Differential Tumor Expression of Inhibitor of Differentiation-1 in Prostate Cancer Patients With Extreme Clinical Phenotypes and Prognostic Implications

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Abstract

Inhibitor of differentiation-1 (Id1) might constitute a novel prognostic factor able to differentiate indolent from aggressive prostate tumors. In this study, 2 cohorts of 52 and 79 prostate cancer patients were selected for Id1 expression analysis. Higher levels of Id1 protein in advanced poor-prognosis patients and a correlation of higher Id1 mRNA expression levels with a lower survival in stage I to III patients were observed.

Background: In the prostate-specific antigen era, potentially indolent prostate tumors are radically treated, causing overtreatment. Molecular prognostic factors might differentiate indolent from aggressive tumors, allowing avoidance of unnecessary treatment. **Patients and Methods:** Fifty-two prostate cancer patients (20 organ-confined and 32 metastatic) were selected. All formalin-fixed and paraffin-embedded primary biopsies and matched metastases of 15 of them were evaluated for tumor and endothelial cell Id1 protein expression. Seventy-nine additional patients with organ-confined prostate cancer were selected for Id1 mRNA in silico analysis. **Results:** Among metastatic cancer subjects, 48% of primary tumors and 38% of metastases showed Id1 tumor cell expression, and 79% of primary tumors and 81% of metastases showed endothelial immunoreactivity. In the organ-confined group none of them showed Id1 protein tumor cell expression and 50% displayed endothelial expression. In the metastatic patients group, lower levels of Id1 protein predicted a nonsignificant longer overall survival (13 months vs. 7 months; $P = .79$). In the in silico analysis, however, lower levels of Id1 mRNA predicted a longer disease-free survival (61 months vs. not-reached; $P = .018$) and the hazard ratio for progression was 0.451 ($P = .022$) in favor of patients showing lower levels. **Conclusion:** In our cohort, it seems to be a differential epithelial expression of Id1 protein according to the prognostic features (metastatic/poor prognosis vs. organ-confined/good prognosis). In localized tumors treated with radical prostatectomy, higher Id1 mRNA expression levels might predict a higher hazard ratio for progression and a shorter disease-free survival. Further validation of these results in larger prospective series is warranted.

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Introduction

Prostate cancer currently represents the first cause of cancer among men in the United States and Europe. Furthermore, this condition was estimated to account for 20% of all new cancer cases in the developed countries in 2008, causing more than 136,000 deaths.[1]

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However, in the prostate-specific antigen (PSA) era, low-risk organ-confined prostate cancer, defined as a Gleason score of 6 or less, PSA inferior to 10 ng/mL, and T1c to T2a stage now constitutes approximately 50% of all newly diagnosed prostate tumors.[2] Most of these will in fact behave as indolent cancers that will never show clinical progression during the patient's lifetime.

Several clinical and pathological factors help to predict the prognosis of these patients.[3] Nevertheless, to recommend active surveillance to subjects who are unlikely to experience significant progression, and offer radical treatment only to those who are at relevant risk is still a difficult task. Thus, most patients diagnosed with organ-confined prostate cancer undergo some kind of radical treatment on a regular practice basis. This increase in the overtreatment rate results in a significant acute and/or chronic toxicity rate.[4]

A number of molecular factors, such as the presence of the transmembrane protease serine 2:E-twenty six (TMPRSS2:ETS) fusion, along with immunodetection of p27, histone-lysine N-methyltransferase (EZH2), and c-avian myelocytomatosis gene (C-MYC) levels, have been postulated to play an important role in predicting clinical aggressiveness of prostate cancer.[5] Nevertheless, none of these markers have been validated for routine use in the therapeutic decision-making process.

Inhibitor of differentiation-1 (Id1), a family member of the helix-loop-helix proteins, has been shown to be expressed in a variety of tumor types of different origin. For the past several years, the effect of Id1 expression in endothelial and tumor cells of different cancers has been investigated.[6]

Some studies on prostate cancer cell lines and animal models observed that Id1 plays a key role in tumor differentiation,[7] progression,[8] and angiogenesis.[9,10] Most of these functions seem to be exerted by Id1 through the activation of the epidermal growth factor receptor,[11] nuclear factor kappa-light-chain-enhancer of activated B cells (NFK-B),[12] or mitogen-activated protein kinase (MAPK)[13] signaling pathways or the inhibition of others, such as the cyclin-dependent kinase inhibitor 2A (CDKN2A) or p16(INK4a)/retinoblastoma protein (pRB) pathway.[14]

In the case of prostate cancer, Id1 seems to be closely related to tumor cell proliferation, according to Asirvatham et al.[7] Moreover, in vitro assays showed that constitutive expression of Id1, and to a lesser extent Id2, converted cells of the nonaggressive human adenocarcinoma cell line (LNCaP) into more proliferative and invasive cells and increased their secretion of matrix metalloproteinases. In addition, using stable Id1 transfectants, Ling et al found that the expression of Id1 was able to reduce androgen-stimulated growth and S phase fraction of the cell cycle in LNCaP cells, indicating that Id1 might be involved in the development of androgen independency in those cells. Moreover, the Id1-induced androgen-independent prostate cancer cell growth was shown to be correlated with an upregulation of epidermal growth factor receptor and PSA expression.[11]

The analysis of Id1 expression in human prostate cancer specimens has produced, however, controversial results. In most studies, the antibody used for immunohistochemistry (IHC) was a nonspecific polyclonal antibody,[15-17] the only commercially available antibody until recently. In such studies it was often difficult to determine which particular cell type shows Id1 expression, because of the lack of specificity of the antibody and the differences observed in antibody performance between product lots. Thus, the correlation observed between Id1 expression in human prostate cancer samples and prognosis might not be reliable. A highly specific monoclonal antibody against Id1 was further developed. This antibody has been validated using immunohistochemistry, showing that it renders consistent and reliable results.[18,19] In the first publication using this new antibody and according to the results in breast tumors, it was observed that although most of the unselected organ-confined human prostate cancer samples from radical prostatectomies (97%) showed high levels of Id1 on endothelial cells, only a small proportion of tumor samples (3%) showed Id1 expression.[18] This observation was also made in human breast cancer specimens. However, when samples from poorly differentiated and highly aggressive breast carcinoma with metaplastic morphology were selected, the tumor cell expression of Id1 was much greater (22%), suggesting a potential correlation between Id1 breast cancer cell expression and tumor prognosis.[18]

To the best of our knowledge, no studies using the novel antibody have been performed to reassess the potential correlation of Id1 expression with prostate cancer prognosis. Moreover, the correlation of Id1 expression between primary tumors and matched metastases has never been explored. Therefore, the use of this antibody might help to clarify the clinical relevance of Id1 as a prognostic marker.

According to the extreme phenotype selection theory developed by others,[20,21] in the current study we intentionally selected 2 groups of patients showing clinical and pathological opposite prostate cancer prognostic characteristics. We therefore tested the Id1 expression pattern in their tumor diagnostic biopsy samples using a monoclonal antibody in immunohistochemistry analysis. Furthermore, in some of them, metastatic tissues were also available and the expression of Id1 was also studied. In addition, an in silico analysis was also performed to assess the expression of Id1 mRNA in a subset of organ-confined stage I to III prostate cancer patients.

Patients and Methods

Patient Selection

A total of 52 patients diagnosed with prostate adenocarcinoma were selected. All diagnoses were biopsy-confirmed and all patients received standard care at our institution, according to clinical stage. To investigate the effect of prognostic features on Id1 expression pattern, we intentionally selected 2 diverse groups of patients showing clinical and pathological opposite prostate cancer characteristics.

The chosen variables to discriminate extreme clinicopathological phenotypes (poor- vs. good-prognosis patients) were PSA (< 20 vs. ≥ 20 ng/mL), Gleason score (< 8 vs. ≥ 8) and clinical stage (T1-T2 vs. T3-T4). In the metastatic group, the number of metastatic sites and the tumor resistance to docetaxel were also analyzed.

Using these criteria, 20 patients constituted the organ-confined prostate cancer cohort and the metastatic cohort included 32 subjects.

In addition, a third cohort of 79 patients diagnosed with stage I to III primary prostate adenocarcinomas were obtained from Glinsky et al[22] and used for the in silico analysis.

The study protocol was approved by the ethical committee. Reporting recommendations for tumor marker prognostic studies (REMARK) criteria were followed throughout the study.