

Genetic polymorphisms in cytokine genes and urinary bladder cancer risk

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ABSTRACT

Urinary bladder cancer (UBC) is the ninth most common cancer worldwide. Cytokines play dual roles in cancer development. On one hand, they are involved in the activation of the immune response to control tumor growth. On the other hand, they may be involved in malignant transformation and tumor proliferation. Genetic variations of cytokines genes are thought to play an important role in individual's susceptibility to any cancer and have emerged in recent years as significant determinants of cancer susceptibility and prognosis of cancer. In this review, the literature investigating the association between genetic polymorphisms of cytokine genes and the risk of UBC are summarized. We have thoroughly reviewed the genetic polymorphism studies on IL-6, IL-4, TGF- β , TNF- α , PPARs and COX-2 genes in relation with UBC. Overall, it appears that genetic polymorphisms in the cytokine genes play an important role in determining susceptibility to UBC.

KEYWORDS: urinary bladder cancer, cytokine genes polymorphisms, genetic polymorphisms, inflammation

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INTRODUCTION

Urinary bladder cancer (UBC) is the ninth most common cancer worldwide. The incidence of UBC varies highly along with different regions worldwide with higher occurrence in males as compared to females (Jemal et al., 2011). Transitional cell carcinoma (TCC) of UBC comprises 90% of all primary tumors of UBC. Approximately, 70% of newly diagnosed TCC of UBC are non-muscle invasive and have high recurrence and progression rate despite local therapy, while remaining 25% of newly diagnosed are muscle invasive UBC and have poor survival rate despite systemic therapy (Babjuk et al., 2011; Stenzl et al., 2011). The incidence rate of UBC is highest in southern Europe, North America and Western Asia while lowest incidence rate is found in Melanesia, Middle Africa and Western Africa. In 2017, there were an estimated 79,030 new cases of UBC and 16,870 UBC related deaths in the United State (Bethesda MD, 2017).

Cytokines have been described as 'a vast array of relatively low molecular weight, pharmacologically active proteins that are secreted by a cell for the purpose of altering either its own functions (autocrine effect) or those of the adjacent cells (paracrine effect)' (McDermott et al., 2001). The secretion of cytokines from cells is a crucial response to any damage and infection in the body. Cytokines include chemokines, lymphokines, interferons, interleukins, tumor necrosis factors (TNF) and growth factors. They are produced by immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells as well as endothelial cells, fibroblasts, and various stromal cells (Ibelgaufts et al., 2013). In many instances, individual cytokines have various biological functions. Different cytokines can have the same function, which provides for biological redundancy within the inflammatory and immune systems. As a result, it is rare that loss or neutralization of one

cytokine will markedly interfere with either of these systems. This fact has great significance in the development of therapeutic strategies (Coppack et al., 2001).

Metabolism of cytokines

Cytokines act via receptors and have a key role in the immune system and regulate maturation of cell, differentiation, growth and cell death (McDermott et al., 2001; De Luca et al., 2008). Cytokines are released by various cells as a fundamental response to cellular damage or invasion of harmful pathogens in the body. Epithelial and endothelial cells are the foremost cells to release cytokines in order to signal other innate immune cells. Epithelial cells act in coordination with innate immune cells, such as eosinophils, neutrophils, mast cells, macrophages, dendritic cells (DC), natural killer (NK) cells etc., to exert appropriate inflammatory and adaptive immune response against bacterial, viral and parasitic invasion (Saenz et al., 2008).

Epithelial cells and innate immune cells release cytokines through various discrete pathways involving distinct organelles, carriers and molecules. Uniqueness of different pathways is crucial in order to apply a fine control on the release of these important immune-regulatory messengers as it is essential for mounting appropriate and essential immune response (Stanley and Lacy, 2010). Cytokine release from these cells acts as a medium of communication with other innate immune cells and thus helps in regulating the amplification of inflammation and expansion of T cells and B cells with associated production of antibodies and cytotoxic responses (Jolly and Sattentau, 2007).

Cytokine secretion pathways from cells can broadly be categorized as: Classical and Non-classical pathways. In classical pathways, cytokines, after synthesis in nucleus, are transported to Golgi

complex through Endoplasmic Reticulum (ER) for packaging. While in non-classical pathways, cytokines secretion does not follow ER-Golgi trafficking. Most cytokines are released through classical pathways, IL-2, IL-3 and IL-7 possess a classical signal peptide allowing their synthesis and packaging through ER-Golgi trafficking and thus represent classical pathway (Stanley and Lacy, 2010). On the other hand, IL-1 β , IL-15, IL-18 and MIF do not have a signal sequence and appear to be released from cytoplasm directly, independent of the ER-Golgi trafficking (Nickel, 2003). However exact mechanisms of non-classical secretion are yet to be studied for more clarity.

Classical pathways of cytokine secretion can be further categorized as- Regulated exocytosis and Constitutive exocytosis. In regulated exocytosis, cytokines are packaged in Golgi body in the form of small membrane bound secretory granules for storage and are released only during receptor-mediated stimulation (Stinchcombe and Griffiths, 2007). On the other hand, in constitutive exocytosis cytokines are not stored but are released through recycling endosomes (REs) and small secretory vesicles soon after their synthesis which may or may not be triggered by receptor stimulation (Stow et al., 2009). Granulocytes such as eosinophils, neutrophils, mast cells are the best representative of regulated exocytosis as these innate immune cells possess various types of granules which are used for cytokine secretion. Constitutive exocytosis pathway is best defined in macrophages for their ability to package and release of TNF and IL-6. Studies based on morphological and bio-chemical analysis of their secretory organelles have shown that macrophages contain LROs (lysosome-related-organelles) instead of granules. LROs do not store cytokines but instead continually export their contents to cell membrane (Murray et al., 2005). Exact mechanism of cytokine secretion from

epithelial cells is not yet established but most likely it is believed to be through constitutive exocytosis (Stanley and Lacy, 2010).

Trafficking of cytokines or of any protein in general, through vesicles in secretory cells is dependent on various unique sets of molecules which are distinct on the basis of type of cell, type of cytokines and type of receptors in order to develop an appropriate innate and adaptive immune response. Thus, various components involved in cytokine packaging and trafficking are specific to the cell type as well as the cytokine. Hence, study of any cytokine in one type of cell can neither be applied for other cytokines in same type of cells nor to the same cytokine in other type of cells. Hence, much remains to be studied before establishing exact mechanisms of regulation of cytokines in innate immune cells as well as other cells involved in immune response (Stanley and Lacy, 2010).

Genetic polymorphisms in cytokine genes and UBC risk

Cytokines have dual roles in cancer development. On one hand, they are involved in the activation of the immune response to control tumor growth. On the other hand, they may be involved in malignant transformation and tumor proliferation. Cytokine can become deregulated and pathologic in inflammatory condition. Virchow in 1863 initially hypothesized that cancer arises at the site of inflammation because prolonged irritation, tissue injury and activated local host response eventually favored cell proliferation (Balkwill and Mantovani, 2001). A large number of cancers are derived from the site of chronic inflammation. Among 1.2 million global cases each year, more than 15% of malignancies can be attributed to infection (Wang et al., 2002). In cancerous cell, sustained cell proliferation and progression is supported by environment rich in inflammatory cell, growth

factors, and DNA-damaging agents (Balkwill and Mantovani, 2001). Pro-inflammatory cytokines, growth factors, chemokines and ROS interact in a complex manner in the development and progression of cancer (Macarthur et al., 2004).

In the condition of cell damage, infection and oxidative stress, several cytokines produce inflammatory response. The fact that cytokines themselves can trigger the release of other cytokines leads to increased oxidant stress that results in chronic inflammation. Inflammation is observed as a “secret killer”, and an inflammatory component is present in the microenvironment of most neoplastic tissue, including some in which a direct relationship with inflammation has not yet been proven (Zhu et al., 2012). Chronic inflammatory condition of unknown region can favor the development of tumor, as everlasting inflammation of bladder caused due to schistosomiasis and indwelling catheters usually associates with bladder cancer.

Genetic polymorphisms of cytokines and inflammatory mediators have emerged in recent years as significant determinants of cancer susceptibility and prognosis (Macarthur et al., 2004). Some of these polymorphisms are associated with the risk of UBC. In the following section studies based on relation between the risk of UBC and genetic polymorphisms in genes encoding cytokines have been summarized.

Interleukins genes

Interleukine-6 (IL-6) is a pleiotropic and multifunctional cytokine, particularly involved in immune inflammatory response. It plays a key role in the initiation of different intracellular pathways like JAK/STAT, MAPK, PI3K that concomitantly activate expression of other genes resulting in enduring inflammation and cancer development (Tawara et al., 2011). In acute inflammatory conditions, IL-6 regulates the production of acute

phase proteins, and simultaneously, it also affects the level of inflammatory response by regulating anti-inflammatory cytokines (Ferrari et al., 2003). IL-6 is known to possess both pro-inflammatory and anti-inflammatory effects (Scheller et al., 2011). In the urinary system, IL-6 may transform urothelial cell and provide selective growth advantage to urothelial cancerous cells (Okamoto et al., 1997). In vitro study demonstrated that urothelial malignant cells secrete a large amount of IL-6 as compared to normal urothelium and treatment with anti-IL-6 antibody and antisense oligo-nucleotide was found to exert anti-tumor effect via the induction of apoptosis in mouse bladder carcinoma (Li et al., 2010). Besides inflammatory response, IL-6 is also involved in cancer cell differentiation, tumor growth and change in the microenvironment of tissue, which may further result in neo-angiogenesis and inhibit the processes of apoptosis and acquired cell defense (Wojcik et al., 2010). An increased level of IL6 was found in the serum and urine of urinary bladder cancer patients (Andrews et al., 2002; Seguchi et al., 1992). Whereas in immuno-histochemistry studies, IL-6 immuno-positivity was seen in 80% of the cases (Naik et al., 2011) and IL-6 has been suggested to be a prognostic marker and a target for anti-cancer therapy (Leibovici et al., 2005). The levels of IL-6 with pre-operative and post-operative resection of bladder and local staining of bladder cancer specimens with IL6 showed a possible though confined role of IL6 in UBC.

Several studies have observed an association between IL-6 gene polymorphisms and the risk of different cancers, including UBC (Andrews et al., 2002; Gautam et al., 2016; Seguchi et al., 1992; Naik et al., 2011). IL-6 -174 G>C variation affects gene transcription and the level of IL-6 protein (Fishman et al., 1998). Gautam et al. in a case-control study observed that the CC genotype at IL-6 -174 G>C site confers a protective association

against UBC risk. However, a study on North Indian and Caucasian population reported contrasting results (Leibovici et al., 2005; Aben et al., 2002). Wang et al. and Ebadi et al. observed that IL-6 -174 G>C variant genotype was significantly associated with an increased risk of UBC (Wang et al., 2014; Ebadi et al., 2014). Further, a study on an East-Asian population did not find any association between genotypes at -174 G>C polymorphic site and UBC risk (Ahirwar et al., 2008). Few studies (Leibovici et al., 2005, Aben et al., 2002), investigated the association of -174 G>C with smoking and UBC risk and found that the frequency of variant allele was higher in smokers than never smokers (Leibovici et al., 2005; Aben et al., 2002). Gautam et al. did not find any association between IL-6 -572 G>C, -596 A>G polymorphisms and UBC risk (Gautam et al., 2016).

Interleukin-4 (IL-4) is an anti-inflammatory cytokine by virtue of its ability to suppress genes of pro-inflammatory cytokines such as TNF- α and IL-1. IL-4 chiefly produced by activated CD4⁺ T cells, mast cells and basophils. IL-4 plays a key role in surveillance and elimination of transformed cells by Th2 development, eliminating extracellular pathogens and inhibiting Th1 (Muller-Hermelink et al., 2008). Several studies have explored the association between IL-4 -590 C>T polymorphism and risk of cancers (Gaur et al., 2011; Gomes et al., 2011). Chu et al. in a case-control study on Chinese population observed that genetic variant IL-4 -590 C>T might modulate the risk of UBC (Chu et al., 2012). Bozdogan et al. in a case-control study found that allele distribution frequencies of IL-4 were significantly different between two groups (Bozdogan et al., 2015). Tsai et al. observed that the IL-4 intron 3 variable numbers of tandem repeats (VNTR) is associated with UBC risk (Tsai et al., 2005).

Transforming growth factor- β (TGF- β) gene

TGF- β pathway is a signal transduction protein that normally controls cellular homeostasis in normal cell and early stages of cancer; however, in late stages, the pathway is believed to help in tumor proliferation and metastasis. There are three isoforms of TGF- β : TGF- β 1, TGF- β 2, and TGF- β 3; each of them being encoded by distinct genes. TGF- β 1 is most abundant and located on chromosome 19q13.1, harbors many genetic variations that influence TGF- β 1 protein expression (Watanabe et al., 2002). TGF- β 1 is expressed in endothelial cells, hematopoietic, and connective tissue cells (Massague et al., 1998). TGF- β 1 is a potent inhibitor of proliferation in epithelial cells and acts as tumor suppressor, it controls cell proliferation by reducing the ability of cell to enter S-phase (signals cell cycle arrest) (Alexandrow et al., 1995), while loss of this response associates with continuous expression of TGF- β by cancerous cells which helps aggressive progression of cancer (Davis et al., 2008; Massague et al., 1992; Parsons et al., 1995). Since TGF- β 1 plays dual roles in cellular processes, its expression and association with different carcinomas has been found depending on cellular content and tumor stage. Elevated plasma level of TGF- β 1 has been found in advanced stages of hepatocellular carcinoma, prostate, breast, and colon cancer (Teicher et al., 2001). Cancerous cells become resistant to inhibitory effects of TGF- β 1 through mutations or inactivation of TGF- β 1 receptors. In the late stages of cancer, TGF- β 1 makes the microenvironment like angiogenesis, evasion of apoptosis and proliferation that favors the progression and metastases of cancer (Noordhuis et al., 2011; Ding et al., 2011; Kubiczkovz et al., 2012). Accordingly, TGF- β 1 polymorphisms have been studied in a variety of carcinomas and linked with cancer risk (Castillejo et al., 2009; Joshi et al., 2011; Wu et al., 2010).

In an in-vitro study on TGF- β 1 mRNA expression in different cell lines (non-tumorigenic, tumorigenic, metastatic) of clonally derived rat urinary bladder carcinoma, it was observed that mRNA expression in tumorigenic/metastatic cell line was thirty times more than that of non-tumorigenic cell line and the level of expression was proportional to the aggressiveness of the urinary bladder cancer (Hitoshi et al., 1992).

The association of TGF- β 1 has been studied in a number of different carcinomas, such as breast cancer (Shin et al., 2005; Joshi et al., 2011), prostate cancer (Shahrokh et al., 2001), and others (Yu et al., 2010). A study investigating association of 356 SNPs in the TGF- β 1 pathway observed an association of forty-one SNPs with the risk of UBC (Wei et al., 2012). Castillejo et al. analyzed three polymorphisms in the TGF- β 1 and four polymorphisms in TGF β R1 genes however, all the SNPs investigated showed a similar genotype distribution between cases and controls (Castillejo et al., 2009). Another study found similar result and the distribution was statistically insignificant (Bozdogan et al., 2015). In contrast, Gautam et al. observed a significant association of TGF- β 1 c.29 C>T substitution with the risk of UBC (Gautam et al., 2015).

Tumor necrosis factor alpha (TNF- α) gene

TNF- α is a multifunctional cytokine and a primary mediator of inflammation, host defense and tissue homeostasis/cellular organization. Depending upon its concentration and duration of cell exposed, TNF- α have beneficial or harmful consequences including destruction of blood vessels and cell-mediated killing of certain tumors as well as acting as a tumor promoter (Balkwill et al., 2002). TNF- α is majorly secreted by macrophages in response to infection, inflammation and environmental stress. An individual's resistance ability in response to these

risk factors may be changed due to variation in genetic composition of TNF- α . Furthermore, TNF- α has been linked to all steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis (Sethi, et al., 2008). Tumor cells secrete their own TNF- α in autocrine manner which further enhance the expression of other growth factors such as transforming growth factor-alpha (TGF- α) and epidermal growth factor receptor (EGFR), both of which mediate proliferation (Schmiegel et al., 1993).

Further developments suggest that the biological effects of the TNF- α cytokine may be regulated by soluble TNF- α receptor, however alteration of receptor levels may modulate physiological role of TNF- α . There has been a notion that the ratio of TNF- α /TNF- α -receptor can predict the disease outcome. The discovery of functional regulatory polymorphisms of TNF- α gene and its receptors have led to a conceptual breakthrough in our understanding of the genetic control of inflammation and its other functions (McDermott et al., 2001). Single nucleotide polymorphisms of TNF- α promoter region have been largely investigated for genetic variation in different carcinomas including UBC, some studies with an association of polymorphism and risk of cancer while others are with contradictory results (Azmy et al., 2004; Gupta et al., 2008; Kohaar et al., 2009; Oh et al., 2000, Saenz-Lopez et al., 2008). However, the connection between TNF- α gene polymorphisms and UBC is controversial. Although, Marsh et al. investigated seven genetic variations in TNF- α gene and found that TNF- α -859T and +488A polymorphisms were significantly associated with UBC risk (Marsh et al., 2003). A study on the Asian population analyzed three SNPs of TNF- α gene and found that TNF- α -1031 T>C polymorphism showed an overall 2.23-fold higher risk of UBC in cases than controls (Ahrwar

et al., 2009). Several studies have investigated the link between TNF- α -308 G>A polymorphism; however, the genotype distribution was similar between cases and controls (Jeong et al., 2004; Kim et al., 2005; Leibovici et al., 2005; Nomomura et al., 2006; Tsai et al., 2001; Wu et al., 2013). Contradictory to these results, Lima et al. found that carriers of TNF- α -308 A alleles had a significant association with UBC risk (OR = 2.80) (Lima et al., 2011)

Peroxisome proliferator-activated receptors (PPARs) gene

PPARs are ligand-activated intracellular transcription factors regulating the expression of genes. PPARs have a significant role in cellular differentiation, development and metabolism (Dunning et al., 2014). There are three known types of PPARs; alpha (α), beta (β) and gamma (γ). A remarkable attention has been given to PPARgamma for its important role in anti-inflammatory response. In addition, PPARgamma has been shown to be expressed in several malignancies considering prostate, lung, breast, bladder and colon and its ligand induces growth arrest through apoptosis in malignant cells (Mansure et al., 2009). Genetic polymorphism in the coding region of PPARgamma c.34 C>G results in an amino acid change (Proline to Alanine at codon 12) that affects PPARgamma's receptor activity. Wang et al. observed that variant PPARgamma CG+GG along with IL-6 CC had a higher risk of developing UBC (Wang et al., 2004). Yoshimura et al. studied PPARgamma expression in human bladder tumor (BT) and normal bladder (NB) tissues and observed that its expression was statistically higher in BT and NB tissue. In addition, a correlation between PPARgamma expression and progression of UBC was also established (Yoshimura et al., 2003). Keeping this relationship in mind, agonists of PPARgamma have been used

as promising therapeutic agents (Mansure et al., 2009; Yoshimura et al., 2003).

Cyclooxygenase-2 (COX-2) gene

COX-2 also known as prostaglandin-endoperoxide synthase (PTGS) has a fundamental role in inflammatory response. COX-2 serves as a mediator of acute and chronic response to inflammation and other actions involved in cellular repair and proliferation (Vane et al., 1998). Therefore pharmaceutical inhibition of COX-2 can provide aid from pain and inflammation. Genetic variations of COX-2 result in aberrant expression of COX-2 that has been found to be associated with carcinogenesis (Tsuji et al., 1998). Kang et al. investigated two polymorphisms, -1166C>G and -1186 T>G promoter region of COX-2 and found that the -1186 T>G was associated with increased risk of UBC (Kang et al., 2005). Gangwar et al. investigated three polymorphisms of COX-2 and observed that COX-2 -765 G>C in the promoter region showed UBC risk particularly in smokers and in patients with aggressive tumor (Gangwar et al., 2011). In a meta-analysis, Wan et al. studied three polymorphisms of COX-2 (-765 G>C, -1195 G>A and +8473 C>T) comprising 4,138 cases and 4,269 controls and found that Chinese and American population having +8473 C>T variation showed a protective association against UBC susceptibility while -765 G>C variation was significantly associated with a higher risk of UBC in Chinese and Indian populations. In Indian population, -1195 G>A polymorphism showed a protective role against the development of UBC (Wan et al., 2015).

Conclusion

In current scenario of molecular epidemiology, several studies have identified genetic risk factors that predispose individuals to higher risk of UBC. However, to explore the cytokines functions and their association with UBC susceptibility much work

is needed to be done. Considering the current understanding and further investigations, the data may be useful for the construction of genetic panels for the risk of UBC and screening of individuals who are at higher risk of UBC as well as differentiation of patients with aggressive and non-aggressive UBC. As cytokines also have a significant role in immunology and inflammation and cancer microenvironment is rich in inflammatory cytokines, studies on genetic polymorphisms in these genes and response to treatments such as chemo and radiotherapy will help to distinguish the treatment responders and non-responders.

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Conflict of interest

The authors have declared that no competing or conflict of interests exist. The funders had no role in study design, writing of the manuscript and decision to publish.

Authors' contributions

KAG and ANS contributed to the concept and writing of the manuscript.

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