Epigenetic events, remodelling enzymes and their relationship to chromatin organization in prostatic intraepithelial neoplasia and prostatic adenocarcinoma

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OBJECTIVE

To explore the nuclear chromatin phenotype, overall epigenetic mechanisms, chromatin remodelling enzymes and their role as diagnostic biomarkers in prostate lesions, using high-resolution computerized quantitative digital image analysis (DIA).

MATERIALS AND METHODS

A tissue microarray (TMA) was constructed using paraffin wax-embedded prostatic tissues from 78 patients, containing 30 cores of benign prostatic hyperplasia (BPH), 10 of low-grade prostatic intraepithelial neoplasia (LGPIN), 38 of prostate adenocarcinoma, 20 of BPH tissue excised at 0.6–1 mm from LGPIN lesions, and 10 of BPH prostatic tissues obtained 0.6–1 mm from high-grade PIN (HGPIN) lesions. Chromatin phenotype was assessed on haematoxylin-stained sections using high-resolution texture analysis. For quantitative immunohistochemistry, antibodies raised against acetylated histone H3 lysine 9 (AcH3K9), 5′methylcytidine (5MeC) and the chromatin remodelling ATPase ISWI (SNF2H and SNF2L) were used. The immunodensity was measured using DIA to determine the epigenetic profile of the cases. At least 60 nuclei were measured from each case.

RESULTS

There were many statistically significant differences in staining intensity and nuclear distribution patterns in chromatin phenotype and immunostaining (p ≤ 0.001). These changes allowed the differentiation between the various pathological subgroups with a classification accuracy of 76–100% using chromatin phenotype or immunostaining (5MeC, AcH3K9 and ISWI). In PIN lesions, there was a high chromatin content with DNA-hypermethylation, while in prostatic adenocarcinoma there was a lower chromatin content with DNA-hypomethylation and H3K9-hypacetylation. There was significantly more ISWI protein in neoplastic tissues. There were malignancy-associated changes (MAC) in chromatin phenotype and overall epigenetic events in BPH tissues adjacent to PIN lesions.

CONCLUSIONS

The present study confirms the ability of high-resolution computerized digital imaging of nuclear texture features to detect changes in chromatin phenotype, epigenetic events and the presence of chromatin remodelling, factors that can be used to distinguish between different prostatic pathologies, i.e. BPH, LGPIN, HGPIN and prostate adenocarcinoma, and further allow the detection of MAC near PIN lesions. These results provide a base for future diagnostic applications of DIA combined with immunohistochemistry. Our experiments underscore the importance of epigenetic mechanisms during carcinogenesis. Further studies are needed to elucidate the complex interplay between chromatin structure, its modifications and the progression of prostate cancer.

KEYWORDS

prostate cancer, hormonal deprivation therapy, chromatin, texture analysis, epigenetic, methylation, acetylation, chromatin remodelling

INTRODUCTION

Texture is one of the important characteristics used in identifying objects or regions of interest in an image [1]. Nuclear-texture features describe DNA-distribution patterns in cell nuclei, and can be measured reliably by high-resolution image analysis in tissue sections and in single-cell preparations. With the rapid improvement in computer technology and the recent developments in genetics, molecular biology and informatics, quantitative image analysis has emerged as a reliable diagnostic and prognostic tool in several tumour types, including prostate cancer. Digital image analysis (DIA) of texture features can accurately, rapidly and repeatedly measure the quantity and properties of these features objectively. The objectivity and reproducibility of the computerized quantitative DIA technique were the main reasons for its selection to analyse different prostatic tissues in the present study [1,2].

The organization of nuclear chromatin changes according to the metabolic state of the cells, which might correlate with the prognosis of the individual patient [3]. Therefore, studying the nuclear chromatin phenotype might be helpful in understanding the genetic, molecular and biological changes involved in prostate cancer development.
and progression, as it is a sensitive indicator of cell development phenotype. While the chromatin pattern is a powerful biomarker in prostate cancer, little is understood about the mechanisms that control remodelling of chromatin at the morphological level. It is proposed that these subtle morphological changes are controlled by changes in chromatin organization caused by remodelling enzymes as well as epigenetic events, such as CpG methylation and histone acetylation.

DNA methylation in the promoter region of a gene generally correlates with transcriptional silence of this gene, and changes in DNA methylation patterns, in particular hypermethylation of tumour-suppressor genes, have been reported in prostate cancer [4,5]. Antibodies against 5′methyl-cytidine (5MeC) have been used clinically as a prognostic marker in patients with cancer, by detecting nucleoside alterations in urinary excretions [6]. It was further used to quantify in situ differences between normal and malignant cells by visualizing the distribution of methyl-rich regions along the human chromosomes [7]. Moreover, the 5MeC antibody can be used to quantify DNA methylation in prostate cancer tissues. Therefore, it was chosen in the present study to assess changes in overall DNA-methylation in different prostatic lesions.

Histone acetylation is another important epigenetic marker. Histone proteins are the fundamental building blocks of eukaryotic chromatin. Several studies have diversely reported the post-translational modifications that often occur on the tail domains of these proteins. Although the exact function of these highly conserved modifications has remained elusive, there is biochemical and genetic evidence that changes in several chromatin-based processes are related to histone modifications [8,11,12]. Histone H3 is primarily acetylated at lysine 9, 14, 18 and 23. Modification at lysine 9 is thought to play a dominant role in histone mobilization and transcriptional regulation, because it can be either acetylated or methylated. While H3K9 acetylation is mostly associated with transcriptional active genes, methylated H3K9 indicates a chromatin structure that is rather repressive for the genes that it comprises. Interestingly, the histone methyltransferase EZH2 was found to be over-expressed in prostate cancer with poor prognosis, and overall histone-modification patterns have prognostic value for prostatic malignancy [9,10]. This was the basis for choosing the epigenetic key modification acetylated histone H3 lysine 9 (AcH3K9) on the histone level, as a diagnostic marker in the present study.

Chromatin-remodelling complexes use the energy of ATP-hydrolysis to change nucleosomal positions, so that distinct regions of DNA become accessible for interaction with regulating factors [13]. One class of such remodelling factors contains the ATPase ISWI as a catalytic subunit. ISWI-chromatin remodelling activity is important for gene expression, DNA replication and the maintenance of higher order chromatin structure. It was discovered in Drosophila (fruit fly) and in humans it is presented by the two isoforms SNF2H and SNF2L. Studies of their differential expression showed that SNF2H is prevalent in proliferative cell populations, whereas SNF2L is predominantly expressed in terminally differentiated cells and abundant in neurones [14]. Experimental down-regulation of SNF2H delays replication and stops proliferation of haematopoetic progenitor cells. However, little is known about the role of ISWI enzymes in carcinogenesis. Therefore, ISWI immunoreactivity, as a potential marker for proliferation and neoplastic change in prostate cancer, was explored in the present study.

The present study was designed to explore the overall nuclear chromatin phenotype, epigenetic markers and the presence of chromatin-remodelling enzymes, and their role as diagnostic biomarkers in prostate lesions, using high-resolution computerized quantitative DIA.

**MATERIALS AND METHODS**

A tissue microarray (TMA) was constructed using paraffin wax-embedded prostatic tissues from 78 patients (Fig. 1). Each core was 0.6 mm in diameter with 1 mm between cores. The TMA was divided into primary and independent datasets. The primary dataset contained 30 patients (60 cores/sample) with BPH, 10 (10 cores) with low-grade prostatic intraepithelial neoplasia (LGPIN), 10 (10 cores)
with high-grade PIN (HGPIN) and 19 (35 cores) with prostate adenocarcinoma, classified as three cores of Gleason grade 3, 26 cores of Gleason grade 4, three cores of Gleason grade 3 and 4, and three cores of Gleason grade 5. Additionally, 20 cores (from 20 patients) of BPH tissue were taken at 0.6–1 mm from LGPIN lesions, and 10 cores (from 10 patients) of BPH tissues were taken at 0.6–1 mm from HGPIN lesions. An independent dataset was randomly selected and contained seven patients (seven cores) with BPH, nine (18 cores) with LGPIN, 10 (20 cores) with HGPIN and 30 (57 cores) with prostate adenocarcinoma (all Gleason grade 3).

Immunohistochemical (IHC) staining was performed using antibodies raised against AcH3K9 (Cell Signalling Technology Laboratories), 5MeC (Gift from Dr Alain Niveleau, Grenoble, France) and ISWI SNF2L (Gift from Patrick Varga Weisz and described in Poot et al. [15]). Before immunolocalization, AcH3K9 and ISWI sections were pre-treated in pH 6.0 citrate buffer in a microwave oven (at 750 W) for 22 min. 5MeC sections were pre-treated in pH 3.5 citrate buffer in a microwave oven (at 750 W) for 20 min followed by 90 min incubation in HCl at 37 ºC. Localization was according to the manufacturer’s instructions, using an Envision™ Peroxidase System horseradish peroxidase (HRP)-labelled polymer (Dako, Ely, UK). For AcH3K9 and ISWI antibodies, HRP-labelled polymer conjugated to antibodies against rabbit immunoglobulins was used. For 5MeC antibody localization, HRP-labelled polymer conjugated to antibodies against mouse immunoglobulins was used and visualized using liquid diaminobenzidine chromogen for 2 min (Dako). Negative controls were used for all the tested antibodies; the primary antibody was replaced by rabbit serum or IgG1.

For DIA we used a calibrated digital video photometer consisting of a Leitz light microscope (Orthoplan), Sony 3 chip CCD (RGB) colour video camera, IRIS model DX-930P and a Kingshill CL6123 regulated power supply as a stable light source. For microscope adjustment, the Koehler illumination technique for control of light path and illumination beam was used. In-house software regulated the light supply at a fixed level, measuring 250 mean pixel intensity over the image, thus maximizing the dynamic range. The spatial domain was calibrated and calculation showed that one pixel was equivalent to 0.0196 µm². Using 13 optical density images, a calibration reference table for the imaging system was constructed, and a calibration curve for image conversion was created with in-house software. All images were captured at ×100 objective magnification under oil, in colour (24-bit Tiff images), at an image size of 768 × 576 pixels. After completing the image capture, all images were subjected to a pre-processing operation of background subtraction to remove artefacts and any variation in shading, using a macro developed on the KS400 imaging system (Carl Zeiss Vision). Manual segmentation by tracing the nuclear boundaries used the KS400 software (Fig. 2A). Only glandular nuclei were selected for segmentation. At least 30 nuclei were segmented from each core; the numbers segmented in each TMA are summarized in Table 1. The analysis was in the red channel for chromatin phenotype and in the blue channel for IHC. A KS400 macro was used to extract texture features for each nucleus. Nine geometric features quantified the nuclear morphology, 10 densitometric features assessed the nuclear-staining intensity, and 24 discrete features assessed the nuclear chromatin clumping and its distribution [1]; 22 Harrlick features assessed the staining intensity was measured by computing the sum (total or integrated), (SumD) and mean of optical density (MeanD) for each nucleus. Mean correlated to the visual assessment of staining intensity and SumD takes into account the nuclear size.

**RESULTS**

There were numerous significant changes ($p < 0.001$) in chromatin, immuno-intensities and distribution patterns among the different pathological groups (Mann–Whitney independent samples test, $p ≤ 0.001$). There were significant differences for all markers with significant disruption of chromatin phenotype, overall DNA methylation, H3K9 acetylation and ISWI ATPase expression in neoplasia.

To assess the nuclear chromatin content and overall DNA methylation, and AcH3K9 and ISWI ATPase nuclear content, staining intensity was measured by computing the sum (total or integrated) and mean optical density for each nucleus (Fig. 2B). The mean optical density is strongly correlated with the visual assessment of staining intensity. It assesses the staining intensity with little regard to the nuclear size. The sum or integrated optical density is a different measurement that takes the nuclear size into account. Both features were found to be important in assessing nuclear-staining intensity in IHC.
Analysis of chromatin nuclear and IHC staining intensity is shown in Fig. 3. The chromatin phenotype is clearly altered in PIN lesions and adenocarcinoma. The mean chromatin density was higher in LGPIN, HGPIN and cancer lesions (Fig. 3A, left).

Notably, the chromatin disturbance in HGPIN was higher than in cancer samples. However, these changes seem to be related to the associated change in nuclear size. Figure 3A (right) shows the sum of the optical density, and emphasizes the major change in density and nuclear size in the invasive lesions.

Figure 3B shows hypermethylation in LGPIN, reflected in both density mean and density sum nuclear measurements. Again, there is a trend for overall hypomethylation in cancer samples, but again this appears to reflect the massive increase in nuclear size in these lesions.

Figure 3C shows the results for H3K9 acetylation; here the density mean and density sum values appear to conflict with each other, although it is evident that nuclear size is very important in determining overall acetylation density, as detected by other research in our department. The distribution of H3K9 acetylation-positive pixels over an increasing nuclear area suggests that acetylation is being progressively reduced in neoplastic lesions. However, examination of the density sum, which is dependent of the mean nuclear size, shows that H3K9 acetylation actually increases monotonically from LGPIN through HGPIN to prostate adenocarcinoma. This emphasizes the shortfalls in the visual assessment of these variables and the value of quantitative evaluation of both the mean and sum of the nuclear optical density.

Finally, Fig. 3D shows the results for ISWI ATPase; these results are less influenced by the nuclear size, as seen from the similarities in the density mean and density sum graphs. There was ubiquitous over-expression of ISWI ATPase in all the neoplastic lesion groups, with high values in LGPIN. A discriminant analysis test between the pathological subgroups was used to identify the key texture features that are able to differentiate between independent pathological subgroups. This was done separately for different group comparisons to identify the important discriminatory features and investigate the potential classification accuracy that might be achieved using
blocks that did not contain PIN or prostate samples from BPH paraffin wax-embedded.

No differences between them and BPH examination under light microscopy showed at 0.6–1 mm from PIN lesions, visual

In the 30 cores of prostatic tissue sampled detected in BPH tissues adjacent to PIN lesions. Changes in chromatin organization, overall DNA methylation, histone acetylation and ATP-dependent chromatin remodelling. Such changes in chromatin structure not only affect the overall chromatin organization inside the nucleus, but also contribute to the development and progression of malignant disease. In the present study, we measured these morphological and molecular changes using texture analysis to evaluate their potential as diagnostic markers.

As texture-feature analysis is based on statistics, it is recommended to measure as many cells as possible. However, for data analysis the reasonable approach is to compute the entire feature set, and then select features for analysis based on their ability to discriminate between particular cell populations. The variable selection procedure in stepwise discriminant function analysis is considered to be well suited to this task [1]. By using stepwise discriminant analysis, the changes in chromatin organization led to the ability to differentiate between the different prostatic pathologies in the present study with an accuracy of >80%. However, by applying the same test to features measuring ISWI remodelling enzyme, overall DNA methylation, histone acetylation and ATP-dependent chromatin remodelling.

The use of 5MeC and AcH3K9 markers was much more effective at discriminating these two groups, providing 100% correct classification based on the combination of only five features. ISWI also shows remarkable discriminatory power for distinguishing benign and malignant prostate nuclei, with an overall correct classification of 91%. The results of the other comparisons are given in Table 2. Of particular note is the ability of chromatin phenotype to accurately discriminate HGPIN from prostate adenocarcinoma nuclei, and the role that the range of epigenetic and chromatin markers have in distinguishing between LGPIN and HGPIN.

Changes in chromatin organization, overall methylation, overall acetylation and chromatin remodelling by ISWI were also detected in BPH tissues adjacent to PIN lesions.

In the 30 cores of prostatic tissue sampled at 0.6–1 mm from PIN lesions, visual examination under light microscopy showed no differences between them and BPH samples from BPH paraffin wax-embedded blocks that did not contain PIN or prostate cancer lesions (N/BPH). However, digital image texture analysis of chromatin phenotype detected significant differences (p < 0.001) in 51 of 65 features between BPH samples adjacent to LGPIN lesions (BPH/LGPIN) and N/BPH samples. Moreover, 54 of 65 features were significantly different (p < 0.001) between BPH samples taken adjacent to HGPIN lesions (BPH/HGPIN) and N/BPH samples (Mann–Whitney nonparametric independent samples test, p < 0.001). On the other hand, none of the features were significantly different between BPH/LGPIN lesions and LGPIN samples or between the BPH/HGPIN samples and LGPIN samples. Epigenetic changes were also identified in overall DNA methylation and H3K9 acetylation in BPH tissues adjacent to PIN lesions. The number of significantly different features between BPH/LGPIN and N/BPH, BPH/LGPIN and LGPIN, BPH/HGPIN and N/BPH and BPH/HGPIN and HGPIN nuclei for both 5MeC and AcH3K9 are listed in Table 3.

**DISCUSSION**

During gene regulation, chromatin structure is controlled by several mechanisms, including...
methylatio and overall acetylation status of the AcH3K9 loci, the classification accuracy was higher among the different prostatic pathologies, particularly in H3K9 overall acetylation. This would mean that this variable is a sensitive diagnostic marker in prostatic lesions.

Other studies have shown the ability of quantitative image analysis to detect the presence of changes in chromatin organization, but only between BPH and prostate cancer. Christen et al. [3] reported an overall successful classification rate of 93% between BPH and adenocarcinoma samples using carefully selected nuclei generated by tissue disaggregation. Changes in chromatin texture were also used to assess disease progression in PIN and prostate cancer tissues. Bartels et al. [16] reported distinct and statistically significant texture and spatial changes in nuclear chromatin of secretory PIN and prostate adenocarcinoma cells.

In the present study, we showed the potential value of DIA of nuclear texture in quantifying nuclear DNA content, and for the first time, IHC staining in prostate lesions. Measuring DNA content using both the sum and mean of optical density revealed a monotonically increasing trend in nuclear chromatin density from BPH to LGPIN to HGPIN, followed by a reduction in the chromatin density in prostate adenocarcinoma nuclei [Fig. 3A]. This could be consistent with the presence of higher levels of condensed heterochromatin in proliferative lesions than the higher levels of dispersed euchromatin in prostate cancer.

The changes in chromatin phenotype staining intensity in prostatic lesions are consistent with other reported measurements of the total optical density values for nuclei in prostate tissue that range from normal to adenocarcinoma. Those studies showed that PIN nuclei had a higher sum (total) optical density than BPH and adenocarcinoma nuclei, and constitute a separate group [17–19].

By contrast with other studies, which only used the sum of optical density to measure staining intensity in texture analysis [16,17,19,20], we found that measuring both the mean and sum of the optical density are valuable in assessing staining intensity (Fig. 2B). The mean was a more accurate representation of the strength and depth of staining intensity, as it accounts for variation in nuclear size and effect on staining intensity. However, the sum of staining intensity has also been used to quantify the total amount of staining intensity pixels and thus the total nuclear chromatin content. These two features were used to quantify the immunoreactivity of epigenetic biomarkers and chromatin remodelling enzymes in prostate tissue. By using TMA we ensured that the same conditions were applied equally and simultaneously to all tissue samples during immunostaining and quantitative image analysis.

Measuring the 5meC staining intensity in prostate tissues using quantitative image analysis identified the overall DNA methylation status in these tissues. The overall DNA hypermethylation identified in PIN lesions, particularly in LGPIN, together with the overall DNA hypomethylation in prostatic adenocarcinoma, were consistent with reported measurements of DNA methylation in cancer in general and in prostate cancer in particular [7,21] (Fig. 3B).

Genome-wide DNA hypomethylation was reported to be most pronounced in urothelial carcinoma and occurs in prostatic cancer [22]. Published studies report that overall DNA hypomethylation accompanied by region-specific DNA hypermethylation is a common characteristic among tumour cells [23,24], with substantial DNA hypomethylation in genes of cancer cells compared with their normal counterparts [25]. DNA hypomethylation increases instability of the chromosome complement of a cell, which leads to the activation of tumour-promoting genes by mechanisms that might include among others, altered heterochromatin-euchromatin interactions causing oncogenesis. As it is generally thought that DNA hypermethylation is associated with heterochromatcondensed DNA, the development of hypomethylation in these regions might lead to decreased heterochromatin condensation. This makes it prone to chromosomal rearrangement and genomic instability [26]. Therefore, the reported DNA hypermethylation in PIN lesions could be consistent with the high levels of heterochromatin identified in these lesions in chromatin phenotype. Similarly the overall DNA hypomethylation in adenocarcinoma nuclei could be consistent with a higher content of the less condensed euchromatin identified in these lesions.

In the present study, the overall acetylation status of the H3K9 locus in prostatic lesions was also assessed by measuring the nuclear staining intensity, using both the sum and the mean of optical density (Fig. 3C). There was a reduction in mean optical density in AcH3K9 staining intensity from BPH to LGPIN and HGPIN nuclei, followed by subsequent reduction in AcH3K9 staining intensity to its lowest levels in prostate cancer lesions. However, the sum of optical density showed a higher content of weakly stained AcH3K9 loci in adenocarcinoma nuclei than in both BPH and PIN nuclei. This could indicate an absolute increase of both histone H3 and acetyl groups at lysine 9, combined with loss of acetyl groups at certain genomic loci in cancer cells, which could be responsible for the overall H3K9 hypoacetylation in adenocarcinoma nuclei. Up-regulation of histone deacetylases and histone methyltransferases, in particular EZH2, might be causing this phenotype.

Several studies showed that core histone acetylation is essential for the expression of tumour suppressor genes in many tumours [27–31]. Therefore, the overall H3K9 hypoacetylation seen in adenocarcinoma nuclei would be consistent with a down-regulation of the tumour suppressor genes. This could lead to the acceleration of the malignant process and development of prostate cancer. The successful application of histone deacetylase inhibitors as antitumour drugs underscores the importance of this mechanism. However, acetylation in specific promoter regions of individual genes in prostatic lesions remains to be investigated.

ISWI chromatin-remodelling ATPases are the catalytic core of various multi-subunit protein complexes in the nucleus. Their activity lends fluidity to chromatin, which is required for fundamental processes in the nucleus, such as gene expression, DNA replication and maintenance of the higher order chromatin structure. ISWI complexes were originally identified in the fruit fly. The human homologues of ISWI are the two isoforms SNF2H and SNF2L. Studies of their differential expression showed that SNF2H is prevalent in proliferative cell populations, whereas SNF2L is predominantly expressed in terminally differentiated cells and is abundant in neurones [14]. Studies with
leukaemia cells suggest that both in vitro and in vivo induction of differentiation is followed by down-regulation of SNF2H expression [32]. Likewise, blocking the expression of SNF2H can stop proliferation [32]. Moreover, an ACF1–SNF2H complex was shown to accelerate DNA replication in heterochromatin [33]. Although the ISWI antibody used in the present study was raised against SNF2L, it recognizes both SNF2H and SNF2L [15]. Although the antibody detects both isoforms, it could be postulated that the increase of the ISWI signal in prostate cancer represents SNF2H rather than SNF2L, due to the above mentioned role of SNF2H in cell proliferation and the neure-specific expression pattern of SNF2L. However, expression of SNF2L in prostatic tissues cannot be excluded.

Biochemical assays showed that the action of ATP-dependent chromatin-remodelling activities increases the accessibility of DNA within chromatin templates. Considering the function of ISWI for the maintenance of higher order chromatin structure, it is likely that more ISWI activity is required to pack increased amounts of aneuploid DNA into cancer cells [34]. More recent functional studies suggest that many ATP-dependent chromatin-remodelling activities can also regulate transcription [13,35–37]. In the present study, ISWI ATPase staining intensity was low in BPH nuclei and higher in LGPIN nuclei (Fig. 3D). The increased amounts of ISWI ATPase in LGPIN nuclei gradually declined towards HGPIN nuclei, with a subsequent decline of overall ISWI ATPase amounts in cancer nuclei. However, the overall amounts of ISWI ATPase in PIN and cancer nuclei were much greater than in BPH nuclei.

Based on previous observations of epigenetic biomarker expression in prostate lesions, in the present study it was noted that the high levels of ISWI ATPase staining intensity in LGPIN nuclei were similar to that detected in 5MeC phenotype (Fig. 3B,D). The simultaneous increase of both DNA methylation and ISWI ATPase expression in LGPIN nuclei, and the subsequent decrease in prostate adenocarcinoma nuclei, could be consistent with a role for ISWI in repressing gene transcription [33]. It is widely recognized that chromatin remodelling, histone de-acetylation and DNA methylation are consecutive steps of gene inactivation.

Interestingly, in the H3K9 acetylation phenotype, the highest sum of the ACh3K9 staining intensity signal correlated with a high sum of the ISWI staining intensity in prostate adenocarcinoma, as compared to the BPH controls. Regulatory subunits of ISWI protein complexes such as ACF1 contain a bromo-domain, which binds specifically to acetylated lysine residues. Therefore, histone acetylation is a possible mechanism of ISWI recruitment, but further functional studies are needed to understand the complex interaction of chromatin-modifying enzymes.

In malignancy, there are dramatic changes in chromatin appearance. Promising examples, for the value of nuclear texture feature measurement in the classification and detection of malignancy-associated changes (MAC) in a variety of human tissues, including prostatic, colonic and oesophageal lesions, have been established [38].

Using high-resolution DIA, the present study identified similarities in the measured texture features between PIN lesions and BPH tissues sampled 0.6–1 mm from these lesions (Table 3). There were also similarities, but to a lesser extent, in overall DNA methylation and H3K9 acetylation between the PIN lesions and the adjacent BPH tissues. This would indicate not only the presence of MAC in chromatin organization but also in overall epigenetic events in the BPH tissues adjacent to PIN lesions.

Several studies showed the ability of texture analysis of chromatin phenotype to identify changes in tissues adjacent to prostate cancer or PIN lesions. A discriminant function score for these features was able to identify PIN and MAC in secretory cell nuclei from visually normal—appearing tissue regions of prostates harbouring PIN or adenocarcinoma [19]. These findings were confirmed in the present study using chromatin phenotype. However, the present study shows for the first time the use of overall DNA methylation and H3K9 acetylation to identify MAC near PIN lesions with high accuracy, using high-resolution computerized DIA.

In conclusion, chromatin phenotype, epigenetic events together with chromatin-remodelling enzymes, quantified by DIA in different prostatic lesions, are successful diagnostic markers in prostate tissues. The staining intensity and content of these markers were assessed by a reliable reproducible objective technique. Our experiments underscore the importance of epigenetic mechanisms during carcinogenesis and highlight their potential for medical applications. Further studies are needed to elucidate the interplay between chromatin structure, its modifications and the progression of prostate cancer.

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CONFLICT OF INTEREST

None declared.

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Abbreviations: DIA, digital image analysis; TMA, tissue microarray; (LG)(HG)PIN, (low-grade) (high-grade) prostatic intraepithelial neoplasia; Ach3K9, acetylated histone H3 lysine 9; 5MeC, 5′methylcytidine; SNF2H, SNF2L, chromatin remodelling ATPase ISWI isoforms; MAC, malignancy-associated changes; HIC, immunohistochemistry; HRP, horseradish peroxidase; N/BPH, samples obtained from BPH paraffin-embedded blocks that contained no PIN or prostate cancer lesions; BPH/LGPIN, BPH, samples obtained 0.6–1 mm from LGPIN lesions; BPH/HGPIN, BPH samples obtained 0.6–1 mm from HGPIN lesions; H&E, haematoxylin and eosin.