

Diagnostic and Therapeutic Potential of a Human Antibody Cloned from a Cancer Patient That Binds to a Tumor-Specific Variant of Transcription Factor TAF15

Nicole Schatz¹, Stephanie Brändlein¹, Kilian Rückl¹, Frank Hensel², and H. Peter Vollmers¹

Abstract

Human hybridoma technologies permit the cloning of patient antibodies that may have desirable qualities. In this study, we report the isolation of a natural IgG antibody from a stomach cancer patient that illustrates novel diagnostic and therapeutic uses. Human antibody PAT-BA4 recognizes a tumor-specific variant of the transcription factor TATA-binding protein-associated factor 15 (TAF15) that is expressed on the plasma membrane of stomach cancer and melanoma cells but not healthy tissues. TAF15 is a member of the multifunctional TET protein family involved in mRNA transcription, splicing, and transport that is normally expressed only in the cytoplasm and nucleus of fetal or adult tissue cells. However, in malignant cells, TET family members including TAF15 seem to be involved in cell adhesion and spreading. In support of this likelihood, we found that PAT-BA4 inhibited tumor cell motility and tumor cell adhesion. Our findings define a role for a tumor-specific TAF15 antigen in malignant processes. *Cancer Res*; 70(1); 398–408. ©2010 AACR.

Introduction

The innate immunity is responsible not only for the first-line defense against infections but also for the removal of cellular waste and malignant cells (1–3). In addition, the natural immunity triggers the second immune response. On humoral level, most of the therein involved antibodies are natural immunoglobulin M (IgM) antibodies. They represent >99% of the Igs. A series of natural monoclonal IgM antibodies with tumor-specific properties have been isolated from cancer patients and healthy people. They are germ-line coded and possess low affinity, and their targets are post-translationally modified cell surface antigens. The epitopes are, in most cases, carbohydrate structures (4, 5).

In this article, we describe a natural IgG, PAT-BA4, which defines a new tumor-specific antigen, a 78-kDa TATA-binding protein-associated factor 15 (TAF15) variant. TAF15 protein [also known as TAF(II)68, TAF2N, or RNA-binding protein 56] is a member of the TET (TLS-EWS-TAF15) family. This multifunctional family belongs to the RNA-binding proteins and consists of human EWS (Ewing's sarcoma), TLS [translocated in liposarcoma, also known as FUS (fusion) protein], TAF15, and the closely related *Drosophila* cabaza (also called sarcoma-associated RNA-binding fly homologue;

refs. 6–9). A structural and functional similarity was observed between all four proteins. They all possess well-conserved structures and are therefore supposed to originate from the same ancestor gene (10).

About the functions of human TET proteins, they are still poorly described, although they are involved in many cellular processes. TAF15, EWS, and TLS proteins are almost ubiquitously expressed in human fetal and adult tissues, with only few cell types where no TET expression is detected (11, 12). The three proteins are located in the cytoplasm and nucleus of normal human cells, where they are involved in transcription, splicing, mRNA transport, microRNA processing, signaling, and maintenance of genomic integrity (13–15). TAF15 is a multifunctional RNA- and ssDNA-binding protein, which shows unique as well as overlapping functions with the other family members (7, 12). Beside their wild-type functions, the TET proteins are also known to be involved in tumor pathogenesis. Chromosomal translocation of their genes, resulting in fusion oncoproteins, has been found in several types of tumors (16–18). Remarkably, the chimera proteins always contain the NH₂-terminal part of the TET proteins. The fusion breakpoints in the TET members were identified between the Q-rich region and the RRM domain (6). A characteristic translocation t(9;17)(q22;q11.2) resulting in gene fusion of TAF15 and nuclear receptor NOR1 (also known as TEC or CHN) was found in extraskeletal myxoid chondrosarcomas (19–21). Another common translocation t(12;17)(p13;q11) leads to a gene fusion of TAF15 with the transcription factor CIZ (also known as NMP4 and ZNF384) detected in acute leukemia (22). We show here that a most likely modified version of TAF15 is located in the membrane of malignant tissue and is absent in nontransformed cells.

Authors' Affiliations: ¹Institute of Pathology, University of Würzburg; ²Patrys GmbH, Würzburg, Germany

Corresponding Author: H. Peter Vollmers, Institute of Pathology, University of Würzburg, Josef-Schneider Str. 2, D-97080 Würzburg, Germany. Phone: 49-931-20147898; Fax: 49-931-20147798; E-mail: path027@mail.uni-wuerzburg.de.

doi: 10.1158/0008-5472.CAN-09-2186

©2010 American Association for Cancer Research.

Materials and Methods

Cell culture. PAT-BA4–producing hybridoma cells and pancreatic adenocarcinoma cell line BxPC-3 were generated and cultured as described previously (5, 23, 24).

Purification of PAT-BA4 antibody. The cell culture supernatant of hybridoma cells was purified using a protein A column (GE Healthcare). The antibody was eluted with a solution containing 0.1 mol/L arginine and 0.1 mol/L NaCl (pH 3.8) and immediately neutralized with 10% of 1 mol/L Tris (pH 8). Buffer exchange with PBS was performed using a PD10 column (GE Healthcare).

Membrane protein extraction. Membrane proteins were isolated from adherent pancreas carcinoma cell line BxPC-3 as described elsewhere (25). Briefly, harvested cells were resuspended in hypotonic buffer [20 mmol/L HEPES (pH 7.4), 3 mmol/L KCl, 3 mmol/L MgCl₂, Complete tablet (Roche Diagnostics)], incubated on ice (30 min), and sonicated (5 min). The nuclei were pelleted by centrifugation (13,000 rpm, 10 min). A further centrifugation step (40,000 rpm, 45 min) was done to pellet the membrane proteins, which were resuspended in lysis buffer [50 mmol/L Tris-HCl (pH 7.4), 1% NP40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 µg/mL pepstatin, Complete tablet].

Antigen purification and identification. PAT-BA4 antigen was purified by affinity chromatography using a PAT-BA4–coupled Sepharose column with subsequent SDS-PAGE and Western blotting of the bound protein. For purification, pooled membrane protein extracts of BxPC-3 cell were used. The antigen was eluted with 0.1 mol/L glycine buffer (pH 2.2) and neutralized immediately with 1 mol/L Tris (pH 9.0). Western blot analysis was performed with a PAT-BA4 concentration of 300 µg/mL. To exclude unspecific protein bindings, unrelated human IgG (Chrompure, Dianova) served as isotype control. Column bleeding was checked by a control using only secondary antibody (rabbit anti-human IgG horseradish peroxidase conjugated; Dako).

To identify PAT-BA4 antigen, the positive band was cut out of a Coomassie-stained gel. The band with an estimated molecular mass of 78 kDa was completely sequenced in an external lab (TopLab) using matrix-assisted laser desorption/ionization (MALDI) mass spectroscopy. The peptide masses were compared with human sequences in National Center for Biotechnology Information database by ProFound.

Transfection of TAF15 small interfering RNA. The transfection experiment was performed as described in the transfection reagent protocol (siLentFect Lipid Reagent; Bio-Rad). BxPC-3 cell line was used for the assay. siGENOME SMART-pool small interfering RNA (siRNA), a pre-designed and validated siRNA for silencing the target molecule TAF15, was purchased from Dharmacon. Four oligonucleotide duplex strands were used for gene expression downregulation of TAF15. The sense sequences of the used strands were (a) GAACUUUGCUCGAAGGAAUUU, (b) GAUGAGCAGUCAAUUUAUGUU, (c) CAAUUAGAAGACCGGAAUUU, and (d) AUGAUGAGCAGUCAAUUUAUU. The cells were transfected with a final concentration of 100 nmol/L siRNA. As serum-free medium, Opti-MEM I + GlutaMAX-I (1×) medium

(Invitrogen) was used. To exclude nonspecific or cytotoxic effects on protein knockdown, Silencer negative control siRNA (Ambion) and mock-transfected and nontransfected cells served as control. The negative control siRNA consists of a 19-bp scrambled sequence with no significant homology to known gene sequences of mouse, rat, or human.

Detection of TAF15 protein knockdown by fluorescence-activated cell sorting and cytospin analyses. For fluorescence-activated cell sorting (FACS) analysis, BxPC-3 cells transfected with siGENOME SMARTpool TAF15 siRNA, Silencer negative control siRNA, and nontransfected cells were harvested 48 h after transfection. An amount of 2×10^5 cells were incubated on ice with PAT-BA4 antibody (300 µg/mL) for 20 min. Mouse anti-human CD55 antibody [decay-accelerating factor (DAF), 1:1,000; Acris] was used to control cytotoxicity. Samples were incubated in the dark with secondary FITC-labeled antibodies [rabbit anti-human IgG (Dako) and rabbit anti-mouse IgG (Dianova)] for 20 min on ice. As negative control, cells only incubated in secondary FITC-labeled antibody were used. The cells were analyzed by flow cytometry (FACS-can, Becton Dickinson) and evaluated with WinMDI software.

For cytospin analysis, cells transfected with siGENOME SMARTpool TAF15 siRNA, Silencer negative control siRNA, and mock-transfected and nontransfected cells were harvested 48 h after transfection. The immunohistochemical staining of the cells was performed as described elsewhere (5). The cytospins were incubated with PAT-BA4 (100 µg/mL) and peroxidase-labeled secondary antibody (rabbit anti-human IgG, 1:50).

Immunohistochemical comparison of PAT-BA4 and commercial anti-TAF15 antibody. The staining on fixed tissue was performed as described elsewhere (5). Briefly, the sections were stained with either PAT-BA4 antibody (100 µg/mL) or anti-TAF15 antibody (1:25; GeneTex, Inc.). For isotype controls, human Chrompure IgG and rabbit serum IgG (Sigma-Aldrich) were used. Anti-cytokeratin 5.2 antibody (Dako) served as positive control. As peroxidase-labeled secondary antibodies, rabbit anti-human IgG, goat anti-rabbit IgG (Dianova), and rabbit anti-mouse IgG (Dako) were used, all in a ratio of 1:50. After mounting with Aquatex (Merck), the sections were analyzed using light microscopy.

RNA isolation, reverse transcription, and semiquantitative PCR. Total RNA from normal and cancerous lung tissue was isolated using the phenol-guanidine-isothiocyanate method with Trizol reagent (Invitrogen) as described elsewhere (26). The isolated RNA was paraphrased in cDNA using standard protocols. An amount of 5 µg total RNA was reverse transcribed in the presence of oligo-dTMP and random primers (ratio, 1:1) as well as SuperScript II Reverse Transcriptase (M-MLVRT, Invitrogen). TAF15 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were detected by subsequent PCR analysis. The amplification of cDNA template was performed with primers (10 pmol/µL) of TAF15 and GAPDH in a PCR Mix [sterile aqua bidest, 2.5 µL of 10× PCR buffer, 1 µL of 50 mmol/L MgCl₂, 2.5 µL of 2.5 mmol/L nucleotide triphosphates, 0.13 µL Taq DNA Polymerase (Invitrogen), and 5 µg cDNA]. The assay was adjusted to a volume of 25 µL. The primers were designed on

their reported sequences and synthesized by MWG-Biotech. The used oligonucleotide sequences for TAF15 primers were 5'-GCCTGGCTTTTCGATTCGTTG-3' and 5'-CGACGGT-CATCTTGTGTGTGG-3' and for GAPDH primers were 5'-AGATGATGACCCTTTTGGCTC-3' and 5'-AAGGTCG-GAGTCAACGATTT-3'. TAF15 and GAPDH were amplified by a hot start at 94°C (5 min) and 40 cycles at 94°C (30 s), 57.3°C (30 s), and 72°C (30 s), with 72°C (7 min). For negative control, the PCR was performed without cDNA. The PCR products were detected by a UV transilluminator.

Adhesion assay. A total of 200 BxPC-3 cells were incubated on ice for 30 min and subsequently transferred in 50 μ L PAT-BA4 (200 μ g/mL). As controls, complete RPMI 1640 and Chrompure human IgG (200 μ g/mL) were used. The adherent cells were fixed in 60% isopropanol after an incubation time of 30, 45, and 60 min. Then, the cells were stained with hematoxylin and counted.

Motility assay. A 24-well plate was inoculated with 1×10^5 BxPC-3 cells. After 6 h, a cross was scratched into the cells (80% confluent). One well was then incubated with PAT-BA4 (400 μ g/mL) for 24 h. Complete RPMI 1640 and Chrompure human IgG (400 μ g/mL) served as negative controls. The cells were fixed with 5% glutaraldehyde, stained with hematoxylin, and evaluated by light microscope.

Statistical analysis. All values are expressed as the mean \pm SD. The significance between the mean values was evaluated by two-tailed unpaired Student's *t* test. Asterisk indicates $P < 0.05$. Statistical analysis was performed with the statistical software R.³

Results

Tumor specificity of PAT-BA4 antibody. Immunohistochemically stained paraffin sections showed that PAT-BA4 antibody binds specifically to malignant but not to nonmalignant tissues (Table 1A and B). Unspecific binding was checked by isotype control (Chrompure IgG). As positive control, anti-cytokeratin antibody was used. In contrast to PAT-BA4 antibody and positive control, there was no binding detectable in the isotype control (Fig. 1).

PAT-BA4 target protein purification and identification. For PAT-BA4 target protein purification, an affinity chromatography was performed with pooled BxPC-3 membrane extracts. In Western blot analysis of the purified membrane extracts, PAT-BA4 binds to a protein with a relative molecular weight of \sim 78 kDa. An unspecific binding could be excluded by using an isotype control (Chrompure IgG). The unrelated human IgG showed no band at 78 kDa. Furthermore, column bleeding was checked by a control with only secondary antibody. Thus, remaining bands in the PAT-BA4 blot could be assigned to antibody fragments (Fig. 2A). To identify the detected protein, the corresponding band was excised from a Coomassie-stained gel (Fig. 2A) and analyzed by MALDI mass spectroscopy.

Sequence database search with the trypsin-digested peptide masses identified human TAF15 protein (NP_631961.1 isoform 1/NP_003478.1 isoform 2) as the highest-ranking candidate. The amino acid coverage lies at a minimum of 22% and the peptide mass error was <30 ppm. In the peptide mass map, the matched peptide masses obtained by MALDI mass spectroscopy fingerprint analysis were marked with asterisks (Fig. 2B). So, the PAT-BA4 antigen could be identified as TAF15 protein.

Validation of PAT-BA4 target protein TAF15. To validate TAF15 as PAT-BA4 target protein, a transient knockdown experiment with siRNA was performed. The siRNA cleaves the TAF15 mRNA, which leads to a blocked protein expression on the cell surface. A FACS analysis was conducted to investigate the binding behavior of PAT-BA4 on tumor cells after silencing with siRNA. Therefore, BxPC-3 cells were transfected with siRNA targeting human TAF15 (siGENOME SMARTpool siRNA). Nontransfected cells grown in complete RPMI 1640 served as control and showed a positive PAT-BA4 binding 48 hours after transfection. To exclude nonspecific effects on TAF15 expression, caused by siRNA uptake in cells, Silencer negative control siRNA (Ambion) was used as a negative control. PAT-BA4 antibody showed a positive binding on cells transfected with scrambled siRNA (Fig. 3A). The cytotoxic effect of all cells was controlled by using human anti-CD55 antibody (Fig. 3B). There was no toxic effect detectable. A positive binding behavior of all cells treated with anti-CD55 antibody was regarded. FACS analysis with PAT-BA4 of the cells treated with siRNA against human TAF15 implicated a decreased binding affinity 48 hours after transfection, accompanied with cell surface downregulation of TAF15 protein (Fig. 3A). The binding was reduced over 40% (Fig. 3C). Percentages of anti-CD55 antibody binding are shown in Fig. 3D. In addition to FACS analysis, cytospin preparations were performed to detect PAT-BA4 binding behavior of siRNA-transfected BxPC-3 tumor cells. As reference, untreated cells were used. They showed a positive PAT-BA4 binding, visible as a brown staining. Nonspecific effects of the transfection could be excluded by scrambled Silencer negative control siRNA, whereas cytotoxicity of the assay was tested with mock-transfected cells [cells treated with transfection reagents (siLentFect Lipid Reagent) but not with siRNA]. In both cases, the staining intensity was similar to that of the untreated cells. In contrast to all these controls, cells transfected with TAF15 silencing siRNA showed a decreased PAT-BA4 binding after 48 hours (data not shown). The TAF15 protein downregulation led to a lower PAT-BA4 antibody binding, visible as a pale staining. Both assays, FACS analysis and cytospin preparations, showed that PAT-BA4 antibody binding depends on TAF15 expression. This indicates that TAF15 is the target of PAT-BA4 protein.

Comparison of TAF15^{PAT-BA4} and "Wild-Type" TAF15. Western blot analysis with PAT-BA4 and anti-TAF15 antibody of purified BxPC-3 cell membrane extract showed the same binding pattern. Both antibodies bind to the 78-kDa TAF15 protein. To figure out if the antibodies also have the same epitope, a Western blot inhibition assay was performed. Two different assays of each antibody (PAT-BA4 or

³ R.D.C. Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2005.

Table 1. Expression of PAT-BA4 antigen on human malignant and nonmalignant tissues

A. Malignant tissue		
Tested tissue	Tumor type	+/-
Stomach	Adenocarcinoma (intestinal)	5/0
Lung	Primary tumor: Neuroendocrine carcinoma, squamous cell carcinoma, adenocarcinoma	9/0
	Metastasis: Lymph node	1/0
Colon	Primary tumor: Adenocarcinoma	3/0
	Metastasis: Liver	0/1
Pancreas	Primary tumor: Adenocarcinoma	5/0
	Metastasis: Lymph node	1/0
Esophagus	Adenocarcinoma (Barrett carcinoma), squamous carcinoma	4/0
Prostate	Adenocarcinoma	5/0
Breast	Primary tumor: Invasive ductal carcinoma	4/0
	Metastases: Lung, lymph node	2/0
Melanoma	Metastasis: Malignant melanoma	5/0
Bladder	Urothelial carcinoma	3/0
Testis	Seminoma	3/0
B. Nonmalignant tissue		
Tested tissue	Cell type	+/-
Lung	Glandular, alveolar	0/4
Breast	Glandular	0/3
Colon	Glandular	0/5
Stomach	Glandular	0/3
Pancreas	Glandular	0/5
Esophagus	Epithelial	0/4
Prostate	Glandular	0/3
Lymph node	Lymphocytes	0/2
Uterus	Epithelial	0/3
Testis	Glandular	0/3

anti-TAF15) were carried out. One assay was preincubated with the opponent antibody to check binding interactions, and the other one without the opponent antibody. By comparing the different assays, there was no blocking effect detected. Both antibodies showed a constant band (Fig. 4A). This means that PAT-BA4 and TAF15 do not have the same epitope.

Another aspect is the location of the 78-kDa TAF15. Thus far, TAF15 protein has been found only in nucleus and cytoplasm of numerous cell types (12). This distribution seems to be adapted to its cellular function in normal cells, as already described. In our Western blot assay, TAF15 protein was iso-

lated from the membrane protein fraction. Therefore, it is supposed to be located on the cell surface. A FACS analysis with living BxPC-3 cells was performed to prove the surface location of TAF15. The cells were incubated with either PAT-BA4 or anti-TAF15 antibody. An anti-CD55 antibody (DAF) served as positive control (data not shown). FACS analyses with PAT-BA4 and anti-TAF15 showed a peak shift in contrast to isotype controls based on tumor cell membrane binding (Fig. 4B). The same FACS assay was performed with living lymphocytes to check TAF15 location on nonmalignant cells. Anti-CD45 antibody (Dako) was used as positive control (data not shown). In contrast to malignant cells, FACS

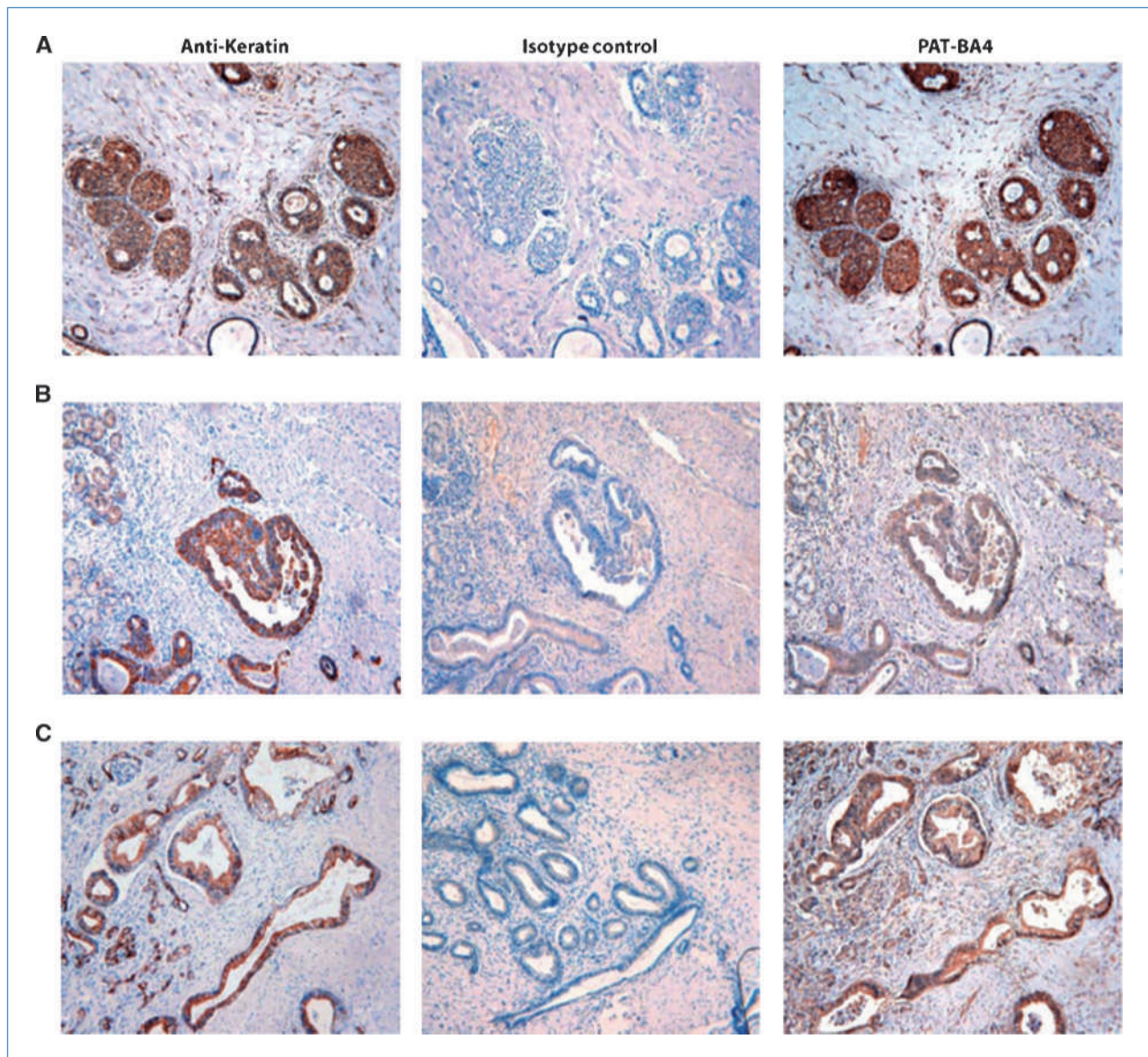


Figure 1. Immunohistochemical staining of different human adenocarcinoma tissues. Paraffin-embedded sections were incubated with PAT-BA4 antibody or unrelated human IgG antibody as isotype control. Anti-cytokeratin antibody was used for morphologic studies and served as positive control. A, breast adenocarcinoma tissue. B, stomach adenocarcinoma tissue. C, pancreas adenocarcinoma tissue.

analysis with lymphocytes showed no PAT-BA4 and anti-TAF15 antibody binding (Fig. 4B). These results imply that TAF15 protein is present on the cell surface of malignant cells but absent on the cell surface of nonmalignant cells.

To get closer information about TAF15 protein distribution pattern in malignant and nonmalignant cells, an immunohistochemical staining with paraffin sections was accomplished. Therefore, nonmalignant and malignant pancreas tissue was stained with either PAT-BA4 or anti-TAF15 antibody (Fig. 4C). The following differences could be observed: The tumor-specific PAT-BA4 does not bind to normal tissue but to cell membrane of cancer tissue. Commercial

anti-TAF15 binds to nuclei of normal tissue and to nuclei and cell membranes of malignant tissue. This might indicate that PAT-BA4, in contrast to commercial antibody, binds to a tumor-specific variant of TAF15. PAT-BA4 probably binds to a modified TAF15 protein, which does not exist in normal tissue. The nuclei of normal and malignant cells, stained by commercial anti-TAF15 antibody, might contain the wild-type variant lacking the PAT-BA4 epitope. Therefore, anti-TAF15 antibody can bind to both the nuclear as well as the membrane TAF15 form.

Overexpression of TAF15 in malignant cells and tissue. The protein expression of TAF15 on malignant and

nonmalignant cells was determined by Western blot analysis. Cell lysates, made of an identical amount (1.2×10^6) of BxPC-3 tumor cells or lymphocytes, were used. On both blots, the 78-kDa band was detected with anti-TAF15 antibody ($3 \mu\text{g}/\text{mL}$; AVIVA Systems Biology). The malignant cells have, in contrast to the nonmalignant cells, a stronger band based on a higher TAF15 protein expression (Fig. 5A). Due to the fact that nonmalignant cells also have a 78-kDa TAF15 band, there is no further clue for a modification in this assay. These results lead to the assumption that there is no mod-

ification or else a small modification is not detectable by Western blot analysis.

On mRNA level, the TAF15 expression was determined by semiquantitative PCR. Human malignant and nonmalignant lung tissue was used for mRNA isolation. The constitutively expressed "housekeeping" gene *GAPDH* served as internal standard for gene expression of TAF15 in malignant compared with nonmalignant lung tissue. The TAF15 mRNA level was increased in malignant tissue in comparison with nonmalignant tissue (Fig. 5B).

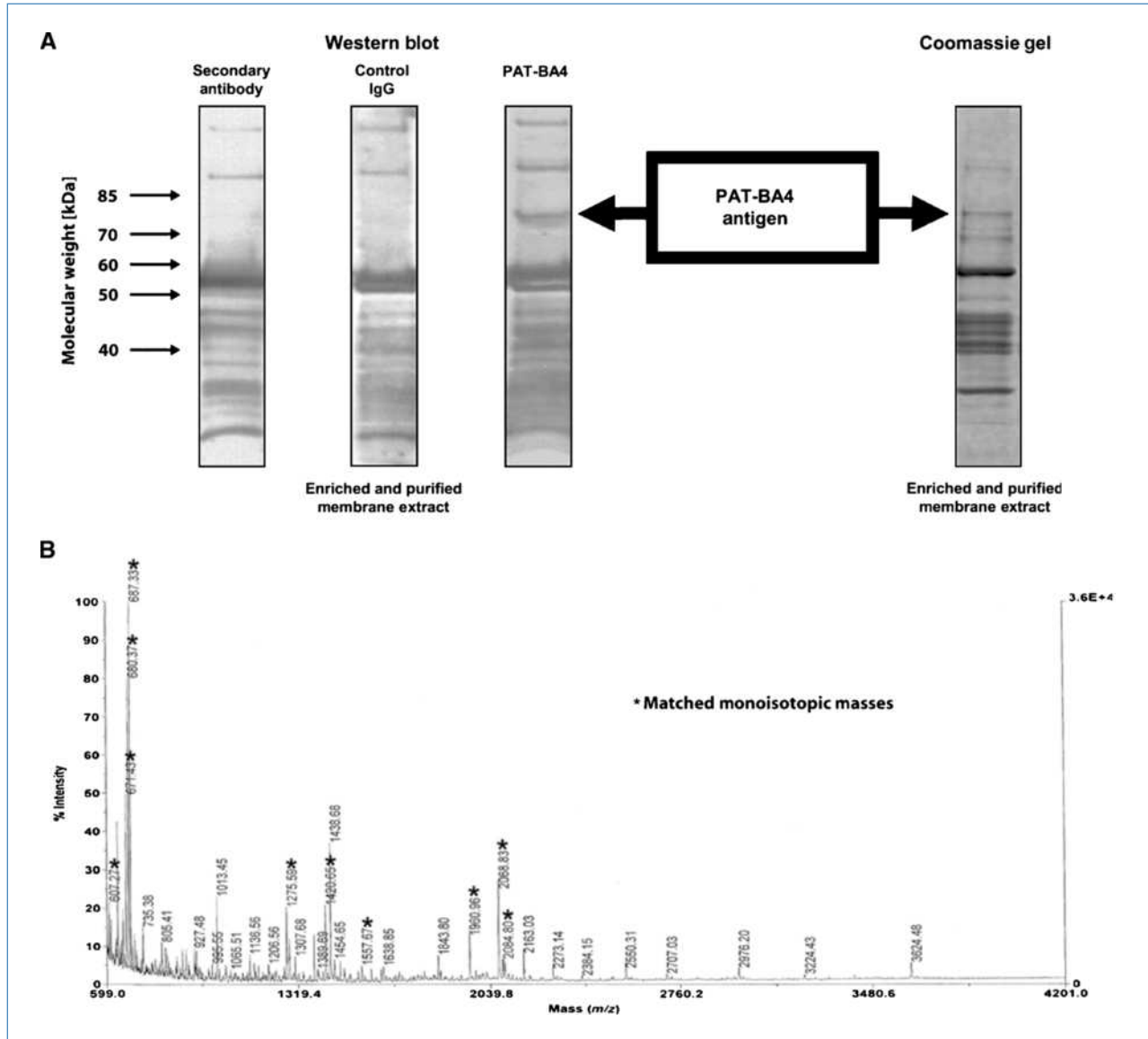


Figure 2. Purification and identification of PAT-BA4 antigen by affinity chromatography and MALDI mass spectrometry. **A**, protein purification of PAT-BA4 antigen from pooled membrane extracts of pancreas carcinoma cell line BxPC-3. Purified protein eluate was gained by affinity chromatography with crude membrane extracts. Western blot analysis was done with only secondary antibody, control IgG, and PAT-BA4 antibody. A Coomassie gel of purified protein eluate was performed. The protein band marked with an arrow was excised from Coomassie-stained gel. **B**, identification of the excised 78-kDa protein band by MALDI peptide mass mapping. The peaks with asterisks matched the calculated masses of tryptic peptides of human TAF15 (NP_631961.1 isoform 1/NP_003478.1 isoform 2). Thereby, the peptide mass error was <30 ppm and the minimum sequence coverage of the corresponding amino acids equates 22%.

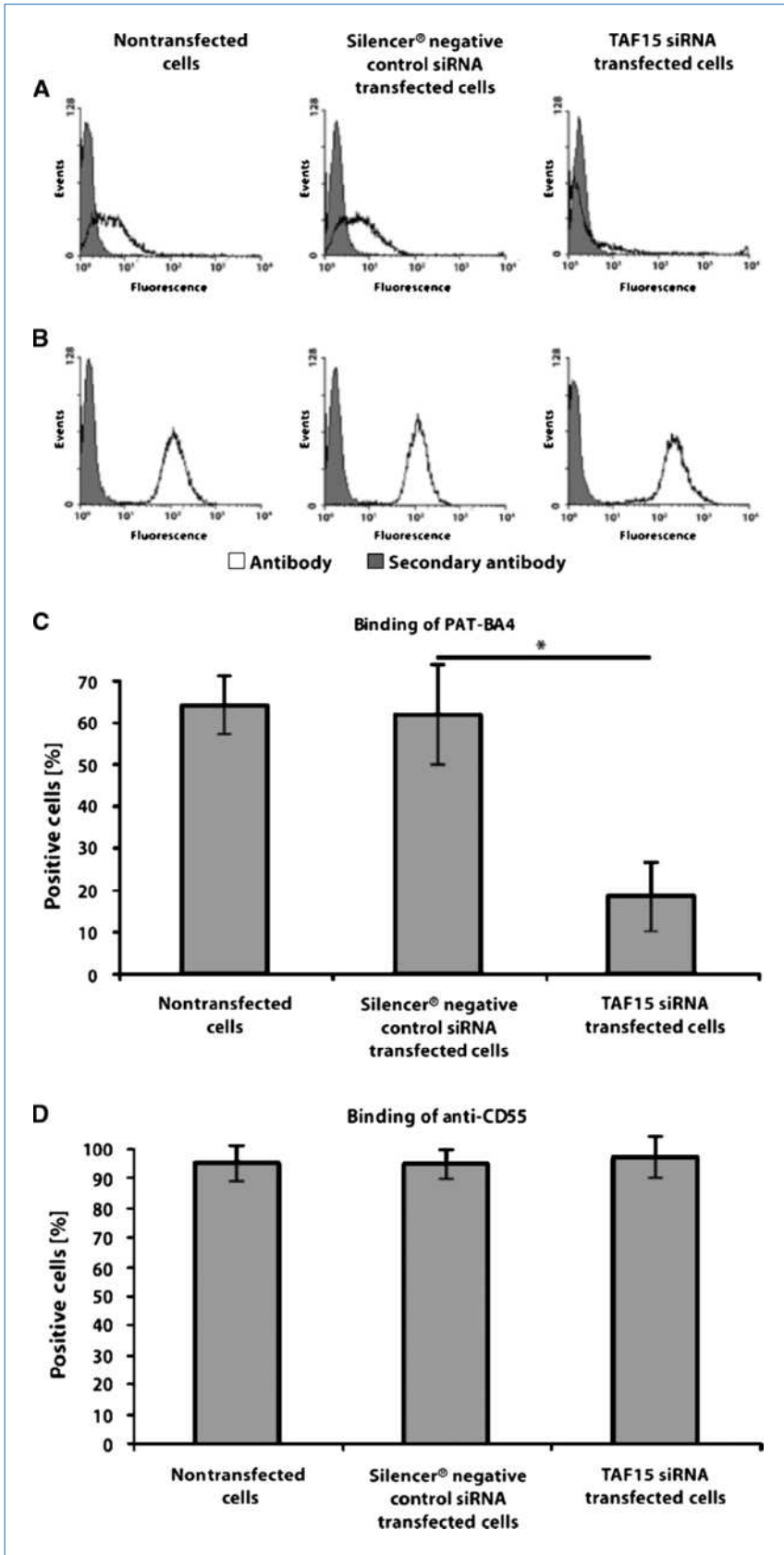
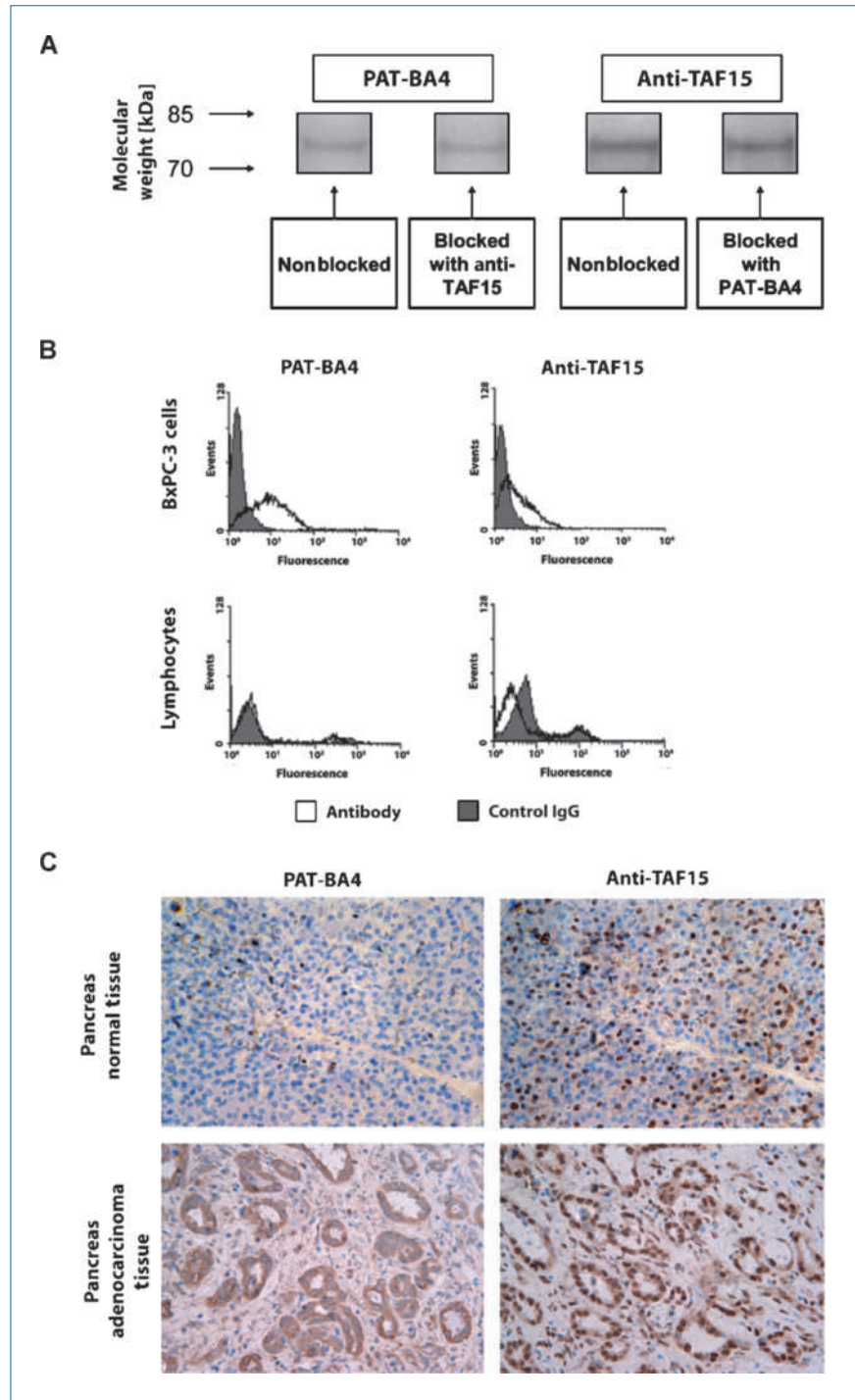


Figure 3. Validation of TAF15 as target for PAT-BA4 antibody by siRNA technology. Pancreas carcinoma cell line BxPC-3 was transfected with siRNA against human TAF15. Forty-eight hours after transfection, protein level of TAF15 was monitored by FACS analysis. Nontransfected cells and cells transfected with scrambled siRNA showed a positive binding of (A) PAT-BA4 antibody and (B) anti-CD55 antibody. TAF15 siRNA-transfected cells showed a reduced PAT-BA4 antibody binding but an unchanged binding of anti-CD55 antibody. Percentages of cell surface antigens of (C) PAT-BA4 and (D) anti-CD55 antibody are depicted. Columns, mean; bars, SD. *, $P < 0.05$.

Figure 4. Comparison of TAF15^{PAT-BA4} and wild-type TAF15 by Western blotting, FACS analysis, and immunohistochemical staining. **A**, Western blot inhibition assay with PAT-BA4 (300 µg/mL) and anti-TAF15 (3 µg/mL) antibody. One assay of each antibody was preincubated with the opponent antibody to check blocking effects; the other one was performed without the opponent antibody. **B**, FACS analyses with either PAT-BA4 (300 µg/mL) or commercial anti-TAF15 antibody (30 µg/mL; Santa Cruz Biotechnology) were performed on BxPC-3 cells and lymphocytes. Both assays were done with the same cell amount (2×10^5) and the same antibody concentrations. **C**, immunohistochemical staining of pancreas normal tissue and pancreas adenocarcinoma tissue. The paraffin-embedded sections were incubated with either PAT-BA4 (100 µg/mL) or anti-TAF15 antibody (1:25).



PAT-BA4 function in tumor cell spreading and tumor cell motility. TAF15 protein has been described in correlation with cell spreading and cell adhesion (12), important features for malignancy. To check the influence of PAT-BA4 antibody on these malignant processes, two different assays were performed. The cell adhesion was checked using adherent growing BxPC-3 cells. For engraftment, the tumor

cells were immediately incubated with PAT-BA4 antibody. The adherent cells were counted after different periods of time. As negative controls, cells in complete RPMI 1640 or unrelated Chrompure human IgG were used. The cells grown in complete RPMI 1640 were adjusted to 100%. In the PAT-BA4 assay, the cell amount was reduced over 20% after 45 minutes compared with cells grown in complete RPMI

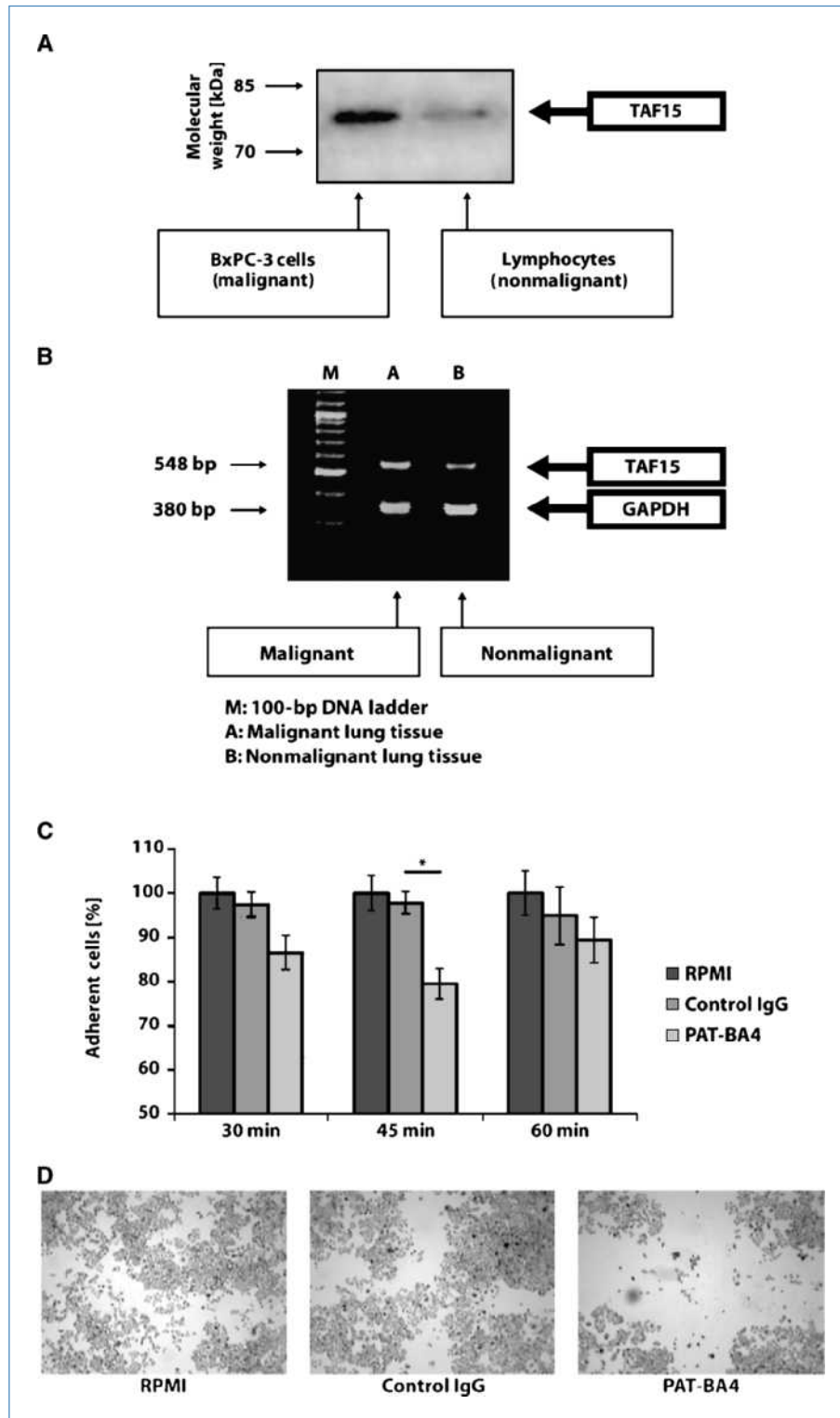


Figure 5. Important characteristics of TAF15 protein and PAT-BA4 antibody. Expression of TAF15 on (A) protein and (B) mRNA level. A, Western blot analysis with anti-TAF15 (3 µg/mL) antibody on cell lysates of BxPC-3 cells and lymphocytes made of the same cell amount (1.2×10^6). B, semiquantitative PCR with mRNA from malignant and nonmalignant lung tissue. GAPDH mRNA level was used as internal standard. Functional assays with PAT-BA4 on BxPC-3 cells. C, histogram of adhesion assay with BxPC-3 cells. For tumor cell engraftment, the BxPC-3 cells were incubated with PAT-BA4 antibody. Chrompure human IgG served as isotype control. The amount of adherent cells was determined after different periods of time. Untreated cells grown in complete RPMI 1640 were adjusted to 100%. D, motility assay with BxPC-3 cells. The tumor cells were grown in 24-well plates to a confluence of 80%. A cross was then scratched into the cell monolayer and PAT-BA4 antibody was added. Complete RPMI 1640 and unrelated human IgG served as negative controls. Columns, mean; bars, SD. *, $P < 0.05$.

1640 (Fig. 5C). This shows an inhibition of PAT-BA4 in tumor cell adhesion.

To check tumor cell mobility, BxPC-3 cells were cultured in a 24-well plate. After a confluence of 80%, a cross was

scratched into the cells and immediately incubated with PAT-BA4. Complete RPMI 1640 and Chrompure human IgG served as negative controls. In contrast to both controls, the cross was still clearly visible in the PAT-BA4 assay 24 hours

after incubation. These results confirm that PAT-BA4 inhibits the migration of the tumor cells into the cross by blocking tumor cell movement (Fig. 5D).

Discussion

In this article, we showed that the natural IgG antibody PAT-BA4 binds to TAF15, a member of the TET family. PAT-BA4 is a germ-line-coded IgG antibody and therefore belongs to the innate immune system. It binds tumor specifically to the detected 78-kDa TAF15 variant. This variant is only located on the cell surface of malignant cells as shown in FACS analyses of cancer cells and lymphocytes. About the functions of TAF15 in normal cells, this is not surprising. Whether the antigen is additionally tumor specifically modified like some IgM antigens is not clarified thus far (5).

The multifunctional TAF15 protein is, as already described, implemented in cellular processes concerning malignant cells. We found an overexpression of TAF15 in malignant cells on the protein level and on the mRNA level. Therefore, an amazing functional aspect is that TAF15 protein seems to be involved in cellular stress response (12). Cells overexpressing TAF15 formed cytoplasmatic aggregates that were colocalized with stress granule marker TIA-1. The protein has also been detected in stress granules together with TIA-1 after exposing cells to heat shock and oxidative stress (12, 27). Environmental stress plays a very important role in malignant cells. Due to a poor vascularization (28–30) and immunologic attacks (30–32), malignant cells are permanently exposed to stress. Therefore, it is not surprising that TAF15 is overexpressed in malignant cells.

Furthermore, TAF15 is also supposed to be involved in cell spreading and cell adhesion. The protein was found colocalized together with the focal adhesion/spreading initiation center markers vinculin, FAK, and RACK1 in large cytoplasmatic granules near the plasma membrane (12, 33). Cell spreading and cell adhesion are known to play an important role in cancerogenesis, especially in metastasis (34). Cell spreading is necessary for tumor cell migration into the bloodstream and tumor cell invasion to new tissue areas. Cell adhesion helps the intravascularly located tumor cells in the blood to affix them onto vascular endothelia. In functional assays, it was shown that PAT-BA4 antibody inhibits both features of malignancy. This aspect provides PAT-BA4 a potent antitumor effect by directly attacking characteristic cancer properties.

Over millions of years, nature has created an excellent immune surveillance system that guarantees elimination of most dangerous cells and particles at an early stage. Natural Igs play a very important role in these processes. Due to their low affinity, these antibodies are able to detect not a single epitope but instead a specific set of antigens. IgMs represent the major group; only a small percentage is IgG or IgA (together <1%; refs. 5, 31).

Several natural IgM antibodies and the corresponding antigens have yet been described. The antibody SC-1 detects a post-translationally modified version of CD55 (also known as DAF). This glycosylphosphatidylinositol-linked membrane molecule is responsible for the protection against comple-

ment. On cancer cells, in addition to the “wild-type CD55,” the modified version is coexpressed. This increases the protection capability against autologous complement (25). The antigen of the natural human antibody PAM-1 is a membrane-bound growth factor receptor called CFR-1. A “normal” counterpart has thus far not been detected, but it can be assumed that new receptors for growth factors positively influence the capacity of cellular growth (35). Another IgM antibody, SAM6, binds to the heat shock protein (HSP) GRP78, a member of the HSP70 family. These HSP molecules are involved in protection against cellular stress factors, and malignant cells often overexpress them on the surface (30). In all three cases, the epitopes are N- or O-linked sugars and the molecules are permanently expressed on the surface of malignant cells and are, in this specific version, absent on normal cells.

In contrast to this IgM target molecules, TAF15 seems to play a role in cellular adhesion and movement, processes that are not permanently used by cells. Another potentially important difference is the fact that all thus far isolated IgM antibodies bind to carbohydrate epitopes. The epitope for PAT-BA4 is unknown, but it is not a sugar (data not shown).

The heterogeneity of Ig classes seems to follow the need of different localization and function of the innate immunity. It is therefore likely that the small set of IgGs plays a different role in this game compared with other Ig classes. However, this remains speculative and more information is needed to support any explanation.

Taken our findings together, we have isolated a natural monoclonal IgG antibody from a cancer patient. This antibody detects a tumor-specific molecule that is normally localized in the nucleus and cytoplasm. But when expressed on malignant cells, it seems to be involved in adhesion and motility processes. These results show that human monoclonal antibodies, directly isolated from cancer patients, are good tools for the identification of new tumor-specific targets.

Disclosure of Potential Conflicts of Interest

F. Hensel, employee of Patrys, has supported the study with purified PAT-BA4 antibody. S. Brändlein and H.P. Vollmers are consultants of Patrys. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The other authors disclosed no potential conflicts of interest.

Acknowledgments

We thank Annette Vollmers for improving the manuscript.

Grant Support

Patrys, Inc. (Melbourne, Victoria, Australia).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 6/16/09; revised 9/24/09; accepted 10/22/09; published online 1/4/10.

References

1. Moretta L, Biassoni R, Bottino C, Mingari MC, Moretta A. Natural killer cells: a mystery no more. *Scand J Immunol* 2002;55:229–32.
2. Greenberg S, Grinstein S. Phagocytosis and innate immunity. *Curr Opin Immunol* 2002;14:136–45.
3. Mekori YA, Metcalfe DD. Mast cells in innate immunity. *Immunol Rev* 2000;173:131–40.
4. Vollmers HP, Brändlein S. Nature's best weapons to fight cancer. Revival of human monoclonal IgM antibodies. *Hum Antibodies* 2002;11:131–42.
5. Brändlein S, Pohle T, Ruoff N, Wozniak E, Müller-Hermelink HK, Vollmers HP. Natural IgM antibodies and immunosurveillance mechanisms against epithelial cancer cells in humans. *Cancer Res* 2003;63:7995–8005.
6. Guipaud O, Guillonnet F, Labas V, et al. An *in vitro* enzymatic assay coupled to proteomics analysis reveals a new DNA processing activity for Ewing sarcoma and TAF(II)68 proteins. *Proteomics* 2006;6:5962–72.
7. Bertolotti A, Lutz Y, Heard DJ, Chambon P, Tora L. hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncogenes TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. *EMBO J* 1996;15:5022–31.
8. Stolow DT, Haynes SR. Cabeza, a *Drosophila* gene encoding a novel RNA binding protein, shares homology with EWS and TLS, two genes involved in human sarcoma formation. *Nucleic Acids Res* 1995;23:835–43.
9. Zinszner H, Immanuel D, Yin Y, Liang FX, Ron D. A topogenic role for the oncogenic N-terminus of TLS: nucleolar localization when transcription is inhibited. *Oncogene* 1997;14:451–61.
10. Morohoshi F, Ootsuka Y, Arai K, et al. Genomic structure of the human RBP56/hTAFII68 and FUS/TLS genes. *Gene* 1998;221:191–8.
11. Morohoshi F, Arai K, Takahashi EI, Tanigami A, Ohki M. Cloning and mapping of a human RBP56 gene encoding a putative RNA binding protein similar to FUS/TLS and EWS proteins. *Genomics* 1996;38:51–7.
12. Andersson MK, Ståhlberg A, Arvidsson Y, et al. The multifunctional FUS, EWS and TAF15 proto-oncogenes show cell type-specific expression patterns and involvement in cell spreading and stress response. *BMC Cell Biol* 2008;9:37.
13. Law WJ, Cann KL, Hicks GG. TLS, EWS and TAF15: a model for transcriptional integration of gene expression. *Brief Funct Genomic Proteomic* 2006;5:8–14.
14. Gregory RI, Yan KP, Amuthan G, et al. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 2004;432:235–40.
15. Shiohama A, Sasaki T, Noda S, Minoshima S, Shimizu N. Nucleolar localization of DGCR8 and identification of eleven DGCR8-associated proteins. *Exp Cell Res* 2007;313:4196–207.
16. Crozat A, Aman P, Mandahl N, Ron D. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature* 1993;363:640–4.
17. Rabbitts TH, Forster A, Larson R, Nathan P. Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. *Nat Genet* 1993;4:175–80.
18. Delattre O, Zucman J, Plougastel B, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature* 1992;359:162–5.
19. Hisaoka M, Ishida T, Imamura T, Hashimoto H. TFG is a novel fusion partner of NOR1 in extraskeletal myxoid chondrosarcoma. *Genes Chromosomes Cancer* 2004;40:325–8.
20. Sjögren H, Meis-Kindblom J, Kindblom LG, Aman P, Stenman G. Fusion of the EWS-related gene TAF2N to TEC in extraskeletal myxoid chondrosarcoma. *Cancer Res* 1999;59:5064–7.
21. Attwooll C, Tariq M, Harris M, Coyne JD, Telford N, Varley JM. Identification of a novel fusion gene involving hTAFII68 and CHN from a t(9;17)(q22;q11.2) translocation in an extraskeletal myxoid chondrosarcoma. *Oncogene* 1999;18:7599–601.
22. Martini A, La Starza R, Janssen H, et al. Recurrent rearrangement of the Ewing's sarcoma gene, EWSR1, or its homologue, TAF15, with the transcription factor CIZ/NMP4 in acute leukemia. *Cancer Res* 2002;62:5408–12.
23. Faller G, Vollmers HP, Weiglein I, et al. HAB-1, a new heteromyeloma for continuous production of human monoclonal antibodies. *Br J Cancer* 1990;62:595–8.
24. Brändlein S, Rauschert N, Rasche L, et al. The human IgM antibody SAM-6 induces tumor-specific apoptosis with oxidized low-density lipoprotein. *Mol Cancer Ther* 2007;6:326–33.
25. Hensel F, Hermann R, Schubert C, et al. Characterization of glycosylphosphatidylinositol-linked molecule CD55/decay-accelerating factor as the receptor for antibody SC-1-induced apoptosis. *Cancer Res* 1999;59:5299–306.
26. Brändlein S, Beyer I, Eck M, et al. Cysteine-rich fibroblast growth factor receptor 1, a new marker for precancerous epithelial lesions defined by the human monoclonal antibody PAM-1. *Cancer Res* 2003;63:2052–61.
27. Kedersha NL, Gupta M, Li W, Miller I, Anderson P. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 α to the assembly of mammalian stress granules. *J Cell Biol* 1999;147:1431–42.
28. Feldman DE, Chauhan V, Koong AC. The unfolded protein response: a novel component of the hypoxic stress response in tumors. *Mol Cancer Res* 2005;3:597–605.
29. Koumenis C. ER stress, hypoxia tolerance and tumor progression. *Curr Mol Med* 2006;6:55–69.
30. Rauschert N, Brändlein S, Holzinger E, Hensel F, Müller-Hermelink HK, Vollmers HP. A new tumor-specific variant of GRP78 as target for antibody-based therapy. *Lab Invest* 2008;88:375–86.
31. Vollmers HP, Brändlein S. The "early birds": natural IgM antibodies and immune surveillance. *Histol Histopathol* 2005;20:927–37.
32. Vollmers HP, Brändlein S. Death by stress: natural IgM-induced apoptosis. *Methods Find Exp Clin Pharmacol* 2005;27:185–91.
33. de Hoog CL, Foster LJ, Mann M. RNA and RNA binding proteins participate in early stages of cell spreading through spreading initiation centers. *Cell* 2004;117:649–62.
34. Vollmers HP, Birchmeier W. Monoclonal antibodies inhibit the adhesion of mouse B16 melanoma cells *in vitro* and block lung metastasis *in vivo*. *Proc Natl Acad Sci U S A* 1983;80:3729–33.
35. Hensel F, Brändlein S, Eck M, et al. A novel proliferation-associated variant of CFR-1 defined by a human monoclonal antibody. *Lab Invest* 2001;81:1097–108.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Diagnostic and Therapeutic Potential of a Human Antibody Cloned from a Cancer Patient That Binds to a Tumor-Specific Variant of Transcription Factor TAF15

Nicole Schatz, Stephanie Brändlein, Kilian Rückl, et al.

Cancer Res 2010;70:398-408.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/70/1/398>

Cited articles This article cites 35 articles, 9 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/70/1/398.full#ref-list-1>

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/70/1/398.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/70/1/398>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.