Effect of Radiotherapy on Non-Neoplastic and Malignant Prostate

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Abstract: Pathologists nowadays are frequently confronted with post-radiotherapy biopsies of the prostate. It is critical to recognize the changes of benign prostatic tissue with radiation effect, which include frequent acinar atrophy, acinar distortion, basal cell hyperplasia, decreased acinar/stromal ratio, and stromal fibrosis. The non-neoplastic irradiated prostate can have nuclear enlargement and bizarre nuclei, changes overlapping or exceeding those commonly seen in prostatic adenocarcinoma. The presence of high-grade PIN adjacent to acini in question favors a cancer diagnosis. Ceratin immunostains, particularly cytokeratin 34ßE12, in conjunction with selected other markers, can help the pathologist determine whether residual cancer is present in the most difficult cases.

Keywords: Irradiation, therapy-induced changes, prostate, biopsy, immunohistochemistry.

INTRODUCTION

There are more than 30 articles in the published literature about the pathology of irradiated prostate cancer, representing 0.3% of all Medline citations on prostate cancer over the past 30 years. Changes after brachytherapy resemble those after external beam therapy. The rate of postradiation therapy positive biopsy varies widely, ranging from 19% to 93% based on patient selection factors, the interval from treatment, the number of biopsy samples obtained, the use of other therapies, and, perhaps most importantly, histologic interpretation [1]. Factors that determine the likelihood of a positive biopsy include pretreatment clinical stage, cancer grade, post-treatment serum PSA, and digital rectal examination. There are three main problems with interpretation: 1) false-negative biopsies resulting from sampling variation; 2) false positive biopsies due to slow regression of tumor; and 3) biopsies showing residual tumor of indeterminate viability. In this report I evaluate the diagnostic criteria for a positive biopsy after radiotherapy and the prognostic significance of these criteria. Consideration of the effects of irradiation on the benign prostate serves as a baseline for interpreting changes in cancer.

PATHOLOGIC FINDINGS FOLLOWING RADIOTHERAPY

The diversity of histopathologic changes in the prostate after radiotherapy have been well-described [2-6], but treated specimens continue to challenge the surgical pathologist. The difficulty of biopsy interpretation after treatment is multifactorial and includes separation of carcinoma from its many mimics, identification of small foci of carcinoma, and separation of treatment effects in normal tissue from recurrent or persistent carcinoma [2, 3, 7-13]. As more patients choose radiotherapy, particularly brachytherapy, and as these patients are observed for longer intervals, pathologists bear an increasing burden to discriminate irradiated benign acini from irradiated adenocarcinoma [1, 14, 15].

Benign Tissue, Including Hyperplasia

The degree of histologic change caused by radiation in benign or hyperplastic acini varies with the dose and duration of irradiation and interval from therapy onset (Table **1**) [11, 16]. Changes include acinar atrophy, distortion with loss of cytoplasm, and decreased ratio of acini to stroma. Nuclear changes include nuclear enlargement (86% of cases) (Fig. **1**) and prominent nucleoli (50%) (Fig. **2**) [3]. The basal cells are preserved at least focally (Fig. **3**), since the acinar secretory cells are more sensitive to irradiation necrosis and atrophy. Consequently, atypical basal cell hyperplasia is seen in 57% of cases (Fig. **4**) [3], defined as basal cell proliferation with nuclear enlargement and prominent nucleoli in > 10% of cells.

More atypia of benign glands was noted after brachytherapy than after external beam therapy in a comparative study of 44 cases, and our experience verifies this. This atypia seems to persist longer after brachytherapy as well. With external beam therapy, there was less atypia in men biopsied 48 months after treatment compared to those biopsied at a shorter interval after treatment. In some cases, however, abnormal findings persisted to a variable degree for ten years. In contrast, no decrease in atypia over time was noted in men treated with brachytherapy [17].

Adenocarcinoma

Just as most prostate cancer grows slowly, it is slow to regress, with histologic changes evolving at least 12 months after the completion of irradiation therapy. Ongoing cell death limits the value of needle biopsy prior to about 12-18 months [2]. Slow tumor death is attributed to the fact that radiotherapy causes necrosis only after a prostate cell has gone through cell division and to long tumor doubling time. Sampling variation is minimized by obtaining multiple

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specimens [2, 18-25]. After 12-18 months, biopsy is a good method for assessing local tumor control, but complete histologic resolution of cancer may take 2-3 years [19].

Table 1. Histopathologic Findings in Benign Prostatic Tissue in Postirradiation Needle Biopsies at the Time of PSA (Biochemical) Failure [7]

Histopathologic Findings	Percentage of Cases
Inflammation	39
Atrophy	79
Postatrophic hyperplasia	18
Acinar distortion	54
Decreased acinar/stroma ratio	86
Basal cell hyperplasia	68
Atypical basal cell hyperplasia	57
Hyperplastic (proliferative) change	11
Squamous metaplasia	0
Eosinophilic metaplasia	21
Stromal Changes	
Stromal fibrosis	93
Stromal edema	21
Stromal calcification	21
Hemosiderin deposition	0
Atypical fibroblasts	25
Necrosis	0
Granulation tissue formation	0
Myointimal proliferation	11
Cytologic Changes	
Nuclear pyknosis	75
Nuclear enlargement	86
Prominent nucleoli	50
Bizarre nuclei	54
Cytoplasmic vacuolization	29
Intraluminal Contents	
Crystalloids	0
Mucin	4
Eosinophilic granular secretions	39
Corpora amylacea	32

The therapeutic success of radiotherapy for prostate cancer requires complete or near-complete eradication of tumor. Conventional external beam radiotherapy misses 20% to 35% of the target volume when compared with three-dimensional conformal planning with dose escalation [26]. Brachytherapy techniques will probably improve local cancer control and prolong survival [27]. Evaluation of local tumor control is assisted by digital rectal examination and transrectal ultrasound. Post-therapy serum PSA correlates

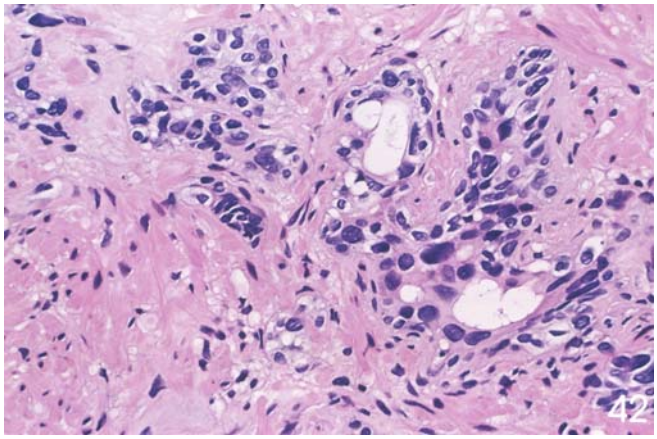

Fig. (1). In the *non-neoplastic* irradiated prostate, nuclear enlargement and smudged chromatin are the most notable changes.

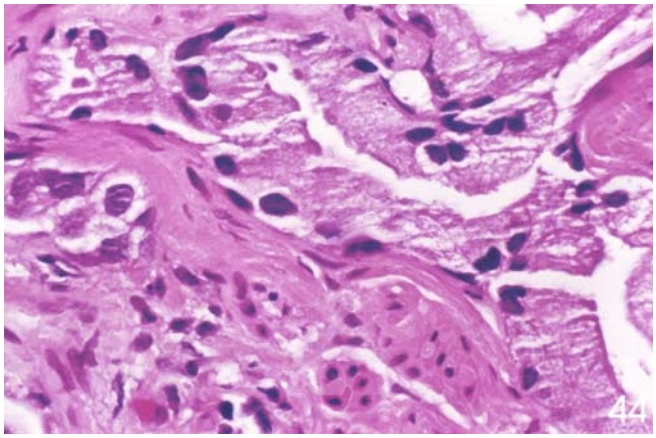

Fig. (2). Benign acini may have extreme nuclear enlargement, but there are no prominent secretory cell nucleoli.

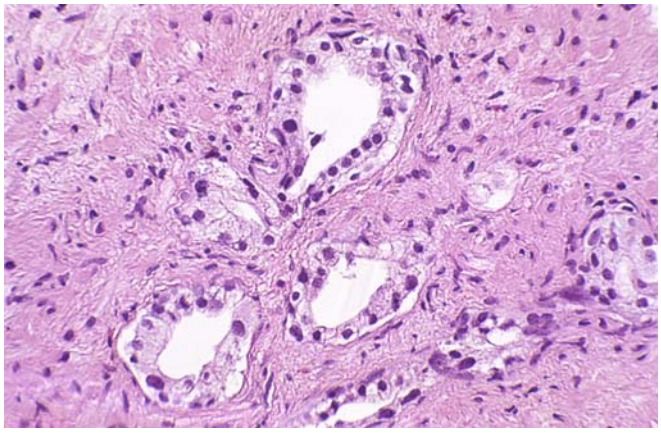

Fig. (3). Preservation of at least a focal basal cell layer (**lower right**) is a key finding of benign acini.

with post-therapy biopsy results, including degree of radiation effect [11]. Crook *et al*. diagnosed postradiotherapy biopsies as indeterminate in 33% of first biopsies (median 13 months), 24% of second biopsies (28 months), 18% of third biopsies (36 months) and 7% of fourth biopsies (44 months) [19]. These figures are higher than the 1.5-9.0% [7, 12] of biopsies with atypical (ASAP) findings in unselected non-irradiated series, highlighting the increased diagnostic challenge after radiotherapy. The identification of cancer in

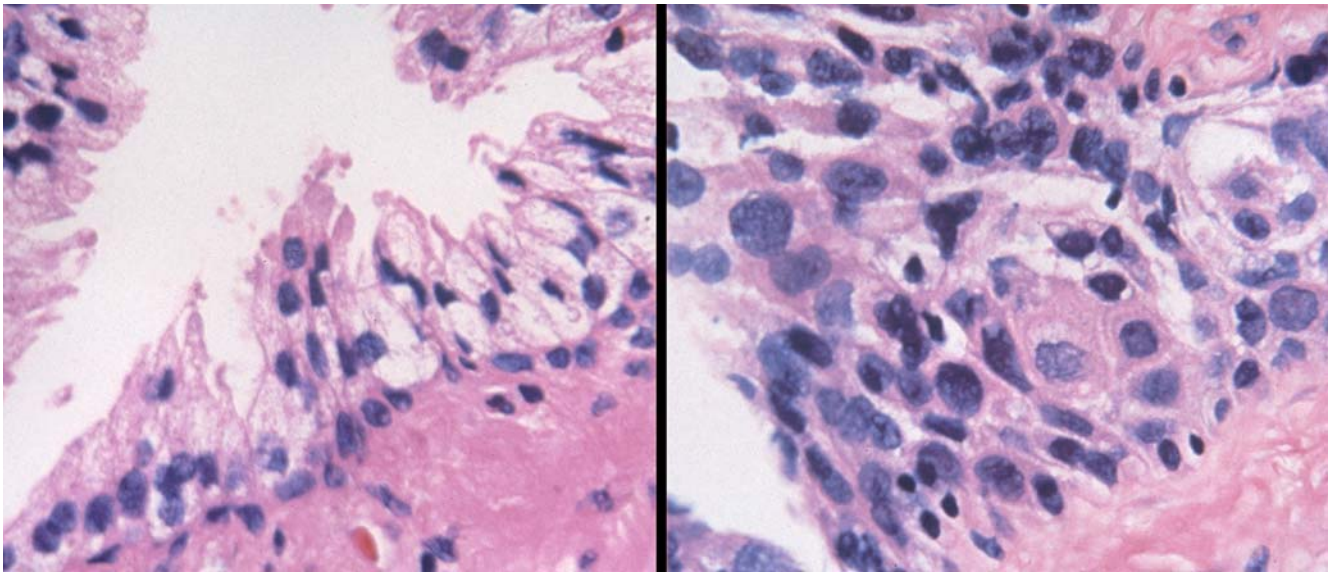

Fig. (4). In basal cells, normally a single layer (**left**), there is nuclear enlargement and they may become so hyperplastic that they form several layers and secretory cells are inconspicuous (**right**). Irregular, potato-shaped nuclei are pathognomonic for basal cells.

needle biopsy specimens after radiotherapy has a significant impact on patient management; positive needle biopsies portend a worse prognosis [1, 15, 28-33].

The histologic diagnosis of cancer without radiation effect relies on both architectural and cytoplasmic atypia. Changes vary widely among patients [11]. Radiotherapy causes cytologic atypia of benign glands, forcing the pathologist to discriminate cancer almost totally on architectural findings (Table **2**), although cytologic changes such as vacuoles and loss of nucleoli appear later (Fig. **5**). Early radiotherapy changes include cytomegaly, with preservation of nucleomegaly and prominent nucleoli. Occasionally, cytologic findings such as double nucleoli in a secretory cell can be helpful. However, the main post-treatment change may be atrophy, often with disappearance of the nucleoli (Fig. **6**). Compared with pretreatment cancer, cancer after radiotherapy *retains the architectural features of infiltrative growth, perineural invasion, intraluminal crystalloids, blue mucin secretions, and the absence of corpora amylacea.* The presence of concomitant high-grade PIN is an important clue (Fig. **7**). Grade 3 cancer acini may take on a ragged, poorly formed shape, causing a resemblance to grade 4 (Fig. **8**). Grade 4 cancer may lose its acinar luminal structure, creating the appearance of invasive single cells characteristic of grade 5 (Fig. **9**). Some cases have increased Paneth cells [5] and these are noted in 32% of biopsies [11].

High-Grade Prostatic Intraepithelial Neoplasia (PIN)

After radiotherapy, PIN retains characteristic features of untreated PIN and is readily recognized in biopsy and prostatectomy specimens (Fig. **10**). The salient microscopic features include nuclear crowding, nuclear overlapping and stratification, nuclear hyperchromasia, and prominent nucleoli. The basal cell layer is present but often fragmented. Sometimes PIN is obvious at low power by its darkness and will alert the pathologist to adjacent cancer. The most common

Table 2. Histopathologic Findings in Prostatic Adenocarcinoma in Postirradiation Biopsies [7]

Histopathologic Findings	Percentage of Cases
Gleason Score	
< 7	17
7	48
> 7	35
Percentage of Cancer Involvement	
≤ 10	31
11-40	28
41-80	35
81-100	6
Number of Cancer Foci	
1	36
2-4	50
> 5	14
Combined Score of Radiation Effect*	
0-2 (minimal)	52
3-4 (moderate)	38
5-6 (severe)	10
Infiltrative growth	100
Perineural invasion	31
Atrophic change	10
Nuclear pyknosis	72
Nuclear enlargement	93
Prominent nucleoli	79
Percentage of Cytoplasmic Vacuolization	
< 10	45
10-50	45
> 50	10
Inflammation	0
Stromal desmoplasia	76
Necrosis	0
Intraluminal Contents	
Crystalloids	3
Mucin	21
Eosinophilic secretions	24
Corpora amylacea	0
Concomitant high-grade PIN	7

*Radiation effect was quantified using the scoring system described by Crook and co-workers [22].

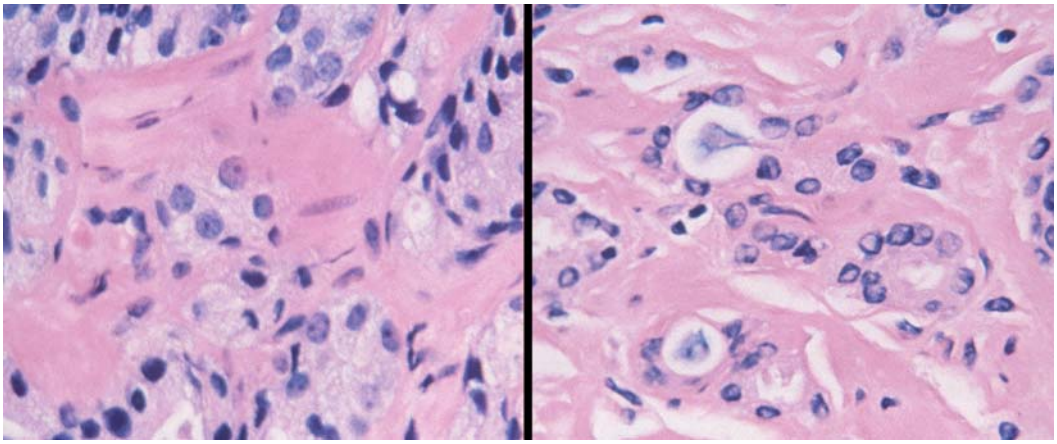

Fig. (5). Identification of *irradiated cancer* is a problematic area in pathology, now that increased numbers of post-treatment biopsies are being done. Early changes include cytomegaly, vacuoles, and nucleomegaly with persistent single and occasionally double nucleoli in each nucleus (**left**). Later changes include atrophy and sometimes cytoplasmic vacuolation, with the nucleoli now being inconspicuous (**right**).

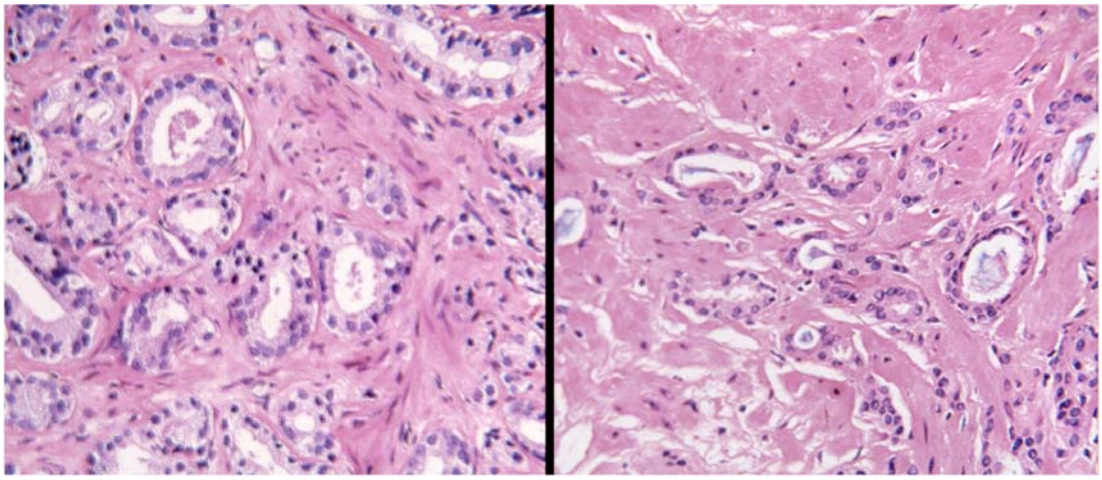

Fig. (6). In this matched set of photomicrographs from the same patient, compared with pre-treatment grade 3 cancer (**left**), the main post-treatment change is atrophy (**right**). Note, however, the maintenance of infiltrative pattern, angulated acini, absence of basal cells, and inspissated luminal blue mucin characteristic of cancer. Depending on the duration of irradiation, one may see all atrophic cancer acini, unchanged acini, or a combination of atrophic and unchanged acini.

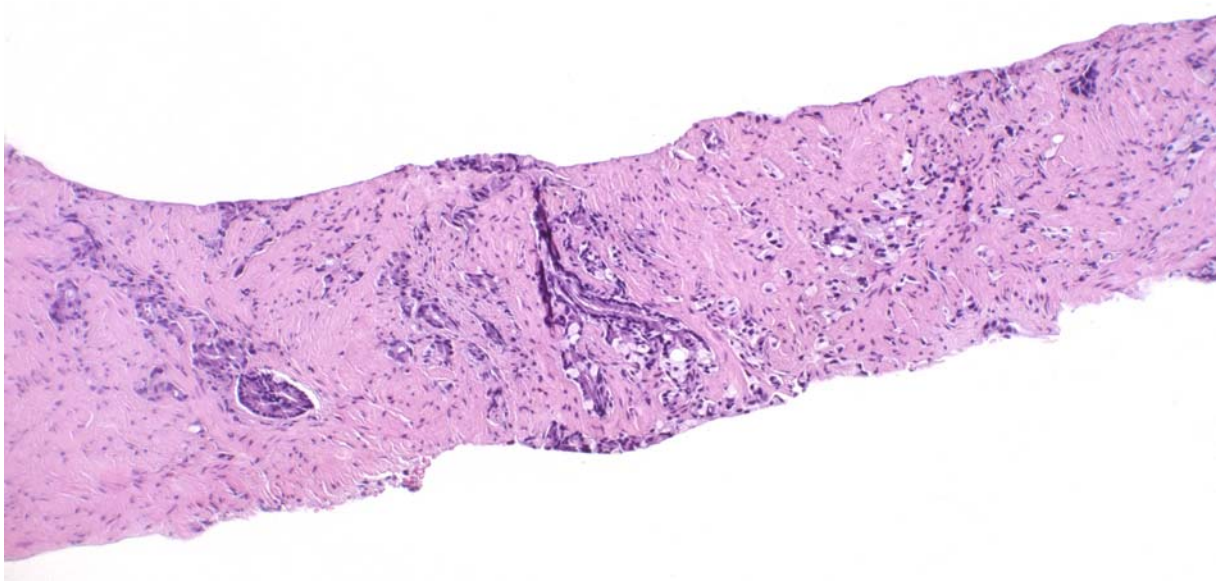

Fig. (7). This needle biopsy contains post-irradiation grade 3 cancer (**left**), a focus of high-grade PIN (**center**), and grade 4 cancer (**right**). The residual neoplasm loses architectural differentiation while retaining the cytologic features of cancer.

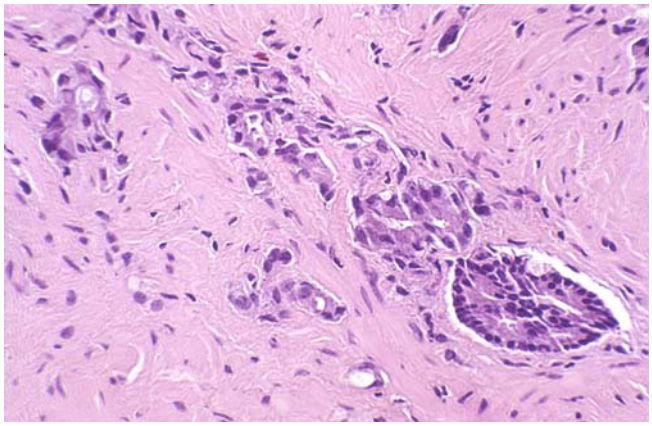

Fig. (8). Gleason grade 3 cancer after radiotherapy. The acini are decreased in number and smaller in size, with a haphazard arrangement.

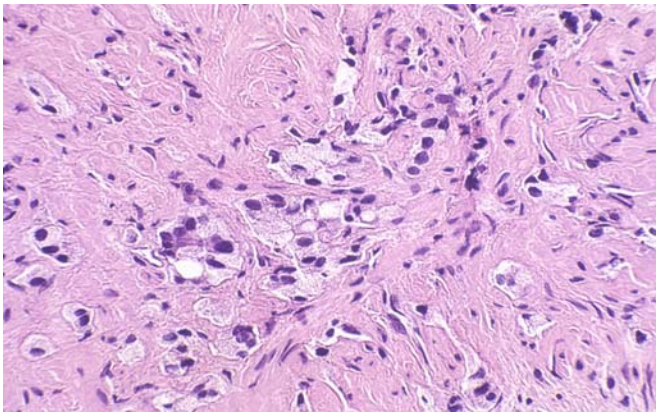

Fig. (9). Gleason grade 4 cancer after radiotherapy. The acinar luminal structure breaks down, making it tempting to overgrade the apparent single cells as grade 5. Nucleoli have disappeared, indicating maximal effect.

patterns of PIN after treatment, the tufting and micropapillary patterns, are similar to those reported in untreated prostates [34]. The only radiotherapy-related observations were occasional cytoplasmic vacuolation or sloughing of epithelium into the lumen [16, 18].

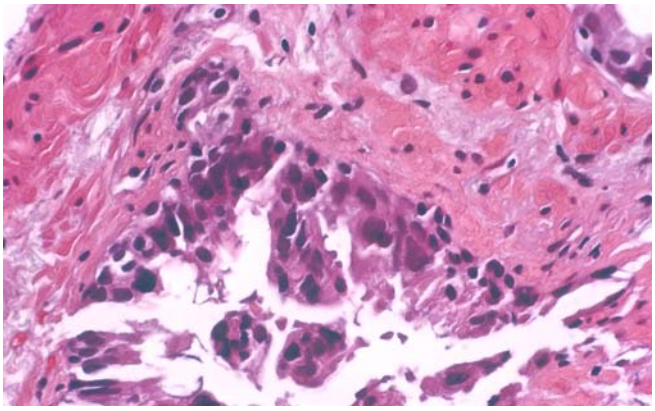

Fig. (10). In this biopsy, high grade PIN with radiation effect is a helpful feature that should prompt a search for cancer. However, the frequency of PIN in irradiated prostate cancer (based on salvage prostatectomy findings) is decreased to 62% of cases [18]. In contrast, 82-86% of non-irradiated, step-sectioned prostates show high grade PIN [34].

The prevalence of high-grade PIN accompanying cancer is 82-100% of non-irradiated radical prostatectomy specimens [34, 35]. It was noted in only 62% of cases after radiotherapy [18], a decreased prevalence, similar to that seen after androgen ablation (50%) [35]. Volume of PIN without radiotherapy [18] averaged 1.32 cm^3 compared to 0.12 cm^3 after radiotherapy [34]. One study paradoxically noted a higher prevalence (70%) of PIN after radiotherapy than expected [36] but these investigators failed to employ accepted diagnostic criteria for PIN, so their results are not comparable with those of the authors [18] or others. High-grade PIN was reported in 9% of post-therapy biopsies [19], but sampling limitation underestimates the prevalence. It is possible that radiation alters the phenotype of PIN such that recognition is obscured.

No significant correlation was seen between PIN in post-irradiation salvage prostatectomy specimens and cancer-specific survival or other clinicopathologic data [18]. For isolated high-grade PIN in needle biopsies, the general recommendation has been to perform repeat biopsies in order to rule out cancer. Use of 12-core sampling rather than sextant sampling, however, diminishes the positive predictive value of isolated high-grade PIN for cancer, possibly obviating the need for repeat biopsy unless clinical suspicion is high [37].

Stroma and Vessels

Stroma may be fibrotic, with paucicellular scarring, and vascular changes include intimal thickening and medial fibrosis (Fig. **11**) [2]. Pathologists must be aware of these changes because they preclude the usual reliance on nuclear and nucleolar size to help identify prostate cancer.

CANCER GRADE, STAGE, AND DNA PLOIDY AFTER RADIOTHERAPY

In 2009, M.D. Anderson Cancer Center reported a series of salvage prostatectomy cases. Tumor was bilateral in 74% of cases; 71% of tumors involved the apex and 65% the base. Gleason score was 8 or above in over half the cases, and stage was 3a or 3b in more than half the cases. 12% of patients had lymph node metastasis [21]. Similarly, others [16, 25] found an increase in tumor grade following irradiation. Whether any of this is artifactual is controversial since particularly in grade 4 cancer, radiotherapy may cause disappearance of glandular lumina, resulting in grade 5 morphology (Fig. **6**). Some investigators recommend grading of cancer in specimens after radiotherapy, recognizing that the biologic significance of grade may be different from that in untreated cancer [22]. Cheng *et al*. believe that Gleason grade in postirradiation needle biopsy specimens provides useful predictive information and recommend its use in this setting [3] despite suggestions to the contrary [38]. Taken together, the findings suggest that cancer after treatment frequently is related to a process of clonal evolution that results in cancer progression and tumor dedifferentiation. Thus most authors believe persistent cancer post-radiation demonstrates a shift to higher Gleason grade and higher tumor stage, indicating increasing biologic aggressiveness and cancer dedifferentiation after radiation [22, 25, 26, 30, 39].

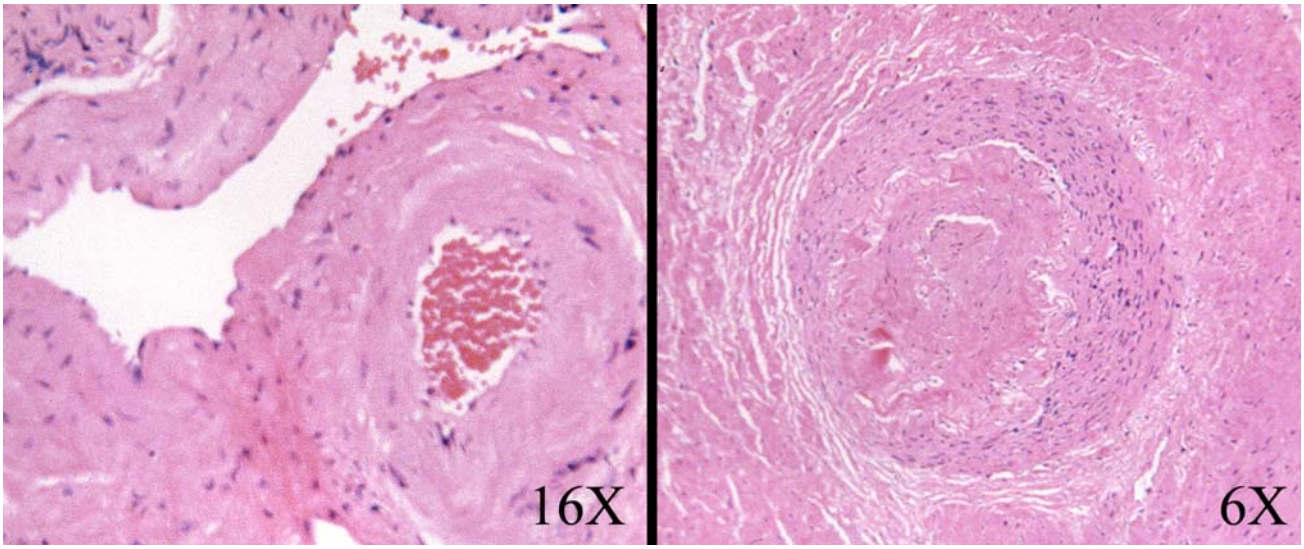

Fig. (11). In cases where the history of irradiation is not given or is uncertain, stromal and vascular changes can cue the pathologist to recognize radiation effect. The stroma becomes fibrotic, and the cellularity of normal vessel walls (**left**) increases because of smooth muscle proliferation (**right**).

The authors found a good correlation of Gleason grade between post-irradiation salvage prostatectomy and treated biopsy specimens [22, 40]. Needle biopsies underestimated prostatectomy Gleason grade in 35% of cases and overestimated grade in 14% of cases, similar to the findings in studies of patients who were not treated by radiotherapy [33, 40-45]. By comparison, in 316 patients who underwent radical prostatectomy without prior androgen deprivation or radiotherapy, Gleason grade in needle biopsies underestimated prostatectomy grade in 40% of cases and overestimated grade in 25% of cases [40].

There is also a shift after therapy toward nondiploid cancer. Siders and Lee [5] evaluated matched pre-radiation and postradiation specimens from 58 patients and found a 24% increase in the number of poorly differentiated cancers (Gleason score 8-10) and a shift toward aneuploid cancer in 31% of pretreatment diploid cancers [5]. Postirradiation Gleason grade and DNA ploidy are independent prognostic factors in patients with prostate cancer who fail radiotherapy [21].

CLINICAL SIGNIFICANCE OF POSTRADIATION BIOPSY CANCER

Digital rectal examination for the detection of radiation failure is imprecise unless there is gross cancer recurrence [46]. Consequently, some clinicians favor postradiation biopsy for the preclinical detection of recurrence, thereby allowing earlier intervention with salvage therapy; others consider routine postradiation biopsy justifiable only in a research setting. Zapatero *et al*. [47] determined that 21% of patients had a positive biopsy result at 24-36 months. Studies suggest that if prostatic carcinoma is not histologically ablated by radiotherapy after 12 months, it is probably biologically active [2, 28, 40]. The rate of positive findings on biopsy varies from 5% to 55% following brachytherapy [48] and from 20% to 93% following external beam radiotherapy [22, 29, 49-52]. This wide variation is attributable to selection of patients with broad ranges of pretreatment serum PSA, stage and grade of tumor, number of biopsy cores taken (more in contemporary studies), and radiation dosage. Interobserver variability may be an extra source of variation, as discussed below.

A positive biopsy result within 12 to 18 months of external beam radiotherapy may contain cancer in regression, and 30% of patients show eventual clearance of tumor at a mean time of 30 months after radiotherapy [53]. Kuban and Schellhammer have shown that a positive biopsy result after 12-18 months predicted clinical recurrence in approximately 80% of patients; remarkably, approximately 20% had no evidence of cancer at 10 years' follow-up [1]. However, Crook *et al*. [19] extended this interval to 24 months, eliminating biopsies prior to that from their study because delayed tumor progression was seen in 30% of patients. At 24-36 months, the biopsy result was one of two independent predictors of outcome, along with PSA nadir. Perineural invasion of cancer, however, was not an independent prognosticator in patients undergoing brachytherapy [54]. Conversely, 30% of patients with local or distant failure had negative findings on biopsy [49]. An identical 30% positive rebiopsy rate was found in men suspected of having cancer but whose initial TRUS-guided biopsy was negative [55]. This underscores the role of *sampling variation*: the false-negative rate of biopsy is 23% based on repeat biopsies in untreated men with prior positive biopsy [56].

Interobserver reproducibility in the diagnosis of cancer in postradiation biopsies varies moderately. Miller *et al*. found a "false positive" rate of 15% (4/26 specimens) and a "false negative" rate of 3% (2/70 specimens) [57]. Jones *et al*. classified 107 cases signed out by non-subspecialty pathologists and found one false positive and nine false negative cases [58]. However, 5 of 6 cases classified as suspicious by non-subspecialty pathologists were negative according to at least 2 of a panel of 3 specialty urologic pathologists, again showing some tendency toward overdiagnosis. Urologic pathologists disagreed with each

other in 3% (3/107) cases; two of three agreed with 23% of cases and all agreed with 74% of cases. Mean Kappa value was 0.66, indicating only moderate reproducibility.

Radiotherapy Combined with Androgen Ablation

Neoadjuvant androgen deprivation therapy (ADT) appears to have an additive or synergistic effect with external beam radiotherapy. In a recent study, 31 patients were treated with ADT before radiotherapy, and only 3 (10%) had cancer on post-therapy biopsy compared to 44 of 106 men (41%) treated with radiotherapy alone ($p = 0.004$) [16]. Androgen ablation probably also potentiates brachytherapy.

SCORING RADIATION EFFECT IN THE BENIGN PROSTATE

To determine whether the severity and extent of radiation changes in the prostate are of prognostic value, Crook *et al*. [19] graded nuclear and cytoplasmic changes in biopsy specimens following external beam radiotherapy. Cytoplasmic and nuclear changes were each graded on a 0-3 scale, and added together for a score of 0-6. They found that grading of radiation effect in the noncancerous prostate correlated with serum PSA nadir, immunoreactivity for PCNA, and local cancer recurrence [19]. Patients did poorly if there was little or no evidence of radiation change in the needle biopsy, suggesting incomplete coverage of the prostate by the therapeutic field or radiation-resistant foci as the source of local failure. Goldstein *et al*. [48] consider grading nuclear and cytoplasmic changes useful in a three-year prospective study of patients receiving brachytherapy. They also note that the presence of adenocarcinoma on post-treatment biopsy was an important predictor of failure.

Salvage radical prostatectomy specimens, conversely, demonstrated great discrepancy with biopsies in the scoring of radiation effect after external beam radiotherapy [22]. Forty-eight percent of needle biopsy specimens had moderate or severe radiation effect compared with only 6% of radical prostatectomy specimens. These findings suggest that scoring of radiation effect in needle biopsies may also overestimate the effectiveness of brachytherapy and could be misleading. This discrepancy could also explain why cytologic atypia in benign glands was observed in 98% of postradiation biopsies [11] and 77% of prostates in cystoprostatectomy specimens after radiotherapy for urothelial carcinoma [59]. Quantification of radiation effect is of questionable relevance in patients who fail to be cured by radiotherapy.

DIFFERENTIAL DIAGNOSIS OF PROSTATE CANCER AFTER RADIOTHERAPY

In the author's experience, atypical basal cell hyperplasia most frequently mimics treated cancer following irradiation. Atypical basal cell hyperplasia is defined as basal cell proliferation with more than 10% of cells exhibiting prominent nucleoli. These cells were present in 57% of cases in a study of salvage prostatectomies [18] and seemed to represent a nonspecific host response to radiation injury.

IMMUNOHISTOCHEMISTRY, VESSELS AND NUCLEI AFTER RADIOTHERAPY

Prostate-Specific Antigen (PSA), Keratin 34βE12, and α-Methylacyl-CoA Racemase

No definite method exists to assess tumor viability after irradiation. Presence of secretory cells can be documented by reactivity for PAP, leading one group of investigators to suggest that tumor cells capable of protein production probably retain the potential for cell division and consequent metastatic spread [60]. Expression of PSA and pan-cytokeratin often persists after therapy. In a recent small study, residual carcinoma was present in 6 of 14 cases after brachytherapy. PSA reactivity was noted to be decreased in glands that show radiation effect [61].

Basal cell cytokeratin (34βE12) expression also persists after radiotherapy in benign and atrophic glands, helping to visualize treated adenocarcinoma. Some authors report an indeterminate rate of 33% on first post-therapy biopsy, decreasing to 7% on fourth biopsy [54]. However, Brawer *et al.* found indeterminate findings in fewer than 10% of cases with the use of this immunostain on serial sections [20]. Particularly with use of the steam-EDTA optimized method [62] basal cell cytokeratin helps exclude the cancer mimics mentioned above: atypical basal cell hyperplasia, atypical adenomatous hyperplasia, sclerosing adenosis, and postatrophic hyperplasia.

Yang *et al*. tested the new marker α-methylacyl-CoA racemase (AMACR, P504S) to determine its usefulness in diagnosing post-irradiation cancer [63]. It was consistently reactive in 28 cancers and non-reactive in 12 benign post-biopsy cases. However, used in conjunction with cytokeratin 34ßE12, P504S was considered not to increase recognition of post-irradiation cancer compared with cytokeratin 34ßE12 alone [64].

Proliferation Markers

MIB-1 immunoreactivity in pretreatment needle biopsies independently predicts post-radiation recurrence [65] and helps determine optimal radiation dose. In post-radiotherapy prostate biopsy specimens, retention of proliferating cell nuclear antigen (PCNA) [53, 66] or Mib-1 (Ki-67) [26, 52] immunoreactivity correlates with local cancer recurrence ($p = 0.004$). After brachytherapy, residual carcinoma that shows radiation injury also has a minimal (< 5%) Ki-67 reactivity [61]. Furthermore, prostate cancer in salvage prostatectomies is proliferative in 96% of cases, showing increased Mib-1 immunostaining [22]. The mean Ki-67 labeling index in recurrent prostate cancer after radiation is increased (mean, 7.0%) compared to the index in prostatectomy series without prior radiotherapy (mean, 2.7%).

Oncogenes and Tumor Suppressor Genes

Prostate cancer after radiotherapy has increased p53 nuclear accumulation [67], although some other results suggest no significant difference [68]. Abnormal p53 (in 20% or more cells) was detected in 168 of 777 (21.6%)

cases, and was significantly and in dependently associated with cause-specific mortality [45]. These findings suggest that p53 alterations are present before radiotherapy and serve as a pre-therapy predictor of cancer recurrence [67, 68].

Glutathione S-transferase pi (GST-π) is a detoxifying enzyme that inactivates reactive oxygen free radical species by conjugation with glutathione. Most prostate cancers do not express GST-π, and loss of GST-π expression is considered as a phenotype associated with malignant transformation [69].

$p21^{WAF1}$ and $p27^{Kip1}$ are members of KIP family of cell cycle proteins and inhibit several cyclin-dependent kinase complexes. Functional loss of the cycle-dependent inhibitors has been implicated in carcinogenesis and cancer progression. Loss of $p27^{Kip1}$ expression in prostatic and nonprostatic malignancies is associated with a more aggressive phenotype [70]. Loss of $p21^{WAF1}$ function has been implicated in the failure of irradiation response, and p21 has been shown to be an independent prognostic factor in prostate carcinoma. The authors detected $p21^{WAF1}$ nuclear immunoreactivity in cancer cells in 39 (75%), of 52 patients (median nuclear immunoreactivity, 5%; range 0% to 80%); $p27^{Kip1}$ nuclear immunoreactivity was detected in all 52 patients (median nuclear immunoreactivity, 50%; range, 5% to 90%). Five-year distant metastasis-free and cancer-specific survival rates were 71% and 82% for patients with low expression of p21 (5%), compared with 94% and 100% for patients with high expression of p21 (>5%) (p = 0.02 and 0.01, respectively) [70]. Five-year distant metastasis-free survival and cancer-specific survival rates were 91% and 82% for patients with low expression of p27 (< 50%), compared with 88% and 96% for patients with high expression of p27 (≥ 50%) (p = 0.06 and 0.01, respectively).

Anti-Apoptosis Genes

Early growth response-1 (Egr-1) gene is an early response gene, in the family of c-jun and c-fos. Egr-1 activation is required for the cellular response to radiation injury. The authors noted overexpression of Egr-1 in prostate cancer, which increased with Gleason grade [71]. Ahmed *et al*. later found that Egr-1 immunohistochemical expression correlated with treatment failure. The overexpressed Egr-1 is in a mutant form which does not transactivate the usual target genes TP53, pRB, and Bax [72]. Egr-1 may come to be used as part of a panel with a proliferation marker to predict prognosis.

Microvessel Density

In a study by Hall and co-workers [73] microvessel density was higher in cancer specimens from patients who failed radiotherapy than in patients who did not fail; however, the results were not analyzed independently of cancer grade.

Nuclear Morphometry

The degree to which nuclei deviate from circularity predicts the prognosis of patients with stage 2 prostate cancer. This observation was applied to biopsies from men treated with external beam irradiation. A prognostic factor score incorporating 2 parameters, suboptimal circle fit and feret-diameter ratio, predicted cancer-free survival (p = 0.0014) [74].

SUMMARY

Substantial and characteristic changes occur in the microscopic appearance and immunophenotype of the hyperplastic prostate and adenocarcinoma following androgen deprivation therapy and radiotherapy. These changes are rarely seen in untreated cancer, and the combinations of features following therapy are sufficiently distinctive that pathologists can usually recognize them. Pathologists must be aware of these distinct changes because of the reliance placed on nuclear and nucleolar size in the identification of prostate cancer, particularly in small specimens and lymph node metastases.

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