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STAG2 is a Biomarker for Prediction of Recurrence and Progression in Papillary Non-Muscle Invasive Bladder Cancer

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Abstract

Purpose—Most bladder cancers are early stage tumors known as papillary non-muscle invasive bladder cancer (NMIBC). After resection, up to 70% of NMIBCs recur locally, and up to 20% of these recurrences progress to muscle invasion. There is an unmet need for additional biomarkers for stratifying tumors based on their risk of recurrence and progression. We previously identified STAG2 as among the most commonly mutated genes in NMIBC and provided initial evidence in a pilot cohort that STAG2 mutant tumors recurred less frequently than STAG2 wild-type tumors. Here we report a STAG2 biomarker validation study using two independent cohorts of clinically-annotated papillary NMIBC tumors from the US and Europe.

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Conflict of Interest: The authors declare no potential conflicts of interest.

Experimental Design—The value of STAG2 immunostaining for prediction of recurrence was initially evaluated in a cohort of 82 patients with papillary NMIBC ("Georgetown cohort"). Next, the value of STAG2 immunostaining for prediction of progression to muscle invasion was evaluated in a progressor-enriched cohort of 253 patients with papillary NMIBC ("Aarhus cohort").

Results—In the Georgetown cohort, 52% of NMIBC tumors with intact STAG2 expression recurred, whereas 25% of STAG2-deficient tumors recurred (p=0.02). Multivariable analysis identified STAG2 expression as an independent predictor of recurrence (HR=2.4; p=0.05). In the progressor-enriched Aarhus cohort, 38% of tumors with intact STAG2 expression progressed within five years, versus 16% of STAG2-deficient tumors (p<0.01). Multivariable analysis identified intact STAG2 expression as an independent predictor of progression (HR=1.86; p=0.05).

Conclusions—STAG2 IHC is a simple, binary, new assay for risk stratification in papillary NMIBC.

INTRODUCTION

Bladder cancer is the fifth most common cancer in the United States and the fourth most common in men, with >80,000 new cases annually (1). Most of these tumors (~75%) are polyp-like outgrowths referred to as papillary non-muscle invasive bladder cancer (NMIBC) (2). NMIBC can generally be completely surgically resected via an outpatient cystoscopy-based procedure known as transurethral resection of bladder tumor (TURBT). A subset of patients are then treated with adjuvant intravesical Bacillus Calmette-Guerin (BCG) immunotherapy or intravesical chemotherapy, depending on risk parameters (3). Despite these treatments, as many as 70% of these tumors will recur, and ~20% of these recurrences will progress to invasion of the surrounding detrusor muscle (3).

Because it is not possible to accurately predict which NMIBCs are most likely to recur and progress, clinicians err on the side of caution and perform an extremely intensive post-resection surveillance regimen. For example, for superficially invasive ("pT1") NMIBC, the American Urological Association recommends surveillance by cystoscopy as frequently as every 3 months for 2 years after removal of the tumor, every 6–12 months for the next two years, and at least yearly thereafter for the rest of the patient's life (3). Such a surveillance regimen is invasive, inconvenient, and uncomfortable for patients. Furthermore, it is extremely expensive – remarkably, the clinical management of NMIBC imposes the highest per patient costs on the US health care system than the management of any other cancer type (4,5,6,7).

Another current challenge in the care of patients with NMIBC is the decision whether or not to treat with adjuvant intravesical BCG immunotherapy and/or intravesical chemotherapy. These treatments are known to be effective in reducing the risk of recurrence and progression, but are sometimes avoided because of the potential for side effects (8,9).

We and others discovered mutations of the STAG2 tumor suppressor gene in ~35% of NMIBC, identifying it as among the most commonly mutated genes in NMIBC (10,11,12). The STAG2 protein is a component of the cohesin complex, which plays important roles in

diverse cellular processes including chromosome segregation, chromatin structure, gene expression, and DNA repair (reviewed in refs.13,14,15).

In our initial study identifying STAG2 mutations in bladder cancer, we found that STAG2 mutations were much more common in NMIBC (~32%) than in tumors that had progressed to muscle invasion (~12%) (10). Similar findings were reported by several other groups (11,12,16). This observation suggested that NMIBCs with mutant STAG2 genes may be less likely to recur and progress to muscle invasion than NMIBCs with wild-type STAG2 genes, pointing to STAG2 as a potentially useful biomarker for predicting recurrence and progression in NMIBC.

In order to test this hypothesis, we developed an IHC-based assay for identifying tumors harboring STAG2 mutations. This assay is particularly robust because (i) it utilizes a monoclonal antibody whose epitope is at the extreme carboxyl-terminus of STAG2, and ~85% of tumor derived mutations of STAG2 are truncating (i.e., nonsense, frameshift, or splice site mutations that result in the complete absence of the carboxyl-terminus epitope); (ii) STAG2 is on the X-chromosome, so only a single mutation is required for complete gene inactivation (i.e., there is no intermediate heterozygote effect); and (iii) STAG2 is among the most abundant proteins in the human proteome. This IHC assay for STAG2 inactivation has been validated internally by staining formalin-fixed, paraffin-embedded pellets of gene edited isogenic sets of STAG-proficient and deficient cultured human cancer cells, as well as externally by several groups evaluating STAG2 inactivation in Ewing sarcoma, bladder cancer, pancreatic cancer, and other tumor types (10,11,12,17,18,19,20,21).

Using this assay, we reported the results of a pilot study on 34 clinically-annotated papillary NMIBCs from the MD Anderson Cancer Center (10). In that initial cohort, 12% of STAG2-deficient NMIBCs recurred (1/8), whereas 58% of STAG2-expressing NMIBCs recurred (15/26; p=0.05). These data provided initial support for the hypothesis that STAG2-deficient NMIBC are less likely to recur and progress to muscle invasion than STAG2-expressing NMIBC. However, those findings clearly required validation in independent, larger cohorts of patients. Therefore, the study reported herein was performed.

MATERIALS AND METHODS

Georgetown Cohort

The Lombardi Comprehensive Cancer Center (LCCC) Cancer Registry was searched to identify all cases of NMIBC (i.e., pathological stage pTa and pT1) treated at Medstar Georgetown University Hospital prior to 12/31/2012. Cohort discovery revealed that there were 150 unique patients who met this criteria (Supplemental Fig. 1). For each of these patients we obtained the complete clinical abstract from the Cancer Registry and the pathology report from the Cerner database. Tumor blocks were located for 124 of the 150 patients. Thirteen patients were excluded because tumor blocks displayed poor tissue integrity. For the remaining 111 patients, slides were cut, stained with H&E, and examined by two genitourinary pathologists (DS, JS). Re-grading was performed in accordance with the 2016 WHO Classification.

Once patients were evaluated against exclusion criteria (Supplemental Fig. 1), the final cohort was comprised of 82 patients. Exclusions included patients: (i) whose cut tumor block displayed poor integrity on the slide mount (n=2), (ii) whose cut slides had no identifiable tumor (n=7), (iii) whose tumor was carcinoma in situ (CIS; n=2), (iv) whose tumor was adenocarcinoma (n=1), (v) who had a cystectomy (n=9), (vi) who had CIS and had a cystectomy (n=5), and (vii) whose clinical information in the cancer registry was sufficiently ambiguous that the presence or absence of recurrence could not be determined (n=3). All of these exclusions were performed prior to data analysis. Median follow up was 75 months. All tumor samples studied were TURBT specimens from the initial diagnosis; none were recurrences. Recurrence was defined as tumor visualization by cystoscopy followed by a positive biopsy. 69/82 patients received no adjuvant therapy, 9/82 patients received adjuvant BCG immunotherapy, 3/82 patients were treated with mitomycin C, and 1/82 patients was treated with Thiotepa. No patients were treated with radiation therapy. 4/82 tumors had variant histology (3 nested, 1 plasmacytoid). In this cohort, 35/82 (43%) of tumors recurred. Studies were conducted in accordance with the Belmont Report and the U.S. Common Rule. This study was approved by the Institutional Review Board at Georgetown University. A waiver of consent was approved by the IRB for this study.

Aarhus Cohort

283 papillary NMIBC tumors (stage pTa or pT1) were collected from Aarhus University hospital from 1979 to 2007. Median follow up was 74 months. One biopsy from each tumor was taken and used to construct a tissue microarray (TMA) using the method developed by Kononen (22). All tumors were re-graded in accordance with the 2004 WHO Classification. 66 patients received adjuvant BCG immunotherapy, three patients received intravesical chemotherapy, and one patient received both during the course of their treatment. All tumor samples studied were TURBT specimens from the initial diagnosis; none were recurrences. Additional details regarding criteria used for patient selection and construction of the TMA are described in refs. 23,24,25,26. Use of the TMA sections was approved by the National Committee on Health Research Ethics, Denmark.

Once patients were evaluated against exclusion criteria (Supplemental Fig. 2), the final Aarhus cohort was comprised of 253 patients. Exclusions included: (i) absent cores (15), and (ii) tissue with poor integrity after staining (15). These exclusions were performed prior to data analysis. Recurrence was defined as tumor visualization by cystoscopy followed by a positive biopsy. Progression from NMIBC to muscle invasive bladder cancer (MIBC) was defined by pathological examination of resected tissue. In this cohort, virtually all patients recurred (235/253; 93%), and 103/253 (41%) patients progressed to MIBC. As such, this cohort is enriched for patients who progressed, and is therefore particularly well suited for the purpose of providing substantial statistical power to evaluate the predictive value of biomarkers on progression.

Immunohistochemistry

Immunohistochemistry was performed in the Georgetown University Medical Center Histopathology and Tissue Shared Resource. Five micron sections from formalin fixed paraffin embedded tissues were de-paraffinized with xylene and rehydrated through a graded

alcohol series. Heat induced epitope retrieval (HIER) was performed by immersing the tissue sections at 98°C for 20 minutes in Target retrieval solution, high pH (Dako). Immunohistochemical staining was performed using the VectaStain Kit from Vector Labs according to manufacturer's instructions. Briefly, slides were treated with 3% hydrogen peroxide for 10 minutes. Endogenous biotin was blocked using an avidin/biotin blocking kit from Invitrogen. The slides were then treated with 10% normal goat serum for 10 minutes and exposed to primary antibody for STAG2 (1:50, Santa Cruz, sc81852) for 1 hour at room temperature. Slides were then exposed to biotin-conjugated mouse secondary antibody (Vector Labs), Vectastain ABC reagent and DAB chromagen (Dako). Slides were counterstained with hematoxylin (Fisher, Harris Modified Hematoxylin) at a 1:8 dilution for 2 minutes at room temperature, blued in 1% ammonium hydroxide for 1 minute at room temperature, dehydrated, and mounted with Acrymount.

Pathologic Evaluation

STAG2 staining was determined in a blinded fashion by two independent pathologists at two different medical centers (BH, DS). Slides and cores were read as either positive, negative, or mosaic for STAG2 expression. Since staining for STAG2 is largely binary, there was 99% agreement between the two pathologists. Disagreements were resolved by a third observer (TW).

For the Georgetown cohort, tumor stage and grade were determined by two genitourinary pathologists (DS, JS) in accordance with the 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs. High grade includes both uniformly and focally high grade papillary urothelial carcinomas. Low grade includes both low grade papillary urothelial carcinomas and Papillary Urothelial Neoplasms of Low Malignant Potential (PUNLMPs). For the Aarhus cohort, tumor stage and grade were determined by an experienced genitourinary pathologist in accordance with the 2004 WHO Classification of Tumors of the Urinary System and Male Genital Organs, as described in detail in ref. 23.

Statistical Analysis

Statistical analyses were conducted using R (27). Power analyses were conducted. Demographic information is presented in the form of means (standard deviations) for continuous variables and counts (percentages) for categorical variables. Survival analysis was performed using Kaplan Meier curves, the Peto-Peto modification of the Wilcoxon Test, and Cox Proportional Hazards models. Statistical significance was assessed at p=0.05.

RESULTS

As described above, in 2013 we and others reported the discovery of STAG2 mutations in bladder cancer (10,11,12,16). In that initial study, we performed a pilot IHC study on a cohort of 34 patients and demonstrated that while 58% (15/26) of STAG2-expressing NMIBCs recurred, only 12% (1/8) of STAG2-deficient NMIBCs recurred (p=0.05). Based on that initial pilot data, we performed a power analysis to estimate the sample size needed in a validation study to achieve 80% power with significance level = 0.05. Considering the ~3:1 ratio of STAG2 positive:negative samples in the pilot cohort, a total of 56 samples,

where 42 were positive and 14 were negative, would be required to achieve the desired power in a validation study, based on a two-sided logrank test.

We next identified a cohort of 82 patients with papillary NMIBC whose initial tumor was treated by TURBT at Medstar Georgetown University Hospital prior to 12/31/2012, and whose clinical information was available in the LCCC Cancer Registry. The details of cohort discovery are described in Materials and Methods and depicted in Supplemental Fig. 1. Clinical and pathological characteristics of the cohort are listed in Table 1.

STAG2 IHC and pathologic evaluation was performed as described in Materials and Methods. 54/82 tumors were uniformly STAG2 positive (66%); 19/82 were uniformly STAG2 negative (23%); and 9/82 tumors were mosaic (11%), with discrete patches of STAG2 negativity. Since there were >42 STAG2 positive tumors and >14 negative tumors, this cohort was powered at >80%. Three examples of each staining profile are shown in Fig. 1. This 34% frequency of complete or mosaic STAG2 inactivation is similar to the 32% frequency of STAG2 mutations in NMIBC reported in our initial study (10). Of note, we have previously shown that tumors with mosaic inactivation of STAG2 harbor inactivating mutations of the gene in their STAG2 non-expressing cells (10).

The clinical and pathological characteristics of the cohort were then correlated with STAG2 expression (Table 1). Uniform and mosaic loss of STAG2 expression was highly correlated with low grade tumors ($p<0.01$; Fisher's Exact Test). This relationship between loss of STAG2 expression and low pathological grade confirms previous work by Taylor et al. (16).

We then performed a univariate analysis to evaluate whether STAG2 staining was significantly associated with recurrence. Initially, univariate analysis was performed on three groups: (i) tumors with uniform retention of STAG2 expression, (ii) tumors with uniform loss of STAG2 expression, and (iii) tumors with mosaic loss of STAG2 expression. This analysis revealed that ~52% of the STAG2-expressing tumors recurred (28/54), whereas 22% of STAG2-deficient tumors recurred (2/9) and 26% of STAG2 mosaic tumors recurred (5/19; $p=0.05$; Fisher's Exact Test). The Peto-Peto modification of the Wilcoxon test showed that the two survival curves in the Kaplan-Meier curve presented in Fig. 2A were statistically different ($p=0.02$).

Since bladder cancers have been shown to display substantial intratumoral heterogeneity (2,28), it was not unexpected that a subset of tumors showed mosaic inactivation of STAG2. Since both uniform and mosaic inactivation of STAG2 were both highly correlated with low grade tumors (Table 1), and because tumors with uniform and mosaic inactivation both harbor inactivating mutations of the gene, we considered combining tumors with uniform and mosaic loss of expression into a single group. Moreover, to test whether this biological justification for combining groups was also statistically justified, we conducted a likelihood ratio test, which demonstrated significant improvements in model fit by collapsing negative and mosaic tumors into a single group.

Therefore, we performed a second univariate analysis comparing the likelihood of recurrence of STAG2 expressing tumors to tumors with either uniform or mosaic loss of STAG2 expression. This analysis revealed that ~52% of uniformly STAG2-expressing

tumors recurred (28/54), whereas only ~25% of the tumors with complete or mosaic loss of STAG2 expression recurred (7/28, p=0.02; Fisher's Exact Test). The Peto-Peto modification of the Wilcoxon test showed that the two survival curves in the Kaplan-Meier curve presented in Fig. 2B were statistically different with a p-value of 0.02.

Once we had demonstrated the prognostic value of STAG2 on recurrence in a univariate analysis, we performed additional univariate analyses to evaluate the prognostic value for recurrence of the clinical and pathological characteristics listed in Table 1 for which sufficient clinical information was available. These results are shown in Table 2.

Next, we tested whether STAG2 was an independent predictor of recurrence in a multivariable analysis (Table 3). When considered together in a multivariable analysis with pathological grade, pathological stage, and the presence or absence of adjuvant therapy, STAG2 was an independent predictor of recurrence (p=0.05). The hazard ratio for recurrence in patients with STAG2 expressing tumors was 2.4 times that of patients with STAG2 negative or mosaic tumors. Note, however, that due to the limited number of patients in this cohort, and issues of data sparsity for some parameters (smoking, tumor size, tumor multiplicity), we were unable to include all clinical and pathological characteristics in this multivariable analysis. However, this statistical limitation was rectified in our second, much larger cohort (see below).

An ideal biomarker would be one predicting not only recurrence, but tumor progression as well. Unfortunately, the Georgetown cohort lacked the statistical power to assess the prognostic value of STAG2 expression on progression because only 5/82 (6%) of the patients progressed to muscle invasion during the follow up period. Therefore, in order to evaluate the prognostic value of STAG2 expression on progression, we analyzed a second cohort of 253 patients with papillary NMIBC (the "Aarhus cohort") that was heavily enriched for NMIBC that later progressed to MIBC (103/253; 41%). The details of this cohort are described in Materials and Methods and in refs. 22,23,24,25. The clinical and pathological characteristics of this cohort are shown in Table 4.

STAG2 IHC and pathologic evaluation were performed as described in Materials and Methods. 186/253 tumors were uniformly STAG2 positive (74%); 61/253 were uniformly STAG2 negative (24%); and 6/253 tumors were mosaic (2%).

The clinical and pathological characteristics of the cohort were then correlated with STAG2 expression (Table 4). The six tumors with mosaic loss of STAG2 expression were combined with the 61 tumors with uniform loss of STAG2 expression, as described above for the Georgetown cohort.

In this cohort, loss of STAG2 expression was correlated with female gender (p<0.01) and lower pathological stage (p=0.04). These trends were also present in the Georgetown cohort, but did not reach statistical significance, probably because of the limited size of the Georgetown cohort. In the Aarhus cohort there was also trend towards loss of STAG2 expression correlating with low grade tumors (a correlation that was statistically significant above for the Georgetown cohort), but this did not reach statistical significance in the Aarhus cohort (p=0.06).

We performed univariate analyses to evaluate whether STAG2 staining was significantly associated with progression to MIBC. In the Aarhus cohort, 38% of the STAG2-expressing tumors progressed to muscle invasion within five years (70/186), whereas only 16% of STAG2-deficient tumors progressed within five years (11/67; $p<0.01$; chi-squared test). In the entire 12.5 year window of follow-up, 45% of the STAG2-expressing tumors progressed to muscle invasion (84/186), whereas only 28% of STAG-deficient tumors progressed (19/67; $p=0.02$; chi-squared test). The two survival curves in the Kaplan-Meier curve presented in Fig. 3 were statistically different with $p<0.01$. Unlike in the Georgetown cohort, STAG2 expression was not significantly associated with recurrence in the progressor-enriched Aarhus cohort ($p=0.33$) because virtually all tumors in the Aarhus cohort recurred (235/253; 93%).

Once we had demonstrated the predictive value of STAG2 on progression in a univariate analysis, we next evaluated the prognostic value for progression of gender, age, adjuvant therapy, tumor grade, and tumor stage (Table 5). This analysis indicated that in addition to STAG2 staining, age, pathological grade, and pathological stage were significant predictors of progression in this cohort at $p<0.05$.

Next, we tested whether STAG2 was an independent predictor of progression in a multivariable analysis when including all the factors tested in univariate analysis (Table 6). STAG2 expression was an independent predictor of progression in this multivariable analysis ($p=0.05$), as were increasing age at diagnosis ($p=0.03$) and high grade ($p=0.01$). The hazard ratio for progression in patients with STAG2 expressing tumors was 1.86 times that of patients with STAG2 negative or mosaic tumors.

DISCUSSION

Here we demonstrate that a simple, robust, virtually binary assay for identifying STAG2-mutant tumors is useful for risk stratification in papillary NMIBC. We show that tumors that uniformly express STAG2 are twice as likely to recur and progress to muscle invasion as tumors that display complete or mosaic loss of STAG2 expression. This increased risk was maintained in multivariable analysis, demonstrating that it is independent of other variables currently used for risk stratification in NMIBC.

In addition to demonstrating the prognostic value of STAG2 IHC for recurrence and progression, the frequency of STAG2 inactivation reported here confirms our previous work in which we reported that ~32% of NMIBCs harbor STAG2 mutations. A similar high frequency of STAG2 mutations in NMIBC was observed by Taylor et al. (16), but other groups have reported somewhat lower frequencies (11,12). In the Georgetown cohort reported here, the frequency of inactivation was 34%. In the Aarhus cohort the frequency of STAG2 inactivation was only 26% because the cohort is enriched for progressors, who as we shown in this study are less likely to harbor mutations of STAG2. We expect that the actual percentage of STAG2 inactivated papillary NMIBC tumors is probably slightly higher than the 34% seen in the Georgetown cohort, since this IHC assay misclassifies tumors with missense mutations as wild-type and therefore undercounts STAG2 mutant tumors by ~15%.

The data presented here are in general agreement with a recent study by Qiao et al., who evaluated the effect of STAG2 immunostaining on recurrence in a group of 125 patients with NMIBC (91 patients) and muscle invasive bladder cancer (MIBC; 34 patients) in China (29). In their study, NMIBC tumors displaying loss of STAG2 expression were less likely to recur than STAG2-expressing NMIBC tumors. However, this trend was statistically significant only when NMIBC and MIBCs were combined into a single group, perhaps in part because their reported frequency of STAG2 inactivation in NMIBC was 23%, substantially less than the 34% identified here, the 32% we previously reported (10), and the 34% observed by Taylor et al (16).

In the Georgetown cohort, 9/82 (11%) of tumors showed mosaic (ie. patchy) loss of STAG2 expression. This relatively high frequency of mosaicism is similar to that reported in a previous study (16). Here we show that these mosaic tumors (which harbor STAG2 mutations in only a subset of the tumor cells) recur at a similarly low frequency as those tumors with uniform loss of STAG2 expression. Of note, the Aarhus cohort had a lower frequency of mosaic tumors (6/253, 2%) than the Georgetown cohort (11%) because it is less likely that intratumoral heterogeneity will be captured in a 0.6 mm TMA core (Aarhus) than in a cross section of an entire tumor on a whole slide (Georgetown).

This study has several limitations. First, although properly powered, the Georgetown cohort is relatively small (82 patients). Furthermore, the Georgetown cohort, while having complete information for recurrence, lacked complete information for several clinicopathological parameters such as tumor size and multiplicity that are currently used to stratify patients into high and low risk groups. Because of these data scarcity issues, we were unable to include these parameters in a multivariable analysis of the Georgetown cohort (although they were included in the Aarhus cohort). We are currently performing a biomarker validation study with a prospectively collected cohort of NMIBC tumors to resolve these limitations.

Another limitation is that the IHC assay reported herein does not identify 100% of STAG2 mutant tumors; instead, it identifies the ~85% of STAG2 mutant tumors harboring truncating mutations of the gene (10). As such, the ~15% of STAG2 mutant tumors harboring missense mutations of STAG2 are misclassified as STAG2 wild-type when using this assay. As we enter an era in which all human tumors are routinely subjected to DNA sequencing, it may be feasible to sequence all NMIBCs. By combining STAG2 IHC with confirmatory STAG2 tumor DNA sequencing, we predict that ~15% more tumors would be classified as STAG2-mutant, resulting in an assay with even stronger predictive power for the prediction of recurrence and progression.

It is also worth noting that in the initial 2013 papers describing mutations of STAG2 in bladder cancer, there was some disagreement regarding the potential prognostic value of STAG2 mutations on recurrence and progression in NMIBC. The results in our initial study (10) as well as in Balbas-Martinez et al. (12) were concordant with both the data shown here and with the data presented in Qiao et al. (29), in that loss of STAG2 expression was associated with a lower risk of tumor recurrence and progression (although in Balbas-Matinez et al. the correlation was not statistically significant in multivariable analysis). However, Guo et al. (11) reported the opposite association – that STAG2 mutant papillary

NMIBC bladder cancers were more likely to recur and progress than STAG2 wild-type tumors. However, this finding was performed in a relatively small cohort and has not been confirmed in subsequent analysis.

Finally, the clinical observation presented in this study is consistent with our other work showing that the introduction of tumor-derived mutations by gene editing into a STAG2 wild-type cultured human cells causes them to proliferate more slowly (30). Since STAG2 has been shown to modulate differentiation state in cultured myeloid precursors, (31,32,33), we speculate that STAG2 mutations might similarly modulate the differentiation state of the stem-like cells that give rise to bladder epithelial cells. Such altered differentiation could result in an aberrant papillary growth pattern but reduced ability to proliferate and progress, as compared with papillary NMIBC tumors initiated by genetic events other than STAG2 mutation. This hypothesis will require further laboratory-based studies to test.

In summary, here we validate STAG2 IHC as a potentially clinically useful biomarker assay for predicting the likelihood of recurrence and progression in papillary NMIBC. If the power to predict recurrence and progression holds up in larger, prospective studies, STAG2 IHC, when combined with other factors currently used for risk stratification, may prove to be a simple assay to help individualize medical decision-making in patients with papillary NMIBC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRANSLATIONAL RELEVANCE

Bladder cancer is the fifth most common human cancer in the U.S., and the fourth most common cancer in men. While most patients present with early stage lesions known as papillary non-muscle invasive bladder cancer (NMIBC) that are surgically resectable by a cystoscopy-based procedure, up to 70% of these tumors will recur. In ~20% of cases, these recurrences invade the muscular wall of the bladder. Because of this unpredictable clinical course, a diagnosis of NMIBC is fraught with uncertainty regarding the need for adjuvant therapy and the necessary frequency of post-operative surveillance by cystoscopy. Therefore, there is a substantial unmet need for more precise risk stratification to provide patients and their physicians additional information regarding the likelihood that any given NMIBC will recur and progress to muscle invasion. In this manuscript, we validate a new prognostic biomarker in NMIBC using a simple, binary, immunohistochemistry-based assay that reliably identifies mutational inactivation of the STAG2 gene, one of the most commonly mutated genes in papillary NMIBC.

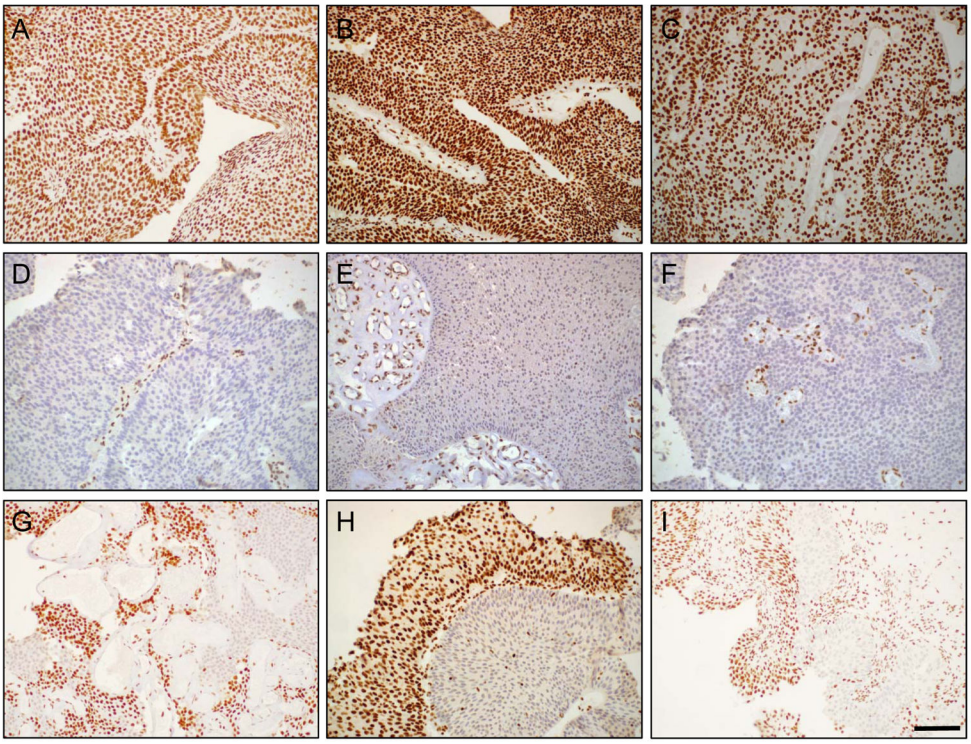

Figure 1.

STAG2 immunohistochemistry. Three representative examples each are shown for tumors with uniform STAG2 expression (A–C), uniform absence of STAG2 expression (D–F), and mosaic STAG2 expression (G–I), all at 20x magnification. In each panel, non-neoplastic cells stain positively for STAG2 and serve as an internal positive control. Scale bar = 100 μm.

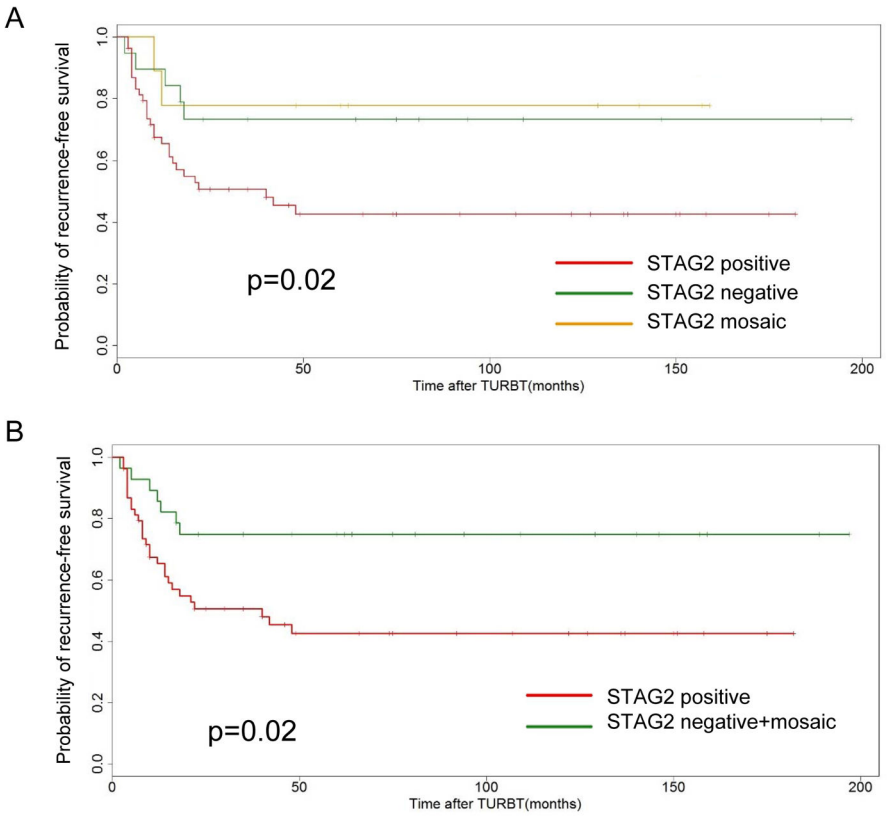

Figure 2.

Likelihood of recurrence after TURBT stratified by STAG2 staining. (**A**) Kaplan-Meier survival estimates as a function of STAG2 staining, with mosaic tumors considered as a separate group. (**B**) Same as (A) with negative and mosaic tumors collapsed into a single group. Examples of each staining profile are shown in Figure 1.

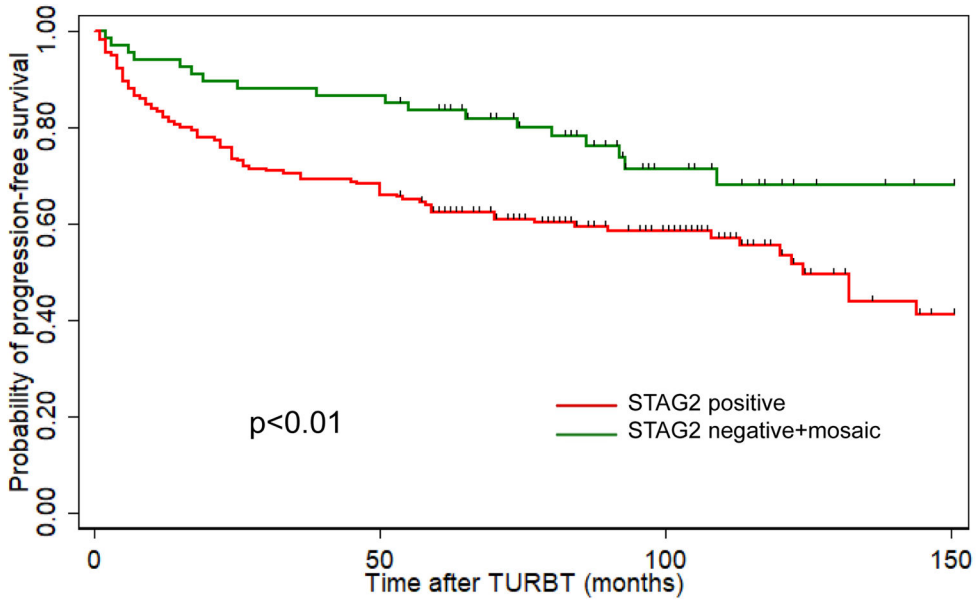

Figure 3.
Kaplan-Meier estimates of progression free survival as a function of STAG2 staining in the Aarhus cohort.

Table 1

Clinical and pathological characteristics of Georgetown cohort

Variable	Total	STAG2 Negative (n=19)	STAG2 Mosaic (n=9)	STAG2 Positive (n=54)	*P*
Gender					
Male	65 (79%)	15 (79%)	6 (67%)	44 (81%)	0.59
Female	17 (21%)	4 (21%)	3 (33%)	10 (19%)	
Race					
White	65 (79%)	15 (79%)	6 (67%)	44 (81%)	
Asian	1 (1%)	0 (0%)	0 (0%)	1 (2%)	0.61
Black	10 (12%)	3 (16%)	1 (11%)	6 (11%)	
Unknown	6 (8%)	1 (5%)	2 (22%)	3 (6%)	
Age at Diagnosis (Mean)	70	66	71	71	0.06
Adjuvant Therapy					
No	68 (83%)	17 (89%)	9 (100%)	42 (78%)	0.24
Yes	14 (17%)	2 (11%)	0 (0%)	12 (22%)	
Smoking					
No	18 (22%)	5 (26%)	2 (22%)	11 (20%)	0.78
Unknown	32 (39%)	7 (37%)	2 (22%)	23 (43%)	
Yes	32 (39%)	7 (37%)	5 (56%)	20 (37%)	
Grade					
Low Grade	44 (54%)	15 (79%)	7 (78%)	22 (41%)	<0.01
High Grade	38 (46%)	4 (21%)	2 (22%)	32 (59%)	
Stage					
Ta	69 (84%)	16 (84%)	9 (100%)	44 (81%)	0.52
T1	13 (16%)	3 (16%)	0 (0%)	10 (19%)	
Tumor Size					
≤ 3 cm	2 (2%)	7 (37%)	2 (22%)	8 (15%)	0.26
> 3 cm	8 (10%)	0 (0%)	0 (0%)	3 (6%)	

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Variable	Total	STAG2 Negative (n=19)	STAG2 Mosaic (n=9)	STAG2 Positive (n=54)	*P*
Unknown	72 (88%)	12 (63%)	7 (78%)	43 (79%)	
Multiplicity					0.72
1	14 (17%)	4 (21%)	1 (11%)	9 (17%)	
>1	11 (13%)	4 (21%)	1 (11%)	6 (11%)	
Unknown	57 (70%)	11 (58%)	7 (78%)	39 (72%)	

Table 2

Univariate Cox proportional hazard regression analysis for prediction of recurrence in Georgetown cohort

Variable	Hazard Ratio	*P*	Lower CL	Upper CL
Positive STAG2 Staining (vs. negative+mosaic)	1.69	0.03	1.05	2.70
Male Gender (vs. female)	1.17	0.57	0.68	2.00
Increasing Age	1.02	0.02	1.00	1.04
Adjuvant Therapy (vs. none)	2.22	0.01	1.20	4.09
High Grade (vs. low grade)	1.34	0.20	0.86	2.09
Pathological Stage T1 (vs. Ta)	1.81	0.05	0.99	3.30

Table 3

Multivariable Cox proportional hazard regression analysis for prediction of recurrence in Georgetown cohort

Variable	Hazard Ratio	*P*	Lower CL	Upper CL
Positive STAG2 staining (vs. negative+mosaic)	2.41	0.05	1.00	5.81
High Grade (vs. low grade)	1.03	0.93	0.49	2.17
Adjuvant Therapy (vs. none)	1.62	0.23	0.73	3.59
Pathological Stage T1 (vs. Ta)	1.59	0.29	0.68	3.75

Table 4

Clinical and pathological characteristics of Aarhus cohort

Variable	Total	STAG2 Negative (61) + Mosaic (6) (n=67)	STAG2 Positive (n=186)	*P*
Gender				
Male	199 (79%)	45 (67%)	154 (83%)	<0.01
Female	54 (21%)	22 (33%)	32 (17%)	
Age at diagnosis		64.82	67.32	0.09
Adjuvant Therapy				
No	183 (72%)	50 (75 %)	133 (72 %)	0.62
Yes	70 (28%)	17 (25 %)	53 (28 %)	
Grade				
Low grade	161 (64%)	49 (73 %)	112 (60 %)	0.06
High Grade	92 (36%)	18 (27 %)	74 (40 %)	
Stage				
Ta	159 (63%)	49 (73 %)	110 (59 %)	0.04
T1	94 (37%)	18 (27 %)	76 (41 %)	
Size				
≤ 3 cm	154 (61%)	39 (58 %)	115 (62 %)	0.73
> 3 cm	69 (27%)	19 (28 %)	50 (27 %)	
Unknown	30 (12%)	9 (13 %)	21 (11 %)	
Multiplicity				
1	153 (60%)	37 (55 %)	116 (62 %)	0.44
>1	76 (30%)	22 (33 %)	54 (29 %)	
Unknown	24 (10%)	8 (12 %)	16 (9 %)	

Table 5

Univariate Cox proportional hazard regression analysis for prediction of progression in Aarhus cohort

Variable	Hazard Ratio	*P*	Lower CL	Upper CL
Positive STAG2 Staining (vs. negative+mosaic)	2.02	<0.01	1.22	3.37
Male Gender (vs. female)	1.06	0.81	0.66	1.70
Increasing Age	1.36	<0.01	1.11	1.67
Adjuvant Therapy (vs. none)	0.96	0.86	0.63	1.48
High Grade (vs. low grade)	3.21	<0.01	2.16	4.77
Pathological Stage T1 (vs. Ta)	2.77	<0.01	1.87	4.11
Size >3 cm (vs. ≤3 cm)	0.97	0.90	0.60	1.56
Multiplicity >1 (vs. =1)	1.05	0.83	0.67	1.64

Table 6

Multivariable Cox proportional hazard regression analysis for prediction of progression in Aarhus cohort

Variable	Hazard Ratio	*P*	Lower CL	Upper CL
Positive STAG2 Staining (vs. negative+mosaic)	1.86	0.05	1.00	3.43
Male Gender (vs. female)	1.19	0.55	0.68	2.09
Increasing Age	1.31	0.03	1.03	1.65
Adjuvant Therapy (vs. none)	0.87	0.59	0.51	1.46
High Grade (vs. low grade)	3.81	0.01	1.33	10.86
Pathological Stage T1 (vs. Ta)	0.67	0.45	0.24	1.91
Size >3 cm (vs. ≤3 cm)	0.88	0.61	0.53	1.45
Multiplicity >1 (vs. =1)	1.15	0.58	0.71	1.86