglia@unito.it (F. Porpiglia), marcello.tucci@gmail.com (M. Tucci), mauro.papotti@unito.it (M. Papotti), alfredo.berruti@gmail.com (A. Berruti), ida.rapa@unito.it (I. Rapa).

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1. Introduction

Prostate cancer (PCa) is among the most common adult malignancies. Androgen deprivation therapy (ADT) is the standard of care for metastatic PCa and initially induces tumor regression in most patients. However, ADT inevitably results in castration-resistant PCa (CRPC), which at least initially still remains androgen receptor (*AR*)–driven, through various mechanisms, such as aberrant expression, de novo intraprostatic androgen production, cross-talk with other oncogenic pathways, and reactivation of epithelial-mesenchymal transition processes that may facilitate tumor progression and therapeutic resistance [1].

The effect of ADT on the modulation of other molecules involved in pathogenesis and progression of PCa is largely unknown. Among these, Ets-related gene (*ERG*), a member of the Ets transcription factors, involved in many cell processes such as proliferation, apoptosis, angiogenesis, and metastasis, and is most frequently fused with transmembrane-protease-serine2, an androgen-dependent protease (*TMPRSS2*) in 40% to 50% of PCa [2]. The prognostic role of this fusion gene in PCa is controversial; some studies showed that the presence of the translocation *TMPRSS2*:*ERG* seems to predict cancer recurrence after surgery [3], whereas a null or positive prognostic effects were found by others [4,5]. About 10% of *TMPRSS2*:*ERG*-negative PCas express high levels of serine peptidase inhibitor, kazal type 1 (*SPINK1*) and are associated with poor prognosis [6]. Another Ets family member, SAM-pointed domain containing ETS transcription factor (*SPDEF*), which positively regulates *PSA* gene in normal prostate cells, is lost in more aggressive and metastatic tumors [7].

Among the mechanisms modulated by ADT and associated with the onset of CRPC, neuroendocrine differentiation (NED) has been widely investigated in vitro, at the tissue level, or as biochemical markers [8,9]. Literature evidence demonstrated that NED is associated with higher Gleason score and stage as well as poor prognosis [10], although this latter observation has been challenged by other authors, with special reference to its prognostic impact in patients with hormone-naïve disease [11]. Indeed, the transdifferentiation process from an epithelial-like to an NE-like phenotype can be considered a consequence of the selective pressure induced by androgen deprivation. The driving events in the pathogenesis of NED include loss of *AR* and androgen-regulated protein expression and induction of NED and neural programs. Therefore, NED is a hormone-refractory and not castration-resistant phenotype.

This scenario underlines that CRPC is a complex and heterogeneous disease whose heterogeneity is destined to increase over time as a consequence of specific antineoplastic therapies such as newer endocrine therapies (such as abiraterone and enzalutamide) and chemotherapy.

Therefore, it is of major interest to discover and validate biomarkers associated with the response to ADT to improve patients' selection and prevent the onset of a more aggressive hormone-resistant tumor.

In the past, one of the major limitations for studying PCa has been the lack of posttreatment biopsies. The collection of tumor samples for molecular profiling at various time points during therapy and progression is relevant in identifying patients undergoing transformation and in understanding the molecular mechanisms underlying transdifferentiation.

Neoadjuvant therapy offers the unique opportunity to collect tumor samples at diagnosis and after treatment, thus providing important information on the interaction between drugs and tumor biology and on mechanisms of efficacy and resistance of specific therapies.

The present study was designed to (i) analyze the dynamic changes induced by neoadjuvant ADT in genes involved in the pathogenesis and progression of PCa, and (ii) compare the expression profiles in biopsy and prostatectomy specimens to validate their potential role as preclinical biomarkers.

2. Materials and methods

2.1. Case selection

A series of 59 samples of PCa was collected from the archives of the Divisions of Pathology at San Luigi Hospital (Orbassano, Turin) and at the Città della Salute Hospital (Turin), according to the following criteria: histologically confirmed diagnosis of prostate adenocarcinoma and availability of representative tumor tissue from both prostatectomy material and the corresponding diagnostic transrectal biopsy.

The cases were divided into 2 groups: group 1, untreated patients (22 patients; NT), and group 2, patients treated with luteinizing hormone–releasing hormone analogue between the first diagnostic biopsy and surgery (37 patients; ADT+).

For each patient, clinicopathological parameters (age, Gleason score—2014 modification—on diagnostic biopsy and prostatectomy, TNM, and status of surgical margins) were collected. The newly proposed Gleason score–based grading group stratification was also considered [12]. Positive resection margins were subdivided into focal (a single focus $\leq$0.8 mm in greatest extension) and extensive [13]. All tissue samples were anonymized by a staff member of the Pathology Department not involved in the study, and the study was approved by the institutional review board of the hospital.

2.2. Cell line and cultures

To validate in vivo results, the androgen-sensitive LNCaP PCa cell line was purchased from the American Type Culture Collection (Manassas, VA) and maintained in Roswell Park Memorial Institute (RPMI)–1640 (Sigma-Aldrich, St Louis, MO) supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin (25 U/mL), and streptomycin (25 mg/mL; all from Sigma) in a humidified atmosphere containing 5% CO_2 at 37°C. For androgen withdrawal experiments, LNCaP cells