Expression of the Tumor Suppressor Protein 14-3-3σ Is Down-regulated in Invasive Transitional Cell Carcinomas of the Urinary Bladder Undergoing Epithelial-to-Mesenchymal Transition*

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The 14-3-3 proteins constitute a family of abundant, highly conserved and broadly expressed acidic polypeptides that are involved in the regulation of various cellular processes such as cell-cycle progression, cell growth, differentiation, and apoptosis. One member of this family, the 14-3-3 isoform σ, is expressed only in epithelial cells and is frequently down-regulated in a variety of human cancers. To determine the prevalence of 14-3-3σ silencing in bladder cancer progression, we have studied the expression of this protein in normal urothelium and bladder transitional cell carcinomas (TCCs) of various grades and stages using two-dimensional gel electrophoresis in combination with Western blotting and immunohistochemistry. We show that the expression of 14-3-3σ is down-regulated in invasive TCCs, particularly in lesions that are undergoing epithelial-to-mesenchymal conversion. Altered expression of 14-3-3σ in invasive TCCs is not due to increased externalization of the protein nor to an aberrant proliferative potential of neoplastic cells. Furthermore, we found that impaired 14-3-3σ expression is not associated with increased levels of the dominant-negative transcriptional regulator ΔNp63. Down-regulation of 14-3-3σ was confirmed by indirect immunofluorescence using a peptide-based rabbit polyclonal antibody specific for this protein. We also show that the expression of 14-3-3σ is highly up-regulated in pure squamous cell carcinomas. Taken together, these results provide evidence that deregulation of 14-3-3σ may play a key role in bladder cancer progression, in particular in differentiation events leading to epithelial-to-mesenchymal transition and stratified squamous metaplasia. Molecular & Cellular Proteomics 3: 410–419, 2004.

The 14-3-3 proteins comprise a family of abundant, highly conserved and broadly expressed eukaryotic 25- to 33-kDa acidic polypeptides. Their biological role is still somewhat unclear, but it has been demonstrated that these proteins play a regulatory role in cell proliferation, differentiation, cell death, and in modulating signal transduction pathways (1). At least seven isoforms have been identified so far. The σ isoform was originally isolated as an epithelial-specific marker (HME1) whose expression level is drastically decreased in breast cancer cell lines as compared with normal mammary epithelial cells (2). Our group identified and cloned this molecule independently as a transformation-sensitive epithelial marker that we termed stratfin, present in cultured epithelial cells and in stratified squamous keratinizing epithelium (3). There is now evidence that 14-3-3σ (stratifin) is involved in many biological processes, including cell-cycle progression and cell death (4–8), and several studies have confirmed a major regulatory role for this protein in the G2/M checkpoint control. This observation together with its reported down-regulation in various human cancers, including breast, stomach, colon, lung, liver, pancreas, oral cavity, and vulva (9–17), have established this protein as a tumor suppressor likely to be involved in carcinogenesis.

Bladder cancer is the second most common genitourinary tumor and the fourth most common solid malignancy in Denmark. It encompasses a large variety of histological heterogeneous tumor types arising predominantly in the epithelium (urothelium) lining of the urinary bladder and the ureters. Tumor types of the urothelium include transitional cell carcinomas (TCCs), squamous cell carcinomas (SCCs), adenocarcinomas, as well as other less frequent lesions (18). At present, established prognostic criteria for urothelial malignancies are of limited value, underscoring the urgency for discovering new molecular markers for the prognosis of bladder cancer patients, in particular for patients that are at high risk of progression and recurrence.
For many years, we have carried out a systematic and comprehensive proteomic analysis of bladder tumors in an effort to identify protein markers that may form the basis for improved diagnosis, prognosis, and new forms of treatment (19, 20). The strategy we have employed is based on the systematic comparison of the proteome expression profiles of fresh tissue biopsies from normal and malignant urothelium. To date, this approach has revealed a number of proteins, including but not limited to adyopcyto-type fatty acid binding protein, glutathione S-transferase-m, prostaglandin dehydrogenase, and keratin 13, whose rate of synthesis correlates with tumor progression (21, 22). Here we present evidence indicating that deregulation of the tumor suppressor protein 14-3-3σ may play a key role in bladder cancer progression, in particular in events leading to epithelial-to-mesenchymal transition (EMT) and stratified squamous metaplasia.

EXPERIMENTAL PROCEDURES

Bladder Tumor Biopsies—Bladder specimens collected over a period of 5 years at Skejby Hospital, Aarhus, Denmark were analyzed. Tumors were classified by an experienced pathologist according to Bergkvist and colleagues (23). All tumors were evaluated according to TNM stages and morphological grades and are presented according to the nomenclature: stage Ta, grade 1, pTa G1; stage Ta, grade 2, pTa G2; stage T1, grade 3, pTa G3; stage T1, grade 2, pT1 G2; stage T2, grade 3, pT2-4 G3; and stage T2-4, grade 4, pT2-4 G4. The Scientific and Ethical Committee of Aarhus County approved the project.

Labeling of Bladder Samples with [35S]Methionine—Tumor samples clean of clots and contaminating tissue and urothelium random biopsies diagnosed as normal were dissected, split into small pieces with the aid of a scalpel, and subsequently labeled with [35S]methionine in a 10-ml sterile conical plastic tube containing 0.2 ml of modified Eagle’s medium devoid of methionine, 2% dialyzed (against 0.95% NaCl) fetal calf serum, and 100 μCi of [35S]methionine (Sj204; Amersham Biosciences, Uppsala, Sweden). After labeling for 14–16 h, the medium was carefully aspirated, and the pieces were dissolved in 0.3–0.4 ml of lysis solution (8 M urea, 100 mM dithiothreitol, 2% Nonidet P–40, 2% carrier ampholytes 7–9) with the aid of a 1-ml plastic pipette. In the cases where extracellular proteins were examined, the aspirated medium was freeze-dried and resuspended in 0.3–0.4 ml of lysis solution. Samples thus prepared were stored at −20 °C until use.

Proteomic Analysis and Quantitation of the Levels of 14-3-3σ—Two-dimensional gel electrophoresis (2D PAGE) was performed as previously described (24). Gels were stained with silver nitrate or in some cases with Coomassie Brilliant Blue and subjected to autoradiography. Proteins were identified using a combination of procedures that included: matrix-assisted laser desorption/ionization time-of-flight, Biflex (Bruker Daltonics, Billerica, MA); 2D PAGE Western immunoblotting; and comparison with the master 2D gel images of human keratinocyes and TCC proteins (proteomics.cancer.dk). For quantitation, 2D gel autoradiographs were scanned using a Molecular Imager device (Bio-Rad Laboratories, Hercules, CA) and were analyzed using PDQuest 7.1 analysis software (Bio-Rad Laboratories). Only gels presenting well-focused spots and limited amount of protein remaining at the origin were selected for quantitation. Protein levels were normalized to actin, and the average means with corresponding standard deviations were determined.

Cell Cultures—Primary cell cultures were derived from bladder tumors and cultured as previously described (25). Also examined were two established cell lines; RT4, a human bladder cancer cell line derived from a papilloma of the urinary bladder, and MRC5, a human fibroblast cell line. Established cell lines were cultured according to the procedures suggested by the American Type Culture Collection (Manassas, VA).

Indirect Immunofluorescence—All bladder specimens (tumors, random biopsies, and cystectomies) were frozen immediately upon arrival to our laboratory and stored frozen in liquid nitrogen. Immuno-histochemical analysis was performed on 6-μm-thick sections of frozen tissue according to standard methods using either a rabbit polyclonal antibody raised against a C-terminal peptide (H₂N-C-NAGEEGGAEAPQEGS-COH₂) specific for 14-3-3σ (Eurogentec, Brussels, Belgium) and/or a mouse monoclonal antibody against cytokeratin 5 (CK5; Neomarkers, Fremont, CA). Briefly, formaldehyde-fixed sections (3.6% formaldehyde for 4 min) were immersed for 15 min in normal fetal calf serum to block nonspecific staining and then were incubated with the relevant primary antibodies overnight at 4 °C. Next, the formed antibody complexes were detected with a tetramethyl rhodamine isothiocyanate (TRITC)-labeled secondary antibody. The sections were washed three times with cold phosphate-buffered saline between incubations. Normal goat or mouse serum instead of primary antibody was used as a negative control. Double staining, using appropriate fluorescein isothiocyanate (FITC)– and TRITC-labeled secondary antibodies (Dako, Glostrup, Denmark) were performed to investigate the relative localization of 14-3-3σ and CK5. To examine the possible co-localization of p63 and 14-3-3σ, we used isofrom-specific rabbit antibodies reactive against Tp63α or ΔNp63α (kindly provided by Karin Nylander) and 14-3-3σ using the Zenon™ Tricolor Rabbit IgG Labeling Kit (Molecular Probes, Eugene, OR). Sections were imaged using either standard epiluminescence fluorescence microscopy (Leica DMRB; Deerfield, IL) or laser scanning microscopy (Zeiss 510LSM; Oberkochen, Germany).

Western Blot Analysis—Western blotting was performed essentially as previously described (26). Proteins were resolved by 2D PAGE, blotted onto Immobilon-P polyvinylidene difluoride membranes (Millipore, Bedford, MA), and detected with a 14-3-3σ-specific rabbit peptide polyclonal antibody prepared by Eurogentec using Supersignal WestPico detection reagents according to manufacturer’s instructions (Pierce, Rockford, IL).

RESULTS

Expression Analysis of 14-3-3σ in Bladder Tissue Biopsies—Fresh bladder tumors and normal random biopsies were analyzed blindly by 2D PAGE in combination with autoradiography and/or silver nitrate staining. Of the biopsy specimens examined, only those yielding high-quality protein profiles and exhibiting minor contamination with connective and/or muscle tissue, judged by the levels of expression of vimentin and desmin, were chosen for comparison. These include: 42 normal biopsies, 11 pTa G1, 23 pTa G2, 1 pT1 G2, 5 pTa G3, 9 pT1 G3, 12 pT2-4 G3, 5 pT2-4 G4, and 5 pure SCCs. Fig. 1 shows representative 2D PAGE proteome expression profiles of fresh biopsies from normal urothelium (cyst 189) and an invasive TCC (566-1 pT2-4 G3) labeled with [35S]methionine, with the latter exhibiting much lower levels of 14-3-3σ protein. Samples were divided into three groups according to their relative expression levels of 14-3-3σ: low (15–75 relative units; average 46 ± 30), medium (140–250 relative units; average 180 ± 50), and high (300–830 relative units; average 530 ± 190). The ratio of the average means in...
these groups is 1:4:12 (low:medium:high). Some representative gels are shown in Fig. 2. In general, normal biopsies and noninvasive TCCs exhibited high levels of 14-3-3, while 10 out of 36 invasive lesions examined had either medium or low levels of this protein (Table I). Closer analysis of the protein expression profiles of the 10 invasive tumors showing down-
regulation of 14-3-3σ indicated that 6 out of these 10 tumors are undergoing EMT as judged by their expression of the mesenchyme-specific markers vimentin and protein gene product 9.5 (PGP 9.5) (Fig. 3) (27, 28).

To exclude the possibility that the lower levels of 14-3-3σ protein observed in some invasive tumors were caused by increased release of this protein to the medium during the labeling process, we also analyzed the 2D gel patterns of externalized proteins from whole biopsies, normal as well as tumor specimens. Fig. 4 shows representative 2D gels of whole-cellular extracts from a normal biopsy (cyst 473) and an invasive TCC (375-2, pT2-4 G4). High levels of intracellular 14-3-3σ were matched by comparably high levels of this protein in the medium (Fig. 4, upper panels). Likewise, low levels of intracellular 14-3-3σ were accompanied by equally low levels of externalized protein as illustrated in the case of TCC 375-2 (pT2-4 G4) (Fig. 4, lower panels). These results show that the down-regulation of 14-3-3σ observed in some invasive TCCs is not due to increased protein release to the medium during labeling.

2D PAGE and indirect immunofluorescence analysis of pure bladder SCCs revealed very high levels of expression of 14-3-3σ (Fig. 5), comparable to those observed in keratinizing stratified squamous epithelia (3). High expression levels of this protein were also observed in squamous metaplasias (results not shown).

**Immunostaining of 14-3-3σ in Normal and Invasive TCCs**—To confirm the differential expression of 14-3-3σ in invasive TCCs, we raised a peptide-specific antibody against this protein in rabbits in collaboration with Eurogentec. This antibody was tested for cross-reactivity with other 14-3-3σ protein family members by 2D PAGE immunoblotting using both isoelectrofocusing and nonequilibrium pH gradient electrophoresis (24). As depicted in Fig. 6, the antibody shows exquisite isoform specificity as it reacted only with 14-3-3σ in the RT4 bladder epithelial cancer cell line. No cross-reactivity was detected in nonepithelial cells known to express all other isoforms (Fig. 6, human MRC5 fibroblasts).

The 14-3-3σ antibody was subsequently used to perform immunostaining of cryostat sections of specimens examined by 2D gel electrophoresis. As shown in Fig. 7, normal as well as noninvasive TCCs showed strong immunoreactivity for 14-3-3σ in the epithelium lining of the urinary bladder (Fig. 7, A–D). Notably, in normal urothelium the 14-3-3σ immunoreactivity was often weaker in the suprabasal intermediate layer as compared with basal (Fig. 7, B and C, white arrowheads).

**Table I**

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<th>Pathological classification</th>
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*a Levels of expression of 14-3-3σ in SCCs were generally upregulated as compared to normal urothelium, with samples showing very high levels of σ protein content.

**Fig. 3.** IEF 2D gel of [35S]methionine-labeled proteins synthesized by an invasive TCC (569-1 pT2-4 G4) presenting low levels of 14-3-3σ expression. Arrowheads indicate positions for epithelial-specific proteins (CK18 and 14-3-3σ), mesenchymal-specific proteins (vimentin and PGP 9.5), and a ubiquitously expressed protein (actin).
and umbrella cells (Fig. 7, B and C, yellow arrowheads). This differential staining was occasionally not observed in some histological normal specimens derived from patients with invasive TCCs (Fig. 7D).

Differential 14-3-3α immunoreactivity with stronger staining of the basal layers (Fig. 7, E and F, white arrowheads) was observed in some patients bearing superficial (Fig. 7E, 898-3 pTa G1) or invasive lesions (Fig. 7F, 738 pT1 G3), most likely reflecting tumor type differences (20). Highly invasive TCCs, on the other hand, showed homogenous staining (Fig. 7G, 702 pT2-4 G3; H, 697 pT1 G3; and I, 566-1 pT2-4 G4), with immunoreactivity to 14-3-3α corresponding well to the protein profiles obtained by 2D gel analysis (cf. Fig. 7G–I, with corresponding panels in Fig. 2). Staining of SCCs with the 14-3-3α antibody confirmed the high expression of this protein as determined by 2D PAGE (Fig. 5).

The staining phenotypes of both normal urothelium and malignant lesions were confirmed by double immunostaining with 14-3-3α and CK5 antibodies (Fig. 8). In nonmalignant tissue, we observed complete co-distribution between CK5 and 14-3-3α staining of the basal, intermediate, and superficial layers (Fig. 8A, white arrowhead). Invasive tumors that showed low levels of 14-3-3α expression in the 2D PAGE analysis were characterized by the absence of 14-3-3α staining in cells that stained throughout with the CK5 antibody (e.g. Fig. 7B; 569-1 pT2-4 G4). Additionally, we observed heterogeneous staining patterns with aberrant 14-3-3α staining in five lesions that had shown high levels of expression by 2D PAGE analysis. The extent of the staining heterogeneity varied, but it included variants of the two major subtypes shown in Fig. 8, C and D. Thus, in three specimens we could observe single scattered cells and/or areas of abnormal α expression (e.g. Fig. 8C, white arrowheads; 916-1 pTa G1), while in two other cases the heterogeneity was characterized by the pro-
areas of the lesion displaying negative staining (Fig. 8).

Levels of 14-3-3 were analyzed in normal urothelium (Fig. 9, A, cyst 981; B, cyst 981; C, cyst 1004; and D, cyst 975), and invasive TCCs (E, 898-3 pTa G1; F, 738; G, 702 pT2-4 G3; H, 697 pT1 G3; I, 566 pT2-4 G4). All samples showed high levels of 14-3-3 expression by 2D gel analysis, underscoring the importance of complementary immunostaining studies.

Impaired Expression of 14-3-3 Is Not Associated with Altered p63 Expression—In an effort to identify the molecular alterations that could be responsible for the down-regulation of 14-3-3 expression in invasive TCCs, we analyzed the levels of p63 in the invasive lesions, as several studies on human tumors have suggested an oncogenic function for p63.

It has been shown that down-regulation of 14-3-3 expression is a hallmark of bladder cancer (6), presumably due to inhibition of CDK activity and can make primary human epithelial cells grow indefinitely (36) and that overexpression leads to inhibition of cell proliferation and prevents anchorage-independent growth of breast cancer cells (6).

Proliferative Status Is Not a Major Determinant of 14-3-3 Expression—It has been shown that down-regulation of 14-3-3 can make primary human epithelial cells grow indefinitely (36) and that overexpression leads to inhibition of cell proliferation and prevents anchorage-independent growth of breast cancer cells (6), presumably due to inhibition of CDK activity. These results indicate that cell-cycle control is one of the key functions of 14-3-3.

Impaired Expression of 14-3-3 Is Not Associated with Altered p63 Expression—In an effort to identify the molecular alterations that could be responsible for the down-regulation of 14-3-3 expression in invasive TCCs, we analyzed the levels of p63 in the invasive lesions, as several studies on human tumors have suggested an oncogenic function for p63.
regulation of 14-3-3σ was required for increased cell proliferation, we analyzed by 2D PAGE the levels of this protein in papillary tumors (low grade and stage) and in primary cultures (6 days) derived from them. As an example, Fig. 10 shows that papillary tumors (low grade and stage) and in primary cultures, we analyzed by 2D PAGE the levels of this protein in

**FIG. 9.** Laser scanning analysis of the double-labeled immunofluorescence for 14-3-3σ (Alexa Fluor® 594; red) and p63 (Alexa Fluor® 488; green). Original magnification, ×100 (A and D, SCC 536-1), ×800 (B and C, 916-1 pTa G1 and 817-5 pT2-4 G3, respectively), and ×630 (E and F, 916-1 pTa G1 and 817-5 pT2-4 G3, respectively); also ×630 for lower right panel inset in A (corresponding to the framed area in the lower left panel). This figure shows that 14-3-3σ expression in tumors does not correlate with the dominant-negative splice variant ΔNp63 (A–C) nor with the trans-activating variant TAp63α (D–F).

DISCUSSION

The 14-3-3σ is a member of a multifunctional protein family that comprises seven isoforms (β, γ, η, τ, σ, ε, and ζ). These proteins are involved in a plethora of biological processes such as apoptotic cell death, mitogenic signal transduction, and cell-cycle control (1). The latter is particularly relevant in the case of the σ isoform, as this protein has been shown to inhibit G2/M progression in a p53-regulated manner and is critical to upholding G2 arrest upon DNA damage in colorectal cancer cells (4, 7). Furthermore, 14-3-3σ has been associated with Cdk2 and Cdk4, suggesting that it may also regulate G1/S progression (6). In primary human epidermal keratinocytes, down-regulation of 14-3-3σ results in evasion from senescence (36), and inactivation of this protein in various cancers has been generally attributed to hypermethylation of the CpG island present in the promoter area of the 14-3-3σ gene (9–17, 37). Down-regulation of 14-3-3σ, however, is not a general requirement for neoplastic transformation as this protein is overexpressed in the neoplastic epithelium of pancreatic adenocarcinoma as compared with normal pancreas (39). Overall, these lines of evidence indicate that functional inactivation of 14-3-3σ may be linked to carcinogenesis and suggest that silencing of 14-3-3σ may be an important event in tumor progression in specific forms of cancer.

Here we have presented evidence showing that down-regulation of 14-3-3σ in bladder carcinomas occurs mainly in invasive TCCs and that the low levels of 14-3-3σ observed in some tumors are not due to increased externalization of the protein, or to the proliferative status of malignant cells as primary cultures derived from tumors of low grades and stages showed high levels of expression of this protein. We found that 10 out of 36 invasive specimens examined showed down-regulation of this protein, and of these, 6 out of the 10 corresponded to lesions undergoing EMT, a phenomena in which cells dissociate from the epithelia and migrate freely, thus contributing to the invasion and metastatic process (38). The manifestation of the mesenchyme phenotype was not merely due to sample contamination with connective tissue, as we could observe expression of PGP 9.5, a marker associated with mesenchymal neoplasms (28), which is not expressed in connective tissue (27). It is likely that down-regulation of 14-3-3σ in high-grade neoplasms is due to the greater plasticity of these cells to differentiation events, as well as to the effect of the tumor microenvironment. The latter may explain the variability observed in immunoreactivity within the same tumor. Immunofluorescence analysis of cryostat sections confirmed these data, revealing positive staining of bladder epithelia in normal samples, but weak or negative staining of malignant cells in specimens that showed low levels of 14-3-3σ as determined by 2D PAGE (Fig. 7). Additionally, we identified some specimens that albeit showing normal levels of 14-3-3σ in 2D gels possessed heterogeneity areas devoid of 14-3-3σ (mosaicism or progressive loss in the front of invasion of the tumor; Fig. 8), underscoring the im-
demonstrated that 14-3-3 (Fig. 5), an observation that is in line with a recent report that 14-3-3 differentiates toward a squamous cell lineage (39). High levels of stronger in cells destined for squamous epithelium or differentiate to SCCs overall show significantly higher levels of this protein and a primary culture derived from it (6 days in culture), were separated by 2D PAGE (IEF) and visualized by autoradiography. The area of the gel that contains the 14-3-3 was consistently occur during culturing. The position of the proliferation marker PCNA is indicated for reference.

Interestingly, our studies also revealed generally elevated levels of 14-3-3σ in SCCs compared with normal urothelium (Fig. 5), an observation that is in line with a recent report that demonstrated that 14-3-3σ expression had a tendency to be stronger in cells destined for squamous epithelium or differentiating toward a squamous cell lineage (39). High levels of 14-3-3σ have also been reported in hyperproliferative skin diseases, such as psoriasis, condylomata acuminata, and actinic keratoses (40, 41; see also proteomics.cancer.dk/2Dgallery/kerat-t-po270_skerat.html), suggesting again that down-regulation of this protein is not an absolute requirement for cellular proliferation. However, because 14-3-3 proteins are known for their potential to regulate cellular activity by binding and sequestering proteins containing a cognate phosphorylated consensus motif (1), we cannot exclude the possibility that expression of a σ-interacting protein is affected in these cultured cells resulting in impaired cellular activity, functionally equivalent to down-regulation of 14-3-3σ.

In an effort to shed some light into the molecular mechanism underlying the down-regulation of 14-3-3σ in some invasive TCCs, we analyzed the expression of p63 in these lesions, as studies on human tumors have suggested an oncogenic function for p63 isoforms (29, 30) in urothelial lesions (31–33). p63 is a homolog of the p53 gene that encodes two types of isoforms that can either function to trans-activate p53-responsive genes (TAp63 isoforms) or act as a dominant-negative transcription factor (ΔNp63 isoforms) (29, 30). The dominant-negative splice variant ΔNp63 has transcriptional repressor activity and can bind to the 14-3-3σ promoter in vivo (34). Our results showed concomitant expression of ΔNp63α and 14-3-3σ in TCCs having both high and low expression of 14-3-3σ (Fig. 9), ruling out potential inhibitory effects of this isoform on 14-3-3σ transcription as the basis for the loss of expression observed. An alternative mechanism for the down-regulation of 14-3-3σ is the targeting of this protein for proteasomal degradation by the ubiquitin E3-ligase Efp (10). However, we found no evidence of proteolytic fragments of 14-3-3σ in the 2D PAGE and 2D Western analyses we performed, making Efp-targeted proteolysis, if anything, a minor contributing mechanism for down-regulation of 14-3-3σ in invasive bladder carcinomas.

As a general rule one can say that levels of 14-3-3σ are highly cell-type dependent. Thus, squamous epithelial cells are clearly the cell type displaying the highest levels of 14-3-3σ, whereas mesenchymal cells lack this protein. Similarly, SCCs overall show significantly higher levels of this protein than TCCs. In addition, tumor progression with associated epithelial plasticity also leads to altered levels of 14-3-3σ, in particular in tumors undergoing EMTs, which display impaired expression of 14-3-3σ. Consequently, quantification of 14-3-3σ can potentially be of great value for the pathological evaluation of bladder carcinomas. Furthermore, conversion from an epithelial to a mesenchymal phenotype is increasingly being considered as an important event in carcinoma progression and metastasis, and to generally correlate with a poor prognosis of the disease, with loss of epithelial characteristics and acquisition of a migratory, fibroblastoid phenotype being associated with invasive cell behavior (39, 42, 43). Studying the molecular mechanisms involved in EMT might provide new insights into tumor progression and metastasis and may lead to the identification of molecular pathways and key regulators that are important for the progression of carcinoma toward dedifferentiated and more malignant states. Our results provide evidence that deregulation of 14-3-3σ may play a key role in bladder cancer progression, in particu-
ular in differentiation events leading to EMT and stratified squamous metaplasia. In this respect, it is noteworthy to mention that 14-3-3σ has recently been found as a gene associated with transforming growth factor β-induced EMT in Ha-Ras-transformed EpH4 mammary epithelial cells (44). Levels of 14-3-3σ can therefore represent a valuable marker for tumor progression and aggressive behavior.

In summary, determination of functional threshold levels and clarification of the functional role(s) of 14-3-3σ in bladder cancer progression may shed some light as to the molecular mechanism(s) involved in epithelial differentiation and plasticity in normal urothelium and provide a novel therapeutic target for the specific treatment of aggressive bladder carcinomas.

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