Prognostic significance of tumor suppressor protein p53 in prostate cancer

Valeur pronostique de la protéine p53 dans le cancer de la prostate

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Abstract

Backround: The p53 gene mutation is one of the most common genetic alterations in many cancers. In prostate cancer (PCa), it has been associated with a poor prognosis, tumor progression and aggressiveness. P53 mutation induces an abnormal protein expression in related tissues.
Aim: This study aimed to assess p53 expression using immunohistochemistry in PCa and to discuss its prognostic value.
Methods: We have retrospectively collected all cases of PCa diagnosed in our pathology department between 2012 and 2022. An automatized immunohistochemical analysis was performed using monoclonal p53 antibody. For each case, we assessed the proportion of positive cells and the intensity of staining. P53 expression was considered abnormal when it was totally negative or overexpressed (>=50% of positive cells).
Results: Twenty-four cases have been selected. Abnormal p53 expression was found in 42% of cases (P53 was overexpressed in 6cases and totally negative in 4 cases). Mean age of patients with p53 abnormal expression was 70years old. Patients with p53 abnormal expression had Gleason score >7 in 5 cases, ISUP grade >2 in 3 cases, peri-neural invasion in 8cases, capsule invasion in 9cases. All patients with p53 overexpression developed androgen resistance (p<0.01).
Conclusion: An aberrant expression profile of the p53 protein was observed in 42% of cases, and a statistically significant association was found with androgen resistance. Our results suggest a potential prognostic role of p53 in PCa.

Key words: prostate carcinoma, p53, immunohistochemistry

Résumé

Introduction: Les mutations du gène p53 sont parmi les anomalies moléculaires les plus fréquentes dans les cancers et notamment du cancer de la prostate et semblent être associées à un mauvais pronostic.
Objectif: Etudier l'expression de la protéine p53 par immunohistochimie et de discuter sa valeur pronostique.
Méthodes: Il s'agit d'une étude rétrospective descriptive ayant porté sur les cas d'adénocarcinome prostatique diagnostiqués dans notre service de 2012 à 2022. Une étude immunohistochimique automatisée a été effectuée à l'aide de l'anticorps anti-p53. Pour chaque cas, nous avons évalué le pourcentage de cellules marquées et l'intensité du marquage. Le profil d'expression était aberrant en absence totale d'expression ou en cas de surexpression (>=50% de noyaux marqués).
Résultats: Vingt-quatre cas ont été inclus. Un profil d'expression aberrant du p53 a été observé dans 42% des cas (surexpression dans 6cas, absence d'expression dans 4cas). L'âge moyen de ces patients était de 70ans. Parmi ces cas, le score de Gleason était >7 dans 5cas, le grade ISUP>2 dans 3 cas, une invasion péri-nerveuse dans 8cas et une invasion capsulaire dans 8cas. Tous ces patients ont développé une résistance aux androgènes (p<0.01).
Conclusion: Un profil d'expression aberrant de la protéine p53 a été observé dans 42% des cas et une association statistiquement significative a été retrouvée avec la résistance aux androgènes.

Mots clés: carcinome, prostate, p53, immunohistochimie

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Introduction

Prostate adenocarcinoma is a complex and multifactorial disease that continues to be a significant factor in morbidity and mortality; indeed it is the second most common malignancy and the fifth leading cause of death worldwide in men [1]. Although promising progress has been achieved in prostate carcinogenesis comprehension, there are insufficient data on the different pathways and biomarkers involved in prostate cancer development and progression. Among these biomarkers in particular, the prognostic significance of the well-known tumor suppressor gene p53 and the immunohistochemical (IHC) expression of the related protein are not clearly elucidated. Likewise, there is a lack of data regarding the association of p53 expression and resistance to hormonal therapy in prostate cancer. The lack of studies on this field raised the need to focus on p53 expression in prostate adenocarcinoma and to discuss its prognostic significance through its association to progression and treatment resistance.
Hence, in the present study, we aimed to assess the prognostic significance of p53 immunohistochemical abnormal expression in prostate adenocarcinoma and its relation to drug resistance.

Methods

Study design

The study was approved by the Biomedical Research Ethics Committee of our institution. We have retrospectively collected all cases of prostate adenocarcinoma diagnosed in our pathology department during a period of 10 years (October 2012– Januray 2022). The clinical data: age, levels of serum prostate-specific antigen (PSA) and patient outcome (recurrence, androgen resistance, death) were extracted from the patient's medical record. The pathological characteristics of the tumor: Gleason score, ISUP (International Society of Urological Pathology) grade, perineural invasion, and extra-prostatic extension were retrieved from the pathological reports. We have reviewed all the hematoxylin-eosin-stained sections for each case to select samples with sufficient tumor material. The tumor tissue in the corresponding formalin-fixed paraffin-embedded blocks was retrieved.

Immunohistochemical analysis

All cases were tested with p53 monoclonal antibody (LEICA; DO7) used at 1/100 dilution. Automated immunohistochemical technique was performed with an automated immunostainer (Leica Bond MAX), according to the manufacturer's protocol. Immunohistochemical staining was assessed by two pathologists as follows:

- Only nuclear staining was considered positive.
- The percentage of positive tumors cells was assessed semi-quantitatively.
- The intensity of the staining was evaluated: low-moderate, or high.
- P53 expression was considered normal (wild-type) when the staining was scattered and patchy.
- P53 was considered abnormal when it was either totally negative (protein loss) or over expressed (diffuse nuclear staining in ≥ 50% of tumor cells).

Statistical analysis

Qualitative variables were summarized using frequencies and percentages. Quantitative parameters were summarized using medians and standard deviation. We used Fisher test to assess the relation of p53 expression to the clinical and pathological parameters. p-value less than 5% was considered statically significant. All statistical analysis was performed with SPSS Statistics software (version 23).

Results

Twenty-four cases of PCa were included in this study. The mean age was70 years (varying from 55-87 years-old]. On digital rectal examination, the mean estimated prostate weight was 62.4 g (32-177g). 83.3% of patients presented with lower urinary tract symptoms suggestive of prostate disease. The consistency of the prostate gland was hard in 5/24 cases. 11/24 of specimens were biopsies. The mean PSA level of the patients at diagnosis was 38.02 ng/ml, ranging from 0.02 to 238 ng/ml. On pathological examination, the histological subtype was acinar adenocarcinoma in all cases, 62.5% of cases were of Gleason score <=7 and with an ISUP grade<=3. Poor prognostic factors were found in 9 cases. The follow-up period varied from 24 months to 10 years with a median of 4.4years. It was performed by clinical examination, prostate-specific antigen monitoring and imaging (ultrasound, computed tomography scan or MRI). PSA levels decreased in all cases except of one case, where it increased it decreased from 52 to 697ng/ml at 10years. The clinical and pathological characteristics of patients according to p53 expression are summarized in Table 1.
The overall survival rate at 5 years was 95.8% and the disease-free survival rate was 25% at 5years.
On Immunohistochemical analysis, positive staining for the p53 antibody was found in 75% (18/24) of cases. The staining was low in 11/24 cases, moderate in 5/24 cases and high in 2/24 cases. Abnormal p53 expression was found in 42% of cases. P53 overexpression was found in 16,7 % of all cases and protein loss was found in 25% of all cases (Figure 1-2). Clinical and pathological characteristics of patient's according to p53 expression are summarized in table 2.
All patients with abnormal 53 expression developed ADT resistance.
Patients with androgen resistance had abnormal p53 expression in 71.4%, the association was statistically significant ($p<0.01$).

Table 1. Clinical and pathological characteristics of the patients.

Characteristics	N (%)
Mean age	70years old (55-87years old)
PSA :	
• <4ng/ml	3 (12.5%)
• >4ng/ml	21 (87.5%)
Tumor Sample :	
• Biopsy	11
• Prostate Resection	6
• Radical Prostatecomy	3
• Prostate adenoma resection	4
Gleason	➢ 10 (n=1case) ➢ 9 (n= 4cases) ➢ 8(n= 4cases) ➢ 7(n= 9cases) ➢ 6 (n=6 cases)
ISUP	• 5(n=5cases) • 4(n=4cases) • 3(n=4cases) • 2(n=5cases) • 1(n=6cases)
Vascular Invasion	0
Perineural invasion	4
Capsule extension	5
Tumor necrosis	0
Follow-up	
• Androgen resistance	14
• Recurrence	3
• Death	1
• Uneventful	6

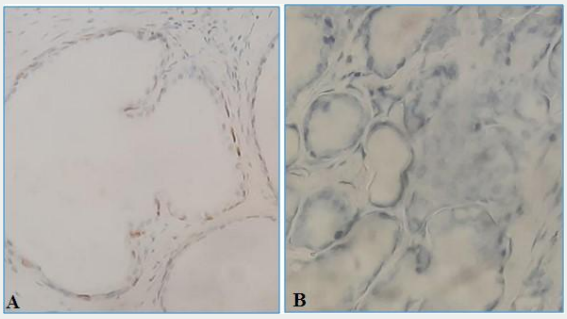

Figure 1. A: IHC x 40: p53 normal patchy staining **B**: IHC x 40: p53 absence of staining

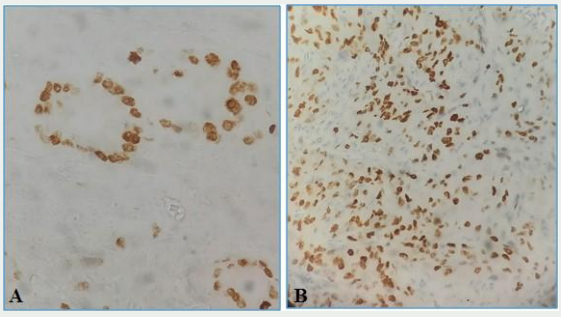

Figure 2. A: IHC x 40: p53 overexpression (50% of positive cells with moderate intensity) **B**: IHC x 40: p53 overexpression (80% of positive cells with high intensity)

Tableau 2. Clinical and pathological characteristics of patients based on p53 expression

Characteristics	P53 normal expression	P53 abnormal expression	P value
Mean age	67 years old	71 years old	0.28
PSA at diagnosis :			0.58
• <4ng/ml	3	0	
• >4ng/ml	11	10	
Gleason :			0.4
• *6-7*	10	5	
• *8-9-10*	4	5	
ISUP :			0.24
• 1-2	6	7	
• 3-4-5	8	3	
Perineural invasion :			1
• ***Positive***	2	2	
• ***Negative***	12	8	
Capsule extension :			0.36
• ***Positive***	4	1	
• ***Negative***	10	9	
Follow-up :			**<0.01**
• ***Recurrence***	3	1	
• ***Androgen resistance***	4	10	
• ***Death***	1	1	
• ***Unremarkable***	6		

Discussion

In the present study, abnormal p53 immunohistochemical expression was found in 42% of cases and the protein was assessed over-expressed in 16,7% of all cases. Indeed, it is well established that p53 is one of the most mutated genes in PCa, described in 50 to 70% of cases [4,5]. Many studies focused on p53 mutations in prostate adenocarcinoma since the '90s [6,7] with variable rates depending on the tumor stage, with the highest rates described in locally advanced and metastatic disease [4,8–11]. Previous studies, based on genome sequencing techniques, reported p53 mutations in metastatic PCa in 31 to 73% of cases [12–14] and in 28% to 36% in castration-naïve metastatic PCa [15–17]. These findings initially suggested that inactivation of p53 is a late event during PCa progression. However, there is an emerging evidence that p53 mutations tends to occur at early stages in primary tumor's subclones [18]. Previously, a p53 mutation rate of 8% in localized PCa was reported [15]. A more recent study reported that 17,6% of primary PCa harbored P53 mutation [19]. P53 alterations can be either protein-stabilizing missense mutations that lead to nuclear accumulation, or truncating mutations leading to total protein loss [15]. Strong nuclear positivity or complete absence by immunohistochemistry has a strong correlation with the presence of an underlying mutation. The abnormal produced protein, accumulated in the cells, can thus be detected using IHC suggesting a growing clinical potential interest for p53 IHC in early prostate cancer [20]. In the present study, an abnormal p53 expression was found in 41.6% of cases which is consistent with previously reported studies that described widely variable rate of p53 expression ranging from 1,1 % in localized tumors [21] to 54,7% in surgically treated PCa [22]. These disparities may be explained by

differences in the methodology of p53 immunostaining assessment [20]. Indeed, to date, no established consensus exists on evaluating p53 "overexpression" in PCa. While some authors only considered the cut-off of 5% of positive cells, others suggested that only marked p53 nuclear intensity should be considered regardless of the percentage of positive cells [20,23,24]. Additionally, nonsense/frameshift TP53 mutations can be hard to detect due to low expression of p53 protein [25]. Finally, this may be due to the differences in the techniques, studied specimens (one section or multiple cores of tumor tissue), patient's stage (primary tumors, metastasis). In the present study, interestingly, all patients with >50% of positive cells showed marked nuclear staining. However, it should be noted that the percentage of positive cells seems more reliable than the intensity of staining, which highly depends on the formalin fixation time, the immunohistochemical method and the antibody characteristics (clone, dilution). In a recent study, Guedes LB et al. [25] proposed a clinically and analytically validated immunohistochemical assay to detect p53 mutation. Accumulation of p53 was defined by a nuclear staining in over 10% of cells. P53 IHC showed high sensitivity for the detection of p53 missense mutations. Despite divergences in these findings, p53 IHC seems to be a reliable indicator for p53 mutations that needs to be standardized [21,23].

In the present study, patients with abnormal p53 expression were slightly older (71years old versus 67 years-old for patients with normal p53 expression). However, no statistically significant association was found between p53 expression and Gleason score or ISUP grade. Evidence of the prognostic value of p53 expression was mainly evaluated in gastric, breast and colorectal cancer [23], with less data in PCa. In their study, Visakorpi et al. [26] were the first to establish a strong correlation between p53 expression and high histological grade and cell proliferation rate. Subsequent studies also reported the correlation between p53 alterations with tumor grade, stage and biochemical relapse [21–24]. Notably, Gesztes et al. [23] found that high p53 expression is significantly associated with vascular invasion, and can thus predict distant metastasis occurrence. In other studies, p53 expression was a significant prognostic indicator of poorer overall survival and distant metastasis free survival [23,25,27]. In the present study, we didn't found association between p53 abnormal expression and the studied clinicopathological characteristics which could be related to the small sample size. However, p53 abnormal expression was significantly associated with androgen therapy resistance ($p<0.001$). This finding is consistent with the previously reported findings. Indeed, Robinson D et al. [28] also suggested that p53 mutations may be associated with resistance to ADT. Mutations of the p53 tumor suppressor gene is a common genetic alteration in prostate cancer and mostly cause changes in p53 protein conformation, leading to loss of p53 function which results in drug resistance [2]. Molecular studies in samples from patients with high grade Pca showed that these tumors are characterized by many alterations involving p53 leading to lineage plasticity and androgen indifference in murine prostate cancer models [29].In the study published by Hientz. K et al, the authors highlighted the role of p53 overexpression in chemotherapy and drug resistance in primary and metastatic PCa [30]. On the other hand, a recent study assessed p53 expression in patients treated with novel therapeutic options: Abiraterone and Enzalutamide [27], p53 accumulation was found to be significantly associated with poorer survival, suggesting that p53 mutations can be predictive of poor response to novel therapies.

Based on these findings, immunohistochemical assessment of p53 expression could have a potential role in predicting outcome, notably to predict response to therapy even in an early setting.

Additionally, p53 seems to be a relevant target for therapeutic intervention in PCa [31]. The first clinical trials were led in hormone refractory PCa, based the administration of mutant p53 targeting molecule APR-246 [32]. APR-246 seemed to be safe with good tolerance and showed biological and clinical effects. The clinical benefit of this molecule needs to be evaluated in further clinical studies.

The strength of this study is that the author's used an automated immunohistochemical technique which offers an objective and reproductible method to assess p53 expression in prostate cancer which is strongly associated with underlying p53 mutations. However, the small size of our sample is a major limitation of our study, but considering the promising results reported in our paper, further analysis on a larger scale is highly recommended.

Conclusions

Our study data revealed that abnormal immunohistochemical expression of p53 is found in 42% of prostatic carcinoma, with protein overexpression found in 16,7 % of cases.P53 abnormal expression was statistically significant associated to androgen therapy resistance. P53 IHC is a widely available tool to predict underlining p53 mutation. However, it is necessary to elaborate a consensus for a standardized immunohistochemical protocol for p53 IHC analysis on PCa. Our study suggests a potential clinical utility of p53 analysis to better risk stratification and to predict response to treatments. Studies on the potential role of p53 as a an effective anti-cancer target for PCa are highly recommended.

List of abbreviations:
PCa: prostate cancer
PSA: prostate-specific antigen
ISUP: International Society of Urological Pathology
IHC: Immunohistochemsitry
ADT: androgen therapy

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