Quantitative assessment of the relationship between RASSF1A gene promoter methylation and bladder cancer

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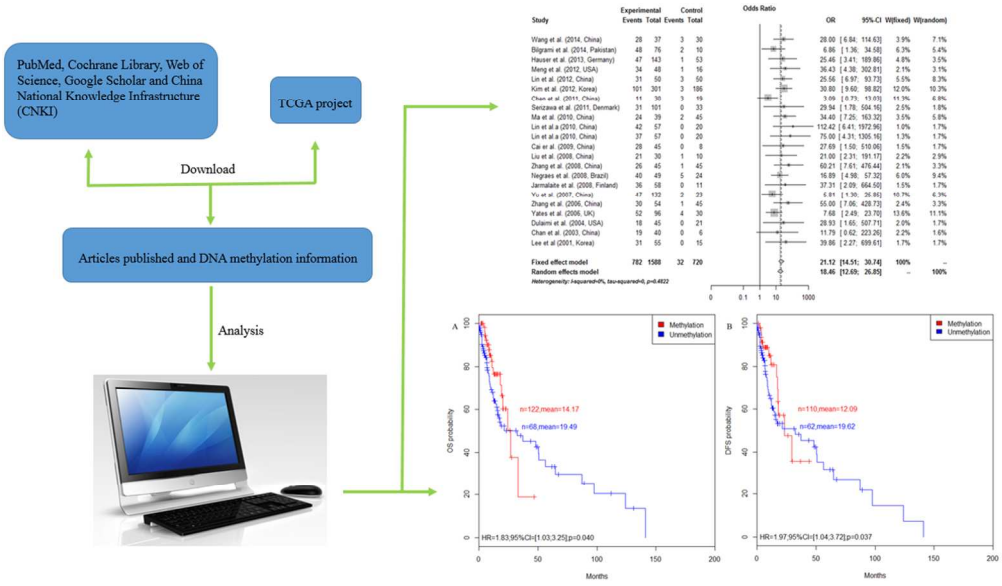

Graphical Abstract
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Full Title: Quantitative assessment of the relationship betweendiagnostic role of RASSF1A gene promoter methylation andin bladder cancer

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Conflict of interest statement

No potential conflicts of interest relevant to this article were reported.We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in the manuscript entitled "Quantitative assessment of the diagnostic role of RASSF1A gene promoter methylation in bladder cancer".

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Abstract

Methylation of the Ras-association domain family 1 isoform A (RASSF1A) promoter region is thought to participate in the initiation and development of many different cancers. However, in bladder cancer the role of RASSF1A methylation is unclear. To evaluate the relationship between RASSF1A methylation and bladder cancer, a quantitative assessment of an independent meta-analysis was performed. In addition, a DNA methylation microarray dataset from the cancer genome atlas (TCGA) project was used to validate the results of the meta-analysis results. We searched published articles from network database and methylation data was extracted from the TCGA project~~TCGA data was extracted from TCGA project~~. All data analyzed~~were analysis~~ by R software. The results of the meta-analysis indicated that the rate of RASSF1A gene methylation in bladder cancer patients was significantly higher than in healthy

controls. The hazard ratio (HR) was 2.24 (95% CI=[1.45; 3.48], p=0.0003) for overall survival (OS), and the *RASSF1A* ~~gene~~ promoter methylation status was strongly associated with the TNM stage and differentiation grade of the tumor~~bladder cancer~~. This result was similar to the data from the~~of~~ TCGA project. There was a significant relationship between the methylation of the *RASSF1A* promoter and bladder cancer risk~~,~~ and prognosis. Therefore, *RASSF1A* promoter methylation ~~methylation~~ could be a potential biomarker for the clinical diagnosis of bladder cancer.

Key words: RASSF1A, promoter methylation, TCGA, meta-analysis

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Introduction

Previous research has shown that allelic loss of~~lack of the allele~~ ~~3p~~ chromosome 3p is frequent ~~appeared frequently~~ in malignant tumors (1). Sekido et al. (2)~~ through~~

~~lung cancer and breast cancer cell lines~~ found that the deleted~~losing~~ area was located at~~in~~ the 3p21.3, which encompasses~~contains~~ 120 kb DNA. Dammann et al. (3) shown that its~~Two hybrid screening methods were used to clone a~~ cDNA ~~which~~ was highly homologous with the NORE1/Maxp1 gene~~Norel and Maxpl~~, and which~~which~~ was named Ras-association domain family 1 (RASSF1)~~ (3)~~. RASSF1 has 8 exons, and one of the RASSF1 family members is RASSF1A. RASSF1A has been closely associated with several different cancers and has been identified as a candidate tumor suppressor gene. Current research shows that the role of the RASSF1A gene is to inhibit cell proliferation, and also to promote cell apoptosis and aging. Functional analysis also shows that RASSF1A has a~~the~~ potential role in~~of~~ maintaining microtubule stability (4). However, the RASSF1A protein is often absent in many tumor cells, as a consequence of the gene being inactivated/silenced. It is believed that the major mechanism of this silencing is ~~a consequence of~~ RASSF1A promoter methylation (5). Such methylation is a common means by which many normal genes are silenced, and indeed silencing of tumor suppressor genes is a part of~~during~~ normal homeostatic mechanisms. Recently, the RASSF1A gene has been highlighted as a~~the~~ gene most commonly methylated in tumors.

The use of biomarkers to detect cancer has attracted much attention in recent years, as it offers many advantages over routine techniques, which rely on biopsies to examine cell morphology and to look for signs of precancerous lesions (6). Methylation of ~~the~~ RASSF1A gene promoter would be an ideal marker of tumor biology for several reasons. Firstly, RASSF1A gene promoter methylation can rarely

be found in normal tissue, so is a fairly unique marker. Secondly, unlike standard histological methods the detection of methylation would rely much less on the individual experience of a practitioner, as such a test could be automated (7). Thirdly, the methylation of RASSF1A gene promoter can occur in many different types of cancer, so it could be developed as a broad-spectrum diagnostic test. Finally, the frequency of RASSF1A gene promoter methylation has been linked to ~~the~~ tumor grade, and thus could provide not only a yes or no answer, but also additional information regarding the tumor stage and prognosis.

Traditional malignant tumor diagnosis was set up under the microscope to check the tumor cell for morphological changes, which are based on visible differences in histopathology between tumor and normal tissue. Since 2000, the ~~world~~ World ~~health~~ Health ~~organization~~ Organization (WHO) has made a major change to its~~their~~ tumor classification by adding more comprehensive information. This included immune phenotype, genetic characteristics and clinical manifestation, as well as imaging to define tissue pathology and morphology, both sorted and graded. Compared to an ordinary histopathological diagnosis, the additional information is ~~now~~ more beneficial and ~~will~~ can allow an individualized treatment of a given tumor. Hence, both oncogene and tumor suppressor genes can~~will~~ be used as a new generation of biomarkers in the clinical diagnosis of tumors.

In recent years, a growing number of studies have shown that the occurrence and development of human tumors correlate with abnormal DNA methylation. This tumor specific abnormal gene methylation can be detected early, even before the clinical

diagnosis. One major advantage of analyzing DNA methylation is that it can be measured in serum and urine samples, as well as in tumor tissue. RASSF1A gene promoter methylation was found to be the most common genetic inactivation event that occurs in human tumors, and it would be a useful and reliable marker for the early detection of cancer. Therefore, in this study we collated relevant data in the published literature, in order to explore the relationship between RASSF1A gene promoter methylation and bladder cancer. As publication bias and heterogeneity can affect meta-analysis results, we downloaded DNA methylation data from the cancer genome atlas (TCGA) project to validate our meta-analysis results. The DNA methylation data from the TCGA project contained genome-wide methylation status, and it would provide no publication bias and no heterogeneity in analyzing the relationship between RASSF1A promoter methylation and bladder cancer.~~Therefore, in this study we collated relevant data in the published literature and TCGA project, in order to explore the relationship between RASSF1A gene promoter methylation and bladder cancer.~~

Materials and Methods

Published articles, ~~Search~~ search strategy, data extraction and meta-analysis

We conducted a literature search (up to and including 20th July 2015) of computerized databases, including PubMed, Cochrane Library, Web of Science, Google Scholar and China National Knowledge Infrastructure (CNKI), for articles published in both English and Chinese. The study used a subject and text word

strategy with 'bladder cancer or carcinoma of bladder or bladder carcinoma or bladder neoplasms or carcinoma of urinary bladder', 'RASSF1A or Ras association domain family 1A or RASSF1', 'methylation or hypermethylation or epigenetic'. In addition, we searched the reference list of relevant original papers and review articles, to identify additional eligible studies. We followed the standard guidelines for conducting and reporting meta-analyses of observational studies (8). The included articles met the following criteria: (1) Original study ~~and~~ with the diagnosis of bladder cancer ~~was~~ based on histopathology; (2) The subjects in every study ~~were~~ comprised ~~of~~ bladder cancer patients and healthy controls; (3) Data were~~was~~ included in the analysis only if the full text of the article was in English or Chinese. We excluded animal studies, clinical trials, cross-sectional studies, reviews, commentaries, letters, and studies that examined other associations. The data were extracted from each study by two independent reviewers, using pre-specified selection criteria. Decisions were made, and disagreements about study selection were resolved, by discussion with a third reviewer. The following information was extracted from each study: the first author's last name, publication year, study location, mean age, TNM stage, differentiation grade, the method and the primers used in the article, ~~and~~ the number of RASSF1A gene promoter methylation cases and controls.

All statistical tests were performed with R software (R version 3.1.2) including meta and ~~metefor~~ metafor packages. The strength of the association between RASSF1A gene promoter methylation and bladder cancer was measured using a pooled odds ratio (OR) and Hazard Ratio (HR) with a 95 % confidence interval (CI),

and withwhen p<0.05 we considered statistically significant. Group analysis was performed and stratified by the study character of age, gender, smoking habit, TNM stages and differentiation grade. The heterogeneity among studies was estimated by the Cochran Q test and I^2 statistic. Heterogeneity was considered statistically significant at P≤0.10. The I^2 statistic describes the percentage of total variation in point estimates that can be attributed to heterogeneity. For the I^2 metric, we considered low, moderate, and high I^2 values to be 25, 50, and 75%, respectively (9). Tau-squared (τ^2) was used to determine how much any heterogeneity could be explained by subgroup differences. The data were pooled using the random effects model (I^2>50%, p≤0.05) or fixed effects model (I^2<50%, p>0.05) according to heterogeneity statistic I^2 (10). If there was no With a lack of heterogeneity among included studies, the pooled odds ratioOR estimates were calculated using the fixed-effects model (11). Otherwise, the random-effects model was used (10). The possibility of publication bias was assessed using the Begg and Egger's regression asymmetry test (12). For sensitivity analysis, we also used the random-effects model for all the above analyses. Additional sensitivity analyses were performed by omitting one study at a time, then calculating a pooled estimate for the remainder of the studies, to evaluate whether the results were markedly affected by a single study. Sensitivity (also called the true positive rate) measures the proportion of positives that are correctly identified as such, e.g., the percentage of sick people who are correctly identified as having the condition; specificity (also called the true negative rate) measures the proportion of negatives that are correctly identified as such, e.g. the

percentage of healthy people who are correctly identified as not having the condition. Therefore, sensitivity and specificity were assessed in the meta-analysis of diagnostic tests.

TCGA data extraction and analysis

DNA methylation information for bladder cancer was download from the TCGA project [http://cancergenome.nih.gov/]. The methylation signals of the 25,978 CpG sites shared by the 450 K datasets were extracted and the methylation status of each probe was defined according to the beta-value~~signals of the 25,978 shared CpG sites by 450 K datasets. The methylation status of each probe was defined according to the beta-value~~ (~~Beta~~beta-value = (intensity value from the methylated bead type)/(the sum of intensity values from the methylated and ~~+ intensity value from~~ unmethylated bead types + 100)). Any ~~beta~~ beta-value equal to, or greater than 0.6, was considered fully methylated, whereas a beta value equal to, or less than 0.2, was considered unmethylated. Beta values between 0.2 and 0.6 were considered partially methylated. To our knowledge, the CpG site will be considered methylated when the beta-value is greater than the empirical threshold of 0.3, for tissue data (13).

Results

Study characteristics

For meta-analysis, twenty-one articles (14-34) were obtained according to the above standards, after we screened 104 potentially relevant articles for inclusion, on the basis of title, abstract and full text (Figure S1A). The characteristics of the 21

articles (published between 2001 and 2014) are shown in ~~Supporting Information~~ Table S1~~-7~~ 8 (Figure S1B). The 21 articles came from China, Korea, Pakistan, Brazil, USA, Germany, Denmark, Finland and the UK (Figure S1C). In total, 1,588 bladder cancer patients and 720 normal controls were collected. We designated all patients from China, Korea and Pakistan as Asian; patients who came from Brazil and the USA were termed Mixed-race and patients from Germany, Denmark, Finland and the UK were termed Caucasian. As an experimental method to assess RASSF1A promoter methylation status, 18 of 21 included articles used methylation-specific polymerase chain reaction (MSP), while others used quantitative methylation specific polymerase chain reaction (QMSP). The primers used in both methods are listed in Table S8. The promoter region and the CpG sites of RASSF1A were previously described (3, 35). Forty-nine percent of bladder cancer patients had the methylated RASSF1A allele, with a frequency ranging from 32.87% to 81.63%, in individual trials. However, only 4.44% of normal controls had the methylated RASSF1A allele, with a frequency ranging from 0% to 20.83%, in individual trials. All the 21 studies focused on the risk of bladder cancer, however, many had a different specific focus: 4 primarily focused on the prognosis in bladder cancer ~~(~~Table S2)~~(15, 19, 21, 36)~~, 6 focused on the patients' age (Table S3)~~(22, 23, 26, 32, 34),~~ 7 focused on gender differences (Table S4) ~~(22, 23, 26, 31 34)~~, 2 examined smoking habits of patients (Table S5) ~~(20, 34)~~, 13 (Table S6)~~(14, 15, 17 20, 22, 23, 27, 28, 31, 33)~~ and 12 (Table S7) examined the TMN stage and differentiation grade ~~(15, 18 23, 26, 28, 32 34)~~, respectively (Figure S1D). The different frequencies observed for RASSF1A gene

promoter methylation between these different groups are listed on Table 1.

According to previous studies (3, ~~35~~) and the different primers used in the articles included in our meta-analysis, we analyzed eleven different probes located in or near the RASSF1A promoter region, and chose four of them (cg10580282, cg14943722, cg11607701, cg06360465), which contained the transcription start site of RASSF1A. Ultimately, bladder~~Bladder~~ cancer tissue samples (260 in total) and 21 adjacent normal cancer tissue samples were obtained from the TCGA project database (~~Supporting Information~~ Table ~~8~~9). Out of the 260 patients, 61.15% had RASSF1A gene promoter methylation, while there was no methylation of RASSF1A in normal tissue. The number of patients classified according to age, gender, smoker or non-smoker, TNM stage and differentiation grade, are shown ~~on~~ in Table 1.

The relationship between RASSF1A gene promoter methylation and bladder cancer risk

The results of this meta-analysis show that the frequency of RASSF1A gene promoter methylation is significantly higher in bladder cancer patients than in ~~that of~~ normal controls, by fixed effect model (OR=21.12; 95% CI=[14.51; 30.74]; z=15.93; p< 0.0001) and by random effects model (OR=18.46; 95% CI=[12.69; 26.85]; z=15.26; p< 0.0001) with τ^2=0 and I^2=0.00% (Figure ~~2~~1~~A~~). This clearly indicates a statistically significant increase in the likelihood of methylation in bladder cancer compared to normal controls, with no heterogeneity in the 21 articles. Subgroup analysis by ethnicity demonstrated that methylation of the RASSF1A gene promoter is positively associated with an increased risk of bladder cancer, among Mixed-race

(OR=23.36; 95% CI=[8.39;65.05], z=15.93), Asians (OR=24.10; 95% CI=[15.01; 38.69], z=15.93) and Caucasians (OR=13.99; 95% CI=[6.47;30.25], z=15.93) (all p<0.0001) (Figure ~~3A~~2A) by the fixed effects model. The similar meta-analysis results of the bladder cancer risk are found in ~~the~~ tissue (OR=18.44; 95% CI=[11.66; 29.16]) and urine samples (OR=19.82; 95% CI=[9.25; 42.45]) (Figure ~~3C~~2B). In addition, subgroup analysis by method and by primers used showed that the OR was 22.68 (95% CI=[15.04; 34.21], p<0.0001) in MSP, 14.11 (95% CI=[5.55; 35.87], p<0.0001) in QMSP, 21.15 (95% CI=[13.23; 33.80], p<0.0001) in primer type I, 21.06 (95% CI=[11.27; 39.33], p<0.0001) in primer type II, with varying heterogeneity (Figure 2C, D).

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Using data obtained from the TCGA project, we were able to compare the ~~difference in~~ frequency of RASSF1A gene promoter methylation in bladder cancer tissue and normal tissue, and found a significant difference (Figure ~~3B~~3A). This significant difference was true for patients who were Asian, Black, African American and White (Figure ~~3D~~3B). This study therefore gave a similar result to that of the meta-analysis.

Next we performed~~For the~~ bias analysis and sensitivity analysis of the 21 articles, which were focused on the relationship between RASSF1A gene promoter methylation and bladder cancer risk. The visual assessment of the Begg~~er's~~ test (t=0.97, df=20, p=0.34) and Egger's test (t=2.12, df=20, p=0.046) did not reveal any evidence of obvious asymmetry in the 21 articles. Therefore, there does not appear to

be any publication bias in the 21 studies (Figure ~~4A~~S2A). Sensitivity analyses were conducted to determine whether modification of the inclusive criteria of the meta-analysis affected the final results, but no single study was found to affect the pooled OR (Figure ~~4B~~S2B). The pooled sensitivity of the 21 articles was 0.96 (95% CI=[0.94-0.98]) and the specificity was 0.47 (95% CI=[0.39-0.55]) (Figure S2C), while the area under the curve (AUC) of the receiver operating characteristic curve (ROC) was 0.93 (95% CI=[0.90-0.95]) (Figure S2C). Hence, the diagnostic accuracy of the included studies was high and the meta-analysis results are overall very reliable.

The relationship between RASSF1A gene promoter methylation and the clinical features of bladder cancer

DNA methylation is thought to be linked to certain clinical characteristics, such as whether the patient was a smoker or not, and the tumor differentiation grade. Therefore, meta-analyses were conducted based on age, gender, smoking status~~habits~~, TNM stages and differentiation grade, and revealed~~found~~ that methylation of the RASSF1A gene promoter was not implicated in the incidence of bladder cancer based on age (OR=1.16, 95% CI=[0.72;1.87], p=0.55) (Figure ~~5A~~4A), gender (OR=1.43, 95% CI=[0.88;2.32], p=0.15) (Figure ~~5C~~4C) and smoking status ~~habit~~ (OR=0.86, 95% CI=[0.37;2.00], p=0.73) (Figure ~~5E~~4E). This~~Similarly, the~~ result was similar to the one obtained from the TCGA project (Figure ~~5B~~4B/D/F). However, when we compared the TNM stage I-II (low grade) and TNM stage III-IV (high grade), by meta-analysis and by using~~in~~ the TCGA project data, a significant difference was

found (Figure ~~5G~~4G-H). The same was true for the differentiation grade (Figure ~~5I~~4I-J), suggesting that advanced bladder cancer would have a high frequency of RASSF1A gene promoter methylation.

Although there was no~~wasn't any~~ heterogeneity between age, gender, smoking habits, TNM stage and differentiation grade (Table 2), a bias and sensitivity analysis for these 5 groups was implemented. As a result of the small amount of data for patients grouped according to age, gender and smoking habits, the assessment was only carried out between patients grouped according to the TNM stage and differentiation grade. Therefore a larger and higher quality systematic review should be undertaken in the future. As a result, we only found~~there was only~~ publication bias in the studies~~articles~~ of TNM stages and differentiation grade; the sensitivity analysis found that no single study could affect the pooled OR of TNM stage and differentiation grade (Table 2).

The relationship between RASSF1A gene promoter methylation and prognosis of bladder cancer patients

The role of RASSF1A gene promoter methylation has been examined for several different tumour types, such as lung cancer (37), breast cancer (38) and liver cancer (39). However, the role of RASSF1A gene promoter methylation in the prognosis of bladder cancer was not known. Here, a systematic review based on 4 articles including 503 bladder cancer patients in total (~~Supplementary~~ Table S2) and data extracted from the TCGA project, ~~were~~ was carried out. The ~~Hazard Ratio (~~HR~~)~~ was found to be 2.24 (95% CI=[1.45; 3.48], p=0.0003) for the 4 articles and ~~1.38~~1.83 for

the TCGA project data (95% CI: 1.03-3.25, p=0.040~~95% CI: 0.82~2.32, p=0.30~~) (Figure ~~6A~~5A) for overall survival (OS) when we used 199 bladder cancer patients analyzed by Kaplan-Meier method, which suggests that bladder cancer patients with RASSF1A gene promoter methylation have a poor prognosis. The HR of 172 bladder cancer patients analyzed for disease-free survival (DFS) was 1.97 (95% CI=[1.04; 3.72], p=0.037), which demonstrates that bladder cancer patients with RASSF1A gene promoter methylation may have a 97% chance of recurrence after surgery or other treatment (such as chemotherapy and combined treatment) (Figure 5B).~~Disease-free survival (DFS) can also demonstrate the prognosis, and the result is similar to the OS (Figure 6B).~~

Discussion

Modern tumor molecular biology studies have shown that tumors can be caused by genetic and epigenetic mechanisms. The instability of the genome has long been considered an important mechanism driving bladder cancer (40). Multiple molecular genetics studies have found that many gene loci experience loss of heterozygosity and lack of homozygosity, and a deficiency in tumor suppressor genes is thought to play an important role in the development of bladder cancer. In addition, epigenetic modifications, such as DNA methylation and histone acetylation, are also responsible for the development of tumors (~~41,~~ 42). Abnormal DNA methylation patterns were identified ten years ago as one of the molecular characteristics all tumors have in common (43), and ~~is~~ are now known to be the most important form of genetic

modification in mammals (44). Many researchers (41, 45) believed that DNA methyltransferase mediated methylation in overall genomic DNA, but those high levels of methylation was were a sign of tumorigenesis. Indeed, DNA methylation was implicated inattributed to the silencing of tumor suppressor genes and was suggestedthought to lead to the development of tumors. Some studies (5, 46) found that gene promoter region methylation patterns were not random, : but that some genes in certain tumor types were commonly methylated, but unmethylated in other tumor types. Tumor suppressor gene promoter regions often showed abnormal methylation, resulting in gene inactivation and thus driving tumorigenesis. The CpG islands within the gene promoter region are the targets for methylation, as this prevents gene transcription. Methylation of tumor suppressor genes will lead to permanent gene silencing, such that the proteins are never expressed and cannot inhibit growth and differentiation.

Methylation leading to epigenetic silencing of tumor suppressor genes, is now known to be common in many human tumors, including bladder cancer (40). RASSF1A is a tumor suppressor gene, and its inactivation can occur due to methylation of the promoter region, gene mutation or loss of heterozygosity and lack of homozygosity, although studies have shown that abnormal methylation of the promoter region is the major mechanism. In most human epithelial tumors, the RASSF1A promotor is highly methylated. For example, 94% of small cell lung cancer (47), 87% of breast cancer (48), 74% of prostate cancer (49), 76% of renal cell carcinoma (50) and 91% of nasopharyngeal carcinoma (51) had abnormal methylation

of the RASSF1A gene promoter. Although a previous study investigated ~~has focus on~~ the relationship between RASSF1A promoter methylation and bladder cancer risk (52), ~~while considering~~ the ~~factors of~~ differences in gender proportion, age distribution, racial composition~~source~~, test methods, and primers used in the studies would result in some differences in the results, and moreover, that study lacked ~~the result lacks~~ quantitative assessment. Therefore, an integrated analysis to quantify the ability to test for such methylation in bladder cancer, was performed. A significant association was identified between methylation of the RASSF1A gene promoter and the risk of ~~developing~~ bladder cancer ($p<0.0001$) as well as prognosis ($p<0.05$), using a meta-analysis ~~and data from the TCGA project~~. Subgroup analysis by race, sample type, method and primers used, also showed that~~of the association between~~ RASSF1A gene promoter methylation was associated with~~and~~ bladder cancer risk~~.~~. ~~showed that patients grouped according to their race and the sample type, had a higher risk than the normal controls.~~

Meta-analysis involves a merger effect on the results between multiple studies, however it should be stressed that only homogeneous studies would merge. Therefore, if the difference between the studies is too big, they cannot merge together. In other words, meta-analysis results may be affected by heterogeneity. The heterogeneity in meta-analysis is mainly caused by methodological differences and biological effects resulting from different subject group characteristics in each study, such as age, gender and race composition. In our meta-analysis, no heterogeneity in the analysis of bladder cancer risk was caused by age, gender and smoking status (including

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subgroup analysis by race, sample source, method and primers), but there was heterogeneity caused by TNM stage and differentiation grade. However, the number of articles matched by age, gender and smoking status was very small, which would affect the veracity of the meta-analysis results. Therefore we decided to also use the data from the TCGA project to further support our meta-analysis results. The data from the TCGA project can avoid the heterogeneity produced by methodological differences and biological effects. Moreover, we did not have to consider the human factor and the bias between different researchers when analyzing the data from the TCGA project. We therefore analyzed the data from the TCGA project and found a significant association between RASSF1A promoter methylation and the risk, the prognosis, the TNM.stage, and the differentiation grade, of bladder cancer. Hence, these results confirm the results of the meta-analysis.

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Analysis was performed to assess the influence of publication bias on the random effects model in the meta-analysis of bladder cancer risk. We found no obvious asymmetry in the 21 articles and no single study was found to affect the pooled OR. The pooled sensitivity of the 21 articles was 0.96 and the specificity was 0.47 with the AUC of 0.93. Hence, the diagnostic accuracy of the included studies was high and the meta-analysis results are overall very reliable. This therefore indicates a strong association between RASSF1A promoter methylation and bladder cancer risk. It should be noted that although no association could be made between RASSF1A gene promoter methylation ~~with~~ and age, gender or smoking ~~habit~~ status of the bladder cancer patients, publication bias could not ~~can't~~ be fully eliminated. This is therefore a

major limitation of the study, although it is re-assuring that the TCGA project data analysis reached the same conclusion.

RASSF1A can inhibit cell proliferation, control cell cycle, promote cell apoptosis and aging, but whether methylation of the RASSF1A gene promoter contributes to TNM stage in tumors, and their differentiation grade, remains unclear. In all of the data selected for this analysis, the TNM stage III-IV groups indicated a higher significance for RASSF1A gene promoter methylation in bladder cancer ($p<0.0001$) than TNM stage 0-II. When high and low grade bladder cancers were compared for RASSF1A gene promoter methylation, a significant difference was identified. These results are similar to the results of several studies previously published (15, 18, 19) indicating that advanced cancer has a higher frequency of RASSF1A gene promoter methylation.

In conclusion, this integrated analysis of pooled data provides strong evidence that the methylation status of the RASSF1A gene promoter is strongly associated with both the risk of developing bladder cancer and patient prognosis. In addition, RASSF1A promoter methylation is strongly associated with an advanced TNM stage and differentiation grade of bladder cancer. Therefore, methylation of the RASSF1A gene promoter could be a promising diagnostic assay for the clinical diagnosis of bladder cancer.

Supporting Information

Table S1-~~7~~8. The articles ~~focuses~~ focusing on the relationship between

RASSF1A gene promoter methylation and bladder cancer, which were included in the meta-analysis.

Table ~~S8~~S9. The characteristics of the data obtained from the TCGA project.

Figure S1. Flow chart of study identification and the number of articles in specific years, countries and groups.

Figure S2. Publication bias, sensitivity analyses, summary receiver operating characteristics (SROC) estimation for the relationship between RASSF1A gene promoter methylation and bladder cancer risk.

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Figure Legends

~~Figure 1. Flow chart of study identification and the number of articles in specific years, countries and groups.~~

Figure ~~2~~1. Combined estimates for the association between RASSF1A gene promoter methylation and bladder cancer risk, with forest plot.

Author, year, country of the studies and methylated (M) and total (T) number ~~of the~~ samples ~~(T)~~ in case and control, combined odds ratio (OR) with 95% confidence region are~~were~~ indicated ~~labeled~~ in the right column of the figure. The DerSimonian-Laird estimator and Mantel-Haenszel method were selected to conduct a combined estimation for the random effects model and fixed effects model, respectively.

Figure ~~3~~2. Subgroup meta-analysis of the relationship between RASSF1A gene promoter methylation and risk of ~~developing~~ bladder cancer.

A~~/C~~-D, Subgroup meta-analysis based on race ~~and~~ , sample, different test methods, different primers, by fixed effects model~~, respectively. B/D, Evaluation of the methylation of RASSF1A gene promoter in bladder cancer, including differences in race, in the TCGA project. The β=0.3 indicates by red dotted line.~~.

Figure 3. Statistical analysis of the relationship between RASSF1A gene promoter

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methylation and risk of bladder cancer in the TCGA project.

A-B, Evaluation of the methylation of RASSF1A gene promoter in bladder cancer, including differences in race, in the TCGA project. The β=0.3 indicates by red dotted line.

~~Figure 4. Publication bias and sensitivity analyses on the relationship between RASSF1A gene promoter methylation and bladder cancer risk.~~

~~A, Funnel plot from 21 studies comparing colorectal cancer with normal controls;~~

~~B, Sensitivity analysis of the summary odds ratio coefficients on the relationship between RASSF1A gene promoter methylation and bladder cancer risk.~~

Figure ~~5~~4. Quantitative assessment of the relationship between RASSF1A gene promoter methylation and patient age, gender and smoking habit, TNM stage and differentiation grade, in bladder cancer.

A/C/E/G/I, Meta-analysis for the relationship between RASSF1A gene promoter methylation and patient age, gender and smoking habit, TNM stages and differentiation grade, in bladder cancer. B/D/F/H/J, ~~Assessed~~ Assessment of the relationship between RASSF1A gene promoter methylation and patient age, gender and smoking habit, TNM stages and differentiation grade, in bladder cancer by TCGA project. The β=0.3 indicates by red dotted line.

Figure ~~6~~5. Association of patient survival and RASSF1A gene promoter methylation status by Kaplan-Meier method.

A, survival curves by methylation status of RASSF1A gene promoter. The number of censored cases with and without methylation ~~were~~ was 122 and 68, respectively, and

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the mean survival time ~~were~~ was 14.17 and 19.49, respectively. B, Kaplan-Meier survival analysis of recurrent bladder cancer showing the association between tumor progression and RASFF1A gene methylation status. The number of censored cases with and without methylation was~~were~~ 110 and 62, respectively, and the mean survival times ~~were~~ was 12.09 and 19.62~~;~~, respectively.

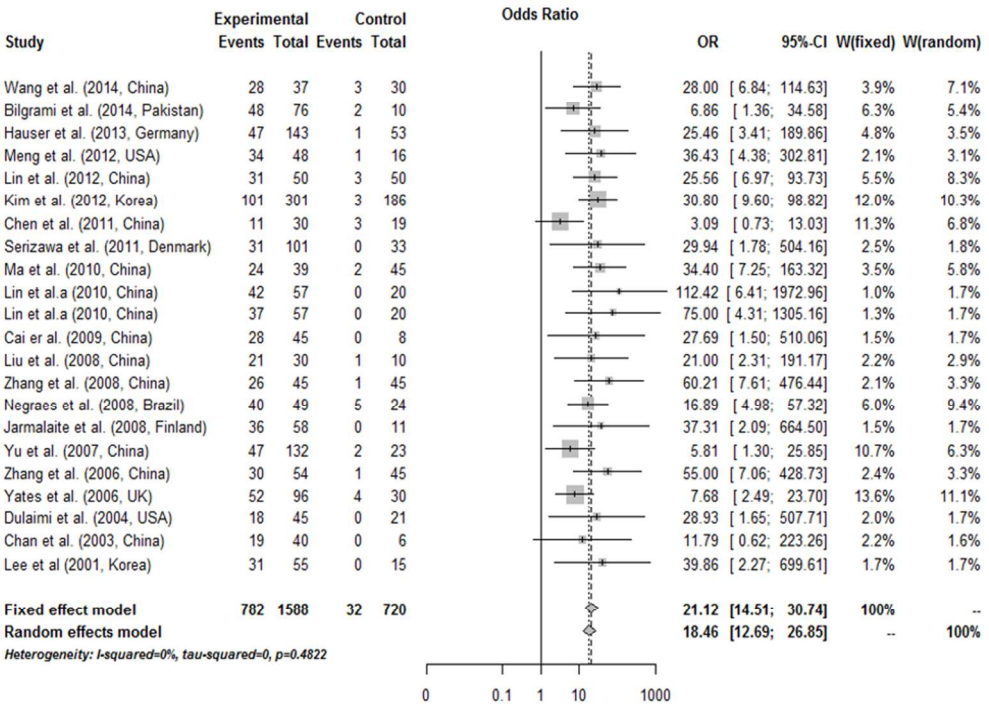

Figure 1. Combined estimates for the association between RASSF1A gene promoter methylation and bladder cancer risk, with forest plots.
Author, year, country of the studies and methylated (M) and total (T) number of samples in case and control, combined odds ratio (OR) with 95% confidence region are indicated in the right column of the figure. The DerSimonian-Laird estimator and Mantel-Haenszel method were selected to conduct a combined estimation for the random effects model and fixed effects model, respectively.
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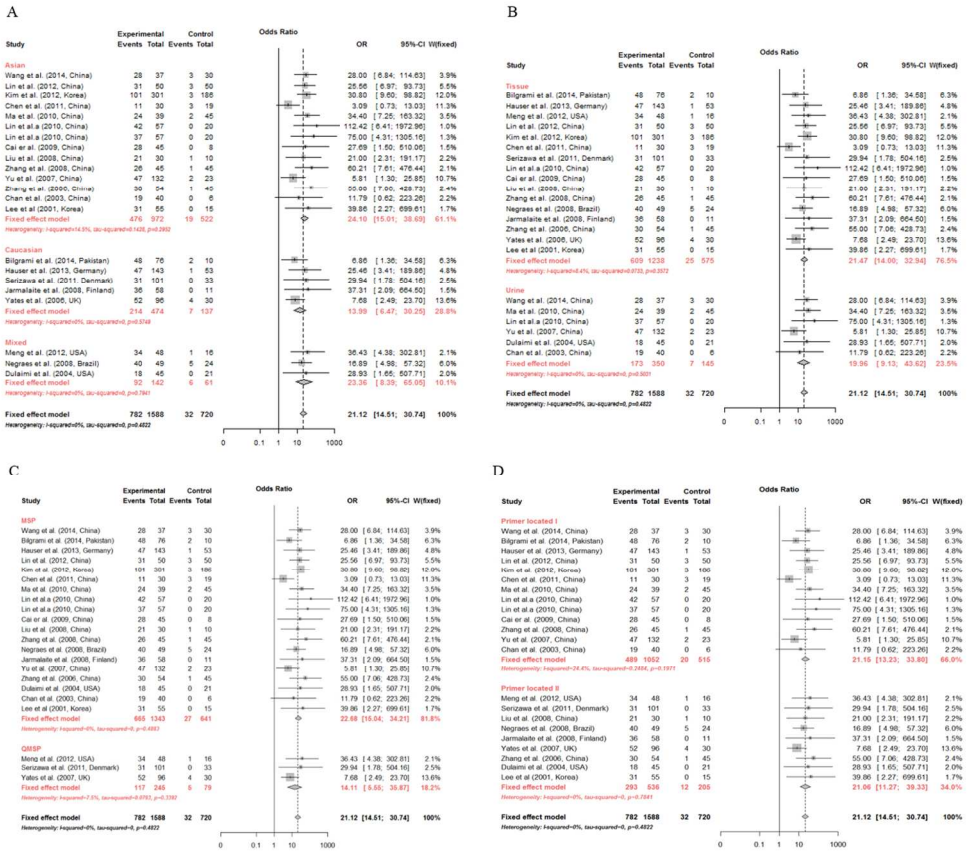

Figure 2. Subgroup meta-analysis of the relationship between RASSF1A gene promoter methylation and risk of bladder cancer.

A-D, Subgroup meta-analysis based on race, sample, different test methods, different primers, by fixed effects model.

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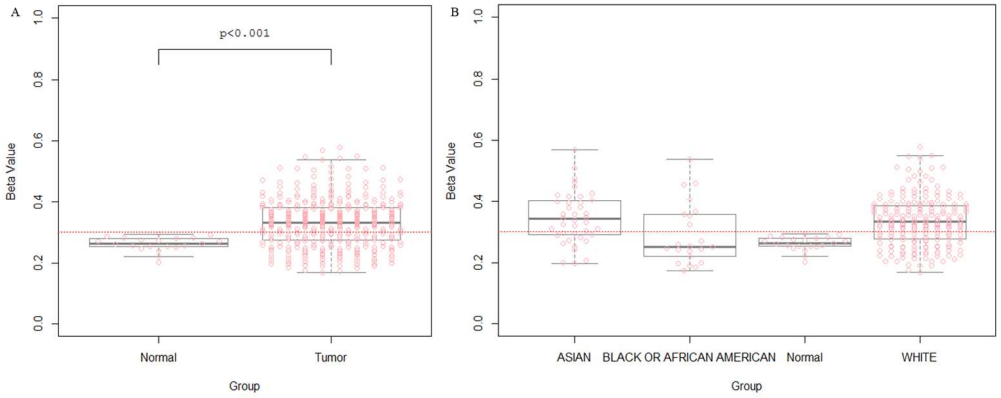

Figure 3. Statistical analysis of the relationship between RASSF1A gene promoter methylation and risk of bladder cancer in the TCGA project.
A-B, Evaluation of the methylation of RASSF1A gene promoter in bladder cancer, including different race, in the TCGA project. The β=0.3 indicates by red dotted line.
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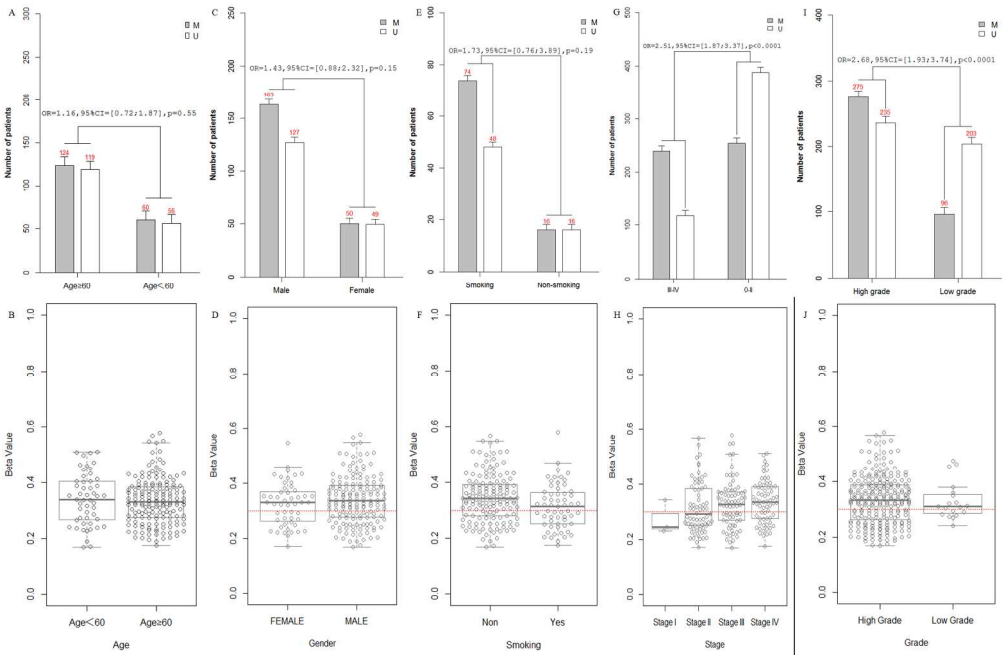

Figure 4. Quantitative assessment of the relationship between RASSF1A gene promoter methylation and patient age, gender and smoking habit, TNM stage and differentiation grade, in bladder cancer. A/C/E/G/I, Meta-analysis for the relationship between RASSF1A gene promoter methylation and patient age, gender and smoking habit, TNM stages and differentiation grade, in bladder cancer. B/D/F/H/J, Assessment of the relationship between RASSF1A gene promoter methylation and patient age, gender and smoking habit, TNM stages and differentiation grade, in bladder cancer by TCGA project. The β=0.3 indicates by red dotted line.
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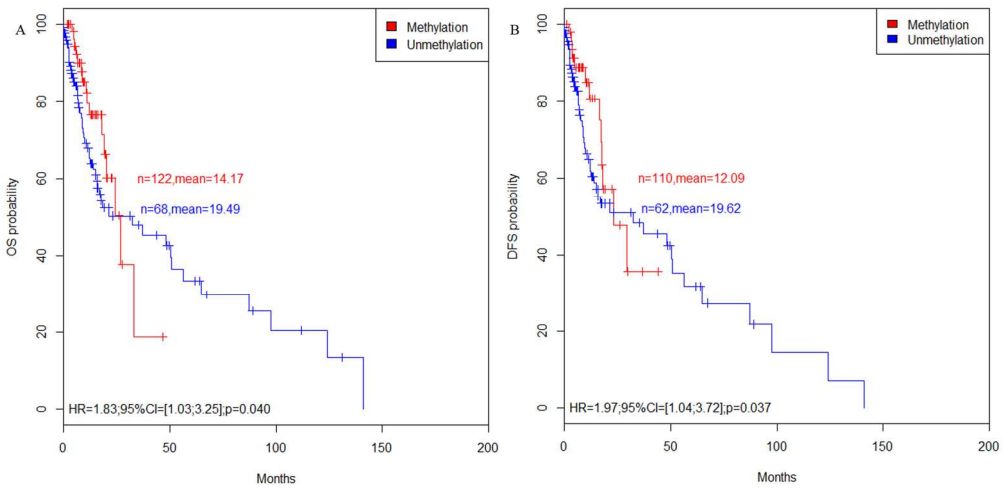

Figure 5. Association of patient survival and RASSF1A gene promoter methylation status by Kaplan-Meier method.

A, survival curves by methylation status of RASSF1A gene promoter. The number of censored cases with and without methylation was 122 and 68, respectively, and the mean survival time was 14.17 and 19.49, respectively. B, Kaplan-Meier survival analysis of recurrent bladder cancer showing the association between tumor progression and RASFF1A gene methylation status. The number of censored cases with and without methylation was 110 and 62, respectively, and the mean survival time was 12.09 and 19.62, respectively.
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Table 1. Characteristics of eligible studies and TCGA project considered in the report

	Meta-analysis			TCGA project		
	N	M	OR; 95%CI; p	N	M	OR; 95%CI; p
Gender			1.43;[0.88;2.32];0.15			0.48;[0.23;1.03];0.062
Male	290	163		164	109	
Female	99	50		51	30	
Age			1.16;[0.72;1.87];0.55			0.99;[0.52;1.92];0.991
Rang age				34-90		
Mean age (year)				67.56		
≥60	243	124		164	106	
＜60	112	60		51	33	
TNM stage			2.51;[1.87;3.37];<0.0001			1.82;[1.01;3.22];0.044
I	64	25		3	1	
II	2	4		69	33	
III	35	23		73	43	
IV	6	9		66	43	
Differentiation grade			2.68;[1.93;3.74];<0.0001			3.38;[1.23;9.32];0.018
Low	299	96		19	7	
High	510	275		241	152	
Smoking status			0.86;[0.37;2.00];0.73			1.40;[0.78;2.51];0.254
Smoking	121	46		105	76	
Non-smoking	32	16		101	71	

Table 2. Heterogeneity, bias analysis and sensitivity analysis

	Test of heterogeneity			Quantifying heterogeneity		Begger's test		Egger's test		Sensitivity analyses	
oup	Q	df	p	tau^2	I^2	t	p	t	p	Low (OR; 95%CI)	High (OR; 95%CI)
k	20.62	21	0.48	0.00	0%	0.97	0.34	2.12	0.05	19.80 [13.34;29.37]	23.42 [15.80;34.72]
nder	5.79	7	0.56	0.00	0%	NA	NA	NA	NA	1.28 [0.76;2.15]	1.61 [0.94;2.76]
e	2.66	6	0.85	0.00	0%	NA	NA	NA	NA	1.07 [0.64;1.78]	1.11 [0.67;1.85]
M stage	21.8	13	0.06	0.24	40.40%	-0.12	0.90	0.71	0.38	2.36 [1.74;3.18]	2.79 [2.05;3.79]
ade	25.31	12	0.01	0.49	52.60%	-1.79	0.101	-1.00	0.34	2.24 [1.57;3.20]	3.10 [2.18;4.40]
oking	0.00	1	0.99	0.00	0%	NA	NA	NA	NA	NA	NA

NA - not available due to the small data.

Table S1: The article features of the relationship between RASSF1A gene promoter methylation and bladder cancer risk

Author	Year	Country	Method	Sample	TNM. stage	Mean age (year)	Male/Female	M1	T1	M2	T2
Wang et al.	2014	China	MSP	Urine	I-IV	65.8	31/6	28	37	3	30
Bilgrami et al.	2014	Pakistan	QSP	Tissue	I-IV	64	63/13	48	76	2	10
Hauser et al.	2013	Germany	MSP	Tissue	I-IV	71.1	106/37	47	143	1	53
Meng et al.	2012	USA	QMSP	Tissue	I-IV	67	35/13	34	48	1	16
Lin et al.	2012	China	MSP	Tissue	I-IV	65	26/24	31	50	3	50
Kim et al.	2012	Korea	MSP	Tissue	I-IV	67	238/63	101	301	3	186
Chen et al.	2011	China	QMSP	Tissue	I-IV	71.5	25/5	11	30	3	19
Serizawa et al.	2011	Denmark	QMSP	Tissue	I-IV	NA	80/21	31	101	0	33
Ma et al.	2010	China	MSP	Urine	I-III	61.4	27/12	24	39	2	45
Lin et al.[a]	2010	China	MSP	Tissue	I-IV	64	38/19	42	57	0	20
Lin et al.[a]	2010	China	MSP	Urine	I-IV	64	38/19	37	57	0	20
Cai er al.	2009	China	MSP	Tissue	NA	63.12	32/13	28	45	0	8
Liu et al.	2008	China	MSP	Tissue	I-IV	55.6	24/6	21	30	1	10
Zhang et al.	2008	China	MSP	Tissue	I-III	62	36/9	26	45	1	45
Negraes et al.	2008	Brazil	MSP	Tissue	I-IV	67.58	44/10	40	49	5	24
Jarmalaite et al.	2008	Finland	MSP	Tissue	I-IV	66	47/11	36	58	0	11
Yu et al.	2007	China	MSP	Urine	I-IV	63.4	107/25	47	132	2	23
Zhang et al.	2006	China	MSP	Tissue	I-III	61.5	44/10	30	54	1	45
Yates et al.	2006	UK	QMSP	Tissue	I-IV	77	66/30	52	96	4	30
Dulaimi et al.	2004	USA	MSP	Urine	I-IV	59.5	33/12	18	45	0	21
Chan et al.	2003	China	MSP	Urine	I-IV	70	29/11	19	40	0	6
Lee et al	2001	Korea	MSP	Tissue	I-IV	NA	47/8	31	55	0	15

MSP, methylation specific polymerase chain reaction; QMSP, quantitative methylation specific polymerase chain reaction; T, the total number of patients in each article; M, the number of patients with methylation; 1, the case group; 2, the control group; NA, not available.

[a]means the data from the same articles.

Table S2: The article features of the relationship between RASSF1A gene promoter methylation and the prognosis in bladder cancer

Author	Year	Country	Method	Sample	TNM. stage	Mean age (year)	Male/ Female	HR	p	Low (95% CI)	High (95% CI)
Bilgrami et al.	2014	Pakistan	QSP	Tissue	I-IV	64	63/13	1.49	0.01	0.94	12.06
Kim et al.	2012	Korea	MSP	Tissue	I-IV	67	238/63	8.559	0.014	1.547	47.364
Chen et al.	2011	China	QMSP	Tissue	I-IV	71.5	25/5	2.08	0.039	1.79	5.09
Yates et al.	2006	UK	QMSP	Tissue	I-IV	77	66/30	2.54	0.002	1.467	21.09

MSP, methylation specific polymerase chain reaction; QMSP, quantitative methylation specific polymerase chain reaction; HR, Hazard Ratio.

Table S3: The article features of the relationship between RASSF1A gene promoter methylation and the age (age≤60,age＞60) of bladder cancer patients

Author	Year	Country	Method	Sample	TNM.stage	Mean age (year)	Male/Female	M1	T1	M2	T2
Ma et al.	2010	China	MSP	Urine	I-III	61.4	27/12	13	22	11	20
Negraes et al.	2008	Brazil	MSP	Tissue	I-IV	67.58	44/10	8	39	3	9
Chan et al.	2003	China	MSP	Urine	I-IV	70	29/11	15	40	3	11
Lee et al	2001	Korea	MSP	Tissue	I-IV	NA	47/8	21	33	13	22
Lin et al.[a]	2010	China	MSP	Tissue	I-IV	64	38/19	25	37	15	20
Lin et al.[a]	2010	China	MSP	Urine	I-IV	64	38/19	25	37	12	20
Dulaimi et al.	2004	USA	MSP	Urine	I-IV	59.5	33/12	17	35	3	10

MSP, methylation specific polymerase chain reaction; T, the total number of patients in each article; M, the number of patients with methylation; 1, the case group; 2, the control group; NA, not available.
[a]means the data from the same articles.

Table S4: The article features of the relationship between RASSF1A gene promoter methylation and the gender of bladder cancer patients

Author	Year	Country	Method	Sample	TNM.stage	Mean age (year)	Male/Female	M1	T1	M2	T2
Zhang et al.	2006	China	MSP	Tissue	I-III	61.5	44/10	23	36	3	9
Ma et al.	2010	China	MSP	Urine	I-III	61.4	27/12	17	27	7	12
Negraes et al.	2008	Brazil	MSP	Tissue	I-IV	67.58	44/10	11	40	0	9
Chan et al.	2003	China	MSP	Urine	I-IV	70	29/11	14	29	5	11
Lee et al	2001	Korea	MSP	Tissue	I-IV	NA	47/8	29	47	5	8
Lin et al.[a]	2010	China	MSP	Tissue	I-IV	64	38/19	27	39	14	19
Lin et al.[a]	2010	China	MSP	Urine	I-IV	64	38/19	25	39	13	19
Dulaimi et al.	2004	USA	MSP	Urine	I-IV	59.5	33/12	17	33	3	12

MSP, methylation specific polymerase chain reaction; T, the total number of patients in each article; M, the number of patients with methylation; 1, the case group; 2, the control group; NA, not available.
[a]means the data from the same articles.

Table S5: The article features of the relationship between RASSF1A gene promoter methylation and the smoking status of bladder cancer patients

Author	Year	Country	Method	Sample	TNM.stage	Mean age (year)	Male/Female	M1	T1	M2	T2
Serizawa et al.	2011	Denmark	QMSP	Urine	I-IV	NA	80/21	27	90	5	15
Lee et al	2001	Korea	MSP	Tissue	I-IV	NA	47/8	19	31	11	17

MSP, methylation specific polymerase chain reaction; QMSP, quantitative methylation specific polymerase chain reaction; T, the total number of patients in each article; M, the number of patients with methylation; 1, the case group; 2, the control group; NA, not available.

Table S6: The article features of the relationship between RASSF1A gene promoter methylation and the TNM.stages of bladder cancer patients

Author	Year	Country	Method	Sample	TNM.stage	Mean age (year)	Male/Female	M1	T1	M2	T2
Wang et al.	2014	China	MSP	Urine	I-IV	65.8	31/6	23	30	5	7
Bilgrami et al.	2014	Pakistan	QSP	Tissue	I-IV	64	63/13	21	43	27	33
Meng et al.	2012	USA	QMSP	Tissue	I-IV	67	35/13	5	9	28	39
Lin et al.	2012	China	MSP	Tissue	I-IV	65	26/24	4	11	27	39
Kim et al.	2012	Korea	MSP	Tissue	I-IV	67	238/63	70	239	31	62
Serizawa et al.	2011	Denmark	QMSP	Urine	I-IV	NA	80/21	21	86	11	19
Lin et al.[a]	2010	China	MSP	Tissue	I-IV	66.5	38/19	25	32	17	25
Lin et al.[a]	2010	China	MSP	Urine	I-IV	66.5	38/19	21	32	16	25
Ma et al.	2010	China	MSP	Urine	I-III	61.4	27/12	14	23	10	16
Liu et al.	2008	China	MSP	Tissue	I-IV	55.6	24/6	4	12	17	18
Jarmalaite et al.	2008	Finland	MSP	Tissue	I-IV	66	47/11	7	38	10	20
Zhang et al.	2006	China	MSP	Tissue	I-III	61.5	44/10	15	31	11	14
Chan et al.	2003	China	MSP	Urine	I-IV	70	29/11	12	29	7	11
Lee et al	2001	Korea	MSP	Tissue	I-IV	NA	47/8	12	27	22	28

MSP, methylation specific polymerase chain reaction; QMSP, quantitative methylation specific polymerase chain reaction; T, the total number of patients in each article; M, the number of patients with methylation; 1, the case group; 2, the control group; NA, not available.

[a]means the data from the same articles.

Table S7: The article features of the relationship between *RASSF1A* promoter methylation and the TNM.stages of bladder cancer patients

Author	Year	Country	Method	Sample	TNM.stage	Mean age (year)	Male/Female	M1	T1	M2	T2
Negraes et al.	2008	Brazil	MSP	Tissue	I-IV	67.58	44/10	3	21	8	28
Jarmalaite et al.	2008	Finland	MSP	Tissue	I-IV	66	47/11	1	10	16	48
Chan et al.	2003	China	MSP	Urine	I-IV	70	29/11	3	10	16	30
Lee et al	2001	Korea	MSP	Tissue	I-IV	NA	47/8	12	18	22	37
Bilgrami et al.	2014	Pakistan	QSP	Tissue	I-IV	64	63/13	21	43	27	33
Lin et al.	2012	China	MSP	Tissue	I-IV	65	26/24	4	11	27	39
Kim et al.	2012	Korea	MSP	Tissue	I-IV	67	238/63	11	70	37	79
Serizawa et al.	2011	Denmark	QMSP	Urine	I-IV	NA	80/21	5	54	26	50
Chen et al.	2011	China	QMSP	Tissue	I-IV	71.5	25/5	2	12	7	18
Ma et al.	2010	China	MSP	Urine	I-III	61.4	27/12	5	8	19	31
Lin et al.[a]	2010	China	MSP	Tissue	I-IV	66.5	38/19	16	19	26	38
Lin et al.[a]	2010	China	MSP	Urine	I-IV	66.5	38/19	12	19	25	38
Dulaimi et al.	2004	USA	MSP	Urine	I-IV	59.5	33/12	1	4	19	41

MSP, methylation specific polymerase chain reaction; QMSP, quantitative methylation specific polymerase chain reaction; T, the total number of patients in each article; M, the number of patients with methylation; 1, the case group; 2, the control group; NA, not available.

[a]means the data from the same articles.

tings of primers of present 21 studies

Year	Method	Primer Type	Forward primers	Reverse primer	Product size (bp)	Genomi
2014	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2014	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2013	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2012	QMSP	Primer located II	ATGTTAAGGGAATTTATTTAGAATGTATTT	AACCTTCACTTAAAATAAAAAAAA	146	chr3:50
2012	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2012	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2011	QMSP	Primer located II	GCGTTGAAGTCGGGGTTC	CCCGTACTTCGCTAACTTTAAACG	75	chr3:50
2011	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2010	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2010	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2009	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2008	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2008	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2008	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2008	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2007	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2007	QMSP	Primer located II	ATTGAGTTGCGGGAGTTGGT	ACACGCTCCAACCGAATACG	135	chr3:50
2006	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2004	MSP	Primer located II	M:GGGTTTTGCGAGAGCGCG U:GGTTTTGTGAGAGTGTGTTTAG	GCTAAGAAACGCGAACCG CACTAACAAACACAAACCAAAC	169 169	chr3:50
2003	MSP	Primer located I	M:GTGTTAACGGGTTGCGTATC U:TTTGCTTGGAGTGTGTYAATGTG	AACCCCGCGAACTAAAAACGA CAAACCCCACAAACTAAAAACA	94 108	chr3:50
2001	MSP	Primer located II	M:TTTTTCCATTTCGCGTCTCT U:TCACCCATTTTTCCATTTCTCT	CGTTTTTGCCCTTTCTTCGC CTTTTTTTCCCTTTCTTCTCTT	192 192	chr3:50

PATIENT ID	Beta Value	Person Gender	Diagnosis A
TCGA-BL-A13I-01	0.350122103	FEMALE	57
TCGA-CF-A1HR-01	0.394922727	MALE	62
TCGA-CF-A1HS-01	0.344362384	FEMALE	75
TCGA-DK-A1A3-01	0.289968136	MALE	60
TCGA-DK-A1A5-01	0.291622194	MALE	79
TCGA-DK-A1A6-01	0.450747608	MALE	53
TCGA-DK-A1A7-01	0.223446222	FEMALE	67
TCGA-DK-A1AA-01	0.336819417	MALE	57
TCGA-DK-A1AB-01	0.223068311	FEMALE	74
TCGA-DK-A1AC-01	0.318083351	MALE	72
TCGA-DK-A1AD-01	0.375078378	MALE	69
TCGA-DK-A1AE-01	0.351571579	MALE	84
TCGA-DK-A1AF-01	0.248952946	FEMALE	84
TCGA-DK-A1AG-01	0.287325915	MALE	65
TCGA-BT-A20J-01	0.191158336	MALE	75
TCGA-BT-A20N-01	0.339491843	MALE	72
TCGA-BT-A20O-01	0.269814407	MALE	75
TCGA-BT-A20P-01	0.548079247	FEMALE	81
TCGA-BT-A20Q-01	0.484813866	MALE	73
TCGA-BT-A20T-01	0.355960725	MALE	63
TCGA-BT-A20U-01	0.336410185	FEMALE	70
TCGA-BT-A20V-01	0.353145126	FEMALE	59
TCGA-BT-A20W-01	0.272528918	MALE	71
TCGA-BT-A20R-01	0.296765254	FEMALE	79
TCGA-BT-A20X-01	0.236067423	MALE	56
TCGA-CF-A27C-01	0.422315575	MALE	52
TCGA-DK-A2I1-01	0.225250372	FEMALE	73
TCGA-DK-A2I2-01	0.329575977	FEMALE	63
TCGA-G2-A2EC-01	0.460471396	FEMALE	58
TCGA-G2-A2EJ-01	0.351825688	FEMALE	56
TCGA-G2-A2EO-01	0.343907262	MALE	69
TCGA-G2-A2ES-01	0.25832977	MALE	85
TCGA-GD-A2C5-01	0.274457903	FEMALE	53
TCGA-H4-A2HO-01	0.379737068	MALE	53
TCGA-H4-A2HQ-01	0.258390769	FEMALE	64
TCGA-BT-A2LA-01	0.388010816	MALE	54
TCGA-BT-A2LB-01	0.324044359	FEMALE	73
TCGA-DK-A2HX-01	0.445221966	FEMALE	80
TCGA-DK-A2I6-01	0.175765252	MALE	81
TCGA-G2-A2EF-01	0.361418359	MALE	50
TCGA-G2-A2EK-01	0.355381597	MALE	57
TCGA-G2-A2EL-01	0.204129644	MALE	77
TCGA-E5-A2PC-01	0.386860458	FEMALE	61
TCGA-FD-A3B3-01	0.321290025	FEMALE	74
TCGA-FD-A3B4-01	0.269459836	FEMALE	55
TCGA-FT-A3EE-01	0.433930831	FEMALE	80
TCGA-HQ-A2OE-01	0.455943171	MALE	69
TCGA-BT-A2LD-01	0.353302993	FEMALE	78
TCGA-CF-A3MG-01	0.358043869	MALE	48

TCGA-CF-A3MH-01	0.281316104	MALE	75
TCGA-CF-A3MI-01	0.203726654	MALE	62
TCGA-DK-A3IL-01	0.365230098	FEMALE	79
TCGA-DK-A3IM-01	0.357152327	MALE	76
TCGA-DK-A3IN-01	0.359114507	MALE	72
TCGA-DK-A3IQ-01	0.321185685	MALE	74
TCGA-DK-A3IT-01	0.213753104	MALE	62
TCGA-DK-A3IU-01	0.206413568	MALE	58
TCGA-FD-A3B5-01	0.241201587	MALE	86
TCGA-FD-A3B6-01	0.213746203	MALE	75
TCGA-FD-A3B7-01	0.199975658	MALE	66
TCGA-FD-A3B8-01	0.239499483	MALE	56
TCGA-G2-A3IB-01	0.245988504	MALE	66
TCGA-G2-A3IE-01	0.23347257	MALE	51
TCGA-GC-A3I6-01	0.190849686	MALE	45
TCGA-GV-A3JW-01	0.203605278	MALE	74
TCGA-GV-A3JX-01	0.411350071	MALE	59
TCGA-BL-A3JM-01	0.365925168	MALE	62
TCGA-CF-A3MF-01	0.464683582	MALE	34
TCGA-CU-A3KJ-01	0.249691973	MALE	75
TCGA-DK-A2I4-01	0.293023426	MALE	79
TCGA-DK-A3IK-01	0.320907153	MALE	87
TCGA-DK-A3IS-01	0.204050293	MALE	68
TCGA-DK-A3IV-01	0.325977179	MALE	60
TCGA-FD-A3N5-01	0.365920736	MALE	69
TCGA-FD-A3N6-01	0.173154236	FEMALE	43
TCGA-FD-A3NA-01	0.417986223	MALE	60
TCGA-GV-A3JZ-01	0.33602892	MALE	55
TCGA-BT-A3PH-01	0.513058476	MALE	76
TCGA-BT-A3PJ-01	0.320623288	MALE	76
TCGA-BT-A3PK-01	0.37706798	MALE	80
TCGA-GC-A3RB-01	0.337536862	MALE	54
TCGA-GD-A3OP-01	0.422628511	FEMALE	84
TCGA-GD-A3OQ-01	0.387007984	MALE	48
TCGA-GD-A3OS-01	0.226701522	FEMALE	54
TCGA-GV-A3JV-01	0.465223457	MALE	66
TCGA-GV-A3QG-01	0.331820486	MALE	65
TCGA-GV-A3QH-01	0.42316733	MALE	67
TCGA-GV-A3QI-01	0.511693639	MALE	47
TCGA-CU-A3QU-01	0.402606658	MALE	58
TCGA-CU-A3YL-01	0.32594245	MALE	67
TCGA-DK-A3WX-01	0.296623882	FEMALE	67
TCGA-DK-A3WY-01	0.225965508	FEMALE	67
TCGA-DK-A3X1-01	0.26663169	FEMALE	78
TCGA-DK-A3X2-01	0.214525592	MALE	85
TCGA-E7-A3X6-01	0.20822377	MALE	70
TCGA-E7-A3Y1-01	0.292989609	MALE	57
TCGA-FD-A3SJ-01	0.231110078	MALE	59
TCGA-FD-A3SL-01	0.332419862	MALE	60
TCGA-FD-A3SM-01	0.219788398	MALE	70

TCGA-FD-A3SN-01	0.372232644	MALE	79
TCGA-FD-A3SO-01	0.334883651	MALE	68
TCGA-FD-A3SP-01	0.370400193	MALE	60
TCGA-FD-A3SQ-01	0.391877104	MALE	62
TCGA-FD-A3SR-01	0.341535196	MALE	68
TCGA-FD-A3SS-01	0.359840608	MALE	66
TCGA-G2-A3VY-01	0.417616497	MALE	66
TCGA-GC-A3BM-01	0.272126172	MALE	70
TCGA-GC-A3OO-01	0.382382659	MALE	79
TCGA-GC-A3RC-01	0.499465362	MALE	59
TCGA-GC-A3RD-01	0.291003605	FEMALE	83
TCGA-GC-A3WC-01	0.320144384	FEMALE	80
TCGA-GV-A3QF-01	0.321651286	MALE	79
TCGA-K4-A3WS-01	0.296544226	MALE	66
TCGA-K4-A3WV-01	0.218196287	FEMALE	77
TCGA-BT-A42B-01	0.418425442		
TCGA-BT-A42C-01	0.250066134		
TCGA-DK-A3WW-01	0.169430073	MALE	57
TCGA-FJ-A3Z7-01	0.391272561	MALE	76
TCGA-FJ-A3ZE-01	0.275393159	MALE	65
TCGA-FJ-A3ZF-01	0.509930604	MALE	73
TCGA-GC-A3YS-01	0.327964281	MALE	61
TCGA-GU-A42R-01	0.390144411	MALE	68
TCGA-GV-A3QK-01	0.400478856	FEMALE	56
TCGA-GV-A40E-01	0.402601615	MALE	75
TCGA-GV-A40G-01	0.543320916	MALE	77
TCGA-K4-A3WU-01	0.360708476	MALE	87
TCGA-BT-A42E-01	0.251956773	MALE	74
TCGA-BT-A42F-01	0.215783835		
TCGA-CF-A47S-01	0.379073632	MALE	41
TCGA-CF-A47T-01	0.273236694	FEMALE	58
TCGA-CF-A47V-01	0.509637858	MALE	52
TCGA-CF-A47W-01	0.325311473	MALE	42
TCGA-CF-A47X-01	0.319709364	MALE	60
TCGA-CF-A47Y-01	0.309315577	MALE	55
TCGA-FD-A43N-01	0.431306463	MALE	76
TCGA-FD-A43P-01	0.358293002	MALE	74
TCGA-FD-A43S-01	0.25190552	FEMALE	71
TCGA-FD-A43U-01	0.385535708	MALE	70
TCGA-FD-A43X-01	0.407963922	MALE	84
TCGA-GU-A42P-01	0.342877412	MALE	72
TCGA-GU-A42Q-01	0.289856602	MALE	67
TCGA-E7-A4IJ-01	0.403483262	MALE	56
TCGA-E7-A4XJ-01	0.568189645	MALE	66
TCGA-E7-A519-01	0.389225348	MALE	72
TCGA-E7-A541-01	0.439917586	MALE	66
TCGA-FD-A43Y-01	0.280464883	MALE	65
TCGA-FD-A5BR-01	0.315380551	MALE	57
TCGA-FD-A5BS-01	0.230323469	MALE	68
TCGA-FD-A5BT-01	0.281578508	MALE	84

TCGA-FD-A5BU-01	0.325284494	FEMALE	76
TCGA-FD-A5BV-01	0.432802709	FEMALE	47
TCGA-FD-A5BX-01	0.262435054	MALE	82
TCGA-FJ-A3Z9-01	0.242736843	MALE	72
TCGA-HQ-A2OF-01	0.250878338		
TCGA-HQ-A5ND-01	0.191072661		
TCGA-K4-A4AC-01	0.310620638	MALE	83
TCGA-K4-A54R-01	0.326862887	MALE	59
TCGA-MV-A51V-01	0.365668543	MALE	75
TCGA-CF-A5U8-01	0.289709378		
TCGA-CF-A5UA-01	0.348013242		
TCGA-CU-A5W6-01	0.334968323	MALE	70
TCGA-E7-A5KE-01	0.429060838	FEMALE	78
TCGA-E7-A5KF-01	0.311260213	MALE	67
TCGA-FD-A5BY-01	0.233685418	FEMALE	63
TCGA-FD-A5BZ-01	0.348559335	FEMALE	77
TCGA-FD-A5C0-01	0.336889891	MALE	61
TCGA-FD-A5C1-01	0.349201643	FEMALE	61
TCGA-HQ-A5NE-01	0.405704992		
TCGA-K4-A4AB-01	0.227540126	MALE	76
TCGA-K4-A5RJ-01	0.276371428	MALE	75
TCGA-LT-A5Z6-01	0.253948831	MALE	56
TCGA-BL-A5ZZ-01	0.366040634	FEMALE	80
TCGA-DK-A6AV-01	0.230917023	FEMALE	82
TCGA-DK-A6AW-01	0.34184658	MALE	69
TCGA-DK-A6B1-01	0.432167479	MALE	67
TCGA-DK-A6B2-01	0.419943969	MALE	70
TCGA-DK-A6B6-01	0.471918945	MALE	57
TCGA-E7-A677-01	0.476734666	MALE	81
TCGA-E7-A678-01	0.240856021	MALE	55
TCGA-FD-A62N-01	0.356380598	MALE	69
TCGA-FD-A62O-01	0.549878791	MALE	74
TCGA-FD-A62P-01	0.312662922	MALE	76
TCGA-FD-A62S-01	0.221671864	FEMALE	60
TCGA-FT-A61P-01	0.419432047	MALE	76
TCGA-LC-A66R-01	0.313206183	MALE	78
TCGA-R3-A69X-01	0.204555729		
TCGA-DK-A6B0-01	0.25109315	MALE	61
TCGA-DK-A6B5-01	0.252467542	MALE	45
TCGA-E5-A4TZ-01	0.389805994	MALE	64
TCGA-E5-A4U1-01	0.237910175	MALE	72
TCGA-GC-A6I1-01	0.241353498	FEMALE	90
TCGA-GD-A6C6-01	0.432484571	MALE	64
TCGA-K4-A6FZ-01	0.372604489	FEMALE	75
TCGA-K4-A6MB-01	0.29831698	MALE	64
TCGA-PQ-A6FI-01	0.281947515	MALE	70
TCGA-PQ-A6FN-01	0.210217956	FEMALE	78
TCGA-S5-A6DX-01	0.234622483		
TCGA-E7-A6ME-01	0.448530305		
TCGA-E7-A6MF-01	0.269329219		

TCGA-FD-A6TF-01	0.343511096		
TCGA-FD-A6TG-01	0.251981934	MALE	73
TCGA-FD-A6TH-01	0.362489764	MALE	63
TCGA-FD-A6TI-01	0.473111726	MALE	73
TCGA-GD-A76B-01	0.315674507		
TCGA-GU-A763-01	0.317263109		
TCGA-GU-A766-01	0.351586841		
TCGA-GU-A767-01	0.295827769		
TCGA-CU-A72E-01	0.239172039	MALE	76
TCGA-E7-A7DV-01	0.285403451	MALE	44
TCGA-FD-A6TA-01	0.243410681	MALE	58
TCGA-FD-A6TB-01	0.349312034		
TCGA-FD-A6TC-01	0.291343803	FEMALE	79
TCGA-FD-A6TD-01	0.396644441	MALE	77
TCGA-FD-A6TE-01	0.506226147	MALE	54
TCGA-FD-A6TK-01	0.330968721		
TCGA-GU-A762-01	0.408046769		
TCGA-GV-A6ZA-01	0.245071569	MALE	54
TCGA-E7-A7XN-01	0.20029255		
TCGA-K4-A83P-01	0.278202764	MALE	77
TCGA-LT-A8JT-01	0.485622735		
TCGA-XF-A8HB-01	0.186059172		
TCGA-XF-A8HC-01	0.293126874		
TCGA-XF-A8HD-01	0.376578826	MALE	77
TCGA-XF-A8HE-01	0.265557736	MALE	47
TCGA-XF-A8HF-01	0.293067485	MALE	80
TCGA-XF-A8HG-01	0.578721632	MALE	69
TCGA-YC-A89H-01	0.38297901		
TCGA-2F-A9KO-01	0.310637744		
TCGA-2F-A9KP-01	0.398608883		
TCGA-2F-A9KQ-01	0.238761247		
TCGA-2F-A9KR-01	0.252574791		
TCGA-2F-A9KT-01	0.380976591		
TCGA-2F-A9KW-01	0.363615228		
TCGA-S5-AA26-01	0.197259132		
TCGA-XF-A8HH-01	0.42276298		
TCGA-XF-A8HI-01	0.309016627		
TCGA-YC-A8S6-01	0.272609853	MALE	71
TCGA-YF-AA3L-01	0.538425364		
TCGA-G2-AA3B-01	0.424430981		
TCGA-G2-AA3C-01	0.322579566		
TCGA-GU-AATO-01	0.327739102		
TCGA-XF-A9SH-01	0.263101922		
TCGA-XF-A9SI-01	0.206137526		
TCGA-XF-A9SX-01	0.380451928		
TCGA-XF-A9SZ-01	0.251270203		
TCGA-XF-A9T0-01	0.276630933		
TCGA-XF-A9T4-01	0.3669131		
TCGA-YC-A9TC-01	0.189000639		
TCGA-BL-A0C8-01	0.431614254	MALE	73

TCGA-BL-A13J-01	0.304261344	MALE	65
TCGA-BT-A0S7-01	0.220930013	MALE	75
TCGA-BT-A0YX-01	0.331007838	FEMALE	70
TCGA-C4-A0EZ-01	0.40057534	FEMALE	69
TCGA-C4-A0F0-01	0.252180973	MALE	60
TCGA-C4-A0F1-01	0.197079965	MALE	71
TCGA-C4-A0F6-01	0.380639945	FEMALE	82
TCGA-C4-A0F7-01	0.394800351	MALE	77
TCGA-CU-A0YN-01	0.376356394	MALE	60
TCGA-CU-A0YO-01	0.275643612	MALE	84
TCGA-CU-A0YR-01	0.317509298	MALE	83
TCGA-BL-A13J-11A	0.253117293	MALE	65
TCGA-CU-A0YN-11A	0.251131786	MALE	60
TCGA-CU-A0YR-11A	0.256807657	MALE	83
TCGA-GD-A3OP-11A	0.282027018	FEMALE	84
TCGA-GD-A3OQ-11A	0.202470625	MALE	48
TCGA-K4-A54R-11A	0.294095769	MALE	59
TCGA-K4-A5RI-11A	0.253546375		
TCGA-GC-A6I3-11A	0.274181338		
TCGA-BT-A20J-11A	0.281045812	MALE	75
TCGA-BT-A20N-11A	0.269387489	MALE	72
TCGA-BT-A20P-11A	0.271889922	FEMALE	81
TCGA-BT-A20U-11A	0.238268271	FEMALE	70
TCGA-BT-A20V-11A	0.291620439	FEMALE	59
TCGA-BT-A20W-11A	0.278405246	MALE	71
TCGA-BT-A20R-11A	0.245385837	FEMALE	79
TCGA-BT-A20X-11A	0.261422275	MALE	56
TCGA-GD-A2C5-11A	0.269931042	FEMALE	53
TCGA-BT-A2LA-11A	0.220842477	MALE	54
TCGA-GC-A3BM-11A	0.262187777	MALE	70
TCGA-GC-A3WC-11A	0.254746275	FEMALE	80
TCGA-K4-A3WV-11A	0.285561778	FEMALE	77

Race Category	Neoplasm Histologic Grade	Neoplasm Disease Stage Am	Patient Sm
WHITE	High Grade	Stage III	Current sm
ASIAN	High Grade		Lifelong No
ASIAN	High Grade		Lifelong No
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage II	Current ref
	High Grade	Stage III	Current sm
WHITE	High Grade	Stage III	
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Lifelong No
BLACK OR AFRICAN AMERICAN	High Grade	Stage IV	
WHITE	High Grade	Stage II	
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	
ASIAN	High Grade		Lifelong No
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage IV	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current sm
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current sm
BLACK OR AFRICAN AMERICAN	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage II	Current sm
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage III	Current sm
WHITE	High Grade	Stage III	Lifelong No
[Not Evaluated]	Low Grade		Current ref
WHITE	High Grade	Stage IV	Lifelong No
ASIAN	Low Grade	Stage II	Lifelong No

ASIAN	Low Grade	Stage II	Current sm
ASIAN	Low Grade	Stage II	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage II	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage II	Current sm
BLACK OR AFRICAN AMERICAN	High Grade	Stage III	Current ref
WHITE	High Grade	Stage II	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current sm
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
ASIAN	Low Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage II	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage IV	[Unknown]
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage II	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current Ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	Low Grade	Stage II	Lifelong No
WHITE	Low Grade	Stage II	Lifelong No
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	Current ref

WHITE	High Grade	Stage IV	Current Ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current ref
ASIAN	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current sm
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current ref
	High Grade	Stage III	Current ref
	High Grade	Stage II	Current ref
	High Grade		
	High Grade		
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current ref
	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Current Ref
	High Grade		
ASIAN	Low Grade	Stage II	Lifelong No
ASIAN	Low Grade	Stage II	Lifelong No
ASIAN	High Grade	Stage II	Lifelong No
ASIAN	Low Grade	Stage II	Current sm
ASIAN	Low Grade	Stage II	Current sm
ASIAN	Low Grade	Stage II	Lifelong No
WHITE	High Grade	Stage III	Current sm
BLACK OR AFRICAN AMERICAN	High Grade	Stage II	Current ref
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current sm
ASIAN	High Grade	Stage II	Current sm
ASIAN	High Grade	Stage II	Current sm
WHITE	High Grade	Stage II	Current sm
WHITE	High Grade	Stage II	[Unknown]
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Current ref

BLACK OR AFRICAN AMERICAN	High Grade	Stage II	Current sm
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage I	Current ref
	High Grade		
	High Grade		
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	[Unknown]
WHITE	High Grade	Stage III	Lifelong No
	Low Grade		
	Low Grade		
WHITE	High Grade	Stage III	Current sm
ASIAN	High Grade	Stage II	Lifelong No
ASIAN	Low Grade	Stage II	Current sm
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage III	Lifelong No
	High Grade		
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage III	Current ref
ASIAN	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage II	Current ref
ASIAN	Low Grade	Stage II	Lifelong No
ASIAN	Low Grade	Stage III	Current sm
ASIAN	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage II	Current ref
BLACK OR AFRICAN AMERICAN	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current ref
	High Grade		
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	Current ref
[Unknown]	High Grade	Stage II	Current ref
WHITE	High Grade	Stage II	Lifelong No
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage IV	Current Ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage III	Current ref
	High Grade		
	High Grade		
	Low Grade		

	High Grade		
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	Current Ref
	High Grade		
	High Grade		
	High Grade		
	High Grade		
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	[Unknown]
[Not Evaluated]	High Grade	Stage IV	Lifelong No
	High Grade		
	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage IV	Current sm
	High Grade		
	High Grade		
WHITE	High Grade	Stage I	Current sm
	High Grade		
WHITE	Low Grade	Stage IV	Current ref
	High Grade		
	High Grade		
	High Grade		
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Current ref
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
WHITE	High Grade	Stage II	Current Ref
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
	High Grade		
WHITE	High Grade	Stage I	Current ref

WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage III	Lifelong No
	High Grade	Stage IV	Current sm
	High Grade	Stage II	Current sm
	High Grade	Stage III	Current sm
	High Grade	Stage III	Current sm
	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage III	Current sm
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage IV	[Unknown]
WHITE	High Grade	Stage IV	Current sm
WHITE	High Grade	Stage II	[Unknown]
WHITE	High Grade	Stage II	Current ref
	High Grade	Stage III	Current sm
WHITE	High Grade	Stage III	Lifelong No
WHITE	High Grade	Stage III	Lifelong No
BLACK OR AFRICAN AMERICAN	High Grade	Stage IV	
WHITE	High Grade	Stage II	
WHITE	High Grade	Stage IV	Lifelong No
WHITE	High Grade	Stage IV	
WHITE	High Grade	Stage IV	Current ref
WHITE	High Grade	Stage III	Current ref
WHITE	High Grade	Stage II	Current ref
WHITE	High Grade	Stage III	Current ref
	High Grade	Stage II	Current ref

Disease Fre	Disease Fre	Overall Sur	Overall Survival Status
		7.33	DECEASED
0.46	DiseaseFre	0.46	LIVING
0.43	DiseaseFre	0.43	LIVING
2.53	DiseaseFre	2.53	LIVING
		2.14	DECEASED
31.4	DiseaseFre	31.4	LIVING
3.68	Recurred/P	18.4	LIVING
		19	LIVING
8.9	DiseaseFre	8.9	LIVING
		131	LIVING
		112	LIVING
6.93	Recurred/P	16.1	LIVING
17.4	Recurred/P	17.6	LIVING
		15.6	LIVING
		19	DECEASED
		26.1	DECEASED
		12.2	DECEASED
		17.9	DECEASED
		19.5	DECEASED
		14.9	DECEASED
		8.64	DECEASED
		5.06	DECEASED
		8.34	DECEASED
		5.06	DECEASED
		8.25	DECEASED
0.69	DiseaseFre	0.69	LIVING
10.6	DiseaseFre	10.6	LIVING
5.35	Recurred/P	7.79	DECEASED
22.3	Recurred/P	22.9	DECEASED
29.9	Recurred/P		DECEASED
35.2	DiseaseFre	35.2	LIVING
29.5	Recurred/P	33	DECEASED
7.72	DiseaseFre	7.72	LIVING
1.51	DiseaseFre	1.51	LIVING
6.73	DiseaseFre	6.73	LIVING
17.1	DiseaseFre	17.1	LIVING
		16.2	DECEASED
			DECEASED
87.3	DiseaseFre	87.3	LIVING
61.9	DiseaseFre	61.9	LIVING
5.68	DiseaseFre	5.68	LIVING
3.45	Recurred/P	26.9	DECEASED
43.6	DiseaseFre	43.6	LIVING
12.9	DiseaseFre	12.9	LIVING
11.8	Recurred/P		DECEASED
		3.25	DECEASED
	DiseaseFre		LIVING
18.2	Recurred/P	20.5	DECEASED
0	DiseaseFre	0	LIVING

0	DiseaseFre	0	LIVING
0	DiseaseFre	0	LIVING
		13.6	DECEASED
6.6	Recurred/P		DECEASED
		8.21	DECEASED
8.21	Recurred/P	17.7	DECEASED
21.3	DiseaseFre	21.3	LIVING
19.1	Recurred/P		DECEASED
		8.94	DECEASED
6.87	DiseaseFre	6.87	LIVING
		4.01	DECEASED
12.4	DiseaseFre	12.4	LIVING
7.03	DiseaseFre	7.03	LIVING
		20.1	DECEASED
2.23	DiseaseFre	2.23	LIVING
6.41	DiseaseFre	6.41	LIVING
6.14	DiseaseFre	6.14	LIVING
3.65	Recurred/P	6.73	DECEASED
0	DiseaseFre	0	LIVING
5.45	DiseaseFre	5.45	LIVING
124	DiseaseFre	124	LIVING
2.2	DiseaseFre	2.2	LIVING
50.2	DiseaseFre	50.2	LIVING
6.96	Recurred/P	9.66	DECEASED
11.4	Recurred/P	22.5	DECEASED
15.9	DiseaseFre	15.9	LIVING
44	Recurred/P		DECEASED
5.68	DiseaseFre	5.68	LIVING
		4.66	DECEASED
12.8	DiseaseFre	12.8	LIVING
		9.95	DECEASED
4.5	DiseaseFre	4.5	LIVING
2.1	DiseaseFre	2.1	LIVING
3.12	DiseaseFre	3.12	LIVING
16.4	Recurred/P		DECEASED
4.4	Recurred/P	14.3	DECEASED
2.76	Recurred/P		DECEASED
4.11	Recurred/P	8.48	DECEASED
4.14	DiseaseFre	4.14	LIVING
5.19	DiseaseFre	5.19	LIVING
12.2	Recurred/P		DECEASED
9.95	Recurred/P	10.6	DECEASED
141	DiseaseFre	141	LIVING
48	DiseaseFre	48	LIVING
17.5	Recurred/P	18	DECEASED
12.1	Recurred/P		DECEASED
0.1	DiseaseFre	0.1	LIVING
18	Recurred/P	24.3	DECEASED
8.54	Recurred/P		DECEASED
4.73	Recurred/P		DECEASED

3.61	Recurred/P		DECEASED
4.7	Recurred/P	5.52	DECEASED
13.2	DiseaseFre	13.2	LIVING
36.9	Recurred/P	46.8	DECEASED
12.2	Recurred/P	19.8	DECEASED
10.2	Recurred/P	12.8	DECEASED
0.85	DiseaseFre	0.85	LIVING
2.89	DiseaseFre	2.89	LIVING
15.8	DiseaseFre	15.8	LIVING
15.9	DiseaseFre	15.9	LIVING
14.1	DiseaseFre	14.1	LIVING
17.7	DiseaseFre	17.7	LIVING
		20.3	DECEASED
8.74	DiseaseFre	8.74	LIVING
4.66	DiseaseFre	4.66	LIVING
8.28	DiseaseFre	8.28	LIVING
15.2	DiseaseFre	15.2	LIVING
3.48	DiseaseFre	3.48	LIVING
4.07	DiseaseFre	4.07	LIVING
1.12	DiseaseFre	1.12	LIVING
2.99	Recurred/P		DECEASED
6.67	DiseaseFre	6.67	LIVING
7.46	Recurred/P	8.57	DECEASED
19.1	DiseaseFre	19.1	LIVING
3.45	DiseaseFre	3.45	LIVING
9.99	DiseaseFre	9.99	LIVING
0.13	DiseaseFre	0.13	LIVING
0.07	DiseaseFre	0.07	LIVING
0.1	DiseaseFre	0.1	LIVING
0.07	DiseaseFre	0.07	LIVING
0.1	DiseaseFre	0.1	LIVING
0.1	DiseaseFre	0.1	LIVING
23	DiseaseFre	23	LIVING
14.9	DiseaseFre	14.9	LIVING
14.9	DiseaseFre	14.9	LIVING
14.9	DiseaseFre	14.9	LIVING
3.61	DiseaseFre	3.61	LIVING
		10.9	DECEASED
			DECEASED
0.49	DiseaseFre	0.49	LIVING
		2.23	DECEASED
0	DiseaseFre	0	LIVING
0.59	DiseaseFre	0.59	LIVING
12.1	DiseaseFre	12.1	LIVING
13.4	DiseaseFre	13.4	LIVING
37.1	DiseaseFre	37.1	LIVING
		11	DECEASED

6.87	DiseaseFre	6.87	LIVING
4.4	Recurred/P	5.35	DECEASED
3.25	Recurred/P	5.68	DECEASED
7.13	DiseaseFre	7.13	LIVING
5.58	Recurred/P		DECEASED
10.5	DiseaseFre	10.5	LIVING
13.5	DiseaseFre	13.5	LIVING
		1.84	DECEASED
0.56	DiseaseFre	0.56	LIVING
0.66	DiseaseFre	0.66	LIVING
6.83	DiseaseFre	6.83	LIVING
17.5	Recurred/P	27.4	DECEASED
0.89	Recurred/P		DECEASED
49.6	DiseaseFre	49.6	LIVING
2.5	DiseaseFre	2.5	LIVING
1.74	DiseaseFre	1.74	LIVING
3.45	DiseaseFre	3.45	LIVING
7.95	Recurred/P		DECEASED
64.1	DiseaseFre	64.1	LIVING
48.5	DiseaseFre	48.5	LIVING
67.3	DiseaseFre	67.3	LIVING
8.8	Recurred/P		DECEASED
21.2	DiseaseFre	21.2	LIVING
1.38	DiseaseFre	1.38	LIVING
0.82	DiseaseFre	0.82	LIVING
2.66	DiseaseFre	2.66	LIVING
4.83	DiseaseFre	4.83	LIVING
		6.27	DECEASED
6.37	DiseaseFre	6.37	LIVING
6.47	DiseaseFre	6.47	LIVING
6.34	DiseaseFre	6.34	LIVING
64.6	DiseaseFre	64.6	LIVING
32.3	DiseaseFre	32.3	LIVING
14.3	Recurred/P	15.3	DECEASED
23.2	Recurred/P		DECEASED
2.66	DiseaseFre	2.66	LIVING
2.2	DiseaseFre	2.2	LIVING
1.81	DiseaseFre	1.81	LIVING
4.27	DiseaseFre	4.27	LIVING
12.2	DiseaseFre	12.2	LIVING
16.7	DiseaseFre	16.7	LIVING

2.66	DiseaseFre	2.66	LIVING
2.2	DiseaseFre	2.2	LIVING
4.01	DiseaseFre	4.01	LIVING
9.33	DiseaseFre	9.33	LIVING
1.22	DiseaseFre	1.22	LIVING
50.6	DiseaseFre	50.6	LIVING
9.3	DiseaseFre	9.3	LIVING
6.7	DiseaseFre	6.7	LIVING
4.11	DiseaseFre	4.11	LIVING
11.5	DiseaseFre	11.5	LIVING
2.3	Recurred/P		DECEASED
88.9	DiseaseFre	88.9	LIVING
97.4	DiseaseFre	97.4	LIVING
56.1	DiseaseFre	56.1	LIVING
13.1	Recurred/P	15.3	DECEASED
4.83	DiseaseFre	4.83	LIVING
26	Recurred/P		DECEASED

		2.66	DECEASED
		6.57	DECEASED
10.7	Recurred/P	13.1	DECEASED
		8.97	DECEASED
			DECEASED
			DECEASED
23	DiseaseFre	23	LIVING
		2.04	DECEASED
		12.9	DECEASED
		4.89	DECEASED
7.19	Recurred/P	7.19	LIVING
		2.66	DECEASED
		12.9	DECEASED
7.19	Recurred/P	7.19	LIVING
2.1	DiseaseFre	2.1	LIVING
3.12	DiseaseFre	3.12	LIVING
10.5	DiseaseFre	10.5	LIVING
		19	DECEASED
		26.1	DECEASED
		17.9	DECEASED
		8.64	DECEASED
		5.06	DECEASED
		8.34	DECEASED
		5.06	DECEASED
		8.25	DECEASED
7.72	DiseaseFre	7.72	LIVING
17.1	DiseaseFre	17.1	LIVING
2.89	DiseaseFre	2.89	LIVING
17.7	DiseaseFre	17.7	LIVING
4.66	DiseaseFre	4.66	LIVING

Figure Legends

Figure S1. Flow chart of study identification and the number of articles in specific years, countries and groups.

A, Flow chart shows study selection procedure. Twenty-one studies were included in the meta-analysis. B, The distribution of the number of literatures in the electronic database from 2001 to 2014. C, The distribution of the number of literatures in different countries. D, The distribution of the number of literatures in different group.

Figure S2. Publication bias, sensitivity analyses, summary receiver operating characteristics (SROC) estimation for the relationship between RASSF1A gene promoter methylation and bladder cancer risk.

A, Funnel plot from 21 studies comparing colorectal cancer with normal controls; B, Sensitivity analysis of the summary odds ratio coefficients on the relationship between RASSF1A gene promoter methylation and bladder cancer risk. C, SROC of RASSF1A promoter region methylation test in bladder cancer risk.

Figure S1

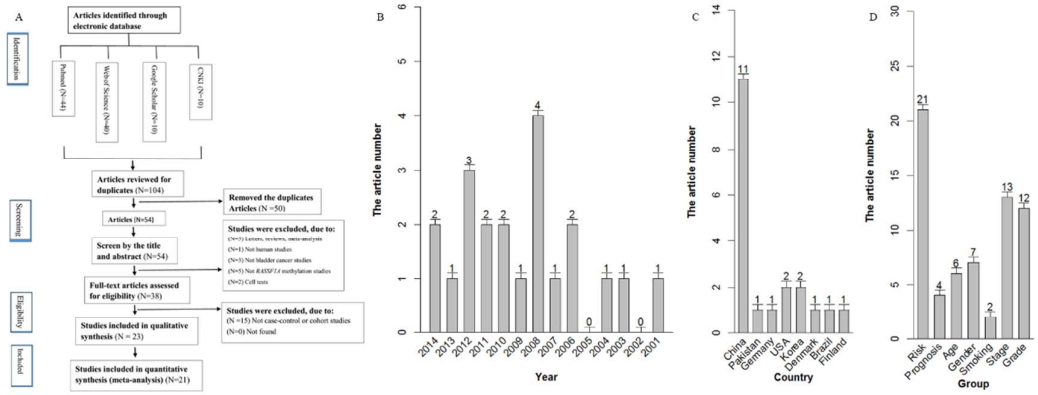

Figure S2