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TRIM29 as a novel prostate basal cell marker for diagnosis of prostate cancer

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Short title: TRIM29 as a prostate basal cell marker

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Abstract

Tripartite motif protein 29 (TRIM29) is one of the TRIM family proteins, some of which function as E3 ubiquitin ligases. In this study, we investigated the usefulness of TRIM29 for diagnosis of prostate cancer. Prostate tissues including carcinoma and non-carcinoma tissues obtained by needle biopsy and radical prostatectomy were used. Immunohistochemistry was performed according to standard procedures using an antibody against TRIM29. Immunohistochemical staining with an antibody against 34βE12, which recognizes cytokeratins 1, 5, 10 and 14, was performed as a control. Basal cells of normal prostatic glands were stained with anti-TRIM29 antibody in all cases, whereas prostate cancer tissues had no or little staining with anti-TRIM29 antibody. TRIM29 is selectively expressed in basal cells of the normal prostate gland, and immunohistochemical staining with anti-TRIM29 antibody showed the same expression pattern as that with 34βE12 in prostate cancer and its benign mimics. Our data indicate that TRIM29 may be useful for distinguishing prostate cancers from benign tissues.

Keywords: TRIM29, Prostate cancer, Basal cell, Human.

Introduction

The normal prostate epithelium contains three different types of cells: secretory, basal, and neuroendocrine cells. Each subset of cells is morphologically and immunophenotypically identified within normal prostate tissues (Verhagen et al., 1988). With regard to the origin of secretory cells and basal cells, it has been hypothesized that basal cells represent the precursors of secretory cells (Robinson et al., 1998). On the other hand, cell kinetic studies using prostates of rats suggested that basal and secretory cells are independent lineages with self-renewal abilities (English et al., 1987). Therefore, it is now controversial whether the putative prostate stem cells are able to produce both basal and secretory cells.

Diagnosis of prostate cancers relies on observations of architectural, cytological, and immunohistochemical features of the tissues. Recently, pathologists must evaluate many prostate needle biopsy specimens because of the extensive use of prostate-specific antigen (PSA) screening. It is relatively easy to diagnose prostate cancer tissues that have diffuse individual cell infiltration, a cribriform pattern or closely packed medium-sized glands, but it is sometimes difficult to diagnose those with small gland types as malignant carcinomas. Malignant transformation of prostate cancers is associated with the absence of basal cell layers in the epithelium of the acini. Immunohistochemical analysis is an essential and important tool in the evaluation to confirm the presence of basal cells. Two commonly used basal cell markers are cytokeratin 1/5/10/14 (34βE12: anti-high molecular weight cytokeratin (HMCK) antibody) and p63, which is one of the p53 family proteins based on their structural similarity (Ramnani and Bostwick, 1999; Signoretti et al., 2000; Varma et al., 1999).

Actually, basal cell-specific anti-keratin antibody 34βE12 is useful for distinguishing benign prostate and cancer, leading to validation of the diagnosis of malignancy.

TRIM29, also known as ataxia-telangiectasia group D-associated protein (ATDC), is a member of the tripartite motif (TRIM) family of proteins (Hatakeyama, 2011; Kapp et al., 1992). Several recent reports have shown the role of TRIM29 in tumorigenesis examined on the basis of functional studies. It has been reported that TRIM29 is highly expressed in gastric cancer with poor histological grade, large tumor, great extent of tumor invasion and lymph node metastasis, suggesting that TRIM29 play a pivotal role in differentiation, proliferation, and progression of gastric cancer cells (Kosaka et al., 2007). Recently, TRIM29 has been shown to be an important positive regulator of β-catenin-dependent signaling in pancreatic cancer (Wang et al., 2009). Others have reported that the amount of TRIM29 increased in keratinocytes after exposure to UVB and that knockdown of TRIM29 induces decrease in viability of keratinocytes after UVB exposure (Yuan et al., 2010). We previously revealed that TRIM29 negatively regulates p53 via inhibition of Tip60 and suppresses apoptosis induced by UV irradiation in HCT 116 cell lines (Sho et al., 2011).

In the present study, we showed that TRIM29 represents a selective marker of basal cells within the prostatic epithelium by analyzing TRIM29 expression in a series of normal prostates and prostate cancers.

Materials and methods

Human tissue samples

This study was approved by the Hokkaido University Hospital Ethics Committee, Sapporo, Japan. Tissues from patients who gave informed consent under the guidelines of the Hokkaido University Hospital Ethics Committee were used for this study.

Excised samples from tumor lesions and adjacent normal tissues were obtained within 3 h after the operation. Fifteen cases of formalin-fixed, paraffin-embedded sections of specimens of prostate tissues consisting of prostate cancers (8 Gleason's score 3 + 3 = 6, 3 Gleason's score 3 + 4 = 7, 1 Gleason's score 4 + 3 = 7, 2 Gleason's score 4 + 4 = 8 and 1 Gleason's score 4 + 5 = 9) and benign lesions obtained by needle biopsy and radical prostatectomy were used (Table 1). Tissues were fixed in 4% formaldehyde in 0.1 M phosphate buffered saline (pH 7.4) for 3 days and then embedded in paraffin wax. Paraffin-embedded sections (3 μm in thickness) were mounted on silane-coated slides (Matsunami Glass Ind., Ltd., Osaka, Japan).

Immunohistochemical studies

Immunohistochemistry was performed according to standard procedures. Briefly, tissues sectioned to 3 μm were deparaffinized with xylene and rehydrated through a graded ethanol series. Antigen retrieval was carried out using a pressure cooker with citrate buffer for 3 min. Endogenous peroxidase activity was quenched by incubating sections in 3% hydrogen peroxide for 5 min. Subsequently, tissue sections were incubated with anti-TRIM29 monoclonal antibody (1:200, anti-ATDC (A-5), Santa

Cruz Biotechnology, Santa Cruz, CA, USA) or anti-cytokeratin 1/5/10/14 monoclonal antibody (34βE12, Nichirei, Tokyo, Japan) at 37ºC for 30 min. An EnVision kit (Dako, Glostrup, Denmark) was used to detect the staining. The tissue sections were then photographed with a CCD camera (DP71, Olympus, Tokyo, Japan) attached to an Olympus BX51 microscope. Immunopositivities of the sections were evaluated by two independent investigators under a microscope.

Results

Selective expression of TRIM29 in basal cells in the prostate epithelium

It has been reported that TRIM29 regulates β-catenin-dependent signaling in pancreatic cancer and that TRIM29 expression affects viability after UVB exposure (Sho et al., 2011; Wang et al., 2009; Yuan et al., 2010). To clarify the tissue expression pattern of TRIM29 protein, we performed immunohistochemical staining using several human tissues. In immunosistochemical analysis using anti-TRIM29 antibody, we found selective and specific expression of TRIM29 in the prostate. Immunohistochemical analysis showed that TRIM29 was exclusively expressed in the nuclei of basal cells in the prostate epithelium, whereas no staining was observed in secretory cells or in the stroma (Figs. 1A and C). Antibody for basal cell-specific anti-keratin antibody such as 34βE12 was used as a control for basal cells in the prostate (Figs. 1B and D). Actually, the basilar phenotypes were verified by positive staining with 34βE12. TRIM29 expression in basal cells was observed in all normal prostatic glands that we used in this assay.

Loss of TRIM29 expression in prostate cancers

It has been reported that prostate cancers lack a basal cell layer of epithelium in the prostatic acini (Ramnani and Bostwick, 1999; Signoretti et al., 2000; Totten, 1953; Varma et al., 1999). Therefore, we evaluated the expression levels of TRIM29 in human prostatic intraepithelial neoplasms (PIN) and adenocarcinomas. Immunohistochemical analysis showed that TRIM29 is highly and specifically expressed in PIN as was shown by staining using 34βE12 (Figs. 2A and B, Table 2). On the other hand, malignant acini in prostate cancers lost the positive staining with anti-TRIM29 antibody (Figs. 2C and D, Table 2). These findings showed that TRIM29 has specificity to that of 34βE12 in terms of the discrimination of benign and malignant lesion of prostate by immunohistochemical stainings.

Discussion

In our study, we showed that TRIM29 is selectively expressed in basal cells of normal prostatic glands but that TRIM29-positive cells disappear in prostate cancers. DNA microarray analysis indicated the absence of ATDC expression in prostate cancers on the basis of mRNA expression level (Ernst et al., 2002). So far, results of several expression analyses of TRIM29 using human normal and cancer tissues have been reported with TRIM29 being highly expressed in the lung, bladder, colorectal, ovarian, endometrial cancers and multiple myeloma (Dyrskjot et al., 2004; Glebov et al., 2006; Hawthorn et al., 2006; Loewen et al., 2006; Mutter et al., 2001; Santin et al., 2004; Zhan et al., 2002). Previous analyses also suggested that TRIM29 is involved in the response to DNA damage through p53 and functions as an oncogene that promotes tumorigenesis (Sho et al., 2011; Yuan et al., 2010). On the other hand, TRIM29

functions as a tumor suppressor in non-tumorigenic breast cells and invasive estrogen receptor-positive breast cancer, and low expression level of TRIM 29 in breast cancer has been reported (Liu et al., 2012; Nacht et al., 1999). Therefore, the expression levels and functions of TRIM29 are likely differ depending on the cell or tissue types.

Prostate cancer is easily recognized by nuclear abnormality, atypical architecture of acini and cancer cell infiltration. However, it is difficult to diagnose highly differentiated adenocarcinomas. Therefore, in pathological analysis of prostate cancers, it is important to distinguish the cell types, including secretory, basal and neuroendocrine cells, in the prostate epithelium. Previous studies showed that prostate adenocarcinoma loses a basal cell layer of the epithelium in the prostatic acinus (Totten, 1953). These findings lead to identification of adenocarcinoma-specific or basal cell-specific biomarkers. Recent clinical diagnosis showed that an enzyme involved in β-oxidation of branched-chain fatty acids, α-methylacyl-CoA racemase (AMACR), is upregulated in prostatic adenocarcinoma and that its sensitivity is very high among different Gleason grades (Jiang et al., 2001; Rubin et al., 2002). AMACR is highly expressed in the cytosol of prostate cancer cells with granular quality, whereas AMACR staining is negative or weakly positive in benign prostate glands.

In some cases, immunohistochemistry has been performed to judge whether basal cells of acini are present or not. Anti-cytokeratin antibody 34βE12 is specific to basal cells for distinguishing benign prostate and cancer (Ramnani and Bostwick, 1999; Varma et al., 1999). Broadly, anti-keratin 34βE12 relies on the absence of basal cell staining to confirm the diagnosis of malignancy in prostate cancers. As with anti-keratin 34βE12 antibody, an antibody against p63, one of the p53 family proteins, is commonly used for identification of another basal cell marker (Signoretti et al., 2000).

Although loss of the basal cell layer has long been the hallmark of prostatic acinar adenocarcinoma, there has been a very small number of cases of unequivocal Gleason pattern 3 adenocarcinoma in which basal cells remain (Oliai et al., 2002). Careful attention to the distribution of 34βE12 staining can minimize potential mislabeling by abnormal expression of 34βE12 as benign. It is likely p63 has higher specificity than 34βE12 for basal cells, showing less non-specific reactions with cancer cells. In order to obtain an accurate diagnosis of prostate cancers, a more reliable basal cell marker in addition to 34βE12 and p63 is needed. To clarify the specificity of TRIM29 for basal cells, detailed analysis of TRIM29 expression should be performed using unequivocal Gleason pattern 3 adenocarcinoma in which basal cells remain.

In conclusion, TRIM29 immunohistochemistry may be a useful procedure for morphological analysis in surgical pathology for prostate cancers.

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Table 1. Clinicopathologic findings of 15 cases of prostate cancers

Parameters	Category	No. of cases (percent)
Age (years)	<65	2 (13.3)
	65–75	7 (46.7)
	>75	6 (40)
PSA (ng/ml)	4<PSA<6	5 (33.3)
	6<PSA<10	8 (53.3)
	10<	2 (13.3)
T (Primary tumor extent)	T1c	12(80)
	T2a	2 (13.3)
	T2b	0 (0)
	T2c	1 (6.7)
N (Lymph node metastasis)	N0	15(100.0)
	N1<	0 (0)
M (Distant metastasis)	M0	15 (100)
	M1<	0 (0)
TNM Stage	I	0 (0)
	II	15 (100)
	III	0 (0)
	IV	0 (0)

Table 2. Extent of TRIM29 staining in prostate cancers and PIN

Gleason's score	Percentage of TRIM29-positive acini for all acini (%)			
	0	0-30	30-60	60-100
Gleason's score 3 + 3 = 6 (n=8)	8	0	0	0
Gleason's score 3 + 4 = 7 (n=3)	3	0	0	0
Gleason's score 4 + 3 = 7 (n=1)	1	0	0	0
Gleason's score 4 + 4 = 8 (n=2)	2	0	0	0
Gleason's score 4 + 5 = 9 (n=1)	1	0	0	0
PIN (n=1)	0	0	0	1
Normal lesion (n=15)	0	0	0	15

Legends to Figures

Fig. 1. Immunohistochemical analysis of the human prostate with an antibody to TRIM29. (A) TRIM29 staining in the normal prostate (low-level amplification). (B) 34βE12 staining in the normal prostate (low-level amplification). (C) TRIM29 staining in the normal prostate (high-level amplification). (D) 34βE12 staining in the normal prostate (high-level amplification). Micrographs in A and B were obtained at low magnification, and each black rectangle in A and B is magnified in C and D. Arrows indicate the cells stained with antibodies. Scale bar =100 μm.

Fig. 2. TRIM29 expressions in prostatic dysplasia and adenocarcinoma. (A) TRIM29 expression in PIN. (B) 34βE12 expression in PIN. (C) TRIM29 expression in prostatic adenocarcinoma. (D) 34βE12 expression in prostatic adenocarcinoma. Arrows indicate the cells stained with antibodies. Scale bar =100 μm.

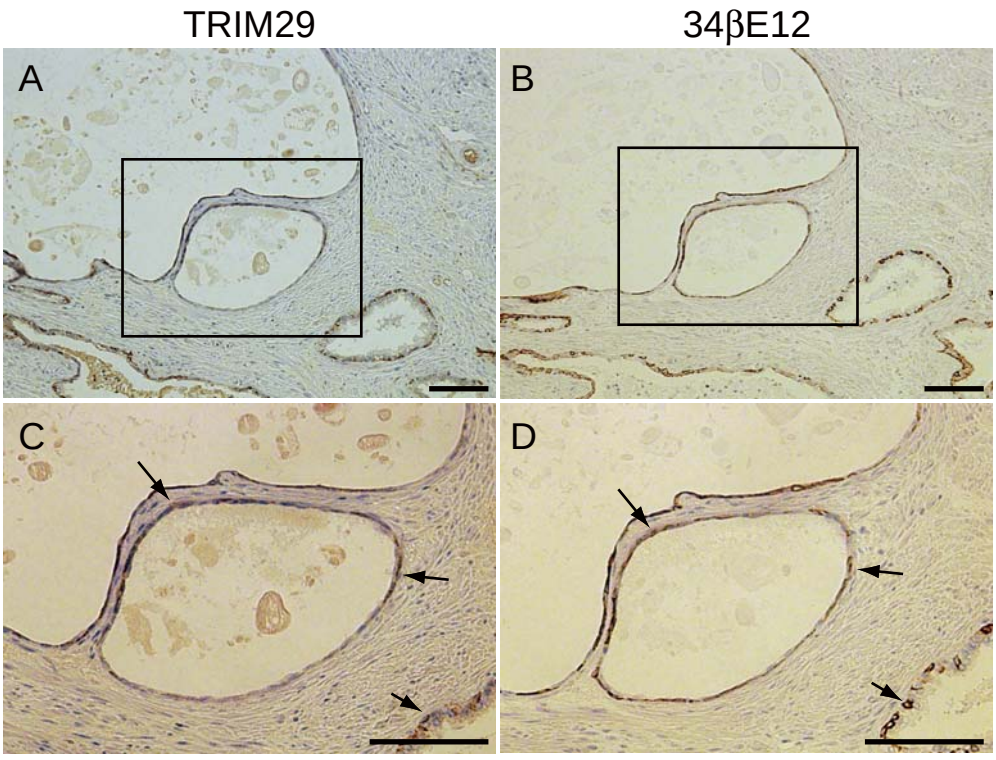


Fig. 1 Kanno

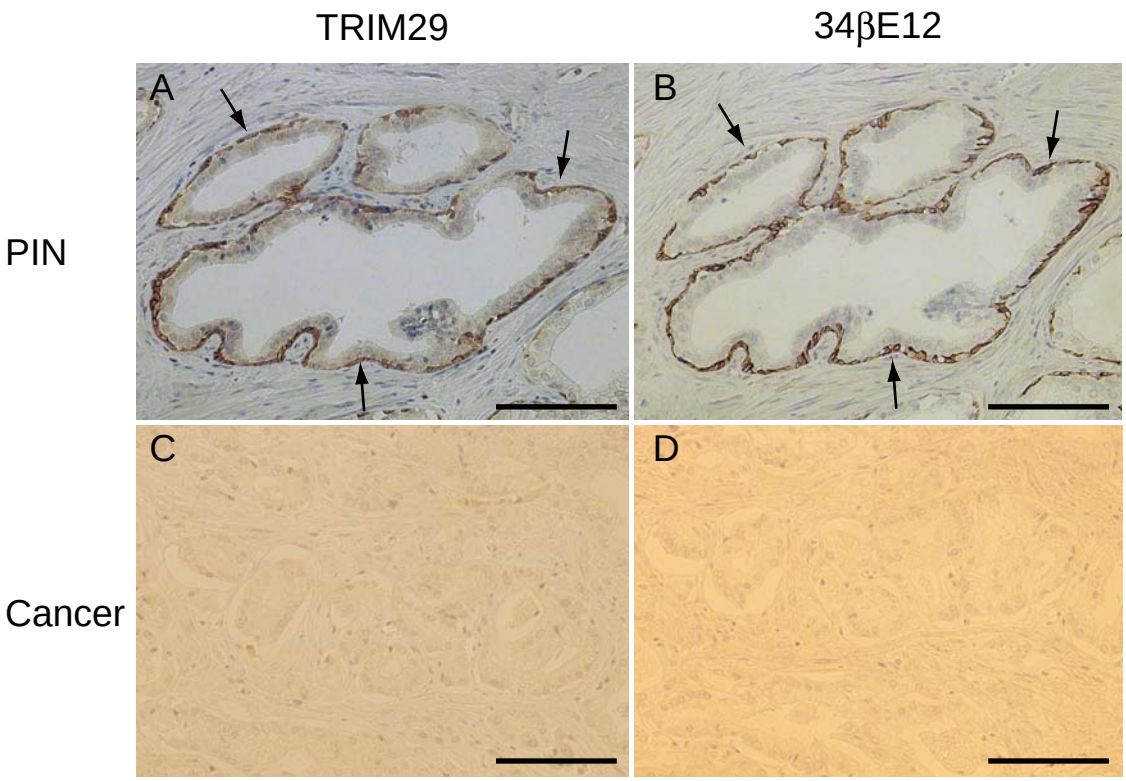


Fig. 2 Kanno