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Germline Prognostic Markers for Urinary Bladder Cancer: Obstacles and Opportunities

David W Chang, Jian Gu, and Xifeng Wu*

Department of Epidemiology, Unit 1340, The University of Texas M. D. Anderson Cancer Center, 1155 Pressler Blvd., Houston, TX 77030

Abstract

Urinary bladder cancer is a heterogeneous disease with diverse genetic and environmental risk factors that can influence disease risk or clinical course for recurrence, progression, and survival. Therefore, identification of these factors is paramount for disease prevention and optimal clinical management of bladder cancer patients. Of particular interest is the need to identify molecular biomarkers that can give accurate assessment of tumor biological potential and to predict treatment response. Recent advances in molecular biology, cytogenetic and genomic research have spurred discovery efforts for novel genetic, epigenetic, and proteomic biomarkers that are prognostic for cancer. This review focuses on some of the important germline polymorphisms found to be correlated with clinical outcomes in bladder cancer. So far the majority of the identified candidate loci was based on prior knowledge of pathogenesis and had not been validated for clinical applications. The future challenges are to analyze the wealth of information from whole-genome studies, to understand the underlying biological mechanisms of these associations, the network of gene-gene and gene-environment interactions, and to apply these markers for the identification of high-risk population for targeted, personalized therapy.

Keywords

Polymorphisms; prognostic markers; clinical outcome; bladder cancer

Introduction

Urinary bladder cancer (UBC) is one of the most common malignancies among men in Western countries accounting for an estimated 70,500 total new cases (52,700 men) and 14,700 new deaths (10,400 men) in the United States in 2010 [1]. The men-to-women incidence ratio is about four to one. Cigarette smoking is the predominant risk factor followed by occupational exposure to aromatic amines and other industrial chemicals [2,3], which may partly explain the higher prevalence among men than women. Nevertheless only a small percentage of the individuals with environmental exposure develop UBC, suggesting that genetic predisposition may also contribute to the disease. Indeed, a large-scale classical twin study estimated that inherited genetic predisposition may contribute to one third of the risk, ranked fourth among all sporadic cancers [4]. In addition, candidate gene analysis of genetic polymorphisms has identified *GSTM1*-null and *NAT2* slow acetylator genotypes as

*Corresponding Author: Xifeng Wu, MD, PhD, Department of Epidemiology, Unit 1340, The University of Texas M. D. Anderson Cancer Center, 1155 Pressler Blvd., Houston, TX 77030; phone: 713-745-2485; fax: 713-792-4657; xwu@mdanderson.org.

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predisposing risk factors [5]. Recent genome-wide association study (GWAS) has identified at least 8 novel susceptibility loci for UBC, including single nucleotide polymorphisms (SNPs) in 8q24.21 (near *MYC*), 3q26 (*TP63*), 8q24.3 (*PSCA*), 5p15.33 (*TERT-CLPTM1L*), 4p16.3 (*MEM129-TACC3-FGFR3*), 22q13.1 (*CBX6*, *APOBEC3A*), 19q12 (*CCNE1*), and 2q37.1 (*UGT1A cluster*) [6–8]. In addition, there was a strong gene-smoking interaction between NAT2 slow acetylator genotype and smoking, as well as between GSTM1-null genotype and smoking [8]. These data support the notion of low-penetrance genetic polymorphisms interacting with environmental factors to influence the risk for development of UBC.

The majority of UBC are urothelial cell carcinomas (UCC) in Western countries, which include 90% or more of the cases, with the remaining comprising squamous cell carcinoma, adenocarcinoma, and other rare undifferentiated subtypes [9]. The natural history of UBC is complex and may involve divergent biological pathways, but two general forms are recognized: non-muscle invasive bladder cancer (NMIBC) and muscle-invasive form (MIBC). At presentation, 75–80% of UBC cases are NMIBC and display a relatively indolent, low grade tumor [10]. However, this “benign” phenotype betrays the relatively high recurrence rate. Indeed, 50–70% of the patients treated for NMIBC have recurrent disease and 5–20% progress to a more advanced stage within 5 years [11,12]. Based on the tumor, node, and metastasis (TNM) staging system, NMIBC can be further classified as Ta, T1, and Tis types. The papillary form (Ta) has a tendency to recur locally but rarely invades the basement membrane or metastasizes. The other types, carcinoma in situ (Tis or *CIS*) and T1 (invading the sub-epithelial connective tissue) are high-grade tumors that have increased potential for invasion and/or metastasis [13]. Standard treatment for NMIBC includes transurethral resection (TUR) with or without intravesical immunotherapy [14]. Instillation of intravesicle Bacille Calmette-Guerin (BCG) therapy has been found to significantly reduce the recurrence and progression of NMIBC. Nevertheless, about one third of these responders later displayed tumor recurrence or progressed to muscle-invasive cancer [15,16]. The ability to identify patients who are at high risk for recurrence or progression will have tremendous clinical value to benefit patient treatment, which may allow a more tailored approach to the clinical management of UBC by providing individualized treatment that is most likely to be effective.

Currently, clinicopathological variables (including age, clinical stage, tumor grade, tumor size, and histology) remain the most common prognostic predictors in the clinical decision making for UBC patients. The use of nomograms, which incorporate multiple clinicopathological variables, has increased predictive accuracy, especially for cancer recurrence after cystectomy [17]. However, the predictive accuracy of clinical outcome based on pathological variables is not entirely satisfactory, particularly for patients treated with BCG therapy. Therefore, more objective biomarkers are needed to complement the conventional prognostic methods for more accurate assessment of clinical outcome and better management of disease. Not unlike other cancers, UBC is a polygenic disease which involves multiple biological pathways. Recent studies have identified several genetic and biological alterations with an extensive repertoire of candidate genes and pathways that have been implicated in UBC development and progression, including anti-apoptotic genes, cell cycle regulators, various immune, nuclear, and proliferative markers, and cellular growth factors[9]. Changes in DNA ploidy and microsatellite instability have been detected in the voided urine of bladder cancer patients with recurrent tumors and are reported to be potential prognostic markers for recurrence [18–21]. However, these genetic and proteins markers may indicate changes in disease state after the patients’ tumor has already recurred or progressed and may not explain the variability in the clinical course of NMIBC patients.

Previous reviews on bladder cancer have extensively discussed the various classes of prognostic biomarkers, which are mostly tumor based. Blood-based prognostic biomarkers such as SNPs have been less well studied. This review summarizes the current state of literature regarding SNPs and their association with UBC recurrence, progression, treatment response, and survival. The SNPs examined are either potentially functional SNPs (affecting gene expression or protein function) or tagSNPs. A tagSNP represents a particular chromosomal region with high linkage disequilibrium. TagSNPs have been used extensively in GWAS for non-biased, efficient coverage of genome variations. It should be noted that hitherto very few of the candidate biomarkers have been confirmed or validated in independent and prospective studies.

Candidate genes and pathways associated with UBC recurrence and survival

The prime candidates in cancer susceptibility studies are usually involved in carcinogenesis-related processes, including cell cycle, DNA repair, apoptosis, oxidative stress, and signal transduction pathways [22]. Naturally, these candidates are also investigated for clinical outcome studies. So far, genetic variants of several candidate genes and pathways have been associated with UBC recurrence, progression, and survival as summarized in Table 1. However, most of the candidate gene/pathway studies were of limited sample size and had not been validated in independent populations, which necessitate caution in interpretation.

TP53 and MDM2

The TP53 tumor suppressor located on chromosome 17p13 is one of the most well-studied genes in cancer, whose alterations have been implicated in 50% of the human malignancies. Inactivation of TP53 gene could promote tumorigenesis in human bladder cells and was correlated with poor survival in human tumors [23]. As “guardian of the genome”, TP53 encodes a transcription factor that is involved in cell cycle arrest, initiation of apoptosis, and DNA repair during response to DNA damage. Its antagonist is the MDM2 proto-oncogene whose protein product binds p53, inhibits its transcriptional activity, and targets it for proteasomal degradation. The p53 protein has already been extensively studied as an immunohistochemical marker for prognosis [21]. For genetic variations, two functional SNPs of TP53 (Arg72Pro) and MDM2 (SNP309) were shown to be significantly associated with UBC recurrence and survival in patients treated with cystectomy or chemotherapy [24,25]. In one report, Horikawa et al. found that patients carrying the variant allele of TP53 (Pro72) had lower risk of recurrence but poorer survival compared with the wildtype allele (Arg72)[24], while another study by Shinohara et al. found association between MDM2 genotype with treatment-specific survival in patients with muscle-invasive bladder cancer [25]. In addition, Sanchez-Carbayo and colleagues reported the association between the MDM2 genotype and early onset of tumors and overall survival in patients with muscle-invasive bladder cancer [26]. More large-scale studies of independent populations are needed to confirm some of these associations.

ERCC6, XPD, XPF, XPG, XRCC1, and MSH6

Genes of the DNA repair pathway may be involved in the pathogenesis, progression, and treatment response of cancer. Defects in DNA repair genes may lead to increased mutagenic events in the genome and greater susceptibility to cancer risk [27]. However, lower DNA repair capacity (DRC) may be associated with better treatment response to radiation or chemotherapy since tumors cells would have lower potential to repair DNA damages generated by genotoxic agents [28–30]. There are at least four different pathways involved in DNA repair, including base excision repair (BER), nucleotide excision repair (NER), double-strand-break repair (DSB), and mismatch repair (MMR). Each pathway acts on

distinct types of DNA damage and involves multiple factors. As shown in Table 1, genetic variants in several DNA repair genes were found to be associated with recurrence or survival. A functional SNP in ERCC6 (Met109Val), a gene of the NER pathway, was associated with recurrence but not progression in NMIBC patients [31]. In addition, increased number of high risk variants in NER pathway was associated with higher recurrence rates and shorter recurrence-free survival time [31]. In a study involving 311 UBC patients, Sanyal et al., reported that several genes of the DNA repair pathway, including XPD, XPG, MSH6, and OGG1 were associated with disease stage, progression, or survival [32]. In particular, the variant allele of XPD (Lys751Gln) correlated with reduced risk of progression and increased survival in UBC patients, while variant allele of OGG1, a BER gene, was associated with increased death following radiotherapy. A study by Sakano et al. has found an association between a XPG variant (ASP1104His) and higher tumor stage (Ta vs. T1), but not with overall or cancer-specific survival [33]. In another study involving 78 patients with MIBC, Sakano et al. reported that the combined genotypes of XPD (Lys751Gln) and XRCC1 (Arg399Gln) were associated with disease-specific survival [34]. Lastly, in a two-stage analysis of 364 UBC cases and 400 controls from a Chinese population, Wang et al. found genetic variants of *XPF* gene were associated with UBC risk and recurrence-free survival [35]. Importantly, the authors identified the causal link of a significant tagSNP (rs744154) with a functional polymorphism (−357A>C) in the XPF promoter, which affected transcription factor binding and gene expression. Taken together, genetic variations of the DNA repair pathway might influence clinical outcome of recurrence, treatment response, and survival. Although most of the findings were not robust and have not been replicated, they yielded clues which might point to the underlying mechanism of pathogenesis allowing better design for future therapy.

EGFR, TGFβ1, and TGFβR

Growth factor signaling plays an essential role in the growth, differentiation, and survival of the organism. However, cancer cells hijack this developmental mechanism to gain nonproductive growth advantage over normal cells. Epidermal growth factor (EGF) and transforming growth factor beta (TGF-β) signaling generate proliferative and anti-proliferative signals which can affect tumor development and progression [36–38]. These pathways can affect cell proliferation, differentiation, apoptosis, tumor metastasis, angiogenesis, and immune response. In a large case-control study of 857 UBC cases and 1191 controls from New Hampshire, Mason et al. found genetic variants of EGFR were associated with increased UBC risk [40]. Moreover, several of the variants also correlated with overall survival in the cases. One of the EGFR SNPs (R521K; rs11543848) is a known functional SNP that alters ligand binding and transmembrane signaling [39]. The variant allele of this SNP decreases receptor tyrosine kinase activation and was associated with increased UBC survival [40]. In another large case-control study of 1157 UBC cases and 1157 matched controls, Castillejo et al. examined the association of three SNPs in *TGFβ1* and three SNPs and one insertion/deletion polymorphism of *TGFβR1* with UBC risk and clinical outcome (tumor relapse, progression, and survival) [41]. They found the *TGFβ1* and *TGFβR1* SNPs were not associated with overall disease risk or disease outcome. However, in stratified analysis among patients with MIBC, rs868 and a linked SNP (rs334358) of *TGFβR1* were significantly associated with survival. These data need to be validated in independent populations; nevertheless they suggest that genetic variations of growth signaling pathways could influence UBC risk and clinical outcome.

PI3K-AKT-mTOR and Sonic hedgehog pathways

The phosphoinositide-3-kinase (PI3K) with its downstream effector and target, AKT serine-threonine kinase and mammalian target of rapamycin (mTOR), is an important cell signaling pathway regulating growth and survival and may impact tumorigenesis and clinical

response. Somatic alterations of all three genes and PTEN, a tumor suppressor and negative regulator of the pathway, have been found in UBC tumors (42–44) and could influence tumor behavior and disease outcome. In a recent study of 319 MIBC patients, Chen et al. found that three SNPs from this pathway (AKT2: rs3730050, PIK3R1: rs10515074, and RAPTOR: rs9906827) were significantly associated with survival [45]. Moreover, there was a combined effect of these three SNPs on survival with significant trend toward higher risk of death with increasing number of unfavorable genotypes.

Sonic hedgehog (Shh) is a developmental and stem cell pathway that has been implicated in several types of human cancers. Inherited or sporadic mutations of Shh components, such as PTCH receptor and SMO, were found in basal cell carcinoma and medulloblastoma [46]. Deletions of PTCH1, a putative tumor suppressor on chromosome 9q22, have been discovered in UCC [47,48]. In a study of 419 NMIBC and 318 MIBC patients, Chen et al. [49] identified two SNPs that were associated with recurrence of NMIBC in patients treated with TUR only (SHH:rs1233560 and GLI2:rs11685068), which were replicated in 356 independent TUR-only NMIBC patients. Another two SNPs in GLI3 (rs6463089 and rs3801192) were significantly correlated with recurrence in NMIBC patients treated with BCG after adjusting for multiple comparisons. Three SNPs in GLI2 and SHH were associated with survival in MIBC patients; however, they were non-significant after adjustment for multiple comparisons. These results emphasize the potential utility of modifying cell signaling pathways to affect disease course and clinical outcome for bladder cancer.

IL-6, PPARG, and NFKB1

The premise that inflammation may be a basis for cancer has been put forth since the days of Rudolf Virchow in the 19th century, who first proposed that the origin of cancer was at sites of chronic inflammation [50]. Since then, it has been firmly established that prolonged tissue injury and inflammatory response could contribute to the development and progression of various disease processes, including cancer. Chronic inflammation from sources such as urinary tract infections, indwelling catheter, and schistosomiasis has been associated with carcinoma of the bladder [51]. Our group has previously examined the association of several functional SNPs of the inflammation-related candidate genes in IL-6 (G-174C), IL-8 (T-251A), TNF (G-308A), and PPARG (Pro12Ala) with recurrence risk, progression, and survival [52]. We found that the variant *PPARG* genotype was associated with reduced recurrence risk among TUR only NMIBC patients. The variant *IL-6* genotype was associated with an increased recurrence risk in NMIBC patients receiving maintenance BCG, while the variant allele of the same SNP was associated with an increased progression risk in NMIBC patients, and an improved 5-year overall and disease-specific survival in MIBC patients. Recently, a group from Germany reported a genotype-phenotype correlation study of a functional, promoter insertion/deletion polymorphism in the *NFKB1* gene and its association with recurrence risk for NMIBC [53]. The investigators found that patients with the homozygous deletion genotype had higher recurrence risk than those with the homozygous insertion. As expected, the homozygous insertion genotype was associated with higher NFKB1 expression. The above results suggest that while prolonged inflammatory response might predispose to carcinogenesis, the elevated expression of immune genes could protect against tumor recurrence. Additional independent studies with larger sample size and more polymorphisms are needed to examine the genotypic and phenotypic correlation of inflammation genes and UBC clinical outcome.

CDKN2A, E-cadherin, and HIF-1 α

Other miscellaneous genes whose genetic variants were reported to be prognostic for UBC include those involved in cell cycle (*CDKN2A*), cell adhesion (*E-cadherin*), and hypoxia

response (*HIF-1*) pathways. *CDKN2A* is a unique gene locus that encodes two cell-cycle-regulating protein products, p16 and p14^{ARF}, by an alternative splicing mechanism. P16 is a cyclin dependent kinase (CDK) inhibitor which prevents cell cycle progression, while p14^{ARF} has been shown to interact with MDM2 and block MDM2-dependent degradation of p53 which is involved in cell cycle arrest and apoptosis after DNA damage. *CDKN2A* is frequently mutated or deleted in a wide variety of tumors including UCC [55], and is known to be an important tumor suppressor gene. In a hospital-based prospective study of 309 NMIBC patients, Sakano et al. examined the correlation of two 3'untranslated region (UTR) SNPs of *CDKN2A* (500C>G and 540C>T) with disease-specific survival [56]. They found that patients with the variant allele of either SNP had significantly shorter survival than those with the wildtype genotype. These SNPs did not correlate with tumor stage or disease progression.

E-cadherin (*CDH1* gene) is Ca²⁺-dependent homophilic cell adhesion molecule that is essential for the establishment and maintenance of cell junctions during development. Loss of E-cadherin expression can lead to derangement of tissue architecture, increased tumor invasiveness, and is a hallmark event of epithelial-to-mesenchymal transition (EMT) [21]. Our group previously investigated the association of a promoter SNP of *CDH1*(C-160A) and risk of UBC recurrence in a study of 274 Caucasian NMIBC cases [56]. We found that patients carrying at least one variant allele of this SNP had 32% reduction in recurrence risk and longer median recurrence-free survival time than those with the homozygous wild type genotype.

Tumor development often encounters low oxygen environment which requires induction of hypoxia response pathway. The presence of hypoxic lesions in solid tumors is often associated with more aggressive phenotype, resistance to treatment, and poor survival [57]. Nadaoka et al. examined the association of two functional hypoxia-inducible factor-1 (*HIF-1*) SNPs (Pro582Ser and Ala588Thr) with the incidence and progression of UCC in 219 Japanese cases and 464 healthy controls [58]. The investigators also evaluated the genotype-phenotype correlation between *HIF-1* genotypes and tissue expression of vascular endothelial growth factor (VEGF) and microvessel density in 73 cystectomy specimens. The results showed that the *HIF-1* genotype did not significantly influence the incidence or disease status. However, among patients who underwent radical cystectomy, those with a variant allele had significantly worse disease-free survival and disease-specific survival [58]. The variant *HIF-1* genotypes also correlated with higher VEGF expression with a borderline significance but were not associated with microvessel density. Taken together, there is biological support for the association of functional genetic variants in candidate genes involving cell cycle progression, cell-cell interaction, and hypoxic response with clinical outcome in UBC patients, although more studies are clearly needed for replication and validation and to explore the detailed biological mechanisms.

Genome-wide assessment of polymorphic loci for clinical outcome

Unlike candidate gene studies, whole-genome scan of genetic loci associated with clinical response and disease outcome presents an unbiased approach to identify novel prognostic factors without *a priori* knowledge or assumptions. The GWAS approach has been applied extensively to assess factors associated with cancer risk. Because of the requirement for large sample size of cases with more or less uniform clinical characteristics, the GWAS has not been adopted as widely for prognostic study. Nevertheless, the use of GWAS to study disease recurrence, overall survival, and treatment outcome will accelerate in the coming years. So far, a few GWAS have been applied to identify novel SNPs associated with survival in non-small cell lung cancer (NSCLC) [59,60], prostate cancer, [61], and breast cancer [62]. For the prostate cancer and breast cancer studies, there were no significant

associations identified suggesting the low risk effect of most common germline variants, which would require much larger sample size to detect. Alternatively, the common variants associated with survival may be specific to tumor subtype, disease stage, or treatment regimen, therefore, further stratification of the study population is necessary to detect the association. For the GWAS of clinical outcome in UBC, efforts are already underway and a report has been recently submitted for review by us and our collaborators¹.

MicroRNA as prognostic marker

MicroRNAs (MiRNAs) are a class of small noncoding RNAs that regulate gene expression by targeting mRNA transcripts for degradation or translational repression. The involvements of miRNA in normal cellular processes and in tumorigenesis have been researched extensively in recent years. As many as a third of the human genes are miRNA targets, and miRNA expression profiles have been used to classify human cancers [63,64]. Catto et al. have recently characterized the distinct alterations of miRNA expressions for high- and low-grade UBC [65]. They found that altered miRNA is common in UBC and occurs early during tumorigenesis. In high-grade tumor, there was upregulation of miR-21, which suppresses p53 function, while in low-grade tumor, several miRNAs were downregulated, particularly miR-99a/100. In addition, Dyrskjot et al. has performed genomic profiling of miRNAs and found miR-129 as being prognostic for UBC [66]. Our work has previously found that genetic variants of miRNA biosynthesis and miRNA genes were associated with increased UBC risk [67]. SNPs in miRNA binding sites, which can affect target gene expression, also could serve as predictors of cancer risk and clinical outcome [68]. These studies point to the potential utility of miRNA markers as prognostic indicators.

Pharmacogenetics of UBC clinical outcome

Genetic factors play an important role in variations to drug response [69]. In the past few decades, the influence of certain monogenetic traits, especially those involving drug metabolism, on the response to certain drug treatments have been extensively explored. These candidates include genes of the cytochrome P450 system (e.g. *CYP1A2*, *CYP2A6*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4*) [70], drug transporter (*OATP-C*) [71,72], and drug metabolism (*TPMT*, *UGT1A1*, *SULT1A1*, *DPYD*, *MTHFR*, and *TYMS* (thymidylate kinase) [73–77]. In cancer therapy, chemotherapeutic agents are characterized by a narrow therapeutic index and high toxicity. Genetic variations of these genes may affect protein function and/or protein level leading to altered drug metabolism and adverse clinical response. The involvement of these gene variations in the toxicity of various chemotherapeutic drugs have been reported for leukemias [73] and cancers of the colon, lung, breast, and prostate [77–81]. For UBC, there were little or no report of genetic variants of drug metabolic pathway associated with treatment response, although the *NAT2* slow acetylator and *GSTM1* null genotypes have been associated with increased risk of UBC [82]. Nevertheless, our previous study has found that SNPs in inflammation genes were associated with recurrence in NMIBC patients receiving BCG [52] and Sakano et al. reported that genetic variants in DNA repair genes might be prognostic factors in MIBC treated with radiation and platinum-based chemotherapy [34], suggesting that other cellular pathways could also impact the pharmacogenetics of drug treatment in UBC.

¹Malat N, Dinney C, Chanock S, et al. Inherited genetic prognostic factors in patients with non-muscle-invasive bladder cancer: results from a genome-wide assessment. Submitted.

Opportunities and challenges

High through-put genotyping and next-generation sequencing

Previous candidate gene and pathway studies have identified many potential genetic variants influencing clinical outcome of UBC patients. However, as mentioned earlier, most of these studies had limited sample size and required prior knowledge of genes and pathways associated with cancer development. Moreover, very few of these studies have been prospectively confirmed or replicated in independent populations so that the possibility of false discovery remains. The recent developments of new high-throughput technologies for genotyping and next generation sequencing have accelerated the pace of genomic research. Now hundreds of thousands to more than a million genetic variants could be queried simultaneously in a matter of days. However, most of the SNPs selected for whole-genome coverage are tagSNPs so that the real functional or causal SNP might be missed. The sample size requirement for GWAS of clinical outcomes is enormous, which necessitates multi-institutional collaborations. In addition, the current GWAS approach is designed to detect common variants with modest biological effects, and is likely to miss rare alleles with stronger association. Newer sequencing technologies allow the possibility to obtain whole-genome sequences *en masse* in a high-throughput manner, which will identify all rare alleles that might be associated with disease risk or clinical outcome [83]. The sequence reads are generated in massive parallel manner and assembled using computer software. The drawback of next generation sequencing is the current cost of these technologies which will prohibit large-scale sample analysis [22], although targeted resequencing of candidate genes and pathways are more feasible and have produced significant results [84].

Gene-gene and gene-environment interactions

The development of cancer is a complex and multifactorial process. For most cancers, it is unlikely that any one gene will assume the majority of the risk for carcinogenesis or effect for clinical response. Rather an interplay of genetic and environmental risk factors helps to determine the process from exposure to disease and finally to outcome. This is particularly relevant for tobacco-related cancers, where the exposure to carcinogens in cigarette smoke is the main driving force for tumorigenesis, which can be modified by polymorphisms of various cellular pathways [85,86]. Similarly, gene-gene interactions could affect the susceptibility risk of different cancers as seen in the metabolic genes in lung cancer [87], DNA repair genes in bladder cancer [88–90], estrogen metabolism genes in breast cancer [91], and angiogenic genes in prostate cancer [92]. So far, there are few reports of gene-gene or gene-environment interactions for the clinical outcomes of human cancers. Various statistical methods have been developed to analyze gene-gene and gene-environment interactions including multifactor dimensionality reduction (MDR) [91] and classification analysis of regression tree (CART) [93]. Recently, Gui et al. has developed a novel survival MDR method for detecting gene-gene interactions that can be applied to UBC prognosis [94]. The challenge now is how to use these and other methods to analyze large sets of data emerging from GWAS and to minimize false discoveries.

Translational applications

The ultimate goal of prognostic marker discovery is to apply it in clinical setting, whereby patients with high risk for beneficial and adverse outcome can be identified. Currently, the conventional histopathological characterization of disease stage and tumor grade allows only a gross stratification of clinical outcomes for UBC. The advantage of germline biomarkers is that they are more readily accessible compared to tumor biomarkers, and they are relatively stable unlike tumor biomarkers whose status could evolve during the course of disease. Despite the significant progress in the identification of germline biomarkers, none of them has been vigorously validated and the molecular basis of most candidate prognostic markers

remains unknown. The advent of high-throughput query of genome-wide information should enable the unbiased discovery of even more potential prognostic markers. The future task is to validate the most promising of these markers in larger sample size and in prospective clinical trials. Once confirmed, the genes hosting these germline biomarkers are potential targets for pharmaceutical intervention, which may form the basis for more directed, personalized medicine.

Conclusions

Bladder cancer is a multifactorial disease, whose initiation and progression involve a complex network of genes and pathways. The recent discoveries of germline prognostic markers and the advent of high-throughput technologies may facilitate efforts for improved prediction of response to therapy to guide targeted therapy. It is likely that no single marker will be sufficient for prognosis but a combination of markers from several genes or pathways may be required, which will necessitate an understanding of the underlying biological mechanism as well as the potential gene-gene and gene-environment interactions. The key to success is to identify which of these markers are important, so that they may be evaluated in future prospective clinical trials. This will require careful consideration of many caveats in study designs, independent validations, and sophisticated statistical methods to weed out false discoveries. Despite the challenges, substantial progress has already been made, and we should expect even more concerted efforts in the future to improve the prognosis and treatment of UBC patients.

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Table 1
Genetic variants of candidate genes and pathways associated with clinical outcomes in bladder cancer

Candidate pathway	Candidate gene	SNP position	rs no.	Outcome measured	Number of cases	Genetic model used	HR (95% CI) ^a	P value	Reference
Cell cycle, apoptosis	<i>TP53</i>	Arg72Pro	rs1042522	Recurrence	87	Recessive	0.36 (0.14–0.93)	0.035	24
	<i>TP53</i>	Arg72Pro	rs1042522	Survival	86	Recessive	2.76 (1.11–6.84)	0.019	24
Cell cycle	<i>MDM2</i>	309T>G	rs2870820	Survival	96	Dominant	0.57 (0.36–0.95)	0.031	25
	<i>MDM2</i>	309T>G	rs2870820	Survival	141 ^b	Dominant	Protective ^c	<0.05 ^d	26
	<i>CDKN2A</i>	540C>T	rs2811711	Survival	211	Dominant	3.3 (1.1–9.9)	0.03	55
				Survival	211	Dominant	3.3 (1.1–9.9)	0.03	55
DNA repair	<i>ERCC6</i>	Met1097Val	rs2228526	Recurrence	288	Dominant	1.54 (1.02–2.33)	<0.05	31
	<i>XPD</i>	Lys751Gln	rs13181	Survival	260	Dominant	0.6 (0.4–1.0)	0.04	32
	<i>XPD</i>	Lys751Gln	rs13181	Progression	201	Dominant	0.4 (0.2–1.0)	0.06	32
	<i>XPG</i>	Asp1104His	rs17655	pT1 stage	113	Dominant	4.9 (2.0–12.9)	<1 × 10 ⁻⁴	33
	<i>XPF</i>	–350A>C	rs6498486	Recurrence	117	Dominant	Increased risk ^c	0.0245 ^d	35
	<i>EGFR</i>	Arg521Lys	rs11543848	Survival	NA	Additive	Protective ^c	<0.05 ^d	40
Growth factor signaling	<i>TGFBR1</i>	3' UTR	rs868	Survival	92	Additive	3.0 (1.15–7.82)	3 × 10 ⁻³	41
	<i>TGFBR1</i>	Intron	rs334358	Survival	93	Additive	2.83 (1.09–7.34)	9 × 10 ⁻³	41
PI3K-AKT signaling	<i>AKT2</i>	Intron	rs3730050	Survival	302	Additive	1.68 (1.16–2.44)	4 × 10 ⁻⁴	45
	<i>PIK3R1</i>	Intron	rs10515074	Survival	300	Dominant	1.83 (1.24–2.69)	2 × 10 ⁻³	45
	<i>RAPTOR</i>	Intron	rs9906827	Survival	299	Dominant	0.55 (0.37–0.81)	2 × 10 ⁻³	45
Stem cell signaling (Sonic hedgehog)	<i>GLI2</i>	3' flanking	rs11685068	Recurrence	497 ^e	Dominant	2.07 (1.33–3.21)	1.3 × 10 ⁻³	49
	<i>SHH</i>	5' flanking	rs1233560	Recurrence	497 ^e	Additive	1.39 (1.14–1.70)	1.0 × 10 ⁻³	49
	<i>GLI3</i>	Intron	rs6463089	Recurrence	201 ^f	Dominant	2.40 (1.50–3.84)	2 × 10 ⁻⁴	49
	<i>GLI3</i>	Intron	rs3801192	Recurrence	200 ^f	Dominant	2.54 (1.47–4.39)	9 × 10 ⁻⁴	49
Inflammation	<i>PPARG</i>	Pro12Ala	rs1805192	Recurrence	89 ^g	Dominant	0.41 (0.20–0.86)	NA	52
	<i>IL-6</i>	–174G>C	rs1800796	Survival	149	Dominant	0.43 (0.19–0.94)	NA	52
	<i>NFKB1</i>	–94 indel ATTG	rs28720239	Recurrence	101	+/- vs -/-	2.86	5 × 10 ⁻³	53

Candidate pathway	Candidate gene	SNP position	rs no.	Outcome measured	Number of cases	Genetic model used	HR (95% CI) ^a	P value	Reference
Cell Adhesion	<i>CDH1</i>	-160C>A	rs16260	Recurrence	274	Dominant	0.68 (0.48–0.96)	0.04 ^d	56
Oxidative stress	<i>HIF1A</i>	P582S or A588T	rs11549462 or rs11549467	Survival	73	Dominant	3.10 (1.38–6.99)	6 × 10 ⁻³	58

^aAdjusted hazard ratio using multivariate logistic regression analysis with homozygous wildtype genotype as reference

^bMeasured in tumors instead of peripheral blood

^cAs shown by Kaplan Meier survival estimate

^dLog rank P value

^eIn TUR treated patients from two independent studies

^fIn BCG treated patients

^gIn patients with no BCG treatment

NA - no information available