Original Research

In silico Modulation of the Interaction Between VEGF and eNOS Proteins in Atherosclerosis as a Future Diagnostic and Therapeutic Approach

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Abstract

Atherosclerosis is related to other cardiovascular diseases such as angina, infarction, hypertension and cardiac death. Several risk factors influence atherosclerosis onset and progression, the main ones are diabetes, sedentary lifestyle, high blood pressure, obesity, alcohol consumption, smoking and cholesterol. Atherosclerosis is a globally serious health problem, affecting over millions of people worldwide. Endothelial dysfunction and angiogenesis may be associated with genetic polymorphisms that take place in genes and proteins with essential roles in maintaining endothelial and cardiovascular homeostasis. Among these genes, eNOS and VEGF stand out, being responsible for the synthesis of nitric oxide and angiogenesis, respectively. Dysfunction affecting any of those proteins, their structures or the protein interaction patterns they establish may increase atherosclerosis and other diseases susceptibility. Here, we perform an in silico analysis of the interaction interfaces between angiogenesis-related protein VEGF and the nitric oxide production protein eNOS. VEGF and eNOS play a role in the onset of atherosclerosis and they present polymorphisms that could increase the susceptibility to cardiovascular diseases such as atherosclerosis. We identified important hot spots within the interaction interface of VEGF and eNOS. We also compared hot spot residues with clinical significant polymorphisms of those proteins regarding the onset and progression of atherosclerosis. Finally, we designed peptides that could modulate the interaction of VEGF and eNOS as a perspective of future treatment and better prognostic for atherosclerotic patients.

Keywords

Atherosclerosis; Hot Spots Systems Biology; eNOS; VEGF

Introduction

Atherosclerosis is a multifactorial disorder with a complex etiology influenced by genetic [1], physiological [2,3], immunological [4] and environmental [5] aspects. The incidence of cardiovascular diseases has increased over the years due to non-traditional risk factors such as inflammation [6,7], oxidative stress [8,9], infectious agents [7,10]. The development of atheromatous plaques arteries and the consequent reduced supply of oxygen-rich blood to the affected tissues defines the atherosclerotic disease. The so-called traditional risk factors, such as hypertension
[11,12], smoking [13,14], increased glycaemia [15] and dyslipidemia [11], also influence the atherosclerosis onset and development. Atherosclerosis risk factors induce the expression of inflammatory cytokine genes [4], Reactive Oxygen Species (ROS) [8] and lipid oxidation [16,17] leading to an altered endothelium metabolism.

Atherosclerosis lead to severe clinical complications such as thrombosis [18,19], stenosis [20], infarction [21], stroke [22] and kidney failure [23]. The incidence and high mortality rates among patients with atherosclerosis are well established. Genetic [1], hormonal [24], environmental factors [5] and the phenotype of the individual influence the onset and development of the disease [25,26]. Regarding the genetic factors, hundreds of genes have been described in association with the disease and several influence the regulation of the endothelial function [25], angiogenesis [27], inflammation [6,7] and the metabolism of organic molecules such carbohydrates, lipids and as amino acids [28]. Endothelial dysfunction leads to major changes in the endothelial phenotype, which is a result of variation in the expression of certain genes such as eNOS (endothelial Nitric Oxide Synthase) and VEGF (Vascular Endothelial Growth Factor).

The eNOS coding gene is located on chromosome 7. The eNOS gene is responsible for the synthesis of nitric oxide, which has lipophilic substance highly active in a great variety of physiological processes [29,30]. The nitric oxide produced by eNOS protein activity regulates the vascular tone [31,32], cell cycle progression [33,34], immune system cell adhesion [35] and platelet aggregation [36,37]. Anomalies in the eNOS gene lead to endothelial dysfunction, which influences atherogenesis through regulation of the endothelial metabolism, reduction in nitric oxide levels and increased production of ROS. Nitric oxide is a promising therapeutic agent as it shows protective and anti-atherogenic effects. Genetic polymorphisms of eNOS alter nitric oxide levels in plasma, influencing the onset of several diseases such coronary artery disease [38], infarction [39], diabetes [40], hypertension [41] and atherosclerosis [42].

Angiogenesis provides energy supply in order to maintain physiological homeostasis in tissues. The process is also important for disease development such as chronic inflammation [7], infarction [21], cancer [43], degenerative diseases [44], cardiovascular diseases [45] and atherosclerosis [46]. VEGF is a growth factor featuring a signal protein that regulates the blood vessel formation, which supplies cells with oxygen and nutrients [47]. Blood concentration of VEGF is elevated in certain disease such as asthma [48], diabetes [49,50], cancer [51] and atherosclerosis [52,53].

Here, we perform an in silico analysis of the interaction interfaces between angiogenesis-related protein VEGF and the nitric oxide production protein eNOS. VEGF and eNOS influence the onset of atherosclerosis and they present polymorphisms that could increase the susceptibility to cardiovascular diseases such atherosclerosis. We identified important hot spots within the interaction interface of VEGF and eNOS. We also compared hot spot residues with clinical significant polymorphisms of those proteins regarding the onset and progression of atherosclerosis. Finally, we designed peptides that could modulate the interaction of VEGF and eNOS as a perspective of future treatment and better prognostic for atherosclerotic patients.

Materials and Methods

Briefly, the 3-D structure of the protein VEGF used in the analysis were retrieved from the PDB (Protein Databank; https://www.rcsb.org/). The 3-D structure of the protein eNOS was modeled by the I-TASSER server. We used KBDOCK to identify protein domains and interaction between protein domains [54]. ClusPro server was used in order to identify the best stable protein complex conformation between VEGF and eNOS [55]. We used PyMol (https://pymol.org) for the analysis of the interaction interface, hot spots, the polymorphic residues and the manual design of modulating peptides.

Hot spots residues were identified by the KFC2 server. KFC2 analyzes the biochemical environment around amino acid residues within the interface of interaction and compares energetically favorable hot spots with residues that were previously experimentally identified. There are two scores for the KFC2, K-FADE and K-CON. Those scores are used to predict the hot spots and they are based on conformation energy and biochemical properties of possible hot spot amino acid residues [56]. Polymorphic residues that are clinically important for atherosclerosis were identified by the dbSNP databank (database of Single Nucleotide Polymorphism; https://www.ncbi.nlm.nih.gov/SNP).

Results and Discussion

The VEGF protein binds to protein partners and thus activates intracellular pathways in order to perform its functions related to angiogenesis [57]. It has been shown that VEGF is a mediator of nitric oxide and eNOS is regulated by VEGF receptor [58]. Here, we propose an in silico mode of interaction between VEGF and eNOS (Figure 1). Figure 1 shows the interface of interaction between VEGF and eNOS. The stability of the protein complex accounts for a normal function of eNOS and nitric oxide production [58]. The identification of the binding regions and the chemical properties of residues within the
interaction interface shed some light on the relation between polymorphism and atherosclerosis susceptibility.

Figure 1: The interaction between VEGF and eNOS proteins.

The 3-D structure represented by pink corresponds to eNOS and the green one to VEGF. The blue region is the interface of interaction between VEGF and eNOS. The contact region between these proteins are stabilized by intermolecular interactions, it is a large region that comprises several hot spot amino acid residues helping to maintain the stability of the conformational structure of the complex.

We found 15 hot spot amino acid residues within the VEGF-eNOS interaction interface (Table 1). Single Nucleotide Polymorphism (SNP) within the interface of interaction interfere with protein-protein interactions and affect the stability of the complex, thus, altering the efficacy of the biological function performed by the protein complex [59]. Among the hot spots, 60% are polymorphic and present clinically important SNPs (Figure 2). Genetic variation might increase the susceptibility to atherosclerosis due to loss of the protein complex function due to conformational anomalies and hence inefficient interaction with partners [60].

<table>
<thead>
<tr>
<th>Hot Spot</th>
<th>Residue number</th>
<th>Score 1’</th>
<th>Score 2’</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARG</td>
<td>98</td>
<td>0.47</td>
<td>0.05</td>
<td>CYS, HIS</td>
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<tr>
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<td>0.18</td>
<td>GLN, TRP,</td>
</tr>
<tr>
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<td>0.14</td>
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<td>187</td>
<td>1.76</td>
<td>0.36</td>
<td>GLN, GLY, TRP</td>
</tr>
<tr>
<td>TRP</td>
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<td>1.40</td>
<td>0.27</td>
<td>nonsense</td>
</tr>
<tr>
<td>LEU</td>
<td>32</td>
<td>1.02</td>
<td>0.23</td>
<td>-</td>
</tr>
<tr>
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<td>33</td>
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<td>0.07</td>
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<tr>
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<td>0.03</td>
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</tr>
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<tr>
<td>HIS</td>
<td>99</td>
<td>0.97</td>
<td>0.26</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Hot spot residues identified on the VEGF-eNOS interaction.
*Scores refer to values related to conformational states and biochemistry of the amino acid residues classified as hot spots.
Some of the polymorphic hot spots located in the interface of interaction between VEGF and eNOS are represented in yellow. The red residues correspond to hot spots that do not present clinically important genetic variation. The interface of interaction is represented in blue, the pink protein is eNOS and the green one is VEGF.

SNPs that alter hot spot residues may change the conformational structure of the hot spot region of a protein, as shown in figure 3. The wild type complex between VEGF and eNOS do not establish a bond between TRP 190 and LEU 32, as the distance between the atoms of those residues are higher than 2.5Å. When one of those hot spots residues vary, or even neighbor residues vary, there is a conformational change that implicate in a less stable complex that affects the normal function of VEGF and eNOS. The variation in their expression may lead to high levels of angiogenesis and low levels of nitric oxide production and then increasing the susceptibility to atherosclerosis and other diseases [42,52].
The top of the image shows that for the normal 3-D conformation of the VEGF-eNOS complex the residues TRP 190 and LEU 32 do not bind through hydrogen bonds. If there is an SNP affecting these hot spot residues or neighbor residues the pattern of amino acid binding to each other changes and can affect the whole complex and consequently the function it performs.

Peptides are small bioactive molecules that are highly selective. Bioinformatic assays have designed relatively stable peptides that have been used in diagnosis and therapeutics of several diseases [61-63]. We have designed 20 peptides (data not shown) and we selected four of them to modulate the interaction between VEGF and eNOS. The four selected peptides were energetically optimized and they interact with important residues that favor the interaction of VEGF with eNOS. We used the eNOS side of the interface of interaction in order to design the peptides and we built an in silico model of interaction taking VEGF as the anchor (Figure 4). The results are bioinformatically satisfactory, the next step is to test the four main peptides in vitro and to validate the in silico model.

Figure 4: Modulating peptides of the interaction between VEGF and eNOS.

Four peptides out of 20 were energetically selected to modulate the interaction between VEGF and eNOS. All of the four peptides resulted in biochemistry favorable models that could modulate the target protein complex and help to establish stable expression of the protein levels.

Concluding Remarks

The understanding of protein-protein interaction has many clinical applications. Several diseases develop from anomalies in the PPI patterns they perform. The rational design of small bioactive molecules such as peptides has increased as important new therapeutic and diagnostic approaches. The peptides mimic the functional motif of a given protein within a multiprotein complex. Here, we performed an in silico analysis of the interaction interfaces between angiogenesis-related protein VEGF and the nitric oxide production protein eNOS. VEGF and eNOS influence the onset of atherosclerosis and they present polymorphisms that could increase the susceptibility to cardiovascular diseases such as atherosclerosis. We designed four optimized peptides that could modulate the interaction...
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