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Full Length Research Paper

Mapping Angiogenic Cells CD31 (*PECAM1*) and CD45 in PCa and BPH Biopsies

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Molecular characterization of PCa and BPH using the angiogenic tissue markers CD31 and CD45 to differentiate between the two major types of prostate gland disorders is investigated. 8 clinically characterized biopsies were analyzed at the Histopathology Laboratory of the Ekiti State University Teaching Hospital; the tissue sections were processed for antigen retrieval and were labeled with *anti-CD45* and *anti-CD31* to map the location of angiogenic cells and vascularization in BPH and PCa. The results showed that PCa biopsies expressed embryonic stem cell status of CD31+/CD45+, while BPH showed CD31-/CD45+. This suggests the presence of migrating blood cells (vascularization) in PCa and the absence of such in BPH. It also confirms the role of platelets in endothelial activation that can result to cell proliferation and apoptosis in the prostate gland of either BPH or PCa diagnosed patients.

Key words: CD31, CD45, Prostate, Endothelioma, Vascularization, Angiogenesis

INTRODUCTION

Angiogenesis is a very important factor in the progression of benign tumors and malignancies. As the tumors get vascularized, mapping the angiogenesis markers is an important tool in detection of prostate benign neoplasm progression and malignancy (Ting et al., 2013). Specific protein targeting and labeling are important for this detection. The cluster of differentiations- CD31 and CD45 were selected as the angiogenic tumor markers for PCa as they are important parameters in the determination of micro vessel density within the prostate gland (Li et al., 2013). CD31 (PECAM 1) is a platelet endothelial factor responsible for cell-to-cell adhesion and plays important (Cooper et al., 2002). CD31 can be localized on a wide variety of cells including T-lymphocytes. Of importance is its presence in the endothelial cells and intercellular junctions; this protein functions within a larger family of immunoglobin (Ig's) involved in angiogenesis.

It is also expressed in certain types of tumors especially in the glandular tissue of the prostate (Maeshima et al., 2001). Immunodetection of CD31 can help determine the index of vascularization as in PCa and BPH progression in otherwise what is called tumor angiogenesis or tissue angiogenesis which may be roles in removing aged neutrophils from the body. It is also important in the activation of alpha intergrin receptors required to hold leukocytes to the endothelium caused by inflammation and other fibrous tissue changes within glandular tissue (Gravina et al., 2013). This is also

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important in the demonstration of prostatic angiomas and angiosacromas (Jin et al., 2013; Tafani et al., 2011). CD45 is a protein tyrosine phosphate (PTP) located in hematopetic cells; the enzymes initiates dephosphorylation of phosphotyrosine residue and are characterized by homologous cyclic domains (Carvalho et al., 2013). The CD45 has several isoform peculiar to specific cell types; *CD45* is a protein expressed in mature lymphocytes that are activated as a form of response to apoptosis; apoptosis not programmed cell death, but physiological cell death in this context (Brennen et al., 2013). This study evaluates prostatic cellular disorders, (BPH and PCa), where CD31 is mapped against CD45 to determine the angiogenesis index in PCa and BPH.

MATERIALS AND METHODS

Tissue processing

BPH and PCa samples (biopsies) were obtained from patients clinically diagnosed and histologically confirmed to have the condition(s) following ethical guidelines approved by the Ekiti State University Teaching Hospital, Ado-Ekiti. The biopsies were fixed in formolcalcium (4BPH and 4Pca) and processed histologically to obtain paraffin wax embedded sections at the Pathology Laboratory of Ekiti State University Teaching Hospital, Ado-Ekiti.

Histology and immunohistochemistry

Tissue sections were processed for routine hematoxylin and eosin following the methods of Zhang et al. (2012) to demonstrate the general morphology of the tissues and vessels in the tissue (small arteries).

CD31/PECAM 1

The following proteins were labeled in the BPH and PCa tissue biopsies; *CD31* in the glandular tissue of the prostate, *CD31* in the muscular part of the prostrate and *CD31* in the endothelium of blood vessels [anti Human-*CD31* monoclonal diluted in Tris buffer saline (TBS) 1:500].

Lymphocytic marker (*CD45+)*

This was demonstrated in the overall prostate tissue (glandular and muscular part) as an indirect measure of lymphocytic cell response at the onset of tumorgenesis (if any; progression of physiological cell death). [*CD45* Anti-human monoclonal diluted in PBS at 1:100]

Procedure

The paraffin wax embedded sections were mounted on a glass slide in preparation for antigen retrieval where the slides were immersed in urea overnight and then placed in a microwave for 45 min to re-activate the antigens and proteins in the tissue sections. Primary antibody treatment involved treating the sections with biotinylated goat serum for one hour following which the sections were transferred to 1% bovine serum albumin (BSA) to block non-specific protein reactions. Secondary treatment involved the use of diluted anti-CD31 and anti-*CD45* on the pre-treated sections for 1 h. The immunopositive reactions were developed using a polymer 3'3' diaminobenzidine tetrachloride (DAB) with colour intensification involving the use of mathenamine silver kit. The sections were counterstained in coomasie-G250 (brilliant blue) and treated in 1% acid alcohol (freshly prepared).

Transformation

Mathenamine silver intensification is used on the immunoperoxidase preparation after the peroxidase/H_20_2/DAB reaction has been carried out to give a brown deposit. The sections were then counterstained in Hematoxylin. The counterstained sections were washed in running tap water, thoroughly rinsed in distilled water, and placed in preheated methenamine silver solution at 60°C for 5 min. Although it could be occasionally longer if the intensification had been carried out at room temperature. In this study, to further increase the clarity, hematoxylin was removed from counterstained nuclei with 1% acid alcohol before the silver intensification was carried out. The composition of the stock solution was 0.125% silver nitrate in 1.5% hexamine. The solution was stored at 4^oC. Prior to use, 2 ml of 5% tetra borate was added to 50 ml of the stock silver solution giving a pH of 8.0, which was then filtered into a coupling jar and protected from sunlight.

RESULTS AND DISCUSSION

The study involves characterization of BPH and PCa biopsies from human prostate by staining the biopsies immunohistochemically using surface antigens for tumor angiogenic cells CD31 and CD45. The nuclei were stained blue following hematoxylin counter stain (Figures 1 to 3). Over the years the controversy of a possible link between BPH and PCa has been extensively discussed; researchers have described the possibility of BPH progressing to PCa. To clarify the essence of the controversy in the first instance, BPH tissue cells will later become tumorgenic or secondly, the presence of symptoms of BPH may predispose to PCa by cellular activation of glandular tissue (Chen et al., 2012).

A major feature peculiar to both BPH and PCa is an increase in the rate of cell proliferation. The clinical manifestations of prostate cancer result from the effects of local growth of the tumor, the spread to regional lymph nodes via the lymphatic system, and the hematogenous dissemination to distant metastatic sites. Although most patients with early-stage prostate cancer are asymptomatic, locally advanced disease can lead to obstructive or irritative voiding symptoms that result from local tumor growth into the urethra or bladder neck, extension into the trigone of the bladder or both making it difficult to differentiate from symptomatic BPH. However, BPH arises as spherical masses of epithelial and stromal elements from the glands lining the proximal prostatic urethra. The ratio of epithelium to smooth muscle in the prostate can vary among individuals, from 1:3 to 4:1.

However, larger prostates may contain more androgen-dependent epithelial elements than smaller glands, which contain a higher proportion of smooth muscle. In either case, the outcome of BPH may be urethral obstruction induced mechanically by epithelial overgrowth and

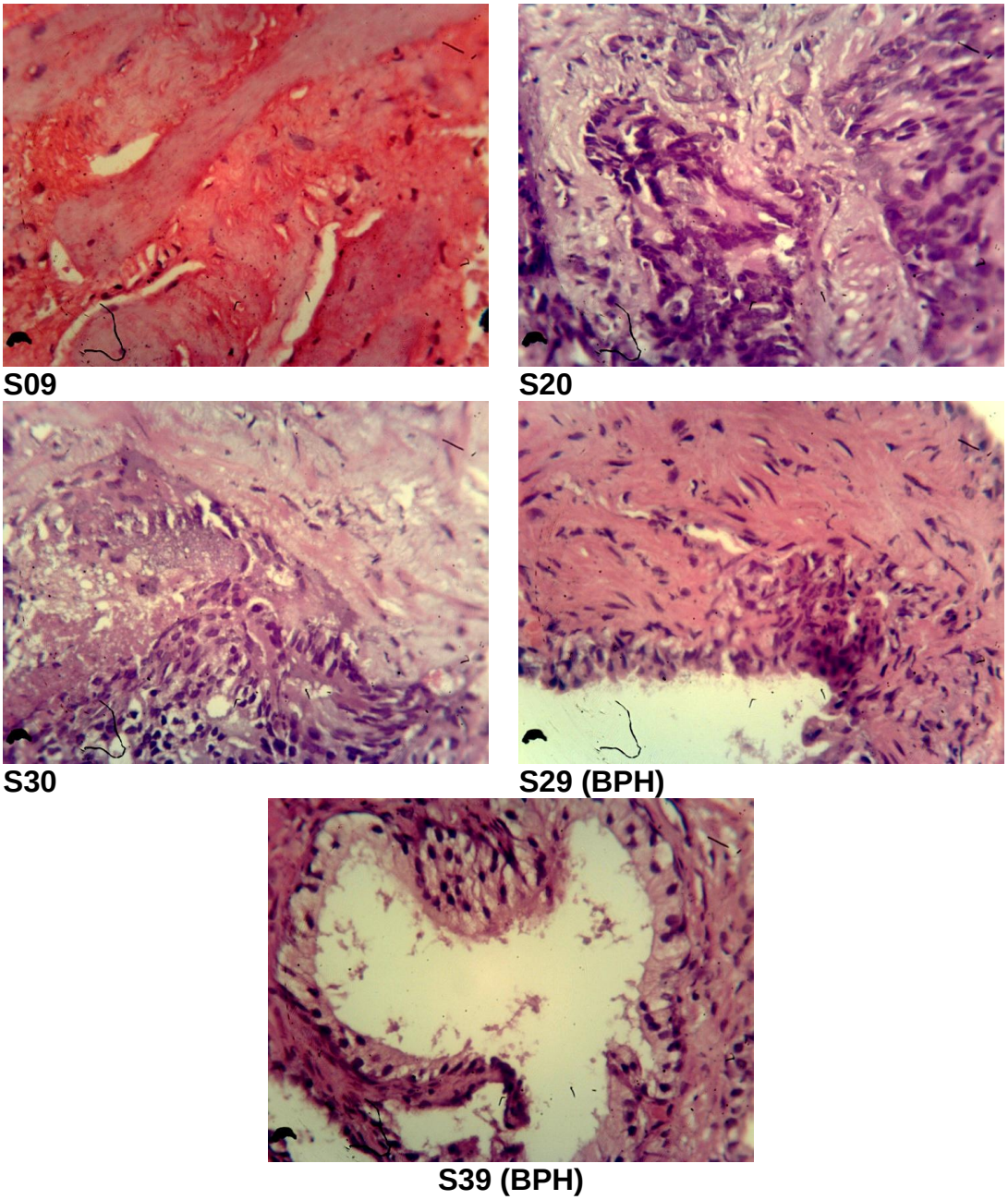

Figure 1. Histological demonstration of the general morphology in human prostate tissue S09, S20 and S30 for PCa while S29 and S39 represent the general morphology of BPH tissues. Cell aggregation can be observed in both PCa and BPH; PCa cell aggregations are located at random in glandular tissue or close to the ductal epithelium, while BPH forms an arranged layered mass of cells in the fibromuscular layer. (Magnification ×400).

dynamically by prostatic smooth muscle contraction, or a combination of the two (Kyprianou et al., 1996). In both cases, increased cell population is resultant while PCa contains cell populations clumped to form a tumor at random locations along the epithelium or glandular tissue, resulting in nodular swellings (Figures 1 and 2). The BPH appears to be a more coordinated proliferative process where cell detachment is not observed and the resultant cell mass have a defined layer like appearance.

A major difference between BPH and PCa is that having acquired the cell mass, the cell aggregations in PCa becomes highly vascularized as seen in the distribution of CD31 (CD31+) in 3 PCa biopsies while in BPH no vascularization is observed (CD31-) (Figure 2).

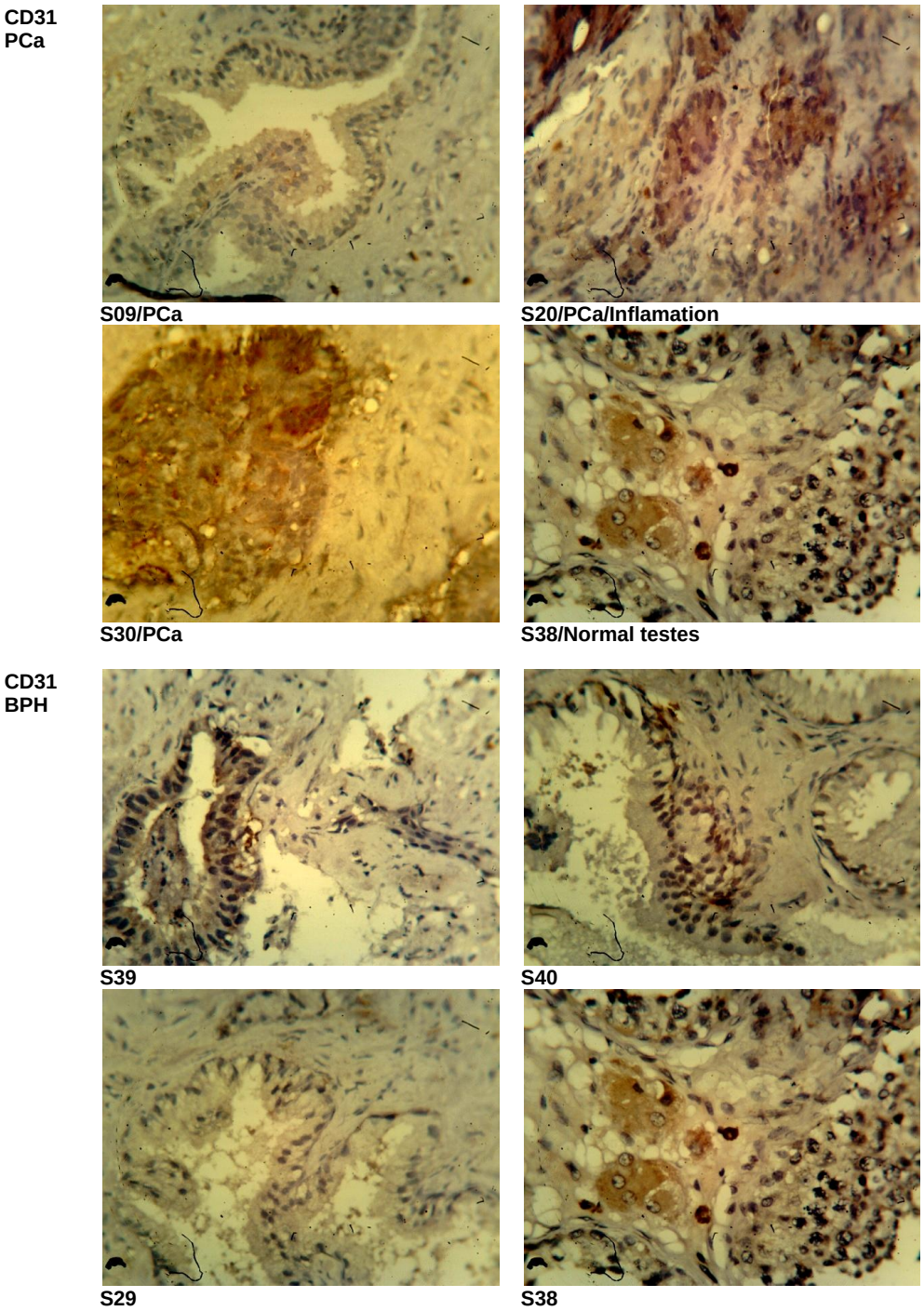

Figure 2. Immunohistochemical demonstration of CD31 in human prostate tissue S09, S20, S30 (PCa). S38 represents the control human testicular tissue. Immunopositivity was observed S30, S09 and S20. Although the level of expression is lower and sparse in S09, this can be accounted for a function of the difference in sites where the tumor cells are localized. While S09 is epithelia, S20 and S30 are glandular and are accessible to vascular supply compared to S09. In the BPH biopsies (S39,S40 and S29), CD31 immunopositivity shows vascularisation of the BPH tissue mass. A feature that seem to accompany cell proliferation. (Magnification ×400).

This is similar to the findings of Wong and Chen (2012) and Chen et al. (2012). The platelet derived factor CD45 are activated as a form of response to apoptosis, the distribution of CD45 was restricted to specific tissue sites

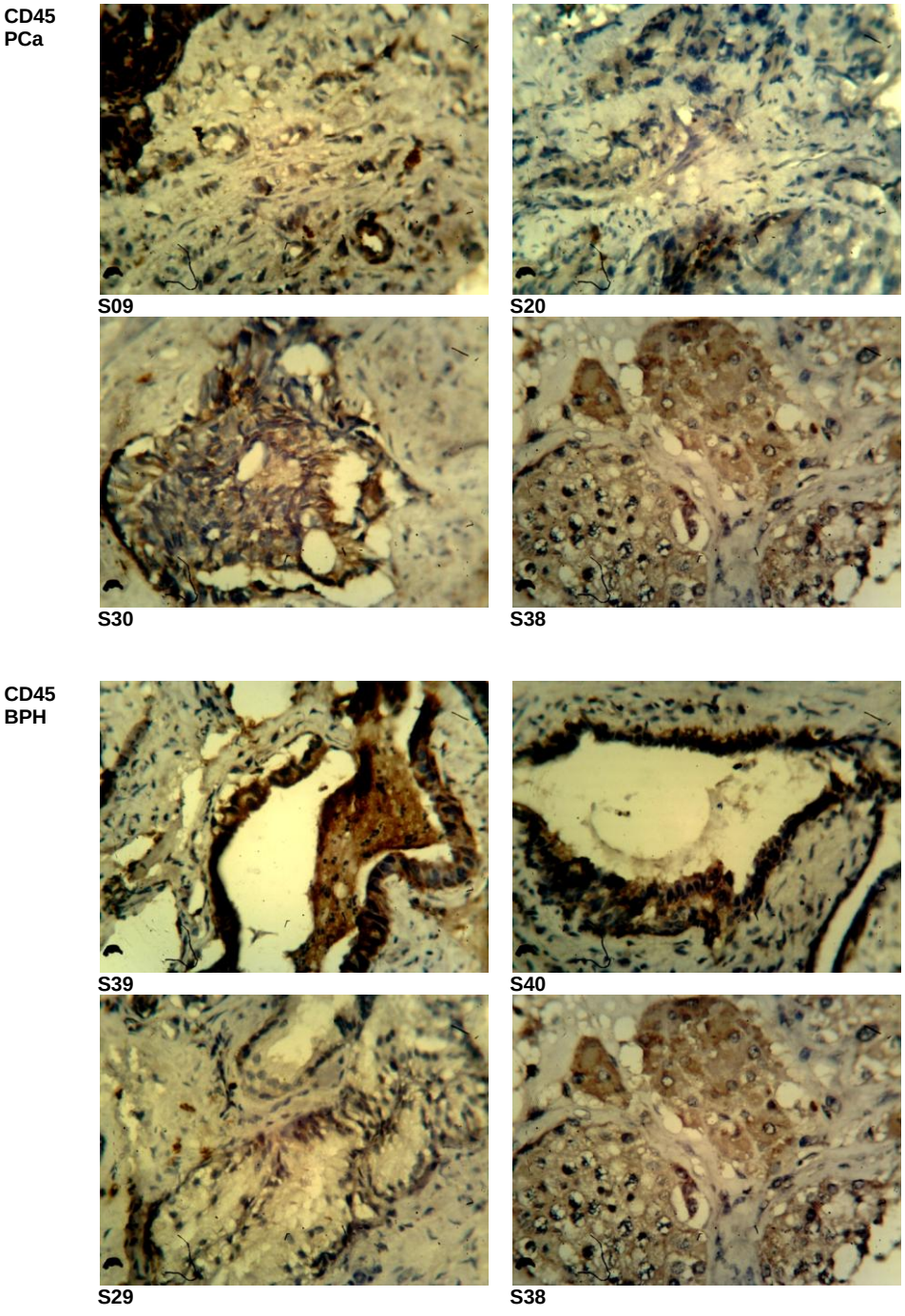

Figure 3. Immunohistochemical demonstration of CD45 in human prostate tissue S09, S20, S30. S38 represents the control human testicular tissue. BPH tissue biopsies S39, S29 and S40). Growing evidence has implicated white adipose tissue as a source of stromal progenitor cell recruited to the tumor microenvironment. S09 (+), S20 (+), S30 (+). In BPH tissues CD45 positivity was observed around the epithelium and was absent in S29 biopsy (Magnification ×400).

within the glandular tissue but negative for the epithelium in PCa biopsies while in BPH, the CD45 was found both around the epithelium of the gland and the fibromuscular layer (Figure 3). CD45 thus seem to be associated with massive cell proliferation (PCa/CD45+ and BPH/CD45+).

This is in agreement with the findings of Morelli et al. (2013) and Jones et al. (2013). Most of the cell death (CD45+) observed were restricted to the surface of the

Table 1. CD31 and CD45 distribution in 4BHP and 4PCa Biopsies following immunohistochemistry using monoclonal antibodies against Human CD31 and CD45.

PCa	*CD31*	*CD45*	BPH	*CD31*	*CD45*
S09	-/+	+	S39	-	+
S20	+	+	S40	-	+
S30	+	+	S29	-	-
S38	+	+	S38	-	+

tumor mass in PCa or tissue mass in BPH. It is characterized by extensive proliferation and inflammation of fibromuscular layer cells which has also been reported to be an important predisposing factor to PCa (Lazar et al., 2012).

In this study PCa recorded CD31+/CD45+ while BPH showed CD31-/CD45+ (Table 1). This result may be useful in predicting the management of carcinoma-*in-situ* as CD31+/CD45+ may suggest progression to PCa. These outcomes may also indicate infiltration of tissue sites by macrophages like eosinophils. There is also a possibility of having the presence of tumor cells from more than one source that is lymphomas and adenocarcinomas. The CD45+ elevated levels in PCa and BPH show activation and aggregation of platelets and according to the studies of Jin et al. (2013), it is a predisposing factor to recurrence of the tumor.

Combined immunodetection of CD31/CD45 also maps the distribution of stimulating proliferating cell populations. Studies on muscles showed that CD31+/CD45- characterized cells constitute about 5 to 6% of cell population in the muscles while CD31-/CD45+ represents the status of 90% of the muscle cells (Figure 3 and Table 1). As a form of response to inflammation, it is possible that CD31+/CD45+ cell population were recruited from elsewhere (Taffani et al., 2011). Tissue sites where CD31+/CD45+ have been identified includes embryonic prostate tissue containing stem cells programmed to form both glandular and muscle tissue of the prostate gland and this is also an evidence of re-expression of embryonic proteins in PCa cells. While the CD31-/CD45+ are characteristic of muscles (90%), it confirms the immune mapping in BPH where the status was CD31-/CD45 (Table 1). CD45 is also peculiar to cells that are capable of active proliferation such as epithelial cells which most of the time undergo exfoliative cytology as part of the routine of the tissue where these cells are localized (Mackern Oberti *et al.,* 2011).

Previous studies already established that CD31 is characteristic of angiogenic cells originating from the bone marrow and such CD31 cells will under defined conditions give rise to endothelial cells and as such it is important to identify cells with increased angiogenic activity (Poveda et al., 2011; Blann et al., 2011). The CD31/CD45 immuno-mapping can also be used as a distinguishing factor for blood cell cancer proper or tumor cells that move within the blood cells (Wong et al., 2012). The role of endothelial cells mapped with CD31 is important in determining the rate of progression of BPH especially those linked with inflammation (Ribeiro et al., 2012). Studies on other endothelial factors like VCAM-1 suggests active chemical interaction in blood vessels (endothelium) to recruit lymphocytic cell lines into the area and induce apoptosis by physiological cell death in the BPH tissue mass or PCa cells (Lawson et al., 2007). In conclusion, the relative distribution of CD31/CD45 is an important molecular marker in determining angiogenesis in PCa progression and also a major tool in distinguishing PCa from BPH.

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Conflict of interest statement

The Authors hereby declare there is no conflict of interest associated with this study or any of the procedures and materials used for the purpose of the study

ABBREVIATIONS

PCa; (Prostate Cancer)**, BPH**; (Benign Prostatic Hyperplasia)**, LUTS**; (Lower Urinary Tract Symptoms)**, CD**; (clusters of differentiation).

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