Stat3 activation in urothelial stem cells leads to direct progression to invasive bladder cancer

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Abstract

Two subtypes of human bladder cancer, non-invasive papillary and muscle-invasive cancer, develop through independent pathologic and molecular pathways. Human invasive bladder cancer frequently develops without prior clinical evidence of a non-invasive tumor stage. However, an animal model that recapitulates this unique clinical progression of invasive bladder cancer has not yet been developed. In this study we created a novel transgenic mouse model of invasive bladder cancer by targeting an active dimerized form of Stat3 to the basal cells of bladder epithelium. When exposed to the carcinogen nitrosamine, Stat3 transgenic mice developed invasive cancer directly from carcinoma in situ (CIS), bypassing the non-invasive papillary tumor stage. Remarkably, invasive bladder cancer driven by active Stat3 was predominantly comprised of stem cells, which were characterized by cytokeratin 14 (CK14) staining and enhanced tumor sphere-forming ability. Active Stat3 was also demonstrated to localize to the nucleus of human invasive bladder cancers that were primarily composed of CK14+ stem cells. Together, our findings demonstrate that Stat3-induced stem cell expansion plays a critical role in the unique clinical progression of invasive bladder cancer through the CIS pathway.
Introduction

Bladder cancer is the fifth most common cancer with 69,250 new cases annually in the United States. Urothelial carcinoma (UC) represents approximately 90% of bladder cancers, which arise from an epithelial origin. Two subtypes of bladder UCs exist: non-invasive papillary and muscle-invasive cancer. Evidence supports that these two subtypes develop through their own independent pathologic and molecular pathways, although certain overlap does exist (1-4). The vast majority of muscle-invasive cancers arise de novo from carcinoma in situ (CIS) without prior clinical progression through non-invasive papillary lesions (2, 4). Muscle-invasive bladder cancer is clinically unfavorable with only a 5-year overall survival of 48-67% even after radical cystectomy (removal of entire bladder) for localized disease (5).

Several signaling pathways, such as p53, pRB, PTEN and their downstream interacting proteins, have been described in mediating the development of invasive bladder cancer (6-9). For instance, TP53 mutation and RB inactivation is common in human bladder CIS (7, 8) and invasive cancer (6), and were shown to be associated with poor prognosis (10, 11). However, mouse model carrying urothelial-specific deletions of p53 and pRB only produced late-onset hyperplasia and low-grade non-invasive papillary bladder tumors (12). Exposure of these urothelial-specific p53/pRB deficient mice to subcarcinogenic dose of the carcinogen, N-Butyl-N-(4-Hydroxybutyl)nitrosamine (BBN), led to 50% incidence of invasive bladder cancer, while single knock out mice of p53 or pRB did not develop invasive cancer in response to BBN (12). Independently, lack of PTEN protein in human bladder cancer is shown to associate with poor outcome, and mouse urothelial-specific deletions of PTEN and p53 via an adeno-Cre delivery system to the bladder induces invasive cancer development (9). Despite the current understanding of signaling pathways and mouse models for invasive bladder cancer, a mouse model representing the unique clinical progression of
invasive bladder cancer directly from CIS, without prior pathological appearance as non-invasive papillary tumor, is lacking. In the current study, we describe a novel mouse model that targets an active dimerized form of Signal transducer and activator of transcription 3 (Stat3) to the basal cells of bladder epithelium (urothelium). When Stat3 is constitutively active, the carcinogen BBN induced rapid progression of urothelial progenitor cells to atypical CIS formation and subsequent muscle-invasive cancer. This mouse model implicates a role for Stat3 in predisposing urothelial cells toward de novo CIS formation and invasive cancer development, which closely resembles the clinical pathogenesis of human invasive bladder cancer.

Materials and Methods

**K5.Stat3 transgenic mice and Nitrosamine (BBN) treatment protocol.** K5.Stat3 transgenic mice were characterized as previously described (13). Adult transgenic mice and wild-type littermates at 6-8 weeks of age were treated with 0.05% BBN in drinking water for 12 weeks, followed by regular drinking water. Mice were sacrificed at 1wk (n=4), 2wk (n=4), 4wk (n=4), 6wk (n=4), 13wk (n=4) and 20wk (n=42) after first BBN treatment. Mouse bladders were either fixed in 10% formalin and paraffin embedded for histological analyses or freshly dissociated for *in vitro* tumor-sphere forming assay.

**Immunostaining and western blotting.** Tumor sections were analyzed following standard H&E procedures or immunohistochemical analysis protocols (DAKO) (14). Nikon microscopy system and NIS Elements software were used for imaging and semi-automated quantification of CK14+ and CK18+ cells. Primary antibodies used are listed as follows, Flag (Sigma F1804), Stat3 (Cell Signaling 9139), pTyrStat3 (Cell Signaling 4113), CK14 (Convance PRB-155P), CK5 (Abcam ab75869), CK18 (Abcam ab668), and cleaved Caspase-3 (Cell Signaling 9661).
**Tumor-sphere forming assay.** Bladder tumors were enzymatically dissociated into single cell suspension as previously described (14) and their ability to generate sphere-forming stem cell colonies was analyzed in an *in vitro* assay as previously described (15). In brief, viable single cell suspension of tumor cells were resuspended in 1:1 ratio of serum-free Keratinocyte Growth Media (Gibco/Invitrogen) and Growth Factor Reduced Matrigel™ (BD Biosciences, 356231). Tumor spheres formation were assayed 12 days after first plated.

**Animal care and patient materials.** All animal procedures were approved under protocol AN-5529 and all patient materials were approved under IRB protocol H-26809.

**Results and Discussion**

**Urothelial characterization of Stat3 transgenic mice.**

Stat3 is a latent transcription factor that normally resides in the cytoplasm. Upon growth factor/cytokine receptor or non-receptor tyrosine kinase-mediated activation, Stat3 rapidly translocates into the nucleus where it binds to consensus promoter region and activates target gene transcription (16). The association of Stat3 to human invasive bladder cancer and poor survival has been reported (14, 17). However, its biological role in *de novo* bladder tumorigenesis has not been fully explored. We previously reported the generation of transgenic mice overexpressing a dimerized form of active Stat3 (Stat3C) (18) by targeting its expression to epithelial basal cells via the keratin 5 promoter (13) (Supplemental Figure 1A). Here, we investigated the relevance of these transgenic mice in modeling the pathogenesis of human invasive bladder cancer by exposing them to a well-established chemical carcinogen regimen [N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)] for inducing rodent bladder cancer (19). Transgene expression in the mouse bladder of Stat3 transgenic mice was first validated by western blot analysis of Flag protein expression, a polypeptide that was tagged onto the Stat3
transgene (Supplemental Figure 1B). The urothelial overexpression of Stat3 protein was 5.6-fold greater in Stat3 transgenic mice compared to wild-type littermates, as demonstrated by densitometry analysis (Supplemental Figure 1B). The restricted expression of Stat3 to the nucleus of urothelial basal cells was determined by immunohistochemistry (IHC) (Supplemental Figure 1C & D).

*N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) induces immediate progression into carcinoma-in-situ in Stat3 transgenic mice.*

Although hyperplasia was evident in bladder urothelium of Stat3 transgenic mice, spontaneous bladder tumors were not observed after 14-16 months of age [Figure 1A (TG, n=14) & Figure 1G (WT, n=9)], suggesting active Stat3 signaling alone is not sufficient for driving bladder tumorigenesis. After short exposure to the carcinogen BBN, dysplastic lesions resembling CIS were evident in Stat3 transgenic mice as early as 1 week after carcinogen treatment (Figure 1B). Early invasion into the lamina propria was observed at 2 weeks (Figure 1C) and invasive cancer was evident 4 weeks (Figure 1D) after first carcinogen treatment. These CIS are characterized by atypical cellular morphology from basal, intermediate, to umbrella cells, and almost the entire urothelium analyzed contained typical CIS in Stat3 transgenic mice (Figure 1A-D). Carefully analyses of these early lesions did not reveal evidence of non-invasive tumors. These results implicate a role for Stat3 in predisposing carcinogen-initiated urothelial cells to rapid progression into CIS, which bypassed non-invasive tumor stage. In contrast, in BBN treated wild-type mice, both urothelial hyperplasia and focal CIS were observed as premalignant lesions in the bladder (Figure 1H-J). These results suggest that in wild-type mice, carcinogen-induced bladder tumorigenesis could go through both pathological pathways: (1) step-wise progression from urothelial hyperplasia into non-muscle invasive papillary tumors and eventually muscle-invasive bladder cancer, or (2) CIS into muscle-
invasive bladder cancer. However, due to the limitation of the current model, it is impossible to trace the fate of these early lesions from wild-type mice and specifically pinpoint which pathological pathway followed during tumor progression.

_N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) induces invasive bladder cancer in Stat3 transgenic mice_

At 20 weeks after first BBN treatment, 100% of Stat3 transgenic mice developed grossly visible muscle-invasive bladder cancers (Supplementary Figure 1E), which were confirmed by hematoxylin and eosin (H&E) staining in histological sections showing microscopic invasion into muscle bundles (Figure 1E & F). On the other hand, development of either hyperplasia or non-invasive cancer was seen in 41.38% of age-matched wild-type mice (Figure 1K) while 58.62% developed invasive bladder cancer (Figure 1L). Kaplan-Meier analysis revealed a better survival of wild-type mice in comparison survival Stat3 transgenic mice (Supplementary Figure 1F). The expression of Stat3 has a reported association with invasive properties and poor clinical prognosis in human bladder cancer (14, 17). The distinct bladder tumor phenotype from Stat3 transgenic mice versus wild-type littermates in this carcinogenesis experiment clearly supports an involvement of active Stat3 in _de novo_ development of early CIS and muscle-invasive cancer. Another phenotype worth highlighting in Stat3 transgenic mice is the rapid progression into CIS and invasive cancer, without prior appearance as non-invasive tumors. In human bladder cancer, 70-80% go through a step-wise progression from hyperplasia to non-invasive papillary tumor, but a majority of these tumors do not progress into invasive cancer (Supplemental Figure 2A, D and E). The cancer progression in Stat3 transgenic mice closely resembles the alternative pathway in humans from CIS to invasive bladder cancer (Supplemental Figure 2A, B and C). However, it should be noted that Stat3 is unlikely the only pathway that can drive the
development of BBN-induced invasive bladder cancer, since only ~30% of invasive bladder cancer from wild-type mice showed nuclear localization of Stat3 (data not shown).

**BBN-induced premalignant lesions in Stat3 transgenic mice demonstrated an early expansion of CK14+ stem cells.**

We have previously reported bladder cancer patients with a higher frequency of CK14+ cancer stem cells associate with poor survival outcome in two independent patient cohorts (20). Since bladder cancer with poor survival outcome are primarily high-grade invasive cancer, we hypothesize that active Stat3 may regulate a possible expansion of CK14+ stem cell in this transgenic mouse model, which subsequently lead to early CIS and invasive cancer progression. To test our hypothesis, we set off to examine the relative expression of stem and differentiated cell markers in bladder tumors derived from Stat3 transgenic mice in comparison to that from wild-type littersmates. We recently reported CK14 as a primitive stem cell marker precursor to CK5 in human bladder cancer (20). CK14 is an acidic type I cytokeratin that commonly heterodimerizes with the basic type II cytokeratin CK5, and it is perceived that they co-express within the same urothelial cells. Immunohistochemical staining of urothelium from young adult wild-type muse revealed that CK5 is expressed continuously throughout the basal cell layer (Figure 2E). Interestingly, CK14 expressing cells is scattered and represents only a subpopulation of CK5+ urothelial cells (Figure 2F). These staining patterns agree with the concept that CK14+ cells are a subpopulation and may be precursor to CK5+ urothelial cells, although lineage-tracing experiment is required for definitive proof. We therefore proceeded to use CK14 as a putative stem/progenitor cell marker and CK18 as a more differentiated cell marker to compare the relative frequency of these cellular compartments in premalignant lesions or bladder tumors derived from Stat3 transgenic...
mice and wild-type littermates. Premalignant CIS lesions induced by BBN in Stat3 transgenic mice demonstrated a significant expansion of CK14+ cells into at least 3-6 layers of cells (Figure 2C & J) as compared to early hyperplastic lesions induced by BBN in wild-type mice, which had a smaller expansion of CK14+ cells to form a continuous layer in urothelial basal cells \( (P < 0.0001) \) and a greater single layer of CK18+ differentiated superficial cells (Figure 2G & J, \( P = 0.0002 \)). In non-invasive papillary tumor induced by BBN in wild-type littermates, a significant proportion of tumor cells retain a distribution of more differentiated tumor cells marked by CK18, although a clonal outgrowth of CK14+ cells (20.6%) was also observed (Figure 2H, green color). In invasive bladder tumors induced by BBN in wild-type mice, there is an increase of CK14+ tumor cells to 61.3% with the presence of CK18+ differentiated cells (Fig. 2H, higher magnification in Supplementary Figure 3A - D), while in invasive bladder tumors induced by BBN in Stat3 transgenic mice, CK18+ differentiated cells are completely absent (0%) with predominantly CK14+ stem cells (77.0%, Fig. 2D, higher magnification in Supplementary Figure 3E - H). Statistical analysis revealed a significant difference in the percentage of CK14+ stem cells in invasive cancer derived from Stat3 transgenic mice, in comparison to both non-invasive and invasive tumors derived from wild-type littermates (Figure 2J, \( P < 0.0001 \) & \( P = 0.01 \), respectively). These results revealed that a shift of balance toward CK14+ stem cells is evident in invasive bladder tumors from both Stat3 transgenic mice and wild-type mice, although more significant in that from Stat3 transgenic mice. Therefore, Stat3 is unlikely the only pathway that can drive expansion of CK14+ stem cell in invasive bladder tumors. Nevertheless, active Stat3 clearly contributed to a significant expansion of CK14+ stem cells in early CIS that likely contribute to subsequent invasive tumor formation in Stat3 transgenic mice.

Stat3-driven bladder tumors contain a higher frequency of sphere-forming stem cells.
To examine the validity of CK14+ tumor cells as functional stem cells, we have successfully adapted an *in vitro* assay to analyze the frequencies of sphere-forming stem cells in corresponding bladder tumors derived from BBN treated Stat3 transgenic mice and those from wild-type littermates (15). Interestingly, when equal numbers of tumor cells were analyzed in this assay, Stat3-driven bladder tumor cells generated a significantly higher number of sphere-forming stem cells in comparison to wild-type littermates (Figure 3A, P = 0.02). These results are consistent with earlier immunofluorescence staining, confirming the increase of CK14+ cells in BBN-induced bladder tumors in Stat3 transgenic mice (Figure 2H) are indeed functional stem cells. Although the sphere size difference was not statistically significant (Figure 3B), a small proportion of tumor cells from BBN induced Stat3 transgenic mice generated spheres that are larger in size (>90μm) (Figure 3C & D) in comparison to the regular size (35 - 90μm) generated from BBN induced wild-type tumors (Figure 3E). We attempted to serially passage these spheres *in vitro*. Unfortunately, these primary tumor spheres seemed to secrete extracellular matrix that made it technically impossible to enzymatically retrieve viable cells for serial passaging (data not shown). As a complementary approach, we overexpressed Stat3 in mouse bladder carcinoma cell line MB49, and Stat3 MB49 cells showed higher sphere formation in primary and secondary passages compared to control (Supplemental Figure 4A & B).

*Active Stat3 confers urothelial cell survival in response to carcinogen treatment.*

Next, we explored the potential mechanisms leading to this significant expansion of CK14+ stem cells in lesions from BBN treated Stat3 transgenic mice, in comparison to that from BBN treated wild-type littermates. Since Stat3 has a recognized anti-apoptotic role by mediating downstream expression of Bcl-XL, we hypothesize that Stat3 may
confer better survival of urothelial cells in response to carcinogen treatment. In early lesions treated by BBN, we examined the frequency of caspase-3 positive cells to quantify apoptotic cells. In wild-type and Stat3 transgenic premalignant lesions, we observed a portion of caspase-3 positive cells within the CK14- superficial layers (Figure 3F & G, marked by arrows), as well as some caspase-3 positive cells within other cell layers (Figure 3F - I, marked by asterisk). There was not a significant difference in apoptotic cells within the CK14+ cells of wild-type and Stat3 transgenic mice (Figure 3J). Interestingly, within the CK14- cells, there was an unexpected increase in the percentage of apoptotic cells in the early lesions from Stat3 transgenic mice in comparison to that from wild-type littermates (Figure 3J, P = 0.007). We reasoned that while Stat3 protects CK14+ cells from BBN-induced apoptosis, it is not targeted to CK14- superficial cells (Supplemental Figure 1D). Therefore, BBN might preferentially induce apoptosis of CK14- cells in Stat3 transgenic mice. This might provide a possible mechanistic insight to the significant expansion of CK14+ cell compartment in early lesions from Stat3 transgenic mice, in comparison to wild-type control. In wild-type tumors, there was an overall and statistically significant increase in apoptotic index in invasive versus non-invasive tumors (Figure 3K, P = 0.002), which is consistent with a previous report on BBN-induced rodent bladder tumors (21). Interestingly, invasive tumors from Stat3 transgenic mice exhibit a significant reduction in apoptotic index, in comparison to invasive tumors from wild-type littermates (Figure 3K, P = 0.004). Anti-apoptotic role of Stat3 at this later stage of tumorigenesis may be important for survival of cancer cells that have accumulated additional alterations for malignant progression.


To examine the human relevance of these bladder tumor results from mice, which implicate a role of Stat3 in invasive progression, we analyzed 8 early-stage (6 pTa LG, 2
Ta HG) and 8 advanced stage human bladder cancers (1 TIS, 2 pT2, 5 pT3) (Supplemental Table 1). In early-stage bladder tumors, we observed 37.5% (3 of 8) of which express nuclear active Stat3, while 100% (8 of 8) of higher-stage bladder cancer expressed nuclear active Stat3 (Supplemental Table 1). Immunohistochemical analysis of representative sections from higher-stage tumors with nuclear expression of active Stat3 (Figure 4A & C) revealed that they also predominantly express CK14+ stem cells (Figure 4B & D). In contrast, representative sections from pTa low grade tumors revealed the lack of nuclear Stat3 staining (Figure 4E & G) and CK14 staining (Figure 4F & H).

Collectively, these results from Stat3 transgenic mice and human bladder cancer for the first time implicate a role for Stat3 in predisposing urothelial cells towards the CIS progression pathway into invasive bladder cancer. Since chronic inflammation induced by cigarette smoking and persistent urinary tract infections have been demonstrated to be a promoting factor for bladder cancer (22), Stat3 activation may be an important downstream mediator to inflammatory cytokines such as interleukins (IL-17 and IL-6) during bladder tumorigenesis. Further studies to identify the upstream activators for Stat3 and to investigate its expression in a large human cohort of human CIS and invasive bladder cancer will likely reveal its significance in driving urothelial cells toward the pathway of CIS and invasive cancer progression. This unique mouse model reported in the current study will provide a valuable platform to understand other interacting pathways in driving CIS and invasive bladder cancer progression.

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References


**Figure Legends**

**Figure 1 | Stat3 activation predisposes urothelial basal cells to carcinogen-induced CIS and invasive bladder cancer development.** Hematoxylin and eosin (H&E) staining of (A) bladder urothelium from four untreated, aged Stat3 transgenic (TG) mice (14 to 16 months old); (B) bladder urothelium from four Stat3 TG mice with CIS and invasion into the lamina propria after 1 week of BBN treatment; (C) bladder urothelium from Stat3 transgenic mice with CIS and early invasion into the lamina propria after 2 weeks of BBN treatment; (D) CIS and/or invasive bladder cancer after 4 weeks of BBN treatment from Stat3 transgenic mice; and (E) muscle invasive cancer in Stat3 transgenic mice 20 weeks after BBN at low magnification and (F) high magnification; (G) bladder urothelium from four untreated aged wild-type mice; (H) bladder urothelium from wild-type mice with hyperplasia and carcinoma *in situ* (CIS) after 1 week of BBN treatment; (I) bladder urothelium from wild-type mice with hyperplasia and CIS after 2 weeks of BBN treatment; (J) bladder urothelium from wild-type mice with hyperplasia and CIS after 4 weeks of BBN treatment; (K) non-invasive papillary tumor in wild-type mice 20 weeks after BBN treatment initiation; and (L) muscle invasive cancer in wild-type mice 20 weeks after BBN treatment initiation. Atypical cells are emphasized with Δ, dotted lines mark the basement membrane, and invasion into the lamina propria or muscle is indicated with black arrows. All scale bars represent 100 μm.

**Figure 2 | Stat3 activation leads to expansion of CK14+ stem cells in carcinogen-induced bladder lesions.** Immunohistochemical analysis of untreated adult Stat3 transgenic mice in serial sections with (A) CK14 (marker for stem cells, positive staining indicated with Δ) and (B) CK5 (marker for basal cells). Immunofluorescence staining of cytokeratin 14 (CK14 – green) and cytokeratin 18 (CK18 – red, marker for more
differentiated cells) in (C) BBN-induced carcinoma *in situ* (CIS) from Stat3 transgenic mice (low and high magnification); and (D) BBN-induced invasive bladder tumor from Stat3 transgenic mice (low and high magnification). Immunohistochemical analysis of untreated adult wild-type mice with (E) CK14 and (F) CK5. Immunofluorescence staining of CK14 and CK18 in (G) BBN-induced premalignant lesions from wild-type mice (low and high magnification); (I) BBN-induced non-invasive papillary bladder tumor from wild-type mice (low and high magnification); and (I) BBN-induced invasive bladder tumor from wild-type mice (low and high magnification). (J) Graph quantifying the percentage of CK14+ and CK18+ epithelial cells in premalignant lesions from Stat3 transgenic and wild-type mice. (K) Graph quantifying the percentage of CK14+ and CK18+ epithelial tumor cells in Stat3 transgenic invasive tumors, wild-type invasive tumors, and wild-type non-invasive tumors, (***, P < 0.001; *, P < 0.05). Bladder epithelium is marked with asterisks. Unless otherwise indicated, scale bars represent 100μm.

**Figure 3 | Stat3 activation in bladder tumors leads to expansion of sphere-forming stem cells.** (A & B) Graph quantifying the number and size of sphere-forming stem cells from BBN treated wild-type and BBN treated Stat3 transgenic bladder tumor cells. (C) Bright field images demonstrating a typical sphere formed from Stat3 transgenic bladder tumor cells (<90μm) (low and high magnification). (D) Bright field images demonstrating a relatively larger size sphere formed from Stat3 transgenic bladder tumor cells (>90μm) (low and high magnification). (E) Bright field images demonstrating a typical sphere formed from wild-type bladder tumor cells (low and high magnification). Immunohistochemical analysis of BBN-induced premalignant lesions from Stat3 transgenic mice in serial sections for (F) CK14 and (G) cleaved caspase-3. Immunohistochemical analysis of BBN-induced premalignant lesions from wild-type mice
in serial sections for (H) CK14 and (I) cleaved caspase-3. Asterisks denote active caspase-3 within CK14+ cells, and ▼ denote cleaved caspase-3 within CK14- cells. (J) Bar graph quantifying apoptotic index (CK14+ and CK14- cells) in premalignant lesions from wild-type and Stat3 transgenic. (K) Bar graph quantifying apoptotic index in wild-type non-invasive papillary tumors, wild-type invasive tumors, and Stat3 transgenic invasive tumors. All scale bars represent 100μm. **, P < .001; *, P < 0.05; n.s., not significant.

**Figure 4 | Nuclear Stat3 and CK14 expression in advanced-stage human bladder cancer.** (A, C, E & G) Immunohistochemical analysis of nuclear and cytoplasmic Stat3 in human advanced-stage bladder cancer and early-stage non-invasive papillary tumors. (B, D, F & H) Immunohistochemical analysis of CK14 in serial sections of human advanced-stage bladder cancer and early-stage papillary tumors. Scale bar represents 100μm.
Figure 1

- **Untreated**
  - 14–16 months old
  - 1 wk: CIS/early invasion
  - 2 wk: CIS/early invasion
  - 4 wk: CIS/early invasion
  - 20 wk: Muscle invasive

- **BBN treated**
  - 14–16 months old
  - 1 wk: Hyperplasia & CIS
  - 2 wk: Hyperplasia & CIS
  - 4 wk: Hyperplasia & CIS
  - 20 wk: Non-invasive

**Stat3 TG**

**Wild-type**

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.
Untreated

Premalignant lesion

BBN Treated

Non-invasive papillary tumor

Invasive tumor

Adult urothelium

Premalignant lesion

Non-invasive papillary tumor

Invasive tumor

Stat3 TG

WT

CK14

CK5

CK14

CK5

J

K

Positive Cells (%)

Positive Cells (%)

Premalignant lesion

Tumor

J

K

Stat3 TG invasive tumor
WT invasive tumor
WT non-invasive tumor

CAN-11-3195R

Figure 2
Figure 3

(A) Graph showing the comparison between WT and Stat3 TG groups with a significant difference indicated by *.

(B) Graph showing the comparison between WT and Stat3 TG groups with no significant difference indicated by n.s.

(C) Images of WT Stat3 TG showing cell morphology.

(D) Images of WT Stat3 TG showing cell morphology.

(E) Images of WT Stat3 TG showing cell morphology.

(F) Images of WT Stat3 TG showing protein expression.

(G) Images of WT Stat3 TG showing protein expression.

(H) Images of WT Stat3 TG showing protein expression.

(J) Bar graph showing the percentage of cleaved Caspase-3 positive cells in WT and Stat3 TG groups.

(K) Bar graph showing the percentage of CK14 positive cells in WT and Stat3 TG groups.

Legend:
* Significant difference
n.s. No significant difference
** Significant difference
Figure 4
Stat3 activation in urothelial stem cells leads to direct progression to invasive bladder cancer


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