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Should Gleason 6 be labeled as cancer?

Ibrahim Kulac¹, Michael C. Haffner^{3,4}, Srinivasan Yegnasubramanian^{3,5}, Jonathan I. Epstein^{1,2,3,4,5}, and Angelo M. De Marzo^{1,2,3,4,5}

¹Department of Pathology

²Department of Urology

³Department of Oncology

⁴The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

⁵The Brady Urological Research Institute

Abstract

Purpose—This review covers arguments for and against removing the label of “cancer” Gleason score 6 tumors.

Recent findings—While there are a number of factors that determine whether men elect active surveillance, the most powerful predictor remains the Gleason score. The Gleason grading remains a robust and powerful predictor of outcome in patients with prostate cancer. A pure Gleason score 6 (GS6) tumor is exceedingly unlikely to cause harm in the near-term and there have been discussions regarding whether the term cancer should still be applied. In this manuscript we update the largely clinico-pathological arguments that have led to the suggestion to remove the cancer label from GS6 tumors, and, we provide counter arguments based on practical matters of needle biopsy sampling, classical histopathology, and molecular biology findings.

Summary—The implications are that by retaining the label of cancer and implementing the recently proposed concept of prognostic groups, with patients harboring GS6 tumors placed into the lowest category, there is still a strong rationale in support of the choice of active surveillance or watchful waiting for most patients with GS6 lesions.

Keywords

Prostate cancer; Gleason score; Active surveillance

Introduction

Although the United States Preventative Services Task Force (USPSTF) has recommended against generalized PSA screening, many men are still undergoing routine PSA testing (1, 2). Thus, a key challenge is to determine which men with low risk prostate cancer can safely

The Johns Hopkins University School of Medicine, Department of Pathology, 1550 Orleans Street, CRB 2 Rm 152, Baltimore, Maryland 21231, Phone: 410- 614- 5686, Fax: 410- 502- 9817, ; Email: ademarz@jhmi.edu

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be monitored by active surveillance (AS) (or watchful waiting), and which men require immediate treatment. While there are a number of factors that determine whether men elect active surveillance, the most powerful predictor remains the Gleason score. Although the Gleason grading system has undergone some significant changes over the last decade, and continues to do so (3, 4), it remains a robust and powerful predictor of outcome in patients with prostate cancer. As detailed below, a pure Gleason score 6 (GS6) tumor is exceedingly unlikely to cause harm in the near-term. Given this, there have been discussions regarding whether the term cancer should still be applied for GS6 lesions (5–11), and new potential names such as IDLE (11) (indolent lesion of epithelial origin) have been proposed. In this manuscript we will outline and update the largely clinic-pathological arguments that have led to the suggestion to remove the cancer label from GS6 tumors, and, we will provide counter arguments to support the retention of the label of cancer. A number of these issues, including whether GS6 tumors can be considered invasive and whether they harbor the “hallmarks” of cancer (12, 13) have been covered recently (6, 14) such that key points from those articles will only be briefly discussed here when appropriate. In addition, we will present updated molecular pathology based evidence that in our opinion largely support the retention of the cancer label for this lesion at this time.

Clinco-Pathological Features of GS6 Cancer

This section provides an overview of the clinical and pathology findings that have prompted the call to remove the label of cancer from GS6 tumors.

1. The nearly negligible rate of lymph node metastases in GS6 Tumors

An often employed determinant of whether a neoplasm is considered malignant (and hence the term cancer employed) is the capability for the tumor to undergo metastasis. In prostate cancer, regional metastases occur to the pelvic lymph nodes. It is now known that lymph node metastasis is extremely rare in GS6 prostate cancer especially if the Gleason score was applied after reviewing fully submitted radical prostatectomy (RP) specimens and the modified Gleason system from the ISUP 2005 consensus is utilized (15, 16) (17). For example, Ross et al. (15) identified 14,123 radical prostatectomies from 4 institutions and found lymph node metastases in 22 patients (prevalence of 0.156%). When these positive cases were reviewed using the ISUP 2005 grading system (15), all of the available cases ($n = 19$) showed at least some Gleason pattern 4. The authors concluded that Gleason score of 6, if evaluated in a fully sampled prostatectomy specimen with modern grading, may not be capable of metastasis. Liu et al. (16), reviewed US SEER database results for patients diagnosed with GS6 cancer. Among 21,960 prostatectomy patients, 0.48% had lymph node metastasis. While this number is greater than zero, it is exceedingly low and none of these cases were reviewed by expert genitourinary pathologists and in many cases it is likely that the entire prostate was not submitted, leading to under-grading. Taken together, it is clear that if one could be certain that a given patient's prostate harbored only GS6 tumor(s), then virtually all of these patients could be safely monitored without need for immediate intervention. Further, given its low malignant potential (e.g. in this case the lack of ability to metastasize) it is not unreasonable to suggest that this lesion not be labeled as cancer.

However, since ~20–35% of GS6 tumors on prostate biopsy are upgraded at radical prostatectomy (18), in the absence of a radical prostatectomy it simply cannot be known whether a patient harbors only GS6 cancer. In practical terms, therefore, we submit that it is still most reasonable to retain the cancer label for these GS6 lesions. Recent studies indicating that multi-parametric MRI guided prostate needle biopsies can decrease the upgrading rate (19) suggest that with widespread MRI implementation then this argument against removing the cancer label would be diminished. Further, the addition of molecular prognostic markers to GS6 needle biopsies may help ascertain who is likely to harbor higher grade more aggressive tumors in the prostate (see below).

2. The nearly negligible rate of disease progression in men treated for GS6 cancer; very few men progress with GS6 disease that have been treated by primary surgery

A number of studies have shown that the progression rate after surgery is extremely low if only GS6 prostate cancer was identified in thoroughly examined specimens. For example, the 15-year prostate cancer specific mortality rate for pathological GS6 or less, 3+4, 4+3 and 8–10 was 0.2% to 1.2%, 4.2% to 6.5%, 6.6% to 11% and 26% to 37%, respectively for cases stratified by age at diagnosis (20). Likewise, biochemical recurrence free survival for Gleason score ≤6 prostate cancer is remarkably high; >95% for 4 years as shown in one large cohort (3). Further, most prostate cancer cases from carefully evaluated RP specimens that are truly GS6 and organ confined show very low rates of disease progression (e.g. 0.4% in one study of 2551 men) (21).

The most definitive way to determine whether a given GS6 tumor can progress to a higher grade more aggressive lesion would be to sample it longitudinally over time. Unfortunately, since one cannot be sure there is no higher grade lesion already present without prostate removal, this type of study has been impossible to perform. Further, even if one could be sure they were sampling a pure GS6 tumor and following it over time, the molecular pathology tools to definitively establish clonality are just now becoming available (for example, see (22)). Further, there is no *a priori* biological reason that these lesions cannot progress into higher grade more aggressive tumors if left untreated.

3. Patients on AS patients with GS6 rarely develop metastatic disease or die from prostate cancer

All published AS cohorts have shown excellent outcomes, especially if restricted to low and very low risk men with only GS6 and low volume disease (23–30). In the Johns Hopkins data as of 2012, of 769 patients only 1/6 of the patients underwent a reclassification (23). Most of the reclassifications to higher grade likely resulted from initial under-sampling since 80% of those patients that left AS did so with a median of 2.2 years after study entry. Klotz et al. (31), recently reported on 993 patients with a median follow-up time of 6.4 years. Overall, 2.8% (28 patients) of the entire group developed metastatic disease, 12 (44%) of the 28 patients with metastasis had a Gleason score of 3+4 = 7 at diagnosis. Of the 28 total patients who developed metastatic disease, only two were not upgraded to Gleason score ≥7 prior to developing metastases; and since these patients did not undergo prostatectomy they may have been undergraded.

4. Standard histopathological features of GS6 lesions favor the continued use of the label of carcinoma/cancer

A number of investigators have recently challenged the view that pure GS6 tumors have histopathological properties consistent with the label of cancer ((7, 8); see (14)). However, standard histopathological features of prostate lesions would favor that GS6 tumors retain the label of carcinoma/cancer, and a number of these have been reviewed recently (14). The diagnosis of prostate cancer, like nearly all other cancers, requires changes in nuclear structure. These changes include nuclear enlargement, nucleolar enlargement, nuclear size and shape variability, and nuclear hyperchromasia. There are also often variable alterations in the cytoplasm such as hyperchromasia. These features alone are not sufficient to make a diagnosis of carcinoma, since all of these may also present in high grade prostatic intraepithelial neoplasia (PIN), the likely precursor to many invasive adenocarcinomas of the prostate. The definition of PIN consists of those glands whose luminal cells exhibit nuclear and cytological features of prostate cancer but the atypical cells are present within pre-existing (non-dilated) ducts and/or acini. The key feature required to indicate that an epithelial neoplasm is labeled as a carcinoma/cancer is stromal invasion. In prostate carcinoma, stromal invasion is also accompanied loss of basal cells, which are present around benign glands, as well as, PIN glands. Both loss of basal cells and invasion into the stroma are always seen in GS6 tumors, as well as other higher grade prostate cancer variants. In addition, some GS6 lesions can infiltrate around nerves, and at times extend beyond the prostate into the peri-prostatic fat; clear signs of invasive potential (14).

Molecular biology results largely in favor of retaining the cancer label

This section provides evidence relating to heritable somatic DNA alterations including somatic genetic alterations and somatic epigenetic alterations. It also reviews clonal relationships between different Gleason patterns and the well-known finding that bladder cancer development proceeds along at least two separate lines of molecular alteration.

Somatic Genetic Alterations

While Ahmed et al. provided a compelling review of a number of molecular features that seem to distinguish GS6 tumors from those with Gleason pattern 4 and higher patterns, including many phenotypic gene expression based analyses placed in the context of Hanahan and Weinberg's "Hallmarks" of cancer, most of those changes described to occur more in higher grade lesions were phenotypic and not completely absent in GS6 tumors (7) ; thus the data presented are not fully compelling to suggest that GS6 tumors arise mechanistically from a distinct molecular pathway (s). If we focus more on somatic genomic alterations, the data appears quite compelling to us that GS6 and higher grade lesions share many similar molecular features suggesting often they arise from common heritable pathway alterations.

Genomic rearrangements are a hallmark of prostate and other cancers. For example, ~50% of prostatic adenocarcinomas from men of European decent, regardless of disease grade or stage, harbor a clonal genome rearrangement resulting in *ETS* family member gene fusions, with *TMPRSS2-ERG* being the most common¹. In terms of Gleason score and disease stage, approximately 50% of all primary prostate cancers including tiny "insignificant" GS6

cancers at RP (32), as well as 50% of castrate resistant lethal metastatic prostate adenocarcinomas have *TMPRSS2-ERG* fusions (33). Further, using a whole genome sequencing approach, Baca et al., recently described a common type of prostate cancer genome alteration, referred to as chromoplexy (34). Chromoplexy consists of a series of DNA breakage and joining events whereby a number of DNA segments from various genomic locations become stitched together (34); surprisingly, this alteration occurs similarly in GS6 tumors and higher grade prostate cancers (Fig. 1). A number of other types of genomic rearrangements are found in prostate cancer and these also did not show large differences based on Gleason score (Fig. 2). In terms of recurrent point mutations and small insertions and deletions (indels), which in general occur at low frequencies in known driver genes in prostate cancer, the overall number of such lesions are similar in GS6 and higher prostate tumors (34, 35) (Fig. 2). Taken together, these results suggest common molecular pathways and mechanisms for the development of a number of somatic genetic alterations between GS6 and higher grade tumors.

Some types of rearrangements lead to large scale copy number alterations with gains and losses of segments of genomic material. Such copy number alterations are common in prostate cancer (36), and studies using whole genome array-based comparative genomic hybridization (CGH) analyses have demonstrated that copy number changes tend to increase in extent and number with grade and disease aggressiveness (34, 37, 38). Further, a subset of prostatic carcinomas, predominantly GS6 tumors, have been deemed nearly free of large scale copy number alterations (37, 38). These “quiet” genomes in some GS6 lesions may be molecularly distinct from other GS6 lesions and higher grade more aggressive tumors. Nevertheless, it is also clear that at least some of the GS6 score tumors analyzed to date do show relatively large numbers of copy number alterations such that this feature alone cannot entirely separate prostate cancers by grade (34, 36–38).

Other well-known alterations, such as deletions of a single copy of chromosome 8p resulting in deletions of *NKX3.1*, heterozygous and homozygous deletions involving *PTEN* on chromosome 10q23, and gains of 8q24/*MYC* occur in GS6 lesions, albeit at a reduced rate compared to higher grade and more aggressive lesions (39–48). Other changes, such as *TP53* mutations or deletions, are found relatively infrequently in primary prostate tumors, although when they occur they are more common in higher grade, higher stage tumors (49), with a further increase seen in castrate resistant metastatic disease (33). Thus, these more traditionally examined alterations are more common in higher grade tumors, but do occur with at least some frequency in GS6.

Clonal Relationships Suggest a Common Origin for Gleason Score 7 Tumor Components

There are two main mechanisms by which higher grade prostate cancers may develop which were recently outlined by Lavery and Droller (8) in which Gleason pattern 4 or higher lesions may arise *de novo* from benign prostate cells (clonal model), or, such patterns arise from “de-differentiation” of Gleason pattern 3 lesions (transitional model). Recent studies examining clonal relationships between prostatic tumors that are composed of both Gleason

¹This event is also common in men of African and Asian ancestry, albeit it occurs less commonly in those groups.

patterns 3 and 4 suggest that when these are found together in the same isolated tumor lesion, they are clonally related (50, 51). And, in at least a few cases, it was shown that the Gleason pattern 4 lesions harbored an additional “hit” by showing a deletion in the *PTEN* gene, while the clonally related adjacent Gleason pattern 3 lesion did not show the *PTEN* alteration (50). This suggests (although does not prove) that at least in some cases a Gleason pattern 4 lesion can evolve from a Gleason pattern 3 tumor. This fits well with the now widely seen result that indicates that in prostate cancer *PTEN* alterations tend to occur during disease progression as a subclonal molecular alteration, subsequent to *TMPRSS2-ERG* fusion, when such lesions are found together in the same tumor (34, 52–54). While these results are compelling, this does not preclude the finding that at times it appears that high grade prostate tumors may arise *de novo* in the prostate (55, 56).

Somatic Epigenetic Changes

In terms of heritable somatic epigenomic events, somatic DNA methylation of the CpG island within the *GSTP1* gene occurs in approximately 90% of all prostatic adenocarcinomas, also regardless of grade or stage (57). A number of other genes are also hypermethylated frequently in prostate cancer (e.g. *APC*, *RASSF1*, *MDR1*, *EDNRB*) and, although hypermethylation of some occur more frequently in higher grade lesions (*EDNRB*) or those with biochemical recurrence (*PTGS2*), most occur commonly both in GS6 and higher grade lesions (58). These results further support the overall concept of similar molecular alterations occurring in GS6 and higher grade lesions.

Different Grade Bladder Tumors do Appear to Arise Via Distinct Molecular Pathways

Taken together, our assessment of the current molecular data is that such data do not favor the concept that there are clearly separate independent pathways for low and high grade prostate cancer lesion development. This is in contrast to molecular alterations found in some other organs in which there is evidence that low grade and high grade lesions often develop via independent pathways (e.g. breast and bladder). This is particularly striking in bladder cancer in which a high fraction of low grade papillary tumors (including in situ papillary low grade carcinoma as well as in situ papillary tumors of low malignant potential [PUNLUMP]) harbor somatic clonal point mutations in the *FGFR3* gene yet usually lack alterations in *TP53*, whereas a high fraction of carcinoma in situ (CIS which is high grade by definition) and high grade T1 and muscle invasive bladder carcinomas do not harbor *FGFR3* mutations and do harbor *TP53* mutations (59, 60). Further, since *FGFR3* mutations appear to occur early during tumor development, it is apparent that most high grade invasive urothelial carcinomas lacking *FGFR3* mutations did not start out as low grade papillary lesions. There are exceptions in which some muscle invasive urothelial carcinomas do harbor *FGFR3* mutations, suggesting that some of these tumors may have indeed started out as low grade and then progressed to high grade (61). Yet, in prostate cancer there does not seem to be this type of clear divergence in early somatic DNA alterations based on grade as seen in bladder cancer.

The Future: Can Molecular Markers Help?

Members of our group have recently found that patients harboring pure GS6 tumors in their prostatectomy samples have a lower rate of *PTEN* loss in Gleason pattern 3 areas than patients with Gleason score 7 do in their Gleason pattern 3 regions (either 4+3 = 7 or 3+4 = 7); (62). Further, there was also a greater rate of chromosome 8p loss and chromosome 8q24 gain in Gleason pattern 3 regions from patients with a GS7 tumor. The Gleason pattern 4 regions showed higher rates of changes at all 3 examined loci (62). In a separate study, Lotan et al. recently reported that tumors that were only GS6 on biopsy that had lost *PTEN* by IHC had an increased rate of upgrading at prostatectomy compared to those without *PTEN* loss [odds ratio=3.04 (1.08–8.55; P=0.035)] (63). Taken together, these findings strongly suggest that Gleason pattern 3 lesions are different molecularly depending on whether they are present in the setting of a GS6 tumor or in the setting of a GS7 tumor. These studies also suggest that appropriate molecular markers may be applied to help determine if patients with GS6 biopsies are at higher risk for harboring a previously unsampled higher grade tumor in the prostate. It should be pointed out that IHC for *PTEN* has been extensively validated analytically and is currently ready for clinical application widely in Clinical Laboratory Improvement Amendments 1988 (CLIA) certified anatomic pathology laboratories. Similar types of studies are no doubt being carried out using a number of promising RNA expression-based or protein-based multiplex assay signatures from at least 4 commercial entities. Another possible avenue may also be application of the percent of the genome with copy number alterations as mentioned above (37, 38), if this technology is further developed into a clinical grade test. Therefore, it is hoped that in the future in addition to histological grading, molecular markers along with improved imaging can be applied that will facilitate the overall determination of the aggressiveness of a given GS6 lesion and aid in the selection of patients for AS. If in the future we could be truly confident (e.g. $\geq 95\%$) that a given patient only harbored a GS6 lesion with molecular properties also consistent with indolent disease, then it may be appropriate to reclassify this lesion as a non-cancer.

Conclusions/Summary

The case for removing the label of cancer is largely supported by the following: i) the negligible rate of lymph node metastases in men with GS6 disease in RPs; ii) the very low risk of progression after primary treatment in men with GS6 disease; iii) the general safety of AS and the very low rates of progression of men with GS6 disease in AS; and iv) the lack of convincing molecular pathological longitudinal evidence to date showing that a pure GS6 tumor can progress to a higher grade life threatening lesion over time. In our opinion, however, the arguments for the continuation of using the term cancer for these lesions still overshadow those against removing the label of cancer. For example, there are a number of histological and molecular features of GS6 tumors that support the label of cancer for these lesions and there is no solid molecular evidence suggesting that GS6 vs higher grade tumors commonly arise as unique and distinct molecular subtypes as in the case in other cancers such those in the breast and urinary bladder. In fact, a number of histopathological and molecular alterations are shared between GS6 and higher grade lesions such as nuclear alterations, invasion into the stroma, *ETS* family member gene fusion events, chromoplexy

and somatic CpG hypermethylation of specific genes. While some of these changes are substantially less common in GS6 tumors, they nonetheless support similar pathways of tumor development overall. Further, given the practical issues with sampling leading to frequent under-grading with prostate needle biopsies, we submit that it would be pre-mature to remove the label of cancer from GS6 prostate tumors. Rather, we favor the application of new prognostic groups such that GS6 tumors fall into the lowest of these groups which will provide patients and clinicians with reassurance about the overall favorable prognosis of these tumors, without renaming the lesion to a non-cancerous entity at this time (3, 14).

- The case to be made that pure Gleason score 6 (i.e. only Gleason pattern 3) tumors are not capable of causing significant harm in the vast majority of cases.
- Yet, the issue of Gleason score 6 (pattern 3 only) lesions identified by today's standard prostate needle biopsies to be associated with un-sampled higher grade tumor in up to 35% of cases greatly diminishes the force of the argument for removing the cancer label.
- Further support for retaining the cancer label stems from molecular studies of heritable somatic genomic alterations (e.g. *GSTPI* promoter hypermethylation, *TMPRSS2-ERG* fusion genes and chromoplexy) that suggest common molecular pathways for early lesion develop in Gleason pattern 3 and higher grade lesions.

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Figure 1. Chromoplexy in Prostate Tumors by Gleason Score

A, schematic image illustrating chromoplexy. B, example "circos" plot showing genomic segments joined together. Reproduced with permission from Baca et al., (34), Figure 1.

C, Actual data from Baca et al. (34) Supplemental Figure 3 showing similar extent of chromoplexy in GS6 and higher grade tumors annotated with Gleason score.

Figure 2. Genomic Rearrangements in Prostate Cancer by Gleason Score

Shows similar extent of genomic rearrangements as well as mutations in GS6 and higher grade tumors. Reproduced with permission from Baca et al. (34).