厚生連医誌 第12巻 1号 51~53 2003

Original Article

# Immunohistological identification of basal cells in a diagnosis of adenocarcinoma of prostate: comparison of basal cell specific markers between a high molecular weight cytokeratin (34 $\beta$E12) and a tumor suppressor gene product (p63)

Pathology Center

Toshihiko Ikarashi and Hidehiro Hasegawa

Objective: Histological atypism, permeation, and metastasis were important findings for a diagnosis of carcinomas. With reference to prostatic adenocarcinoma, a disappearance of normal 2-cell-layer structure by a loss of basal cells is histologically a special malignant criteria

Study design: In this article, an immunohistological efficacy of basal cell specific markers was compared in three antibodies against two high molecular weight size cytokeratins, 34$\beta$E12 (DAKO Company and ENZO Company), and one tumor suppressor gene product, p63 (Novocastra Company).

Results and Conclusion: Anti-34$\beta$E12 antibody (DAKO Company) after proteinase K pretreatment for the first reagent was the most reliable method in both a positivity rate and a dyeing intensity. There was no positive basal cell in the adenocarcinoma group except two cases, in both of which there were several positive basal-cells-lining ducts with intraductal tumor replacement. The staining cells were, however, decreased even in benign proliferative lesion as benign prostatic hyperplasia. Furthermore, because a number of basal cells decreased with glandular proliferation, an attention may be necessary in the process of the immunohistological discernment of basal cells in boderline malignant tissues and adenocarcinoma ones.

Key Words: prostatic adenocarcinoma, basal cell, immunohistology, 34$\beta$E12, p63

## Introduction

A prostatic adenocarcinoma was diagnosed histopathologically on the basis of findings as histological atypism, permeation nature, or metastasis. Furthermore, malignancies were suggested by a disappearance of normal two-cell-layer structure because of the loss of basal cells. The immunohistological confirmation of basal cells was reported very useful in indistinct basal cells on routine hematoxylin-eosin staining specimens.[1,3] We re-examined this immunohistological efficacy of three antibodies against high molecular weight cytokeratin 34$\beta$E12 (2 kinds of high molecular weight cytokeratin 34$\beta$E12, produced by both DAKO Company and ENZO Company) and p63 of tumor-suppressor gene product (Novocastra Company), which were reported as more reliable basal-cell-specific markers.1-3)

## Material and Method

Twenty examined cases consisted of (1) pure normal gland group; 4 cases, (2) benign hyperplastic gland group; 8 cases, and (3) malignat group (adenocarcinoma); 8 cases, the latter of which included (a) well differentiated type; 1 case, (b) moderatetly differentiated type; 3 cases, and (c) poorly differentiated type; 4 cases. On the basis of a statistical analysis, the immunohistological staining results of mingled normal glands in malignant cases were added into the normal gland group. In this study the troublesome borderline-malignant glands were not examined because there was no definite histological criterion to differentiate borderline-malignant glands from malignant ones.

Specimens were delivered from transurethral biopsy of prostate and fixed with 10% of neutral formalin and routinely precessed. Necrotic or marked degenerative specimens were excluded from this analysis.

Immunohistochemical primary antibodies consisted of anti-34$\beta$E12 antibodies (DAKO Company and ENZO Company) and anti-p63 antibody (Novocastra Company). The immunohistochemical staining procedure was same as previously reported methods. 1-3) For a retrieval of their antigens, a pretreatment was done by either microwave oven or proteinase digestion with 0.05% proteinase K at room temperature for 10' according to previously reported methods. 1-4)

According to our previously reporting standard, the immunohistological positivity of basal cells in non-malignant glands was judged as follows: (1) strong positivity, demonstrated as "positivity grade 2" for the sake of convenience in a statistical processing; their posivities were easily confirmed microscopically under a low power view analysis or more than half of basal cells

were positively stained under a high power view, (2) weak positivity, described as "positivity grade 1" for statistics; more than 5% or less than half cells in number were dyed under a high power view, (3) negative positivity, expressed as "positivity grade 0"; there was no stained cell or positive cells were less than 5% in number.3) On the other hand, a judgment of basal cells in malignant group was as follows: (1) positive staining, called as "positivity grade 1" for statistical analysis: if any positive cells were confirmed microscopically under a high power view analysis regardless to their cellular numbers, (2) negative staining, expressed as "positivity grade 0"; there was no stainable cell.

## Result

As for a degree of the immunohistological positivity in basal cells of normal glands, anti-34 β E12 antibody (DAKO Company) was supreme and anti-34 β E12 antibody (ENZO Company) was the second one, but there's no statistical significance between them. (Table) The staining intensity became stronger in anti-34 β E12 antibody with a retrieval pretreatment by proteinase K than any other antigen-retrieval methods. No positive cell was confirmed by the use of anti-p63 antibody (Novocastra Company).

The immunohistochemical dyeing nature with anti-34 β E12 antibody fell off in benign glandular hyperplasia group in comparison with normal gland group, but there's no statistical significance between them. (Table)

In two cases in malignant group, there were several immunoreactive basal cells in ducts with intraductal tumor replacement, which suggested intraductal tumor infiltration. No troublesome 34 β E12-positive basal cell was admitted in definitely malignant glands.

## Discussion

Immunohistological study by anti-34 β E12 antibody (DAKO Company) with proteinase K predigestion was excellent for the confirmation of basal cells in prostatic glands.

As to malignant glands, there's several immunoreactive basal cells around an area of intraductal or intraglandular infiltration. But most malignant glands lost immunoreactive basal cells. It was, furthermore, easily predicted that its immunohistological usefulness was limited in proliferative lesions because the dyeing nature fell off even in benign prostatic hyperplasia. This poor stainablility suggested that 'no immunoreactive cell' could not indicate 'complete depletion of basal cells' in malignant group.

We have reported the usefulness of proliferation markers for a genetic and histopathological diagnosis of malignancies, namely, either an immunochistochemical analysis of both p53 and Ki-67 or an inspection with p53-polymerase chain reaction single-strand conformation polymorphism (p53-SSCP). 3, 4) In the present condition that the immunohistological dyeing was imperfect for the identification of basal cells. Thus, the histopathological diagnosis of prostatic adenocarcinoma should be done by the immunostainings not only with anti-high molecular weight cytokeratin antibody 34 β E12 antibody (DAKO Company) but also with anti-p53 and anti-Ki-67 antibodies. 3)

## References

1. Oliari BR, et al. Can basal cells be seen in adenocarcinoma of the prostate? An immunohistochemical study using high molecular weight cytokeratin (clone 34 β E12) antibody. Am J Surg Pathol 2002; 26:1151-60.
2. Shah RB, et al. Comparison of the basal cell-specific markers, 34 β W12 and p63, in the diagnosis of prostate cancer. Am J Srug Pathol 2002;26:1161-8.
3. Immunohistochemistry and PCR. In: Ikarashi T, editor. Obstetrical and Gynecological Pathology ABC- Clinicopathological mechanism and its medical strategy-. 34th ed. Nagaoka: Pimento Press; 2002. (Softs: Windows, Exel, Power Point (Microsoft), Photoshop (Adobe), Ichitaroh (Justsystem), and DocuWorks Desk (Fuji Xerox), total contents of 26.5GB)
4. Manual of genetic pathological diagnosis, using nucleic acids derived from formalin-fixed paraffin-embedded specimens, Ver 1.1, The Gene Analysis Section of Pathology Center in The Niigata Pref. Public Welfare Association, and The Study Meeting of Chu-etu Genetic Diagnosis. available from: URL:Http://www.niigata-kouseiren.jp/hospital/byori2/site%20one/top.htm

## 和 文 抄 録

原著

前立腺癌組織診断における基底細胞の免疫組織学的同定－基底細胞特異的マーカーとしての高分子量サイトケラチン34 β E12と癌抑制遺伝子産物p63の有効性の比較－

病理センター
五十嵐俊彦、長谷川秀浩

目的：前立腺癌組織診断において、一般的悪性所見である異型性や浸潤や転移以外に、基底細胞消失による正常2層構造の消失が悪性根拠とされている。

方法：今回、基底細胞の免疫組織学的同定として、基底細胞特異的マーカーとしての高分子量サイトケラチン34 β E12（DAKO社とENZO社）と癌抑制遺伝子産物p63（Novocastra社）に対する3種類の一次抗体の有効性を比較した。

結果・結論：基底細胞陽性率においては蛋白分解酵素処理後の抗34 β E12抗体（DAKO社）が優れ、癌症例では陽性基底細胞の混在を認めず、良悪性の判定に有効であった。しかしながら、非癌性の増殖性病変では基底細胞陽性度が低下し、境界病変における免疫組

織学的鑑別を困難にし、その判定には十分な注意が必要であろう。

キーワード：前立腺癌，基底細胞，免疫組織学，34βE12，p63

Table. Cytokeratin expression in prostate

| cases | tissue number | sampling place | disease | antibody | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| antibody benign/malignant | | | | 34βE12 (DAKO) | | | | 34βE12 (ENZO) | | | | p63 (Novocastra) | | | |
| | | | | benign | | malignant | benign-malignant | benignmalignant | | benign | | malignant | | benign | |
| retrieval pretreatment | | | | enz | MW | enz | MW | enz | MW | enz | MW | enz | MW | enz | MW |
| 2 | 15741 | R3 | normal | 2 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 3 | 16277 | L1 | normal | 2 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 4 | 16363 | LA | normal | 2 | 2 | | | 0 | 2 | | | 0 | 0 | | |
| 9 | 16013 | LLM | normal | 2 | 2 | | | 0 | 1 | | | 0 | 0 | | |
| 5 | 15150 | RLM | BPH | 2 | 2 | | | 0 | 2 | | | 0 | 0 | | |
| 6 | 15241 | RB | BPH | 2 | 2 | | | 0 | 2 | | | 0 | 0 | | |
| 7 | 15242 | TUR | BPH | 2 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 8 | 15243 | TUR | BPH | 2 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 11 | 16190 | TUR | BPH | 1 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 12 | 16315 | TUR | BPH | 1 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 19 | 15243 | LLT | BPH | 2 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 20 | CTL | * | BPH | 2 | 1 | | | 0 | 1 | | | 0 | 0 | | |
| 13 | 15185 | R3 | malignant, wel | 2 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 15611 | TUR | malignant, mod | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 15644 | R1 | malignant, mod | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 15765 | L1 | malignant, mod | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 15644 | L3 | malignant, por | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 16013 | LM | malignant, por | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 15734 | R1 | malignant, por | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 16014 | * | malignant, por | 2 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| mean | | | | | Σtotal | 1.9 | 1.6 | | | 0 | 1.4 | | | 0 | 0 |
| | | | | | | | | | | | | | | | |
| | | | | | | Σnormal | 1.8 | 1.8 | | | 0 | 1.5 | | | 0 |
| | | | 0 | | | | | | | | | | | | |

cf. positivity benign 0 negative～<5%
1 5%≦～<50%
2 50%≦
malignant 0 no stainable cell
1 stainable cell

BPH benign prostatic hyperplasia
enz 0.05% proteinase K, room temperature 10'
mod moderately differentiated adenocarcinoma
MW microwave antigen retrieval method, 5'x3
por poorly differentiated type adenocarcinoma
wel well differentiated type adenocarcinoma