Pathological Effects of Prostate Cancer Correlate With Neuroendocrine Differentiation and PTEN Expression After Bicalutamide Monotherapy

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Abbreviations and Acronyms

ADT = androgen deprivation therapy
BCR = biochemical recurrence
CgA = chromogranin A
ECE = extracapsular extension
HER = human epidermal receptor
IHC = immunohistochemistry
LN = lymph node
NED = neuroendocrine differentiation
PC = prostate cancer
PI3K = phosphatidylinositol 3-kinase
PSA = prostate specific antigen
PTEN = phosphatase and tensin homologue deleted on chromosome 10
RP = radical prostatectomy
SV = seminal vesicle
TMA = tissue microarray

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Purpose: Androgen deprivation therapy is the primary treatment for advanced prostate cancer but many patients eventually experience progression to hormone refractory status. Understanding the molecular changes after androgen deprivation therapy would help evaluate the efficacy or failure of second line therapies. Therefore, we analyzed the expression of the tumor suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN), the human epidermal receptor-2 and neuroendocrine differentiation after bicalutamide monotherapy, which is emerging as an alternative treatment for locally advanced prostate cancer.

Materials and Methods: Molecular arrangements were evaluated in 107 radical prostatectomy specimens from patients given 150 mg bicalutamide before surgery. Pathological regressive changes, and the correlation of postoperative biochemical failure with the extent of molecular arrangements and pathological effects were analyzed.

Results: Patients with minimal regression effects after bicalutamide therapy had advanced pathological stage disease, and tended to have positive chromogranin A expression and PTEN inactivation. Only 4 (3.7%) prostatectomy specimens showed human epidermal receptor-2 immunostaining. The probability of positive chromogranin A expression in the PTEN inactivation group was 2.5-fold (OR 2.5, 95% CI 1.1–5.6, p = 0.023) higher than in the nonPTEN inactivation group. Cox regression analysis revealed that seminal vesicle invasion, PTEN/chromogranin A expression and lymph node invasion were significant variables for time to biochemical recurrence.

Conclusions: PTEN inactivation and neuroendocrine differentiation were related to refractoriness to bicalutamide therapy. These results support the hypothesis that neuroendocrine differentiation is caused by activation of the serine threonine kinase Akt pathway, which results from PTEN inactivation.

Key Words: prostatic neoplasms, treatment failure, bicalutamide, PTEN phosphohydrolase, epidermal growth factor

ANDROGEN deprivation therapy remains the primary treatment for advanced prostate cancer. Although an initial response rate of ADT is more than 80%, many patients eventually have progression to hormone refractory status and require second line therapies.[1] Knowledge of molecular changes induced by the primary therapy may be informative in the selection and predicted success of subsequent therapies.

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The molecular mechanisms underlying the adaptive phenomenon after ADT have not been determined but the PI3K/Akt signaling pathway,[2,3] HER-2 signaling[4,5] and NED[6,7] are involved. Mutations and the loss of the tumor suppressor PTEN lead to constitutive activation of the Akt pathway, providing a mechanism whereby prostate tumor cells survive after the withdrawal of exogenous trophic factors or androgens because activated Akt inhibits PC cell suicide.[8,9] Constitutive activation of the PI3K-Akt pathway in combination with loss of PTEN has been commonly observed in PC, and results in uncontrolled cell proliferation and decreased apoptosis.[10] High levels of HER-2 can be demonstrated in PC especially after ADT. The increased expression and activation of this receptor start a signaling cascade resulting in tumor proliferation, cell adhesion and invasion.[4,5] Induction and activation of HER-2 have been suggested to occur in an androgen depleted environment or as a result of androgen receptor inactivation, and promote ablation resistant survival of PC cells.[11] NED is also thought to contribute to androgen independent growth of PC.[6,7] ADT is hypothesized to induce NED, and neuroendocrine cells contribute to androgen independent growth of PC in an androgen deprived environment by secreting products that act in a paracrine manner on adjacent nonneuroendocrine tumor cells. Moreover Akt has been shown to be critically involved in NED of prostate cancer after ADT.[12]

Based on these considerations we evaluated these molecular changes in RP specimens in relation to the effects of neoadjuvant bicalutamide therapy because bicalutamide monotherapy at 150 mg daily has been investigated as an alternative treatment for locally advanced PC based on its comparative benefits for quality of life issues and associated morbidity.[13] We analyzed pathological regressive changes, and assessed whether biochemical failure after RP correlated with the extent of molecular changes and pathological effects.

MATERIALS AND METHODS

Patient Cohort and Treatment

After approval from the institutional review board we identified 107 patients who received neoadjuvant bicalutamide therapy (150 mg daily) for 4 months of 685 patients who underwent RP for prostate cancer between August 1995 and June 2007 at our institution. Serum PSA was measured before the start of neoadjuvant bicalutamide therapy and on the day before surgery in all patients. All RPs were performed within 2 weeks of bicalutamide discontinuation. Patients were followed every 3 months during the first 2 years, every 6 months until year 5 and annually thereafter. A detectable postoperative PSA (0.2 ng/ml or greater) at 6 weeks after surgery was defined as persistent PSA and 2 serial detectable PSAs after reaching the nadir or nondetectable level were defined as biochemical failure.

Evaluation of Pathological Regression Effects

The entire surface of the resected prostatectomy specimens was coated with India ink, fixed in 4% buffered formalin and paraffin embedded. Whole mount step sections were cut transversely at 5 mm intervals from the apex of the prostate to the tips of the seminal vesicles. Each section was examined for SV invasion, ECE and surgical margins. The total tumor volume was determined by planimetry using a digitizer tablet (V1—1.0 ml or less, V2—1.1 to 5.0 ml, V3—5.0 ml or greater). All areas of the tumors including index tumor and all satellite tumors were used to determine the total tumor volume. Assessment of neoadjuvant ADT effect was based on criteria reported previously.[14–16] Based on the extent of carcinoma cell degeneration on hematoxylin and eosin sections, pathological regression effects were graded as minimal, moderate or extensive.[16]

Construction of TMAs and IHC

TMAs were prepared using archival formation fixed and paraffin embedded prostatectomy specimens. To minimize misrepresentation due to tumor heterogeneity 3 to 4 cores, 0.6 mm in diameter each, were obtained. An initial screening was performed on each TMA containing 3 areas with definite cancer and on corresponding normal areas. Validation in RP specimens was determined from positively charged slides trimmed from whole mount slides.

After deparaffinization slides were routinely processed for IHC using the conventional avidin-biotin peroxidase complex technique. Antibody against HER-2 was obtained from Invitrogen (Carlsbad, California) and HER-2 evaluation was performed using the HercepTest™. Primary polyclonal antibodies for NED were rabbit anti-human CgA from Dako (Carpinteria, California) and PTEN from Rockland Immunochemicals, Inc. (Gilbertsville, Pennsylvania). Primary antibodies were diluted at 1:100 with Dako antibody diluents, with 0.05M Tris-Hcl buffer containing 0.1% Tween to reduce background, and 15 mM sodium azide. Diaminobenzidine was used as a chromogen with hematoxylin counterstain. Positive control of CgA and PTEN was validated by internal control in normal prostate ducts and glands while HER-2 was used in the breast cancer tissue which had been previously documented HER-2 amplification in fluorescence in situ hybridization analysis.

PTEN immunoreactivity was scored according to the previously described formula, staining index = (cytoplasmic staining intensity $\times$ proportion of immunopositive tumor area).[17] PTEN immunoreactivity was scored on a cytoplasmic staining intensity scale of 0 to 3, and the proportion of immunopositive tumor cells was scored as 10% or less—1, 10% to 50%—2 and 50% or greater—3. A PTEN staining index between 0 and 9 and a PTEN index of 4 or less indicated low expression.

HER-2 immunoreactivity was scored as 0—less than 10% of PC cells stained, 1+— greater than 10% of PC cells had faint and incomplete membranous pattern, 2+— greater than 10% of PC cells had weak to moderate but complete membrane staining, and 3+—greater than 10% of PC cells had strong and complete membrane staining