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Roles of Cell Cycle Regulators [p53, Cathepsin-D and Bax] in Prognostic Determination of Prostate Cancer and Benign Prostatic Hyperplasia

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Abstract

Background: The prostate gland is an almond-shaped gland located directly below the urinary bladder and circling the prostatic urethra. The incidence of prostatic disorders has been found to increase with age; especially in *PCa and BPH*. PCa and BPH are both characterized by cell proliferation and active division at specific tissue sites. The two forms of cell proliferation are regulated by cell cycle and are perhaps created by molecular mechanisms dysregulation that will alter such regulatory mechanisms.

Method: Human prostate biopsies were obtained from clinically diagnosed patients and were studied immunohistochemically to map the distribution of *p53, CathD* and *Bax*.

Results and conclusion: In PCa, the increased levels of *p53* and *Bax* signals pre-apoptotic tendencies for rapidly proliferating un-coordinated cells which can be located at random locations due to loss of matrix and adhesion molecules described in high *CathD* levels. Co-localization of *p53*, *CathD and Bax* can be insightful to further determine the role cell cycle *in BPH and PCa* and in distinguishing the patterns of cell proliferation in both conditions.

Keywords: BPH; Prostate cancer; p53; Bax; Apoptosis; Cell cycle; Cathepsin D

Abbreviations: BPH: Benign Prostatic Hypertrophy; PCa: Prostate cancer

Introduction

Prostate cancer and *BPH* represents the most persistent disorders in males. It has been long discussed whether episodes of *Benign prostatic hyperplasia (BPH)* can lead to or perhaps predispose a person to prostate cancer (PCa) [1]. Both PCa and BPH are characterized by cell proliferation localized in the epithelium *(fibromuscular layer)* or the *glandular tissue* [2]. *PCa* can generally be found localized in both tissue sites whereas *BPH* occurs mainly in the fibromuscular part of the prostate, but can also result from proliferation of glandular tissue or over expression of receptors around the bladder neck [3,4]. It is long established that epithelial cells are characterized by active cell division, and because they are more exposed, especially around the body walls, they record more episodes of neoplasm and dysregulation. The primary concept of cell proliferation, division, death and tumorgenesis is best explained via the cell cycle which controls these series of events via cascades of specific protein switch mechanisms localized within the cell [5].

Of importance is the role of protein 53 (*p53*), *a 53 kDa nucleolase* and cell cycle regulator that suppresses tumor formation that may result from DNA replication processes that fail to respond to checkpoint signals. Expression of *p53* has been found to be greatly increased in tumorgenic proliferation. The role it plays in tumor suppression includes hydrolysis of DNA to direct the cell into a permanent resting phase or apoptosis [6]. The incidence of *p53* mutation appeared lower in prostate cancer than in other cancer cases; although it is possible that mutation occurs, but is least detectable due to its association with a more aggressive form of the disease [7,8]. Although, cell proliferation is a primary factor to consider in *PCa and BPH*, the incidence of *p53* expression in cell proliferation associated with *BPH* has also been found to be a function of Bax (cell cycle marker for senescence) [9]. Hence, over expression of *p53 and Bax* in most cells is a molecular marker for apoptosis. Inactivation of *p53* gene is often observed in *PCa* but not in *BPH*, while over expression of Bax and low *p53* is a factor expected in *BPH*. The latter is characteristic of cell division without tumorgenic tendencies [7]. *Cathepsin D* has been described as an essential component required for the progression of prostate cancer from the tumor state to malignancy. Over expression of *CathD* has been observed in malignant fibroblast from the prostate. Examination of human prostate tissue in the study by Pruitt *et al.*, 2013 shows increased stromal staining of *p53*; *In vitro* studies also show that increased *CathD* expression is required for malignancy in

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neoplastic tissues and tumors (Pruitt *et al.,* 2013; Salama *et al.,* 2013). This study seeks to evaluate and distinguish between factors involved in cell cycle regulatory mechanisms associated with tumorgenesis using comparative immunohistochemistry; expression of *CathD*, *Bax* and *p53* in *BPH* and *PCa* biopsies.

Materials and Methods

Tissue processing

BPH and PCa samples (biopsies) were obtained from patients clinically diagnosed and histologically confirmed to have these condition(s). The biopsies were fixed in formolcalcium *(4BPH and 4Pca)* and processed histologically to obtain paraffin wax embedded sections at the pathology lab of University Teaching Hospital, Ado-Ekiti.

Histology: Tissue sections were processed for routine Hematoxylin and Eosin following the methods of [10] to demonstrate the general architecture of prostate biopsies for PCa and BPH.

Immunohistochemistry

Cell cycle markers (*p53, Cathepsin D* and *Bax*): They were immunolabelled in the glandular and muscular prostate tissue using anti Human-*p53* (polyclonal), Rat anti Human-*Bax* and anti-*Cathepsin D* (Monoclonal) to demonstrate cell cycle dysregulation, cell death and onset tumorgenesis. [Dilutions; *p53* (1:100 in PBS), *Cathepsin D* (1:350 in TBS) and *Bax* (1:1,000 in PBS)].

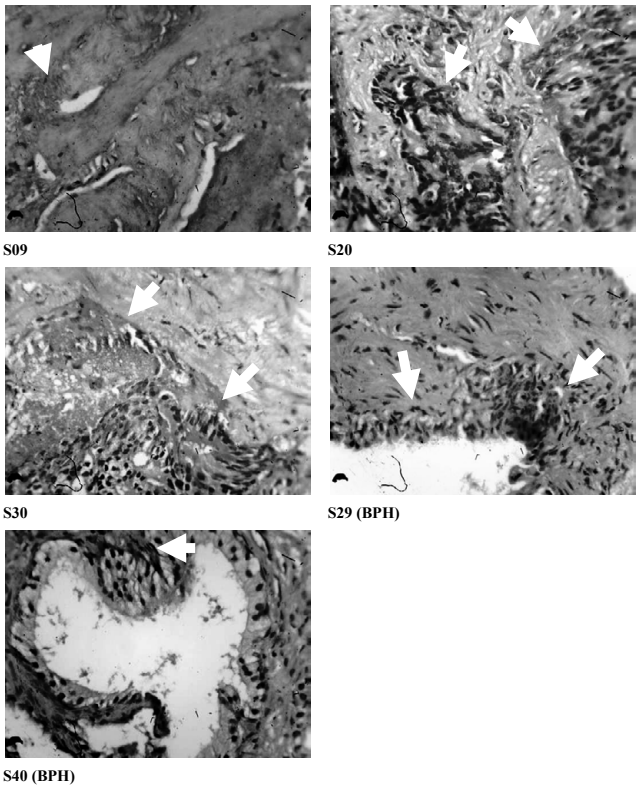

Figure 1: Histological demonstration of human prostate biopsies using Hematoxylin and Eosin stain. S09, 20 and 30 represents *PCa* biopsies, while S29 and 40 represents BPH biopsies. Increased in cell proliferation is characteristic of both tissue types. The distribution of cell mass is uniform and restricted to the epithelium in *BPH*; it is found at random tissue sites in *PCA* (magnification X400).

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 minutes* 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-*p53*, anti- *Cath D*, anti-*Bax* and anti-*CD45* on the pre-treated sections for one hour. 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 Hematoxylin and treated in 1% acid alcohol (freshly prepared).

Transformation: Mathenamine silver intensification was used on the immunoperoxidase preparation after the peroxidase/H_2O_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* five minutes. 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°C. Prior to use, 2 ml of 5% tetraborate 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

Cell cycle describes the cellular control mechanism in place to check and control all the different phases involved in cell reproduction, activities and cell death [11,12]. Each of the different stages of the cell cycle is said to be controlled by several cell switch systems involving the *Cyclins* and *Cyclin dependent kinases (Cdks)* [13]. During the process of rapid cell division, the cell cycle puts in place resting phases (Gap phases) in between the important phases (Synthetic or S-Phase, Mitosis or M-Phase). The cell cycle is characterized by specific resting duration during which the cell proofreads its genome for errors [14]. If such errors are repairable, the cell amends such errors via molecular control mechanisms by literarily stitching the broken DNA material into the rest of the genome. Although the cell is equipped with the metabolic machinery to stitch the broken genetic fragments during replication, it is however not endowed with tools to recognize the actual sites [15-20]. In certain circumstances, the fragmented gene is stitched to a wrong site which might alter gene regulatory region that will prone a cell to over expression of certain proteins that can lead to excessive transcription of genes (cancers) - if the gene regulatory region is altered. In a second mechanism, if the DNA breakage is vast and cannot be repaired easily, the cell quickly sends itself to a permanent resting phase or G_0 (apoptosis). This state is achieved via the increase in the transcription of the p53 gene in response to such genetic errors [16]. The nuclease digests the DNA, hence such cells are believed to be in a state of self-termination (apoptosis). *p53* gene increases the fidelity of *PCa* due to mismatch repair as described by [7,20]. Several studies show that the expression of *p53* corresponds with the progression of *PCa* and has thus been regarded as a prognostic marker and predictor of endocrine therapeutic effect for prostate cancer. The *p53* signaling pathway activates *Bax* as a form of pre apoptotic signal; this accounts

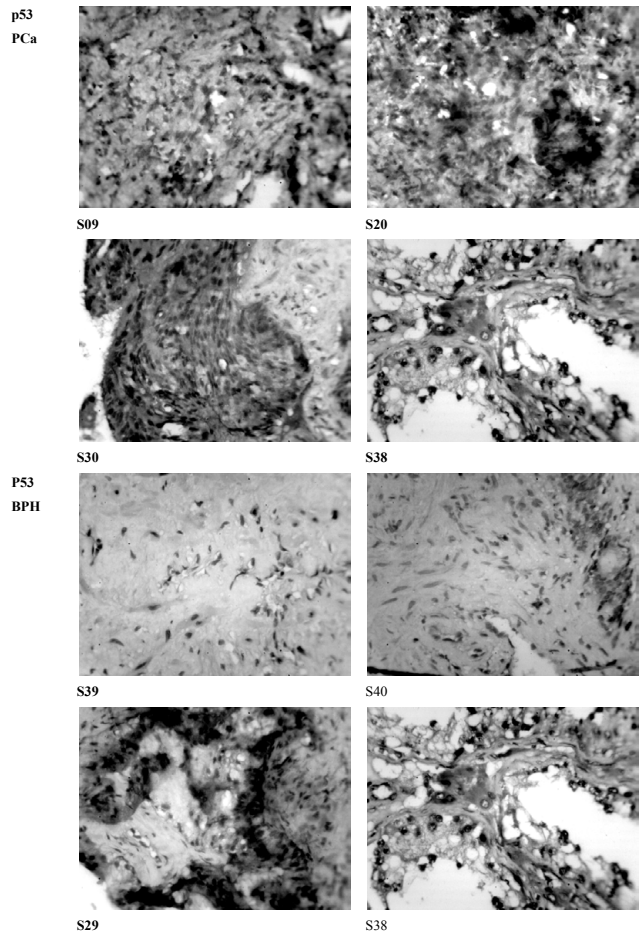

Figure 2: Biopsies treated with anti-Human *p53* (Mab) in immunohistochemistry aimed at demonstrating the expression levels of *p53* in 4 *BPH* and 4 *PCa* biopsies. S38 represents the control having moderate positivity around the basement membrane. This can be likened to *p53* expression in regular cell division and is therefore tagged (-/+). In *PCa* biopsies, p53 expression was immunopositive (+) while it is highly immunonegative in *BPH* (-). Although few cells scattered at wide intervals showed the cytoplasmic inclusions. The biopsy tagged S29, although diagnosed as *BPH*, showed high expression levels of *p53* similar to that observed in clinically diagnosed *PCa* biopsies (S29/+).

PCa				BPH			
	P53	*Bax*	*CathD*		*P53*	*Bax*	*CathD*
S09	+	+	+	*S39*	-	+	±
S20	+	+	+	*S40*	-	+	±
S30	+	+	+	*S29*	+	+	±
S38 (C)	±	±	±	*S38 (C)*	±	±	±

Table 1: Expression levels of p53, CathD and Bax in PCa and BPH Biopsies.

for the over expression of p53 and Bax in the *PCa* biopsies (Figures 1, 2 and Table 1).

The role of lysosomal proteases has been suspected to be involved in malignancy of tumors in the prostate. The enzyme is a protease that caused degeneration of the intercellular matrix, thus facilitating the escape of cancerous cells [17]. This is not a wide spread occurrence in *PCa* as it is restricted to specific tissue sites (Figures 3, 4 and Table 1). The study uses anti-Human *Cath D* (Mab) to map the location of *Cath D* in *BPH* and *PCa* tissues. The BPH biopsies showed moderate Cathepsin D expression which is characteristic of cells found to be undergoing cell proliferation and migration in the basal region of the control testicular tissue (S38). Although, the expression of *CathD* is higher in the *PCa* biopsies, it does not necessarily imply malignancy rather it might be an implication of early onset malignancy. Certain invasive cancer cells also showed *CathD* over expression in isolated cell populations within the glandular tissue (Figure 4 *PCa)*. Kedia and co-workers [18,19] have reportedly observed *CathD* expression in the surface and cytoplasm of tumor cells invading glandular tissue and in single cells involving prostatic stoma. This is equally important in the determination of biological aggressiveness of prostate cancer. Therefore, the importance of CathD over expression is an important prognostic tool for distinguishing *PCa from BPH* in epitheliomas which cannot be isolated unless analyzed with other cell cycle markers like *Bax and p53* to further determine the role of cell cycle regulation in *BPH and PCa*. The reduced *p53 in BPH* is well understood, especially when co-analyzed with Bax as it implies increased senescence not due to tumorgenesis. This scenario describes a well-organized cell cycle but short timed to

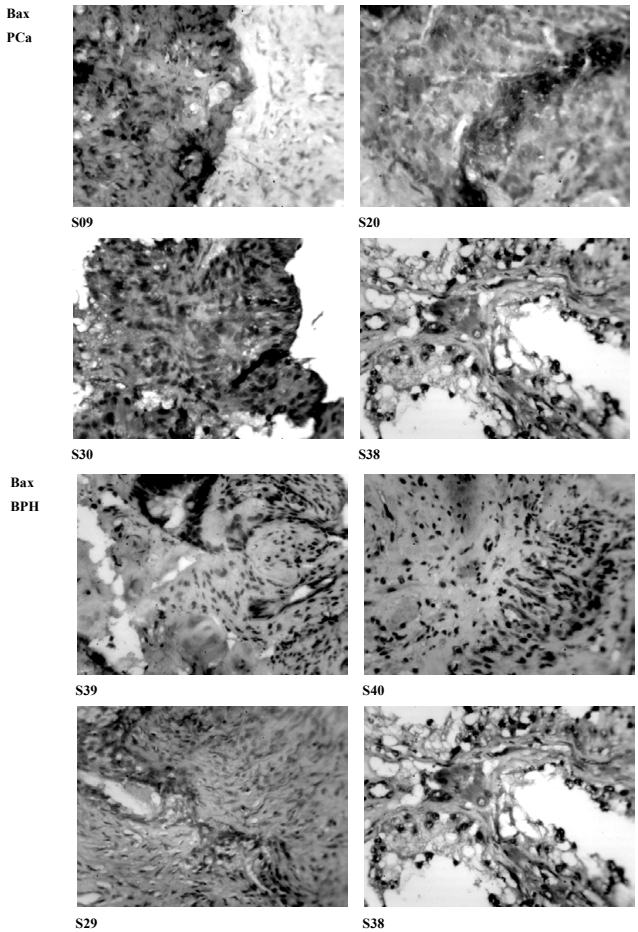

Figure 3: Immunohistochemistry of *BPH and PCa* biopsies using the antigen retrieval method to demonstrate the expression of Bax. This can be mapped against the distribution of *p53* which also shows strong positivity in *PCa* biopsies. The expression of Bax was also strongly immunopositive in BPH biopsies showing evidence of senescence. Comparing this against the *p53* expression levels in Figure 2 above, it shows that Bax play an important role in cell proliferation rather than cell cycle dysregulation. S 29 which showed high *p53* expression is also characterized by the highest *Bax* expression. Over expression of *Bax and P53* might be an evidence of apoptosis in this case rather than tumorgenesis (magnification X400).

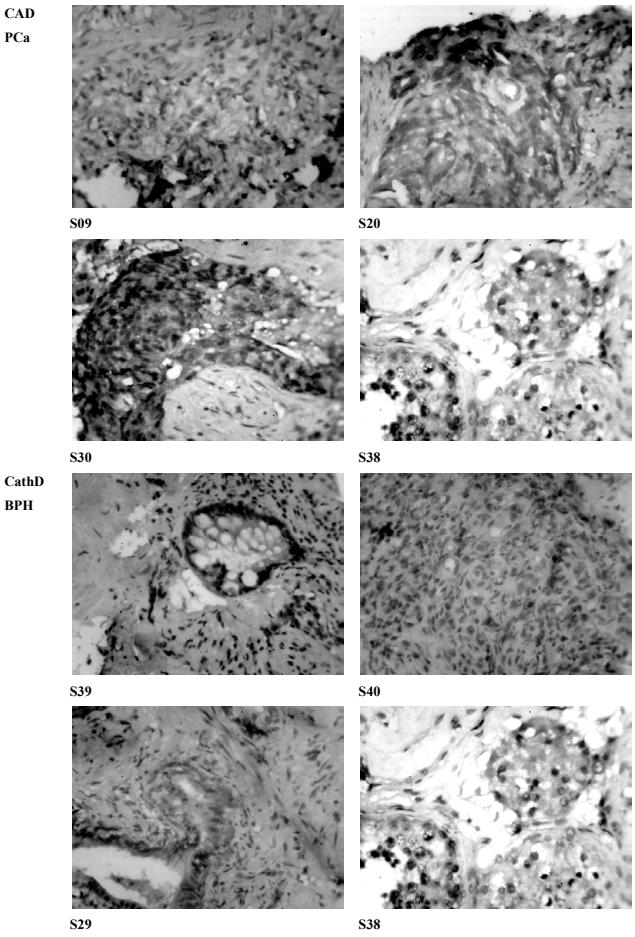

Figure 4: *Cathepsin D* immunohistochemistry demonstrating the specific locations of *CathD* expressing cells in the neoplastic tissue. The expression was restricted to specific tissue sites within the tumor invasion, the positive cell populations were observed sparse in between the glandular tissue also showing that *CathD* plays an important role malignancy. The immunopositivity of this protein was restricted to specific tissue sites especially close to the base of the epithelium. It is important to note that S29 gave the highest immunopositivity, the expression level is higher than that observed in S39 and S40 but not as high as that observed in the control S38 (magnification X400).

give numerous cells over a short period of time. Other studies involving the detection of epithelium protein *E Cadherin* shows intact epithelium with orderly arranged lamellae that is restricted to the fibromuscular epithelium. In PCa, the increased levels of *P53 and Bax* signals pre-apoptotic tendencies for rapidly proliferating un-coordinated cells which can be located at random locations due to loss of matrix and adhesion molecules described in high *CathD* levels. In conclusion, *p53, CathD and Bax* co-localization can be insightful to further determine the role cell cycle *in BPH and PCa* and in distinguishing the patterns of cell proliferation in both conditions.

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Conflict of Interest (COI) 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.

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