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Takashi KAWAHARA, Takahiro KOJIMA, Shuya KANDORI, Masahiro Kurobe, Takayuki Yoshino, Tomokazu KIMURA, Yoshiyuki Nagumo, Ryutaro Ishituka, Koji Mitsuzuka, Shintaro Narita, Takashi Kobayashi, Yoshiyuki Matsui, Osamu OGAWA, Mikio SUGIMOTO, Jun MIYAZAKI, Hiroyuki NISHIYAMA

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TP53 codon 72 polymorphism is associated with FGFR3 and RAS mutation in non-muscle-invasive bladder cancer

Takashi Kawahara1*, Takahiro Kojima1**, Shuya Kandori1, Masahiro Kurobe1‡, Takayuki Yoshino1, Tomokazu Kimura1, Yoshiyuki Nagumo1, Ryutaro Ishituka1, Koji Mitsuzuka2*, Shintaro Narita3*, Takashi Kobayashi4†, Yoshiyuki Matsui4†, Osamu Ogawa4*, Mikio Sugimoto5*, Jun Miyazaki6*, Hiroyuki Nishiyama1*.

1 Department of Urology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan, 2 Department of Urology, Graduate School of Medicine, Tohoku University, Sendai, Japan, 3 Department of Urology, Graduate School of Medicine, Akita University, Akita, Japan, 4 Department of Urology, Graduate School of Medicine, Kyoto University, Kyoto, Japan, 5 Department of Urology, Faculty of Medicine, Kagawa University, Kagawa, Japan, 6 Department of Urology, School of Medicine, International University of Health and Welfare, Narita, Japan.

* These authors contributed equally to this work.
** These authors also contributed equally to this work.
*tkojima@md.tsukuba.ac.jp

Abstract

Objective

TP53, a well-known tumor-suppressor gene in bladder carcinogenesis, has a functional single-nucleotide polymorphism on codon 72. The aim of this study was to elucidate the association between TP53 codon 72 polymorphism and somatic mutations in bladder cancer.

Material and methods

Germline TP53 codon 72 polymorphism and somatic mutations of 50 cancer-associated genes were analyzed in 103 bladder cancer patients (59 non-muscle-invasive and 44 muscle-invasive), using Taqman genotyping assay and target sequencing, respectively. The expression of FGF-FGFR signaling pathway genes was analyzed by RNA sequencing of frozen tissue.

Results

The allele frequency of TP53 codon 72 in our cohort was 37, 42, and 21% for Arg/Arg, Arg/Pro, and Pro/Pro, respectively. Interestingly, the prevalence of FGFR3 mutation was higher in patients with the Arg allele, whereas that of the RAS mutation was higher in patients without the Arg allele. The same association was seen in non-muscle-invasive bladder cancer (NMIBC) patients and no differences were observed in muscle-invasive bladder cancer patients. In NMIBC, FGFR1 expression was higher in patients without the Arg allele and FGFR3 expression was higher in patients with the Arg allele.
Conclusion
The germline TP53 codon 72 polymorphism was associated with mutations of FGFR3 or RAS and expression of FGFR1 and FGFR3 in NMIBC. These findings provide new insight into the molecular mechanisms underlying the influence of the genetic background on carcinogenesis in bladder cancer.

Introduction
Bladder cancer is the fourth and twelfth most common malignancy in men and women, respectively [1]. It is particularly common in the elderly and male population. Cigarette smoking and some Chinese herbs are well-known risk factors [2–5]. Bladder cancer is derived from urothelium and progresses from non-muscle-invasive bladder cancer (NMIBC) to muscle-invasive bladder cancer (MIBC), before becoming metastatic. About 70% of bladder cancer patients have NMIBC, and the remaining 30% have MIBC or metastatic disease [6]. Molecular and histopathological features suggest that bladder cancer can develop along at least two distinct pathways. In one pathway, the papillary NMIBC develop via epithelial hyperplasia and recruitment of branching vasculature, and in the other pathway MIBC develops via flat dysplasia and carcinoma in situ (CIS) [2, 7]. Several important driver genes or tumor suppressor genes involved in carcinogenesis of bladder urothelium have been identified. For example, TP53 mutations are key drivers for CIS or MIBC [8]. On the other hand, NMIBC is characterized by activating point mutations in FGFR3 or RAS [9, 10], and, interestingly, activating RAS mutations are mutually exclusive with FGFR3 mutations [10]. However, the underlying mechanism controlling the selection of specific somatic mutations in bladder cancer remains unknown.

TP53 functions as a transcription factor, regulating the expression of several downstream genes, resulting in cell cycle arrest and apoptosis [11]. TP53 is also known to have a functional single-nucleotide polymorphism (SNP) in codon 72 (rs1042522), which results in the substitution of proline (Pro) for arginine (Arg) in the proline-rich domain. TP3 Arg72 is more potent in apoptosis induction, whereas TP3 Pro72 is better in inducing cell cycle arrest and DNA damage repair [12–15]. TP3 codon 72 polymorphism has been linked to an increased risk of breast cancer, cervical cancer, esophageal cancer, gastric cancer, lung cancer, and skin cancer [16]. However, studies relating to the association between TP3 codon 72 polymorphism and bladder cancer susceptibility have shown inconclusive results [16]. We previously reported that this polymorphism affects the phenotypes or clinical outcomes of bladder cancer [17], but the underlying mechanism remains unknown.

Recently, several studies reported the interesting finding that germline SNPs affect specific somatic mutations. For instance, MC1R polymorphism affects BRAF mutant melanoma [18, 19], a JAK2 germline polymorphism affects JAK2 V617F mutant myeloproliferative neoplasms [20, 21], and TACC3 polymorphism affects FGFR3 mutant bladder cancer [22]. Some reports showed that the TP3 Pro allele is associated with an increased frequency of TP3 mutations in non-small cell lung cancer (NSCLC) [23, 24]. However, there is no report around the relationship between TP3 codon 72 polymorphism and somatic mutations in bladder cancer.

We hypothesized that TP3 codon 72 polymorphism could affect somatic mutations during bladder carcinogenesis and conducted this study to compare germline TP3 codon 72 polymorphism and somatic mutations in bladder cancer. In our cohort, there was no relationship between TP3 codon 72 polymorphism and TP3 mutation. However, mutually exclusive...
mutations of FGFR3 and RAS in NMIBC were significantly related with the TP53 codon 72 polymorphism. This finding provides new insight into the relationship between host germline polymorphism and selection for somatic mutation type in bladder carcinogenesis.

Materials and methods

Patients and tissue samples

This prospective multicenter cohort study included 144 patients with clinical diagnosis of urothelial carcinoma from seven institutions [25]. The research protocol was approved by the Ethics Committee of Tsukuba University Hospital (Approval number: H25-116). It was also reviewed and approved by the Ethics Committees of the following institutes: Tohoku University Hospital, Akita University Hospital, Kyoto University Hospital, Kagawa University Hospital, Hitachi General Hospital, and Tsukuba Medical Center Hospital. Tumor specimens, blood, and clinicopathological information were collected with written informed consent.

Primary bladder cancer tissue samples from 103 patients were stored as formalin-fixed paraffin embedded (FFPE) and frozen tissue. All tissue sections included malignant tumor cell nuclei in 10% or more cells of the whole specimen. The remaining 41 patients were excluded because their tumors originated in the upper urinary tract origin, were without urothelial histology, or fresh frozen tissue could not be obtained.

Hematoxylin and eosin staining was performed, and the slides were evaluated by pathologists at each institute. Tumors were staged according to the 2009 Union for International Cancer Control (UICC) 7th tumor-nodes-metastasis (TNM) classification system.

Tumor DNA extraction from FFPE samples and mutation analysis

Tumor DNA from FFPE was extracted using a QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Limburg, Germany) according to the manufacturer’s instructions. The DNA concentration was assessed using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Tumor DNA with more than 1.5 ng/μL, according to the Qubit fluorometer, was subjected to further analysis. In total, 10 ng DNA was used as template to generate an amplicon library for sequencing. Libraries were prepared using an Ion AmpliSeq Library Kit 2.0 and an Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies, Waltham, MA, USA), which amplifies 207 amplicons covering approximately 2800 COSMIC mutations in the following 50 cancer-associated genes in alphabetical order: ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FRBB4, GNA11, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRα, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL. Adapter ligation, nick repair, and polymerase chain reaction (PCR) amplification were performed according to the manufacturer’s instructions. Emulsion PCR and enrichment steps were carried out using an Ion OneTouch Template Kit and an Ion OneTouch system (Life Technologies), according to the manufacturer’s instructions. Following enrichment, the amplicon libraries were sequenced with an Ion PGM Sequencer (Life Technologies). For data analysis, Torrent Suite 4.0.2 was used, and mutations were detected by the Variant Caller plugin 4.0–6 with somatic/high stringency configuration provided by Ion Torrent (Thermo Fisher Scientific).

Germline DNA extraction and TP53 codon 72 genotyping

Germline DNA was extracted from peripheral blood using the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer’s instructions. Germline DNA samples were
genotyped using TaqMan single-nucleotide polymorphism genotyping assays for rs1042522 (Applied Biosystems, Waltham, MA, USA) according to the manufacturer’s instructions. The results were analyzed on a 7500 real-time PCR system using the allelic discrimination assay program of Sequence Detection software version 1.3 (Applied Biosystems).

RNA extraction and sequence analysis
Total RNA was extracted from frozen tissue using TRIzol (Thermo Fisher Scientific), prepared into messenger RNA (mRNA) libraries, and sequenced using Illumina NextSeq 500. Quality control, ambiguity and length trimming, mapping to the reference genome, normalization of gene expression, and evaluation of differential gene expression were performed using CLC Genomics Workbench version 10 (Qiagen). Default settings were used for quality control and ambiguity and length trimming. RNA-sequence reads were aligned to the reference genome of Homo sapiens GRCh38.p10 (GenBank accession number GCA_000001405.25).

Statistical analysis
Differences were assessed using Kruskal-Wallis test and Mann-Whitney U test with Bonferroni correction. The chi-square test was used to evaluate associations between categorical variables. When the p-value was $p < 0.05$ with chi-square test, residual analysis was performed to identify which category was significant. Gene expression was normalized using transcript per million. Wald test with Benjamini-Hochberg multiple test correction was used for evaluation of differential gene expression. Genes with false discovery rate adjusted $p$-values $< 0.05$ were considered differentially expressed. Adjusted residuals were calculated with js-STAR ver 9.1.7 [26], evaluation of differential gene expression was performed using CLC Genomics Workbench version 10 (Qiagen), and the other statistical analyses were performed using STAT view ver5.0 (SAS Institute Inc. Cary, NC, USA).

Results
Association between germline TP53 codon 72 polymorphism and somatic mutations in bladder cancer
Mutation analysis showed that FGFR3, TP53, PIK3CA, RAS (HRAS, KRAS, or NRAS), AKT1, CTNNB1, ATM, BRAF, and RB1 mutations were present in at least one or more patients (Fig 1 and S1 Table). There were no mutations in the remaining 39 genes. The prevalence of FGFR3 mutation was 33%, followed by TP53 (29%), PIK3CA (25%), and RAS (24%) mutation. FGFR3 mutation was mutually exclusive with RAS mutation ($p < 0.01$; RAS mutation included HRAS, KRAS, and NRAS) and TP53 mutation ($p = 0.02$), but co-existent with PIK3CA mutation ($p < 0.01$). TP53 mutation and RAS mutation were also exclusive ($p < 0.01$). Other mutations were not correlated with each other. The prevalence of mutations in FGFR3 or RAS was higher in NMIBC than in MIBC (FGFR3 was 51% and 25%, while RAS was 27% and 9.4% in NMIBC and MIBC, respectively).

Table 1 shows the frequencies of TP53 codon 72 polymorphism. Three genotypes, Arg/Arg, Arg/Pro, and Pro/Pro, were found in 37% (38/103), 42% (43/103), and 21% (22/103) of the patients, respectively. The results fit the Hardy-Weinberg equilibrium. As shown in Table 1, clinical characteristics, including tumor grade, tumor stage, or smoking status, were not significantly different among the patients. In contrast, there was a significant difference in the prevalence of mutations in FGFR3 and PIK3CA ($p < 0.01$ and $p = 0.04$, respectively) among TP53 codon 72 polymorphisms but not in RAS and TP53. Patients with Pro/Pro had significantly
lower FGFR3 mutation rates and patients with Arg/Arg had higher PIK3CA mutation rates, as evaluated with adjusted residuals.

**Influence of Arg allele of TP53 codon 72 polymorphism on FGFR3 or RAS mutations in NMIBC**

We further analyzed the allele of TP53 polymorphism related to the prevalence of somatic mutations in bladder cancer-related genes (Table 2). When both NMIBC and MIBC patients were analyzed and divided into two groups as having Arg allele (Arg/Arg or Arg/Pro) and not having Arg allele (Pro/Pro), the prevalence of mutations in FGFR3 was significantly higher in patients with the Arg allele (41% vs 4.5%, p < 0.01). In contrast, the prevalence of mutations in RAS was higher in patients without the Arg allele (41% vs 20%, p = 0.04). The prevalence of
mutations in PIK3CA and TP53 was not different between these two groups. When the patients were stratified into groups as having Pro allele (Pro/Pro or Arg/Pro) and not having Pro allele (Arg/Arg), only the prevalence of mutations in PIK3CA was higher in patients without the Pro allele (39% vs 17%, p = 0.01).

Table 1. Patient characteristics and prevalence of somatic mutations with respect to the TP53 codon 72 polymorphism.

| TP53 codon 72 polymorphism | Total, n | Arg/Arg n (%) | Arg/Pro n (%) | Pro/Pro n (%) | p-value |
|----------------------------|----------|----------------|---------------|---------------|---------|
| Age Median (year) (range)  | 103      | 38 (37)        | 43 (42)       | 22 (21)       | 0.24***|
| Sex male/female            |          |                |               |               |        |
| Clinical Stage              |          |                |               |               |        |
| Stage MIBC (T≥T2)          | 44       | 17 (45)        | 15 (35)       | 12 (55)       | 0.30***|
| Grade Low/High             |          |                |               |               |        |
| Smoking status              |          |                |               |               |        |
| Grade Low/High             |          |                |               |               |        |
| Smoking status              |          |                |               |               |        |
| Somatic mutation            |          |                |               |               |        |
| FGFR3                       | 34       | 16 (42)        | 17 (40)       | 1 (4.5)       | <0.01***|
| RAS*                        | 25       | 10 (26)        | 6 (14)        | 9 (41)        | 0.053***|
| PIK3CA                      | 26       | 15 (39)        | 8 (19)        | 3 (14)        | 0.04***|
| TP53                        | 30       | 9 (24)         | 15 (35)       | 6 (27)        | 0.53***|

*RAS: KRAS/HRAS/NRAS
**: Kruskal-Wallis test
***: chi-square test

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Table 2. Frequency of somatic mutations divided according to the clinical stage in TP53 codon 72 polymorphism.

| Somatic Mutation | TP53 codon 72 polymorphism | All cases | NMIBC | MIBC |
|------------------|-----------------------------|-----------|-------|------|
|                  | with Arg allele, n (%)      | without Arg allele, n (%) | p** value | without Pro allele, n (%) | with Pro allele, n (%) | p** value |
| FGFR3            | 33 (41)                     | 1 (4.5)   | <0.01 | 16 (42)                     | 18 (28)                 | 0.13     |
| RAS*             | 16 (20)                     | 9 (41)    | 0.04  | 10 (26)                     | 15 (23)                 | 0.71     |
| PIK3CA           | 23 (28)                     | 3 (14)    | 0.16  | 15 (39)                     | 11 (17)                 | 0.01     |
| TP53             | 24 (30)                     | 6 (27)    | 0.83  | 9 (24)                      | 21 (32)                 | 0.35     |
| NMIBC            | 49                           | 10        |       | 21                           | 38                      |         |
| FGFR3            | 25 (51)                     | 1 (10)    | 0.02  | 11 (52)                     | 15 (19)                 | 0.34     |
| RAS*             | 13 (27)                     | 7 (70)    | <0.01 | 7 (33)                      | 13 (34)                 | 0.95     |
| PIK3CA           | 13 (27)                     | 1 (10)    | 0.26  | 8 (38)                      | 6 (16)                  | 0.054    |
| TP53             | 7 (14)                      | 1 (10)    | 0.72  | 1 (4.8)                     | 7 (18)                  | 0.14     |
| MIBC             | 32                           | 12        |       | 17                           | 27                      |         |
| FGFR3            | 8 (25)                      | 0 (0)     | 0.06  | 5 (29)                      | 3 (11)                  | 0.13     |
| RAS*             | 3 (9.4)                     | 2 (17)    | 0.50  | 3 (18)                      | 2 (7.4)                 | 0.30     |
| PIK3CA           | 10 (31)                     | 2 (17)    | 0.33  | 7 (41)                      | 5 (19)                  | 0.10     |
| TP53             | 17 (53)                     | 5 (42)    | 0.50  | 8 (47)                      | 14 (52)                 | 0.76     |

*RAS: KRAS/HRAS/NRAS
**: chi-square test

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Because mutations in \textit{FGFR3} and \textit{RAS} were more frequently detected in NMIBC, the prevalence of \textit{TP53} codon 72 polymorphism and somatic mutations were separately analyzed in NMIBC and MIBC. In NMIBC, the prevalence of mutations in \textit{FGFR3} was higher in patients with the Arg allele (51\% vs 10\%, $p = 0.02$) and the prevalence of \textit{RAS} mutation was significantly higher in patients without the Arg allele (70\% vs 27\%, $p < 0.01$). In MIBC, no significant differences were identified between \textit{TP53} codon 72 polymorphism and somatic mutations.

**\textit{TP53} codon 72 polymorphism could affect the expression of \textit{FGFR1} and \textit{FGFR3}**

Since fibroblast growth factor (FGF)-FGF receptor (FGFR) signaling pathways are activated not only through \textit{FGFR} gene mutation, but also with overexpression of FGF-FGFR related genes [2], the expression of all 22 subclasses of FGF (\textit{FGF1-FGF14, FGF16-FGF23}) and four subclasses of FGFR (\textit{FGFR1-FGFR4}) was determined. The association of \textit{TP53} codon 72 polymorphism with the expression of these genes was analyzed (S2 Table). Significant differences in the expression of \textit{FGFR1} and \textit{FGFR3} ($p = 0.02$ and $p = 0.04$, respectively) were observed, with no significant difference in the expression of other FGFs and FGFRs (S3 Table). In detail, comparing patients with Arg/Pro vs Pro/Pro, \textit{FGFR1} expression was significantly higher in patients with Pro/Pro, and \textit{FGFR3} expression was higher in patients with Arg/Pro ($p^* = 0.010$ and $p^* = 0.015$, respectively). Comparing patients with Arg/Arg vs Pro/Pro, \textit{FGFR1} expression tended to be higher in patients with Pro/Pro, \textit{FGFR3} expression was not significantly different ($p^* = 0.020$ and $p^* = 0.38$, respectively). Comparing patients with Arg/Arg vs Arg/Pro, neither \textit{FGFR1} nor \textit{FGFR3} was significantly different ($p^* = 0.73$ and $p^* = 0.64$, respectively). $\dagger$: $p$-value was evaluated with Mann-Whitney’s U test with Bonferroni correction.

We further analyzed the allele of \textit{TP53} polymorphisms related to the expression of \textit{FGFR1} and \textit{FGFR3}. As shown in Table 3, when both NMIBC and MIBC patients were analyzed, \textit{FGFR3} expression was higher in patients with the Arg allele (FDR-$p = 0.02$). In NMIBC patients, \textit{FGFR3} expression was higher in patients with the Arg allele (FDR-$p = 0.02$). \textit{FGFR1} expression was higher in patients without the Arg allele (FDR-$p < 0.01$). However, in MIBC patients, no significant differences were identified between \textit{TP53} codon 72 polymorphism and expression of FGFRs.

**Table 3. \textit{FGFR1/3} mRNA expression with respect to the \textit{TP53} codon 72 polymorphism.**

| Clinical stage | \textit{TP53} codon 72 polymorphism | \textit{FGFR1} expression | \textit{FGFR3} expression |
|---------------|------------------------------------|---------------------------|---------------------------|
|               | total, n                           | mean +/- SD | FDR-$p$ value | mean +/- SD | FDR-$p$ value |
| All cases     | with Arg allele                    | 81           | 1.18 +/- 8.11 | 0.14 | 82.85 +/- 128.78 | 0.02 |
|               | without Arg allele                 | 22           | 2.84 +/- 8.64 | 0.42 | 35.66 +/- 77.39 |
|               | without Pro allele                 | 38           | 1.28 +/- 8.99 | 0.66 | 63.36 +/- 97.28 | 1 |
|               | with Pro allele                    | 65           | 1.29 +/- 7.91 | 0.06 | 54.14 +/- 135.48 |
| NMIBC         | with Arg allele                    | 49           | 0.90 +/- 2.17 | $< 0.01$ | 120.07 +/- 144.98 | 0.02 |
|               | without Arg allele                 | 10           | 2.05 +/- 2.50 | 0.79 | 50.83 +/- 28.21 |
|               | without Pro allele                 | 21           | 0.90 +/- 2.32 | 0.79 | 152.85 +/- 107.17 | 1 |
|               | with Pro allele                    | 38           | 1.01 +/- 2.25 | 0.79 | 79.26 +/- 100.57 |
| MIBC          | with Arg allele                    | 32           | 2.10 +/- 12.26 | 1 | 40.01 +/- 83.26 | 1 |
|               | without Arg allele                 | 12           | 4.33 +/- 10.67 | 1 | 21.36 +/- 103.68 |
|               | without Pro allele                 | 17           | 1.90 +/- 13.11 | 1 | 34.67 +/- 79.68 | 1 |
|               | with Pro allele                    | 27           | 3.26 +/- 11.15 | 1 | 25.71 +/- 94.50 |

$\dagger$: Wald test with Benjamini-Hochberg multiple test correction

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Combination of smoking status and TP53 codon 72 polymorphism could affect somatic mutations in NMIBC

Since smoking status has been associated with somatic mutation [27], we analyzed the frequency of FGFR3 or RAS mutations in TP53 codon 72 polymorphism and smoking status. Among NMIBC patients with the Arg allele, the prevalence of RAS mutation was significantly higher in smokers than in non-smokers (34% (10/29) vs 6.7% (1/15), \( p = 0.04 \)). In contrast, among NMIBC patients without the Arg allele, there was no significant difference between the prevalence of RAS mutations and smoking status (smokers 71% (5/7) and non-smokers 67% (2/3); \( p = 0.88 \), Fig 2). There was no significant association between smoking status and FGFR3 or RAS mutations in TP53 codon 72 polymorphism among MIBC patients.

Discussion

In this study, we show that germline TP53 codon 72 polymorphism could affect FGFR3 and RAS mutations in NMIBC. Although there are some reports on the association between TP53 codon 72 polymorphism and TP53 mutation in NSCLC [23, 24], to our knowledge, there is no report on the relationship between TP53 codon 72 polymorphism and somatic mutations, other than TP53 mutation. In our dataset, no association between TP53 mutation and TP53 codon 72 polymorphism was observed (Tables 1 and 2). However, patients with the Arg allele were associated with FGFR3 mutation and patients without the Arg allele were associated with RAS mutation in NMIBC (Table 2). To the best of our knowledge, this is the first report clarifying the relationship between germline TP53 codon 72 polymorphism and somatic mutations in bladder cancer. Several studies have identified that germline polymorphisms are associated with specific somatic mutations [18–22]. Our results also imply that germline background could affect the specific somatic mutations in bladder cancer.

Fig 2. Prevalence of mutations in FGFR3 and RAS in TP53 codon 72 polymorphism and smoking status of NMIBC patients. Black bar and gray bar indicate the frequencies of FGFR3 and RAS mutation, respectively. The number in parentheses indicates the patients’ number.

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The FGF-FGFR signaling pathway is composed of several subtypes of FGFs and FGFRs. Some studies have focused on activating FGFR1 and FGFR3 in bladder cancer [28, 29]; however, other FGFRs and FGF-ligands were not studied. Although the activation and overexpression of FGFR3 have been reported [30], the relationship between other FGF-FGFR subtypes and their expression status have not been documented. We show that the TP53 codon 72 polymorphism is also associated with the expression of FGFR1 and FGFR3. Expression of FGFR1 was higher in patients without the Arg allele, while expression of FGFR3 was higher in patients with the Arg allele. Because FGFR3 mutation activates point mutations [2], its expression was higher in patients with the Arg allele who show higher frequency of the FGFR3 mutation. On the other hand, the reason for higher expression of FGFR1 in patients without the Arg allele is unclear. A previous study showed that silencing FGFR1 expression using small interfering RNA was effective in elevating FGFR3 expression and tumor supportive activity, suggesting that FGFR1 and FGFR3 have an inverse relationship [31].

Several studies and the cohort presented here show that FGFR3 and RAS mutations are mutually exclusive events in bladder cancer [10, 27]. The mutual exclusivity of FGFR3 and RAS gene mutations is thought to reflect activation of the same pathway. The oncogenic role of activated FGFR3 is mediated by the activation of mitogen-activated protein kinase through the RAS signaling pathway [32]. FGFR3 mutations are strongly associated with low-grade and low stage bladder cancer, with lower frequency of recurrence [33]. Unlike FGFR3 mutations, no relation of RAS mutational pattern with tumor grade and stage has been found [10]. FGFR3 mutation was seen in about 70% and RAS mutation in about 20% of low-grade non-invasive papillary tumors [27]. However, whether FGFR3 or RAS mutation is selected in NMIBC is unclear. Our results suggest that TP53 codon 72 polymorphism contributes to the selection of somatic mutations in NMIBC.

Several environmental or habitual factors, including smoking and inflammation, have been associated with bladder carcinogenesis. The dataset presented here suggests that smoking status could affect the somatic mutations in NMIBC, based on TP53 codon 72 polymorphism. In patients with the Arg allele, FGFR3 mutation was higher than RAS mutation. When divided into smoking status, non-smokers with the Arg allele rarely had RAS mutation. On the other hand, patients without the Arg allele had RAS mutation, regardless of smoking status. Previous reports show that smoking is associated with somatic mutations. In NSCLC, EGFR mutations are more frequently found in non-smokers [34, 35]. Unlike EGFR mutations, most KRAS mutated NSCLC patients are former or current smokers [36–38]. In bladder cancer, a few studies have examined the association between smoking and somatic mutations. The cancer genome atlas (TCGA) data shows no statistically significant association between smoking status and somatic mutations [39]. Pandith et al. reported that FGFR3 and RAS mutations were higher in smokers, but no significant association was found [27]. TCGA analyzed only MIBC patients [39], whereas Pandith et al. analyzed both MIBC and NMIBC patients [27]. These discrepancies were probably due to analysis of different clinical background factors in patients. Nonetheless, these results suggest that different types of bladder cancer have specific mutations depending on the TP53 codon 72 polymorphism and smoking status.

Based on the previous bladder carcinogenesis model [7] and our results, we propose a new carcinogenesis model in NMIBC (Fig 3). FGFR3 and RAS mutation could be affected by the TP53 codon 72 polymorphism and smoking status in NMIBC. Non-smokers with the Arg allele show FGFR3 mutation and smokers with the Arg allele show either FGFR3 or RAS mutation. Patients without the Arg allele show RAS mutation regardless of smoking status. Moreover, germline TP53 polymorphisms could affect the expression of FGFR1 and FGFR3 in NMIBC. FGFR3 expression was higher in patients with the Arg allele, and FGFR1 expression was higher in patients without the Arg allele. Taken together these results suggest that TP53...
codon 72 polymorphism and smoking status could affect somatic mutations and FGF-FGFR signaling in bladder carcinogenesis.

Several reports have shown that TP53 codon 72 polymorphism is a response associated with chemotherapy or radiotherapy [40, 41]. The mechanisms underlying the influence of the TP53 codon 72 genotype on anticancer treatment response are still unknown. Although our data show the relationship between TP53 codon 72 polymorphism and mutations in FGF-FGFR gene expression, our results cannot explain the mechanism or the difference in treatment response among TP53 codon 72 polymorphism.

Although our study revealed associations between TP53 codon 72 polymorphisms and somatic mutations in bladder cancer, it was limited by the relatively small sample size. Additionally, the cohort consisted of Japanese bladder cancer patients only; there is still a lack of clarity in the underlying mechanism, and we could not identify a treatment strategy around the TP53 codon 72 polymorphism. Therefore, studies involving larger cohorts and other ethnicities are needed to confirm our results. Nonetheless, our results contribute to the elucidation of the mechanism of bladder carcinogenesis. Further clarification regarding the relation between bladder carcinogenesis and genetic background will aid the development of bladder cancer therapy.

In conclusion, TP53 codon 72 polymorphism is associated with FGFR3 or RAS mutation in NMIBC, suggesting that host germline could affect the selection for somatic mutation type in bladder carcinogenesis.
Supporting information

S1 Table. Patients’ metadata and mutation status.
(XLSX)

S2 Table. FGF-FGFR expression values.
(XLSX)

S3 Table. Comparison between expression of FGF-FGFR signaling pathway genes and TP53 codon 72 polymorphism.
(XLSX)

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Author Contributions

Conceptualization: Takahiro Kojima, Masahiro Kurobe, Koji Mitsuzuka, Shintaro Narita, Takashi Kobayashi, Osamu Ogawa, Mikio Sugimoto, Hiroyuki Nishiyama.

Data curation: Takashi Kawahara, Takahiro Kojima, Masahiro Kurobe, Koji Mitsuzuka, Shintaro Narita, Takashi Kobayashi, Yoshiyuki Matsui, Mikio Sugimoto.

Formal analysis: Takashi Kawahara, Takahiro Kojima, Takashi Kobayashi.

Funding acquisition: Takahiro Kojima, Hiroyuki Nishiyama.

Investigation: Takahiro Kojima, Osamu Ogawa, Hiroyuki Nishiyama.

Methodology: Takahiro Kojima, Osamu Ogawa, Hiroyuki Nishiyama.

Project administration: Takahiro Kojima, Osamu Ogawa, Hiroyuki Nishiyama.

Resources: Takahiro Kojima, Masahiro Kurobe, Koji Mitsuzuka, Shintaro Narita, Takashi Kobayashi, Yoshiyuki Matsui, Mikio Sugimoto, Jun Miyazaki, Hiroyuki Nishiyama.

Supervision: Takahiro Kojima, Osamu Ogawa, Hiroyuki Nishiyama.

Writing – original draft: Takashi Kawahara, Takahiro Kojima, Hiroyuki Nishiyama.

Writing – review & editing: Takashi Kawahara, Takahiro Kojima, Shuya Kandori, Masahiro Kurobe, Takayuki Yoshino, Tomokazu Kimura, Yoshiyuki Nagumo, Ryutaro Ishituka, Koji Mitsuzuka, Shintaro Narita, Takashi Kobayashi, Yoshiyuki Matsui, Mikio Sugimoto, Jun Miyazaki, Hiroyuki Nishiyama.

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