Journal of Cancer Research and Treatment, 2016, Vol. 4, No. 2, 21-25
Available online at http://pubs.sciepub.com/jcrt/4/2/1

DOI:10.12691/jcrt-4-2-1

Histone H1.5 Expression in Prostatic Carcinoma: An Immunohistochemical Study

Mohamed A. El-Rashidy[1], Asmaa E. Bedeer[1], Ahmed M. Kabel[2,3,*]

[1]Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
[2]Pharmacology department, Faculty of Medicine, Tanta University, Tanta, Egypt
[3]Department of clinical pharmacy, College of Pharmacy, Taif University, Taif, Saudi Arabia
*Corresponding author: drakabel@gmail.com

Abstract ***Background and Aim***: Histone H1.5 (HH1.5) is a subtype of histone H1, a family of linker proteins that is known to determine chromatin structure, alter gene expression and DNA repair. It also contributes to regulation of cell proliferation in breast cancer. In this study, we aimed to investigate the immunohistochemical expression of HH1.5 in various prostatic lesions. ***Methods:*** A total 50 cases of various prostatic biopsies were studied. Histone H1.5 expression was evaluated in all cases. HH1.5 expression was scored as negative (<11%), 1+ (11-50%), or 2+ (>50%). Correlations between the intensity and differential localization of these markers and Gleason patterns were evaluated. ***Results***: HH1.5 immunohistochemistry revealed positive nuclear reactivity in all cases (100%) of prostate adenocarcinomas, compared to only 2 (11%) of 18 cases of benign prostatic glands ($P \leq 0.001$). In all positive benign prostate epithelium, HH1.5 was limited to focal and weak reactivity. Similarly, both the two cases of high-grade prostatic intraepithelial neoplasia exhibited focal weak nuclear reactivity. Increased HH1.5 reactivity was observed in Gleason patterns 5 and 4 as compared to Gleason pattern 3, 100%, 64.7% and 50%, respectively ($P \leq 0.002$). ***Conclusion:*** HH1.5 may be a useful diagnostic tool in evaluating prostatic biopsies, particularly with small foci of cancer. Further studies are needed to support these findings and investigate the possible prognostic significance of HH1.5 in prostatic adenocarcinomas.

Keywords: *histone, expression, prostate, carcinoma*

Cite This Article: Mohamed A. El-Rashidy, Asmaa E. Bedeer, and Ahmed M. Kabel, "Histone H1.5 Expression in Prostatic Carcinoma: An Immunohistochemical Study." *Journal of Cancer Research and Treatment*, vol. 4, no. 2 (2016): 21-25. doi: 10.12691/jcrt-4-2-1.

1. Introduction

Prostate cancer is a growing public health problem worldwide. It is the fifth most common incident cancer in the world, one of the most commonly diagnosed male malignancies and the second leading cause of male cancer death in United States [1]. The incidence of prostate cancer is also rising each year, more than 30,000 men are diagnosed with prostate cancer every year [2].

Diagnosis of prostate cancer on core needle biopsy specimens can be challenging because of small foci of cancer or the presence of benign mimickers [3]. Immunohistochemical stains such as high–molecular weight keratin, p63, and α-methyl acyl-CoA racemase (AMACR) have greatly assisted in identifying prostate cancer [4], but they are not without limitations. The occasional absence of basal cells in partial atrophy and adenosis along with outpouching of high grade prostatic intraepithelial neoplasia (HPIN) may yield false-negative results with basal cell markers (high molecular weight keratin and p63) [4,5]. AMACR reactivity is absent in approximately 25%of prostatecancers and 36%of HPIN. Other limitations of AMACR include cytoplasmic staining, which can be ambiguous, and the lack of increased staining intensity with increasing Gleason pattern [5,6].

Histone H1.5 is a family of linker histone proteins that are located in the nucleus and play a role in stabilizing higher order chromatin structure, gene expression, DNA repair, and cell proliferation. There are 11 known subtypes, many with specific and multiple functions that are essential for cell survival [7]. Histone H1.5 (HH1.5) is one of the 7 somatic subtypes, which is expressed in a replication dependent manner and has been found to alter gene expression by regulating transcription through modification of chromatin structure [8]. In a recent study, Li et al [9] found that, in differentiated cells, HH1.5 binds to genes encoding membrane proteins that function in cell to cell communication. Depletion of HH1.5 in fibroblasts resulted in deregulation of genes and alteration of the cell cycle leading to decreased cell growth. Recently, HH1.5 had been immunohistochemically detected in several cancers, including atypical carcinoids and large and small cell neuroendocrine carcinomas [10].

In regards to prostatecancer, histone H1.0, which is a terminally differentiated subtype, showed immunohistochemical expression that is directly associated with Gleason pattern, cell proliferation, and androgen receptor expression [11].

The present study was undertaken to evaluate the immunohistochemical expression of HH1.5 in prostate adenocarcinoma in relation with Gleason pattern

(histological architectural classification of prostate cancer with prognostic feature), benign prostatic glands, and PIN as a potential diagnostic as well as prognostic tool.

2. Materials and Methods

2.1. Clinical and Pathological Data

This retrospective study was carried out in the Department of Pathology, faculty of medicine, Tanta University, Egypt. Cases were collected from archives between the years of 2008 and 2012. A total of 50 cases of prostatic biopsies were taken for study. Brief clinical data were noted from case records.

2.2. Histopathological Evaluation

All prostatic specimens were subjected to careful and detailed gross examination. Tissue sections were fixed in 10% formalin and embedded in paraffin and were used for microscopic study. Sections 4 to 5 µ thick were prepared and stained routinely with hematoxylin and eosin (H&E).

30 cases were prostate adenocarcinoma, 18cases of benign prostatic hyperplasia, and 2 PIN. The diagnosis and grading of prostate adenocarcinoma was made according to the 2005 International Society of Urological Pathology (ISUP) Consensus Conference(12).

2.3. Immunohistochemistry

Prior to immunostaining, epitope retrieval was performed by boiling slides with 10 mmol/L citrate buffer (pH 6) for 4minutes in a pressure cooker at 125°C with slow cooling. Charged slides with 5-µm-thick sections of tissue were incubated with a rabbit polyclonal antibody reactive against HH1.5 (Abcam). The HH1.5antibody was diluted at 1:800 using 5% goat serum in antibody diluent. After washing, tissue sections were treated with horseradish peroxidase (HRP) polymer detection (Thermo scientific). The color was developed with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) as substrate, then counterstained with Mayer's hematoxylin, dehydrated, and mounted. Positive controls consisted of tonsillar and lymph node tissue because lymphocytes consistently demonstrate nuclear expression of HH1.5

2.4. Evaluation of the Immunostaining Sections

HH1.5 immunohistochemical expression was assessed in prostatic adenocarcinoma, PIN, benign prostatic hyperplasia. Only nuclear staining was considered positiveand was scored based on the percentage of reactive nuclei within each individual gland or cluster of reactive glands and staining intensity as negative, 1+ and 2+ as follows [13]: – negative (<11% of sample); 1+ (moderate intensity 11-50% of sample); 2+ (strong intensity >50% of sample). Each Gleason pattern of prostatic adenocarcinoma was separately evaluated for HH1.5 reactivity.

2.5. Statistical Analysis

The results thus obtained were interpreted and correlated statistically. Comparison between multiple groups was made by using Student *t*-test, chi-square test. A value of $P<0.05$ was taken as significant and <0.01 was taken as highly significant, whereas *P*-values of >0.05 were taken as non-significant.

3. Results

A total of 50 cases were used in the present study, which included 18 cases (36%) of benign prostatic hyperplasia (BPH), 2 cases (4%) of prostatic intraepithelial neoplasia (PIN), and 30 cases (60%) of carcinoma. The majority of specimens were collected by needle biopsy (68%), followed by transurethral resection of the prostate (24%) and prostatectomy (8%). The age of the patients ranged from 46 to 95 years with a mean age of 71.3±10.16 years. The majority of cases (11 cases) were in the age group of 76–80 years, which formed 22% of the study group. Patients with carcinoma of the prostate were in the age group of 51–95 years with a mean age of 72.43+10.49 years.

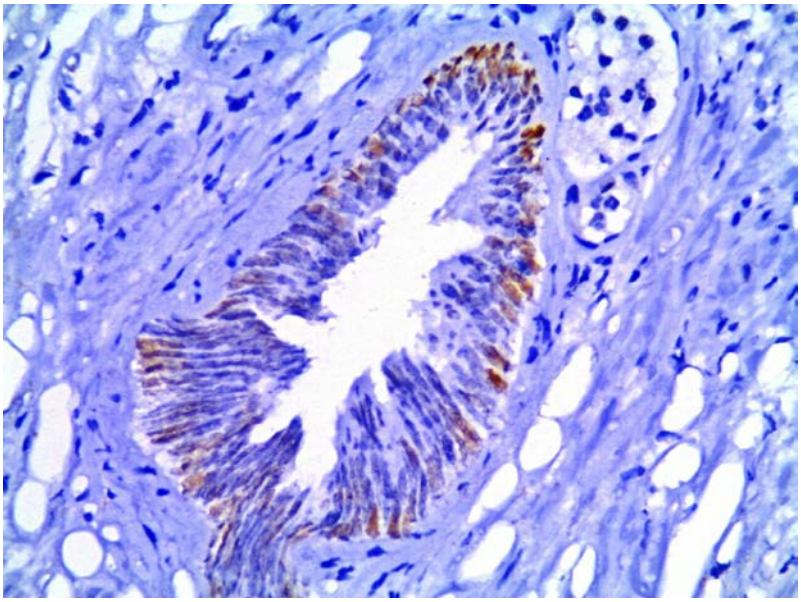


Figure 1. Benign prostatic hyperplasia demonstrating focal weak nuclear reactivity for HH1.5 (immunohistochemistry, original magnification ×400)

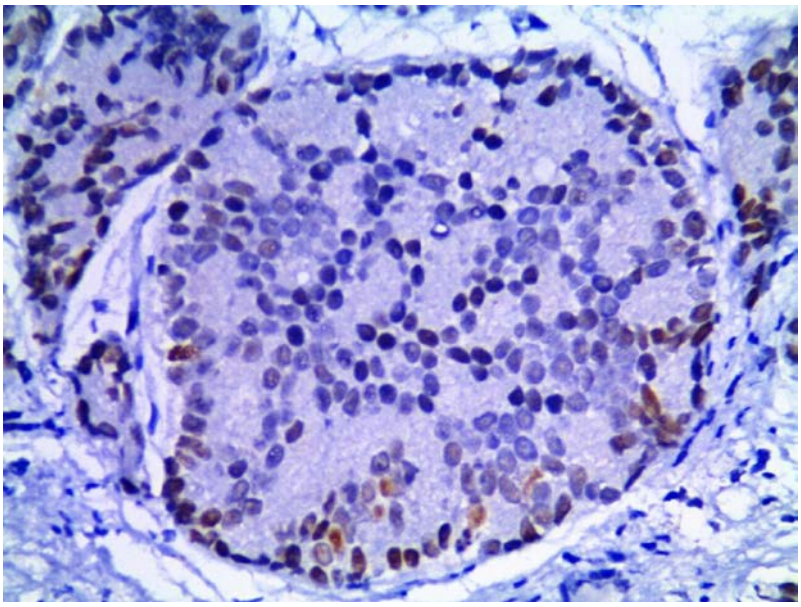
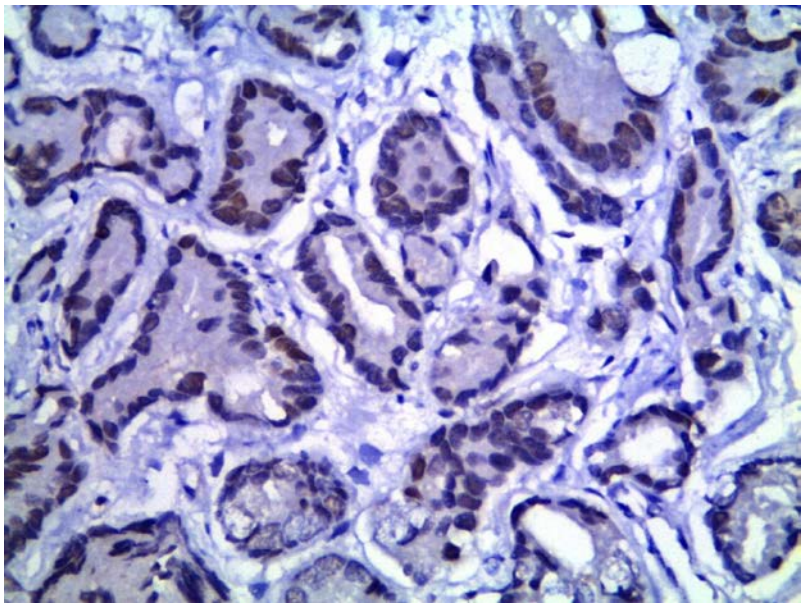
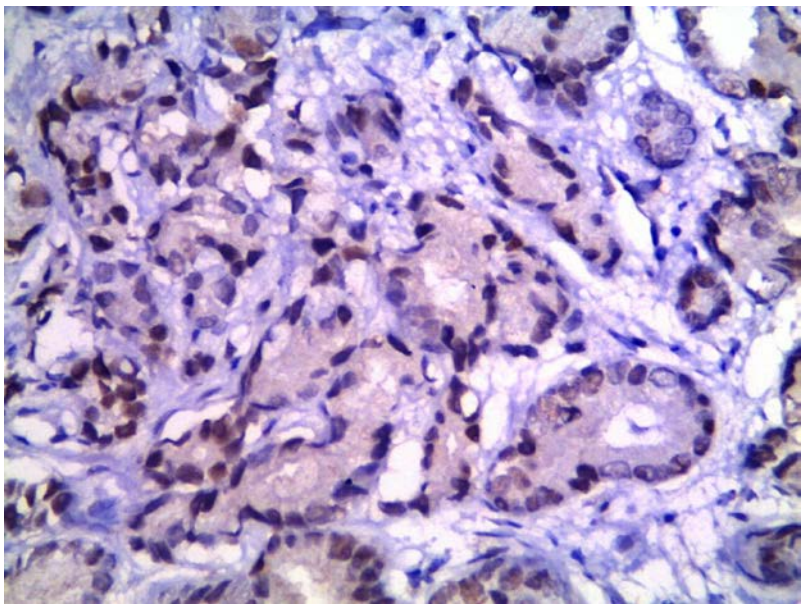


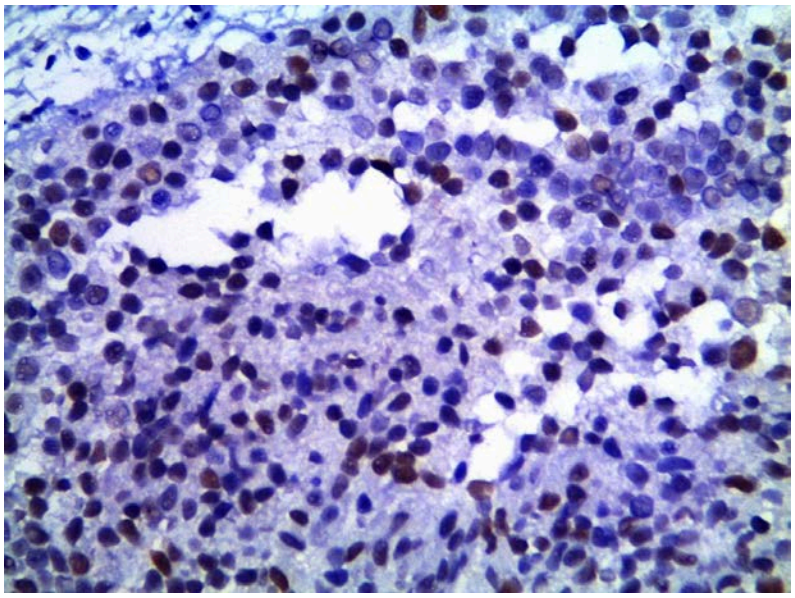
Figure 2. High-grade prostatic intraepithelial neoplasia demonstrating focal and predominantly weak nuclear reactivity for HH1.5 (immunohistochemistry, original magnification ×200)



Gleason pattern 3. Prostate adenocarcinoma showing nuclear expression of HH1.5 (immunohistochemistry, original magnification ×400)



Gleason pattern 4. Prostate adenocarcinoma exhibiting strong diffuse nuclear reactivity for HH1.5 (immunohistochemistry, original magnification ×400)



Gleason pattern 5. Diffuse and strong nuclear expression of HH1.5 in prostate adenocarcinoma (immunohistochemistry, original magnification ×400)

All foci of PIN were of high-grade PIN. Among the 30 cases of prostatic carcinoma 17 cases (56.66%) were Gleason pattern 4, 9 cases (30.0%) were Gleason pattern 5, and only 4 cases (13.33%) were Gleason pattern 3.

HH1.5 was expressed in only2 cases (11%) of BPH and showed focal weak positivity, whereas in PIN and carcinoma it was expressed in 100% of cases. There was a statistically significant difference in expression of HH1.5 between BPH and carcinoma cases, as indicated by a *P*-value of <0.001 (Table 1).

Both the two cases of PIN showed focal weak nuclear reactivity for HH1.5.Of the carcinoma cases, most of cases (22, or 73.3%) showed diffuse strong positivity and 8 cases (26.7%) showed diffuse moderate positivity for HH1.5 as seen in Table 2, and high expression of HH1.5 staining correlated with Gleason pattern (p<0.002).

Table 1. HH1.5 expression in benign glands, HPIN and prostatic carcinoma

HH1.5 expression	BPH (n=18)	HPIN (n=2)	Prostate Carcinoma (n=30)
Positive (34 cases)	2 (11%)	2 (100%)	30 (100%)
Negative (16 cases)	16 (89%)	0	0

P value <0.001 *.

Table 2. Correlation of HH1.5 expression with Gleason pattern in prostatic carcinoma

HH1.5 expression	Gleason pattern 3 (n=4)	Gleason pattern 4 (n=17)	Gleason pattern 5 (n=9)
Moderate (8 cases)	2 (50%)	6 (35.3%)	0
Strong (22 cases)	2 (50%)	11(64.7%)	9 (100%)

P value< 0.002*.

Increased HH1.5 reactivity was observed in higher-grade tumors (Gleason patterns 4, 5) compared to lower-grade tumors (Gleason pattern 3). HH1.5 expression did not correlate with age (p>0.05).

4. Discussion

Histone H1 is a family of linker histone proteins, consisting of 11 subtypes that interact with the DNA between nucleosome particles, their function appears to be of 2 major roles: a general one in forming and stabilizing higher order chromatin structures, which is shared by all subtypes, and a more subtype-specific function involving gene regulation [14]. The latter results from alteration of chromatin structure, DNA methylation, or direct interaction with transcription factors [14,15]. Recently, specific histone subtypes have been implicated in tumorigenesis [16].

HH1.5 is one of 7 somatic subtypes, which is present in allells but with varying expression levels, depending on the cell type and degree of differentiation [17]. Ongoing studies reveal that HH1.5 is not immunohistochemically expressed in all cells, nor is it specific for prostatecancer. Differential immunohistochemical expression ofHH1.5 has been reported in pulmonary neuroendocrine tumors, particularly those of higher grade [10]. Additionally, HH1.5 was one of several mutated genes discovered from sequencing of colorectal carcinoma [18]. This study demonstrates that immunohistochemical detection of HH1.5 is common in prostate cancer cells and rare in benign prostatic epithelium. The positive staining exhibited in rare benign prostatic glands was focal and weak.

Similarly, HPIN showed mostly focal weak reactivity for HH1.5 with occasional cells demonstrating moderate nuclear reactivity. Importantly, the staining observed in benign prostatic glands and HPIN was distinctly different from the strong nuclear reactivity observed in prostatic adenocarcinomas.

There were no cases with moderate-to-strong HH1.5 expression and less than11% involvement of prostatic glands. However, if such cases are encountered, it may be best to regard them as atypical or suspicious for prostatic adenocarcinoma and further work them up.

Interestingly, HH1.5 exhibited an overall increased immunoreactivity in high-grade Gleason patterns 4 and 5 prostate adenocarcinoma as compared to low-grade Gleason pattern 3 prostate cancers. These findings indicate not only a diagnostic but also a potential prognostic role of HH1.5, as the Gleason pattern is one of the major indicators of malignant potential of prostate cancers, often applied as a prognostic factor(11), and since histone H1 positivity was found to increase with increasing Gleason pattern in prostate cancer tissues, histone H1 expression

might also serve as a prognostic marker of prostate cancer. However, because all cases used in the present study were of relatively early stage (Pathologic stage pT2a or pT2b, pN0), no analysis of prognostic potential was possible.

Aside from HH1.5, histone H1, a terminally differentiated histone protein variant, has also been implicated in prostate and breast cancer [8,16]. Histone H1 immunohistochemical expression positively correlated with the Gleason grade, Ki-67 positivity, and androgen receptor expression in prostate cancer [16]. However, its expression in normal luminal cells, basal cells, and stromal cells precludes its diagnostic utility for prostate cancer [16].

Routine examination of prostate core needle biopsies for cancer consists of evaluating 3 main histological parameters: glandular architecture, cytologic features, and absence of a basal cell layer. The presence of the basal cell layer may be difficult to appreciate on routine hematoxylin and eosin sections. Immunohistochemical staining with high-molecular-weight cytokeratins (34βE12) and p63 can highlight the presence or absence of the basal cell layer [4].

Prostate cancer by definition lacks a basal cell layer. However, atypical adenomatous hyperplasia, atrophy, HPIN, and some morphologically benign prostatic glands may show partial or complete absence of basal cell staining [19,20,21,22]. Therefore, absence of basal cell staining in a subset of cases may lead to false diagnosis of malignancy.

AMACR is involved in β-oxidation of branched fatty acids and is overexpressed in prostate cancer [23]. It can be used as an immunohistochemical marker of prostate cancer, but it shows variable sensitivity and specificity [5,23,24].

Additionally, noncancerous lesions such as HPIN, atrophic glands, and AAH have been shown to be positive with AMACR in 64%, 36%, and 18%, respectively [5]. HPIN may show moderate to even strong staining with AMACR [6]. So AMACR immunohistochemical staining limitations may hinder a correct diagnosis in a subset of small foci of prostate cancer or in one of its benign mimickers.

5. Conclusion

This study used HH1.5 immunohistochemistry in distinguishing prostate cancer from benign prostatic glands, with sensitivity and specificity superior to those of AMACR, may prevent false-positive diagnoses . For small foci of prostate cancer, HH1.5 may be a useful tool in combination with hematoxylin and eosin and other immunological markers, particularly cytoplasmic staining with high–molecular weight cytokeratins. Further studies are still needed to confirm these findings and investigate the histoprognostic significance of HH1.5 in prostate cancer.

References

[1] Stein J, Majores M, Rohde M, Lim S, Schneider S, Perner S, Jung K, Kristiansen G and Kirfel J. KDM5C Is Overexpressed in Prostate Cancer and Is a Prognostic Marker for Prostate-Specific Antigen-Relapse Following Radical Prostatectomy .Am J Pathol 2014; 184: 2430-2437.

[2] Lu Z, Qi L, Bo XJ, Liu GD, Wang JM, and Li G. Expression of CD26 and CXCR4 in prostate carcinoma and its relationship with clinical parameters J Res Med Sci 2013; 18(8): 647–652.

[3] Epstein JI. Mimickers of prostatic intraepithelial neoplasia. Int J Surg Pathol 2010; 18:142S-8S.

[4] Hameed O, Sublett J, Humphrey PA. Immunohistochemical stains for p63 and alpha-methyl acyl-CoA racemase, versus a cocktail comprising both, in the diagnosis of prostatic carcinoma a comparison of the immunohistochemical staining of 430 foci in radical prostatectomy and needle biopsy tissues. Am J Surg Pathol 2005;29:579-87.

[5] Evans AJ. α-Methyl acyl CoA racemase (P504S): overview and potential uses in diagnostic pathology as applied to prostate needle biopsies. J Clin Pathol 2003; 56:892-7.

[6] Zhou M, Chinnaiyan AM, Kleer CG, Lucas PC, RubinMA. α-Methyl acyl CoA racemase: a novel tumor marker over-expressed in several human cancers and their precursor lesions. Am J Surg Pathol 2002; 26:926-31.

[7] Happel N, Doenecke D. Histone H1 and its isoforms: contribution to chromatin structure and function. Gene 2008; 431:1-12.

[8] Sancho M, Diani E, Beato M, Jordan A. Depletion of human H1 variants uncovers specific roles in gene expression and cell growth. PLoS Genet 2008;4:1-17.

[9] Li JY, Patterson M, Mikkola HK, Lowry WE, Kurdistani SK. Dynamic distribution of liner histone H1.5 in cellular differentiation. PLoS Genet 2012; 8:1-13.

[10] Hechtman JF, Beasley MB, Kinoshita Y, Ko HM, Hao K, Burstein DE. Promyelocytic leukemia zinc finger and histone H1.5 differentially stain low- and high-grade pulmonary neuroendocrine tumors: a pilot immunohistochemical study. Human Pathol 2013; 44:1400-5.

[11] Sato S, Takahashi S, Asamoto M, et al. Histone H1 expression in human prostate cancer tissues and cell lines. Pathol Int 2012;62:84-92.

[12] Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL, ISUP Grading Committee. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol 2005; 1:228- 42.

[13] Khachaturov V, Xiao GQ, PhD, Kinoshita Y, Unger P, Burstein DE. Histone H1.5, a novel prostatic cancer marker: an immunohistochemical study. Human Pathol 2014; 45: 2115–2119.

[14] Izzo A, Kamieniarz K, Schneider R. The histone H1 family: specific members, specific functions?. Biol Chem 2008; 389:333-43.

[15] Terme JM, Sesé B, Millán-Ariño L, et al. Histone H1 variants are differentially expressed and incorporated into chromatin during differentiation and reprogramming to pluripotency. J Biol Chem 2011; 286:35347-57.

[16] Sato S, Takahashi S, Asamoto M, et al. Histone H1 expression in human prostate cancer tissues and cell lines. Pathol Int 2012; 62:84-92.

[17] Li JY, Patterson M, Mikkola HK, Lowry WE, Kurdistani SK. Dynamic distribution of liner histone H1.5 in cellular differentiation. PLoS Genet 2012; 8:1-13.

[18] Sjöblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006; 314:268-74.

[19] Gaudin PB, Epstein JI. Adenosis of the prostate: histologic features in needle biopsy specimens. Am J Surg Pathol 1995; 19: 737-47.

[20] Beach R, Gown AM, De Peralta-Venturina MN, et al. P504S immunohistochemical detection in 405 prostatic specimens including 376 18-gauge needle biopsies. Am J Surg Pathol 2002; 26: 1588-96.

[21] Jiang Z, Woda BA, Rock KL, et al. P504S: a new molecular marker for the detection of prostate carcinoma. Am J Surg Pathol 2001; 25:1397-404.

[22] Amin MB, Schultz DS, Zarbo RJ. Analysis of cribriform morphology in prostatic neoplasia using antibody to high–molecular weight cytokeratins. Arch Pathol Lab Med 1994; 118:260-4.

[23] Rubin MA, Zhou M, Dhanasekaran SM, et al. α-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA 2002; 287:1662-70.

[24] Sung MT, Jiang Z, Montironi R, MacLennan GT, Mazzucchelli R, Cheng L. alpha-Methyl acyl-CoA racemase (P504S)/34ßE12/p63 triple cocktail stain in prostatic adenocarcinoma after hormonal therapy. Human Pathol 2007;38:332-41.