Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Mar 05; 8(F):31-36.
https://doi.org/10.3889/oamjms.2020.3280
eISSN: 1857-9655
Category: *F - Review Articles*
Section: *Narrative Review Article*

Vascular Endothelial Growth Factor Polymorphism in Bladder Cancer: A Review

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Edited by: Mirko Spiroski
Citation: Siregar GP. Vascular Endothelial Growth Factor Polymorphism in Bladder Cancer: A Review. Open Access Maced J Med Sci. 2020 Mar 05; 8(F):31-36. https://doi.org/10.3889/oamjms.2020.3280
Keywords: Physicochemical properties; Solid dispersion; Irbesartan; Poloxamer 188
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Received: Jun-26-2019
Revised: Feb-20-2020
Accepted: Feb-27-2020

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

Bladder cancer is one of the most common urinary tract cancers. The main risk factors for bladder cancer are tobacco usage, aging, gender, exposure to chemicals and drugs such as cyclophosphamide and chlornaphazine, chronic bladder problems, and genetics. Genetic factors continue to be studied including vascular endothelial growth factor (VEGF) gene polymorphism. Overexpression of VEGF is known to be higher in bladder cancer patient than healthy individual. It is also associated with tumor progression, metastasis, recurrence, and survival since VEGF and its receptor play a key role in angiogenesis. Many studies evaluated the relationship between VEGF polymorphism and the risk of bladder cancer, but the results were inconsistent because of ethnicity and geographical influences. The present study aims to raise knowledge about the role of VEGF polymorphisms on risk of bladder cancer.

Introduction

The incidence of bladder cancer continues to increase. In the US, around 45,000 men and 17,000 women were diagnosed with bladder cancer each year [1]. In 2009, Ploeg *et al*. showed that more than 2.7 million people had a history of bladder cancer and more than 12 million new cases occurred in the world in 2003. A total of 5.4 million cases occurred in developed countries and 6.7 million cases occurred in developing countries [2], [3]. The incidence of bladder cancer is ranked ninth in the world of all types of cancer [2]. An estimated 386,300 bladder cancers are new cases and around 150,200 bladder cancer died in the world in 2008 [4]. Bladder cancer is more common in men than women, with overall a ratio of 3:1, but ratio could reach 5:1 in certain country. In Iran, 83% bladder cancer occurs in male [5]. Five-year survival rate of bladder cancer was around 77.3% [6].

The most common type of bladder cancer occurs in cells lining called transitional cell carcinoma or urothelial carcinoma. In general, the type of transitional cell carcinoma is not invasive. Another type of bladder cancer is squamous cell (carcinoma originating from thin, flat cells due to inflammation or irritation for months or even years) and adenocarcinoma from the gland. In the US, more than 90% of cancer bladder is a type of transitional cell carcinoma. The remaining 3–8% are squamous cell carcinomas and 1% are adenocarcinoma types. Other types such as sarcoma and small cell carcinoma can also occur, but are very rare [7], [8].

Bladder cancer is also classified based on the invasion of the muscularis propria in the bladder wall. Studies show that 75–80% of cases are non-muscle invasive bladder cancer (NMIBC) which consists of Stage Ta (papillary), T1 (invading into the lamina propria), and carcinoma *in situ* [9]. Meanwhile, MIBC is a more severe condition. Patients with NMIBC generally can be managed with intravesical chemotherapy or immunotherapy and neoadjuvant chemotherapy followed by radical cystectomy with bilateral pelvic lymph node dissection which is a standard of care for MIBC patients [10].

Bladder cancer can cause hematuria, dysuria, frequent urination, and feeling the need to urinate but not being able to pass urine. Symptoms of bladder cancer are often not specific. Cystitis can also occur. The etiology of bladder cancer is not fully known. The main risk factors for cancer bladder are smoking; exposure to chemicals and drugs such as phenacetin,

cyclophosphamide, and chlornaphazine; bladder inflammation due to infections of microbes and parasites such as schistosomiasis; and genetics. Genetic factors such as mutation or polymorphism in various genes have been widely studied [1], [11]. Although many people are exposed to these risk factors, only a few people experience bladder cancer. This indicates the presence of genetic factors that cause variations in susceptibility to bladder carcinogenesis. Several studies found that gene polymorphism is associated with an increased risk of developing cancer, both independently and in combination with other carcinogenic factors. Identifying gene polymorphisms can help predict a person's risk of cancer, one of them is vascular endothelial growth factor (VEGF) gene polymorphism [12], [13].This literature evaluates VEGF expression and VEGF polymorphism in bladder cancer.

Methods

This review included *in vitro* and *in vivo* studies that discuss role of VEGF and VEGF polymorphism on bladder cancer. Level or expression of VEGF could be determined by either blood test, immunohistochemistry, or mRNA level while VEGF polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism technique or other molecular techniques. Only VEGF-A (also known as VEGF) publication was included. Therefore, publications that only examine VEGF-B, VEGF-C, and VEGF-D without mentioning VEGF-A were excluded from the study. We also excluded review article, systematic review, and meta-analysis.

We searched PubMed for suitable papers published from January 1, 1999, to January 1, 2019 (20 years). Keywords used were VEGF, polymorphism, bladder cancer, and their synonym. A total 15 publications were reviewed. Role of VEGF on bladder cancer was first reviewed; then, polymorphism of VEGF on bladder cancer risk was discussed. Method of literature searching can be seen in Figure 1.

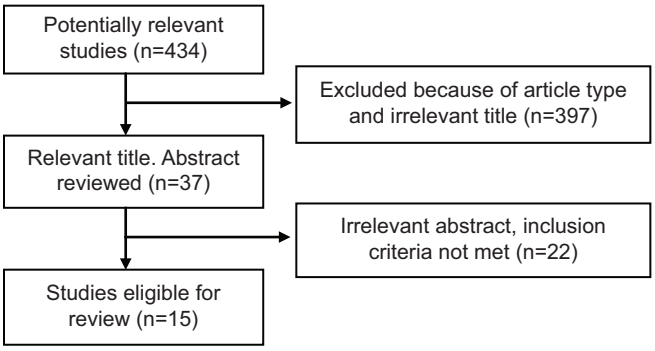

Figure 1: Method of literature searching

VEGF expression in bladder cancer

Angiogenesis is a fundamental process of tumor growth, invasion, and metastasis. The angiogenesis process consists of multiple steps which are controlled by endothelial cells that get an angiogenic stimulus. This endothelial cell migration is accompanied by proliferation and formation of structures that can invade perivascular. Endothelial cell production does not only originate from the division of existing differentiated endothelial cells but also from the influx of bone marrow-derived circulating endothelial progenitor cells [14]. Angiogenesis can occur with sprouting (branching of new capillaries from existing vessels) and non-sprouting processes (cell multiplication within vessel walls) [15]. VEGF is the most potent regulator of angiogenesis [16]. VEGF specifically binds to VEGF receptor tyrosine kinase in endothelial cells to initiate the pathway of intracellular signal transduction that mediates angiogenesis and vascular permeability [13]. The VEGF function is not only for angiogenesis but also increases vascular permeability, induces leukocyte adhesion to the endothelium, and increases chemotaxis of monocyte [17]. In addition, VEGF can also activate NF-κβ and induce the synthesis of various pro-inflammatory cytokines and chemokines [18].

There is an increase of VEGF expression and serum VEGF level in bladder cancer. Compare to healthy individual, patient with bladder cancer has higher serum VEGF level [19]. Expression of VEGF was also higher in bladder cancer specimens than in normal mucosa [20], [21]. Moreover, this higher level of VEGF can also be detected in urine of bladder cancer patient [22]. VEGF signaling is mediated through binding with VEGFR1 and VEGFR2 receptors [23], [24]. Similar to VEGF, increased VEGFR expression is also found in bladder cancer specimen [20], [21]. Therefore, this marker could be used as diagnostic biomarker for patients with bladder cancer [25]. Elevation of both proteins is associated with tumor progression, lymph node or distant metastasis, survival, and recurrence.

VEGF is correlated with both pathologic and histologic state in bladder cancer. Higher VEGF expression tends to have higher T grade (in TMN staging) and higher histologic of bladder cancer [20]. Its serum level is also higher in MIBC than NMIBC and normal patient [19]. Moreover, its overexpression is also associated with lymphovascular invasion and lymph node metastasis [26], [27]. However, other studies also showed the opposite that serum VEGF and its expression were higher in superficial, well-differentiated bladder cancer compared to the invasive, poorly differentiated bladder cancer [21], [28]. This could indicate that elevation of VEGF expression starts since the development of primary tumor. Its level increases until it reaches certain point at advance bladder cancer. After that, there is a reduction in vascular destabilization and decreased formation of new blood vessels, suggesting balance between

vessel regression and vascular growth [29]. VEGF is potential prognostic marker in bladder cancer. Excessive expression of VEGF in tumors is associated with poor prognosis [24], [30]. Furthermore, it is associated with disease-free survival [27]. Patient with overexpression of VEGF has shorter survival without progression [20], [31]. It is also suggested that serum VEGF level could be used as predictor of overall and cancer death and to define high-risk individual that may benefit from prevention therapy [32].

Given the important role of VEGF in bladder cancer pathogenesis, antiangiogenic therapy targeting VEGF and its receptor is developed. Its role continues to be investigated as adjuvant and neoadjuvant therapies for bladder cancer [33], [34], [35].

Role of VEGF polymorphism for bladder cancer risk

Genetic factors continue to be studied for the incidence of bladder cancer. Genetic polymorphism contributes to the risk of developing bladder cancer. Various studies have shown genetic polymorphisms to affect vulnerability and clinicopathological characteristics of bladder cancer, one of them is VEGF gene polymorphism [13], [36]. The VEGF gene is located on chromosome 6p21.3 and consists of 8 exons and 7 introns. More than 30 single-nucleotide polymorphisms (SNPs) of VEGF have been described. The range of encoding genes is approximately 14 kb [13].

Several SNPs have been identified in the VEGF gene. These VEGF SNP positions are shown in Figure 2.

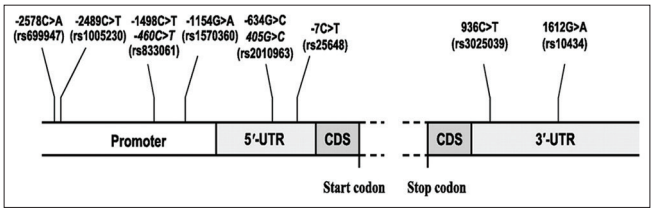

Figure 2: Vascular endothelial growth factor (VEGF) gene structure and VEGF single-nucleotide polymorphisms positions [37]

Some SNPs in the VEGF gene can affect the expression of these genes. Certain allele variations can result in an increase in transcription factors that will bind to the promoter site, this site is the initial site of attachment of the RNA polymerase enzyme that is useful for transcription process. Transcription factors are proteins that control the rate of transcription of genetic information. Transcription factors both alone and together with other proteins in a complex can be as activators of RNA polymerase recruitment, stabilization of RNA polymerase bonds, and catalyzing histone acetyltransferase activity which can increase the rate of transcription so that plasma proteins can increase which can cause a disease or accelerate the progression of the disease [38]. Promoter is a part of DNA that facilitates gene transcription. The base pair sequence of the promoter determines the efficiency of binding with RNA polymerase and thus determines the transcription efficiency. In addition, SNPs located in the 5' untranslated region (UTR) and 3' UTR can also affect VEGF levels. 5'UTR is a regulator DNA region where the genes coding for the protein will be transcribed into mRNA. Polymorphism involving allele changes in 5'UTR of VEGF gene will cause the appearance of SP-1 (CCACC box) which is a transcription factor. The variation of alleles on 5'UTR can cause an increase in transcription factors. Variation of alleles on 3'-UTR of the VEGF gene can affect the stability of mRNA and is associated with the hypoxic induction of VEGF. Human antigen R (HuR) proteins are considered to play a role in mRNA stabilization and prevent mRNA from being attacked by RNAse. HuR proteins also increase the binding of VEGF mRNA to the nucleus and increase the export of VEGF mRNA in hypoxic-induced angiogenesis [39], [40].

Renner *et al*., in Austria (2000), examined the relationship of VEGF +936C>T polymorphism in the 3-UTR of the VEGF gene with VEGF plasma levels in 23 healthy individuals. VEGF plasma levels were significantly lower in carriers of the 936T allele than in non-carriers [41]. Krippl e*t al*.'s study in Austria (2003) of 500 breast cancer patients and 500 healthy controls showed that subjects with the T allele of VEGF +936C>T polymorphism had significantly lower VEGF plasma levels and it was proved that individuals with T allele were protective against breast cancer [42]. A study by Koukourakis on lung cancer patients in Greece reported that the −2578CC, −634GG, −1154AA, and GA genotypes in the VEGF gene were associated with low VEGF expression, while the −2578CA, −634 GC, and −1154GG genotypes were associated with high VEGF expression [43]. Research by Awata *et al*. (2002) in Japan showed that VEGF serum levels increased significantly in 118 healthy individuals with −634 CC genotypes compared with GC and GG genotypes. Meanwhile, VEGF +936C>T and +1612G>A polymorphisms were not associated with VEGF levels [44].

Yang *et al*. reported that AA genotype of VEGF −15,648A>C significantly increased the risk of 1.75 times experiencing bladder cancer in Chinese ethnic [45]. A study by Garcia-Closas *et al*., in Spain, also found that individuals who had AA genotype of VEGF −15,648A>C polymorphism significantly increased the risk of 2.52 times having bladder cancer [46]. This result is similar to the research conducted by Fu *et al*., in China, that AC genotype (odds ratio [OR] = 1.49; 95% confidence interval [CI] = 1.25–1.87; p ≤ 0.001), AA genotype (OR = 2.1; 95% CI = 1.41–2.86; p ≤ 0.001], and CA + AA genotypes (OR = 1.65; 95% CI = 1.23–2.12; p ≤ 0.001] of VEGF −15,648 A>C polymorphism associated with an increased risk of bladder cancer [47].

Garcia-Closas *et al*. found that individuals who had TT genotype of VEGF −7C>T polymorphism increased the risk of 5.11 times getting bladder cancer.

Meanwhile, Jaiswal *et al*., in India, and Fu *et al*., in China, showed that the VEGF −7C>T polymorphism was not associated with the risk of bladder cancer [46], [47], [48].

The study by Longo *et al*., in Italy, of 46 bladder cancer patients and 100 controls found that the combination of TT and CT genotypes in the VEGF +936C>T polymorphism increased the risk of 2.16 times for bladder cancer [49]. However, the results of research conducted by Longo *et al*. different from the results of research conducted by Yang *et al*., in China, Garcia-Closas *et al*., in Spain, and Wafi *et al*., Tunisia, where they found that the VEGF +936C>T polymorphism had no significant association with an increased risk of bladder cancer [45], [46], [50].

Several studies were also conducted to evaluate the relationship between the polymorphism of VEGF −2578C>A and the incidence of bladder cancer. In a study conducted by Fu, in China, it was found that there was a relationship between CA genotype (OR = 1.33; 95% CI: 1.05–1.71; p = 0.012), AA genotype (OR = 2.35; 95% CI = 1.57–3.16; p ≤ 0.001), and CA + AA genotype (OR = 1.70; 95% CI: 1.16–2.31; p ≤ 0.001) of VEGF −2578C>A polymorphism with an increased risk of bladder cancer. This is also in line with Jaiswal's research that there was a significant association between CA genotype of VEGF −2578C>A polymorphism (OR = 1.69; 95% CI = 1.02–2.80; p = 0.044) and an increased risk of bladder cancer [37], [38]. In contrast to those data, Wafi *et al*. found a significant reduction in risk for bladder cancer in subjects with CA genotype (OR = 0.62; 95% CI = 0.41–0.94, p = 0.026) and AA genotype (OR = 0.40, 95% CI = 0.21–0.76, p = 0.005) of VEGF −2578C>A polymorphism [50]. Meanwhile, the research conducted by Henríquez-Hernández *et al*. showed that VEGF −2578C>A polymorphism was not associated with the incidence of bladder cancer [51]. Other VEGF polymorphisms such as TT genotype of VEGF −9228G>T and TT genotype of VEGF −8339A>T were associated with an increased risk of bladder cancer, but CT genotype of VEGF +1378C>T was associated with a reduced risk of bladder cancer [46]. There was no association between VEGF −1498C>T and −634G>C polymorphisms with the risk of bladder cancer [47], [49]. The previous studies indicated potential associations between VEGF polymorphism and risk of bladder cancer; however, the results were inconclusive. Various studies that examined association between VEGF polymorphism and cancer risk showed different results due to ethnicity and geographical factors differences between studies [52].

Conclusion

Angiogenesis is a fundamental process of tumor growth, invasion, and metastasis. VEGF is a potent regulator of angiogenesis. There is an increase of VEGF expression in bladder cancer that associated with tumor progression, metastasis, recurrence, and survival. VEGF gene is a highly polymorphic and VEGF gene polymorphism that has been shown to affect the expression of VEGF proteins, which can affect the risk of cancer, tumor growth, and progression. The previous studies indicated potential associations between VEGF polymorphism and risk of bladder cancer. VEGF polymorphism might be an important risk factor for the initiation and progression of bladder cancer. The result of the previous studies showed inconsistent results due to differences in ethnicity and geographical factors between studies so that research needs to be done on each ethnic group.

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