



Identification of Conserved Residues on the Interaction Interface of VEGF with Urological Cancer-Related Proteins by a Systems Biology Approach

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Abstract

Cancer is a costly and complex public health problem worldwide and urological cancer is responsible for more than 19% of all the types of cancer. The most common urinary cancers affect the prostate, bladder and the kidneys. Tumorous cells rely on oxygen and nutrients availability and the elimination of metabolic products mediated by angiogenesis. Several genes and proteins are related to angiogenesis. VEGF is one of the major proteins that regulate angiogenesis processes and several other genes regulate VEGF. The proteins MDM2, CRYAB and BRCA1 expression interacts with VEGF and dysregulation of such interactions, perhaps through the presence of genetic variation, may bring a higher susceptibility to cancers. Polymorphisms within the coding sequence of those genes might alter expression, protein structure and PPI patterns and consequently diseases. Here, we have shown a systems biology approach for interaction between VEGF and the urological cancer-related proteins MDM2, CRYAB and BRCA1 through the identification of hot spots within the interface of interaction of VEGF and target proteins. Several SNPs identified in clinical practice and deposited on dbSNP were related to the predicted hot spots in the present research. Further analyses should be performed and through the hot spots presented here, the proteins under study could be used as molecular markers and peptide PPI modulators could be design and tested as alternative therapies against urological cancers.

Keywords: VEGF; MDM2; CRYAB; BRCA1; Systems biology; Hot spots

Introduction

Cancer is a costly and complex public health problem worldwide, with an estimation of more than 8.1 million new cancer cases and 9.6 million cancer-related deaths in 2018 [1]. Urological cancer is responsible for more than 19% of all the types of cancer [2]. The most common urinary cancers affect the prostate, bladder and the kidneys. Prostate cancer statistics depends on age and ethnicity. It accounts for 7.1% of male cancers and is the second leading cause of cancer-related deaths in males [1,3]. Bladder cancer is the eighth most frequent carcinoma and the thirteenth most common cause of cancer-related mortality in both males and females [4]. Kidney carcinomas are prevalent over 3% in adults and its incidence depends on factors such as gender, ethnicity, hypertension and chronic kidney disease [2,5]. Overall, urinary cancer brings poor quality of life and high cost to the health care system [6,7].

Tumorous cells rely on oxygen and nutrients availability and the elimination of metabolic products mediated by angiogenesis. Angiogenesis is the process that guarantees energy supply in order to maintain normal levels of biochemical and physiological metabolism in cells and can also be an important factor in disease onset, such as chronic inflammation [8], arthritis [9], cancer [10], and degenerative anomalies [11]. It is long known that tumorous cells use substances that induce angiogenesis from the host vasculature [12]. Interestingly, tumor blood vessels exhibit genetic characteristic markers that are not present in normal blood vessel tissues [13], and polymorphisms in the coding sequence of certain genes, such as VEGF (vascular endothelial growth factor) [14], may play important roles in diseases related to angiogenesis [15].

Tumorous cells signal activating alters the vascular ability of liquids or gases to pass through it [16]. VEGF is a growth factor belonging to the platelet-derived growth factor family. The protein regulates vasculogenesis and angiogenesis. The former plays roles on formation of the embryonic

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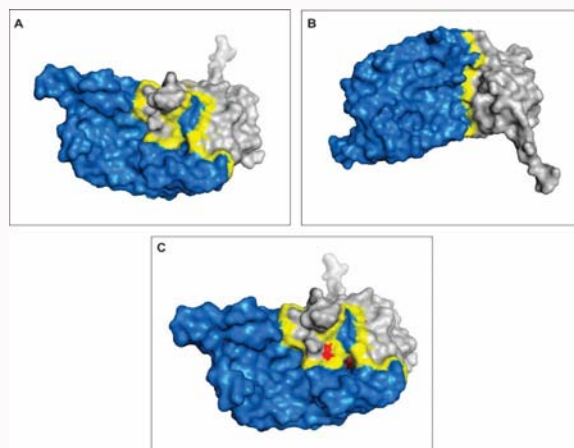


Figure 1: Surface 3-D structure of the VEGF interaction with MDM2. A and B: The interface of interaction (yellow) between VEGF (blue) and MDM2 (gray). C: Hot spots residues on the interface of interaction (red). Hot spots accounts for most of the free binding energy between the interacting proteins.

vascular system while the latter is responsible of the growth of vascular vessels that supplies tissues with oxygen, nutrients and metabolites [17]. VEGF also takes part in process that tries to restore the oxygen and nutrient supply to cells when blood circulation is insufficient during hypoxia, for instance [18]. The VEGF protein comprises two main domains, a VEGF heparin-binding domain and a PDGF (Platelet-Derived Growth Factor)/VEGF domain [19]. The former is located at the C-terminal region of the VEGF protein, the domain is related to the vascular functions of the protein, besides playing a role on neurogenesis and neuron survivability [20-22]. The PDGF/VEGF domain gives rise to a growth factor featuring potent mitogens for cells of mesenchymal origin. The structure is a homodimer with conserved cysteines involved in inter- and intra-molecular interactions [23-25].

Diseases can result from disrupted Protein-Protein Interactions (PPI). PPIs virtually govern all biological functions once proteins almost never function alone. They depend on signaling pathway [26,27], transportation [28], secretion [29] and post-translation modifications in order to accomplish their roles in cells [30]. The interfaces of interaction between proteins that bind are featured by the presence of hot spots. These regions account for the binding free-energy between favoring the interaction [31]. VEGF has been shown to take part in more than 70 physical protein networks [32]. Among VEGF interactors, MDM2 (Mouse Double Minute 2) [33], CRYAB (Alpha-Crystallin B Chain) [34,35] and BRCA1 (Breast Cancer 1) stand out due to their relation to cancer and other diseases [36]. Polymorphisms within the coding sequence of genes might alter expression, protein structure and PPI patterns. Several studies have shown the association between polymorphisms and cancer risk [37-42].

Here, we study an in silico systems biology approach of the interaction interfaces of PPIs between the angiogenesis-related protein VEGF and the proteins MDM2, CRYAB and BRCA1. All of them known to have cancer-related polymorphisms. We identified important hot spots within the interaction interfaces analyzed and compared hot spot residues with clinical significant polymorphisms of those proteins regarding urologic cancers. Such approach is important to design molecules to modulate VEGF interactions and drive diagnosis and treatment into a more efficient prognosis.

Materials and Methods

The 3-D structures of the proteins VEGF, CRYAB and BRCA1

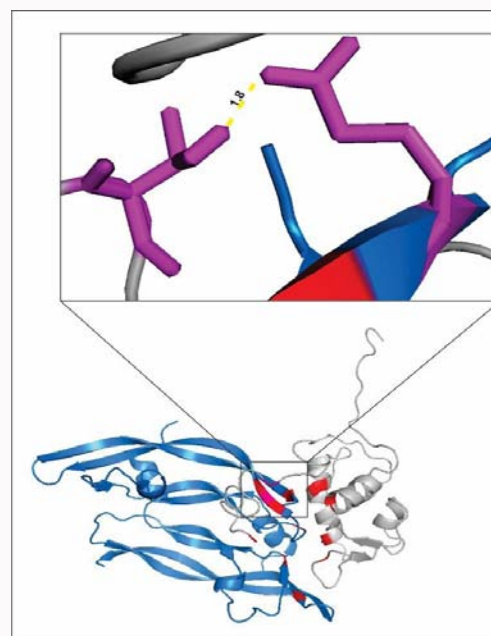


Figure 2: Hot spots residues from the VEGF-MDM2 complex and their interactions. The conformational structure of the binary complex formed by VEGF and MDM2 (bottom). The selected conformation is the one with the lowest energy and shows VEGF (blue) and MDM2 (gray) hot spots within the interface of interaction. Hot spots are represented by the color red and residues in pink are interacting amino acids (top). Here, GLU 67 from VEGF interacts with THR 16 from MDM2, possibly by a hydrogen bond. The distance between the residues is 1.8 Å.

used in the analysis were retrieved from the PDB (protein databank; <https://www.rcsb.org/>). The KBDock server was used to identify protein domains and interaction between protein domains [43]. ClusPro server was used in order to identify the best dual protein complex conformation between VEGF and partner proteins [44]. We used PyMol (<https://pymol.org>) for the analysis of the interaction interface, hot spots and the polymorphic residues.

Hot spots residues were identified by the KFC2 server through analysis and calculations of the biochemical environment around each residue in the binary structure and comparison with hot spots identified experimentally. K-FADE and K-CON were parameters used to predict the hot spots; they are based on conformation specificity

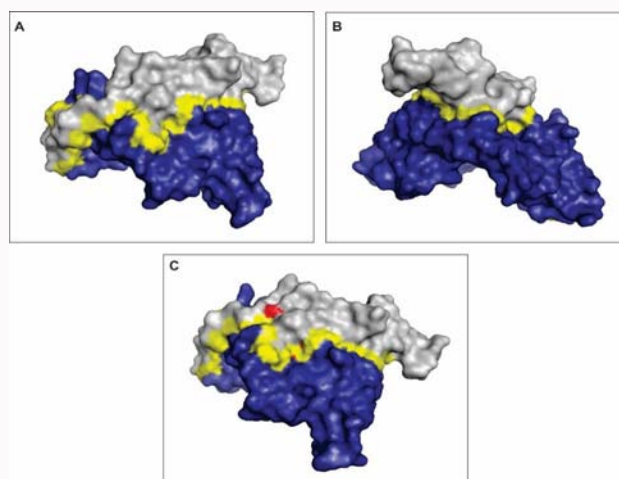


Figure 3: Surface 3-D structure of the VEGF interaction with CRYAB. A and B: The interface of interaction (yellow) between VEGF (blue) and CRYAB (gray). C: Hot spots residues on the interface of interaction are shown red.

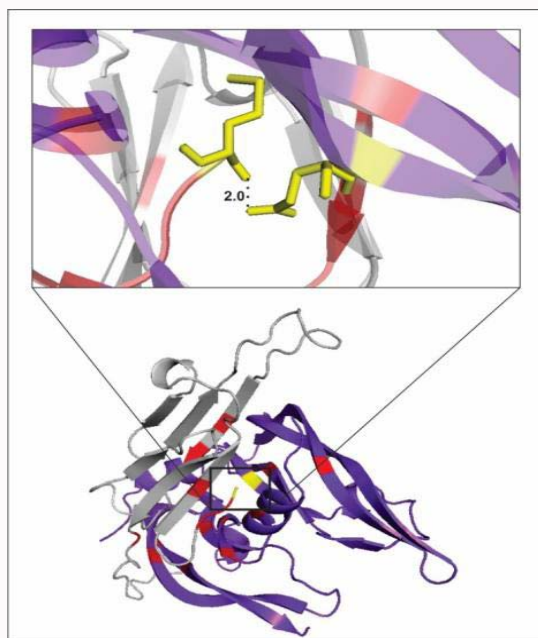


Figure 4: Hot spots residues from the VEGF-CRYAB complex and their interactions. The conformational structure of the binary complex formed by VEGF and CRYAB (bottom). The selected conformation is the one with the lowest energy and shows VEGF (purple) and CRYAB (gray) hot spots within the interface of interaction. Hot spots are represented by red and residues in yellow are interacting amino acids (top). Here, GLU 30 from VEGF interacts with MET 68 from MDM2, possibly by a hydrogen bond. The distance between the residues is 2.0 Å.

and biochemical features, respectively [45]. Polymorphic residues were identified through the dbSNP (database of single nucleotide polymorphism; <https://www.ncbi.nlm.nih.gov/SNP>).

Results and Discussion

Interaction between VEGF and MDM2

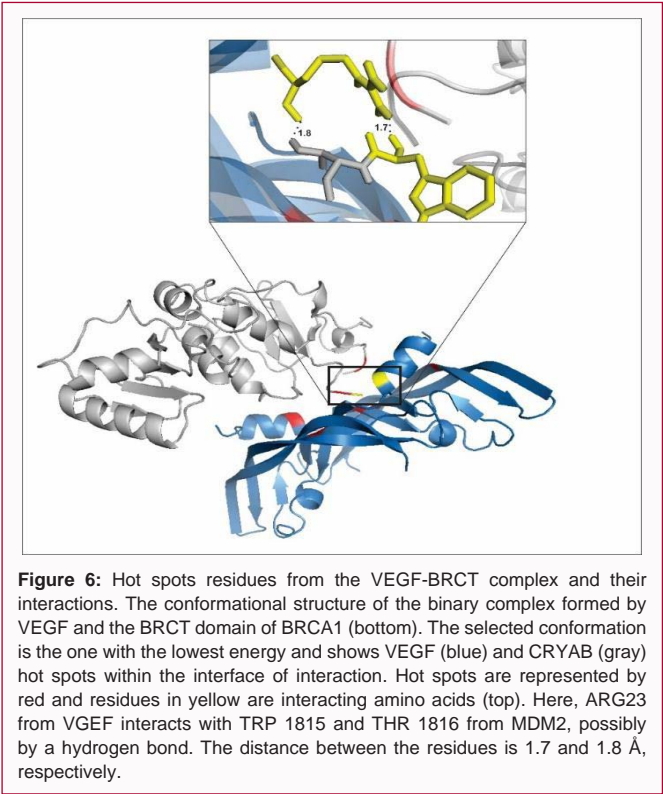
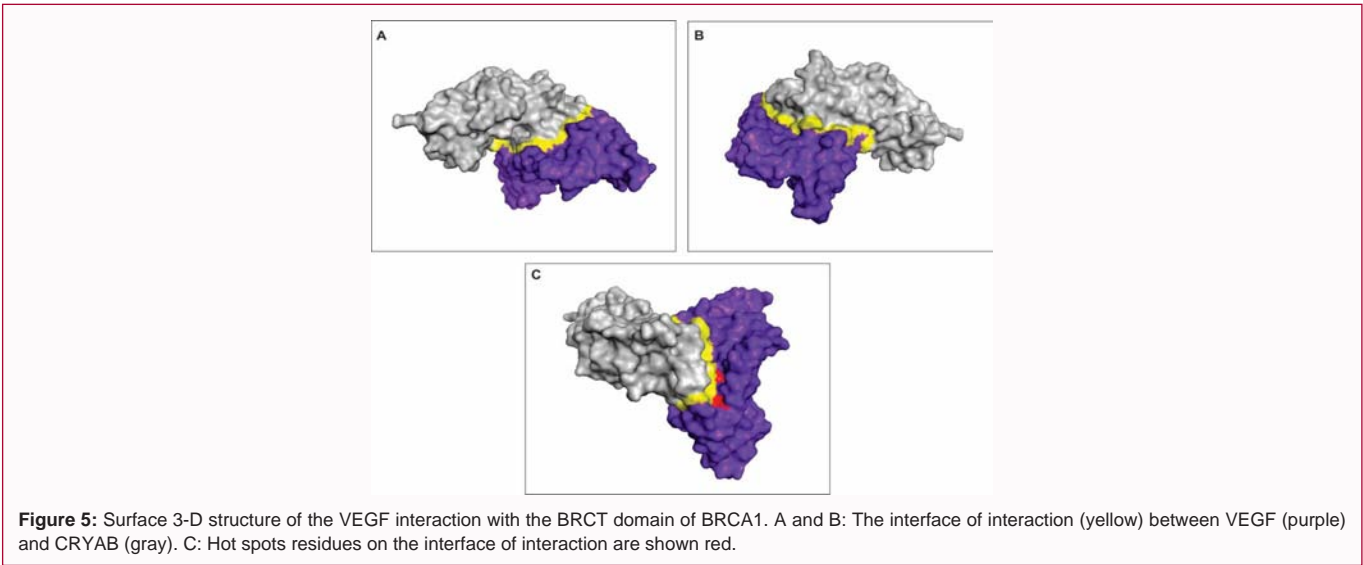
Both proteins are involved in tumor progression [14,46] and they have polymorphisms already described to be related to the disease [47,48]. MDM2 is mainly a nuclear phosphor protein with several conserved functional domains, including two zinc finger domains and a SWIB domain. The latter is an ATP-dependent chromatin-

remodeling protein domain that facilitates transcription activation. It is also related to response to DNA damage, cell cycle arrest and apoptosis [49]. MDM2 is mainly related to brain, breast, ovary, cervix, lung, colon, prostate, and bone cancers. MDM2 expression is also found in cancers associated with a Single Nucleotide Polymorphism (SNP) [50]. MDM2 expression correlates with increased levels of VEGF, which facilitates increased rates of vascularization and metastasis [51,52]. MDM2 is known to induce VEGF expression. During hypoxia, MDM2 is transported into the cytoplasm and induce an increased expression of VEGF in tumorous cells [53]. Moreover, MDM2 and VEGF expression is increased, due to SNPs, in tumors featuring high metastatic and angiogenic activity, such as prostatic [54] and bladder cancers [55].

Figure 1A and 1B shows the interface of interaction between VEGF and MDM2. The stability of the binary protein complex guarantees a normal function of MDM2 regarding regulation of VEGF. Defining the binding regions and biochemical properties of the interaction may give insights of the functional consequences of SNPs and disease susceptibility. We found 13 hot spot within the VEGF-MDM2 interaction interface, some of them are represented in Figure 1C, and 62% of those hot spots are polymorphic (Table 1). SNPS might be related to an increased susceptibility to cancer due to alterations in the conformational stated of the protein and hence inefficient interaction with partners [56]. All the hot spot residues found in the interaction interface of VEGF-MDM2 are represented in Figure 2 (bottom). Figure 2 (top) also shows an example of a hot spot residue from VEGF (GLU 67) interacting with an MDM2 residue (THR16), possibly through a hydrogen bond, since it is shorter than 2.5 Å.

Interaction between VEGF and CRYAB

CRYAB has been identified in various types of cancers and it has been suggested this protein could be a prognostic marker of cancer [57,58]. The CRYAB has also cytoprotective functions as a chaperone responsible for renaturation of structurally unfolded or misfolded proteins [59] and for protection against damage caused by several types of stress [60]. Crystallins interacts with other proteins forming multi-protein complexes with roles on apoptosis, cellular protection, and proteasomal interactions [61]. In addition, CRYAB is another protein that modulates VEGF expression, being intimately related



to VEGF expression and influencing cancer onset and progression [62]. CRYAB induces the VEGF expression, mainly in cancers with poor prognosis and metastasis, once crystallins are related to cell movement as well [63-65]. The expression of CRYAB is active in genitourinary cancers, most frequently in renal carcinomas [66-68].

The region of interaction between VEGF and CRYAB is quite large (Figure 3A and 3B). A stable interaction accounts for a normal activity of both proteins and allows cells to function properly. CRYAB is a VEGF regulator and prevents angiogenesis overexpression [62]. Dysregulation of any of those proteins, such as SNPs within a hot spot region, may interfere with CRYAB regulating VEGF expression and predisposing a cell to turn into a tumorous cell. We found 23 hot spots within the VEGF-CRYAB interface of interaction (Figure

Table 1: Hot spots prediction in the interface of interaction between VEGF and MDM2.

Chain	Residues	KFC ^a	KFC ^b	SNPs ^c	SNP ID
A	MET 1	1.01	0.06	Val	rs1279748757
A	LEU 54	1.2	0.24	synonymous	rs1321937114
A	MET 62	0.78	0.18	-	-
A	ASP 68	0.68	0.04	-	-
A	GLN 72	1.18	0.23	-	-
A	HIS 96	1.1	0.22	-	-
E	ASN 62	1.73	0.14	ASP	rs368814156
E	GLU 67	1.27	0.24	GLY	rs754368592
E	VAL 69	0.92	0.04	synonymous	rs1399664823
E	ARG 105	1.68	0.35	synonymous	rs1424048096
F	LYS 48	0.52	0.13	GLN	rs1359856307
F	MET 81	0.44	0.15	-	-
F	ILE 83	1.33	0.21	LEU	rs767587788

^aHot spot prediction based on structure features; ^bHot spot prediction based on biochemical features; ^cSingle nucleotide polymorphisms

3C) and 65% of those are polymorphic (Table 2). Figure 4 shows the folding 3-D structure of the binary complex formed by CRYAB and VEGF. The top part of Figure 4 highlights a possible way of interaction between residue GLU 30 from the VEGF protein and residue MET 68 from the CRYAB protein (Table 2). Both residues were predicted as hot spots by KFC2. Many other polymorphisms were found for CRYAB and VEGF proteins (data not shown); however, SNPs within the interaction interface are more prone to increase cancer susceptibility.

Interaction between VEGF and BRCT domain of BRCA1

The C-terminal domain of the Breast Cancer Susceptibility Protein (BRCT) has a role on checkpoints of cell cycle, which is essential for DNA repair, homologous recombination [69] and PPI modulation [70]. BRCA1 has been shown to down-regulate the VEGF expression [71]. Suppression of BRCA1 protein, such as in many cancer types, increases intracellular level of VEGF, which could supply oxygen and nutrients to tumorous cells. BRCA1 and VEGF have been implicated in prostate adenocarcinoma [72], and another factor that correlates both proteins is that BRCA1 has a role in hypoxic response, and

Table 2: Hot spots prediction in the interface of interaction between VEGF and CRYAB.

Chain	Residues	KFC ^a	KFC ^b	SNPs ^c	SNP ID
A	MET 68	1.89	0.27	LEU	rs1291858452
A	ARG 69	1.5	0.31	CYS	rs139750142
A	ARG 69	1.5	0.31	HIS	rs987496548
A	LEU 70	1.91	0.18	-	-
A	VAL 77	1.05	0.1	-	-
A	ARG 120	1.25	0.28	GLY	rs104894201
A	TYR 122	0.83	0.32	synonymous	rs1326039597
A	ARG 123	1.75	0.36	TRP	rs534473091
A	ARG 123	1.75	0.36	GLN	rs782206421
A	ARG 149	0.71	0.15	-	-
E	GLU 30	1.78	0.25	rs200030087	rs200030087
E	MET 55	1.41	0.35	LEU	rs1357987443
E	MET 55	1.41	0.35	THR	rs374057152
E	MET 78	0.12	0.17	-	-
F	ARG 23	1.64	0.29	CYS	rs1208889729
F	ARG 23	1.64	0.29	HIS	rs1267535717
F	HIS 27	0.76	0.22	-	-
F	GLU 30	1.07	0.18	synonymous	rs200030087
F	LEU 54	0.12	0.11	-	-
F	MET 94	1.79	0.38	LEU	rs1357987443
F	MET 94	1.79	0.38	THR	rs374057152
F	ILE 76	0.7	0.16	-	-
F	GLN 98	1.02	0.22	-	-

^aHot spot prediction based on structure features; ^bHot spot prediction based on biochemical features; ^cSingle nucleotide polymorphisms

VEGF is induced in such conditions.

Figure 5A and 5B show the interface of interaction between VEGF and the BRCT domain of BRCA1. The stability of the complex is maintained by hydrophobic interactions established between amino acid residues within the interface of interaction, which is predicted to have 12 hot spot residues (Figure 5C). Interestingly, 75% of the hot spots are polymorphic (Table 3) and this genetic variation could be the cause for cancer predisposition, including urological tumors [37]. Figure 6 shows the binary complex formed by VEGF and BRCT domain of BRCA1. The zoomed-in region highlights two sites of interaction that are prone to be hydrogen bonds between hot spots residues presents on both sides of the interaction interface. Again, genetic variation in hot spots clusters could significantly increase risk of cancer.

Conclusion

Cancer is a costly and complex public health problem worldwide. VEGF, MDM2, CRYAB and BRCA1 are potential markers for cancer diagnosis and therapeutic approaches. These proteins are involved in genomic stability controlling cell division, cell cycle progression and arrest, angiogenesis and response to stressors such as hypoxia. PPIs are fundamental factors for a variety of function within cells, including DNA homeostasis and disease onset and progression. Here, we have shown a systems biology approach for interaction between VEGF and the urological cancer-related proteins MDM2, CRYAB and BRCA1 through the identification of hot spots within the

Table 3: Hot spots prediction in the interface of interaction between VEGF and BRCT domain of BRCA1.

Chain	Residues	KFC ^a	KFC ^b	SNPs ^c	SNP ID
E	ARG 23	0.75	0.06	CYS	rs1208889729
E	ARG 23	0.75	0.06	HYS	rs1267535717
E	GLU 30	1.46	0.27	synonymous	rs200030087
E	MET 55	0.6	0	LEU	rs1357987443
E	MET 55	0.6	0	THR	rs374057152
E	MET 78	0.17	0.08	-	-
F	ARG 23	1.64	0.35	CYS	rs1208889729
F	ARG 23	1.64	0.35	HYS	rs1267535717
F	GLU 30	1.23	0.14	synonymous	rs200030087
F	MET 55	0.95	0.09	-	-
X	TRP 1815	1.91	0.27	nonsense	rs80356962
X	PHE 1821	1.65	0.34	-	-

^aHot spot prediction based on structure features; ^bHot spot prediction based on biochemical features; ^cSingle nucleotide polymorphisms

interface of interaction between VEGF and target proteins. Several SNPs identified in clinical practice and deposited on dbSNP were related to the predicted hot spots in the present research. Further analyses should be performed and through the hot spots presented here, the proteins under study could be used as molecular markers and peptides PPI modulator could be design and tested as alternative therapies against urological cancers.

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