Pseudobenign prostate carcinomas: causes of false-negative biopsy results

ALIS DEMA1), SORINA TĂBAN1,2), ELENA LAZĂR1), ANGELA BORDA3), CODRUȚA LĂZUREANU1), DIANA HERMAN2), ANCA MUREȘAN1), MĂRIOARA CORNIANU1), DENISA ANDERCO2), ANDRADA LOGHIN3)

1)Department of Pathology, "Victor Babeș" University of Medicine and Pharmacy, Timisoara
2)Department of Pathology, Emergency County Hospital, Timisoara
3)Department of Histology, University of Medicine and Pharmacy of Targu Mures

Abstract
Prostate carcinomas are continuously surprising the pathologists through their multitude of variants and histological subtypes, some of them being recently described and characterized. Among these are individualized: atrophic carcinoma, foamy gland, pseudohyperplastic, microcystic, certain subtypes of ductal adenocarcinoma and hormone-treated adenocarcinoma, which because of minimal architectural and/or cytological atypia are often under-diagnosed, especially in small tissue fragments. This paper presents the morphological criteria, including information provided by some immunohistochemical markers for positive and differential diagnosis of these variants/subtypes of prostate adenocarcinoma with which the pathologist should be familiar and avoid their confusion with a series of similar histological structures or benign/premalignant lesions.

Keywords: prostate carcinoma, subtypes/variants, differential diagnosis, immunohistochemistry.

Introduction
Despite numerous works dedicated to the subject, prostate cancer continues to surprise the pathologists through various forms of presentation. This explains the fact that in the past 15 years a number of tumor subtypes/variants (some difficult to diagnose, especially in limited tissue fragments amount such as those of needle biopsy (NB), because minor cytological or architectural atypia) have emerged as distinct entities. Among these variants of prostate tumor, some recently described as atrophic, foamy gland, pseudohyperplastic and certain subtypes of ductal adenocarcinoma represent the most common causes of under-diagnosed cancer when the pathologist is not familiar with these entities, and therefore those are called “pseudobenign” carcinomas [1] or carcinomas mimicking benign lesions [2–4].

Foamy gland prostate adenocarcinoma / adenocarcinoma

Foamy gland prostate adenocarcinoma was first described in 1996 [5] as a variant of prostate neoplasm in which tumor cells have foamy cytoplasm [6], and nuclei occupy a small part of the cell surface.

Initial reports indicated this variant, either in pure form or in combination with conventional adenocarcinoma, as consisting of well-individualized glands, corresponding to Gleason pattern 3 [3, 5–7].

Tumor glands having a nodular and/or infiltrative growth pattern are lined by deceptively benign cells, cuboidal or columnar, with abundant foamy, reticular/xanthomatous cytoplasm (Figures 1 and 2), devoid of lipids, glycogen and mucin, but with many cytoplasmic empty vacuoles and polyribosomes; nuclei of tumor cells are usually small, round (Figures 1 and 2), rounder than those of the luminal cells from benign prostate glands, hyperchromatic, with indistinct nucleoli, decreased nucleus to cytoplasm ratio, reasons why these tumors are confused with a number of normal structures or benign/premalignant lesions [3, 5, 6, 8, 9].

In essence, the discrimination of foamy gland adenocarcinoma against similar benign lesions is based on the infiltrative nature of the glands and the presence of dense pink acellular dense intraluminal secretions, reported in over 50% of foamy gland adenocarcinoma cases. These aspects are strong arguments for the malignant nature of glands lined by foamy cells [5].

A series of normal anatomic structures, premalignant or benign prostate lesions as Cowper glands, prostatic mucinous metaplasia, cell clear cribriform hyperplasia, adenosin, foamy High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) or prostatic xanthoma are considered in the differential diagnosis of foamy gland carcinoma. On small tissue fragments, the distinction between these lesions is sometimes difficult and relies primarily on data offered by the usual stains. In this respect the lobular character of the glands advocate for adenosin foci or Cowper glands. In addition, Cowper glands, located near the prostatic apex (Figure 3) are composed of a dimorphic population of mucinous (mucicarmine
and PAS-D – positive), tightly packet rounded acini with basally located uniform nuclei and ducts/ductuli lined by hybrid cells with both mucinous and ductular epithelial features [6, 10]. Immunohistochemically (IHC), Cowper glands are negative for Prostate Specific Acid Phosphatase (PSAP) and variable positive for Prostate Specific Antigen (PSA) [6, 10]. High Molecular Weight Cytokeratin (HMWCK) marks strongly the ductular epithelium of Cowper glands and an attenuated layer of cells at the periphery of the lobules [10], in contrast with foamy gland adenocarcinoma where xanthomatous cells do not contain mucin, and basal cells are missing. Mucinous metaplasia is a focal prostatic lesion characterized through the replacement of principal cells by mucin secreting cells with flattened, basally located nuclei and large amount of cytoplasm (Figure 4). The latter contains neutral and/or acidic mucin (Figure 5) and is PSA-negative [11], a histochemical/IHC profile contrasting with those of foamy gland adenocarcinoma.

Unlike the glands of foamy gland adenocarcinoma, those of clear cell cribriform hyperplasia (Figure 6) present an easily identifiable, sometimes even prominent, basal cell layer at the periphery [12] (Figure 7).

The differential diagnosis between pseudohyperplastic adenocarcinoma with xanthomatous changes and usual pseudohyperplastic adenocarcinoma is based on the presence of nucleomegaly and prominent nucleoli in usual pseudohyperplastic adenocarcinoma [5, 13].

25–38% of foamy gland adenocarcinomas lacks AMACR expression or the reactivity is heterogeneous with negative areas admixed with different intensity stained zones (Figure 8) [14, 15], making the IHC diagnosis of the lesion in some cases to be based solely on the demonstration of basal cells absence at the periphery of glands. The presence of basal cells, usually as a discontinuous layer at the periphery of foamy gland pleads either for foamy HGPIN foci, a rarely seen pattern first described in 2000 (Figures 9 and 10) [6, 16, 17], either for ductal extension of high-grade foamy gland carcinoma – foamy intraductal carcinoma (Figure 11) [18]. Besides, the histologic discrimination of the two entities is almost impossible.

More recent studies report cases of poorly differentiated foamy gland adenocarcinomas, with all the patterns described for conventional high-grade adenocarcinoma, with prominent nuclei present in one third of cases, mitoses in 40% of cases and desmoplastic stroma, an attribute of this variant of high-grade prostate adenocarcinoma [6, 18]. In this category of tumors it was reported a limited aberrant reaction for p63 and especially for HMWCK (34βE12) with a non-basal cell pattern (Figure 12), which complicates the interpretation of IHC reactions for basal cells and the diagnosis of these lesions, too [5, 18]. Poorly differentiated foamy gland prostate adenocarcinoma must be distinguished from prostatic xanthoma, a lesion characterized by the presence of a nodular aggregate, cords or single xanthomatous cells with small, uniform nuclei, indistinct nucleoli, rich vacuolated cytoplasm, scattered in the prostatic stroma. In difficult cases IHC investigation is the one that clarifies the diagnosis, xanthoma presenting the CK-negative, CD68-positive IHC profile [6]. The grading of foamy gland carcinoma is done considering the architectural aspects rather than the cytological ones [19]. Despite its cytological “benign” aspect, this type of carcinoma is sometimes associated with an intermediate or aggressive behavior [5, 8].

Pseudohyperplastic prostate adenocarcinoma

Pseudohyperplastic prostate adenocarcinoma, recently defined as an entity is also a rare variant of prostate epithelial neoplasm with a reported incidence around 2% in NB samples [4, 13], 3% in TURP fragments [20] and 11% in radical prostatectomy (RP) specimens [13]. Areas of pseudohyperplastic carcinoma were identified both in the peripheral and transition zone of the prostate [13], with a nodular or clustered growth pattern. Sometimes in TURP fragments, the tumor appears as small, isolated foci, which may be overlooked [20].

Figure 1 – Foamy gland prostatic adenocarcinoma: tumor cells with abundant, foamy cytoplasm. HE stain, ×400.

Figure 2 – Foamy gland prostatic adenocarcinoma with eosinophilic luminal secretions. HE stain, ×200.
Figure 3 – Cowper glands. HE stain, ×200.

Figure 4 – Prostatic mucinous metaplasia. HE stain, ×400.

Figure 5 – Prostatic mucinous metaplasia: predominantly acidic mucinous secretions. AA–PAS stain, ×400.

Figure 6 – Clear cell cribriform hyperplasia. HE stain, ×400.

Figure 7 – Prominent basal cell layer at the periphery of the clear cell cribriform hyperplasia aggregates. Anti-HMWCK (34βE12), EnVision system, DAB, ×400.

Figure 8 – Foamy gland prostatic adenocarcinoma: heterogeneous reactivity for AMACR. Anti-AMACR, EnVision system, DAB, ×400.
Microscopically, pseudohyperplastic carcinoma has a seemingly benign architectural aspect at low magnification given by the presence of large or medium-sized crowded glands, sometimes arranged back to back, with straight even luminal borders or wavy internal contour with branching papillary projections (Figure 13) or cystically dilated glands, similar to hyperplastic glands [13, 20, 21]. The tumor cells are columnar with amphophilic cytoplasm, basally located enlarged nuclei, with prominent, large nucleoli (Figure 14). Amorphous pink secretions, crystalloids or corpora amylacea are often seen in the lumen of the glands [20–22]. The variant of pseudohyperplastic carcinoma composed of large, obvious dilated glands with straight luminal outline (Figure 15), arranged back to back and lined by abundant cytoplasm; cells is difficult to recognize as malignant [23]. There are cases with combined aspects of foamy gland and pseudohyperplastic features [4].

The most useful criteria for recognizing the malignant nature of the lesion are: crowded glands, absence of dense stroma characteristic for benign prostatic hypertrophy (BPH), tumoral glands infiltrating among obvious benign glands, the continuity of pseudohyperplastic zones with small acinar adenocarcinoma areas, the presence of prominent nucleoli. Only in 25% of cases, the infiltrating character of pseudohyperplastic carcinomatous glands is obvious on NB specimens, making the diagnosis difficult on routine stains [21].

For the cases with minimal glandular crowding and equivocal nuclear atypia, the presence of a large number of glands without basal cells at IHC determinations (p63, CK5/6, HMWCK) (Figure 16) represents a sufficient criteria for the diagnosis of cancer [6]. AMACR has a doubtful value for the positive diagnosis since 23% – 30% of pseudohyperplastic carcinomas lack reactivity for this marker [14] or show a heterogeneous staining pattern (Figures 17 and 18), depending, in the opinion of some authors, of antibody type (mono- vs. polyclonal). These features require caution in assessing the malignant nature of the suspected lesion solely based on AMACR expression.

Although initially included in the category of well-
differentiated carcinoma of the transition zone (Gleason pattern 1–2) [24], more recent data show that this tumor subtype is constantly associated with pattern 3 micro-acinar conventional adenocarcinoma or even with high-grade prostate adenocarcinoma and/or adverse prognostic factors (extraprostatic extension, vascular invasion) [13, 21], which made the International Society of Urological Pathology Consensus Conference on Gleason Grading of prostate adenocarcinoma to recommend grade 3 for pseudohyperplastic adenocarcinoma areas [25]. Despite the apparent benign aspect, the pseudohyperplastic carcinoma may have an aggressive evolution [6].

\[ \text{Atrophic adenocarcinoma of the prostate} \]

Atrophic adenocarcinoma or perhaps more accurately, the atrophic pattern of prostate adenocarcinoma is a rare morphologic subtype of prostate tumor that architecturally resembles the atrophy and post-atrophic hyperplasia, but shows the cytological aspects of prostate adenocarcinoma [22, 26]. Initially described in 1997, this variant of prostatic adenocarcinoma, labeled as such in the absence of hormone therapy history is most often identified in RP and NB specimens, in the peripheral zone of the prostate and more rarely in TURP specimens [26, 27]. The incidence is variably reported, depending on the type of analyzed specimen and the diagnostic criteria (pure form or in association with non-atrophic carcinoma). Kaleem Z et al. [28] reported the presence of atrophic carcinoma areas in 15.8% of fully examined RP specimens, while Egan AJ et al. [26] shows the presence of atrophic carcinoma aspects in 3% of the 202 investigated RP and respectively 2% of 100 analyzed NB.

A malignant prostate epithelial tumor can be labeled as atrophic carcinoma only if atrophic glands occupy at least 50% of the tumor [27]. The diagnostic criteria are: (1) infiltrative appearance with small, atrophic glands, some dilated and distorted, interposed between larger benign glands; tumor glands are lined by flattened cells with scant cytoplasm, so nuclei occupy almost the entire height of the cell (Figure 19) and the nucleus to cytoplasm ratio is increased; (2) simultaneous presence of non-atrophic carcinoma features (Figure 20); (3) more cytological atypia than in benign atrophy, with enlarged nuclei and macronucleoli [6, 26–28]. Inconstantly, there were reported a number of additional microscopic aspects: eosinophilic luminal proteinaceous material, slightly basophilic mucins, crystalloids, apocrine blebs, collagenous micronodules, associated HGPIN lesions, stromal fibrosis and focal chronic inflammatory infiltrate [26].

For several reasons, sometimes is very difficult to discriminate atrophic carcinoma from foci of prostatic atrophy (Figure 21): atrophy is often present in the peripheral zone of the prostate, which is assessed by the NB [19]; on usual stain basal cells are difficult or even impossible to identify at the periphery of atrophic glands; foci of epithelial atrophy may look as collections of apparent infiltrative glands, but unlike atrophic carcinoma, the infiltrating individual glands among benign glands are lacking; pseudoinfiltrative cords are sometimes evident when atrophic glands are tangentially sectioned (Figure 22); some foci of atrophy may present epithelial cells with enlarged nuclei and prominent nucleoli [6]. In difficult cases, IHC makes the difference. The reactions for basal cells markers are proven to be useful (Figure 23) by demonstrating these cells in benign atrophy (Figure 24), but is worthy to remember that basal cells are sometimes only focally present at the periphery of atrophic glands or they are even absent [29, 30]. Some literature data show the absence of AMACR expression in up to 30% of atrophic carcinomas [31] (Figure 25), and on the other hand there are reported cases of prostatic atrophy, especially foci of partial atrophy (Figures 26–28), positive for AMACR [30, 32], so AMACR has a limited discriminatory value in prostate lesions with atrophic glands. Atrophic carcinoma must be differentiated from reactive atypia associated with inflammation.
Figure 15 – Pseudohyperplastic carcinoma: dilated glands with smooth internal contour, cells with abundant cytoplasm and eosinophilic luminal secretions. HE stain, ×200.

Figure 16 – Pseudohyperplastic carcinoma: the absence of basal cells. Anti-p63, EnVision system, DAB, ×200.

Figure 17 – Pseudohyperplastic carcinoma: heterogeneous positive reaction for AMACR including negative areas. Anti-AMACR, EnVision system, DAB, ×200.

Figure 18 – Pseudohyperplastic carcinoma: intense diffuse positive reaction for AMACR. Anti-AMACR, EnVision system, DAB, ×100.

Figure 19 – Atrophic prostatic adenocarcinoma. HE stain, ×400.

Figure 20 – Prostatic adenocarcinoma with atrophic areas. HE stain, ×400.
Figure 21 – Focus of benign prostatic atrophy. HE stain, ×200.

Figure 22 – Focus of epithelial prostatic atrophy with tangentially sectioned glands, apparently infiltrating the stroma. HE stain, ×100.

Figure 23 – Atrophic prostatic adenocarcinoma: the absence of basal cells. Anti-HMWCK (34βE12), EnVision system, DAB, ×400.

Figure 24 – Benign prostatic epithelial atrophy with zonal/focal presence of basal cells at the periphery. Anti-p63, EnVision system, DAB, ×200.

Figure 25 – Prostatic adenocarcinoma with atrophic areas: heterogeneous positive reaction for AMACR, with atrophic glands p63- and AMACR-negative. Anti-cocktail p63/AMACR (Inset), EnVision system, DAB, ×400.

Figure 26 – Focus of partial prostatic epithelial atrophy. HE stain, ×200.
Most of the atrophic adenocarcinomas are moderately differentiated, Gleason score 6 or 7, with a Ki-67 proliferation index lower than that of non-atrophic carcinomas: 4% vs. 5.3% [26–28].

Microcystic adenocarcinoma

Microcystic adenocarcinoma is microscopically characterized by cystic dilatation of the tumoral glands. Microcystic pattern is reported both in atrophic adenocarcinoma [28] and especially in the pseudohyperplastic adenocarcinoma, with an incidence depending on the specimen’s type: 11.2–32% for RP, 80% for TURP and 90% for NB specimens [1, 13, 20, 21]. This variant of carcinoma has an infiltrative, nodular or mixed (nodular-infiltrative) growth pattern. Yaskiw O et al. [1] reserves the microcystic adenocarcinoma denomination for those tumors with cystically dilated glands, larger than non-microcystic adenocarcinoma glands from the same case. Microcystic glands are rounded and lined by cells with moderate or scant cytoplasm, sometimes with apical cytoplasmic snouts, with or without the appearance of flattened nuclei (Figure 29) and show crystalloids and slightly basophilic mucins in the lumen (Figure 30). Tumor cell nuclei are enlarged in size, with prominent nucleoli. Nuclear atypia is difficult to assess in the cystically dilated atrophic glands in which the nuclei appear compressed. The mitoses and perineural invasion are rarely reported. At low magnification the exclusively microcystic foci are extremely difficult to be diagnosed as malignant, because these can be mistaken for benign hyperplasia nodules with cystically dilated glands or with cystic atrophy [33]. The presence of small infiltrative glands in combination with or located near the cystically dilated glands and nuclear atypia are the most useful elements for positive diagnosis of carcinoma. IHC, basal cells are lacking and AMACR is expressed in most cases (Figure 31), feature that facilitates a lot the diagnosis in difficult cases on usual stains [1]. Microcystic adenocarcinomas should not be confused with prostate’s carcinomas with grossly or radiologically identified cystic features, which are classified as ductal adenocarcinomas [34] or as cystadenocarcinomas, the latter being considered also variant of ductal adenocarcinomas [35]. It is estimated that the foci of microcystic adenocarcinoma correspond to Gleason grade 2 or 3.

Ductal adenocarcinoma

Besides the classical subtypes, there are less known and recognized new variants of prostatic ductal adenocarcinoma described in recent years, which mimic a number of benign or premalignant lesions. HGPIN-like adenocarcinoma is most often but not exclusively considered a variant of ductal adenocarcinoma of the prostate mimicking foci of HGPIN, and therefore it could be under-diagnosed and forward incorrectly treated. This variant of prostatic adenocarcinoma signaled for the first time, although not as a ductal adenocarcinoma’s subtype, in 2003 by Amin MB et al. [36], which have described aspects of circumferential perineural invasion by malignant glands similar to micropapillary HGPIN (Figure 32). In 2006, Hameed O and Humphrey PA [37] have used the term PIN-like carcinomas for tumors characterized by the presence of non-cribriform glands lined by stratified columnar cells with oval nuclei or cubic tumor cells with round nuclei (Figure 33) and assessed a 1.3% incidence for this tumor subtype in the consecutive prostatic NB specimens. At that time, the cases described by them were not labeled as variant of ductal adenocarcinoma, although the authors have noticed some morphological similarities with prostatic ductal adenocarcinomas not excluding the possibility that these tumors represent samples of ductal adenocarcinoma.

The prostatic tumors with stratified cells in the glands – HGPIN-like adenocarcinomas – exist either in pure form or in combination with conventional adenocarcinoma with tumor cells arranged in a single row.
Microscopically, the HGPIN-like ductal adenocarcinomas are characterized by individual glands, widely variable in size, some conspicuously cystic dilated and lined by columnar pseudostratified cells, resulting architectural models alike flat, tufting and micropapillary HGPIN [37, 38]. Often the mentioned patterns associate themselves, most commonly the flat with the tufted. The cells have amphophilic cytoplasm and basally located, oval or spindle nuclei without noticeable pleomorphism and mitotic activity; there are no solid areas or necrosis [38]. There have been reported prominent Paneth-like cells in some cases of HGPIN-like ductal adenocarcinoma [39]. The crowded and/or distorted aspect of glands, flat pattern, striking dilated glands, more marked overlapping of cells, less prominent nucleoli and the absence of basal cells at the periphery of the glands are the main criteria to differentiate the lesion from HGPIN foci [38, 40]. AMACR expression in HGPIN-like ductal adenocarcinoma is diffuse, intense or focally positive but can be sometimes absent [38], which limits the value of the marker for the differential diagnosis with the HGPIN foci, that also present a variable reaction to AMACR [41]. This differential diagnosis is crucial since the
therapeutic approach in the two situations is completely different.

If ductal adenocarcinomas are generally considered tumors with Gleason score 8 (pattern 4), it is recommended not to grade the HGPIN-like ductal adenocarcinoma subtype, but to add a comment on the less aggressive evolution of this tumor compared to typical ductal adenocarcinomas, similar to score 6 carcinoma (pattern 3) [38].

Along with HGPIN-like ductal adenocarcinoma was recently described a variant of ductal adenocarcinoma mimicking the prostatic villous adenoma, characterized by the presence of papillary projections with a fibrovascular core lined by columnar cells with abundant cytoplasm and pseudostratified nuclei with minor atypia. The positive reaction for PSA, the absence of mucins on PAS and Alcian Blue stains and the lack of reactivity for CK7, CK20 and CEA, differentiate this form of prostate tumor from villous adenoma of the urethra [42].

The micropapillary, cystic papillary and foamy gland represent other subtypes of ductal adenocarcinoma that may be confused with a number of benign lesions [39].

Prostatic carcinomas with hormone therapy-induced changes

Hormonal treatment given to patients with prostate cancer may result in microscopic changes in both benign and malignant compartment: the non-neoplastic compartment shows atrophy of the glands which are lined by epithelial cells with clear cytoplasm, small nuclei with condensd chromatin, indistinct nucleoli; there are noticed features of basal cell hyperplasia, transitional and/or squamous metaplasia, glandular disruption with secretions extravasation, edema, stromal fibrosis, sometimes rich chronic inflammatory infiltrate; in the neoplastic compartment there are described changes that fall into three histological patterns: (1) the malignant acini become atrophic, lined by cells with pyknotic, hyperchromatic nuclei, similar to their benign counterparts, the only criterion of malignancy being the crowded and/or infiltrative appearance of the glands (Figure 34); (2) the nuclei of tumor cells with therapy-induced changes are small, round, condensed, hyperchromatic, centrally located, usually without nucleoli, rarely with large nucleoli and the cytoplasm is abundant, xanthomatous or clear; these cells may exfoliate into the lumen of malignant glands resembling histiocytes or lymphocytes that may be confused with; the presence of minimally modified tumor cells or without hormone therapy-induced changes advocates for the tumoral nature of the cells with xanthomatous cytoplasm; (3) sometimes the malignant glands completely disappear, leaving residual empty spaces or isolated tumor cells floating within these areas which contain an alcianophilic mucinous substance; there were also reported hemangiopericytoma-like stromal proliferation [6, 22, 43–49].

Using the low magnification, the residual tumor may be overlooked (Figure 35), the microscopic field being dominated by fibrosis and chronic inflammatory infiltrate consisting of lymphocytes, plasma cells, mast cells, eosinophils and sometimes-foamy histiocytes. In such situations it is recommended to examine with high power objective, but sometimes only IHC methods (CK, PSA, protein, AMACR, CD68, LCA) enable the identification of the scattered individual tumor cells (Figures 36 and 37) and facilitate their discrimination from lymphocytes, myocytes and fibroblasts, some degenerated or with artifactual changes [6, 48–50]. Hormonally treated prostate carcinoma shows the architectural aspects corresponding to a Gleason pattern 4 or 5 with fused glands, sheets, small groups or single-file cells [22], which constitute the reasons for recommending not grading these tumors [6].


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References


Figure 36 – Residual carcinomatous cells positive for AMACR in a case of hormonally treated prostatic adenocarcinoma. Anti-AMACR, EnVision system, ×400.

Figure 37 – Positive reaction for PSA in residual carcinomatous cells from a hormonally treated prostatic adenocarcinoma. Anti-PSA, DAB, EnVision system, ×400.


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