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Original article

Significance and outcome of nuclear anaplasia and mitotic index in prostatic adenocarcinomas

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Abstract

Objectives: The Gleason grading system measures architectural differentiation and disregards nuclear atypia and the cell proliferation index. Several studies have reported that nuclear grade and mitotic index (MI) are prognostically useful.

Patients and methods: This study included 232 radical prostatectomy specimens. Nuclear anaplasia (NA) was determined on the basis of nucleomegali (at least 20 μm); vesicular chromatin; eosinophilic macronucleoli, nuclear lobulation, and irregular thickened nuclear membranei. The proportion of area of NA was recorded in each tumor in 10% increments. The MI was defined as the number of mitotic figures in 10 consecutive high-power fields (HPF).

Results: In univariate analysis, significant differences included associations between biochemical prostate-specific antigen recurrence (BCR) and Gleason score, extraprostatic extension, positive surgical margin, the presence of high-pathologic stage, NA $\geq$ 10% of tumor area, MI $\geq$ 3/10 HPF, and preoperative prostate-specific antigen. In a stepwise Cox regression model, a positive surgical margin, the presence of a NA $\geq$ 10% of tumor area, and a MI of $\geq$ 3/10 HPF were independent predictors of BCR after radical prostatectomy. NA $\geq$ 10% of tumor area appeared to have a stronger association with outcome than MI $\geq$ 3/10 HPF, as still associated with BCR when Gleason score was in the model.

Conclusions: The results of our study showed that, in addition to the conventional Gleason grading system, NA, and MI are useful prognostic parameters while evaluating long-term prognosis in prostatic adenocarcinoma.

Keywords: Gleason score; Prostate; Prostate-specific antigen

1. Introduction

Radical prostatectomy (RP) remains one of the choices of treatment for clinically localized prostate carcinoma [1]. However, many patients have disease progression in the form of isolated prostate-specific antigen (PSA) recurrence, local recurrence, and distant metastasis after surgery [2]. Many factors, including preoperative serum PSA, pathologic stage, and surgical margin (SM) status are strongly associated with disease progression [3,4]. Gleason score (GS), which measures the degree of differentiation of prostate carcinoma, also provides prognostic information [4,5]. But Gleason grading system measures architectural differentiation and disregards nuclear atypia and cell proliferation index [6–8]. Several authors have reported that nuclear grade is prognostically useful, such that it can stratify prostate carcinoma cases into several groups [9–12].

One of the simplest ways to directly measure cell proliferation is to quantitate the mitotic activity on light microscopy [13–16]. In a recent study, the prognostic value of the mitotic indices was tested in a series of 303 patients followed up for 13 years [8]. The results showed that, in addition to the conventional Gleason grading system, mitotic indices are useful prognostic parameters while evaluating the long-term prognosis in prostate carcinoma. But the effect on the prognosis and clinical outcomes of nuclear atypia and mitotic indices has not been well studied. We have assessed nuclear anaplasia (NA) and mitotic index (MI) in prostatic adenocarcinomas by use of light microscopy and have correlated the NA and MI with other prognostic factors and the progression of the disease.

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2. Patients and methods

The aim of the study was to analyze the significance and outcome of NA and MI in prostatic adenocarcinomas in RP specimens. The study was based on consecutive 235 RP specimens, comprising 210 robotic, and 25 radical retropubic prostatectomy specimens collected at the Department of Pathology, Ümraniye Education and Research Hospital, from January 2006 to January 2013. None of the patients had a history of receiving cryotherapy, radiotherapy, chemotherapy, nor androgen deprivation. A postoperative serum PSA level $\geq$0.2 ng/ml was considered a biochemical prostate-specific antigen recurrence (BCR) [17]. Standard follow-up protocol at our institution recommended PSA measurement quarterly for the first year postoperatively, semiannually for the second year, and annually thereafter.

Each prostate was sampled according to a standardized laboratory protocol. All specimens were embedded fully. Cancer volume was determined using the grid method [18] and was calculated as the sum of the volume of individual cancer foci. We stained 6 equivocal cases with p63. In total, 4 of them were unstained and 2 of them were stained with p63 (basal cells). These cases were evaluated as prostatic intraepithelial neoplasia and excluded from the study. There was not any case with intraductal carcinoma. All slides and paraffin blocks of a case were sent to another pathology laboratory for consultation. Finally, the study included 232 RP specimens (207 robotic and 25 radical retropubic prostatectomy). Each case was reevaluated by G.K. and B.C.S. and assigned a Gleason grade according to the 2005 International Society of Urological Pathology criteria [19]. But based on the modified Epstein criteria, we assessed all cribriform foci as pattern 4 (G.K. and B.C.S.) [20]. G.K. and B.C.S. analyzed NA on all tumor areas of all slides (8–18 whole mount slides per case). All tumor areas were evaluated. Extraprostatic extension (EPE), microscopic bladder neck invasion, pathologic stage, seminal vesicle invasion, and SM status were defined according to the 2005 International Society of Urological Pathology Consensus Conference Working Groups 3 to 5 (G.K. and B.C.S.) [21–23]. We assessed NA by light microscopy. NA was determined based on the nucleomegali (nuclear enlargement to at least $\times$2 that of a benign epithelial cell, vascular endothelial, and smooth muscle cell); irregular nuclear membrane; lobulation of nuclei; and one or multiple eosinophilic macronucleoli (nucleoli were clearly visible at $\times$100 magnification) similar to nucleoli of ovarian high-grade serous carcinoma cell. NA was determined based on the presence of all of these criteria. In addition, analysis of NA was done in comparison with benign glands and vascular endothelial or smooth muscle cells in the specimens to reduce variability caused by differences in fixation, section thickness, and staining (Fig. 1A–C). NA was observed at almost all cells within individual tumor glands. All slides of 232 cases were reviewed independently by 2 pathologists who were unaware of the clinical data (G.K. and B.C.S.). The final NA was determined by consensus. In addition, the proportion of area of NA was recorded in each tumor in 10% increments. The mitotic indices were determined by an investigator (G.K.). The mitotic figures identified from most cellular areas of the tumor at the tumor periphery near the site of invasive growth. The counting of mitotic figures was done by using an objective magnification of $\times$40 (field diameter = 490 μm). The mitotic figures were identified using the standard criteria as previously described [13]. The MI was defined as the number of mitotic figures in 10 consecutive high-power fields (HPF) (objective magnification $\times$40, field diameter = 490 μm).

2.1. Statistical analysis

IBM SPSS Statistic 22 program was used for statistical analysis. Cutoff values of percentage area of NA and MI were calculated with receiver operating characteristic (ROC) analysis by calculating the area under the curve. ROC curves were for the outcome of BCR. To compare

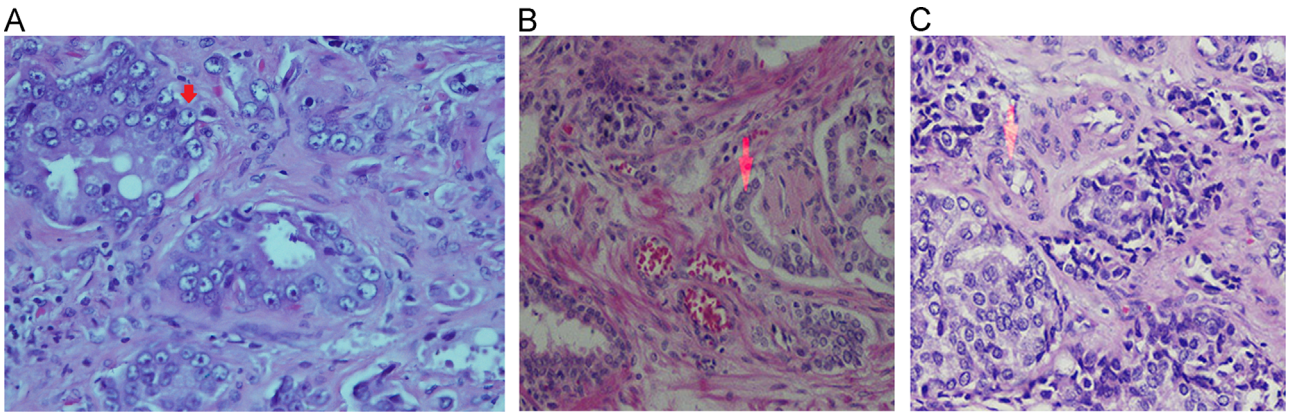

Fig. 1. (A) Prostate adenocarcinoma with nuclear anaplasia (H and E, $\times$400). (B) Prostate adenocarcinoma with nucleomegali, but without nuclear anaplasia (because there is no eosinophilic macronucleoli and nuclear lobulation) (H and E, $\times$200). (C) Prostate adenocarcinoma with nuclear anaplasia (because there is nucleomegali together with eosinophilic macronucleoli and nuclear lobulation) in comparison with benign glands and vascular endothelial cells (H and E, $\times$200).