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Review

Mutational Landscape and Environmental Effects in Bladder Cancer

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Abstract: Bladder cancer is the most common cancer of the urinary tract. Although nonmuscle-invasive bladder cancers have a good prognosis, muscle-invasive bladder cancers promote metastases and have a poor prognosis. Comprehensive analyses using RNA sequence of clinical tumor samples in bladder cancer have been reported. These reports implicated the candidate genes and pathways that play important roles in carcinogenesis and/or progression of bladder cancer. Further investigations for the function of each mutation are warranted. There is suggestive evidence for several environmental factors as risk factors of bladder cancer. Environmental factors such as cigarette smoking, exposure to chemicals and gases, bladder inflammation due to microbial and parasitic infections, diet, and nutrition could induce several genetic mutations and alter the tumor microenvironment, such as immune cells and fibroblasts. The detailed mechanism of how these environmental factors induce carcinogenesis and/or progression of bladder cancer remains unclear. To identify the relationship between the mutations and the lifestyle could be useful for prevention and treatment of bladder cancer.

Keywords: bladder cancer; mutation; smoking; lifestyle; diet; inflammation

1. Introduction

Bladder cancer is the most common cancer of the urinary tract with 430,000 new cases and 165,000 deaths per year worldwide [1]. At diagnosis, the majority of bladder cancers are nonmuscle-invasive papillary tumors of low grade, which are termed as nonmuscle-invasive bladder cancer (NMIBC). NMIBC includes stage Ta, T1 tumors and carcinoma in situ (CIS). NMIBCs frequently recur (50–70%) but infrequently progress to invasion (10–15%) and have a good prognosis [2]. High-grade papillary tumors and flat dysplastic lesions, designated CIS, may progress to muscle-invasive bladder cancer (MIBC). MIBCs (of stage T2 and above) have less favorable prognosis, with five-year survival <50% and common progression to metastasis [3]. Although men are more likely to develop bladder cancer, women often present with more advanced disease and have unfavorable prognosis [4].

Comprehensive analyses using RNA sequence of clinical tumor samples in NMIBCs and MIBCs have been reported [5–10]. These analyses revealed that MIBCs have heterogeneity and can be divided into several molecular subtypes. At the highest level, MIBCs could be divided into basal and luminal subtypes [11]. These reports implicated the candidate genes and pathways that play important roles in

carcinogenesis and/or progression of bladder cancer. DNA mutational patterns of bladder cancer were identified that are predominantly comprised of APOBEC (apolipoprotein B mRNA editing enzyme catalytic polypeptide) mutational signatures. Epithelial-to-mesenchymal transition (EMT), a process increasing invasion and migration, characterized by loss of homotypic adhesion and cell polarity, is one of the key factors for drug sensitivity and metastasis in bladder cancer [12].

Age, gender, cigarette smoking, exposures to chemicals and gases, certain medications, radiation, and genetic factors are established risk factors for initiation and progression of bladder cancer. There is suggestive evidence for several other environmental factors including diet, nutrition, and metabolic syndrome [13,14]. Inflammation is one of the key factors for initiation and/or progression of various types of cancer. Lifestyle, especially dietary habits, is the basis of chronic systemic inflammation, which also constitutes a risk for diabetes mellitus, cardiovascular disease, neurodegenerative diseases, and certain cancers including breast, colon, prostate, and pancreatic cancer [15,16]. Several epidemiological studies about association of pro-inflammatory diet with bladder cancer have been reported [17]. Environmental factors could induce several genetic mutations as smoking damages DNA and reduces repair activity [18]. The mechanism of how environmental factors induce carcinogenesis and progression of bladder cancer remains unclear.

In this review, we discuss the representative genetic mutations in bladder cancer and the potential effects of environmental factors on initiation and/or progression of bladder cancer (Figure 1).

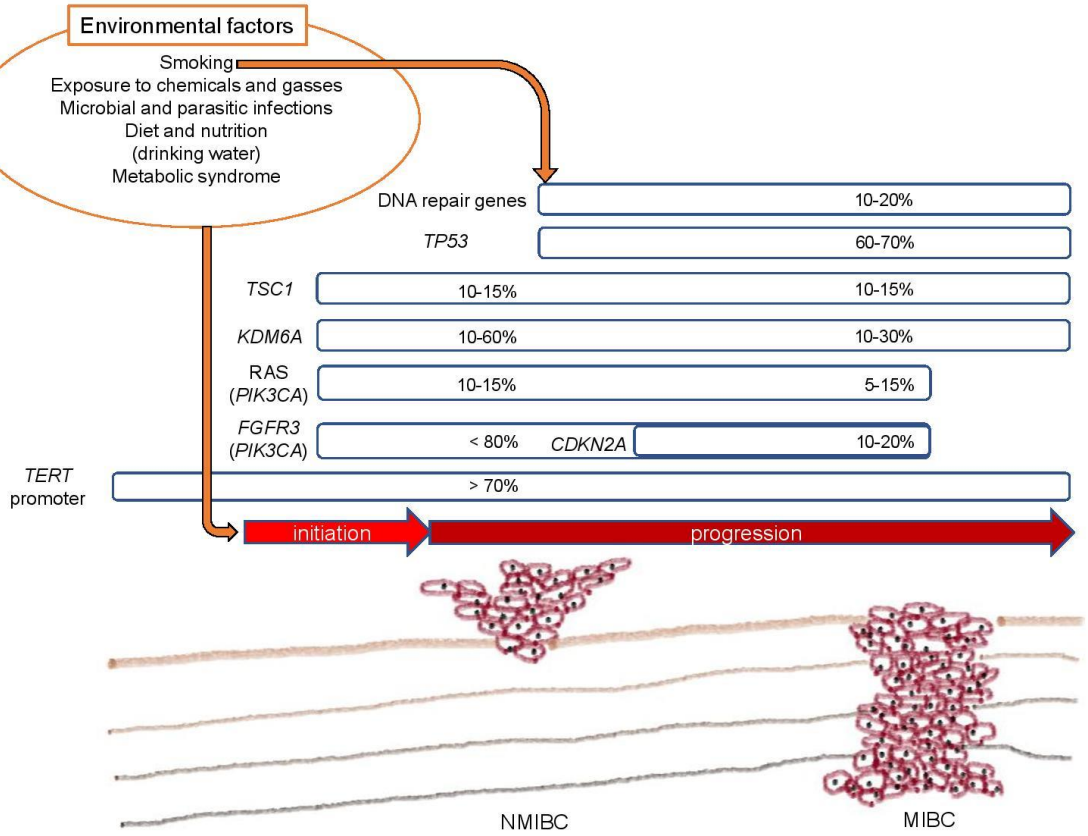

Figure 1. An overview of mutational landscape and environmental effects in bladder cancer. NMIBC, nonmuscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer.

2. Representative Genetic Mutations in Bladder Cancer

The results of comprehensive analyses using RNA sequence revealed several representative genetic mutations in bladder cancer. These mutations suggested candidate genes that are key factors for carcinogenesis and/or progression from NMIBC to MIBC.

2.1. TERT Promoter

Activating mutations in the promoter of the telomerase reverse transcriptase gene *TERT* lead to elevated telomerase expression and allow several cancers to overcome the end-replication problem and avoid senescence. Mutations in *TERT* promoter are the most frequent events identified in bladder cancers of all grades and stages. More than 70% of Ta and T1 tumors have mutations, largely confined to two hotspot nucleotides at positions −124 bp (base pair) and −146 bp upstream from the ATG translation initiation codon [5]. The mutations have also been found in 60% of CIS samples [19], suggesting that this is a universal and early event in the development of both NMIBC and MIBC. While the mutation of *TERT* promoter is an early event in bladder carcinogenesis, hypermethylation of *TERT* promoter is a dynamic process that contributes to disease progression [20].

These mutations are identified in both the normal tissues, which may be precancerous lesion, and the tumor samples of bladder cancer including various rare histological variants (micropapillary, plasmacytoid, adenocarcinoma, squamous cell carcinoma) [21–26]. These mutations can also be detected with ease in both the pellet and cell-free DNA from patient urine samples, promising application in urine-based disease detection and prediction of progression [27–30].

2.2. FGFR3

Fibroblast growth factor receptor, *FGFR3*, point mutation is more common in Ta (up to 80%) than in T1 (10–30%) and MIBC (10–20%). In MIBC, most of the tumors having the mutations are included in a subgroup of luminal subtype, luminal-papillary subtype [31]. The mutations are located in several hotspots, with the most common generating novel cysteine residues that are predicted to drive ligand-independent dimerization. FGFR3 can also be activated by the generation of fusion proteins that retain the kinase domain of the gene fused with another gene, commonly *TACC3* [32].

FGFR inhibitors have been evaluated in clinical trials [33]. It was reported that erdafitinib, one of tyrosine kinase inhibitors of FGFR1–4, had strong clinical activity in locally advanced and metastatic bladder cancer with *FGFR* alterations [34].

2.3. RAS Gene (KRAS or HRAS)

RAS gene (*KRAS* or *HRAS* oncogene) mutations are found in 10–15% of NMIBC and mutually exclusive with *FGFR3* mutations. Ras superfamily of monomeric G proteins participates in bladder cancer progression with other molecules such as epidermal growth factor receptor, p53, and PTEN (phosphatase and tensin homolog) [35]. Multiple components of the Ras-MAPK (mitogen-activated protein kinase) pathway have been identified as potential targets for bladder cancer [36].

2.4. PIK3CA

PIK3CA is activated by point mutation at higher frequency in NMIBC than in MIBC. *PIK3CA* mutations are most commonly found with *FGFR3* or RAS mutations. The *PIK3CA* mutation spectrum in bladder cancer differs significantly from that in other cancers. Mutations E542K and E545K in the helical domain are most common and the kinase domain mutation, H1047R, which is the most common mutation in other cancers, is less common in bladder cancer [37]. PIK3CA have domains for binding to RAS. There may be cooperation between PIK3CA mutant proteins and other events that are known to activate RAS [37].

2.5. KDM6A

KDM6A (Lysine (K)-specific demethylase 6A, H3K27 (Lysine 27 on histone H3) demethylase), a histone modifier, is commonly mutated in both NMIBC and MIBC (among all molecular subtypes). Loss of KDM6A function is expected to lead to gene silencing via transcriptional repression. *KDM6A* is located on the X-chromosome and both alleles are transcribed in females [38]. In contrast to MIBC, a strong gender bias that more mutations of *KDM6A* were present in females than males was observed in

NMIBC [39]. Approximately half of the mutations were accompanied by loss of the second gene copy or were co-heterozygotic. Although bladder cancer is much more prevalent in males, female patients have a worse prognosis [4]. This gender difference persists even after correction for environmental factors such as smoking. While it has been ascribed to differences in sex hormones, particularly androgens, it could also result from the protective effect of two functional *KDM6A* copies in females.

The study using *KDM6A*-knockout bladder cancer cells and patient-derived xenograft model suggested that EZH2 (enhancer of zeste homolog 2, H3K27 methylase) inhibition is a potential therapeutic target for bladder cancer with the mutations [40]. In the recent study using the mice lacking *Kdm6a* in the urothelium, *Kdm6a* deficiency activates inflammatory pathways, promotes M2 macrophage polarization, and causes bladder cancer in cooperation with *p53* dysfunction [41].

2.6. TSC1

Chromosome 9 deletions are found in more than 50% of bladder tumors of all grades and stages. In NMIBC, loss of the long arm (9q) is most common. Mutations of *TSC1* (tuberous sclerosis 1, 9q34) are found in 10–15% of bladder cancers with no clear association with grade or stage. *TSC1* is one of two genes that, when mutated in the germline, cause the syndrome tuberous sclerosis complex, an autosomal-dominant, tumor-suppressor gene syndrome characterized by the development of hamartomas in the kidneys, heart, brain, and skin.

Missense mutations of *TSC1* found in bladder cancer were shown to cause loss of function through aberrant splicing, protein instability, or protein mislocalisation [42]. Loss of TSC1 leads to hypoacetylation of Heat shock protein 90 (Hsp90) and subsequent decreased binding to the Hsp90 inhibitor [43]. Combined Hsp90/mTOR (mammalian target of rapamycin) inhibition is a promising therapeutic approach for *TSC1*-mutant bladder cancer [44].

2.7. CDKN2A

Loss of the short arm (9p) is found at lower frequency than loss of 9q in NMIBC. The 9p deletions are focused on *CDKN2A* (cyclin-dependent kinase inhibitor 2A) locus (encoding p16 and $p14^{ARF}$ (ADP (adenosine diphosphate) -ribosylation factor). Loss of *CDKN2A* is implicated to play an important role in progression of *FGFR3*-mutant NMIBC to MIBC [45]. In the TCGA (The Cancer Genome Atlas) data, *CDKN2A* expression was significantly higher in basal subtype than in luminal subtype [46]. MIBC patients with high expression of *CDKN2A* have poor prognosis. Further investigations into the function and potential for biomarkers of *CDKN2A* are warranted in both NMIBC and MIBC.

2.8. TP53

Mutation frequency of *TP53*, which is one of major tumor suppressor genes, is very low in low-grade Ta but higher in T1 and MIBC. *TP53* mutations are considered an early event in the development of CIS lesions. Inactivation of p53 may lead to increased propensity of CIS lesions to progress to invasive tumors, compared to other superficial tumors [47]. A p53-like molecular subtype of MIBC characterized by wild-type p53 gene expression signatures shows primary and acquired resistance to chemotherapy [7].

TP53 mutations were enriched in tumors with genome-doubling events, suggesting that loss of *TP53* activity facilitates genome doubling [48]. The oncogene MDM2 (murine double minute 2) protein inhibits p53 transactivation function by binding to a region of the *TP53* transactivation domain. The p53 gene family members p53, p73, and p63 display several isoforms derived from the presence of internal promoters and alternative splicing events. Several agents targeting p53 pathway such as synthetic peptides derived from the p53 C-terminal domain have been developed for the treatment of bladder cancer.

The heterozygous p53 knockout mice develop tumors within six months of exposure to genotoxic carcinogens. P53 function does not affect cytotoxicity or cell proliferation induced by *p*-cresidine,

bladder carcinogen [49]. P53 involvement in mouse tumorigenesis may be more important in facilitating the malignant progression of neoplastic foci rather than in the initiation or promotion stage.

2.9. DNA Repair Genes (ERCC2, ATM, ATR, BRCA1, BRCA2, POLE, and FANCA)

Several DNA repair genes (*ERCC2*, *ATM*, *ATR*, *BRCA1*, *BRCA2*, *POLE*, and *FANCA*) are more frequently mutated in high-grade NMIBC and MIBC [5].

ERCC2 (excision repair cross-complementation group 2) is a DNA helicase and a member of the nucleotide excision repair pathway, which repairs intrastrand crosslinks created by genotoxins such as UV irradiation and platinum chemotherapies [50]. In two cohorts of cisplatin-based chemotherapy in MIBC, patients with *ERCC2*-mutated tumors had improved survival compared to those with *ERCC2* wild-type tumors [51,52]. Feki-Tounsi M. et al. showed that slight protective effect of polymorphism of *ERCC2* codon 751 Gln/Gln genotype increased with age in a case-control study [53]. Additionally, the polymorphism seems to reduce bladder cancer risk among smoker and/or alcohol consumers. Further studies about the relation of smoking with the function of *ERCC2* are needed.

2.10. Others

STAG2 (stromal antigen 2), a subunit of cohesion, was significantly and commonly mutated or lost in bladder cancer, mainly in tumors of low stage or grade, and its loss was associated with improved outcome [54].

PTEN and *FOXA1* are downregulated by allelic loss and site-specific DNA hypermethylation, respectively. Conditional inactivation of both *Pten* and *Foxa1* in intermediate/luminal cells in mice resulted in development of bladder cancer exhibiting squamous features, which suggested that hypermethylation of *FOXA1* and allelic loss of *PTEN* drives squamous differentiation and promotes heterogeneity [55].

3. Potential Effects of Environmental Factors on Bladder Cancer

Cigarette smoking, exposures to chemicals and gases, certain medications, radiation, and genetic factors are established risk factors for initiation and progression of bladder cancer. There is suggestive evidence for several other environmental factors including diet, nutrition, and metabolic syndrome. Environmental factors could induce several genetic mutations and alter the tumor microenvironment such as immune cells and fibroblasts.

3.1. Smoking

Cigarette smoking is the major environmental risk factors for bladder cancer. It is estimated that almost half of male patients and a quarter of female patients of bladder cancer can be attributed to smoking [56].

ERCC2 signature mutations were at higher levels in smokers than nonsmokers [30]. Fantoni F. et al. reported molecular footprints of MIBCs in smoking and nonsmoking patients [57]. In the TCGA data, only *GRP15* expression was significantly upregulated in smokers (independent from luminal and basal subtypes). *GRP15* is an orphan G protein-coupled receptor expressed by lymphocytes and mediates recruitment of effector T cells to inflamed tissue. *FLG*, *SPTAN1*, *USH2A*, *LYST*, and *MED13* were more frequently mutated in nonsmokers, whereas *SPTA1* was more frequently mutated in smokers. *TP53*, *TIN*, *KDM6A*, *MLL2* (*KMT2D*), and *MUC16* were the most frequently mutated genes in smokers. Mutational signatures of smokers were similar to those of BBN (N-butyl-N-(4-hydroxybutyl) nitrosamine) mouse model, where tumors are induced by a nitrosamine compound related to the carcinogens found in smoke. This model is known to have similarity with basal subtype [58]. Using this model, the methods to detect bladder cancer in early stage are developed [59]. Analyses of gene expression and mutations identified only a limited set of differences between smokers and nonsmokers, suggesting that tumors originating as the consequence of smoking or other causes can progress in a similar fashion, resulting in similar transcriptional defects.

Exposure of human normal urothelial cells to smoke induced morphological change, along with EMT and MAPK activation [60]. ERK1/2 and p38 inhibitors attenuated smoke-triggered urocytic EMT. Cigarette smoke extract exposure induced morphological change of human bladder cancer cells and enhanced EMT via activation of ERK1/2 pathway [61]. These results suggest that cigarette smoke induces initiation and progression of bladder cancer, mediating EMT and activating ERK1/2 pathway. Kispert S. et al. reported that the exposure of bladder cancer cells to cigarette smoke extract results in increased platelet-activating factor accumulation and increased expression of the platelet-activating factor receptor [62].

3.2. Exposure to Chemicals and Gases

It has been reported that there is occupational carcinogen exposure as a part of risk factors of bladder cancer. Agents with a suspected/established role as occupational bladder carcinogen include 2-naphthylamine, 4-aminobiphenyl, toluene, 4,4′-methylenebis (2-chloroaniline), metal-working fluids, polyaromatic hydrocarbons, perchloroethylene, and diesel exhaust [63]. The detailed mechanism by which each carcinogen induces bladder cancer remains unclear.

In order to discriminate carcinogens and noncarcinogens, in vitro genotoxicity tests are considered useful tools [64]. Isothiocyanates are highly biologically active compounds formed upon enzymatic hydrolysis of glucosinolates, naturally occurring thioglycosides contained in a variety of cruciferous vegetables. Although these compounds are effective chemopreventive agents against the carcinogenic effect of different compounds, including nitrosamines and polycyclic hydrocarbons, the results of the test for genotoxic effects indicated that the compounds are genotoxic, and probably carcinogenic [65]. Reactive oxygen species might be involved in the genotoxic effect of the isothiocynates.

3.3. Bladder Inflammation Due to Microbial and Parasitic Infections

Chronic local inflammation by recurrent urinary tract infections is associated with an increased bladder cancer risk, particularly squamous cell carcinoma [66,67]. The association between bladder schistosomiasis infections, inflammation, and bladder cancer risk has been well established [63]. It was reported that several urinary taxa such as *Fusobacterium*, *Sphingobacterium*, and *Enterococcus* distinguished urogenital schistosomiasis-induced bladder pathologies from urogenital schistosomiasis infection alone and from healthy persons [68]. Strains of bacteria that can mediate the formation of N-nitrosamines have been proposed to contribute to schistosomiasis-induced bladder cancer as well [69].

There is a hypothesis that urinary microbiome may alter the extracellular matrix, which may promote or inhibit urothelial carcinogenesis [70]. The association of genitourinary cancer including bladder cancer with urinary microbiome has been investigated [71].

From microbe analysis in the TCGA data, viral infection such as human papilloma virus and polyoma virus may contribute to a small percentage of bladder cancer [31].

3.4. Diet and Nutrition

3.4.1. Pro-Inflammatory Diet

Diet can induce systemic and/or local inflammation. The association between the inflammatory potential of diet and the risk of urologic cancer including bladder cancer has been investigated in a small number of studies [18]. Dietary inflammatory index (DII), which is computed based on dietary intake assessed using a reproducible and valid 80-item food frequency questionnaire, is used as the indication of pro-inflammatory diet. Although one case-control study demonstrated the positive relation of the diet with the risk of bladder cancer [72], three prospective cohort studies showed no relation [73–75].

A case-control study of 670 bladder cancer cases and 665 controls demonstrated that the highest quartile of DII scores had twofold excess risk of bladder cancer compared to the lowest quartile [72].

There are possibilities of recall, selection, and reverse causation bias as well as incomplete control of confounding in this study. In a prospective cohort of more than 41,000 participants, a diet with pro-inflammatory potential was not associated with the overall bladder cancer risk [73]. In a prospective cohort study with 172,802 women and 45,272 men in the United States, high empirical dietary inflammatory pattern scores were not associated with a higher risk of bladder cancer (RR (relative ratio) 0.92, 95% CI (confidence interval) 0.75–1.12, P_{trend} (P-value for trend analysis) 0.67) [74]. These results were similar regardless of smoking status. In a prospective cohort with 101,721 participants, Luo J. et al. reported that energy-adjusted DII scores were not associated with bladder cancer risk in the multivariate models [75]. The HRs (hazard ratios) (95% CIs) of one-unit increment were 0.99 (0.96–1.02) and 1.01 (0.94–1.10) for men and women, respectively. It was suggested that local rather than systemic inflammation might have a role in the etiology of bladder cancer.

Dietary cholesterol was positively associated with the risk of various types of cancer including bladder cancer in a case-control study [76]. In another case-control study, statistically significant reduced odds of bladder cancer were observed for high intakes (highest quartile vs. lowest quartile) of α-linolenic acid (OR (odds ratio) 0.26, 95% CI 0.19–0.98, P_{trend} 0.01) and vegetable fat (OR 0.39, 95% CI 0.18–0.86, P_{trend} 0.06) [77].

3.4.2. Fruit and Vegetable

Overall fruit and vegetable intake showed a significant protective effect on bladder cancer (RR 0.81, 95% CI 0.67–0.99) in a meta-analysis [78]. The dose–response analysis demonstrated that the risk of bladder cancer decreased by 9% (RR 0.91, 95% CI 0.83–0.99) and 8% (RR 0.92, 95% CI 0.87–0.97) for every 200 g/day increment in fruit and vegetable consumption, respectively [79]. A case-control study showed that the protective effect of vegetable consumption, especially cruciferous vegetables, may be modified by genetic variants of *GSTM1* and *NAT2*, carcinogen-detoxification genes [80]. Further well-designed prospective studies are warranted to confirm these findings.

Dietary açai fruit pulp reduced bladder cancer incidence, tumor cell proliferation, and p63 expression in mice, probably due to its potential antioxidant action [81]. Açai pulp presented a significant reduction in DNA damage induced by H_2O_2, a notable oxidant agent.

3.4.3. Others (Alcohol, Coffee, Arsenic, Drinking Water, Meat, Vitamins)

Although those who reported high alcohol intake have an increased risk of bladder cancer, there was no dose response. These results suggest possible confounding lifestyle factors [63].

Current evidence remains equivocal whether coffee consumption may be associated with risk of bladder cancer. Some analyses showed positive association [82–84], while other analyses demonstrated inverse association [85,86]. Some reports concluded that no statistically significant association was observed [13,87]. A prospective study revealed that positive association between coffee and bladder cancer was attenuated after adjustment for smoking and other potential confounders [84]. In an international, pooled study, positive association was observed in different subgroups, only in never smokers [88] or only in male smokers [89]. These conflicting results suggest that the association between coffee and bladder cancer may be affected by gender and smoking status.

The exposure to arsenic in drinking water is a recognized cause of bladder cancer. A systematic review and a meta-analysis showed a risk effect of 2.7 (95% CI 1.2–4.1) for 10 μg/L and 5.8 (95% CI 2.9–8.7) for 140 μg/L [63]. Detoxification of arsenic may occur through a methylation pathway. The organic methylated arsenicals are much less potent as mutagenic agents than the inorganic arsenicals [90]. Several epidemiological studies suggested that the exposure to disinfection byproducts of chlorinated water such as trihalomethanes [91] and nitrosamines [92] was associated with bladder cancer. There are other potential candidates of bladder carcinogen in chlorinated drinking water, haloamides, halocyclopentenoic acids, furans, and haloquinones [93]. Further studies are needed to determine the causal agents in chlorinated drinking water.

Li F. et al. found an increased risk of bladder cancer associated with processed meat (RR 1.22, 95% CI 1.04–1.43) but not with red meat (RR 1.15, 95% CI 0.97–1.36) in a meta-analysis [94]. Processed meat may produce a carcinogen related to inflammation. Several meta-analyses demonstrated that dietary intake of vitamin A, C, D, and E could reduce a risk of bladder cancer, respectively [13,14,63,95].

3.5. Metabolic Syndrome

In a case-control study with 690 incident bladder cancer patients and 665 cancer-free matched patients, metabolic syndrome was at a twofold higher risk of bladder cancer (95% CI 1.38–3.19) [96]. In a retrospective analysis of 169 patients who underwent transurethral resection, metabolic syndrome was significantly associated with a high histological grade [97]. No association between metabolic syndrome and risk of NMIBC recurrence was found in a cohort of 1485 older (age ≥ 60 years) NMIBC patients [98].

Metabolic syndrome is defined as the presence of three of the following: Hypertension, hyperlipidemia, diabetes, or body mass index >30. Cantiello F. et al. revealed that obesity suggests a possible correlation with pathological factors and prognosis of bladder cancer and that diabetes seems to be associated with worse oncological outcomes [99]. Diabetes also showed a significantly higher relative risk (1.36–1.51) after adjustment for age, sex, and other potential cofounders [100]. Hyperlipidemia exhibited a 37–51% increased risk of bladder cancer compared with nonhyperlipidemia [101].

3.6. Others

Cyclophosphamide, an alkylating agent, and pioglitazone, an antidiabetic drug of the thiazolidinedione class, have been suggested to have a relation to bladder cancer incidence with long-term use [102].

A large cohort study demonstrated a reduced risk of bladder cancer with the use of oral antidiabetic agents, metformin and sulphonylurea [103]. Another cohort study found no association of insulin with bladder cancer risk [104].

Radiotherapy to treat various cancers located in the pelvic region, especially prostate cancer, has been associated with developing second malignancies of the bladder. In a meta-analysis, bladder cancer risk was elevated with a hazard ratio of 1.67 (95% CI 1.55–1.80) after radiation of prostate [105]. Histologically, the number of non-urothelial cell carcinoma was greater than usual, and CIS was more common [106]. Anatomically, tumors were more frequently found at the trigone.

4. Conclusions

Comprehensive analyses revealed mutational landscape of bladder cancer. Further investigations for the function of each mutation are warranted. Cigarette smoking, exposures to chemicals and gases, certain medications, radiation, and genetic factors are established risk factors for bladder cancer. Diet, nutrition, and metabolic syndrome, which are linked to lifestyle, are also suggested to be risk factors for bladder cancer. Identifying the relationship between the mutations and the lifestyle could be useful for prevention and treatment of bladder cancer.

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