Changes in Histologic Grade and Argyrophilic Nucleolar Organizer Regions during Progression of Prostate Cancer

Takemasa Ohki 1, Koichiro Akakura 1, Takeshi Ueda 1, Susumu Akimoto 1, Ryuichi Yatani 2 and Jun Shimazaki 1

1Department of Urology, School of Medicine, Chiba University, Chiba and 2Department of Pathology, Faculty of Medicine, Mie University, Mie

To determine the changes in histologic features during the course of prostate cancer under long-term endocrine therapy, histologic grade and argyrophilic nucleolar organizer regions (AgNORs) were examined in specimens before treatment, at relapse, and at cancer death. A total of 29 patients who had received endocrine therapy and died of prostate cancer were evaluated. Among the 29 cases, biopsy tissues before treatment (25 cases) and during progression from endocrine therapy (10 cases) were compared with autopsy specimens. Histologic grade was determined by the method of Gleason, and the number of AgNORs in cancer cells was counted. Survival of the patients was compared with the histologic features. There was a tendency for a higher grade of cancer during the clinical course. Moreover, a statistically significant increase in the number of AgNORs was observed from pretreatment biopsy to autopsy. Upon comparison of metastatic sites with local cancer at autopsy, no significant difference was noticed in terms of histologic grade or AgNOR count. Although there was no correlation between the number of AgNORs and survival after initial treatment, an inverse relationship was demonstrated between the number of AgNORs and survival in patients with systemic progression after endocrine therapy. In conclusion, prostate cancer shows an increase of malignant potential, as assessed by histologic grade and the number of AgNORs. Patients with cancer of higher proliferative ability showing high grade and greater numbers of AgNORs have poorer prognosis from progression.

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Key words: Prostate cancer—Progression—Autopsy—Histologic change—Argyrophilic nucleolar organizer regions

Introduction

A variety of therapeutic modalities including surgery, radiotherapy, chemotherapy and endocrine therapy have been used for the management of prostate cancer, depending on the stage of the disease. However, endocrine therapy has almost always been conducted during the clinical course by the end stage of the disease. Therefore, examination of autopsy specimens from patients who have died of prostate cancer after relapse following endocrine therapy, focusing particularly on the biologic features of the tumor, is useful for understanding the long-term course of the disease. In prostate cancer patients receiving endocrine therapy, it is generally accepted that the histologic grade of the tumor is related to prognosis. 2-3 Moreover, tumors with a large volume are known to be less differentiated cancers than small tumors. 6 However, few studies have investigated the histologic changes in prostate cancers during their clinical course. 7-9

A number of previous reports have indicated that the frequency of argyrophilic nucleolar organizer regions (AgNORs) in prostate cancer is correlated with the histologic grade, 5-10 although it remains uncertain whether AgNORs represent a reliable prognostic factor in patients with untreated prostate cancer. 5, 13, 14, 16, 17 However, it has become generally accepted that AgNORs are useful for evaluating the proliferative potential of tumors, even by the use of paraffin-embedded specimens. 18

In the present study, to shed some light on the progression of prostate cancer, an attempt was made to correlate histologic grade and the number...
of AgNORs using biopsy and autopsy specimens, focusing on the relationship between the number of AgNORs and prognosis.

Materials and Methods

A total of 29 patients who initially responded to endocrine therapy, thereafter relapsed, died of prostate cancer, and were examined at autopsy during 1966–1993, were included in the present study. Among these cases, biopsy specimens before treatment were obtained in 25 and during progression in 10. Biopsy tissues were obtained from both lobes of the prostate using a Silverman needle. As endocrine therapy, all the patients underwent surgical castration plus treatment with diethylstilbestrol diphosphate, ethynylestradiol or chlormadinone acetate.

The histologic grade of the tumor was evaluated by the same pathologist (R.Y.) using the Gleason score. The number of AgNORs in cancer cells was calculated according to the previous report. Since AgNORs were stained as separate granules within a nucleus or in gathered forms forming clusters, as many individual dots as possible were counted in the latter case, and the average number of dots per cell was obtained from 100 cancer cells. When counting the number of AgNORs, the same lesion used for determination of the Gleason grade was selected.

Progression following endocrine therapy was defined as the appearance of at least one of the following: new or worsened bone metastasis, more than 25% increase in local or soft tissue disease, and more than doubling of the level of serum prostatic acid phosphatase or prostate specific antigen from the stabilized level. The survival period was estimated, and compared with the histologic grade and number of AgNORs in the tumor.

Statistical significance was examined by Student's t test and the Cox-Mantel method.

Results

Histologic Grade and AgNORs

In the pretreatment prostatic biopsies, high-grade cancers had significantly greater numbers of AgNORs than low-grade ones. A similar tendency was observed in autopsy specimens (Table I).

Changes in the Gleason score between the pretreatment biopsy and autopsy samples of the prostate were examined (Table II). High-grade cancers remained at a high grade, whereas the lower-grade ones tended to develop a higher grade, suggesting a trend of dedifferentiation during the overall clinical course. Comparison of the Gleason

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<th>Table I. Histologic Grade and Number of AgNORs</th>
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<td>Biopsy before treatment</td>
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The mean value±SD is shown. *, P<0.01 from Gleason score 7.

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<th>Table II. Changes in Gleason Score of Biopsy Samples Obtained before Treatment and at Autopsy</th>
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<th>Table III. Changes in Gleason Score of Biopsy Samples Obtained during Progression and at Autopsy</th>
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Fig. 1. Changes in the number of AgNORs between biopsy samples obtained before treatment or during progression after endocrine therapy and those obtained at autopsy. Each connected pair of solid circles indicates an individual case. The mean values ± SD of biopsy samples before treatment and at autopsy are 7.24 ± 1.73 and 7.98 ± 1.58 (n = 25), respectively, the difference being significant (p < 0.05). The mean values ± SD of biopsy samples obtained during progression and at autopsy are 6.86 ± 1.39 and 8.28 ± 2.09 (n = 10), respectively, without a significant difference.

Fig. 2. Comparison of the number of AgNORs between primary and metastatic lesions at autopsy. The highest number among metastatic sites in each patient is used. Each connected pair of solid circles indicates an individual case. The mean values ± SD of primary and metastatic tissues are 8.14 ± 1.55 and 8.35 ± 1.37 (n = 25), respectively, without a significant difference.

Fig. 3. The number of AgNORs at autopsy and survival after initial treatment (a) or at the time of diagnosis of systemic progression after endocrine therapy (b). Each solid circle indicates an individual case. (a): not significant, (b): statistically significant (y = -0.16x + 9.37, r = -0.74, P < 0.001).
scores of prostatic tissues between biopsy and autopsy indicated a similar trend towards higher-grade cancer (Table III).

There was a significant increase in the number of AgNORs in localized cancers between pretreatment biopsy and autopsy (Fig. 1). The mean number of AgNORs in the prostate increased during the period from progression to autopsy, although the difference was not statistically significant, perhaps due to the small sample size (Fig. 1).

The metastatic cancer foci were compared with the primary lesions at autopsy in terms of the Gleason score and the number of AgNORs. Even though the highest-grade lesions among the metastatic sites were used for analysis, the Gleason scores and the numbers of AgNORs were not markedly altered (Table IV and Fig. 2).

**Relationship between Prognosis and the Number of AgNORs and Histologic Grade**

When the Gleason scores before treatment and prognosis were compared, the mean survival periods (months ± SD) of patients with Gleason 7 and Gleason 8–10 cancers were 36.1 ± 25.7 and 24.6 ± 22.3, respectively, although the difference was not significant, probably because of the limited number of cases. The number of AgNORs before treatment was not related to survival from the start of treatment to death (data not shown). Since the number of biopsy samples taken during progression was limited, and the numbers of AgNORs were not significantly altered between biopsy during progression and autopsy (Fig. 1), the number of AgNORs at autopsy was compared with prognosis. Although there was no correlation between the number of AgNORs at autopsy and survival after the start of initial treatment (Fig. 3a), an inverse relationship was observed between the number of AgNORs at autopsy and survival in patients with systemic progression after endocrine therapy (Fig. 3b), indicating the significance of proliferative ability as a prognostic factor once a tumor had become refractory to endocrine therapy.

**Discussion**

There have been few reports on histologic changes in prostate cancer during the clinical course in the same patient, due mainly to the difficulty in obtaining tissue samples at different time points. A tendency of dedifferentiation in hepatocellular carcinoma has been reported during the course of treatment. Similarly, it has been reported that dedifferentiation of prostate cancer occurs along with progression, and the present study confirmed this tendency. Genetic instability, often observed in cancer cells, would lead to alterations of genes such as overexpression of oncogenes or loss of tumor suppressor genes. Along with these genetic as well as epigenetic changes, cancer cells may progress to a high grade with greater malignancy during the clinical course. Alternatively, progression to high-grade cancer may be explained by the existence of androgen-insensitive cancer cells in an androgen-sensitive tumor, and such cells can survive androgen ablation, resulting in enrichment of androgen-insensitive, high-grade cancer cells during endocrine therapy.

It has been clarified that more genetic changes in proteins such as androgen receptor and p53 are accumulated in metastatic lesions than in localized tumors. However, in the present study and others, histologic grade and number of AgNORs in metastatic tumors were not significantly different from those in tumors localized to the prostate. This may be because changes in biologic characteristics other than grade or AgNOR number are easily recognized using molecular biological techniques. Alternatively, histologic grade and AgNOR number in primary and metastatic foci may already have progressed significantly by the end stage of the disease.

Although AgNORs are thought to reflect the proliferative potential of a tumor, the number of AgNORs is not related to overall survival. In fact, a previous study conducted in our laboratory indicated that there was no relationship between AgNOR number and response to endocrine therapy in patients with previously untreated prostate cancer. This may be attributable to the fact that response to endocrine therapy at the initial stage is a more important prognostic indicator than proliferative ability, and that since good responders to endocrine therapy show good prognosis, the therapy...
could modify the speed of tumor growth. In this context, it is conceivable that prostate cancer patients with greater numbers of AgNORs show poorer prognosis after radiotherapy. The present study did not show any relationship between the number of AgNORs at autopsy and survival after initial treatment, and this may be explained by changes in the main clones during disease progression. On the other hand, survival after the diagnosis of progression was related to the number of AgNORs found at autopsy. Thus, it is suggested that survival after confirmation of progression is influenced by proliferative activity as reflected by the increase in AgNOR number, and that the biologic features of prostate cancer may change during the clinical course. In fact, the tumor marker doubling time during progression has been shown to correlate well with survival.

It is concluded that during the clinical course of prostate cancer, the tumor accumulates malignant potential, and that this can be evaluated by histologic grade and the number of AgNORs. Patients with cancers of high proliferative ability show a poor prognosis from the time of diagnosis of progression after endocrine therapy.

Acknowledgments

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