ABSTRACT

The aetiology of bladder cancer (BC) is still not fully understood. Genetic factors and many different pathways could be involved in the formation and progression of the BC. Some investigators have reported genetic polymorphisms (GPMs) in various genes which might be associated with BC. As summarised below, we have seen an explosion of literature reporting an association between genetic variation and BC risk, as well as between GPM and clinical outcome. In this review GPMs are categorised based on their primary cellular functions: genes in carcinogen metabolism, DNA repair, cell cycle control, inflammation, apoptosis, methylation, genes functioning as G proteins, and cell adhesion molecules. A pathway-based genotyping approach, which assesses the combined effects of a panel of polymorphisms that act in the same pathway, may amplify the effects of individual polymorphisms and should be more advantageous to association study than the candidate gene approach.

Keywords: Bladder cancer, gene, polymorphism.

INTRODUCTION

Bladder cancer (BC) is the most common malignancy of the urinary tract, the fourth most common cancer in men, and seventeenth most common cancer in women. 74,690 total cases were diagnosed in the United States in 2014, accounting for 4% of all cancers.1 Tobacco is the main known cause for urothelial cancer (UC) formation. In addition, following the skin and lungs, the bladder is the main internal organ affected by occupational carcinogens. In general, there is a long latency period of 10-20 years between the industrial exposure and the formation of the BC, thus proving a definitive causative relationship is difficult. However, there are a variety of occupations statistically associated with BC formation, and all are industrial in nature. 20-27% of all BCs are associated with industrial exposure of some type, primarily in areas with a heavy concentration of chemical industries.2

It is increasingly clear that genetic factors play a critical role in determining the risk of BC. First-degree relatives of patients with BC have a 2-fold increased risk of developing UC themselves, but high-risk of UC families are relatively rare. The inherited risk of BC formation appears to affect all stages of urothelial carcinoma and is not associated with BC formation at an earlier age. Unfortunately, there are no clear Mendelian inheritance patterns, making classic linkage studies impossible.

GENETIC POLYMORPHISM (GPM)

GPM is the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency.3 Geneticists use the term GPMs to describe the inter-individual, functionally silent differences in DNA sequence that make each human genome unique. There are several polymorphisms that seem to be related to the formation of BC, in particular the susceptibility to environmental carcinogens. Many different mechanisms such as, metabolism of carcinogens, DNA repair, cell cycle checkpoint control, apoptosis, and other interconnected cellular processes constitute a network that mediates the toxicologic response of the bladder micro ecosystem. In the following sections, the association between GPMs...
of these key cellular mechanisms, BC risk, and disease progression is described.

Carcinogen Metabolism

Individual differences in cancer susceptibility may be explained to a certain extent by genetic differences in metabolic activation and detoxification of carcinogens. The dynamic equilibrium between carcinogen-activating enzymes and detoxifying enzymes might be fundamental to determine the cell fate after exposure. Cytochromes P-450 (CYPs) is the key metabolic enzyme family capable of metabolising drugs and chemicals. The metabolism of a toxicant consists of two phases: Phase I enzymes, mainly CYPs, typically involved in the activation of carcinogens, whereas multiple Phase II enzymes generally function to detoxify carcinogens. The balance between Phase I and II enzymes often determines the accumulation of reactive intermediates, which may cause oxidative stress and toxicity.

CYP1A1 is an important Phase I xenobiotic metabolising enzyme, well known for its involvement in the metabolic activation of tobacco procarcinogens such as polycyclic aromatic hydrocarbons and aromatic amines. It is a highly polymorphic gene with more than 11 alleles thought to lead to amino acid changes. The majority of the eligible studies observed no significant association between CYP1A1 mutations and BC risk. No significant association was found between CYP1B1 polymorphisms and BC risk in previous published studies. Hepatic CYP1A2 is believed to play an important role in the metabolic activation of arylamines. Humans exhibit considerable inter-individual variability in CYP1A2 activity. This inter-individual variation is most likely caused by both environmentally and genetically determined factors. Studies failed to show direct association between CYP1A2 polymorphisms and BC risk, however we have findings that the carcinogenic potential of this metabolic gene may depend upon the presence of its major inducer, cigarette smoking, and it is associated with increased risk of BC in subjects who are exposed to tobacco smoke.

CYP2D6 encodes debrisoquine hydroxylase, whose substrates include aromatic amines, tobacco nitrosamines, and a wide range of commonly prescribed drugs such as antiarrhythmics, antihypertensives, alpha-blockers, monoamine oxidase inhibitors, morphine derivatives, antipsychotics, and tricyclic antidepressants. Current studies show CYP2D6 did not appear to influence BC susceptibility. CYP2E1 catalyses the metabolic activation of various tobacco-related N-nitrosamines, such as N-nitrosodimethylamine and N-nitrosornicotine, both of which are potent bladder carcinogens in experimental animals. The current studies suggested that the CYP2E1 polymorphism may be associated with BC susceptibility, especially in Caucasians.

NADPH quinone oxidoreductase-1 (NQO1), a chemoprotective enzyme, plays an important role in protection against endogenous and exogenous quinines by catalysing two or four-electron reductions of these substrates. Rich researches suggest that NQO1 GPM contribute to BC development, especially for NQO1 C609T polymorphism.

Glutathione S-transferases (GST) comprises a major group of Phase II enzymes that play the key role in the detoxification of xenobiotics, environmental substances, and carcinogenic compounds. GSTM1 and GSTT1 are two extensively studied GST genes for their association with BC risk. A majority of the studies suggest that the null genotypes of GSTM1 are significantly associated with increased risk of BC. Also Ha et al. concluded GSTM1 tissue genotype has a predictive value for determining recurrence in non-muscle invasive BC. The results from many studies, which indicate increased BC risk is associated with GSTT1 genotypes, are controversial.

There are studies suggesting GSTT1 genotype as a prognostic indicator, independent of traditional pathologic prognostic parameters, for recurrence, progression, and Bacillus Calmette-Guérin (BCG) therapy response. Studies did not find any significant association between BC and GSTA1 and GSTP1 polymorphisms.

SULT1A1 appears to be the principle human SULT (soluble sulfotransferases) form involved in the elimination of most phenolic xenobiotics, as well as some other substrates. The Arg213His polymorphism in SULT1A1 has a strong influence on the activity and stability of the enzyme. Li et al. described a statistically significant protective role of the variant His allele. UDP-glucuronosyltransferases (UGTs) represents another major Phase II drug-metabolising enzyme family sharing roles in detoxification and elimination of endo and xenobiotics. Contrary to previous studies, Zimmermann et al. documented that there is no relationship between UGT2B7 polymorphism and BC.
N-acetyltransferases (NAT) catalyse the metabolic activation of aromatic and heterocyclic amine carcinogens by acetylation. There are two distinct NAT isozymes existing in the human population NAT1 and NAT2. The NAT2 gene is subject to extensive polymorphism, which segregates the populations into rapid, intermediate, and slow acetylator phenotypes. Controversial results exist for the NAT1 polymorphism and BC relationship but the majority highlight that an association is found between the NAT1 polymorphisms investigated, and BC risk.\textsuperscript{33,34} NAT2 polymorphisms and their association with BC have been extensively studied. There are consistent reports on the connection of the NAT2 slow acetylator polymorphisms with higher BC risk, both independently and in association with smoking or occupational exposures, especially arylamine. Also there are some papers demonstrating conflicting results. Selinski et al.\textsuperscript{35} identified an ‘ultra-slow’ acetylator phenotype associated with BC risk, though slow acetylators in general were not associated with BC risk.\textsuperscript{35} Also, Pesch et al.\textsuperscript{36} found that no interaction was detected between NAT2 and any occupational exposure. The combined effect of NAT1 and NAT2 genotypes were also addressed in some of the studies.\textsuperscript{37}

Myeloperoxidase (MPO), catechol-O-methyltransferase (COMT), manganese superoxide dismutase (MnSOD), and glutathione peroxidase 1 (GPX1) are single genes that encode four critical Phase II enzymes modulating carcinogen metabolism. MPO produces a strong oxidant, hypochlorous acid, and also activates procarcinogens in tobacco smoke. COMT catalyses the methylation of various endobiotic and xenobiotic substances, preventing quinine formation and redox cycling. MnSOD is one of the primary enzymes that directly scavenge potential harmful oxidising species and can be induced by free radical challenge and cigarette smoke. GPX1 is a selenium-dependent enzyme that participates in the detoxification of hydrogen peroxide and a wide range of organic peroxides with reduced glutathione. Huang et al.\textsuperscript{38} concluded The MPO GPMs might modify the arsenic methylation profile and BC progression. No effect was observed for BC risk with COMT polymorphism.\textsuperscript{39} GPX1 Pro198Leu polymorphism significantly increased susceptibility to BC, while the MnSOD Ala-9Val polymorphism was not associated with BC risk.\textsuperscript{40,41}

DNA Repair

DNA damage, via constant attack from numerous chemical and physical agents, can initiate cancer. About 10,000 lesions are introduced in each cell every day. Our DNA repair mechanisms prevent the accumulation of the undesirable DNA injuries. Nucleotide-excision repair (NER), base-excision repair (BER), homologous recombination, non-homologous end-join, and mismatch repair are the main DNA repair systems. Each of these repair systems can recognise and fix an array of damage. In the meantime, these repair systems form an intertwining network that functions cooperatively. GPMs of DNA repair proteins with a suboptimal DNA repair capacity have been linked to increased cancer risk.

NER is the most versatile DNA repair pathway. It operates primarily on bulky lesions caused by environmental mutagens, such as UV light and polycyclic aromatic hydrocarbons. Xeroderma pigmentosum complementation group C (XPC) and excision repair cross-complementation group 6 are essential in the NER damage recognition step with different target specificity. Dou et al.\textsuperscript{42} indicates that XPC Lys939Gln polymorphism may contribute to the development of BC risk. Meta-analysis of Liu et al.\textsuperscript{43} suggested that XPG Asp1104His polymorphism was not associated with BC risk. Up to now, many polymorphisms in the XPD gene have been identified, and the Lys751Gln is one of the most important polymorphisms. There are meta-analyses indicating XPD Lys751Gln polymorphism might contribute to the risk of BC.\textsuperscript{44}

BER proteins mainly work on damaged DNA bases arising from endogenous oxidative and hydrolytic decay of DNA. Apurinic/apyrimidinic endonuclease 1, a rate-limiting enzyme of BER, has endonuclease function. Its relationship with BC is still suspicious.\textsuperscript{45} Hundreds of single nucleotide polymorphisms (SNPs) of XRCC1 have been validated and three of them were most extensively investigated: Arg194Trp in Exon 6 (rs1799782), Arg280His in Exon 9 (rs25489), and Arg399Gln in Exon 10 (rs25487). The overall results for these investigations suggest that XRCC1 Arg399Gln polymorphism might be a moderate risk factor for BC.

Cell Cycle (CC) Control

CC controls are biochemical pathways that regulate CC progression in response to DNA damage. Losses of CC control appear to be early steps in the development of carcinogenesis and, ultimately,
cancer progression. The regulation of the CC is governed by both positive and negative CC regulatory factors. p53 is a transcription factor that acts as a fundamental regulator of CC arrest in the cell. This is supported by the fact that p53 is the most frequently inactivated in malignantly transformed cells. p53 elicits CC arrest through activation of downstream genes such as p21. Genetic variants in some of the CC regulators were studied for their associations with BC risk. p53 mutations have been described in more than 50% of human cancers. In particular, p53 loss of function has been related to the development of high-grade muscle-invasive disease. Also Piantino et al. tested Prima-1 molecule as a new therapeutic agent for urothelial carcinomas of the bladder, which characteristically harbours p53 mutations.

Inflammation Genes

There has been compelling evidence supporting the hypothesis that chronic inflammation contributes to cancer development. A substantial number of cancers derive from sites of chronic inflammation. Proinflammatory cytokines, growth factors, chemokines, reactive oxygen species, and COX-2 interact in a complex manner in the development and progression of an inflammatory environment. Genetic variants of inflammatory mediators have emerged in recent years as important determinants of cancer susceptibility and prognosis. Some of these polymorphisms have been linked to BC.

Cytokine proteins have key roles in carcinogenesis. On one hand, they are involved in the activation of the immune system to limit tumour growth. On the other, they may be involved in malignant transformation and tumour growth. The interleukin-1 (IL-1), one of the most potent proinflammatory cytokines, influences nearly every cell type and functions in the inflammation, cell growth, and tissue repair. IL-4 is a key cytokine produced by T cells and has an impact on B cell differentiation and proliferation. IL-4 inhibits macrophage activation and may be involved in cancer formation. Many papers exist which propose a strong relationship between IL-1 and IL-4, and BC risk.\(^47\)

Tumour necrosis factor-alpha (TNF-\(\alpha\)), a multifunctional cytokine, is key in inflammation, immunity, and cellular organisation. TNF-\(\alpha\) has paradoxical roles in cancer, inducing destruction of blood vessels and cell-mediated killing of certain tumours as well as acting as a tumour promoter. The results of several studies do not reach a certain conclusion, and the relationship between TNF-\(\alpha\) and BC remains unclear. Additionally research exists, showing an association of tumour stage\(^48\) and outcome after BCG immunotherapy.\(^49\) Transforming growth factor beta is a potent inhibitor of epithelial cell proliferation and it belongs to the group of tumour-derived cytokines. Castillejo et al.\(^50\) concluded that the genetic variants analysed were not associated with an increased risk of BC.\(^50\)

Apoptosis

Apoptosis plays a central role in cancer development. Two separate pathways (intrinsic and extrinsic) are able to trigger the caspase cascade of the apoptotic pathway. The extrinsic pathway is activated by the ligation of cell surface death receptors by their corresponding ligands, while the intrinsic pathway is triggered by disruption of mitochondrial membrane. Mittal et al.\(^51\) and Wang et al.\(^52\) found an association of Death Receptor 4 in BC development.

G Proteins

G proteins are guanine-nucleotide-binding proteins that form a super-family of signal transduction proteins. The RAS family of monomeric G proteins are small GTPases cycling between a GTP-bound active state and an inactive GDP-bound state. Three of the five human RAS genes - including HRAS, KRAS, and NRAS are known to be associated with human cancer through mutation and/or over expression in tumours. Studies show that HRAS T81C SNP moderately increases BC risk.\(^53\)-\(^55\)

Cell Adhesion Molecules

Cell adhesion is essential in all aspects of cell growth, cell migration, and cell differentiation. A growing body of evidence suggests that alterations in the adhesion properties of neoplastic cells may be pivotal in the development and progression of the malignant phenotype in a range of tumours, including BC. E-cadherin (CDH1), a member of the cadherin family, interacts with cytoskeletal proteins through the catenin complex. E-cadherin seems to function as a tumour-suppressor; loss of expression and/or abnormal function of E-cadherin leads to a loss of cell polarity and derangement of normal tissue architecture. Wang et al.\(^56\) indicates that promoter polymorphism and methylation of CDH1 gene may be involved in the development and progression of BC. CDH1 gene promoter polymorphism and methylation might be promising biomarkers for the diagnosis and prognosis of BC.\(^57\)
Methylation Gene

Genome-wide hypomethylation in human cancer might be a consequence of decreased S-adenosylmethionine (SAM) level. Cancer risk might be modified by polymorphisms in methyl group metabolism genes that affect intracellular concentration of SAM, such as methylenetetrahydrofolate reductase and methionine synthase. Shi et al.18 present no evidence of an association between this polymorphism and BC risk.

REFERENCES

17. Lajin B, Alachkar A. The NQO1 PERSPECTIVE

The ultimate goals of molecular epidemiology studies are to provide a practical risk-assessment model that predicts if an individual is at a higher risk of cancer or to tailor cancer therapy (preventive or treatment) based on each individual’s genetic profile. Unfortunately, we still have a long way to go. Hypothesis-driven genetic association studies, using either a candidate gene approach or a pathway-based approach, have given and will continue to provide us with very valuable information. However, our expectations should not exceed what these studies can provide. The magnitude of associations by these studies will have limited value in public health and clinical care. Continued efforts to exhaustively search and genotype all identified SNPs (single GPM) with potential functional significance in so many genes are costly and impractical. Anyone who has a predominant slow acetylation phenotype should not take up an occupation working with chemicals or dyes. Another example might be suggesting inhibition of a pathway associated with higher recurrence rates. If this is done, an oral therapy might be more attractive than catheterisation and administration of intravesical chemotherapy.

CONCLUSION

A more serious challenge to current association studies is to bypass the inherent limitation of the predominantly used candidate gene approach. Cancer is a complex multigenic and multistage disease involving the interplay of many genetic and environmental factors. It is unlikely that any single GPM would have a dramatic effect on cancer risk. The modest effect of each individual polymorphism, although providing valuable information, would have very limited value in predicting risk in the general population. Therefore, the future of risk assessment for multigenic complex diseases needs to move beyond the candidate gene approach. A pathway-based genotyping approach, which assesses the combined effects of a panel of polymorphisms that act in the same pathway, may amplify the effects of individual polymorphisms and should be more advantageous to association study than the candidate gene approach.