

Gene Expression of P53 and Bcl-2 as Markers of Prostate Cancer Progression

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Abstract

Introduction: Apoptotic genes regulate apoptosis by the action of their pro- and antiapoptotic products. Among the most important proteins are p53 and Bcl-2 proteins. The rate of tumor growth is dependent on the balance between proliferation and apoptosis at all stages of carcinogenesis. The differential expression of these apoptotic genes was analyzed in relation to clinicopathological criteria as Gleason Score in man with Prostate Cancer. Every prostate cancer patient who underwent histopathology examination which fulfill the criteria were included in the study. Samples then rearrange into low Gleason Score (2-6) and high Gleason Score (7-10). All samples then underwent gene expression assay for p53 and bcl-2 to be analyzed for each's relationship with each group. The aim of this study was to evaluate tumor proliferation and the expression of the proto-oncogene Bcl-2 and tumor suppressor gene p53 in patients undergoing transurethral prostatectomy for prostate cancer, and to define their prognostic roles through correlation with final Gleason score, using qRTPCR.. A total of 40 samples consisting of 28 GS (2-6) and 12 GS (7-10) samples were included in the study. There was a significant mean difference between p53 mRNA in the GS 2-6 and 7-10 groups ($p < 0.001$), but not for bcl-2 mRNA. Based on a multivariate analysis, only p53 mRNA had a significant association with the GS group in Adenocarcinoma Prostate with $p = 0.014$, 95% CI 0.926-0.991 with a predicted value of 77.5%. We conclude that p53 mRNA is an independent predictive factor of Gleason Score-based progression of adenocarcinoma Prostate.

Keywords: Prostate cancer; p53; bcl-2; gene expression; gleason score.

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1. Introduction

Mutated p53 is one of the most common genetic abnormalities detected in human cancers. As a major tumor suppressor gene, p53 regulates the cell cycle, controls DNA repair mechanisms and activates apoptotic pathways [1]. The clinical correlates of p53 mutations malignancies have not been fully delineated but have shown that p53 mutation accelerates cancer progression and increases tumor invasiveness and metastasis, which is not, however, the entire picture [2,3]. Translocation the Bcl-2 oncogene on chromosome 18, giving rise to activation of the Bcl-2 gene, with increased production of mRNA and protein [4]. Bcl-2 is an oncogene with anti-apoptotic activity. It has been related to androgen independent prostate adenocarcinomas [5]. Proliferative activity tends to be low in prostate cancer and higher rates have been related to higher tumor stage and disease-specific survival [6]. The activity of proliferating cell nuclear antigen has been evaluated in a number of tumors and has been related to prognosis [7].

The diagnosis of prostatic carcinoma is based on histopathological examination of tissue obtained from the prostate gland. A key feature in a histopathological report of cancer is the grading of the aggressiveness. For prostatic carcinoma, the most widely used method for this grading was developed by Donald Gleason, and is known as the Gleason scoring system [8,9]. The Gleason grading system remains one of the important prognostic and predicting factors for prostate carcinoma [10]. More recently, the emergence of molecular medicine has resulted in the increased use of techniques able to quantitate levels of RNA in clinical diagnostics [11]. Quantitative real-time PCR (qPCR) has become a useful tool for validation and reduction of data produced from microarray studies [12,13].

Many PCa biomarker studies employ microarrays to identify profiles of the disease and its progression [14,15,16]. The aim of this study was to evaluate tumor proliferation and the expression of the proto-oncogen Bcl-2 and tumor suppressor gene p53 in patients undergoing transurethral prostatectomy for prostate carcinoma, and to define their prognostic roles through correlation with final Gleason score, using qRT-PCR.

2. Material and Method

Forty patients who were treated by transurethral resection of the prostate in 2015 to 2016 for adenocarcinoma prostate were analyzed according to histopathological standard methods. Fixation of tissue sample using normal buffer formalin 10%. Tissue preparation from FFPE as previous describe [17]. Total RNA extraction using QIA-symphony instrument with the RNeasy[®]FFPE Kit (Qiagen) following the instructions suggested by the manufacturer. Reverse transcription and quantitative real-time polymerase chain reaction (QRT-PCR) in one step using Rotor Gen Q (Qiagen) perform by adding 2µl total RNA to Master Mix (Qiagen) (15 µl) consisting of the following components: 5.0 µl of 5 reaction buffer, 0.5 µl of dNTP, 0.1 µl of M-MLV reverse transcriptase, (Moloney Murine Leukemia Virus reverse transcriptase; 200 U/µl; USB), and 9.4 µl of dH₂O. 10 µl of SYBR Green (Qiagen), 1.5 µl of primers (see table 1). The housekeeping genes like GAPDH should be used as controls for normalization (see table 1). Running amplification as described previously [18]. Analysis procedures supported by Rotor-Gene Q software.

Table 1: The primer used for reverse transcription

Gen	Primer
p53	F5'GAG CTG AAT GAG GCC TTGG A3' R : 5'CTG AGT CAG GCC CTT CCG TCT T3'
bcl-2	F : 5'TTG TGG CCT TCT TTG AGT TCG GTG3' R : 5'GGT GCC GGT TCA GGT ACT CAG TCA3'
GAPGH	F : 5'TGA TGA CAT CAA GAA GGT GGT GAA3' R : 5'TTC TTG GAG GCC ATG TGG GCC AT3'

3. Result

In 40 sample divided into two groups, low Gleason score (2-6) is 28 (70%) and high Gleason score (7-10) is 12(30%). Median age in low Gleason score is 70 years and high Gleason score is 67.5 years. Median PSA before TURP in low Gs is 12,15 ng% and high Gleason score is 12,05 ng%. Most of clinical staging tumor is T1c. Mean of mRNA Bcl-2 expression in low Gleason score is 80.34 ± 117.60 and in high Gleason score is 31.01 ± 23.48 .

Bivariate analysis mRNA Bcl-2 correlate not significant to Gleason score although the trend is rising in high Gleason score. Mean of mRNA P53 expression in low Gleason score is 243.52 ± 490.52 and in high Gleason score is 20.47 ± 24.08 . Bivariate analysis of mRNA P53 and Gleason score refer to significant correlation (p value < 0,001).

Table 2: Sample Characteristic

Variable	Group	
	GS 2-6 N=28	GS 7-10 N=12
Age		
Median	70	67.5
Min-Max	60-82	57-78
PSA		
Median	12.15	12.05
Min-max	4.5-100	5.4-76.4
Tumor		
T1c	13 (46.4%)	6 (50%)
T2a	2 (7.1%)	1 (8.3%)
T2b	5 (55.6%)	4 33.3%)
T2c	5 (17.9%)	0 (0%)
T3a	0 (0%)	1 (8.3%)
T4	3 (10.7%)	0 (0%)

Table 3: Bivariate analysis between mRNA p53 and low GS (2-6) and high GS (7-10)

Variable	Group		P Value
	GS 2-6	GS 7-10	
	N=28	N=12	
mRNA p53	243.52 ± 490.52	20.47 ± 24.08	<0.001*

Multivariate analysis mRNA Bcl-2 and mRNAP53 result only P53 has significant correlation to Gleason score (p value < 0,014).

Table 4: Multivariate analysis mRNA P53, Bcl-2 and Gleason score

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1 ^a	Age	-0.017	0.088	0.037	1	0.847	0.983	0.828	1.167
	mRNAP53	-0.038	0.018	4.214	1	0.040	0.963	0.929	0.998
	mRNABcl2	-0.007	0.008	0.836	1	0.361	0.993	0.977	1.008
	Constant	2.454	5.970	0.169	1	0.681	11.631		
Step 2 ^a	mRNAP53	-0.039	0.018	4.697	1	0.030	0.962	0.929	0.996
	mRNABcl2	-0.007	0.008	0.807	1	0.369	0.993	0.977	1.009
	Constant	1.310	0.715	3.354	1	0.067	3.705		
Step 3 ^a	mRNAP53	-0.043	0.017	6.027	1	0.014	0.958	0.926	0.991
	Constant	1.098	0.651	2.841	1	0.092	2.997		

4. Discussion

PCa is one of the malignancies with the highest frequency of genetic variations [19]. Renan has calculated a probability of 12 mutated genes from epidemiological data [20]. It has been suggested that a subset of mutant alleles acquired by a sub clone of tumor cells early in tumorigenesis leads not only to a selected replication advantage, but also, later in tumorigenesis, to the ability to metastasize [21]. Clinical tumor stage, Gleason score, and pretherapy serum prostate-specific antigen (PSA) in descending order were independently associated with clinical or biochemical relapse of PCa [22]. mRNA P53 expression is an absolute value result from transcription promoter gen P53. It has been suggested that a subset of mutation and the protein caused apoptosis not function, cell survival and DNA repaired not occurred. The cancer cell become progressive and prognosis is worse, cancer undergo metastasis and castration resistant. Although not only apoptosis has a role, perhaps there is multiple mutation follow the first mutation [23,24]. The antiapoptotic protein Bcl-2 is overexpressed in about

half of all prostate cancers, particularly in androgen-independent cases [25]. Second factor in diminished apoptosis in prostate cancers is Bcl-2. Bcl-2 is not expressed in normal secretory epithelial cells of the prostate, but starting from PIN, Bcl-2 is frequently expressed throughout the epithelium [25]. Interestingly, in the androgen-dependent LNCaP prostate carcinoma cell line Bcl-2 expression is androgen-dependent [26].

TP53 tumor suppressor gene is one of the most frequently mutated genes in human malignancies [27]. However, the mutation frequency of TP53 in PCa has a low level of about 30% [28]. Furthermore, the TP53 mutation frequency in prostate tumor tissue does not show a significant rising level in correlation with rising tumor grading and staging, as for example in bladder cancer. Mutations of TP53 influence the activation of cell proliferation and suppression of DNA repair, and apoptosis [29]. Therefore, an acceleration of tumor progression by TP53 mutations was claimed [30,31]. Kuczyk and his colleagues have described a correlation between overexpression of p53 protein and tumor progression in PCa patients during univariate analysis. P53 overexpression, histological grading, and tumor stage were significant prognostic factors for survival, among which only P53 overexpression remained an independent significant predictor in multivariate analysis [32].

In this study gen P53 expression, histopathological Gleason score during univariate and multivariate analysis were significant correlated.

5. Limitations

Although the research has reached its aims, there were some unavoidable limitations. First, because of the time limit, this research was conducted only on a small size of population who underwent TURP surgery. As mentioned before, the median age was 70 years old. And on that age, the amount of patient who were medically eligible for the TURP operation was considerably low. Another challenge faced was the level of education each patient had. In terms of understanding, the majority of our patients were below average. Therefore, their misguided concern about patient's safety discouraged our patients and their relatives to be enlisted in the experiment.

6. Conclusion

It was found that P53 gene expression was significantly correlated with the progression of prostate cancer.

7. Recommendation

With that result, P53 gene expression can be used as an independent prognostic factor for prostate cancer.

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