the objective response [43]. Our experience of GDC as the third-line chemotherapy in a patient with CDDP-resistant bladder cancer was compatible with the partial response (Fig. 3). When emphasizing the healthy balance between chemotherapeutic efficacy and maintenance of QOL, the combination chemotherapy of taxanes and GEM might be more acceptable than CDDP-based chemotherapy, because of the therapeutic superiority coupled with lower toxicity involving gastrointestinal tract and/or kidney function in the former.

A phase II trial of IFM monotherapy as second-line chemotherapy showed an overall RR of 20%, while the combination of IFM and GEM in cases with

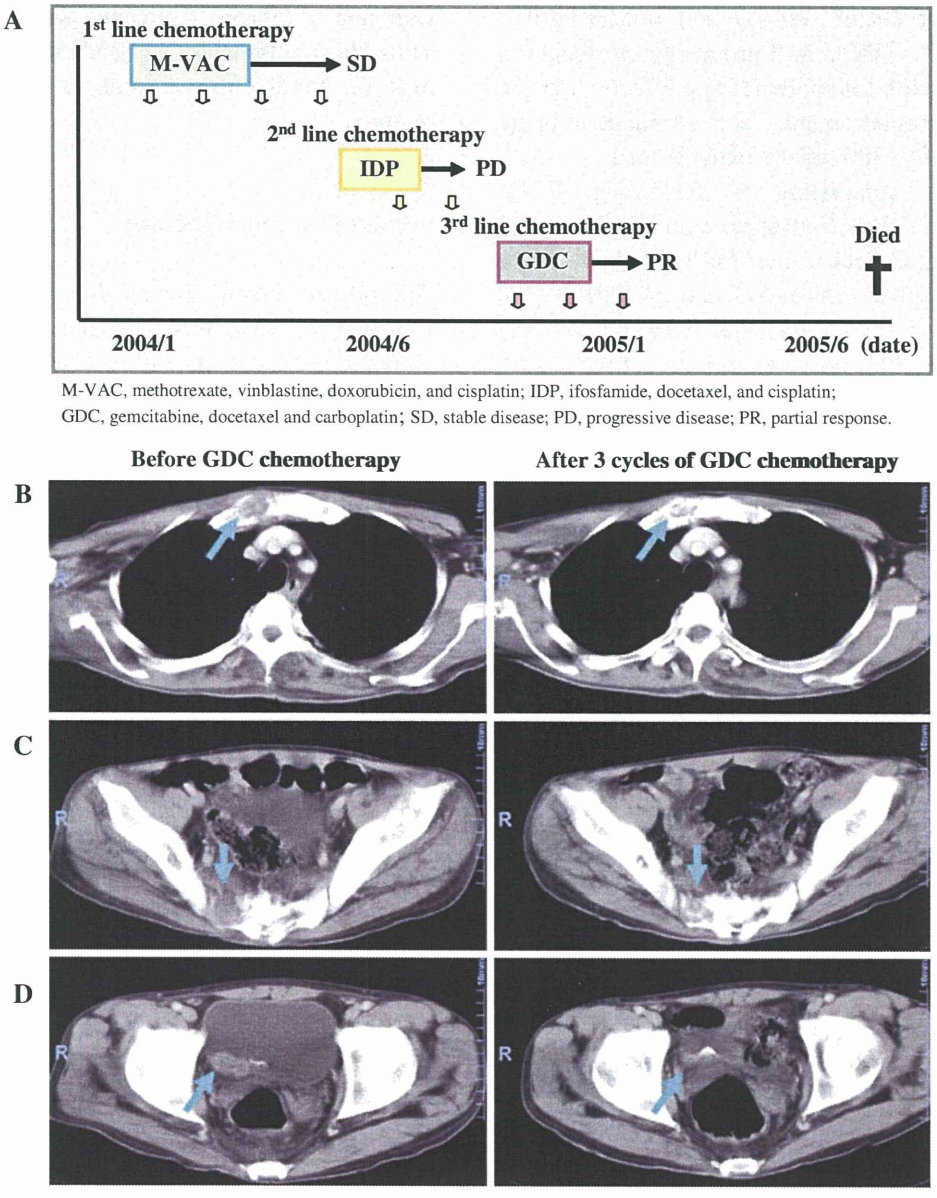

Fig. 3 Representative case of having GDC chemotherapy was shown. A 50-year-old man with diagnosis of bladder cancer (T3aN0M1) was treated with 3 cycles of GDC chemotherapy as third-line treatment, following CDDP-based chemotherapy [M-VAC and IDP (IFM, DOC, and CDDP)]. Schema of clinical course with depiction of chemotherapeutic strategy was shown (**a**). As was evident on CT images, metastatic spread to the sternum (**b**) and sacrum (**c**) was decreased in size after 3 cycles of GDC therapy. Likewise, primary lesion showed partial response after 3 cycles of GDC therapy (**d**); however, progressive chemoresistant disease resulted in multiple organ failure 7 months after GDC chemotherapy

recurrence after CDDP/CBDCA or taxane-based chemotherapy had an overall RR of 21%, with a median TTP of 4 months and survival period of 9 months [44]. In this trial, it is noteworthy that the majority of cases showed subjective improvements in cancer-related symptoms, despite grade 3/4 myelosuppression [36]. Nedaplatin (CDGP), an analog of CDDP developed in Japan, does not exhibit cross-resistance to CDDP and does not show significant adverse effects on kidney and gastrointestinal tract functions [45]. On the basis of the superior clinical effects of IFM as second-line therapy in addition to the excellent overall RR of ITP therapy for urothelial cancer, as noted previously, several trials have been conducted to validate the clinical benefits of a combination approach of CDGP, PTX, and IFM (PIN therapy). Although only a small number of cases were studied, this second-line PIN therapy for chemoresistant cases demonstrated an excellent overall RR of 82% [46]. Also, no chemotherapy-related death was encountered in that study, in spite of the fact that all cases exhibited grade 4 neutrocytopenia, indicating second-line PIN therapy may be applicable for CDDP-resistant cases [47].

Vinflunine is a novel microtubule inhibitor of the vinca alkaloid class that has more activity than vinblastine or vinorelbine [48–50]. Several phase II trials of vinflunine monotherapy after first-line platinum-containing regimen showed an overall RR of 15–18%, with a median TTP of 2.8–3.0 months and OS of 6.6–8.2 months [51, 52]. This regimen included several adverse effects such as grade 3/4 neutropenia, constipation, and asthenia/fatigue, which frequently occurred, but every toxicity was not severe and tolerable. These results indicate that vinflunine is moderately active and has a manageable toxicity in platinum-pretreated patients with advanced bladder cancer, and further randomized study is required.

Chemotherapy for cases with impaired renal function or unfit cases

The majority of bladder cancer patients are elderly; thus, they may already have impaired renal, cardiac, and respiratory functions, and/or potential reductions in bone marrow function or general condition, as reflected by worsened PS. In these cases, administration of CDDP appears to be contraindicated. To overcome the negative impact of standard chemotherapy, CBDCA, with less nephrotoxicity, or GEM which is metabolized in the liver, can be well substituted for CDDP. In a chemotherapeutic strategy of CBDCA, GEM, or taxanes in either a monotherapy or combination approach, an inferior overall RR remains an issue of concern. However, it has not been confirmed whether an inferior overall RR is a reflection of merely impaired renal function or potential problems relating to the combination of chemotherapeutic agents. A small trial of combination chemotherapy with GEM and CBDCA showed an overall RR of 44–56%, as well as treatable myelosuppression [53, 54]. More recently, the phase II/II EORTC-30986 trial including patients with worsened PS and/or impaired renal function showed that the combination chemotherapies of GEM with CBDCA (GCA) and CBDA + MTX + VBL (M-CAVI) provided good overall RR results (GCA: 42%, M-CAVI: 30%) in these so-called "unfit" patients, though patients with both worsened PS and impaired renal function did not benefit [55]. Thus far, investigations into alternative treatment regimens for such unfit cases remain challenging.

Combination with molecular target therapies

Systemic chemotherapy for progressive bladder cancer has made steady progress since M-VAC therapy was first reported in 1983, though that has not translated into improved DFS. In recent years, based on the understanding of cancer pathogenesis at the molecular level, some studies have investigated the efficacy of molecular target therapies in patients with progressive advanced urothelial cancer (Table 1).

Epidermal growth factor receptor (EGFR), which has been identified in certain bladder cancers, is a tyrosine kinase transmembrane receptor that facilitates tumor growth and represses tumor apoptosis in vivo [56]. Gefitinib is an orally active EGFR tyrosine kinase inhibitor. In a phase II trial of gefitinib, an overall RR of 3% was shown, along with rash, general fatigue, diarrhea, anemia, cerebral ischemic disorder, and elevation of serum creatinine level [57]. In addition, a phase II trial of the combination approach of CDDP, GEM, and gefitinib for first-line chemotherapy demonstrated an overall RR of 43%, with a median TTP of 7.4 months, which was not confirmed as a result of substantial benefits added by

Table 1 Phase II trial of molecular target therapy for advanced and/or metastatic urothelial cancer

Source	Regimens	Setting	No. of patients	ORR (%)	MPFS (mo)	MST (mo)
Petrylak et al. [57]	Gefitinib (500 mg/day orally)	2nd line	31	3.0	2.0	3.0
Philips et al. [58]	Gefitinib (500 mg/day orally)	1st line	58	42.6	7.4	15.1
	Cisplatin (70 mg/m^2 day 1 i.v.)					
	Gemcitabine (1,000 mg/m^2 day 1, 8 i.v.)					
Wülfing et al. [62]	Lapatinib (1,250 mg/day orally)	2nd line	59	1.7	2.2	4.5
Hussain et al. [63]	Trastuzumab (4 mg/kg day 1, 8, 15 i.v.)	1st line	44	70	9.3	14.1
	Paclitaxel (200 mg/m^2 day 1 i.v.)					
	Carboplatin (AUC5 day 1 i.v.)					
	Gemcitabine (800 mg/m^2 day 1, 8 i.v.)					
Dreicer et al. [66]	Sorafenib (400 mg × 2/day orally)	2nd line	27	0	2.2	6.8
Sridhar et al. [67]	Sorafenib (400 mg × 2/day orally)	1st line	17	0	1.9	5.9
Gallagher et al. [68]	Group A	2nd line	45	7.0	2.4	7.1
	Sunitinib (50 mg/day for 4 weeks on and 2 weeks off orally)					
	Group B	2nd line	32	3.0	2.3	6.0
	Sunitinib (37.5 mg/day continuously)					
Hahn et al. [69]	Bevacizumab (15 mg/kg day 1 i.v.)	1st line	45	72	8.2	19.1
	Cisplatin (70 mg/m^2 day 1 i.v.)					
	Gemcitabine (1,000 mg/m^2 day 1, 8 i.v.)					

ORR overall response rate, *MPFS* median progression-free survival, *MST* median survival time, *mo* month

gefitinib [58]. This study demonstrated that gefitinib used with conventional chemotherapeutic agents did not bring additional therapeutic effects and may be ineffective for bladder cancer.

HER-2/neu is another transmembrane tyrosine kinase receptor, which is frequently overexpressed in bladder cancer and associated with poor prognosis [59, 60]. Lapatinib (GW572016) is a dual tyrosine kinase inhibitor that inhibits both EGFR and HER-2/neu. In bladder cancer cell lines, lapatinib was found to be a potent inhibitor of EGF-induced activation of HER-2/neu signaling [61]. A phase II trial of lapatinib monotherapy as second-line therapy found an overall RR of 2%, with a median TTP of 8.6 weeks and OS of 17.9 weeks, though there was not significant correlation between clinical benefits and EGFR and/or HER-2/neu expression [62]. On the other hand, a multicenter phase II trial of combination chemotherapy with PTX, CBDCA, GEM, and trastuzumab (a humanized monoclonal antibody that binds to HER-2/neu) reported an overall RR of 70.0%, with a median TTP of 9.3 months and OS of 14.1 months [63]. However, adverse effects included grade 3/4 neutropenia (86%), thrombocytopenia (70%), and two chemotherapy-related deaths.

Vascular endothelial growth factor receptor (VEGFR) is expressed in urothelial cancer, and recent studies suggest that signaling through VEGFR-2 may directly enhance tumorigenesis [64, 65]. Sorafenib is an oral multikinase inhibitor that blocks angiogenesis by targeting VEGFR-2 and 3, and the platelet-derived growth factor receptor-beta. In a phase II study of sorafenib monotherapy as first- or second-line therapy, no response was observed, while adverse events included fatigue, hand–foot reactions, and grade 4 pulmonary embolism [66, 67]. Sunitinib is also a multitargeted tyrosine kinase inhibitor that selectively inhibits VEGFR-1, 2, and 3. A phase II study of sunitinib monotherapy as second-line therapy demonstrated an overall RR of 7%, with a median TTP of 2.4 months [68]. Bevacizumab is a recombinant monoclonal antibody for circulating VEGF-A. Although the Hoosier Oncology Group showed significant antitumor efficacy of bevacizumab under combination strategy with GC as first-line therapy for

patients with metastatic UC, the additional effect of bevacizumab with GC on metastatic UC appears to be limited owing to unexpectedly significant treatment-related toxicity [69].

Some of the major molecular targeting drugs currently available seem to be effective on advanced urothelial cancer. Management strategies might be based on the individualized molecular alternations in addition to histopathologic features.

Conclusion

M-VAC therapy has been established as a standard systemic chemotherapy treatment for bladder cancer, though its adverse effects and poor long-term outcome results remain challenging problems. In addition, effective treatment for the cases showing resistance to M-VAC or recurrent cases after first-line chemotherapy has not been established. Considering efficacy and tolerance, GC therapy is thought to be a promising substitution as compared to M-VAC. Furthermore, a combination of taxanes with GEM and/or platinum-based agents likely provides clinical benefits following M-VAC or GC therapy. In the future, additional investigations using various trial designs may lead to new therapeutic strategies with molecular target agents.

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Requirement for FBP17 in Invadopodia Formation by Invasive Bladder Tumor Cells

Hayato Yamamoto, Mihoko Sutoh, Shingo Hatakeyama, Yasuhiro Hashimoto, Takahiro Yoneyama, Takuya Koie, Hisao Saitoh, Kanemitsu Yamaya, Tomihisa Funyu, Toshiya Nakamura, Chikara Ohyama and Shigeru Tsuboi*

From the Department of Urology, Graduate School of Medicine (HY, SH, TY, TK, CO, ST) and Department of Biomedical Sciences, Graduate School of Health Sciences (TN), Hirosaki University and Departments of Urology (YH, HS, TF) and Biochemistry (MS, KY, TF), Oyokyo Kidney Research Institute, Hirosaki, Japan

Abbreviations and Acronyms

Arp3 = actin-related protein 3
dSH3 = SH3 domain deletion
EFC = extended FER-CIP4 homology
F-actin = filamentous actin
FBP = formin-binding protein
GFP = green fluorescent protein
HA = hemagglutinin
HEK293 = human embryonic kidney 293
K33E = lysine at 33 to glutamine substitution
PMSF = phenylmethylsulfonyl fluoride
SH3 = Src homology 3
WT = wild-type FBP17

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* Correspondence: Department of Urology, Hirosaki University Graduate School of Medicine, 5 Zaifucho, Hirosaki 036-8562, Japan (telephone: 81-172-39-5091; FAX: 81-172-39-5092; e-mail: urology@cc.hirosaki-u.ac.jp).

Purpose: Invadopodia (protrusions of the plasma membrane formed by invasive tumor cells) have an essential role in bladder tumor invasion. To understand the process of bladder tumor invasion it is crucial to investigate the molecular mechanisms of invadopodia formation. We found that invasive bladder tumor cells express FBP17. In this study we examined the role of FBP17 in bladder tumor cell invadopodia formation and invasion.

Materials and Methods: We used the 3 bladder tumor cell lines YTS-1, T24 and RT4 (ATCC®), and primary culture of bladder tumors from patients. Cells were stained with phalloidin for invadopodia formation. FBP17 knockdown cells were tested for invadopodia formation and subjected to invasion assay using a Transwell® cell culture chamber. We also examined the role of the extended FER-CIP4 homology and Src homology 3 domains of FBP17 in invadopodia formation in FBP17 mutant constructs.

Results: Invadopodia formation was observed in invasive bladder tumor cells and FBP17 was localized to invadopodia in invasive cells. FBP17 knockdown decreased invadopodia formation in invasive cells to 13% to 14% ($p < 0.0005$) and decreased their invasive capacity to 14% to 16% ($p < 0.001$). The extended FER-CIP4 homology and Src homology 3 domains of FBP17 were necessary for invadopodia formation and invasion.

Conclusions: Invadopodia formation requires membrane deformation activity and recruitment of dynamin-2 mediated by FBP17. FBP17 has a critical role in the process of bladder tumor cell invasion by mediating invadopodia formation.

Key Words: urinary bladder; urinary bladder neoplasms; neoplasm invasiveness; FNBP1 protein, human; cell surface extensions

BLADDER tumor is the fifth common tumor and the ninth leading cause of cancer death in males in the United States.[1] A large population of bladder tumor cases consists of noninvasive and superficial tumors that can be effectively treated with transurethral resection of the malignant lesions. Most deaths occur in patients with invasive bladder tumors since tumor invasion results in recurrence with metastasis.

Two types of actin based membrane protrusions, blebs and invadopodia, are implicated in tumor invasion. Tumor invasion involves the disruption of anatomical barriers and migration of tumor cells into normal adjacent host tissues. Blebs only mediate the latter process[2] but invado-

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podia mediate each process.[3] Invadopodia are the membrane protrusions enriched by F-actin, actin-binding proteins, matrix metalloproteases, separase and cell signaling molecules.[4,5] Invadopodia formation was observed in various types of cancer cells, such as melanoma, breast cancer, colon cancer, prostate cancer, and head and neck squamous cell carcinoma.[6–9] Recently we identified invadopodia formation by invasive bladder tumors.[10] To understand and control the tumor cell invasion process it is crucial to investigate the molecular mechanisms of invadopodia formation.

Formins function as actin nucleaters and polymerization factors of actin filaments.[11] FBPs regulate formin dependent actin assembly in vivo.[12] Of the 32 mammalian FBPs FBP17 mediates endocytosis by its membrane deformation activity.[13–15] We tested the possibility that FBP17 is involved in the formation of invadopodia and invasion of bladder tumor cells since invadopodia formation includes deformation of the plasma membrane.

MATERIALS AND METHODS

Reagents and Antibodies

Phenylmethylsulfonyl fluoride, leupeptin, pepstatin A, aprotinin, IGEPAL® CA-630, paraformaldehyde, saponin, bovine serum albumin, collagenase, fibronectin, Anti-FLAG® monoclonal antibody and anti-β-actin antibody were obtained from Sigma-Aldrich®. Anti-HA antibody (clone 12CA5) was obtained from Boehringer Ingelheim, Ridgefield, Connecticut. Anti-dynamin-2 and anti-Arp3 antibodies were obtained from BD Pharmingen™. Alexa Fluor® 488 labeled secondary antibodies, Alexa Fluor 568 labeled phalloidin and anti-FBP17 polyclonal antibody[14] were also used.

Cell Culture

We used the human bladder tumor cell lines YTS-1,[16] T24, RT4[17] and HEK293. The bladder tumor cell lines were maintained in RPMI-1640 medium (Sigma-Aldrich). HEK293 cells were cultured in DME high glucose medium (Invitrogen™). Medium was supplemented with 10% fetal bovine serum (PAA Laboratories, Pasching, Austria), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml Fungizone™ amphotericin B. Cells were grown in a humidified incubator with 5% CO_2 at 37C. Using the Myco-Probe® Mycoplasma Detection Kit we confirmed that no mycoplasmal contamination was detected before we started the experiments.

Primary Culture of Bladder Tumors

Invasive bladder tumors were removed by transurethral resection from several genetically independent patients at the Department of Urology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan. Tumors were incubated with RPMI-1640 medium containing 5% fetal bovine serum and 0.1% collagenase at 37C for 16 hours to prepare single cell suspensions. Cells were cultured on coverslips in a humidified incubator with 5% CO_2 at 37C. Tumor stage was determined according to the 2002 American Joint Committee for Cancer staging system. Written consent was obtained from all patients in this study. The Department of Urology, Hirosaki University Graduate School of Medicine institutional review board approved the experiments. The success rate of our primary culture of bladder tumors was 83%.

Transfection and RNA Interference

Cells were co-transfected with the FBP17 constructs and with the GFP expressing plasmid pmaxGFP™ (5:1 molar ratio) using Lipofectamine™ 2000. The targeting sequence for FBP17 was 5′-CCCACTTCATATGTCGAAGTCTGTT-3′.[13] Cells were co-transfected with siRNA and pmaxGFP (5:1 molar ratio) using Lipofectamine 2000 with pmaxGFP serving as a transfection marker. Transfection efficiency measured using pmaxGFP was 50% to 70%.

Immunofluorescence Microscopy

Cells seeded on coverslips were fixed in 4% paraformaldehyde and permeabilized with 0.1% saponin. Cells were incubated with Alexa 568 labeled phalloidin (1:50 dilution) and/or first antibodies (1:100 dilution) for 1 hour at room temperature. After extensive washing with phosphate buffered saline cells were incubated with Alexa 488 labeled secondary antibodies (1:100 dilution). Coverslips were placed on the slides (76 × 26 mm). Cell staining was examined using an IX-71 fluorescence microscope (Olympus®) and an LSM 710 confocal laser scanning microscope (Carl Zeiss, Oberkochen, Germany).

Immunoprecipitation

For FLAG-FBP17 immunoprecipitation 1×10^7 transfected cells were lysed in buffer A, composed of 50 mM tris-HCl (pH 7.5), 150 mM NaCl, 1% IGEPAL CA-630, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A and 1 μg/ml aprotinin. Total lysates were centrifuged at 13,000 rpm at 4C for 15 minutes. Supernatant was incubated with anti-FLAG monoclonal antibody-agarose (Sigma-Aldrich). The resin binding the immune complex was washed 3 times with 0.5 ml buffer A and the complex was then eluted with 1 × Laemmli sodium dodecylsulfate-polyacrylamide gel electrophoresis sample buffer. Eluted proteins were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis and analyzed by Western blot.

Invasion Assay

Transwell cell culture chambers were used for in vitro invasion assay.[18,19] The upper face of the filter was covered with 1 mg/ml Matrigel™ and the lower face was covered with 100 μg/ml fibronectin. Cells (5×10^4) were placed in the upper chamber and incubated for 24 hours. Cells that remained on the upper face of the membrane were removed with a cotton swab. Cells on the lower face of the membrane were fixed with paraformaldehyde and examined by fluorescence microscopy for counting. The total invaded cell number per filter was calculated using transfection efficiency.

Statistical Analysis

We used SPSS® 12.0. Statistically significant differences were determined using the Student's t test with differences considered significant at $p < 0.05$.

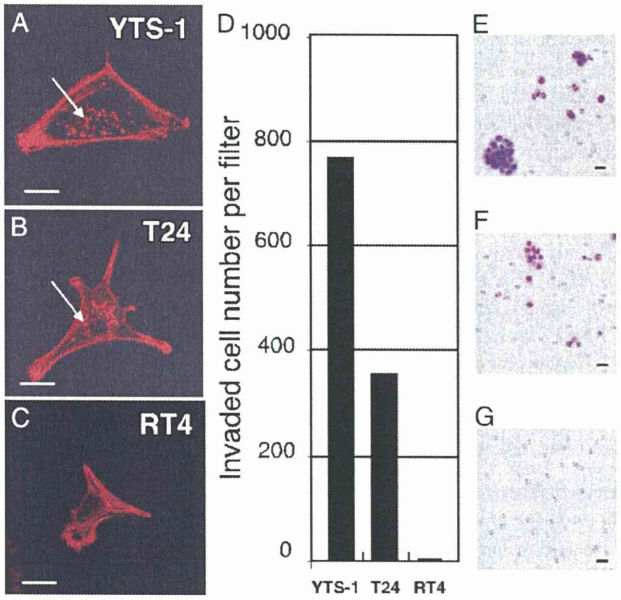

Figure 1. Invadopodia formation and invasion capacity of bladder tumor cell lines. Note invasive YTS-1 (*A*) and T24 (*B*), and noninvasive RT4 (*C*) bladder tumor cells. Scale bars indicate 10 μm. (*D*), in vitro invasion capacity of bladder tumor cells. In vitro invasion assay shows representative fields on bottom of Matrigel coated membrane insert, including YTS-1 (*E*), T24 (*F*) and RT4 (*G*) cells. Bars indicate 25 μm.

RESULTS

We first examined the invasive bladder tumor cell lines YTS-1 and T24, and the noninvasive bladder tumor cell line RT4 for invadopodia formation and in vitro invasion capacity. Cells were stained with phalloidin to visualize F-actin cores of invadopodia. A number of F-actin puncta was observed in YTS-1 and T24 cells but no F-actin puncta were observed in RT4 cells (fig. 1, *A* to *C*). YTS-1 and T24 cells showed high invasive capacity but the invasive capacity of RT4 was undetectable (fig. 1, *D* to *G*). Our previous results confirmed that the F-actin puncta in the YTS-1 and T24 cells were functionally active invadopodia.[10] Results suggest that invadopodia correlate with the invasive capacity of bladder tumor cells.

FBP17 is an 80 kDa cytosolic protein consisting of several functional domains, including an N-terminal EFC domain and an SH3 domain at the C-terminus (fig. 2, *A*). Previous studies showed that FBP17 is involved in endocytosis due to the membrane deformation activity of the EFC domain.[13–15] This activity led us to hypothesize that FBP17 is involved in invadopodia formation since the invadopodia formation process includes deformation of the plasma membrane. To test this hypothesis we first determined whether the bladder tumor cells expressed FBP17. Total lysates were prepared from the 3 bladder tumor cell lines YTS-1, T24 and RT4, and the bladder tumor specimens from patients 1 and 2 with pathological stage pT2 bladder tumors, and analyzed by Western blot using anti-FBP17 polyclonal antibody. All bladder tumor cells expressed FBP17 at similar levels (fig. 2, *B*, *lanes 1* to *5*).

We performed immunofluorescence experiments to examine FBP17 localization in invasive bladder tumor cells. Phalloidin staining revealed a number of invadopodia in YTS-1 and T24 cells (fig. 3, *A* and *B*). To validate the results of the experiments in cell lines using physiologically relevant materials we prepared primary culture cells from the bladder tumor specimens of 2 patients as described, and tested the cells for invadopodia formation. Phalloidin staining revealed that the primary culture cells also formed invadopodia (fig. 3, *C* and *D*). To examine FBP17 localization we used the HA tagged FBP17 construct HA-FBP17 and anti-HA monoclonal antibody since anti-FBP17 polyclonal antibody is not suitable for immunofluorescence microscopy.[13,14,20] Invasive bladder tumor cells, including YTS-1 and T24 cells, and cells from the 2 patients, were transfected with HA-FBP17 and then double stained with

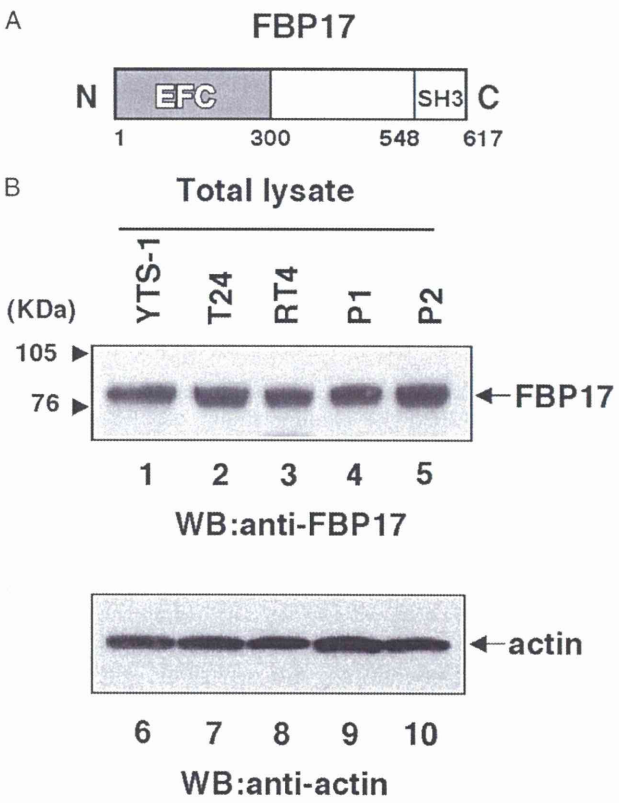

Figure 2. FBP17 domain organization (*A*), and total lysates from bladder tumor cell lines and invasive bladder tumor specimens from patients 1 (*P1*) and 2 (*P2*) (*B*). *N*, N-terminus. *C*, C-terminus. Lysates were analyzed by Western blot (*WB*) with β-actin as internal control.

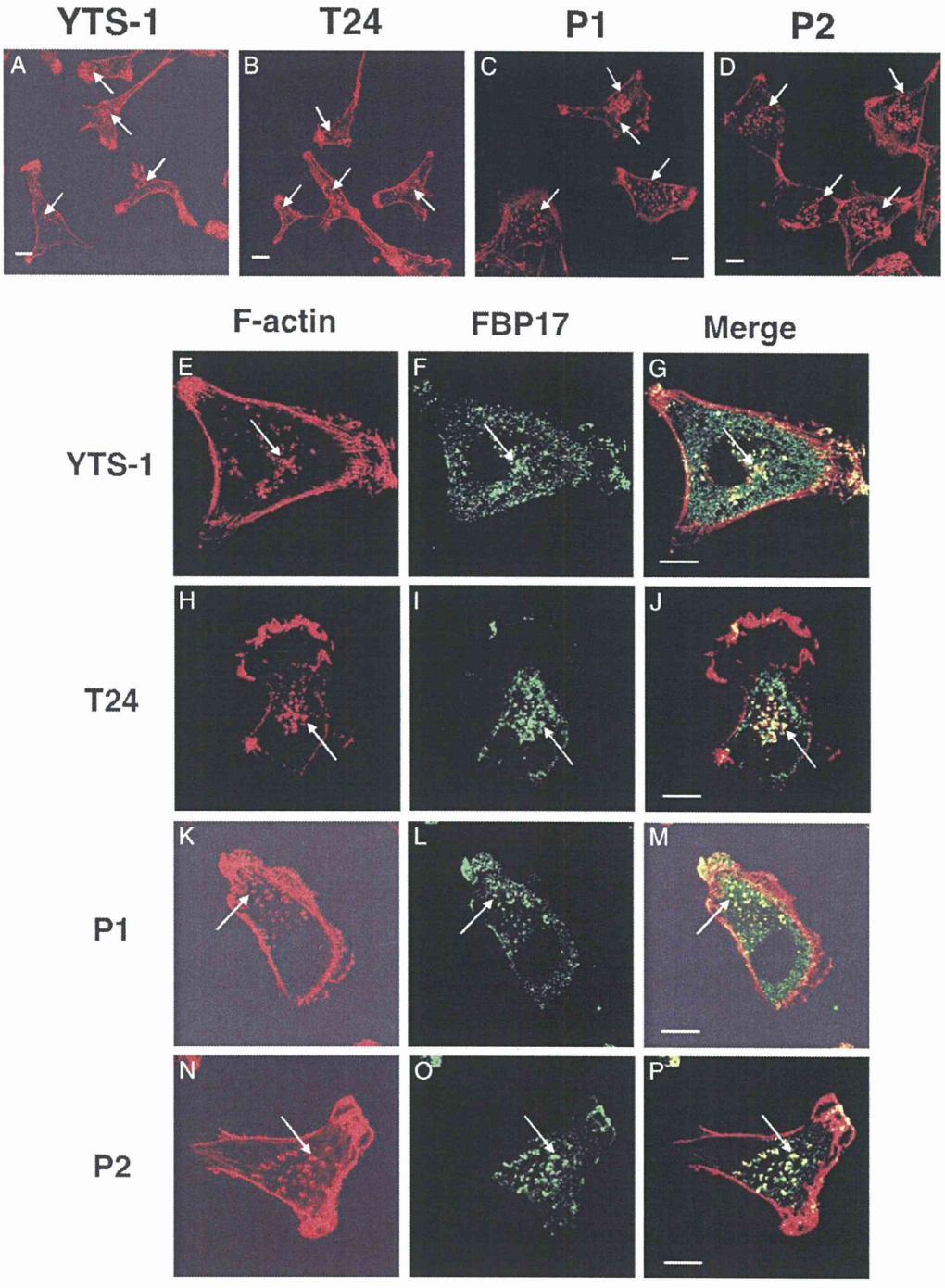

Figure 3. FBP17 localized at invadopodia. Note invadopodia formation by invasive bladder tumor cells YTS-1 (*A*) and T24 (*B*), and primary culture tumor cells from patients 1 (*P1, C*) and 2 (*P2, D*). Arrows indicate representative invadopodia. Confocal laser scanning micrography reveals YTS-1 (*E* to *G*), T24 (*H* to *J*), patient 1 (*K* to *N*) and patient 2 (*N* to *P*). Yellow areas (*G, J, M* and *P*) indicate co-localization of HA-FBP17 (green areas) (*E, H, K* and *N*) and invadopodia (red areas) (*F, I, L* and *O*). Scale bars indicate 10 μm.

phalloidin and anti-HA. Merged images of phalloidin and anti-HA staining revealed that HA-FBP17 co-localized with F-actin puncta in YTS-1, T24 cells and primary culture tumor cells (fig. 3, *G, J, M* and *P*), indicating that FBP17 localizes at invadopodia.

In invasive bladder tumor cells FBP17 co-localized with Arp3, another invadopodia marker in bladder tumor cell lines and primary culture tumor cells (fig. 4). This confirmed that FBP17 localizes at invadopodia.

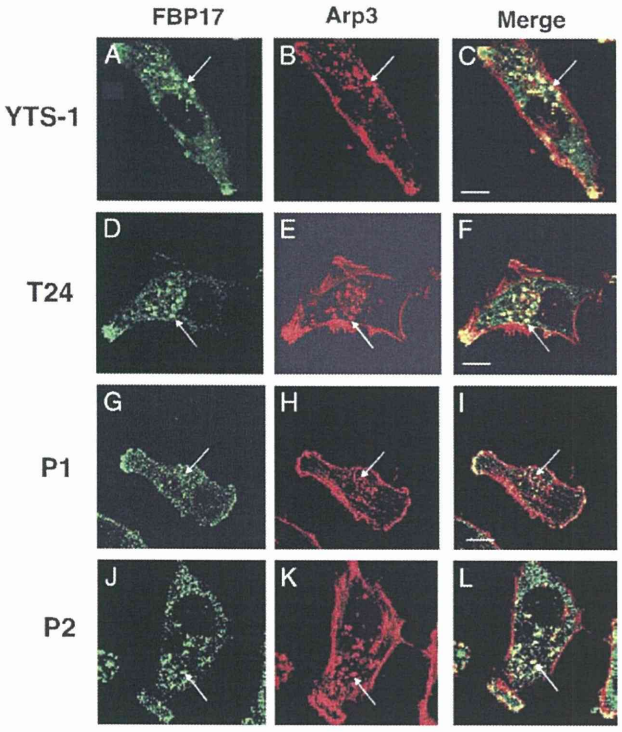

Figure 4. Confocal laser scanning shows FBP17 and Arp3 co-localization in YTS-1, T24, and patient 1 (P1) and 2 (P2) samples. Yellow areas (*C, F, I* and *L*) indicate co-localization of HA-FBP17 (green areas) (*A, D, G* and *J*) and Arp3 (red areas) (*B, E, H* and *K*). Arrows indicate representative invadopodia. Scale bars indicate 10 μm.

To determine the importance of FBP17 in invadopodia formation in bladder tumor cells we knocked down FBP17 using siRNA. To confirm that FBP17 expression was knocked down in cells we transfected YTS-1 cells with siRNA and analyzed the expression levels of FBP17 in cells by Western blot. YTS-1 cells transfected with FBP17 siRNA expressed approximately 80% less FBP17 than cells transfected with its scrambled control siRNA but the 2 cells expressed almost the same β-actin levels (fig. 5, *A*). This indicates that FBP17 expression was efficiently knocked down in most transfected cells. YTS-1 and T24 cells were co-transfected with FBP17 siRNA and pmaxGFP. Two days after transfection cells were stained with Alexa 568 labeled phalloidin. GFP positive cells were examined for invadopodia formation by fluorescence microscopy. To quantify invadopodia formation we scored the percent of cells with invadopodia. Invadopodia formation in YTS-1 and T24 cells was significantly impaired in FBP17 knockdown cells (fig. 5, *B*). This suggests that FBP17 is required for invadopodia formation in invasive bladder tumor cells. Figure 5, *C* to *F* shows several representative cells from each experiment.

To determine the role of FBP17 in invadopodia function we assayed tumor cell invasion in the FBP17 knockdown cells. We tested the transfected cells for in vitro invasion. We counted the number of invaded GFP positive cells through the Matrigel and calculated the total number of invaded cells per filter. Invasion of YTS-1 and T24 bladder tumor cells was significantly decreased in the FBP17 knockdown cells (fig. 5, *G* to *K*). Results suggest that FBP17 has a critical role in tumor cell invasion by mediating invadopodia formation.

FBP17 contains the functionally important EFC domain[13] and SH3 domain (fig. 2, *A*). The EFC domain has membrane deformation activity by binding to the membrane phospholipid phosphatidylinositol 4,5-bisphosphate.[13,15] The SH3 domain binds to dynamin-2 to recruit dynamin-2 to the plasma membrane.[13,14,21] Dynamin-2 has an important role in endocytosis by regulating vesicle recruitment to the plasma membrane.[22] Previous studies showed that FBP17 has an essential role in endocytosis and the regulation of neuronal morphology through EFC and SH3 domain activity.[15,21]

We then asked whether the activity of these 2 domains is needed for invadopodia formation. To address this question we constructed FBP17 mutants defective in these activities and examined whether FBP17 mutant over expression would affect invadopodia formation. To determine the roles of the EFC and SH3 domains we made 3 FBP17 constructs, including WT, K33E and dSH3 (fig. 6, *A*). All constructs were N-terminally FLAG tagged. To assess and confirm the activity of each domain of the FBP17 mutants, we performed membrane tubulation assay for membrane deformation activity and FBP17 immunoprecipitation for FBP17 binding to dynamin-2 (fig. 6, *A* and *B*).[13,15,20] Membrane tubulation in cells transfected with the FLAG-FBP17 constructs is an indicator of the membrane deformation activity of FBP17.[13–15,20] We transfected HEK293 cells with FLAG-FBP17 constructs and stained cells with anti-FLAG monoclonal antibody. The relative membrane tubulation activity of each mutant was expressed as very strong (+++) to almost nothing (−). A number of tubular structures was observed in cells expressing WT and dSH3 but not in cells expressing K33E, indicating that K33E membrane deformation activity was undetectable (fig. 6, *A*).[13,20]

To examine whether the FBP17 mutants would bind to dynamin-2 in cells we transfected YTS-1 cells with the FBP17 constructs. Western blot confirmed that the transfected cells expressed FLAG-FBP17 and its mutants, and endogenous dynamin-2 (fig. 6, *B*, *lanes 1* to *6*). FLAG-FBP17 was immuno-

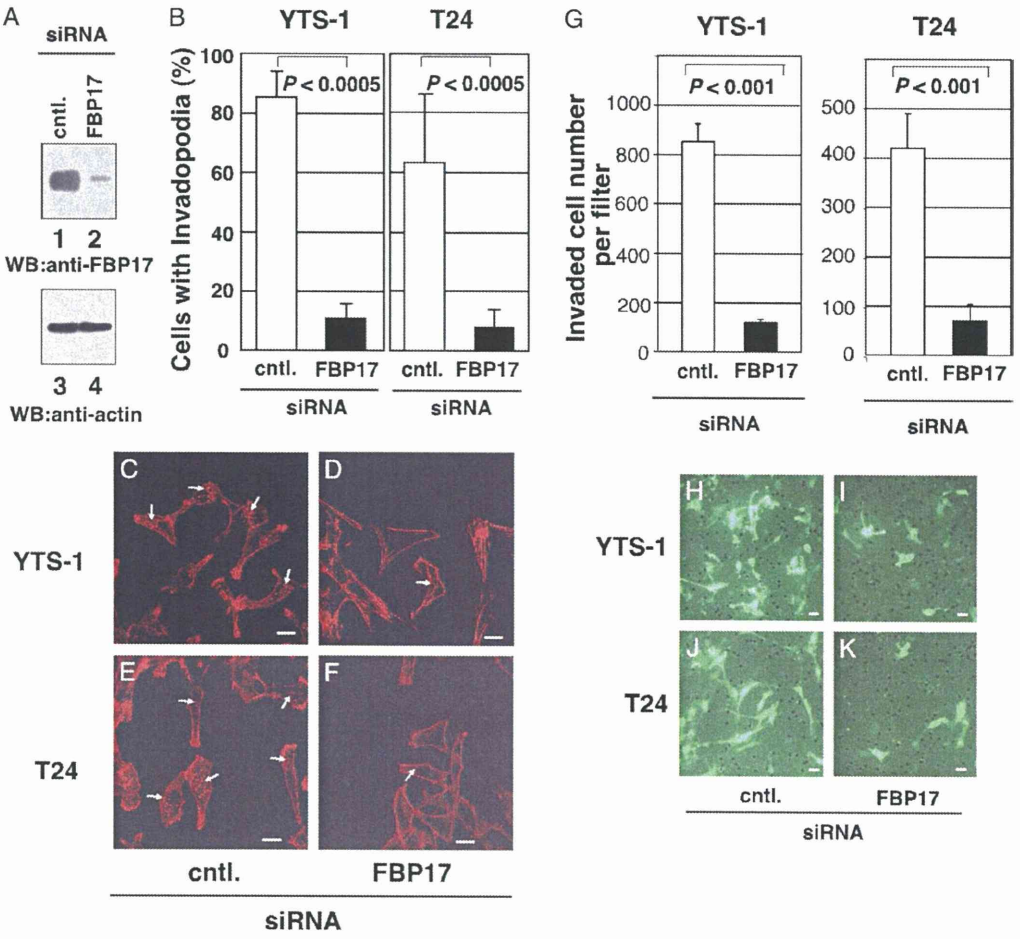

Figure 5. FBP17 role in invadopodia formation. Western blot (*WB*) of FBP17 (*lanes 1* and *2*) and β-actin (*lanes 3* and *4*) (*A*). *cntl.*, control. Invadopodia formation in transfected cells examined by fluorescence microscopy in 3 randomly selected fields (*B* to *F*). Percent with invadopodia was scored per transfected cells in total of 200 to 300 cells (*B*). Invadopodia formation was determined in cells transfected with scrambled control and FBP17 siRNA. Data represent mean ± SD of triplicate experiments (*B* and *G*). Transfected cells were examined for invadopodia formation (*C* to *F*). Arrows indicate representative invadopodia. Scale bars indicate 10 μm. Invasion of cells transfected (*G* to *K*) with scrambled control and FBP17 siRNA (*G*). Representative fields on bottom of Matrigel coated membrane insert (*H* to *K*). Scale bars indicate 25 μm.

precipitated from transfected cell lysates with anti-FLAG monoclonal antibody (fig. 6, *B, lanes 7* to *9*). Endogenous dynamin-2 co-immunoprecipitated with WT and K33E but not with dSH3 (fig. 6, *B, lanes 10* to *12*). We also confirmed that FBP17 interacted with dynamin-2 in RT4 noninvasive bladder tumor cells (fig. 6, *C*).

Cells co-transfected with the FLAG-FBP17 mutant constructs and pmaxGFP were stained with Alexa 568-phalloidin for invadopodia formation. The percent of cells with invadopodia was scored. Invadopodia formation by invasive bladder tumor cells was severely impaired in cells expressing K33E and dSH3 (fig. 7, *A*). Figure 7, *B* to *G* shows representative cells from each experiment. We also tested the transfected cells for in vitro invasion. Bladder tumor cell invasion capacity was significantly decreased in the 2 cells expressing K33E and dSH3, respectively (fig. 7, *H* to *N*). Results suggest that each activity of the membrane deformation and dynamin-2 recruitment of FBP17 is needed for invadopodia formation and invasion.

DISCUSSION

FBP17 is required for invadopodia formation and bladder tumor cell invasion. To our knowledge we report the first study providing evidence that FBP17 has an essential role in invadopodia formation, although invadopodia formation was observed in various types of cancer cells.[6–9]

Previous studies of the molecular basis of invadopodia formation focused on the regulation mecha-

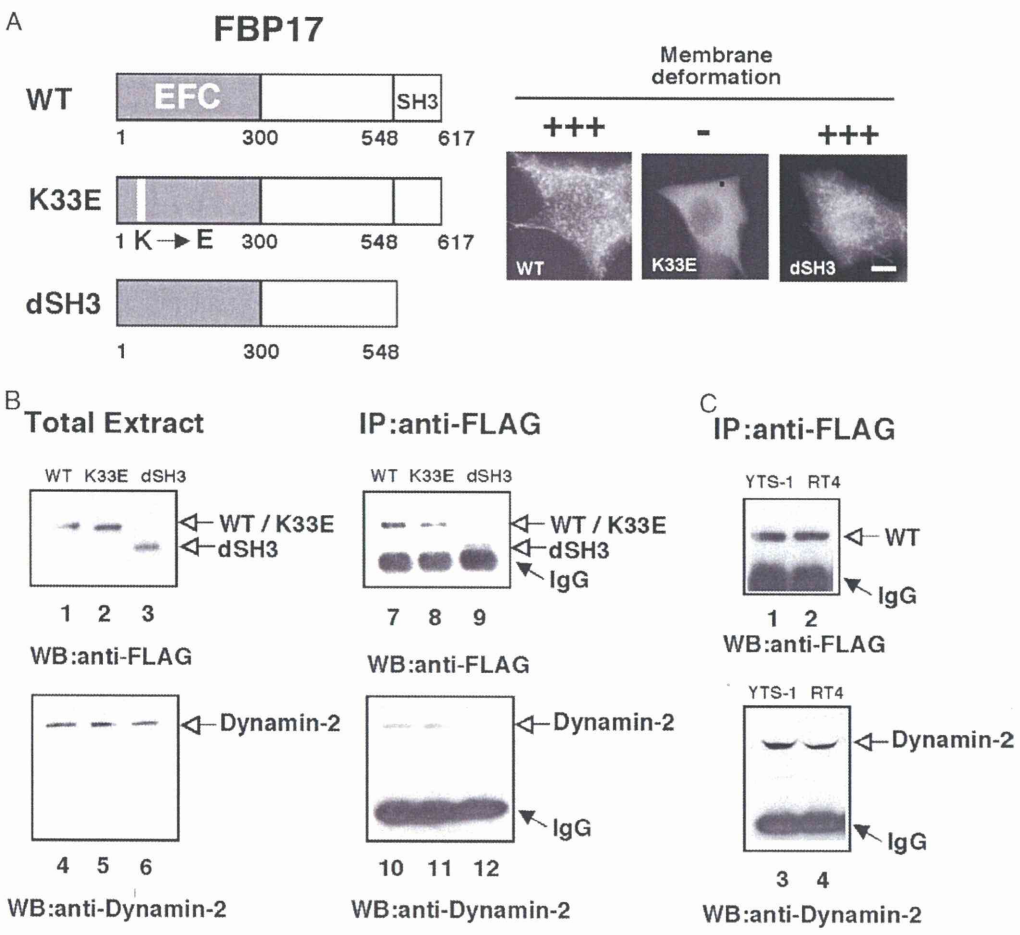

Figure 6. FBP17 EFC and SH3 domain activity in cells. Domain organization and membrane deformation activity of FBP17 mutants using FBP17 constructs WT, K33E and dSH3 (*A*). Note representative transfected cells. Scale bar indicates 10 μm. Relative membrane tubulation activity of each mutant was determined (+++ and −). Western blots (*WB*) show FBP17 binding to dynamin-2 through SH3 domain in cells for expression of FBP17 mutants (*lanes 1* to *3*) and endogenous dynamin-2 (*lanes 4* to *6*) (*B*). Mutants were immunoprecipitated (*IP*) from transfected cell lysates with anti-FLAG, followed by Western blot with anti-FLAG (*lanes 7* to *9*) and anti-dynamin-2 (*lanes 10* to *12*). Open arrows indicate FLAG tagged FBP17 mutants and dynamin-2. Black arrows indicate IgG from anti-FLAG. Binding of FBP17 to dynamin-2 in RT4 cells (*C*). YTS-1 and RT4 cells were transfected with WT immunoprecipitated from total lysates (lanes *1* and *2*), followed by Western blot for dynamin-2 (*lanes 3* and *4*).

nisms of the actin cytoskeleton since invadopodia are F-actin rich organelles.[23–27] To our knowledge the current study provides the first evidence that the membrane deformation activity of the EFC domain of FBP17 is critical for invadopodia formation (fig. 7).

We also noted that dynamin-2 recruitment to the plasma membrane by the SH3 domain of FBP17 is needed for invadopodia formation (fig. 7). This suggests that dynamin-2 probably has an essential role in invadopodia formation by regulating the recruitment of vesicles to the plasma membrane since membrane protrusion requires the delivery of new membrane materials.[28]

Invadopodia formation was not observed in RT4 cells, although FBP17 interacted with dynamin-2 in RT4 and in YTS-1 cells (figs. 2, *B* and 6, *C*). Results suggest that RT4 cells are deficient in some molecular steps other than the interaction of FBP17 with dynamin-2 in the whole molecular process for invadopodia formation and such deficiency causes the lack of invadopodia in RT4 cells.

A large population of patients with bladder tumors diagnosed before muscle invasion can be effectively treated with surgery.[29] However, muscle invasion by bladder tumors has a great impact on prognosis and postoperative quality of life in patients with bladder tumors since muscle invasion leads directly to metastasis and tumor recurrence. Future studies of the molecular basis of invadopodia formation, including this study, may lead to the discovery or development of new agents to control the process of muscle invasion of bladder tumors by suppressing invadopodia formation.

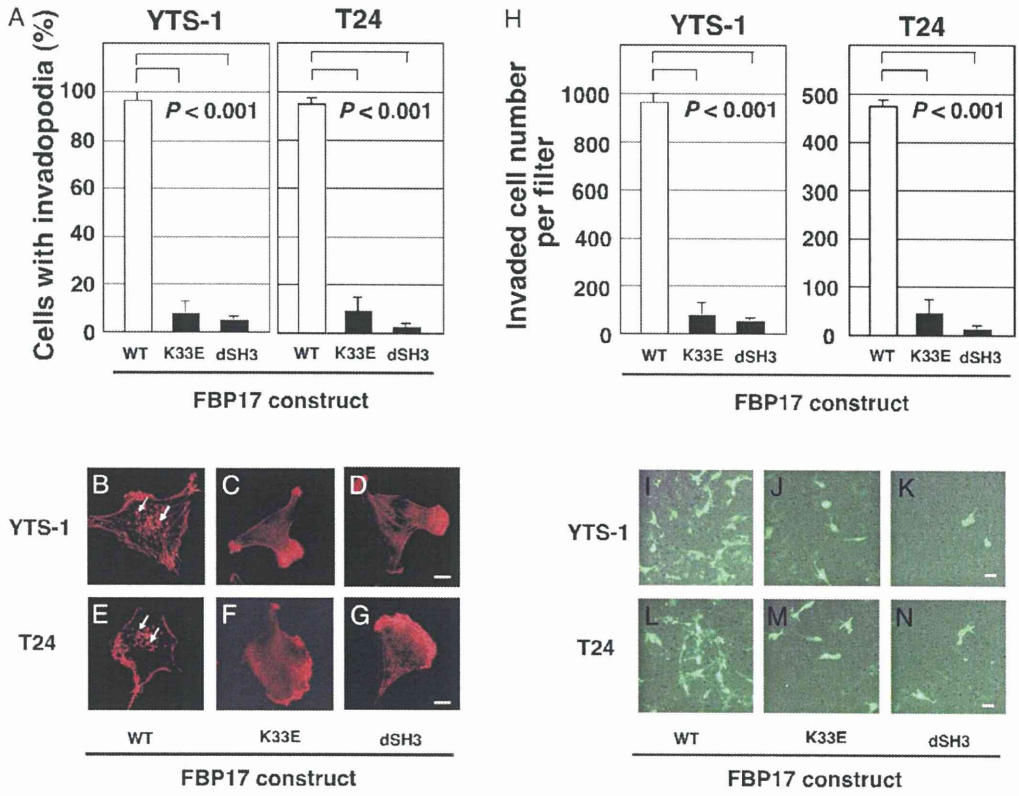

Figure 7. Invadopodia formation and invasion by cells expressing FBP17 mutants. Transfected cells were examined for invadopodia formation (*A* to *G*) and assayed for in vitro invasion (*H* to *N*). Data represent mean ± SD of triplicate experiments (*A* and *H*). Representative cells of each experiment, including YTS-1 cells expressing WT (*B*), K33E (*C*) and dSH3 (*D*), and T24 cells expressing WT (*E*), K33E (*F*) and dSH3 (*G*). Arrows indicate representative invadopodia. Scale bar indicates 10 μm. Invasion of cells expressing WT, and mutants K33E and dSH3 (*H*). Representative fields on the bottom surfaces of the membrane of Matrigel coated insert, including YTS-1 cells expressing WT (*I*), K33E (*J*) and dSH3 (*K*), and T24 cells expressing WT (*L*), K33E (*M*) and dSH3 (*N*). Scale bars indicate 25 μm.

CONCLUSIONS

Bladder tumor invasion is mediated by invadopodia, which are the membrane protrusions rich in F-actin formed by invasive bladder tumor cells. We report that FBP17 is required for invadopodia formation and bladder tumor cell invasion. Our results contribute to the elucidation of the molecular mechanisms of invadopodia formation and may lead to the discovery of new therapeutic strategies to block bladder tumor invasion.

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原　　著

尿中剥離細胞 survivin mRNA 測定による膀胱癌の診断

坂本佑子*1　西岡淳二*2　野間桂*3　安田和成*4
中谷中*5　木瀬英明*6　杉村芳樹*7　登勉*8

The Usefulness of Survivin/Glyceraldehyde-3-Phosphate Dehydrogenase Ratio in Urine Exfoliated Cells for the Detection of Bladder Tumor

Yuko SAKAMOTO[*1], *Junji NISHIOKA*, PhD[*2], *Katsura NOMA*[*3], *Kazunari YASUDA*[*4], *Kaname NAKATANI*, MD[*5], *Hideaki KISE*, MD[*6], *Yoshiki SUGIMURA*, MD[*7] and *Tsutomu NOBORI*, MD[*8]

Objective: Survivin is one of the apoptosis inhibitor proteins and is rarely expressed in adult normal tissues. However, survivin expression has been detected in various tumors. In this study, we evaluated the usefulness of urinary survivin/glyceraldehyde 3 phosphate dehydrogenase (GAPDH) ratio as a marker for bladder tumor.

Patients and methods: Urine samples were obtained from 72 patients with bladder tumor, 36 with urinary tract inflammation as controls. Survivin and GAPDH mRNA expression was measured by quantitative real-time PCR assay in urine cells. The GAPDH housekeeping gene was used for normalization of survivin expression. We also analyzed survivin protein levels using urine samples and recombinant protein by western blotting.

Results: High expression of survivin was confirmed on the protein level using urine samples of bladder tumor by western blotting. Survivin/GAPDH mRNA ratios of bladder tumor quantified by real-time PCR was significantly higher than those of controls ($p=0.001$). In pathological stage of bladder tumor, survivin/GAPDH mRNA ratio of pTis was significantly high compared with pTa and pT1 ($p<0.001$, $p=0.001$, respectively). Grade3 tumors expressed high level of survivin/GAPDH mRNA ratio compared with Grade1 and Grade2 tumors ($p=0.03$). The sensitivity, the specificity and AUC (area under the curve) of survivin/GAPDH mRNA ratio was 83.3%, 86.1% and 0.898, respectively.

Conclusion: Measuring survivin/GAPDH mRNA ratio in urine is non invasive and high sensitive examination. Therefore, survivin/GAPDH mRNA ratio is useful marker for the detection of bladder tumor, especially to detect carcinoma *in situ*.

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Corresponding author: *Kaname NAKATANI*, Personalized Medicine/Clinical Laboratories, Mie University Hospital, Tsu 514-8507, Japan. E-mail: nakatani@clin.medic.mie-u.ac.jp

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*1～4 三重大学医学部附属病院中央検査部，*5 同　オーダーメイド医療部/中央検査部，
*6,7 同　大学院医学系研究科腎泌尿器外科学，*8 同　大学院検査医学（〒514-8507 津市江戸橋 2-174）

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【Key Words】survivin（サバイビン），glyceraldehyde-3-phosphate dehydrogenase: GAPDH（グリセルアルデヒド3リン酸脱水素酵素），bladder tumor（膀胱癌），real-time PCR（リアルタイムPCR）

膀胱癌は，50～70歳代の男性に好発する腫瘍であり，年間罹患数は一万二千人程度と推測されている[1]。膀胱癌は，時間的，空間的に多発する傾向にあり，外科的摘出手術後も再発を繰り返しやすいという特徴をもっている。

膀胱癌の診断や経過観察には，尿細胞診や膀胱鏡による生検・組織診断が一般的に用いられている。しかし，尿細胞診は診断率が低く，膀胱鏡は侵襲的であり，出血や炎症などの合併症を惹き起こしうるという欠点がある。そこで，膀胱癌の早期診断，早期治療のために，スクリーニングとして活用できる高感度かつ非侵襲的な膀胱癌特異的マーカーの検索が続けられている。

近年，IAP（Inhibitor of apoptosis protein）ファミリーに属するアポトーシス阻害分子であるsurvivinの機能が明らかにされつつある。survivinは，抗アポトーシス作用をもつbaculovirus IAP repeat（BIR）ドメインを1個有し[2]，このBIRドメインがcaspaseに直接結合しcaspase活性を阻害することで，アポトーシスを抑制する[3]。また，ユビキチン化に関わるRING fingerドメインを含まず，種々のアポトーシス阻害分子の中でも最も単純な構造をしている[2]。survivinは，細胞死の制御に加え，細胞分裂においてもその重要性が示唆されており，細胞周期に依存してG2/M期で高発現する特徴をもつ[4]。

さらに発現調節の特徴として，survivinは，成人における正常組織では，胸腺など一部組織を除いて殆ど発現していないが，乳癌，胃癌，大腸癌など様々な悪性腫瘍では過剰発現していることが報告されている[5]。

今回我々は，膀胱癌患者尿中に剥離した癌細胞におけるsurvivin発現量と，ハウスキーピング遺伝子であるglyceraldehyde-3-phosphate dehydrogenase（GAPDH）発現量の測定による膀胱癌診断の有用性について検討を行った。

I. 対象および方法

対象は，三重大学医学部附属病院腎泌尿器外科を受診し，組織学的に膀胱癌と診断された患者72例（年齢71.4±9.6歳）および尿中に白血球が多数認められた尿路系炎症患者36例（年齢55.0±16.4歳）である。尿検体は，基本的には自然排泄尿を使用し，一部の膀胱癌患者では膀胱鏡検査中ならびに摘出手術中に回収した尿検体を用いた。

72例の膀胱癌は，膀胱癌取扱い規約1997年度TNM分類に従い分類した。72例の膀胱癌を腫瘍の深達の程度を表わす組織学的深達度別に分類すると，上皮内癌であるpTisが16例，乳頭状非浸潤癌であるpTaが27例，粘膜固有層までの癌の進展がみられるpT1が19例，膀胱周囲組織など筋層以上への癌浸潤が認められるpT2，pT3，pT4があわせて10例である。また，細胞異型度，構造異型度により分類した組織学的異型度別では，軽度異型であるGrade1と中等度異型であるGrade2があわせて41例，高度異型であるGrade3が31例である。

なお，本研究は，三重大学医学部臨床研究倫理委員会に申請・承認され，担当医から対象者に文書によって説明し，文書による同意書を取得した後，実施した。

A. ウエスタンブロット法によるsurvivin蛋白の検出

膀胱癌患者および尿路系炎症患者の尿15ml中の沈渣蛋白をBCA法にて測定した結果，おおよそ1mg程度であったので，ウエスタンブロット法によりsurvivin蛋白の検出を試みた。尿沈渣をlysis buffer（0.05M Tris/HCl，0.1M NaCl，0.1% SDS）にて可溶化した蛋白，ならびにリコンビナントsurvivin蛋白の希釈系列を試料として，20%ポリアクリルアミドゲルでSDS-PAGE後，ニトロセルロースメンブレンにブロットした。リコンビナントsurvivin蛋白は，ヒト肝癌由来細胞株HepG2より，pQE-30ベクター（QIAGEN）を使用してクローニングを行い，大腸菌により発現させた後，Ni-NTA Spin Kit（QIAGEN）により精製した。検出には，化学発光を利用したBMケミルミネッセンスウエスタンブロッティングキット（Roche Diagnostics）を使用し，一次抗体には250倍希釈した抗survivin抗体（Santa Cruz Biotechnology）を，二次抗体には1,000倍希釈したHRP標識抗マウスIgG抗体（Bio Rad Laboratories）を使用した。撮影・定量解析には，ルミノイメージアナライザーLAS-3000（GE Healthcare）を使用した。

Table 1　Primers and probes for quantitative real-time PCR

Gene		Sequence
survivin (185bp)	Forward primer	5'-AAGAACTGGCCCTTCTTGGA-3'
	Reverse primer	5'-CAACCGGACGAATGCTTTT-3'
	Probe	5'-FAM-CCAGATGACGACCCCATAGAGGAACA-TAMRA-3'
GAPDH (226bp)	Forward primer	5'-GAAGGTGAAGGTCGGAGTC-3'
	Reverse primer	5'-GAAGATGGTGATGGGATTTC-3'
	Probe	5'-VIC-TTGCCATCAATGACCCCTTCATTGAC-TAMRA-3'

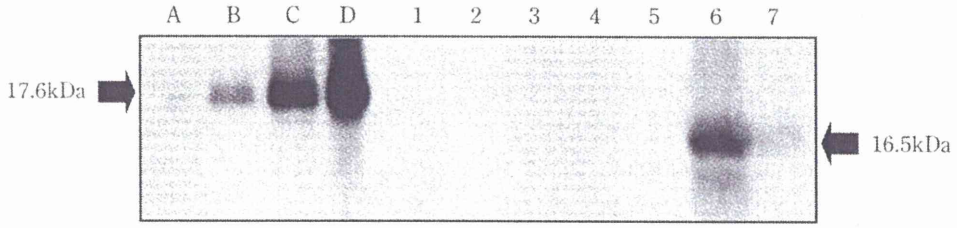

Figure 1　Expressions of survivin protein were detected by western blotting.
Lane A, B, C and D were dilution series of recombinant survivin protein expressed by *E. coli*.
Survivin protein levels of lane A, B, C and D were 10ng, 50ng, 100ng and 200ng, respectively.
Lane 1, 2 and 3 were urine samples obtained from patients with urinary tract inflammation, lane 4, 5, 6 and 7 were those with bladder tumor.
Survivin protein levels were determined using MultiGauge, those of lane 6 and 7 were 845ng and 360ng, respectively.
Molecular weight of recombinant survivin was larger than that of urinary survivin because of His-Tag.

B. Real-time PCR 法による発現定量

尿検体を 1,500 回転，10 分間遠心処理し，収集した細胞より QIAamp RNA Blood Mini Kit (QIAGEN) を使用し RNA を抽出し，Ready-To-Go You-Prime First Strand Beads (GE Healthcare) と Random primer (Takara) を用いて cDNA を合成した。合成した cDNA から，Real-time PCR 法により survivin 発現量を定量し，また内部標準として GAPDH 発現量を同時測定した。GAPDH 発現量は survivin 発現量に比較して大きいため，survivin 発現量を GAPDH 発現量にて除した後，10^5 倍した値にて評価を行った。それぞれの発現量は，QuantiTect Multiplex PCR Master Mix (QIAGEN) を使用し，1 チューブにおいて survivin mRNA と GAPDH mRNA の発現量を同時測定した。**Table 1** にそれぞれのプライマーおよびプローブを示す。Real-time PCR 反応液 25μl を 95℃ 15 分処理し，続いて 94℃ 1 分，60℃ 1 分を 45 サイクルで増幅した。

Real-time PCR は，7900HT Fast Real Time PCR System (Applied Biosystems) を使用し，検量線作成のための標準試料には survivin ならびに GAPDH の断片 cDNA を組み込んだプラスミドを使用した。

Realtime PCR 法では測定毎に，10 倍毎の 5 段階濃度の標準資料を測定し，Ct 値を確認した。また標準資料，測定検体とも二重測定を行い，ほぼ同じ数値を示すことを確認し，平均値を結果に使用した。

C. 統計解析

測定値は，平均値±標準偏差で表示した。統計学的検討は，Dr.SPSS II for Windows 11.0.1 J (SPSS Inc.) を使用して独立 t 検定を行い，危険率 5%未満を有意差ありと判定した。

II. 結　　果

A. ウエスタンブロット法による膀胱癌患者尿中 survivin 蛋白の検出

ウエスタンブロット法の結果を **Fig. 1** に示す。尿中に高度に白血球が検出された尿路系炎症患者 3 例では，いずれの尿沈渣可溶化蛋白質においても survivin 蛋白は検出されなかった。一方，膀胱癌患者の尿沈渣では 4 例中 2 例において survivin 蛋白が検出され，大腸菌により発現させたリコンビナント蛋白から検量線を作成し定量解析したところ，膀胱癌患者尿 15ml 中の survivin 蛋白量はそれぞれ 845ng および 360ng であり，膀胱癌患者の尿中剥離細胞に

survivin 蛋白の発現が確認された。

B. 膀胱癌患者尿中 survivin mRNA の測定

Real-time PCR 法にて，survivin mRNA と GAPDH mRNA との発現量比を測定・算出した結果，膀胱癌群では 64.8±103.4，尿路系炎症群では 3.0±5.6 であった。膀胱癌群での Ct 値(Threshold Cycle)の平均値は，survivin で 33.7，GAPDH で 23.2 であり，尿路系炎症群では，survivin で 39.5，GAPDH で 24.0 であった。膀胱癌群は，尿路系炎症群に比較して有意に高値を示した(**Fig. 2**)。

次に，組織学的異型度別に survivin 発現量を比較検討したところ，Grade1 と Grade2 では 42.6±101.6，Grade3 では 94.1±100.0 であり，異型度が増加するに伴い survivin 発現量の上昇が認められる結果となった(**Fig. 3**)。

続いて組織学的深達度別に survivin 発現量を比較した(**Fig. 4**)。pTis では 170.9±169.8，pTa では 25.1±27.6，pT1 では 30.4±35.6，pT2 以上では 67.4±66.0 であり，pTis は pTa および pT1 と比較して，有意に高値であった。また，pTis を除く pTa，pT1，pT2 以上では，浸潤が深くなるにつれ，survivin 発現量が上昇する傾向が認められた。

尿路系炎症を対照群として，膀胱癌群に対する ROC(Receiver Operating Characteristic curve)解析を実施した結果，カットオフ値は 5.24 と算出され，感度ならびに特異度はそれぞれ 83.3%，86.1%であった。また AUC は 0.898 と良好な値が得られた(**Fig. 5**)。

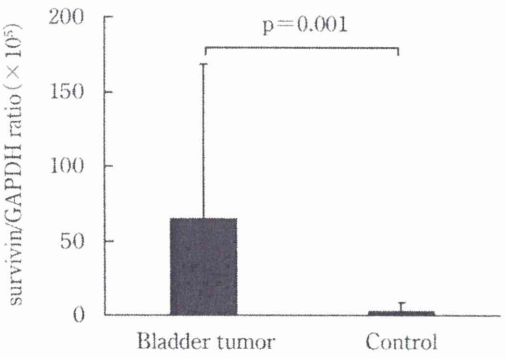

Figure 2 Mean values of survivin/GAPDH ratio for bladder tumor and control.
Results are given as the mean±SD.

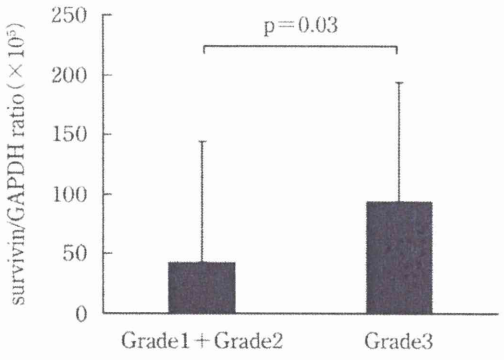

Figure 3 Mean values of survivin/GAPDH ratio for pathological grade of bladder tumor.
Results are given as the mean±SD.

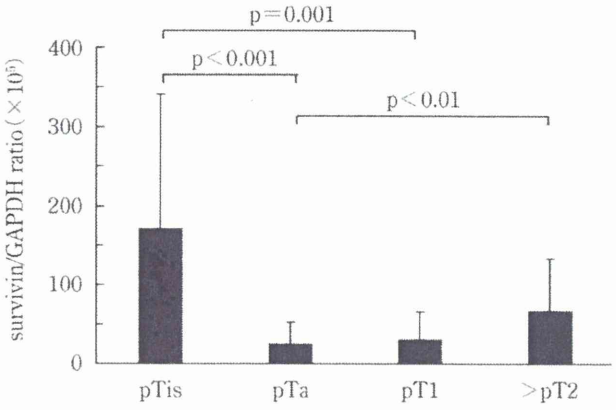

Figure 4 Mean values of survivin/GAPDH ratio for pathological stage of bladder tumor.
Results are given as the mean±SD.

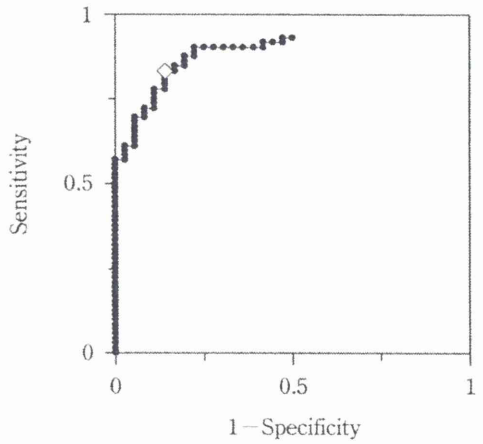

Figure 5 The ROC analysis of survivin/GAPDH ratio (×10⁵) to diagnose patients with bladder tumor.
The calculated cut-off value was 5.24(◇) and the area under the curve was 0.898.
The sensitivity and specificity were 83.3% and 86.1%, respectively.

III. 考　　察

膀胱癌の診断や経過観察には，一般的には尿細胞診や膀胱鏡による生検・組織診断が用いられている。しかし，尿細胞診は，非侵襲的で経済的な検査方法ではあるが，感度が40～60%，特異度が90～95%であると報告されており，特に低異型度の腫瘍では診断率が低いことが課題である[6]。また，膀胱鏡による生検は，膀胱癌の確定診断のために必須の検査であるが，侵襲的であり，出血や炎症などの合併症を惹き起こす場合もある。

隆起性病変であれば，腹部エコー，コンピュータ断層撮影(CT)，磁気共鳴画像(MRI)，膀胱鏡などで診断が可能であるが，上皮内癌である場合，これらの方法では診断が困難であることが多い。そこで，膀胱癌の早期診断，早期治療のために，高感度かつ非侵襲的スクリーニングとなる膀胱癌特異的マーカーの検索が続けられている。

今回，著者らは，survivin mRNA と GAPDH mRNA 発現量の測定による膀胱癌診断の有用性を検討したが，膀胱癌群は尿路系炎症群と比較して著しく高値を示し，有意な差が認められた。

survivin/GAPDH mRNA 定量による膀胱癌診断において，偽陽性，偽陰性となる原因としては，尿中への剥離細胞が少なく，抽出される RNA 量が不十分である場合が考えられ，この場合は，survivin/GAPDH 比の測定精度が低下する。また，survivin は，ほとんどの正常組織では発現していないと考えられているが，末梢血好中球において極微量の survivin が発現していることが報告されている[7]。そこで我々は，末梢白血球を混合した尿の希釈系列を作製し検討したところ，白血球数が 1×10^6 個までは偽陽性とならなかったことから，通常，白血球中の survivin mRNA による偽陽性は除外できると考えられた(data not shown)。

偽陰性となる要因には，尿中への癌細胞の剥離がない場合の他，今回検討に使用したプライマー，プローブでは測定できない survivin の splice variant が著しく高発現している場合が考えられる。survivin には，survivin2B，survivinΔEx3，survivin2α，survivin3B の4種類の splice variant が報告されているが[8]，今回使用したプライマー，プローブでは splice variant のうち，survivin3B は包含して測定されるが，survivin2B，survivinΔEx3，survivin2α は測定されない。膀胱癌においても，wild type survivin の他に survivin2B，survivinΔEx3 などの splice variant の発現が報告されており[9][10]，我々も，survivin cDNA クローニングならびに splice variant 特異的 Real-time PCR にてその発現を確認している。しかし，その発現量は，wild-type survivin に比較して著しく低発現であり，本法による膀胱癌の診断に対して偽陰性に作用するものではないと考えられた(data not shown)。

膀胱癌の組織学的所見と survivin/GAPDH 比との関連性を検討した。組織学的異型度別に比較すると，異型度が増加するに伴い，survivin 発現量の増加が認められ，腫瘍の悪性度に survivin が関与していることが示唆された。さらに，組織学的深達度別での比較では，pTis において survivin 発現量が最も高値を示したことより，膀胱鏡，CT，MRI などでは診断が非常に困難である pTis において，survivin/GAPDH mRNA 発現量比の測定が非常に有用な検査法であると考えられた。また，pTis を除外した pTa，pT1，pT2 以上では，浸潤が深くなるにつれ，survivin 発現量の上昇が認められ，組織学的異型度別での検討と同様に，survivin 発現量が腫瘍の悪性度に関与することが示唆された。

浸潤癌ではない pTis において survivin 発現量が高値であった理由として，pTis は Grade3 の癌細胞が中心となり，放置すると浸潤癌になることが多い悪性度の高い癌であること[11]，また，尿中細胞中の剥離腫瘍細胞の割合が高かったことが要因と考えられる。つまり，上皮内癌である pTis は，腫瘍が粘膜上皮内にとどまり，浸潤することなく広範囲に扁平に広がるので[11]，浸潤腫瘍と比較して，膀胱内へ剥離する癌細胞の割合が高く，本測定法での検出率が高まると考えられた。

尿路系炎症を対照群とした，膀胱癌群に対する ROC 解析の結果，感度ならびに特異度はそれぞれ 83.3%，86.1%であり，また AUC は 0.898 であった。これまでの報告[12][13]における感度・特異度はそれぞれ，68.6～79%，93～100%であり，著者らの方法では特異度は若干劣るものの，非常に高感度な検査法であることが示された。

IV. 結　　語

今回の検討により，尿中剥離細胞の survivin と GAPDH との発現量比の測定は，非侵襲的かつ高感