



Vitamin D Receptor Immunoexpression in Benign and Malignant Prostate Tumors

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Abstract

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BACKGROUND: Prostate cancer is a major health concern of men. Vitamin D can modulate innate or adaptive immune responses. The anticancer and anti-inflammatory effects of Vitamin D are mediated through gene transcription by Vitamin D receptor (VDR), including angiogenesis and tumor cell invasion. Targeted therapy in carcinoma prostate such as is as calcitriol is an alternative therapy for better treatment outcome.

METHODS AND MATERIALS: We investigated VDR immunoexpression in benign prostatic hyperplasia (BPH) and prostate adenocarcinoma. The cross-sectional study, categorical analysis of 60 paraffin blocks consist of 30 samples diagnosed as BPH, and 30 samples of prostate adenocarcinoma were divided three grading groups based on Patterns of Gleason: low, moderate, and high grade. Immunostaining was used to evaluate the VDR immunoexpression by histoscore.

RESULTS: The results showed that strong expression of VDR was 40% in BPH and 33.33% in prostate adenocarcinoma ($p = 0.961$). The strong expression of VDR in low, moderate, and high grade was 10%, 3.33%, and 20%, respectively. Statistically, there is no significant different ($p = 0.906$).

CONCLUSION: Positive immunoexpression of VDR affected the differentiation of prostatic adenocarcinoma.

Introduction

Prostate cancer (Pca) is the second common malignancy of men in the world and is the fifth leading cause of death, an estimated 307,000 deaths in 2012 [1]. In the United States, Pca mortality rates are increasing; in 2013, it was estimated 29,720 men died and in 2014, there will be an estimated 585,720 cancer deaths [2]. Physiologically, men who have age more than 50 years old usually occurred enlargement of the prostate, but not all prostate enlargement is Pca. Screening should be done by ultrasonography and prostatic specific antigen examination to determine whether the condition is benign prostatic hyperplasia (BPH) or Pca [3].

Recently, the role of calcitriol as a target therapy for cancer is often discussed. Target therapy is a specific therapy to inhibit factors that are considered as a cause of carcinogenesis process. Calcitriol is the most active of metabolite Vitamin D [4]. Calcitriol *in vitro* and *in vivo* experiments indicated synergistically with chemotherapy agents. Vitamin D is one of the hormones associated with a lower risk of several types of cancer, including Pca [5].

The prostate gland is an organ that has Vitamin D receptors (VDR) and responds to 1.25 (OH) 2D. In addition, these tissues and cells express the enzyme

25-hydroxyvitamin D-1-hydroxylase which converts 25 (OH) D to 1.25 (OH) 2D. The active form of Vitamin D has been shown to have a strong cell regulation effect in cells besides also being involved in calcium homeostasis. These effects are mediated through VDR. The binding of VDR by 1.25 (OH) D causes several cellular effects, including induced differentiation and apoptosis and inhibited proliferation, angiogenesis, and metastatic potential. Thus, Vitamin D is believed to play an important role in the etiology and treatment of cancer [6], [7].

VDR expression can be over-expression or under-expression (suppressed) in various types of cancer. Over-expression of VDR indicates a large number of VDRs so that it can bind calcitriol. The identification of VDR in tumor tissue by immunohistochemical examination is a good method and is a modality for the development of target therapy [8]. Therefore, this study was conducted to the detected expression of VDR in BPH and prostate adenocarcinoma.

Methods

The sample was a paraffin block from patients diagnosed with BPH and prostate adenocarcinomas at

Grand Medistra Hospital, Lubuk Pakam, Medan. The inclusion criteria were cases diagnosed according to the WHO 2016 [9]. Exclusion criteria were damaged by paraffin blocks and samples that could not be daubed with hematoxylin-eosin and immunohistochemical VDR. Examination of immunohistochemical VDR is carried out at Anatomic Pathology Laboratory, Universitas Sumatera Utara.

This study consisted of two groups, namely, benign and malignant prostate, each with 30 samples. Thus, the malignant group divided three grades; *low* (grade 1 and 2), *moderate* (grade 3), and *high* (grade 4 and 5) according to the WHO criteria. This study was a descriptive-analytic with a cross-sectional approach. Permission and approval were obtained from the Medical Ethics Committee Faculty of Medicine, Universitas Sumatera Utara.

The VDR immunoreactive tumor cells against appearance immunohistochemicals that use antibodies VDR. The assessment of VDR expression is done by the observed intensity and immunohistochemical outward distribution. Primary antibodies used are mouse anti-human VDR (D-6) antibody monoclonal (Medaysis) with a dilution of 1:500 in solution PBS. Intensity consists of negatives (score 0), weak (score 1), moderate (score 2), and strong (score 3). Distribution assessment is done with divide large field into four equal fields as a 25% distribution. Percentage of cells in all four fields then added and obtained into total VDR expression into one large field. The distribution level determined as 0=negative, 1≤10%, 2=10-50% and 3=50%. Based on the intensity value and distribution of VDR, expressions can be calculated histoscore (Intensity × Distribution), that is, weak (1–3), moderate (4–6), and strong (7–9) [10], [11].

Results and Discussion

The sample used is a paraffin block of the 60 patients included in this study (Table 1). The patient with age >70 years old more than age <70 years old. The mean age at diagnosis in this study was 69.2 years, and the youngest patient was 44 years. This is in line with studies done by Gurumurthy *et al.* and Jackson *et al.* [12], [13]. These findings confirm that Pca is a

Table 1: Characteristic clinicopathology of sample

Variable	n (%)
Age	
Median	70
Range	69.2
Benign prostatic hyperplasia	30 (50)
Prostate adenocarcinoma	30 (50)
Low grade	9 (30)
Moderate grade	4 (4.3)
High grade	17 (56.7)
Total sample	60 (100)

disease of elderly men, although young men are not excluded from the study.

Based on the results of immunoreactive analysis showed positively strong VDR immunoreactive expression in BPH groups which were 12 cases (40%), whereas the adenocarcinoma groups were 10 cases (33.3%) (Table 2). Statistically, analysis showed not significant correlation $p = 0.961$.

Table 2: Results of VDR immunoreactive expression in benign and malignant prostate tumors

Immunoreactive expression of VDR	BPH		Prostate adenocarcinoma		Total		p
	N	(%)	N	(%)	n	(%)	
Weak	5	16.7	5	16.7	10	16.7	0.961
Moderate	13	43.3	15	50	28	46.7	
Strong	12	40	10	33.3	22	36.6	
Total	30	100	30	100	60	100	

BPH: Benign Prostatic Hyperplasia.

The results of immunoreactive VDR with the degree of differentiation of prostate adenocarcinoma are shown in Table 3. The data showed low-grade groups give results positively strong VDR immunoreactive expression of three cases (10%), whereas high grade was 6 cases (20%) with $p = 0.906$. Totally, results showed that in all groups (low, moderate, and high), expression VDR in positively moderate was higher than positively strong. This study comparable with studies done by Hendrickson *et al.* showed that protein expression of VDR was highest with Gleason 3+3 tumors and decreased with increasing pathologic Gleason score ($p < 0.001$) [5].

Table 3: Results of VDR immunoreactive expression in prostate adenocarcinoma

Immunoreactive expression of VDR	Prostate adenocarcinoma						Total	p
	Low grade		Moderate grade		High grade			
	n	%	N	%	n	%		
Weak	1	3.3	0	0	4	13.3	5	0.906
Moderate	5	16.7	3	1	7	23.4	15	
Strong	3	10	1	3.3	6	20	10	
Total	9	30	4	4.3	17	56.7	30	

VDR tumor staining was evident intracellular in the cytoplasm, membrane cell, and nuclear [14]. There was considerable variability in staining of VDR across the BPH and prostate adenocarcinoma (Figure 1). The mechanism of VDR affected differentiation can be through genomic pathways in the nucleus that is Vitamin D or, in its active form, calcitriol binds to VDR forming heterodimers with retinoic X receptors (RXR) and with its ligands (nine cisretinoic acids). The dimer is forming complex with Vitamin D response-related elements with gene transcription to produce differentiation function [15], [16]. VDR also has nongenomic pathway that is in the cytoplasm with the mechanism of rapid action VDR binding to calcitriol on the VDR membrane in caveolae plasma membrane. Binding VDR with calcitriol on the plasma membrane activated several second messengers system.

The results of this study indicate that there is no correlation between VDR immunoreactive expression with benign and malignant prostate tumors. Expression VDR in positively moderate was higher than positively strong in benign and malignant prostate tumors, which may

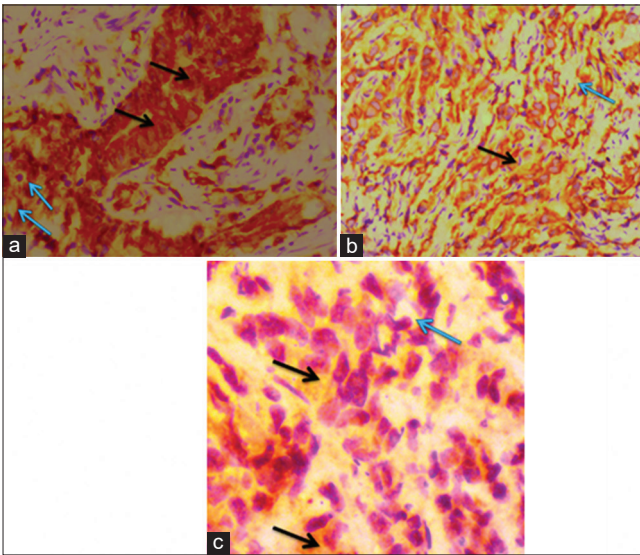


Figure 1: Overview of Vitamin D receptor immunoeexpression. Strong intensity, cytoplasm (black arrow), and tumor cell membrane (blue arrow) dark brown shown in figure (a), while moderate-intensity, cytoplasm, and tumor cells are brown in color younger than the figure (a) is shown in figure (b). Weak intensity, cytoplasm, and tumor cell membrane light brown shown in figure (c). Image was taken 400× magnification

be another factor that influences; further, the study is needed. However, VDR correlates with the high-grade group may be the administration of Vitamin D target therapy or in its active form; calcitriol can be given to the high-grade group.

Conclusion

Positive immunoeexpression of VDR affected the differentiation of benign and malignant prostate tumors.

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