A concise update on prostate pathology

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SOUHRN

Prostate carcinoma is the most common non-cutaneous malignancy in men in developed countries and the incidence has been steadily rising in the developing countries. Active research in recent years has led to tremendous progress in our understanding of the biology and genetics, and marked improvement in diagnosis and treatment of prostate cancer. Gleason grading has remained as the cornerstone for management of patients with prostate cancer. However, the grading system has continuously evolving since its inception in response to changes in the clinical practice of diagnosis and treatment of prostate cancer. The modification of Gleason grading system implemented by the International Society of Urological Pathology in 2005 has profoundly changed the way prostate cancer is graded and consequently how patients are managed. Several prostate cancer histological types with distinct clinical and pathological features have been rediscovered or redefined. Finally, elucidations of the molecular and genetic mechanism helps not only better understand the pathogenesis of prostate cancer, but also identify biomarkers for improved diagnosis, risk stratification and clinical management. This article briefly reviews the most recent advances in the Gleason grading system, new histological types and molecular genetics of prostate cancer.

Keywords: prostate - Gleason grade - intraductal carcinoma - neuroendocrine differentiation - molecular genetics

Novinky v patologii prostaty

SUMMARY

Karcinom prostaty je (mimo kožních nádorů) nejčastější malignitou mužů v rozvinutých zemích a jeho incidence v rozvojových zemích stále roste. Aktivní výzkum v posledních letech výrazně napomohl porozumění biologie a genetiky karcinomu prostaty, vedl ke zlepšení jeho diagnostiky i léčby. Gleasonův grading stále hraje v nastavení léčebné strategie pacientů s karcinomem prostaty zásadní roli. Tento grading se však od začátku vyvíjí a odráží tak postupné změny v klinické praxi. Modifikovaný Gleasonův grading byl zaveden v roce 2005 a výrazně změnil způsob, jakým je grade karcinomu prostaty stanovován, i způsob, jakým je pacient poté léčen. Několik histologických typů karcinomu prostaty s odlišnými klinickými a patologickými znaky bylo nově objeveno nebo předefinováno. Konečně, pochopení molekulárních a genetických mechanismů pomáhá nejen lépe porozumět patogenezi karcinomu prostaty, ale také identifikovat biomarkery pro lepší diagnostiku, stratifikaci rizika a klinický management onemocnění. Tento text stručně shrnuje nejnovější změny v Gleasonově gradingu, nové histologické typy a molekulární genetiku karcinomu prostaty.

Klíčová slova: prostata - Gleasonovo skóre - intraduktální karcinom - neuroendokrinní diferenciace - molekulární genetika

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Prostate carcinoma (PCa) is the most common non-cutaneous malignancy in men in developed countries and the incidence has been steadily rising in the developing countries as well (1). Active research has led to tremendous progress in our understanding of the biology and genetics, and marked improvement in diagnosis and treatment of PCa in the last decade. This article briefly reviews the most recent advances in the Gleason grading system, several PCa histological types that have been recently redefined and molecular genetics of PCa that are most relevant to surgical pathologists' practice.

CONTEMPORARY GLEASON GRADING SYSTEM

Gleason grading system, developed by Dr. Donald Gleason in 1967 (2,3), remains as the cornerstone for the management of prostate cancer. The system is relatively simple and reasonably reproducible to apply (4,5). It is one of the key parameters for planning treatment, and remains as the most important prognostic factor in predicting pathological findings in radical prostatectomy (RP), biochemical failure, local and distant metastasis after therapy and PCa specific mortality.

The Gleason grading system has undergone continuous modification and changes in response to changes in the clinical practice of diagnosis and treatment of prostate cancer since its inception (6). The most significant changes were introduced in 2005 at the auspices of the International Society of Urological Pathology (ISUP) (7) and further modification also ensued (Fig. 1) (4). The resulting contemporary grading system is referred to as "2005 ISUP modified Gleason grading system". However, it is important to stress that the changes put forth by ISUP simply codified what have already been used in practice by many pathologists. It is important for surgical pathologists to be acquainted with and apply the modified grading criteria in their practice.

Important changes in 2005 modified Gleason grading system

Some of the changes are definitional, including precise definition of each Gleason grade and grading criteria for PCa morphological variants. Others are operational, i.e., how to report Gleason grade in special circumstances, including reporting of secondary

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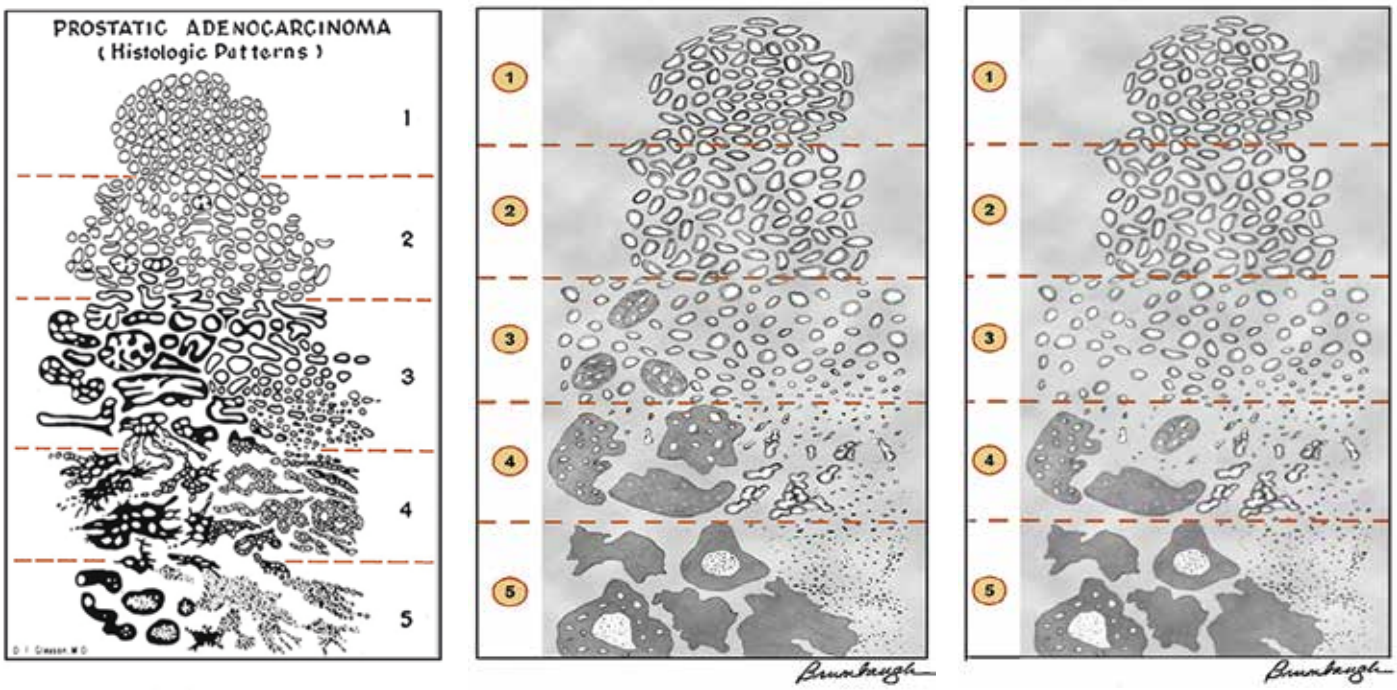

Fig. 1. Gleason grading system. The original system (A) and 2005 International Society Urological Pathology (ISUP) modified system (B) differ significantly in the definition of grades 3 and 4. Poorly formed glands and majority of cribriform glands are graded as grade 4 and only well-formed discrete glands are graded as grade 3 in 2005 ISUP modified system. In the further modification, all cribriform glands are considered grade 4 (C).

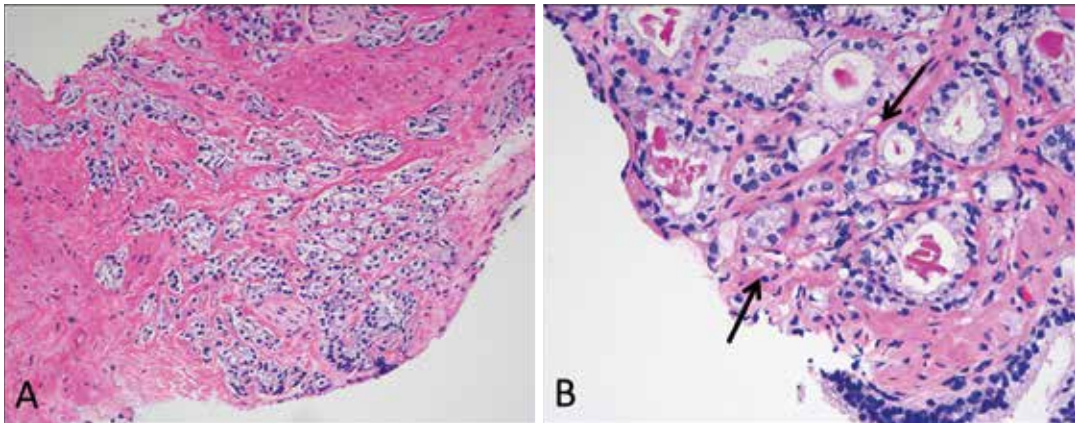

Fig. 2. Grade 4 poorly formed glands (A) should be differentiated from small glands resulting from tangential sectioning (B, arrows). The latter typically encompasses only a few poorly formed glands that are adjacent to or intermingle with other well-formed small glands.

pattern of lower or higher grade when present to a limited extent, tertiary pattern in both biopsy and prostatectomy specimen, etc.

1. Definitional changes

The most important change is perhaps the strict definition of each grade. A Gleason score of 1+1=2 should not be rendered, with only rare exception, regardless of the specimen type. Gleason scores 2-4 should rarely be rendered in needle biopsies, if ever. They should rarely be used in transurethral resection (TURP) and radical prostatectomy (RP) specimens. Therefore, Gleason grade starts at 3 and Gleason score starts at 6 in prostate biopsy specimens and most of TURP and RP specimens.

Grade 3 is strictly defined as discrete, well-formed cancer glands. Ill-defined glands with poorly formed glandular lumens are considered grade 4, together with other grade 4 patterns such as fused, cribriform and hypernephroid glands. However, grade 4 poorly formed glands should be differentiated from small glands resulting from tangential sectioning. The latter typically encompasses only a few poorly formed glands that are adjacent to or intermingle with other well-formed small glands (Fig. 2). A few poorly formed glands adjacent to other

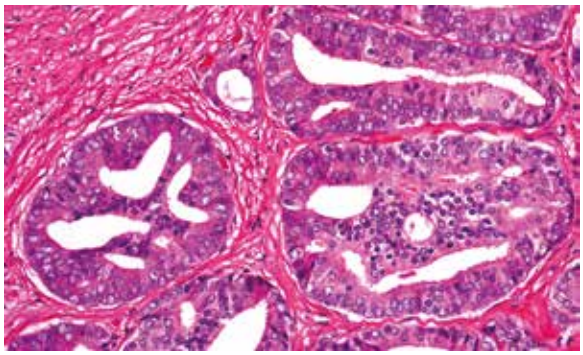

Fig. 3. All cribriform cancer glands are graded as grade 4, including those small ones with smooth counter.

Table 1. Grading of histologic variants and patterns of prostate cancer.

HISTOLOGIC PATTERN OR VARIANT	GLEASON GRADE
Histologic pattern	
Collagenous micronodule	Grading based on underlying architecture
Glomerulation pattern	3 or 4; recent data suggest grade 4
Intracytoplasmic vacuoles	Grade based on underlying architecture
Histologic variant	
Atrophic	3
Foamy gland	Grading based on underlying architecture
Ductal adenocarcinoma	Pattern 4, pattern 5 with necrosis
Mucinous (colloid) carcinoma	Grade based on underlying architecture (3 or 4)
Pseudohyperplastic	Pattern 3
Signet-ring cell carcinoma	Pattern 5
Small cell carcinoma	Not graded
Sarcomatoid carcinoma	Not graded
Nonglandular tumors	
Adenosquamous and squamous carcinoma	Not graded
Basal cell carcinoma	Not graded
Urothelial carcinoma	Not graded

small grade 3 glands are not grade 4. Most cribriform patterns are diagnosed as grade 4 (Fig. 3). A recent study found that all cribriform cancer glands should be diagnosed as grade 4 (8).

Grading of PCa with histologic patterns such as glomerulation and mucinous fibroplasia and the histologic variants (Table 1) is based on the underlying glandular architecture and the peculiar namesake histologic pattern or variation should be ignored.

2. Operational changes

There are several specific issues regarding the grading of prostate needle biopsies. In needle biopsy, high score tumor (grade 4 and 5) of any quantity should be included in the final Gleason score as first or second pattern. Tertiary pattern is not reported for biopsy. Secondary patterns of lower-grade cancer, when present to a limited extent (<5 %) in the setting of a high-grade cancer, should be ignored and not reported. For example, a biopsy containing 98 % of Gleason grade 4 and 2 % Gleason grade 3 cancer is graded as 4+4=8, not 4+3=7.

For biopsies with different cores showing different scores, each core should be assigned an individual score if they are submitted in separate containers or their anatomic site is specified by urologists (by different inking) even when they are submitted in the same container. An overall or global Gleason score is optional. When multiple cores are placed in a container without site specification and two or more cores contain PCa, some pathologists grade each core separately, while others would only provide an overall Gleason score. If, however, the cores are fragmented, an overall score should be given.

In radical prostatectomy specimens, a tertiary pattern higher than the primary and secondary grades should be included in the final Gleason score as the secondary grade when it is > 5 % of the tumor. It can be reported as tertiary pattern if it is < 5 % of the tumor. PCa frequently presents as multifocal disease with heterogeneity in Gleason score (GS) and genetic alterations (9). The concept of dominant, or index, tumor nodules is adopted for the convenience of reporting GS of the entire case and procurement of tissue for research. Most often, the dominant nodule is the largest tumor, and has highest stage and grade. However, in significant number of cases the largest tumor volume, highest GS and staging parameters (such as extraprostatic extension) do not always concur in the same tumor nodule (10). In these cases, pathologists should de-emphasize the concept of dominant tumor nodules. Instead, they should place the emphasis on the multifocal nature of the disease and document the pathological features of all independent tumor foci that have largest tumor size, highest GS and staging parameters.

Implications of modified Gleason grading system

1. Gleason score 6 prostate cancer has become a homogeneous group with uniformly excellent prognosis

Strict definition of Gleason grade 3 cancers and inclusion of any high grade (grade 4 and 5) tertiary pattern in the final Gleason score in prostate biopsy have led to reassigning many GS 6 cancers to GS 7. One immediate effect of such changes is that GS 6 cancers have become more homogeneous in their clinical behavior and have excellent prognosis when diagnosed in both radical and biopsy specimens. Eggener et al. studied the 15-year cancer specific mortality following radical prostatectomy from 1987 to 2005. Of 9557 patients with organ-confined, GS 6 PCa, only 3 (0.03%) died of cancer (11). Since patients in this cohort were enrolled before the modification of Gleason grading system was implemented, authors inferred that "… we may have observed even fewer cancer specific deaths in men with pathological Gleason 6 or less cancer had surgical specimens been subjected to a contemporary pathological review". Similarly, Hernandez et al. found that patients with pathologically organ-confined, Gleason score ≤ 6 PCa, biochemical recurrence and local recurrence following radical prostatectomy were extremely rare and no patients experienced distant metastasis nor prostate cancer specific death (12). GS 6 PCa diagnosed on biopsy also has excellent prognosis despite of sampling issue and potential upgrade to ≥ 7 at radical prostatectomy. Pierorazio et al. studied 5205 patients with GS 6 PCa diagnosed in biopsy (13). Almost 1/3 (31.7 %) cases were upgraded to ≥ GS 7 on radical prostatectomy. However, the 5-year biochemical recurrence free survival was 94.7 % (vs 82.7 % for GS 7 on biopsy). The excellent prognosis of GS 6 PCa sparked a debate whether GS 6 PCa should be labeled as "cancer" in radical prostatectomy (14). Our opinion is that the cancer label should be retained for GS 6 cancer, as these cancers are morphologically and genetically similar to higher grade PCa and can invade extraprostatic tissue. Furthermore, GS 6 PCa in prostate biopsy is upgraded in radical prostatectomy in significant percentage of cases (15).

2. Is the modified Gleason grading system better than the original system?

To claim the modified Gleason system is better than the original one, it has to show that it can improve the inter-observer reproducibility among pathologists who use it, and improve the biopsy and radical prostatectomy Gleason score concordance. Ultimately, it has to demonstrate a better correlation with clinical outcomes.

Studies have so far shown that the inter-observer reproducibility increased from 60 % with the original system to 80 % with the modified system (16-18). The improvement has been in particular impressive for GS 7 PCa. The inter-observer reproducibili-

ty has increased from 27 % in a study conducted in 1997 (19) to 68 % in a study conducted in 2008 (17).

The modified Gleason grading system has also improved the concordance between the biopsy and radical prostatectomy (RP) GS. Before the 2005 modifications, Gleason scores were concordant between biopsy and RP specimens in 28 - 68 % of cases. The discordance was mainly due to biopsy under-grading and accounted for 24 - 60 % of the discordant cases. Biopsy over-grading was less of a problem and accounted for 5 - 32 % of the discordant cases. In general, there was a better concordance in high grade PCa. After the modified Gleason grading system was implemented, there was a 12 - 15 % increase in overall exact concordance between biopsy and RP (17,20). However, biopsy under-grading is still responsible for the majority of the discordance.

The most important question is how the modified Gleason grading system affects the outcome prediction. Biopsy GS is incorporated in several preoperative nomograms to predict pathological findings in RP, such as Partin tables (21) and Kattan nomogram (22), and risk of progression after RP, such as Stephenson model (23) and Han table (24). However, the impact of modified Gleason grading on outcome prediction requires large cumulative data to clarify. So far there are only very limited studies. Several studies have shown that the correlation between biopsy GS and the risk of biochemical recurrence or PCa specific survival was significantly better using the modified grading (25-28). A study by Delahunt et al., however, reported that the original system outperformed the modified one in predicting PSA nadir following external-beam radiation therapy and hormone therapy (29). More studies are needed before a definitive conclusion can be reached.

3. Impact of modified Gleason grading system on patient management

Biopsy GS plays a pivotal role in treatment decision making. For example, the US National Cancer Center Network Practical Guidelines (*http://www.nccn.org/*) stratify PCa patients into 6 recurrence risk groups based on several clinicopathological parameters, including biopsy GS and extent, clinical stage, serum PSA and PSA density. Patients within different risk groups are offered different therapeutic modalities. Therefore, it is expected an upward shift in GS resulting from the modified grading system will impact how patients are managed.

Increasing number of patients are choosing active surveillance (AS), in which patients are monitored closely and definitive treatment such as surgery, radiation and hormonal ablation is withheld until there is sign of progression. The AS criteria vary from institution to institution (30), but require GS $\leq$ 6 in most criteria. With modified Gleason grading system, fewer cases are graded as GS 6 and more cases as GS 7. Therefore, fewer patients would qualify for AS, which will potentially worsen the problem of overtreatment for PCa. However, patients on AS will be safer with less likelihood to progress to definitive treatment as GS 6 PCa constitutes a homogeneous group with excellent prognosis when graded with modified Gleason grading system. Furthermore, recent studies have shown that GS 3+4=7 PCa diagnosed on biopsy is associated with more favorable prognosis and these findings raised the possibility for AS to be a management option for intermediate risk PCa. Bul et al. followed patients with low risk (cT1/2, PSA < 10ng/mL, PSAD < 0.2 ng/mL/mL, GS ≤ 6, positive cores ≤ 2) and intermediate risk PCa (PSA 10 – 20ng/mL, GS = 7) and found that the 10-year metastasis free survival and disease specific survival are similar between low risk and intermediate risk patients, suggesting that AS is a safe approach for intermediate risk PCa (31). Therefore reduced enrollment of patients into AS due to upward grade shift caused by modified Gleason grading system is effectively counter balanced by less progression to definitive treatment for patients already on AS, and more patients with intermediate risk being managed with AS.

4. Limitations of modified Gleason grading system

There have been such significant changes to the Gleason grading system that the modified system is essentially a different system from the original one. It is therefore difficult to compare the outcome data in contemporary series with the historical ones. Another issue is the artificial improvement of prognosis due to grade migration, so called the Will Rogers phenomenon (32). The modified Gleason system has practically eliminated GS 2 – 4. Furthermore, some PCas that were graded as grade 3 in the original system are now graded as grade 4 due to strict definition of grade 3. As the result, some PCa in the lower grade group (GS 6) with better prognosis is moved into a higher grade group (≥ GS 7) and therefore improves the prognosis of the higher grade group.

5. Further modification of Gleason grading system

A very important limitation of the Gleason grading system, both the original and modified systems, is that the numerical scale of Gleason scores does not accurately reflect the biological aggressiveness of the disease. The Gleason scores range from 2 to 10, with 7 further divided to 3+4=7 and 4+3=7. However, the modified Gleason grading system has practically eliminated GS 2-5 in biopsy, and in majority of radical prostatectomy specimens. Therefore the lowest GS in both biopsy and radical prostatectomy is 6. Since 6 is in the middle of the 2-10 numerical scale, patients may therefore reason they have a moderately aggressive cancer despite that GS 6 PCa is the least aggressive tumor. To avoid such confusion, Epstein and his associates proposed a prognostic grouping (13). PCas are stratified into 5 prognostic groups, group I for GS 6, II for GS 3+4=7, III for GS 4+3=7, IV for GS 8 and V for GS 9/10. This approach is supported by other studies (25). It is our opinion that while this prognostic grouping is more closely reflective of the tumor behavior, it cannot replace the Gleason grading system for several reasons. First, this prognostic grouping is still based on Gleason grading, i.e., pathologists have to perform Gleason grading first and then derive the prognostic grouping based on the Gleason scores. Prognostic grouping cannot be rendered *de novo*. Second, Gleason grading system is so entrenched in pathologists, clinicians and patients, replacing it with a similarly numbered system would cause massive confusion. A reasonable approach would be providing the prognostic grouping along with the Gleason scores.

PROSTATE CANCER HISTOLOGICAL TYPES THAT HAVE BEEN REDEFINED

Recently, several PCa histological types with distinct clinicopathological features have been redefined. Two of them will be briefly reviewed here, including ductal carcinoma and prostate cancer with neuroendocrine differentiation.

Intraductal carcinoma of the prostate

Intraductal carcinoma of the prostate (IDC-P) represents spread of invasive carcinoma into preexisting benign ducts and acini (Fig. 4) and is strongly associated with high-grade (Gleason grades 4/5), large-volume invasive prostate cancers.

IDC-P glands are larger than normal peripheral zone glands (33,34), and exhibit markedly irregular and branching contours (Fig. 4A) (33-35). Several architectural patterns are observed in

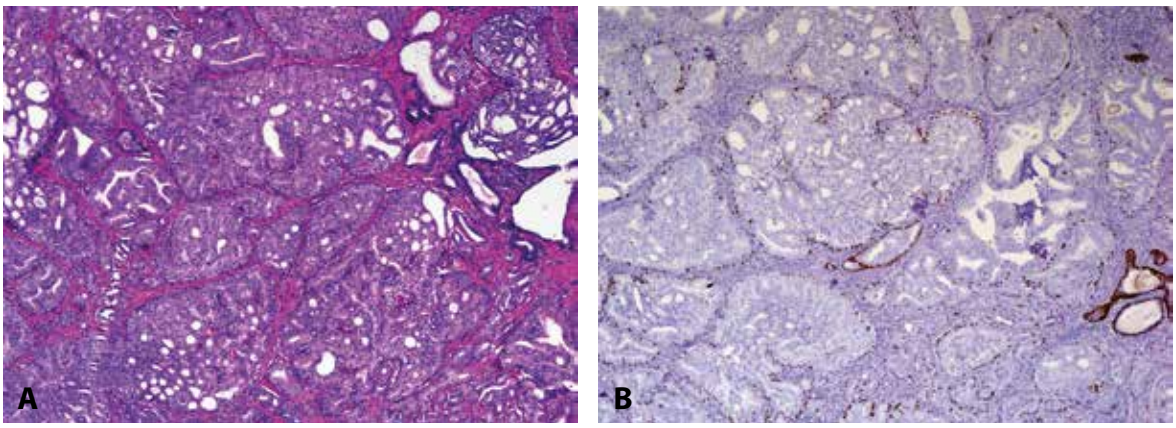

Fig. 4. Intraductal carcinoma of the prostate presents as marked expansile growth of prostate cancer cells (A) within preexisting prostate ducts and acini (A) with at least focally preserved basal cells on P63 immunostain (B).

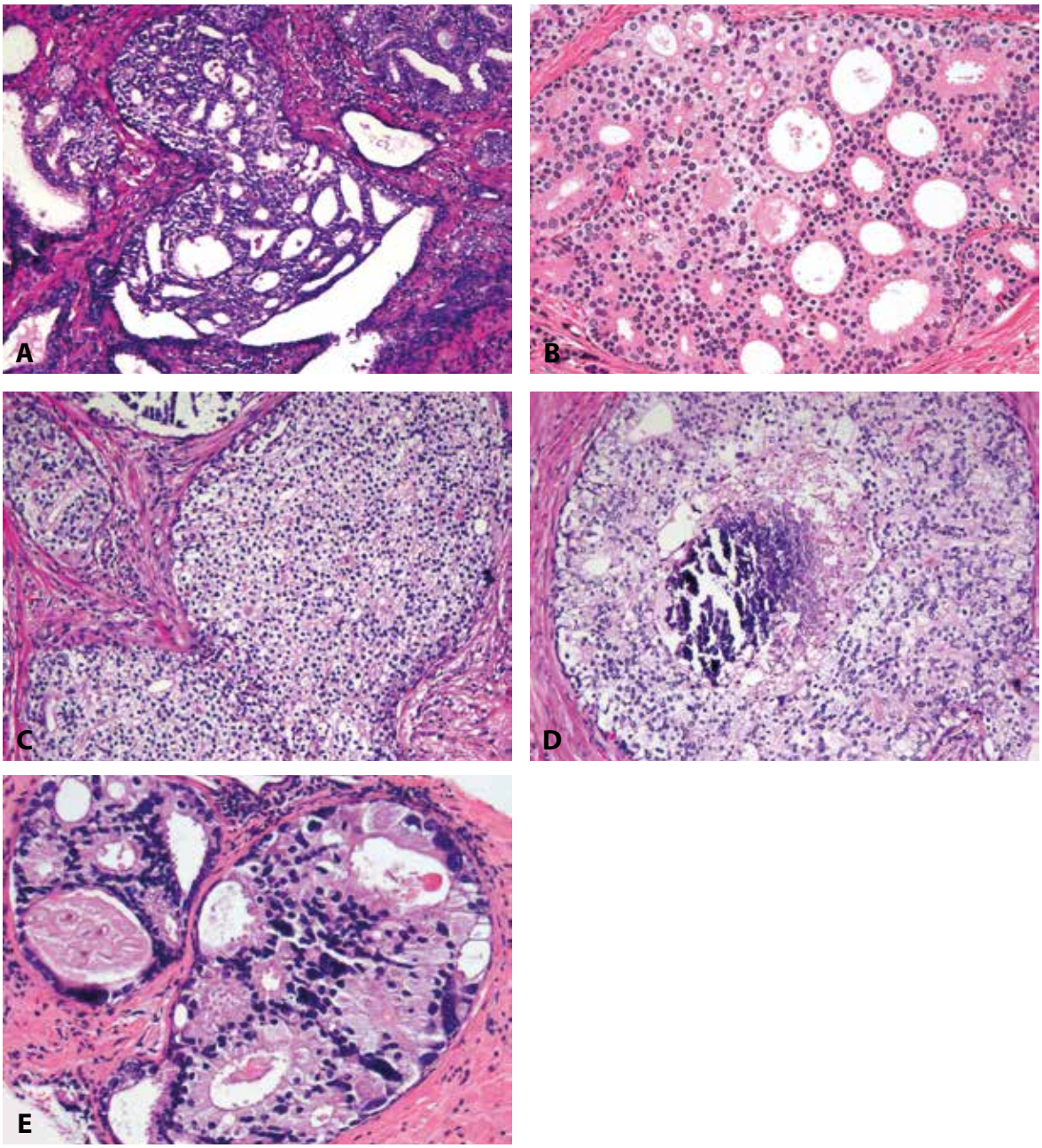

Fig. 5. Histological features of intraductal carcinoma of the prostate (IDC-P) include loose cribriform (A), dense cribriform (B), solid growth (C) patterns. Comedonecrosis (D) and marked nuclear pleomorphism with nuclear size >6× of the adjacent non-neoplastic cells (E) are present in some, but not all, the cases. Of these morphological features, dense cribriform and solid patterns, non-focal comedonecrosis, and marked pleomorphic nuclei are diagnostic of IDC-P.

IDC-P, including trabecular, loose cribriform (Fig. 5A), dense cribriform (Fig. 5B), and solid (Fig. 5C). Comedonecrosis is diagnostic of IDC-P but is not seen in all cases (Fig. 5D). Neoplastic cells in classic IDC-P are pleomorphic, some 6 times larger than the adjacent nonneoplastic nuclei (Fig. 5E).

Several diagnostic criteria were put forth (33,36), but the one proposed by Guo and Epstein is simple, subjective and reproducible (36). This diagnostic approach is summarized in Figure 5. In addition to the presence of malignant epithelial cells filling large acini and prostatic ducts with preservation of basal cells, the diagnosis of IDC-P required the presence of solid or dense cribriform pattern (Fig. 5B, C). If these features are not present, a diagnosis of IDC-P can be made if there is (1) non-focal comedonecrosis involving ≥ 2 glands (Fig. 5D); or (2) marked nuclear atypia, where the nuclei are at least 6 times larger than adjacent benign nuclei (Fig. 5E).

Studies have established that IDC-P represents an aggressive form of PCa and is an adverse pathologic parameter in both radical prostatectomy and needle biopsy specimens. The presence of IDC-P correlated with other adverse pathologic features, including higher Gleason score, larger tumor volume, and greater probability of extraprostatic extension, seminal vesicle invasion, and pelvic lymph node metastasis, in radical prostatectomy. It also correlated with decreased progression-free survival and with postsurgical, biochemical recurrence (34,37-40). IDC-P is uncommon in prostate biopsy; the incidence in a prospective biopsy cohort, i.e., prostate biopsies collected in daily practice, was 2.8 % (41). Isolated IDC-P without concomitant invasive IDC-P was even rarer, seen in 0.26 % cases in the same prospective cohort (41). Increasing body of evidence suggests that IDC-P in prostate biopsies that also contain invasive PCa provides additional prognostic values independent of conventional pathological parameters such as Gleason grade and tumor volume even (41-44); therefore, IDC-P should be reported in biopsy diagnosis.

Epstein and associates reported, in two studies, 66 prostate biopsies in which IDC-P was diagnosed without invasive carcinoma (36,45). They found that the presence of IDC-P, even in the absence of documented invasive carcinoma, was associated with an aggressive clinical course and adverse pathological findings in subsequent radical prostatectomy specimens. Based on their studies of needle biopsy with IDC-P and previous studies in the literature that demonstrated consistent association of IDC-P at radical prostatectomy with multiple adverse prognostic factors, authors recommended definitive therapy in men with IDC-P on needle biopsy, even in the absence of pathologically documented invasive PCa.

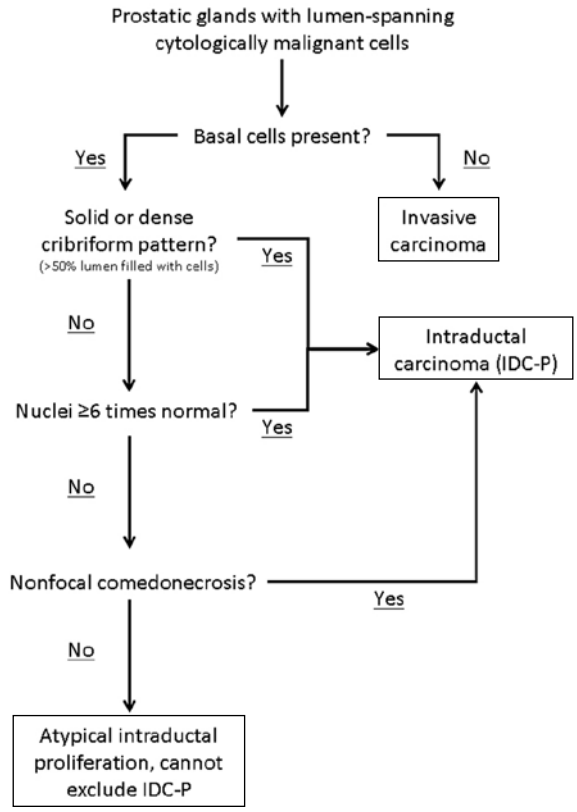

Fig. 6. Schematic representation of the diagnostic criteria for intraductal carcinoma proposed by Guo and Epstein (36).

Prostate cancer with neuroendocrine differentiation

Neuroendocrine (NE) differentiation can occur *de novo* with or without concurrent PCa, or as a treatment-emergent transformed phenotype (46). NE phenotype generally confers a more aggressive clinical behavior and less favorable prognosis than conventional PCa. To standardize the diagnosis and facilitate further study, a morphologic classification of NE differentiation in PCa was proposed recently (47) and consists of six categories: 1) usual prostate adenocarcinoma with NE differentiation, 2) adenocarcinoma with Paneth cell-like NE differentiation, 3) carcinoid tumor, 4) small cell carcinoma, 5) large cell NE carcinoma, and 6) mixed NE carcinoma - acinar adenocarcinoma.

Usual PCa with NE differentiation refers to typical acinar or ductal PCa, in which NE differentiation is demonstrated only by immunohistochemical positivity (synaptophysin, chromogranin and CD 56). The clinical significance of NE differentiation in these tumors is uncertain and most of the studies have shown no effect on outcomes (47). Therefore, routine use of immunohistochemistry to detect NE differentiation in an otherwise typical PCa is not warranted.

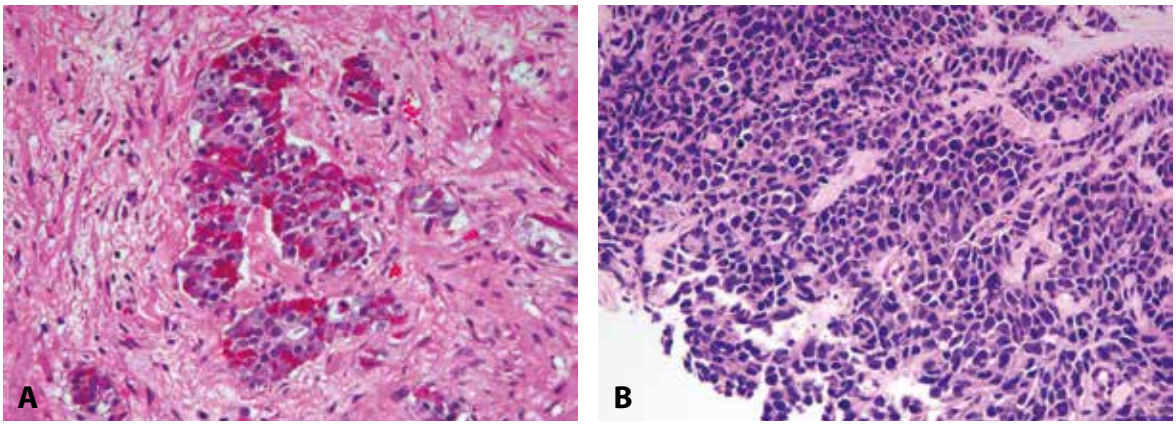

Fig. 7. Prostate carcinoma with neuroendocrine differentiation. (A) Paneth cell-like NE differentiation. (B) Small cell carcinoma.

PCa with Paneth cell-like differentiation is typical PCa containing Paneth cell –like changes with prominent eosinophilic cytoplasmic granules on light microcopy (fig. 7A) and neurosecretory granules by electron microscopy. The Paneth cell-like differentiation may be seen in well-formed cancer glands but can also be present in cords and nests of cancer cells. The clinical significance is not completely understood but studies so far have shown that seemingly poorly differentiated PCa with Paneth cell-like differentiation had favorable prognosis (48). Therefore, it is questionable whether a tumor with Paneth cell-like differentiation and lack of glandular differentiation should be assigned a Gleason score.

Prostate carcinoid tumor is a well-differentiated NE tumor with classical morphology of carcinoid tumor arising in the prostatic parenchyma. It expresses NE markers but not PSA. It is exceedingly rare and strict diagnostic criteria should be used.

Small cell carcinoma is an aggressive NE tumor recognized by the typical morphology similar to small cell carcinoma of the lung. Immunohistochemically, the small cell component is positive for at least one NE marker in almost 90 % of cases (49, 50), positive for prostate markers such as PSA, often focally, in 17 - 25 % of cases. TTF-1 expression is found in over 50 % of cases (49-52). ERG gene fusion is present in 50 % of prostate small cell carcinoma cases by FISH, similar to acinar PCa (53-57). However, IHC for ERG protein is not reliably positive presumably due to lack of androgen receptor expression in small cell carcinoma (54).

Large cell NE carcinoma is a high grade NE tumor with both morphological NE features (large nests with peripheral palisading, non-small cell nuclear features) and extensive NE marker expression. Majority of cases represent progression from a prior typical PCa following long-standing androgen ablation (58).

Mixed NE carcinoma and acinar PCa comprises distinct components of NE (small cell or large cell) carcinoma and typical acinar PCa with abrupt transition. Most, if not all, cases of mixed small cell carcinoma and PCa represent NE transformation after androgen deprivation therapy, and are hormonal resistant with poor prognosis. While the NE component is not graded, the percentage and grade of the acinar component should be provided.

MOLECULAR GENETICS

The most common genetic alteration in PCa is gene rearrangement between members of the E26 transformation specific (ETS) gene family and androgen-regulated genes, and the fusion between ERG, a ETS gene family member, and transmembrane serine protease 2 (TMPRSS2) is the most frequent one (59), present in about 40 - 50 % of PCa cases. Rearrangement involving other ETS genes accounts for additional 15 - 20 % of cases (60). TMPRSS2/ERG gene fusion can be detected by fluorescence in situ hybridization (FISH). The fusion leads to aberrant expression of ERG, which can be detected by immunohistochemistry (61). Positive ERG immunohistochemistry highly correlates with the ERG gene status. Studies of the prognostic significance of ETS gene alterations produced conflicting results. Some studies showed a poor prognosis for cases with TMPRSS2/ERG fusion (60), while others found no association with Gleason score, tumor stage and prognosis (60,62). TMPRSS2/ERG gene fusion was detected in about 20 % of high-grade prostatic intraepithelial neoplasia (HGPIN) intermingled with or in the vicinity of prostate adenocarcinoma that carries the same fusions, but has not yet been demonstrated in isolated HGPIN, benign prostate tissue, or benign cancer mimics (63). The PCa specific nature of TMPRSS2/ERG fusion makes it a useful marker in the diagnosis of minute focus of cancer (64,65) in challenging cases and confirmation of the prostate origin for metastatic carcinoma of unknown primary (66).

Several other molecular alterations are mutually exclusive with ETS gene rearrangement in PCa, and could define distinct molecular subtypes of PCa. Speckle-type POZ (SPOP) mutations is present in 6 - 15 % of cases of PCa that generally lack ETS rearrangement, PTEN inactivation and p53 mutation (67,68). Cases with SPOP mutation tend to have CHD1 deletion. The initial study failed to show significant correlation between SPOP mutation and rate and time of biochemical recurrence due to small sample size. SPINK1 over-expression is another molecular alteration exclusively identified in ETS rearrangement negative cases, and is present in about 10 % of PCa cases (69). SPINK1 positive cases have worse prognosis.

PTEN inactivation by deletion, less frequently by mutation, is an important event in PCa with poor prognosis. It is identified in about 40 % of PCa (70-72). PTEN inactivation activates AKT pathway in PCa, which can also be activated by other genetic changes, including PI3K and mTOR mutations (73). c-myc amplification and over-expression are detected in about 30 % of cases, and are more frequently present in late stage of PCa and associated with worse prognosis (74,75). Androgen receptor (AR) amplification and mutation play an important role in PCa progression and resistance to androgen deprivation therapy (ADT) (76,77). Other genetic changes significantly detected in PCa include NKX3.1 mutation, FOXA1 elevation, and Rb loss. Most of these genetic changes are more frequently identified in late stage of the disease, and are associated with disease progression and poor prognosis (73).

A potential utility of genetic and molecular markers of PCa is to triage patients for appropriate management. In current practice, the management decision is based primarily on clinical and biopsy pathology findings, including clinical stage, serum PSA level and density, digital rectal examination and biopsy Gleason score and tumor extent. Patients are stratified into different risk groups for which different management regimens are offered. However, such stratification scheme is far from perfection and sometimes results in unnecessary treatment in patients with low risk disease and delayed treatment in patients with high risk disease (78). Molecular and genetic markers, used singularly or in combination, can potentially separate indolent PCa from aggressive ones and help identify patients with indolent disease who can therefore be safely followed and patients with aggressive disease who need definitive treatment (79).

There are several commercially available tests marketed for this purpose. One test is Prolaris® by Myriad Genetics (Salt Lake City, UT, USA). This test measures the gene expression of 31 cell-cycle progression genes and 15 housekeeper genes from biopsy specimens to develop a *cell-cycle progression (CCP) score*. This CCP score has been shown to stratify men for 10-year PCa death independent of PSA and Gleason score for PCa managed conservatively (80). Recently this test has also been shown to add independent prognostic information to a standard clinical risk score in a contemporary prostatectomy cohort (81). This recent validation study by Cooperberg et al. may help guide decisions regarding adjuvant treatment and in stratifying men for future adjuvant therapy studies.

Another test, Oncotype Dx Prostate®, is marketed by Genomic Health (Redwood City, CA, USA). It tests the expression of genes representing multiple biological pathways and generates a *genomic prostate score (GPS)*. This test, using prostate biopsy tissue, has been prospectively validated as a predictor of PCa aggressiveness in biopsy tissue despite of tumor heterogeneity, multifocality, and limited sampling at time of biopsy (82). The biopsy-based 17-gene GPS improves prediction of the presence or absence of adverse pathology and may help men with PCa

make more informed decisions between active surveillance and immediate treatment.

Other similar molecular tests are being developed to augment the clinical and pathological parameters in therapeutic decision making. Even though these tests have shown promising results in early studies and started to be requested by physicians and patients, large prospective validation studies are needed on prostate biopsies before they can be recommended in routine clinical practice for risk stratification and patient management.

CONCLUSION

The ISUP 2005 modification of Gleason grading system has profoundly changed the way PCa is graded and patients are managed. Several PCa histological types with distinct clinical and pathological characteristics have been rediscovered or redefined. Further elucidation of the molecular and genetic mechanism not only helps us better understand the pathogenesis of the disease, but also identify biomarkers for improved diagnosis and clinical management.

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