1. ABSTRACT

Retraction artifacts are well known phenomenon in diagnostic surgical pathology for a long time but they were usually considered as artificially produced tissue alteration. Some recent studies of retraction artifact in different tumors have focused on its diagnostic and/or prognostic role. Their presence in prostatic carcinoma may be used in diagnostic and prognostic purposes. Peritumoral retraction artefacts in prostatic carcinoma are more pronounced and more common around neoplastic glands compared to benign glands, gland with prostatic intraepithelial neoplasia and postatrophic hyperplasia that is the one of the most common mimickers of prostatic carcinoma. In addition, the presence of extensive retraction artifact in prostatic carcinomas is clearly associated with tumor features that indicate a more aggressive tumor phenotype and with shorter biochemical recurrence-free survival. Studies at the molecular level demonstrate that retraction artifact may be attributed to molecules produced by reactive cancer stroma. The origin of retraction artifact in tumor specimens is unknown but they are probably the consequence of lack of basal cells and/or stromal changes but not simply artifacts due to laboratory procedures.

2. INTRODUCTION

Retraction artifacts are well known phenomenon in diagnostic surgical pathology for a long time but they have received very little attention in routine daily practice. They were usually considered as artificially produced tissue alteration that interferes with the ability to make an appropriate diagnosis. Peritumoral retraction artifact appears in tissue sections as an empty space partially or completely encircling a nest of tumor cells, usually in conformity with the rounded or angular outline of that particular nest. Different terms were proposed for this phenomenon including peritumoral clefting, periacinar halos, retraction clefting or cleft-like spaces (1, 2).

Recent investigations have stressed their diagnostic and prognostic significance in different tumors (1-13). Periacinar retraction clefting was proposed as an additional and helpful diagnostic criterion in prostate cancer (1-5), breast carcinoma (6), and urothelial carcinoma (especially in cases of urothelial carcinoma with microinvasion) (7-9). Furthermore, retraction artifact could serve as diagnostic criterion for distinguishing noninvasive and invasive extraovarian implants in borderline serous
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Figure 1. A) Periacinar retraction clefting affecting more than 50% of circumference of neoplastic acini in prostatic adenocarcinoma (HE, x400). B) Neoplastic acini in prostatic adenocarcinoma without retraction clefting (HE, x400). C) Periacinar retraction clefting is visible around neoplastic glands but not around benign glands that are present in the same area (HE, x400).

Ovarian tumors. The presence of retraction artifact around solid epithelial nests in extraovarian implants was a sign of invasiveness (10). Retraction clefting may also be observed in other tumor types and can be useful in the differential diagnosis between basal cell carcinoma and adnexal skin tumors (11).

Beside their diagnostic significance, some studies of retraction artifact in tumors have also showed its prognostic role. The presence and/or extent of peritumoral clefting was significantly associated with adverse clinicopathologic tumor features and poor prognosis in borderline serous ovarian tumors with extraovarian implants, breast carcinoma, squamous cell carcinoma of the esophagus and prostate carcinoma (10, 12-15).

Although frequently present in sections of formalin-fixed, paraffin-embedded tissue samples, the origin of and the biologic mechanisms responsible for the retraction artifact or their biologic or clinical significance are still largely unresolved.

3. RETRACTION ARTIFACT IN DIAGNOSIS OF PROSTATE CARCINOMA

Prostatic adenocarcinoma is the most common noncutaneous malignant neoplasm in humans with an estimated prevalence of over 30 percent in males older than 50 years based on histological autopsy studies (16). The diagnosis of prostatic carcinoma is complex, especially in needle core biopsies and is based on a constellation of three major histological criteria: the infiltrative growth pattern, the absence of a basal cell layer and the presence of macronucleoli (nucleoli larger than 1 micrometre in diameter) (1, 16). There are also three strong additional histologic criteria that are considered as diagnostic for prostatic adenocarcinoma including perineural invasion, mucinous fibroplasias (collagenous micronodules) and glomerulations (17). Several supportive diagnostic criteria have been proposed, but only a minority of these supportive features is specific for tumor. Proposed supportive criteria used in routine diagnostic procedure are: marginated nucleoli, multiple nucleoli, wispy, bluish mucinous secretions, intraluminal crystalloids, intraluminal amorphous eosinophilic material, collagenous micronodules (mucinous fibroplasias), glomerulations, peritumoral clefting and others, but many of these supportive criteria may also be present in benign glands or in some nonneoplastic proliferative glandular conditions (1, 16, 18, 19).

One of the proposed additional criteria is peritumoral retraction artifact appearing more frequently around neoplastic glands in comparison with benign glands (1, 20-23). Halpert et co-workers (21, 22) were the first to shortly describe the retraction artifacts in prostatic adenocarcinoma. In their autopsy studies of prostatic adenocarcinoma, halos around tumorous acini were observed. In a study by Varma et al (1) retraction artifacts were found in approximately 40% of cases of prostatic cancer diagnosed by needle core biopsy and in 7% of nonneoplastic glands. They graded retraction artifact as a percentage of gland circumference separated from the stroma using a scale from 0-4. The same authors considered grade 3 (50-75%) and 4 (more than 75%) as positive. We analyzed retraction artifact under high magnification (x40 objective) counting 10 glands in three different fields (2). Retraction artifact affecting more than 50% of circumference in more than 50% of analyzed glands was found in neoplastic glands only. Such strict criteria were fulfilled in about 16% of all cancer cases and were not observed in normal glands. The application of less stringent criteria (one third of glands with more than 50% of circumference) revealed positive retraction artifact in 51.8% of carcinoma cases and 8% of nonneoplastic glands that was also statistically significant. Similar retraction artifact changes should not be present in adjacent normal glands within the same specimens (Figure 1 A, B and C). Retraction artifact could be observed also under lower magnification. Our criteria may be applied in a similar manner to carcinoma cases composed of few neoplastic glands (2).

The presence of retraction artifact in prostatic needle core biopsy was usually attributed to the inadequate laboratory procedure. Therefore, the quantity of retraction artifact was considered as an indicator of quality of laboratory staff. To minimize the influence of laboratory procedure and different technicians an improved
We have further analyzed retraction artifacts in prostatic adenocarcinoma in needle core biopsies and corresponding tumor after prostatectomy to contribute to refinement of the criteria for the determination of retraction artifact and to confirm its diagnostic value (3). There was a significant correlation if the criterion was one third or more of neoplastic glands showing retraction artifact affecting more than 50% of circumference. A stricter criterion that regarded as positive the cases with at least 50% of neoplastic glands (15 or more of 30) with retraction artifact that affected more than 50% of circumference showed retraction artifact in 24 (15.8%) of needle biopsies and 37 (24.3%) of prostatectomy material. (3). There was a correlation of periacinar retraction artifact between needle core and corresponding prostatectomy specimens suggesting that retraction artifacts are not simple artifacts caused by laboratory procedure but in fact may represent a reliable criterion for diagnosis of the prostatic adenocarcinoma (Figure 2A and B). Similar results regarding correspondence of periacinar retraction artifact in needle core biopsies and larger surgical specimens were also reported by Acs et al (12) for breast carcinoma.

There are many different benign conditions that may mimic prostatic carcinoma, like partial and/or complete atrophy, adenosis and many others (16, 27-30). According to recent studies, one of the most common mimickers is postatrophic hyperplasia (PAH) (27, 28). PAH is characterized by hyperplastic glands intermingled with atrophic ones lined with cells with scanty cytoplasm. The degree of nuclear atypia is less than in most carcinomas. The slit-like acini and apical blebs of some cases of PAH are very rare in most carcinomas (16, 27, 28). The dense fibrotic stroma and shrunken muscle cells that may be seen in PAH are quite uncommon in carcinoma. An obvious infiltrative growth pattern is not seen in PAH and lobulation, which is usually present, is helpful architectural feature (27, 28, 31). Adjacent typical atrophy may be a clue to the diagnosis. The basal cell layer is typically present, although it may be difficult to demonstrate it, in some cases even by immunostaining using HMW-cytokeratin or p63 (16, 32).

Numerous features that might help in the differential diagnosis between atrophy and carcinoma are described (16, 27, 28). The presence of retraction clefting around neoplastic glands is an additional criterion favoring prostatic adenocarcinoma (5). Foci of atrophic glands may be classified in two main groups; proliferative atrophy (PA) and proliferative inflammatory atrophy (PIA) and each group may be subclassified into simple atrophy (SA) and postatrophic hyperplasia (PAH), as proposed by the working group for histologic classification of focal prostate atrophy lesions (31). Our study showed no significant difference in the presence and extent of retraction clefts between PA and PIA (5). Prostatic carcinoma showed statistically significant difference in the frequency and extent of retraction clefts, as compared to PA and PIA (Figure 3A and B). According to our knowledge and data from the literature it was not routinely applied to distinguish atrophy from carcinoma (1-3).
Retraction artifact in the prostate

Figure 3. Atrophic glands in focuses of proliferative atrophy (A) and proliferative inflammatory atrophy (B) were mostly without periacinar clefting or clefting was present in less than half of the gland circumference (HE, x200).

4. PROGNOSTIC SIGNIFICANCE OF RETRACTION ARTIFACT IN PROSTATE CARCINOMA

Some recent studies of retraction artifact in tumors have focused on its prognostic role (10, 12-14). The presence and/or extent of peritumoral clefting was significantly associated with adverse clinicopathologic tumor features and poor prognosis in borderline serous ovarian tumors with extraovarian implants, breast carcinoma and squamous cell carcinoma of the esophagus (10, 12-14).

We examined whether the presence and extent of peritumoral retraction artifact could be used to predict biochemical recurrence-free survival in prostatic carcinoma. We also analyzed the correlation between the extent of retraction artifact in the tumors and various clinicopathologic tumor features (15).

Our results revealed that the retraction artifact in prostatic carcinoma correlated with different clinicopathologic tumor features and biochemical recurrence-free survival. Patients with high preoperative PSA and short follow-up time had tumors with a significantly higher percentage of retraction artifacts. While a minimal inclusion criterion for patients without relapse was a follow-up of 60 months, many of the patients with biochemical relapse were included in the study despite a shorter follow-up time. Thus, negative correlation of follow-up time and extent of the retraction artifact indicated that tumors in patients with biochemical recurrence harbored more extensive retraction artifacts. Seminal vesicle invasion and/or extracapsular extension of the tumor (T3 stage tumors) and positive surgical margins were also significantly more common in tumors with extensive retraction artifacts. The extent of retraction artifact showed no significant correlation with lymph node metastasis, but any possible conclusions in this regard are hampered by the fact that only six patients in our study group had nodal metastasis, which is an insufficient number for a reliable statistical analysis. In the group of 46 patients with biochemical recurrence extent of retraction artifact was not significantly different and was not significantly associated with disease free survival time. This fact could indicate that the extent of retraction artifact over some cutoff value had no further implications on biochemical recurrence and disease free survival time. In our investigation, cutoff value was set on retraction artifact involving at least 20% of the tumor glands. The presence of extensive retraction artifact in prostatic carcinomas was clearly associated with tumor features that indicated a more aggressive tumor phenotype. In addition, extensive retraction artifact was associated with poor biochemical recurrence-free survival in both univariate and multivariate analyses (15).

Similar prognostic significance of retraction artifact was reported for breast carcinoma (12, 13). In the study of Acs et al (12) the extent of retraction artifacts showed a significant correlation with increasing tumor size, histologic type, combined histologic grade, and all three components of the histologic grading system. Tumors associated with lymphatic vessel invasion and axillary lymph node metastases showed significantly higher amounts of retraction artifacts compared with tumors without these features. Further, extensive retraction artifact in breast carcinoma was significantly associated with poor overall and disease-free survival (12).

In a more recent study, Acs et al (13) also showed that the presence of extensive retraction artifacts in core needle biopsy samples of invasive ductal carcinoma was significantly associated with the presence of lymph vessel invasion and lymph node metastasis. These findings indicated that the presence of extensive retraction artifacts on core needle biopsy can predict nodal metastasis in the breast carcinoma (13).

In esophageal squamous cell carcinoma, the presence and the extent of retraction artifacts was associated with local tumor penetration and lymph node metastasis. Tumors with extensive retraction artifact mostly presented at a higher stage and with lymph node metastasis. Thus, extensive retraction artifact pertained to a more aggressive tumor behavior and could be viewed as a simple and useful morphological feature of tumor aggressiveness (14).

In addition, as we previously mentioned, Bell et al (10) showed that presence of retraction artifact around solid epithelial nests in extraovarian implants of borderline serous ovarian tumors strongly correlated with adverse outcome (10).

5. POSSIBLE ORIGIN OF RETRACTION ARTIFACT IN PROSTATE CARCINOMA

The origin of retraction artifact in tumor specimens is unknown. Few hypotheses should be taken in
P63, a homologue of the tumor suppressor gene p53, has been shown in the basal cell component of epithelium from a variety of tissues, including prostatic epithelium (39-41). Value of p63 immunostaining in the diagnosis of prostatic adenocarcinoma has been clearly shown (40, 41). However, the presence of clearly identifiable basal cells in a gland or duct does preclude the diagnosis of carcinoma for that structure (39). At least focal high molecular weight cytokeratin (clone34betaE12 antibody) positivity was observed in 1.1% of 3198 prostatic carcinoma cases diagnosed on needle core biopsies (39).

Our investigation performed on 28 core biopsy with PIN and 41 core biopsy with prostatic carcinoma showed that periacinar retraction clefting was significantly more prominent in prostatic carcinoma compared to PIN and nonneoplastic glands. There was no difference between normal glands and PIN regarding clefting. P63 was positive around whole circumference in 12 out of 28 cases with PIN, and discontinuously positive in remaining 16 PIN cases suggesting initial disruption of basal cell layer (Figure 4A and B). P63 immunostaining was also positive in all nonneoplastic glands, and negative in all carcinoma cases. These data suggest that retraction clefting was associated with cancer and lack of basal cells, but not with PIN (4).

Reactive stromal changes that occur in different human cancers are probably involved in local tumor spreading. It seems that stromal components play very important role in enhancement of tumor progression by stimulating angiogenesis and by promoting cancer cell survival, proliferation, and invasion. Studies of human breast, colon and prostatic cancer specimens have identified activated stromal cell phenotypes, modified extracellular matrix (ECM) composition, and increased microvessel density, exhibiting biological markers consistent with stroma at site of wound repair (42-45).

It is well known that stromal cells modulate normal prostatic development, growth, and differentiation through stromal-epithelial interactions (46, 47). In the normal prostate, prostatic smooth muscle cells under the influence of androgen signal to prostatic epithelium control epithelial differentiation and suppress epithelial proliferation. On the other hand, prostatic epithelium sends signals to prostatic smooth muscle cells to maintain smooth muscle differentiation (48-50). In experimental models, prostate stromal cells promote angiogenesis and stimulate the development and rate of human prostate tumorigenesis (51, 52). Furthermore, it is well known that stromal changes in prostatic carcinoma could have implication on prognosis of the disease (53, 54). Ayala et al (53) and Tomas et al (54) showed that intensity of stromal changes could serve as an independent prognostic factor in the assessment of biochemical recurrence-free survival in patients with prostatic carcinoma that underwent radical prostatectomy. In addition, intensity of stromal changes could identify patients with higher risk of disease recurrence in cases of identical Gleason score (54).

Figure 4. P63 immunostaining in prostatic intraepithelial neoplasia (A) and prostatic adenocarcinoma (B). Nonneoplastic glands served as a positive control (p63, x400).
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Ayala et al (53) and Yanagisawa et al (55) also showed that volumes of reactive stroma could predict biochemical recurrence-free survival in prostatectomy specimens and needle core biopsies. Tumors with no reactive stroma and tumors with abundant reactive stroma had a worse prognosis compared to tumors with a moderate quantity of stroma. They explained these findings by two theories. Firstly, the tumors with no stroma had a worse prognosis because in these tumors epithelial cells began to express genes that are normally restricted to stromal cells and became stroma-independent, with a consequent decrease in stroma quantity. Secondly, the tumors with a large amount of reactive stroma had a worse prognosis because abundant stroma could produce larger quantities of growth factors and also could serve as a shield from host immune and inflammatory response (53, 55).

In normal prostate, stroma is predominantly composed of smooth muscle cells with very few fibroblasts, myofibroblasts and collagen fibers. Reactive stroma in cancer is composed of fibroblasts, myofibroblasts, endothelial cells, and immune cells and shows decrease in number of smooth muscle cells (45).

Tuxhorn et al (46) histochemically and immunohistochemically showed increased number of stromal myofibroblasts in prostatic cancer by double-label fluorescent immunohistochemistry. Myofibroblasts as well as peritumoral clefting were most prominent in Gleason grade 3 prostatic carcinoma (46, 47). These findings confirmed that (myo)fibroblastic stromal changes characterized cancer reactive stroma. Cancer growth subsequently leads to increased amount of (myo)fibroblasts and decreased amount of smooth muscle cells as cancer growth progresses. No similar changes were observed in adjacent peritumoral stroma, what was confirmed by similar staining pattern on Mallory method, and similar vimentin, alpha-SMA and desmin expression compared to prostatic hyperplasia (47).

Myofibroblasts in reactive stroma synthesize ECM components such as collagen I, collagen III, fibronectin, tenascin, and versican. In addition, myofibroblasts express proteases, FAP (fibroblast activation protein), and MMPs (matrix metalloproteinases). These products are remodeling ECM and basement membrane and could stimulate cancer cell growth and migration (56). They also stimulate angiogenesis secreting VEGF (vascular endothelial growth factor), especially VEGF-D. VEGFs are expressed in myofibroblasts under hypoxia or in response to growth factors (57). Therefore it seems that retraction artifact may be attributed to some molecules produced by reactive cancer stroma.

Tenascin-C (TN-C) is a large (180–300 kDa), hexameric multidomain glycoprotein located mainly in the ECM that is involved in tissue interactions during embryogenesis, wound healing, inflammation and oncogenesis (58). The most prominent function of TN-C includes anti-adhesion effects, favouring cell motility and growth promotion. These proposed activities suggest a potential role for TN-C in regulation of tumor cells proliferation, invasion and metastasis (59, 60).

To become invasive, prostate cancer cell must first penetrate acinar basement membrane (BM) proteins and extracellular matrix adhesive glycoproteins (61). Structural components of the BM are laminins, type IV and type VII collagens, nidogens, and proteoglycans (61).

Laminins are heterotrimeric molecules made up by one alpha, one beta and one gamma chain. Until today we know of five alpha-chains, three beta-chains and three gamma-chains. These chains combine into at least 14 different laminins. The distribution of these laminin isoforms varies between tissues, but in most BMs more than one laminin is present (62).

Laminins are associated with a variety of biological activities such as cell differentiation, cell shape and movement, maintenance of tissue phenotypes, and promotion of tissue survival and their functions in tumor invasion is under extensive research today (63).

We have shown increased immunostaining of tenascin-C, and decreased staining for laminin in carcinomas compared with peritumoral tissue and benign prostate hyperplasia. Gleason pattern 3 carcinomas showed more pronounced stromal reaction and tenascin-C expression compared with Gleason pattern 4 carcinomas. The main cells in prostate cancer stroma that are responsible for tenascin production are myofibroblasts. Degradation of laminin was not connected with myofibroblastic stromal changes (48). Our study confirmed appearance of myofibroblasts in prostate cancer reactive stroma, especially in Gleason pattern 3 tumors, but not in adjacent peritumourous tissue and stroma in benign prostate hyperplasia. These findings could suggest possible role of tenascin-C in retraction artifact formation (48).

Our results about tenascin-C expression were consistent with results reported by Tuxhorn and co-workers (46). In their study strong periglandular tenascin expression was also shown in cancer cases. However, they studied tenascin expression on 7 cases of Gleason pattern 3 prostate carcinomas only (46).

Furthermore, molecules secreted by epithelial cells, especially molecules involved in cell adhesion such as Ig superfamily of cell adhesion molecules, integrins, cadherins, selectins, syndecans, epithelial cell adhesion molecule (EpCam) and many others could also be implicated in retraction artifact formation (64-70). Over expression or loss of some of these molecules in cancer epithelial cells could aid in artifact formation.

Irie et al (6) proposed that retraction artifact around tumors tissue actually represent lymph vessel compartments and their appearance connected with lymph vessels invasion in breast carcinoma. In order to investigate this hypothesis we analyzed relationship between retraction artifact and expression of immunohistochemical marker of lymph vessels D2-40 in prostatic carcinoma (71). Neoplastic glands were analysed in 25 paraffin sections.
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from radical prostatectomy specimens with prostatic adenocarcinoma diagnosis. Number of lymph vessels inside tumours tissue ranged between 2 and 75 on the one cross section (average number was 17.4). Lymph vessels that mimicked clefs inside tumours tissue ranged between 0 and 10 on the one cross section (average number was 1.33). In 30 tumorous glands with most extended clefs on each cross section number of lymph vessels ranged between 0 and 3 (average number was 0.6). These results suggest that lymph vessels can mimic retraction artifact inside tumor, but retraction artifacts do not represent lymphatic spaces and some other mechanism is responsible for their formation (71).

6. CONCLUSIONS AND PERSPECTIVES

Peritumoral retraction artifacts may be seen in many different types of carcinoma including prostate, breast, skin, ovary, urinary bladder and others. Their presence may be used in diagnostic and prognostic purposes. Peritumoral retraction artifacts are probably the consequence of lack of basal cells and /or stromal changes but not simply artifacts due to laboratory procedure.

The origin of these changes is still unresolved and future studies, particularly at the molecular level, should be performed to better understand this phenomenon.

7. REFERENCES


Retraction artifact in the prostate


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