Overexpression of Cyclin D1 Is Associated with Metastatic Prostate Cancer to Bone

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ABSTRACT

Cyclin D1 is a key regulator of the G1 phase progression of the cell cycle. There is increasing evidence that deregulated cyclin D1 expression is implicated in tumorigenesis and tumor progression in certain neoplasms. Recently, it has been reported that cyclin D1 overexpression might be related to the evolution of androgen-independent disease in prostate cancer. This study was conducted to investigate patterns of cyclin D1 expression in prostate cancer samples representing different points in the natural history and treatment evolution of the disease. Association with clinical outcomes was also explored. Using immunohistochemistry, 86 radical prostatectomy specimens (53 naïve and 33 after androgen deprivation) and 22 androgen-independent bone metastases were studied. We examined the difference in cyclin D1 expression in primary versus metastatic cases. In addition, we examined the association in primary cases between cyclin D1 expression and clinicopathological parameters of poor clinical outcome, including time to prostate-specific antigen relapse and Ki67 proliferative index. Cyclin D1-positive phenotype, defined as identification of positive immunoreactivity in the nuclei of ≥20% of tumor cells, was observed in 10 of 86 (11%) primary cases compared with 15 of 22 (68%) androgen-independent bone metastases (P = 0.001). There was no correlation between cyclin D1 overexpression and either Gleason score, neo-adjuvant hormone treatment, or prostate-specific antigen relapse. We observed a statistical association between cyclin D1 overexpression and high Ki67 proliferative index, defined as ≥20% of positive tumor cells (P = 0.02). These data support the hypothesis that cyclin D1 overexpression may represent an oncogenic event in androgen-independent metastatic prostate cancer to the bone.

INTRODUCTION

Correlating molecular staging with outcome requires a defined clinical framework on which to base prognostication. In prostate cancer, this is achieved by considering the history of the disease, from diagnosis to death, as a series of clinical states that include localized disease, PSA4 relapse after primary therapy, and metastatic androgen-dependent and -independent disease. Factors predictive in the early stage of the disease may not be predictive in late-stage tumors and vice versa. A commonly held belief is that all prostate cancers have a low proliferative index (1–3). This conclusion is based on studies of androgen-dependent primary tumors. However, few investigators have focused on changes in the disease as it progresses to the androgen-independent and the metastatic phenotypes.

In a recent report, we characterized, using a radionuclide bone scan, doubling times of androgen-independent prostate cancers based on serial changes in the percentage of bone involved by tumor. The estimated doubling time was 43 days (4), significantly shorter than estimates based on measurements of proliferative index in the primary tumor and of serial changes in PSA after relapse from primary treatment.

Additional support for a high proliferative index and the potential role of cyclin D1 in androgen-independent disease was derived from both in vitro and in vivo data. In the in vitro data, the transfection of LNCaP cells with a retroviral vector containing the human cyclin D1 produced an increase in S-phase fraction, as well as a decrease in growth factor requirements for proliferation. In the in vivo data, cyclin D1-transduced cells formed tumors more rapidly when implanted into nude mice and were resistant to the growth-inhibitory effect of castration as seen with the nontransfected LNCaP cell. These lesions were observed to be refractory to androgen-ablative treatment by castration (5). In addition, using the CWR22 xenograft prostate cancer model, we developed androgen-independent sublines that emerged after androgen withdrawal. These tumor cells were found uniformly to possess cyclin D1 overexpression (6).

Several studies have suggested that amplification and/or overexpression of cyclin D1 is not a common event in both primary and tumor-derived prostate cell lines (7–9). This study was conducted to investigate patterns of cyclin D1 expression in prostate cancer samples representing different points in the natural history and treatment evolution of the disease. We also correlated cyclin D1 phenotypes with clinicopathological parameters and with proliferative index in an attempt to define

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4 The abbreviation used is: PSA, prostate-specific antigen.
their potential biological and clinical significance in prostate cancer.

**MATERIALS AND METHODS**

**Clinical and Pathological Data.** A cohort of 108 patients with prostatic carcinoma, which included 86 primary and 22 bone metastases to the axial skeleton, were evaluated. All primary tumors (n = 86) represented consecutive cases of patients who underwent radical prostatectomy for prostatic cancer between 1990 and 1991 at Memorial Sloan-Kettering Cancer Center and who were followed up at the center. All metastatic cases (n = 22) were included on the basis of tissue availability in the tumor bank and complete clinical information. Samples were formalin-fixed, paraffin-embedded tissue specimens. Representative H&E-stained sections of each paraffin block were examined microscopically to confirm the presence of tumor, as well as to evaluate the pathological grade and stage of the tumors analyzed. Of the patients with primary carcinoma, 33 of 86 received preoperatively neoadjuvant hormone therapy (a complete androgen blockade: 1–3 months), whereas the remaining 53 patients were not treated with such protocols and were considered hormone-naïve. Hormone-naïve primary cases with sufficient tumor representation on tissue sections were assigned histological grade (n = 47). Cases were grouped as either low Gleason score (<7 (n = 29) or high Gleason score ≥7 (n = 18). Cases were also grouped according to pathological stage, into either early organ-confined tumors, pT2 (n = 51), or advanced tumors extending beyond prostatic capsule, pT ≥3 (n = 35). The response variable time to PSA relapse was defined as the time from radical prostatectomy to the time of the first detectable (nonzero) PSA measurement. To confirm PSA relapse, three consecutive increases of PSA were required; however, the time of relapse was defined as the time of the first detectable PSA measurement. Patients who did not achieve a nonmeasurable PSA after radical prostatectomy were excluded from the analysis.

**Monoclonal Antibodies and Immunohistochemistry.** The two well-characterized antibodies and their corresponding final-working concentrations used for this study were as follows: (a) anticyclin D1 mouse monoclonal antibody (Ab-3, clone DCS-6-IgG1, Oncogene/Calbiochem Laboratories, Cambridge, MA; 1 µg/ml), and (b) anti-Ki67 mouse monoclonal antibody (clone MIB1–IgG1, Immunotech, Marseille, France; 4 µg/ml). A nonspecific mouse IgG1 monoclonal antibody was used as a negative control at similar working concentrations.

**Immunohistochemistry Evaluation.** Nuclear immunoreactivities for both cyclin D1 and Ki67 antigens were recorded as continuous variables; however, they were classified into the following two categories: (a) negative (<20% of tumor cells displaying nuclear immunostaining), and (b) positive (≥20% of tumor cells with nuclear immunostaining), as reported in other studies (10–12).

**Statistical Analysis.** The baseline variables examined were: (a) PSA (in units) at time of diagnosis; (b) Gleason score (divided into two mutually exclusive categories: <7 or ≥7); (c) T stage of disease (2 or ≥3); and (d) percentage of tumor cells with cyclin D1 and Ki67 nuclear expression. Statistical analyses were conducted to assess the following: (a) the correlation between immunophenotypic variables and clinicopathological parameters such as tumor grade, stage, preoperative PSA, hormonal status, and disease status (primary versus metastatic lesions); (b) the correlation among cyclin D1 phenotype and Ki67 proliferative index; and (c) the correlation between cyclin D1 phenotypes and PSA relapse free survival. Fisher’s exact test was used to assess the associations among the different variables, and results were considered significant if the P was < 0.05 (13). The FREQ procedure in SAS was used for this study (14). The univariate associations between time to PSA relapse and the immunophenotypes were evaluated using the log rank test, and survival curves were generated using the Kaplan-Meier estimate (15).

**RESULTS**

Table 1 summarizes immunohistochemical data in relation to clinicopathological parameters. Fig. 1 illustrates the immunohistochemical pattern of cyclin D1 expression and Ki67 proliferative index in representative primary and bone metastatic tumors.

Cyclin D1 positive phenotype, ≥20% of tumor cells displaying nuclear immunostaining, was found in 10 of 86 (11%) radical prostatectomy cases (Fig. 1). This cyclin D1 positive phenotype was found in 3 of 51 (5.9%) pT2 cases compared with 7 of 35 (20%) pT3 lesions. The difference was marginally significant (P = 0.045). We observed that 9 of 40 (22.5%) patients with pretreatment PSA higher than 10 ng/ml had cyclin D1 positive phenotype, compared with 0 of 28 patients with pretreatment PSA between 4 and 10 ng/ml. The association between cyclin D1 and pretreatment PSA level was statistically significant (P = 0.006). However, no association was observed between cyclin D1 positive phenotype and either Gleason score (P = 0.88) or neoadjuvant treatment (P = 0.35). In addition, no association was found between cyclin D1 positive phenotype and time to PSA failure after radical prostatectomy in this cohort of patients (P = 0.2). High proliferative index, ≥20% of tumor cells displaying nuclear Ki67 immunoreactivities, was detected in 11 of 86 (12.7%) radical prostatectomy cases.

Cyclin D1 positive phenotype was observed in 15 of 22 (68.2%) androgen-independent bone metastases compared with 10 of 86 (11.6%) primary cases. This difference was statistically significant (P = 0.001). High proliferative index was identified in 9 of 20 (45%) metastatic evaluable cases compared with 11 of 86 (12.7%) primary tumors (P = 0.002).

A strong association was observed between cyclin D1
positive phenotype and detection of Ki67 high proliferative index in both primary and metastatic cases ($P = 0.02$).

DISCUSSION

This study shows that a significantly greater proportion of androgen-independent metastatic prostate cancers have an increased proliferative activity relative to primary tumors. Associated with this high proliferative index was the overexpression of cyclin D1. No association between cyclin D1 overexpression and time to PSA relapse was demonstrated, but this may have been attributable to the confounding effect of neoadjuvant hormone therapy administered, which is known to delay the time to PSA relapse.

As part of the study, a group of androgen-independent...
metastatic prostate cancer to bone was obtained from our files, based on tissue availability and complete clinicopathological data. This kind of material is difficult to procure, even though prostate cancer has known tropism to bone, because of the fact that it is rarely biopsied. We then examined the potential differences in cyclin D1 expression and proliferative index in both primary and metastatic prostate tumors.

Multiple cyclins have been isolated and characterized, and a temporal map of their expression has been delineated. It is postulated that complexes formed by cyclin D1 and cyclin-dependent kinase-4 govern the G1-S transition in the cell cycle (16). The most frequent genetic abnormality associated with cyclin D1 overexpression is DNA amplification, which usually results in increased gene transcript and protein levels (17). However, studies aimed at the analysis of cyclin D1 expression in primary tumors revealed that protein overexpression frequently occurs in the absence of gene amplification (18,19).

The low frequency (11%) of cyclin D1 positive phenotype in primary prostate cancer observed in this study parallels previous reports dealing with cyclin D1 amplification and/or overexpression in prostate cancer cell lines and primary tumors (7–9). Nevertheless, identification of a positive cyclin D1 phenotype may be significant even in this setting, because identification was associated statistically with higher pretreatment PSA and capsular invasion (pT3 stage). However, it may not predict PSA relapse after radical prostatectomy, which is considered the most sensitive clinical end point of success or failure after definitive treatment. This lack of association has been reported in other studies (8, 20).

The strong association found between cyclin D1 positive phenotype and metastatic bone disease, as well as with high proliferative index, suggests that different cell cycle kinetics govern the growth of metastatic versus primary prostate cancer. We hypothesize that cyclin D1 overexpression acts as an activated oncogenic event and that it might be related to the development of bone metastases and to the evolution of androgen-independent disease.

The distinctive skeletal effect of prostate cancer is its capacity to generate an osteoblastic reaction with new bone formation (21). The link between blastic bone lesions and cyclin D1 overexpression has been observed recently in osteosarcoma, mantle cell lymphoma, and metastatic breast cancer (22–25). Furthermore, in a study investigating osteolytic bone lesions in myeloma patients, cases that were defined as cyclin D1 positive were more frequently associated with extensive bone marrow involvement and increased proliferation (26).

We recently undertook another study to analyze the tumor uptake of fluorinated deoxyglucose, which is known to be correlated with proliferative index (27), in patients with metastatic, progressing prostate cancer. We observed avid fluorinated-deoxyglucose uptake in 70% of metastatic bone lesions (28). In addition, using bone scan index, we observed a relatively short doubling time for metastatic prostate cancer to bone, which could be interpreted as a rapid exponential growth phase of the tumor (4). Taken together, these data strongly support the present observation that metastatic prostate cancer to bone has a high proliferative index.

In vitro and in vivo data substantiate the potential role of sustained overexpression of cyclin D1 in androgen-independent sublines (6, 7). In addition, androgen receptor mutation and amplification has been described in androgen-independent prostate cancer (29). The association between cyclin D1 overexpression and the evolution of hormone-independent disease also has been reported in breast cancer (30). Experimentally overexpressed cyclin D1 can associate with the estrogen receptor and stimulate its transcriptional function in the absence of estrogen. This effect is thought to be separable from the established function of cyclin D1 as a regulator of cyclin-dependent kinases. What remains to be investigated is the mechanism of interaction between cyclin D1 and androgen receptor, although both have been reported to be altered in androgen-independent disease.

We are investigating 17-N-Ally-geldanamycin, an ansamycin antibiotic, which causes G1 arrest in most cancer cell lines. This G1 block is accompanied by down-regulation of D-cyclins and cyclin-dependent kinase-4 expression, rendering pRB on its hypophosphorylated state, which is followed by apoptosis and/or differentiation. This strategy could benefit patients who have androgen-independent prostate cancer with cyclin D1 positive phenotype.

REFERENCES


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