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 tumorigenic 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 tumorigenic 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 tumorigenesis 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)].

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/H2O2/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 literally 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 G0, (apoptosis). This state is achieved via the increase in 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...
The study uses anti-Human Cath D escape of cancerous cells [17]. This is not a wide spread occurrence caused degeneration of the intercellular matrix, thus facilitating the malignancy of tumors in the prostate. The enzyme is a protease that 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 Cath 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 Cath D 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 Cath D over expression in isolated cell populations within the glandular tissue (Figure 4 PCA). Kedia and co-workers [18,19] have reportedly observed Cath D 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 Cath D 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 tumorigenesis. This scenario describes a well-organized cell cycle but short timed to

| P53 | Bax | CathD | P53 | Bax | CathD |
|-----|-----|-------|-----|-----|-------|
| S09 | +   | +     | S39 | -   | +     |
| S29 | +   | +     | S40 | -   | +     |
| S30 | +   | +     | S29 | +   | +     |
| S29 (C) | ± | ± | S38 (C) | ± | ± |

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

![Figure 2: Biopsies treated with anti-Human p53 (Mab) in immunohistochemistry](image)

![Figure 3: Immunochemistry of BPH and PCA biopsies using the antigen retrieval method](image)
give numerous cells over a short period of time. Other studies involving the detection of epithelial 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 and in distinguishing the patterns of apoptotic tendencies for rapidly proliferating un-coordinated cells with orderly arranged lamellae that is restricted to the fibromuscular 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).

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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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