Invasive Bladder Cancer: Genomic Insights and Therapeutic Promise

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Abstract

Invasive bladder cancer, for which there have been few therapeutic advances in the past 20 years, is a significant medical problem associated with metastatic disease and frequent mortality. Although previous studies had identified many genetic alterations in invasive bladder cancer, recent genome-wide studies have provided a more comprehensive view. Here we review those recent findings and suggest therapeutic strategies. Bladder cancer has a high mutation rate, exceeded only by lung cancer and melanoma. About 65% of all mutations are due to APOBEC-mediated mutagenesis. There is a high frequency of mutations and/or genomic amplification or deletion events that affect many of the canonical signaling pathways involved in cancer development: cell cycle, receptor tyrosine kinase, RAS, and PI-3-kinase/mTOR. In addition, mutations in chromatin-modifying genes are unusually frequent in comparison with other cancers, and mutation or amplification of transcription factors is also common. Expression clustering analyses organize bladder cancers into four principal groups, which can be characterized as luminal, immune undifferentiated, luminal immune, and basal. The four groups show markedly different expression patterns for urothelial differentiation (keratins, uroplakins) and immunity genes (CD274, CTLA4), among others. These observations suggest numerous therapeutic opportunities, including kinase inhibitors and antibody therapies for genes in the canonical signaling pathways, histone deacetylase inhibitors, novel molecules for chromatin gene mutations, and immune therapies, which should be targeted to specific patients based on genomic profiling of their cancers.

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Introduction

Bladder cancer is a major cause of morbidity and mortality worldwide, with about 380,000 new cases and 150,000 deaths per year (1). It is notable among the common cancers in that both pre-invasive and invasive forms of the disease are commonly diagnosed. Non-muscle invasive bladder cancer (NMIBC), in which the smooth muscle layer surrounding the bladder is not invaded by tumor, accounts for about 80% of all bladder cancer diagnoses (1). NMIBCs (Ta and T1) include both low and high-grade papillary tumors, and carcinoma in situ, a flat high-grade tumor. NMIBC treatment consists of intravesical chemo- or immunotherapy and requires regular cystoscopic monitoring for early detection of recurrence and/or progression to invasive disease. Muscle-invasive bladder cancer, hereafter termed invasive bladder cancer, is characterized by a high risk of metastases to regional pelvic lymph nodes and visceral sites, and is usually incurable despite systemic chemotherapy. Unfortunately, treatment of invasive bladder cancer has progressed little in the past two decades (2).

Past studies have identified multiple genes as commonly mutated in bladder cancer, including TP53 (3), RB1 (4), TSC1 (5), FGFR3 (6), and PIK3CA (7,8). Many genomic regions of gain and loss have also been identified (1,9).

A comprehensive review of the molecular pathogenesis of bladder cancer was recently published (1). Here we focus on insights derived from the NIH NCI TCGA bladder cancer program (10) and other recent genome-wide analyses that include whole exome sequencing (11–13).

High Mutation Rate in Bladder Cancer Due to APOBEC-Type Mutagenesis

TCGA analysis of 130 invasive bladder cancers revealed a relatively high rate of mutation, a mean of 7.7 and median of 5.5 per Mb within coding regions, amounting to 302 protein-coding mutations per cancer (10). Lung adenocarcinoma, lung squamous cell carcinoma, and melanoma are the only major cancers studied by TCGA that have higher mutation rates. For those cancers the causes are thought to be cigarette carcinogen mutagenesis (lung cancer) and sunlight UV mutagenesis (melanoma) (14). Unexpectedly, the association between smoking history and mutation rate or mutation spectrum in TCGA cohort was rather weak (10), despite the known epidemiologic association between cigarette smoking and bladder cancer. In TCGA data, many mutations seen in bladder cancer were TCW-> TTW or TGW changes (nucleotide subject to change is underlined, W=A/T), a class of mutation probably mediated by one of the DNA cytosine deaminases in the APOBEC gene family (15,16).

To examine mutational categories and processes in greater detail, we performed Bayesian non-negative matrix factorization (Bayesian-NMF) analysis (17) (note that ref. 17 describes the original algorithm; full details of the method and its implementation will be described elsewhere) of the mutations stratified by 96 tri-nucleotide contexts in 238 TCGA bladder cancer specimens (Fig. 1), which were downloaded from Broad GDAC firehose. While conventional NMF requires the number of signatures as an input, Bayesian-NMF

automatically prunes away irrelevant components that do not contribute to explaining observed mutations and effectively determines the appropriate number of signatures and their sample-specific contributions. That analysis identified five distinct patterns of mutagenesis operating among 73301 single nucleotide variants (SNVs) in 238 bladder cancers (Fig. 1A). Two are variations of the APOBEC mutation signature, one consisting of C>T mutations in the TCW context ("APOBEC2" – 17% SNVs), and the other consisting of both C>T and C>G mutations in the consensus ("APOBEC1" – 48% SNVs). In contrast to other signatures the third common mutation pattern ("Unknown") is relatively non-specific in terms of site and context and had a broad spectrum of base changes. 18% SNVs were associated with this signature of uncertain origin. The fourth pattern is the well-known C>T transition at CpG sites ("C>T_CpG" – 10% SNVs). Interestingly, one sample with an ultra-high mutator phenotype (> 4000 SNVs) had a POLE (DNA polymerase epsilon catalytic subunit) mutation commonly seen in colon and endometrial cancers (P286R), and a predominance of C>A mutations at TCT and C>T mutations at TCG sites ("POLE" – 8% SNVs). Figure 1B demonstrates that the number of mutations is highly variable among individual bladder cancers, as is the mutation signature. Overall the APOBEC mutation pattern, with APOBEC1 and APOBEC2 signatures, accounts for about 65% of all point mutations, and is predominant in cancers with high mutation burdens apart from the single POLE hyper-mutated sample. However, there are some cancers with APOBEC mutation signature contribution as low as 5% (Fig. 1B). APOBEC3B is expressed at relatively high levels in all bladder cancers (10), and may be the mediator of APOBEC signature mutations (18). Notably, independent analysis of a smaller data set (n=30), but including whole genome sequencing data for 4 samples, indicated that there was a strong APOBEC signature in 37% of bladder cancer, medium in 28%, and weak in 37% (19).

Genes Commonly Mutated in Bladder Cancer

To identify genes that are statistically significantly mutated in bladder cancer, we combined mutation data sets from TCGA (238 invasive cases) (10), the Beijing Genomics Institute [(BGI); 62 invasive cases and 37 NMIBC] (11), the CNIO (Spanish National Cancer Research Centre), (2 invasive and 15 NMIBC) (12), and the Dana-Farber Cancer Institute/ Broad Institute (50 invasive cases) (13). Thirty-four genes were identified as being significantly mutated using Mutsig 2CV (20) (Fig. 2, Table 1) on this combined set of cohorts (not including the CNIO cohort due to unavailability of synonymous mutations), with rates of mutation varying from a high of 44% in TP53 to a low of 2% in IRS4. Many other large genes had rates of mutation as high as 11% (e.g. CREBBP (11%), MLL3 (11%), ATM (9%), NF1 (7%), and FBXW7 (6%)), but were not identified as statistically significantly mutated since the number of mutations that are expected to be random events ('noise') grows in proportion to the size of a gene. Statistically significantly mutated genes grouped into several different categories (Table 1). Genes related to receptor tyrosine kinase function including several kinases were significantly mutated (n=7), as were those involved in chromatin regulation (n=6), transcription (n=5), and cell cycle regulation (n=4). The list of genes is similar to that reported previously from analysis of individual data sets (10,11). Many pairs of genes from the list showed patterns of co-occurrence of mutations, including TP53 and RB1, STAG2 and FGFR3, MLL2 and NFE2L2, KDM6A and FGFR3, and

ERBB3 and ERBB4, all with q < 0.003 (Fisher exact test with FDR correction used for these and subsequent analyses). Only a few pairs showed patterns of mutual exclusivity, TP53 and any RAS gene, and RB1 and FGFR3, q < 0.02.

The combination of both NMIBC and invasive bladder cancers in this analysis enabled assessment of differences in mutation rate between the two (Table 1). MLL2 mutation was seen at a much higher rate in invasive bladder cancer (19% vs. 6% in NMIBC, p=0.007, q=0.13), as was TP53 mutation (47% in invasive vs. 22% in NMIBC, p=0.0004, q=0.016). Mutations in KDM6A were seen more commonly in NMIBC (43% vs. 23% in invasive bladder cancer, p=0.0032, q=0.12). Many previous studies have investigated differences in mutation frequency between NMIBC and invasive bladder cancer (1). In past studies, FGFR3 mutation was much more common in low grade NMIBC than in invasive cancer (~70% vs. ~12%), while TP53 mutation was much more common in invasive cancer than low grade NMIBC (~40% vs. ~7%) (1). A recent small series identified a higher rate of mutations in KDM6A in NMIBC (65% in 30 NMIBC vs. 33% in 18 MIBC), concordant with our findings, as well as a higher rate of mutations in TP53 in MIBC (56% of 18) vs. NMIBC (5% of 20) (19). (These data were not included in our pooled analysis due to lack of availability of the primary data.) Our observations based on these pooled genome-wide studies support mutation in TP53 as being a key factor differentiating invasive bladder cancer from non-invasive disease. However, differences in FGFR3 mutation were not seen. The observed differences in MLL2 and KDM6A mutation rates between the two stage groups of bladder cancer are relatively novel. They suggest that different chromatin gene mutations contribute to the two different stage groups. However, these observations may be due in part to differences in the histologic characteristics of the NMIBC, or in the patient populations pooled for this analysis, or technical factors in the NGS analysis, and further study is required.

Amplification, Deletion, and Other Genomic Events in Bladder Cancer

Many comprehensive studies have identified numerous genomic amplification and deletion events occurring in bladder cancer (1,9). These findings were confirmed and extended in the recent TCGA analysis based upon Affymetrix SNP profiling and low-pass whole genome sequencing, both analyzed by GISTIC (21). Thirteen genes were targets of focal deletion and 19 were targets of focal amplification (Table 1). The majority of those genes fall into the same categories as those for which mutations are seen, including cell cycle, chromatin regulation, receptor tyrosine kinase signaling, and transcription.

In TCGA data set, 3 (2%) invasive bladder cancers contained FGFR3-TACC3 fusion sequences (11), a chromosomal translocation identified previously in bladder cancer (22). These fusion proteins are highly transforming. They have now been seen in multiple cancer types, and cancers bearing them may be especially sensitive to FGFR3 inhibitors. Four (3%) cancers had fusions involving ERBB2 and various other genomic regions, of uncertain functional significance (11).

Subsets of Bladder Cancer Based upon Expression Profiling

Several recent studies have performed comprehensive gene expression profiling analysis of high grade or muscle invasive bladder cancer and used unsupervised hierarchical clustering to define expression pattern subtypes (10,23–26). Although the findings from those analyses have not been completely uniform, there is considerable similarity. The Sjodahl report (2012) identified 5 expression subtypes, Urobasal A and B, genomically unstable, squamous cell carcinoma-like (SCC-like), and infiltrated (referring to the presence of non-tumor cells) (26). A subtype termed basal was identified by all of the other studies (10,24,25), and is characterized by expression of keratins KRT5, KRT14, and KRT6A/B/C, as well as HES2 and MYC, indicative of a basal or stem cell phenotype, and is similar to the SCC-like subtype of Sjodahl. The three later studies also identified a 'Luminal' expression subtype, so-called because of its similarity to breast cancer luminal subtypes, characterized by high expression of FGFR3, the uroplakin genes, KRT20, and transcription factors PPARG, GATA3, FOXA1, and RXRA. The Luminal subtype was similar to the Urobasal A subtype in the Sjodahl study. Another subtype, p53-like was also identified in one of these studies (24). All of these analyses for which prognostic information was available showed the basal subtype to be associated with poorer prognosis, and the luminal subtype to be associated with more favorable prognosis (23–26).

To examine patterns of expression in invasive bladder cancer in greater detail, we performed unsupervised hierarchical clustering (27) on 238 TCGA bladder cancers for which both RNA-Seq and whole exome sequencing mutational analysis had been performed (Fig. 3). That analysis gave results similar to those published on the 131 samples (10) (Rand index = 0.82), and identified 4 different subtypes, splitting the luminal and basal subtypes into two further subtypes each. 41% of the invasive cancers were in the Luminal subtype (red, Fig. 3) with high expression of KRT20 and UPKs 2/1A/1B/3A, as well as moderate to high expression of multiple pertinent transcription factors (KLF5, PPARG, GRHL5). The Luminal subtype was enriched in male patients, papillary histology, and Stage II tumors, and is similar to the previously identified luminal (10,24,25) and urobasal A (26) subtypes. 29% of the invasive bladder cancers were in the Basal subtype (blue, Fig. 3) with high expression of KRT14, KRT5, KRT6A/B, and KRT16, and low expression of all uroplakins, consistent with a basal or undifferentiated cytokeratin expression pattern. Consistent with previous studies, the basal subtype expressed TP63, TP73, MYC, and EGFR, as well as TGM1 and SCEL, indicative of some degree of squamous differentiation (10,24–26). The Basal subtype was enriched in female patients and non-papillary histology, and also expressed many immune genes at intermediate and somewhat variable levels, including CTLA4 and CD274 (encodes PD-L1), suggestive of immune cell infiltration. 11% of the cancers grouped into a novel subtype that we term Immune Undifferentiated (green, Fig. 3). Those cancers showed very low expression of luminal markers, with variable expression of basal cytokeratins, and relatively high level expression of many immune genes, including CTLA4 and CD274, suggesting significant immune cell infiltration and possible immune evasion (see further below). Last, the Luminal Immune subtype (18%) (orange, Fig. 3) was characterized by expression of Luminal genes (cytokeratins and uroplakins), and intermediate expression of immune genes, and was enriched for Stage N+ tumors. The Luminal subtype was enriched

in cancers with mutations in FGFR3, and amplification events involving PVRL4 and YWHAZ, whereas the Basal subtype was enriched in mutations in NFE2L2 (all with p < 0.02 and q < 0.2) (Fig. 3). Furthermore, both Luminal Immune and Immune Undifferentiated subtypes had a high level of expression of ZEB1, ZEB2, and TWIST1 characteristic of epithelial-to-mesenchymal transition (EMT).

Therapeutic Targets in Invasive Bladder Cancer

Those observations, along with continuing drug development by pharma, has led to a large number of potential therapeutic opportunities for invasive bladder cancer.

First, the high frequency of mutations and genomic deletions affecting chromatin regulatory genes in bladder cancer, higher than in any other epithelial malignancy (11), suggests that therapies targeted at the effects of those mutations could be useful. Mocetinostat, an oral second-generation HDAC inhibitor, is currently being assessed in a clinical trial for invasive bladder cancers with mutations in either EP300 or CREBBP (28). Further pharmaceutical development of agents that target those mutations is needed and is actively being pursued.

Second, mutations and genomic deletion or amplification events that affect the cell cycle are very common in bladder cancer. Those include alterations of TP53 and the cyclin-dependent kinase inhibitors CDKN1A and CDKN2A (Fig. 4A). Both CDNK2A loss and amplification of cyclin D1 (gene symbol CCND1) can be targeted by agents in development that are CDK4/6 inhibitors, including palbociclib (29). MDM2, amplified in 8% of invasive bladder cancer, is also a therapeutic target of several drugs in development. CDKN1A mutation, although extremely rare in other cancer types, is seen in 14% invasive bladder cancer, and occurs with concurrent TP53 mutation about half the time (30). Concurrent loss of CDKN1A and TP53 has been shown in cell line and mouse xenograft models to lead to marked sensitivity to combined treatment with gemcitabine and a CHK1 inhibitor, such as PF477736, suggesting potential clinical utility (30).

Third, the PI3K-AKT-mTOR pathway is commonly subject to mutation in invasive bladder cancer (Fig. 4B). Multiple agents are in clinical development to target PI3-kinase (gene name PIK3CA), one of the genes most commonly mutated in bladder cancer. Though less common than PIK3CA mutations, mutations in TSC1 are well-known in bladder cancer (5), and they have been shown in at least some cases to lead to dramatic sensitivity to treatment with mTOR kinase inhibitors, such as everolimus (31). Further studies are underway to define the precise clinical and genetic characteristics of response to mTOR inhibition in bladder cancer.

Fourth, the extent of involvement of the receptor tyrosine kinase-RAS-ERK signaling pathway involvement in invasive bladder cancer has recently become much more evident (Fig. 4C). Both FGFR3 and all the members of the ERBB family are affected by either activating mutations or amplification events (Fig. 4C). Drugs that target those genetic abnormalities are at various stages of clinical development, and arguably, FGFR3-activating mutations and gene fusions are the most promising targets among those genes. Clinical trials of FGFR3 kinase inhibitors against bladder and other cancers are ongoing (32).

Last, immune therapy has shown considerable promise for invasive bladder cancer. A recent report indicated that one immunomodulatory treatment approach, use of the humanized anti-PD-L1 monoclonal antibody MPDL3280A, had significant activity in bladder cancer (33). Of those patients whose bladder tumors contained high amounts of tumor-infiltrating cells expressing PD-L1 as assessed by immunohistochemistry, 13 of 25 (52%) demonstrated an objective response by 12 weeks on therapy, and the response was ongoing (33). These results build upon a large and growing body of evidence that immune evasion through cancer-induced immunosuppression, often through activation of immune checkpoints, is an important factor in cancer progression (34). For example, both cytotoxic T-lymphocyte associated antigen-4 (CTLA4) and programmed death-1 (PD-1) receptor expressed by T cells can be engaged by corresponding receptor molecules on cancer cells (e.g. PD-L1) or other immune cells, to block lymphocyte activity directed at cancer cells (34). Hence antibodies that block such interaction, directed at either of the interacting molecules, can interfere with cancer checkpoint blockade, leading to native immune cell attack on the cancer, and therefore, to clinical response. The relatively high level of immune gene expression by some bladder cancers, including CTLA4 and CD274 (encoding PD-L1) (Fig. 3) is consistent with the hypothesis that a subset of bladder cancers are characterized by immune suppression, and will be sensitive to immune modulatory therapy. Clinical trials of multiple immune therapy agents are in progress for bladder cancer (2,28). Based upon our current analyses, it appears that the Immune Undifferentiated and Basal subtypes of bladder cancer will be the most promising subtypes for immune checkpoint therapy (Fig. 3). However, further analysis is urgently needed so that these therapies can be applied with the most precision and effectiveness in bladder cancer.

Conclusions

Invasive bladder cancer is characterized by a high overall mutation rate, which appears to be explained mainly by APOBEC-mediated mutagenesis. Both well-known and relatively novel cancer genes are commonly affected in invasive bladder cancer by mutation, genomic amplification/deletion, or both. Genes affected include those involved in transcription, chromatin regulation, receptor tyrosine kinase signaling, PI3K-mTOR signaling, RAS, and the cell cycle. Expression profiling studies are consistent in the identification of two main subtypes of bladder cancer, broadly definable as basal and luminal. Basal tumors are less differentiated, more aggressive, and more lethal; luminal tumors are more differentiated and show higher expression levels of uroplakins and FGFR3. Expression clustering reveals additional subtypes within the two main groups, and, quite significantly, the subtypes differ in immune gene expression and EMT marker expression.

The future is looking bright for therapeutic advances in bladder cancer. There are many promising targets and drugs in development that should be deployed in mutation- and expression-specific fashions, as per the "precision medicine" paradigm. Promising therapeutic agents directed against the cell cycle, receptor tyrosine kinase pathway, and PI3K-mTOR pathway mutations are in hand. Mutations in chromatin regulatory genes are promising targets for which further pharmaceutical development will be required. Immune checkpoint agents, already in the clinic, also show promise, and the expression/mutational subtypes defined above may aid both our pre-clinical and clinical progress with them.

Acknowledgments

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Mutation signatures in TCGA BLCA 238 samples

Signature activity across TCGA BLCA 238 samples
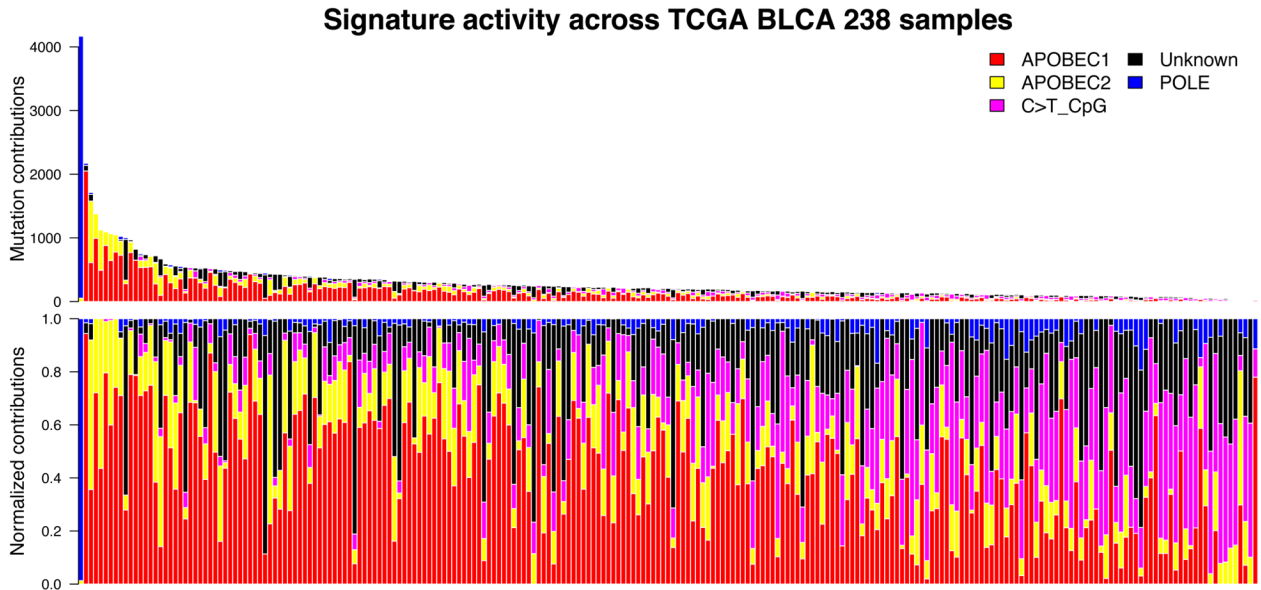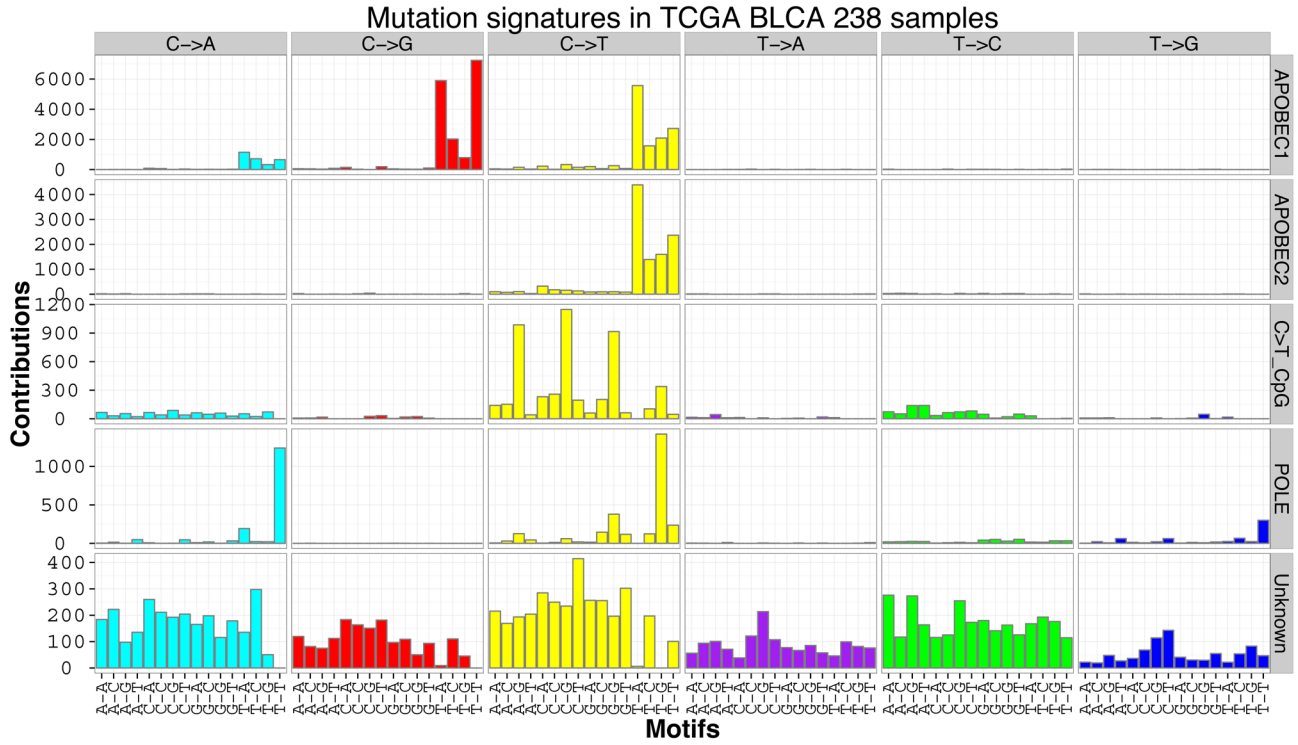
Figure 1.

Mutation signatures in 238 muscle-invasive TCGA bladder cancers. A. Bayesian NMF (17) was used to identify five patterns of mutation that occur in bladder cancer genomes. Two of them match the APOBEC pattern, TCW-> TTW or TGW. The uppermost signature, APOBEC1, consists of both C>T and C>G mutations, whereas the next, APOBEC2, consists of only C>T mutations. The third mutation signature is that of CpG > TpG, the fourth is a POLE signature, and the fifth signature identified by NMF analysis is of

unknown origin. The y axis gives the number of mutations of each type at each specific sequence.

B. Graph of the total number of mutations associated with five mutation signatures (upper panel) and relative proportion of mutation types (lower panels) seen in each TCGA bladder cancer sample.
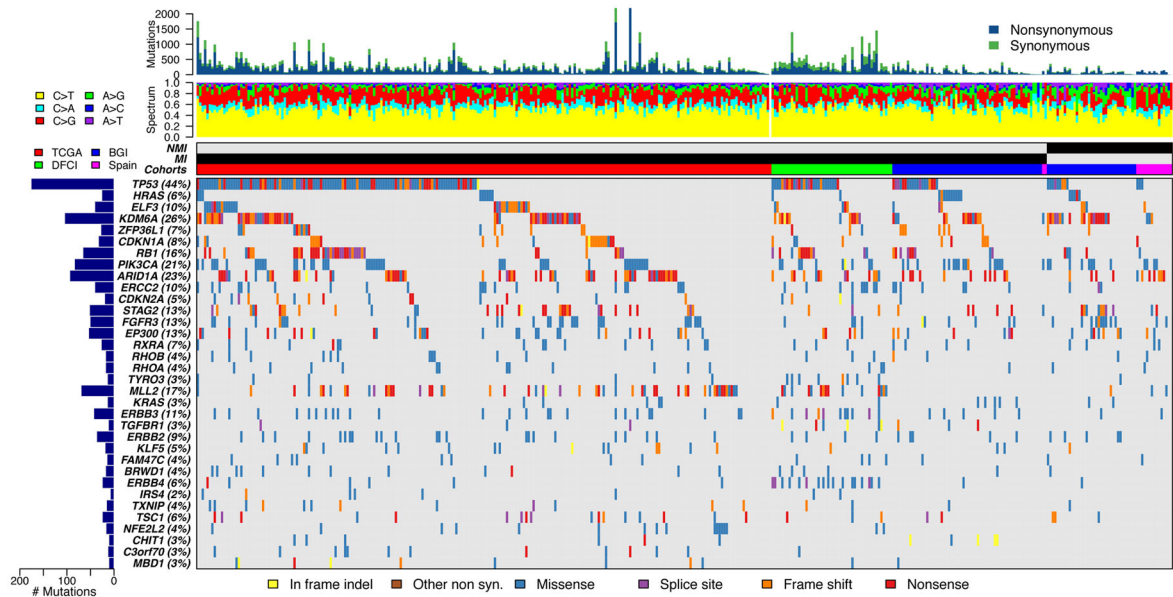
Figure 2.

Significantly mutated genes (SMGs) identified in 404 cases of bladder cancer. Mutation data used was from TCGA (238 invasive cases) (10), the Beijing Genomics Institute (62 invasive cases and 37 NMIBC: 28 T1N0, 2 T1bN0, 1 T1Nx, 6 TaN0) (11), the CNIO (Spanish National Cancer Research Centre), (2 invasive and 15 NMIBC: 3 TaG1, 2 TaG2, 1 TaG3, 3 T1G2, 6 T1G3) (12), and the DFCI/Broad (50 invasive cases) (13). Sequentially from top to bottom: mutation rate, mutation spectrum, non-muscle invasive (NMI) vs. muscle-invasive (MI), source of data, and genes with statistically significant levels of mutation (MutSig 2CV (19) false discovery rate < 0.1) sorted by q value are shown. Colors indicate different mutation types, shown at bottom. The total number of mutations and the percent of samples with mutation in each gene are shown at left. The CNIO data were not included in MutSig analysis to identify SMGs. Mutations seen at allele fraction 5% were not included. Five genes (FAM82A2, STK39, ATP8P2, ZNF83, GLT6D1) identified by Mutsig were also deleted due to suspicious mutation patterns. Note that bar plots at the top are truncated for a few cancers.
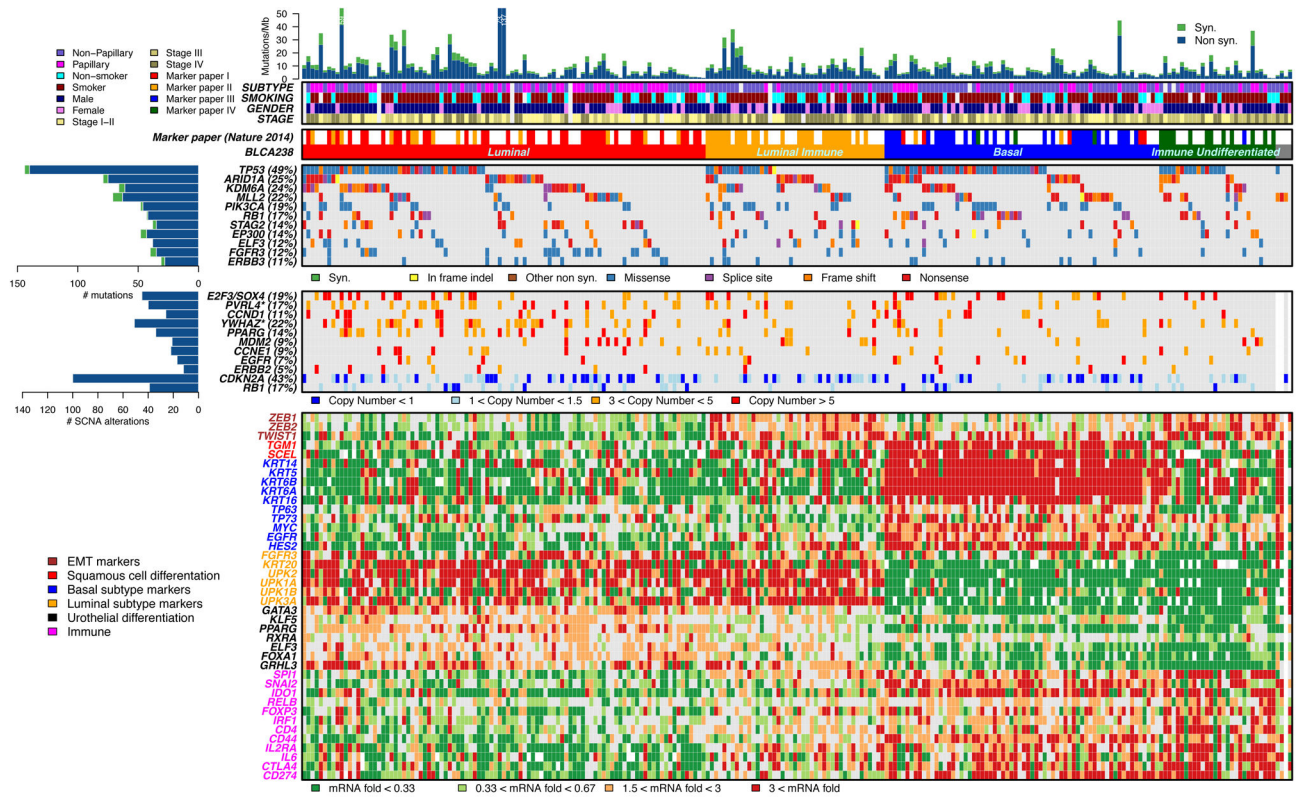
Figure 3. Expression clustering identifies four different types of bladder cancer. Unsupervised hierarchical clustering (27) was performed on 238 TCGA bladder cancers using RNA-Seq RSEM expression values for the 3,000 most variable genes. Mutation and copy number change data were also available for the 238 samples

A. Mutation rate and type, histological subtype, smoking status, gender, and tumor stage are shown.

Four clusters were identified, red (Luminal), orange (Luminal Immune), blue (Basal), and green (Immune Undifferentiated). Four samples without complete data were not included in the clustering and are shown in gray (right).

B. Genes with statistically significant levels of mutation, as identified in Figure 3, and mutation rates > 10% are shown, with mutation types.

C. Genomic regions with statistically significant focal copy number changes (GISTIC2.0) (21) are shown; limited to deletions seen in > 15% of samples, and amplifications seen in 5% of samples. 'Copy number' refers to absolute copy number. The asterisk indicates that the gene listed is one among many within an amplification peak.

D. RNA expression levels for selected genes, chosen to reflect luminal vs. basal differentiation, and for roles in the immune system, are expressed as fold change from the median value for all samples. Gene fonts are color-coded to indicate gene class, and correlate with expression subtypes.

Note that bar plots at the top are truncated for a few cancers.

EMT, epithelial-to-mesenchymal transition; SCN, somatic copy number alterations.

Cell cycle gene mutations and genomic changes in MIBC

MDM2 inhibitors:
RG-7112
RO5503781 DS-
3032b
CGM097
MK-8242
SAR405838

MDM2
8%

TP53
47%

CDKN2A
6% **42%**

CDKN1A
9% **4%**

CHK1 inhibitor
PF477736 +
Gemcitabine

CDK4/6 inhibitors:
Palbociclib
LEE011
LY2835219/abem
aciclib
G1T28-1

CDK4/6

CCND1
11%

CDK2

CCNE1
9%

G1 R S

+P

Figure 4A

RB1
17% **16%**

E2F3
18%

Mutation frequency on left
Copy number change on right
Activating or amplification
Inactivating or deletion

PI3K-AKT-MTOR pathway in MIBC

PI(4,5)P2 → PI(3,4,5)P3 → PI(4,5)P2

RTK -Y-P

PI3K **20%** **3%**

AKT **1%** **7%**

PTEN **3%** **13%**

ERK1/2

+P

TSC2 **2%** **17%**

TSC1 **6%** **12%** TBC1D7

PI3K inhibitors:
GDC-0032
BYL719
INK1117
GDC-0941
buparlisib (BKM120)
XL-147

RHEB-GTP **<1%**

Pi

GDP GTP

RHEB-GDP

Dual PI3K/PI3K inhibitors:
BEZ235
GDC-0980
PKI-587
AZD8055
SAR245409
PF-05212384
PF-04691502
P7170

PRAS40 Deptor
Raptor MTOR mLST8 **1%**

mTORC1

mTOR inhibitors:
Everolimus
Temsirolimus
Sirolimus
MLN0128

Figure 4B

Mutation frequency on left
Copy number change on right
Activating or amplification
Inactivating or deletion

RTK-Ras-ERK pathway in MIBC

FGFR3 inhibitors:
Ponatinib
Dovitinib
BGJ398
JNJ-42756493
E-3810
ARQ 087
R3Mab
Nintedanib

FGFR3 **11%** **4%**

EGFR **0** **7%**

ERBB2 **9%** **4%**

ERBB3 **12% 1%**

ERBB4 **7%**

ERBB4
Dacomitinib

EGFR inhibitors:
Cetuximab
Not erlotinib
GA201
Panitumumab
Afatinib
Neratinib
dacomitinib

HRAS **6%**

KRAS **3%**

ERBB2 inhibitors:
Trastuzumab
Lapatinib
Neratinib
afatinib
ARRY-334543
AZD8931
dacomitinib

ERBB3 Inhibitors:
AV-203 (Ab)
AZD8931
Lapatinib
MEHD7945A

RAF1 BRAF

MEK1/1 Inhibitors:
MEK162
Trametinib
Selumetinib/AZD6244
PD0325901
MSC1936369B

MEK1/2

ERK1/2

Mutation frequency on left
Copy number change on right
Activating or amplification
Inactivating or deletion

Figure 4C

Figure 4. Pathways, potential therapeutic targets, and possible inhibitors for invasive bladder cancer

Genes that drive growth or cancer progression are shown in green. Genes that are tumor suppressors and act to prevent growth or progression are shown in red. Beneath each gene symbol, the number on the left indicates the frequency of inactivating (red) or activating (green) mutation, the number on the right indicates the frequency of copy number loss (red) or amplification (green). Classes of inhibitors and their targets are shown with blunt arrows indicating the components they inhibit.

A. Cell cycle. B. PI3K-AKT-mTOR pathway. C. RTK-RAS-ERK pathway (asterisk indicates not erlotinib).

Table 1

Genes identified as being significantly mutated or subject to focal copy number change in bladder cancer

Genes identified as significantly mutated

Gene	Category	Total # mutations	% samples with mutations	# mutations NMIBC	% NMIBC	# mutations invasive	% invasive	Higher in NMIBC p=	Higher in NMIBC q=	Higher in invasive p=	Higher in invasive q=
TP53	cell cycle	176	44%	12	22%	164	47%	0.9999	1	0.0004	0.01584
HRAS	RAS	26	6%	5	9%	21	6%	0.2577	1	0.8829	0.9586
ELF3	transcription	41	10%	4	7%	37	11%	0.8305	1	0.3318	0.6532
KDM6A	chromatin	105	26%	23	43%	82	23%	0.0032	0.1229	0.9988	0.9988
ZFP36L1	transcription	28	7%	2	4%	26	7%	0.9122	1	0.2477	0.5945
CDKN1A	cell cycle	33	8%	1	2%	32	9%	0.9929	1	0.0468	0.3332
RB1	cell cycle	66	16%	5	9%	61	17%	0.9637	1	0.0894	0.4246
PIK3CA	PI3K-mTOR	84	21%	13	24%	71	20%	0.3162	1	0.7960	0.9452
ARID1A	chromatin	94	23%	11	20%	83	24%	0.7588	1	0.3640	0.6587
ERCC2	DNA repair	41	10%	3	6%	38	11%	0.9354	1	0.1695	0.5482
CDKN2A	cell cycle	20	5%	0	0%	20	6%	1	1	0.0526	0.3332
STAG2	chr. segregation	52	13%	11	20%	41	12%	0.0658	0.8339	0.9713	0.9988
FGFR3	RTK	51	13%	12	22%	39	11%	0.0247	0.4692	0.9907	0.9988
EP300	chromatin	54	13%	9	17%	45	13%	0.2819	1	0.8375	0.9543
RXRA	transcription	27	7%	4	7%	23	7%	0.4997	1	0.7145	0.8776
RHOB	migration	18	4%	2	4%	16	5%	0.7212	1	0.5589	0.8249
RHOA	migration	18	4%	2	4%	16	5%	0.7212	1	0.5589	0.8249
TYRO3	RTK	14	3%	1	2%	13	4%	0.8705	1	0.4199	0.7253
MLL2	chromatin	70	17%	3	6%	67	19%	0.9999	1	0.00704	0.13369
KRAS	RAS	14	3%	2	4%	12	3%	0.5801	1	0.7159	0.8776
ERBB3	RTK	43	11%	2	4%	41	12%	0.9877	1	0.0514	0.3332
TGFBR1	RTK	12	3%	0	0%	12	3%	1	1	0.1742	0.5482
ERBB2	RTK	37	9%	5	9%	32	9%	0.5695	1	0.6284	0.8776
KLF5	transcription	19	5%	1	2%	18	5%	0.9388	1	0.2503	0.5945
FAM47C	other	15	4%	2	4%	13	4%	0.6196	1	0.6764	0.8776
BRWD1	chromatin	18	4%	1	2%	17	5%	0.9289	1	0.2788	0.6233

Genes identified as significantly mutated

Gene	Category	Total # mutations	% samples with mutations	# mutations NMIBC	% NMIBC	# mutations invasive	% invasive	Higher in NMIBC p=	Higher in NMIBC q=	Higher in invasive p=	Higher in invasive q=
ERBB4	RTK	25	6%	1	2%	24	7%	0.9754	1	0.1262	0.5330
IRS4	RTK	8	2%	0	0%	8	2%	1	1	0.3139	0.6532
TXNIP	ROS regulation	16	4%	1	2%	15	4%	0.9039	1	0.3438	0.6532
TSC1	PI3K-mTOR	25	6%	3	6%	22	6%	0.6760	1	0.5644	0.8249
NFE2L2	transcription	17	4%	0	0%	17	5%	1	1	0.0827	0.4246
CHIT1	other	11	3%	0	0%	11	3%	1	1	0.2020	0.5482
C3orf70	other	13	3%	0	0%	13	4%	1	1	0.1502	0.5482
MBD1	chromatin	11	3%	0	0%	11	3%	1	1	0.2020	0.5482
RAS*	RAS	40	10%	7	13%	33	9%	0.2753	1	0.8539	0.9543
RHO*	migration	36	9%	4	7%	32	9%	0.7396	1	0.4572	0.7554

Genes subject to focal copy number change

Gene symbol	Category	Total # focal CN change	% samples with focal CN change
ARID1A	chromatin	12	5
BCL2L1	apoptosis	24	10
BEND3	chromatin	8	3
BIRC3	apoptosis	10	4
CCND1	cell cycle	26	11
CCNE1	cell cycle	22	9
CDKN2A	cell cycle	100	43
CREBBP	chromatin	38	16
E2F3	Elongation factor	43	18
EGFR	RTK	17	7
ERBB2	RTK	12	5
CCSER1	mitosis	36	15
FGFR3	RTK	10	4
FHIT	Fragile site	30	13
FOXQ1	transcription	25	11
GDI2	migration	20	9
IKZF2	transcription	35	15

Genes subject to focal copy number change

Gene symbol	Category	Total # focal CN change	% samples with focal CN change
LRP1B	migration	39	17
MDM2	cell cycle	21	9
MYC	transcription	31	13
MYCL	transcription	13	6
NCOR1	chromatin	57	24
PDE4D	cAMP	52	22
PPARG	transcription	34	14
PRKCI	protein kinase C,	9	4
PTEN	PI3K-MTOR	30	13
PVRL4	Matrix interactions	40	17
RB1	cell cycle	39	17
SOX4	transcription	42	18
WWOX	other	35	15
YWHAZ	14-3-3-zeta	51	22
ZNF703	transcription	24	10

Top: Genes identified as being significantly mutated or subject to focal copy number change in bladder cancer. Genes identified as being significantly mutated in bladder cancer (Mutsig 2CV (20)) from analysis of 404 bladder cancers (350 invasive and 54 non-muscle invasive), as described in the Fig. 1 legend and text. The different colors in the "Category" column for genes identified as being significantly mutated indicate the different functional categories of genes with mutations. The green color in the other columns highlights those p values that are nominally significant (p < 0.05), and those q values that are significant (q < 0.2).

Bottom: Genes identified as involved in copy number change, either amplification or deletion, as identified by GISTIC2.0 (21). The red color in the "Gene symbol" column for genes subject to copy number change denotes amplification; blue denotes deletion. Again, the different colors in the "Category" column highlight the different functional categories of genes with mutations.

The asterisk denotes union of mutations of the types indicated:

RAS means KRAS or HRAS

RHO means RHOA or RHOB

Abbreviations: chr. segregation, chromosome segregation; RTK, receptor tyrosine kinase.