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p63 is more sensitive and specific than 34βE12 to differentiate adenocarcinoma of prostate from cancer mimickers

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ABSTRACT

Objective(s): Prostate cancer is the world's leading cause of cancer and the second cause of cancer-related death in men after lung cancer. Differentiation of prostate adenocarcinoma from benign prostate lesions and hyperplasia sometimes cannot be done on the basis of morphologic findings. Considering the fact that in the prostate adenocarcinoma there is no basal cell layer, basal cell markers can help to differentiate prostate adenocarcinoma from cancer mimickers.
Materials and Methods: We studied 98 prostate biopsy blocks (40 adenocarcinoma and 58 benign lesions) for basal cell marker expression.
Results: p63 and 34βE12 were negative in all prostate adenocarcinoma specimens, but all benign prostate hyperplasia and high grade intraepithelial neoplasia cases expressed them.
Conclusion: Basal cell markers can help to distinguish prostate adenocarcinoma from cancer mimickers.

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Introduction

Prostate cancer is the world's leading cause of cancer and the second cause of cancer-related death in men after lung cancer. Cancer of the prostate is typically a disease of men over age 50 (1). The age adjusted incidence of prostate cancer in the United States is 69 per 100,000. The incidence of latent prostate cancer is even higher. It increases from 20% in men in their fifties to approximately 70% in men between the ages of 70 and 80.

Differentiation of prostate adenocarcinoma from benign prostate lesions and hyperplasia sometimes cannot be done on the sole basis of morphologic findings; In these cases the diagnosis can be made according to the presence or absence of the basal cell layer, considering the fact that in the prostate adenocarcinoma there is no basal cell layer but benign lesion encirclement by this layer. Hence, using basal cell immunohistochemistry markers including p63 and 34βE12 seems useful in distinguishing these two important categories of prostate lesions.

On the other hand, studies have shown that some adenocarcinomas show basal cell layer at least partially by p63 and 34βE12 staining.

As a few cases of adenocarcinoma mimickers and benign prostatic hyperplasia (BPH) don't express basal cell markers, we decided to determine and compare the sensitivity and specificity of these two markers to distinguish adenocarcinoma of prostate from its mimickers.

Materials and Methods

In this cross sectional study we had 98 prostate specimens in the Pathology Department, Ghaem Hospital, Mashhad, Iran that were referred for pathologic assessment. These specimens were collected between April 2009 and March 2010. Out of 98 cases; 40 cases were prostate adenocarcinomas and 58 were benign diseases (cancer mimickers) (Table 1).

Sampling procedures were different, including transurethral resection (TUR), needle biopsy and prostate adenectomy.

Biopsy specimens were fixed in 10% formalin. We reviewed the microscopic slides; confirmed Gleason score and grade in prostatic adenocarcinoma cases; and provided 4 µm slices from paraffin blocks. Immunohistochemical staining was perfor-

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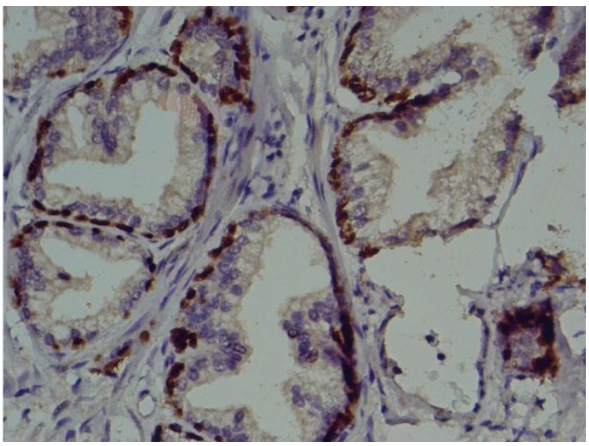

Figure 1. A benign prostate biopsy (BPH) stained with p63. The basal cells show moderate to severe nuclear staining. Nuclei of epithelial cells are negative for p63

Table 1. Frequency of pathologic lesions in prostate specimens

Diagnosis	Number
Adenocarcinoma	40
Benign prostatic hyperplasia	20
High grade prostatic intraepithelial neoplasia	10
Adenosis	12
Partial atrophy	16

med for basal cell markers, including high molecular weight cytokeratin (HMWCK) and p63 (DAKO company).

p63 antibody (Ab) was diluted to 1/100 concentration and 34βE12 Ab was ready to use.

We used two-step polymer method (Envision) for staining the slides. 34βE12 cytoplasmic stain and p63 nuclear stain were accepted as positive. We divided basal cell staining into three categories; <5%, 5–75%, and >75% (2).

Statistical analysis

Chi-square and Fisher's exact tests were used to compare the p63 and 34BE12 percentage and staining intensity data.

Results

All BPH cases were immunoreactive for p63 in more than 75% of the basal cells (sensitivity 100%) (Figure 1). Two out of 40 cases of prostate adenocarcinoma were excluded because of small limited foci. The remaining 38 cases had shown no p63 immunoreactivity (Figure 2).

All cases with high grade prostatic intraepithelial neoplasia (HGPIN) were immunoreactive for p63 in more than 75% of the cells. 8 out of 12 cases of adenosis had 5–75% p63 immunoreactivity. It was less than 5% in the remaining 4 cases.

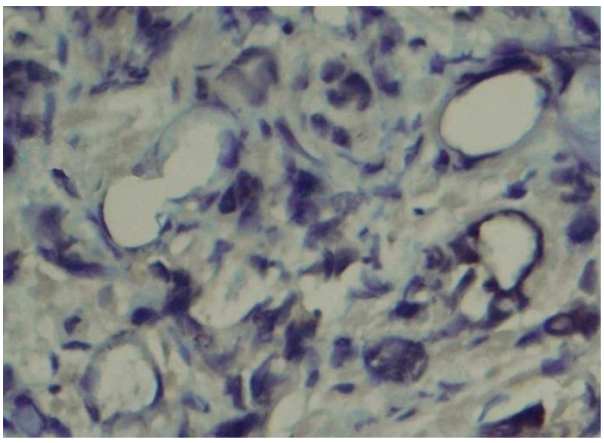

Figure 2. Prostate adenocarcinoma is negative for p63 immunostaining. There is no non-specific staining in cancer cells

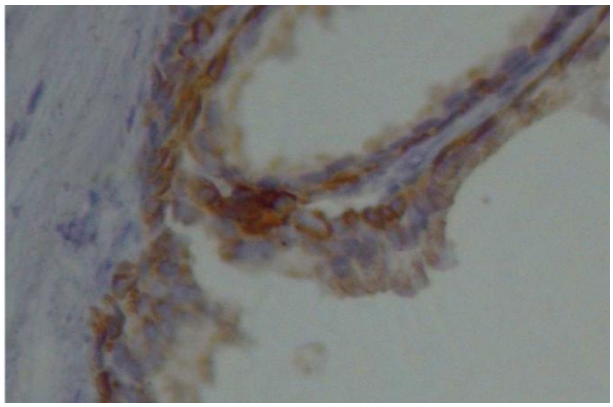

Figure 3. A benign prostate biopsy stained with 34BE12. The basal cells reveal cytoplasmic staining with moderate intensity. The epithelial cells show non-specific cytoplasmic staining with mild intensity

In 16 cases with partial atrophia, 6 cases showed p63 reactivity in 5–75% of the cells and 10 cases were reactive in less than 5% (Table 2).

In all BPH cases, basal cells showed immunoreactivity for 34βE12 in > 75% (Figure 3).

Two out of 40 cases of adenocarcinoma were excluded because of small limited foci; the remaining 38 cases were 34βE12 negative.

All 10 cases of HGPIN were immunoreactive for 34βE12 in >75%. 6 out of 12 cases of adenosis showed reactivity in 5–75% of their cells, 2 cases showed reactivity in <5%, and 2 cases did not show 34βE12 reactivity.

Table 2. p63Immunoreactivity in adenocarcinoma of prostate and cancer mimickers

Diagnosis	Number	p63 reactivity percentage				p63 reactivity intensity
		0%	<5%	5-75%	>75%	
Adenocarcinoma	40	38				
BPH	20				20	+++
HGPIN	10				10	+++
Adenosis	12		4	8		++
Partial atrophy	16		10	6		++/+++

BPH: Benign prostatic hyperplasia; HGPIN: High grade prostatic intraepithelial neoplasia

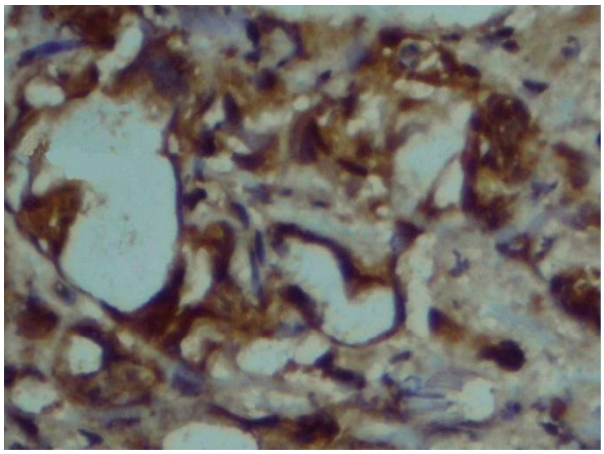

Figure 4. A prostate biopsy harboring cancer stained with 34BE12. The cancer cells show non-specific cytoplasmic staining with moderate intensity

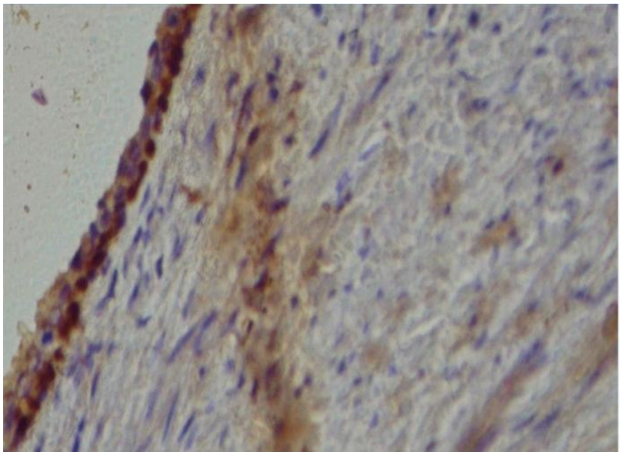

Figure 5. A benign prostate gland (atrophic gland) stained with 34BE12. Both basal cells and epithelial cells show immunoreactivity

Four out of 16 partial atrophy cases had 5–75% reactive cells, while 6 cases were reactive in <5% (partially staining) and 6 cases were negative for 34βE12 immunoreactivity (Table 3).

In 8 cases of BPH , 8 cases of adenocarcinoma, two cases of HGPIN , 4 cases of adenosis, and 8 cases of partial atrophy , the epithelial cell cytoplasms showed non-specific staining for 34βE12 with weak to moderate intensity (Figures 3–5).

Discussion

In 1984, Gown and Vogel reported for the first time that high molecular weight (HMW) anti keratin Ab stained prostatic basal cells specifically (3). Results of later studies showed that basal cells exist only in normal prostatic gland but not in prostatic adenocarcinoma (4–6). Wojno and Epstein showed that in highly suspicious for prostate cancer cases, the proof of basal cell absence by 34βE12 marker is very helpful to confirm prostate adenocarcinoma diagnosis (7).

Signoretti *et al* reported that all basal cells express p63, therefore, this marker can be useful in distinguishing benign lesions from prostate malignancy (8). Person *et al* showed that p63 isexpressed in normal basal cells and benign prostate hyperplasia (BPH). It is focally expressed in prostate atrophy and HGPIN, but there is no p63 expression in the majority of prostate adenocarcinomas (9). However, some studies showed p63 and 34βE12 negativity in some benign lesions such as adenosis, prostate atrophy, and HGPIN (2, 5, 10–15).

In this study, IHC staining for 34βE12 was negative in 4 cases with adenosis and 6 cases with partial atrophy, but they showed patchy positivity for p63. There are different reasons for unexpressed 34βE12, such as unexpressed 34βE12 gene, formalin fixation interval, and long term fixation which may result in 34βE12 Ag deficiency (16).

IHC techniques, especially Ag retrieval method has a role in 34βE12 expression detection (16, 17), but staining differences were more specific in transurethral prostate biopsy (TURP) (18).

Multhaupt *et al* reported that 88% of benign glands in the transitional zone lost 34BE12 antigenicity without Ag retrieval, in TURP samples (19).

In this study like others, adenocarcinoma were p63 and 34βE12 (basal cell markers) negative, while benign lesions expressed them in more than 75%. In some studies basal cell markers were focally positive in morphologically benign lesions such as BPH (13, 18).

In this study, specificity and sensitivity of p63 and 34βE12 were 100% in pure benign lesions and adenocarcinoma.

Our results showed p63 expression in 36 cancer mimicker cases; however, in some, p63 expressed patchy positivity. In some studies cancer mimickers, such as partial atrophy and adenosis, expressed p63 focally, which supports our findings (2).

Table 3.34βE12 Immunoreactivity in adenocarcinoma of prostate and cancer mimickers

Diagnosis	Number	34βeta E12 reactivity percentage				34βeta E12 Reactivity intensity
		0%	<5%	5-75%	>75%	
Adenocarcinoma	40	38				
BPH	20				20	+++
HGPIN	10				10	+++
Adenosis	12	4	2	6		-/++
Partial atrophy	16	6	6	4		-/++

BPH: Benign prostatic hyperplasia; HGPIN: High grade prostatic intraepithelial neoplasia

In the Wang *et al* study, 30% of partial atrophy cases were p63 and 34βE12 negative (cancer pattern) (2).

On the other hand, there are studies showing that p63 expression in some adenocarcinoma cases may be due to trapped benign glands between malignant cells (13, 20–24).

In the Shah *et al* study, 2 out of 27 partial atrophy cases were 34βE12 negative, but p63 was positive (18). In our study 10 out of 28 cases of cancer mimickers (25%) were 34βE12 negative but all (100%) were p63 positive. It seems that p63 is more specific than 34βE12 to distinguish prostate cancer mimickers from adenocarcinoma, which is clinically important.

There was a problem in 34βE12 staining, like in the Shah study (18). This problem was nonspecific epithelial cell staining in 8 (25%) BPH (Figure 3), 8 (21%) adenocarcinoma (Figure 4), and 14 (35%) cancer mimicker cases, which complicates diagnosis.

Other IHC markers, which preferentially are overexpressed in prostate cancer cells but not in basal cells, are α-methyl-acyl-co A (AMACR) (25–28) and recently introduced marker erythroblastosisvirus E26 oncogene (ERG), which can improve distinguishing benign epithelial lesions from prostate adenocarcinoma (18, 29–31).

Conclusion

Other than selection bias in sampling, the specificity and sensitivity of p63 and 34βE12 in true adenocarcinoma and benign lesions (BPH) are high, but in cancer mimickers, especially, when morphologic differentiation is impossible between benign and malignant lesions, p63 sensitivity is significantly higher than 34βE12.

Moreover, 34βE12 nonspecific staining in benign lesions is a problem in interpretation of the results; therefore, in our opinion, p63 is a better marker than 34βE12 for differentiation of benign lesions from prostate adenocarcinoma.

In clinical practice, combination of prostate cancer and basal cell markers is helpful for improved differentiation of prostate cancers from mimickers.

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