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Research Article

Involvement of Protein-Protein Interactions of eNOS and Genetic Polymorphisms in Coronary Artery Disease -

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ABSTRACT

Coronary Artery Disease (CAD) features anomalies such as angina, infarction and cardiac death, among others. Several risk factors are related to CAD onset and progression, the main ones are diabetes, sedentary lifestyle, high blood pressure, obesity, alcohol consumption, smoking and cholesterol. CAD is a globally serious health problem, affecting over 110 million people worldwide and leading to 9 million deaths each year. Endothelial dysfunction may be associated with genetic polymorphisms that take place in genes and proteins with essential roles in maintaining endothelial homeostasis. Among these genes, eNOS stands out, being responsible for the synthesis of nitric oxide, which is a lipophilic substance highly active in a great variety of physiological processes. NOSTRIN, cdc37 and calmodulin interact with eNOS and modulate its activity within endothelial cells in order to maintain vascular integrity and proper homeostasis. Dysfunction affecting any of those proteins, their structures or the PPI patterns they establish may increase CAD susceptibility. Here, we hypothesize that PPIs could be one of the explanations for CAD susceptibility. We have shown an *in silico* approach of interaction between eNOS and the CAD-related proteins NOSTRIN, cdc37 and calmodulin 1 through the identification of hot spots within the interface of interaction between eNOS and target proteins. We also pointed that several SNPs identified in clinical practice and deposited on dbSNP were related to the predicted hot spots and could increase susceptibility to CAD significantly due to alteration of eNOS and partner proteins conformation or loss of function due to impairment of the PPIs they perform. Further studies need to be performed and through the hot spots presented here, eNOS, NOSTRIN, cdc37 and calmodulin 1 could be used as molecular markers and peptides PPI modulator could be design and tested as alternative therapies against CAD progression.

Keywords: eNOS; NOSTRIN; cdc37; Calmodulin 1; PPIs; Hot spots; Polymorphism

INTRODUCTION

Coronary Artery Disease (CAD) comprises a series of anomalies, which includes angina, infarction and cardiac death, among others [1]. The main risk factors related to CADs include diabetes [2-5], sedentary lifestyle [6], high blood pressure [7], obesity [4], alcohol consumption [8], smoking [3] and cholesterol [5]. The onset of CADs occurs because the blood with oxygen and nutrients are unable to reach heart muscles due to fatty deposits inside the arterial walls [9]. CAD is a health problem worldwide, affecting over 110 million people around the globe and leading to 9 million deaths each year [10,11].

Some of the risk factors for CADS, such as, high blood cholesterol, hypertension, diabetes and smoking may lead to endothelial dysfunction, and consequently to a pro-inflammatory and a pro-thrombotic endothelial state [12,13]. Endothelial dysfunction may be associated with genetic polymorphisms that take place in genes and proteins with essential roles in maintaining endothelial homeostasis [14-17]. Among these genes, eNOS (endothelial Nitric Oxide Synthase) is responsible for the synthesis of nitric oxide, which is a lipophilic substance highly active in a great variety of physiological processes [18-21]. The nitric oxide produced by eNOS protein activity regulates the vascular tone [22], cell cycle progression [23,24], immune system cell adhesion [25,26] and platelet aggregation [27,28].

The eNOS protein comprises four main domains (Table 1) [29]. The N-terminal oxygenase domain is featured by heme-thiolate proteins and has a role in the activity of the protein. This family of hemoprotein contains a thiolate anion as the axial ligand to the heme group [30]. The flavodoxin-like domain has a Flavin Mononucleotide (FMN)-binding site, which is related to electron transfer reactions. This domain is important to enable the nitric oxide production, which has a role as a messenger molecule within the metabolism [31]. The FAD binding domain regulates the exchange of reducing equivalents between electron owners [32] and the NAD binding domain regulates the electron flux for ATP synthase and reducing power of cells undergoing active glycolysis [33].

Several other proteins influence endothelial metabolism and they interact with eNOS in order to complement its function and guarantee vascular stability. NOSTRIN (Nitric Oxide Synthase Trafficking) [34], cdc37 (cell division cycle 37) [35], calmodulin [36-39], GCDH (Glutaryl-CoA Dehydrogenase) [35] and SIRT 1 (sirtuin 1) [40] interact with eNOs in order to complement its function and those Protein-Protein Interactions (PPIs) regulate several vascular functions. Dysfunction affecting any of those proteins, their structures or the PPI patterns they establish may increase CAD susceptibility [41-44].

Here, we show the involvement of protein-protein interactions of eNOS and genetic polymorphisms in coronary artery disease by an *in silico* approach. We highlight hot spots residues on the interaction interface between eNOS and the proteins NOSTRIN, cdc37 and calmodulin 1 and polymorphisms that may alter structure, function and PPI patterns between them. The complex and dynamic metabolic processes engaged by those proteins may influence CADs onset, progression and prognosis.

MATERIALS AND METHODS

Briefly, we used the I-TASSER server in order to model the eNOS protein. A series of templates were used in order to find homologous proteins available in the PDB (protein databank) and to build up a 3-D structure that represents a stable conformation of the protein. The most common eNOs, NOSTRIN, cdc37 and calmodulin 1 polymorphisms related to CAD were retrieved from dbSNP (the single nucleotide polymorphism database). The interaction interface was determined by the ClusPro server and all the 3-D structures were visualized and analyzed by PyMol (https://pymol.org/2/). Amino acid residues were classified into hot spots through the KFC2 server [45].

RESULTS AND DISCUSSION

The interaction between eNOS and NOSTRIN

The main function of NOSTRIN is the intracellular trafficking

Table 1: Features of the eNOS protein domains

Family	Description	ID	Bit Score[a]	E-value[b]
NO_synthase	oxygenase domain	-	605.0	4.2e - 182
Flavodoxin	Flavodoxin	CL0042	175.6	5.7e - 52
FAD_binding	FAD binding domain	CL0076	275.7	2.4e - 82
NAD_binding	Oxidoreductase domain	CL0091	66.1	3.6e - 18

[a]Normalized score expressed in bits that estimate the magnitude of the alignment.
[b]Expectation value, which is a correction of the p-value for multiple testing

of eNOS between different compartments. NOSTRIN catalyzes the detachment of eNOS from the plasma membrane and transport the protein to several cellular compartments [46]. eNOS trafficking is essential to maintain normal levels of nitric oxide within intracellular compartments. The stimulation of endothelial cells leads to the transportation of eNOS in vesicles via NOSTRIN activity [47] and by endocytosis [34].

Figure 1 shows the interaction interface between eNOS and NOSTRIN through an *in silico* approach. The 3-D structure of NOSTRIN in figure 1 shows only the SH3 domain, which is the part of the protein that interacts with eNOS. Figure 1 also shows the hot spots predicted in the interaction interface. We found eight hot spots residues (Table 2) that contribute to the interaction between the proteins and also to the stabilization of the complex, as they are energetically favored residues [45]. Among those, five are polymorphic residues and may alter slightly the protein structure, the efficiency of interaction and finally disrupt eNOS transportation to intracellular compartments, reducing the availability of nitric oxide for the cell signaling and general metabolism. Several polymorphism linked to eNOs have been pointed as an increase to CAD susceptibility [15,48-50].

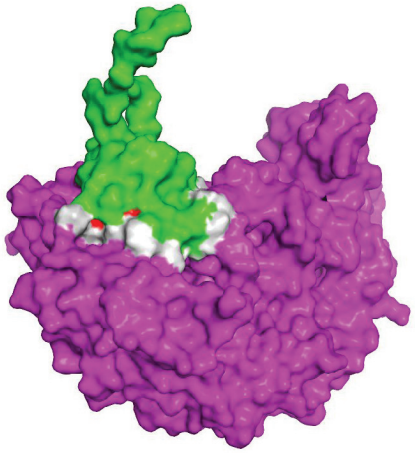

Figure 1: The interaction between eNOS and NOSTRIN. The eNOS protein is the biggest structure represented by pink while the NOSTRIN is represented by green. The interaction interface is represented by the color gray and the red regions are predicted hot spots. Single Nucleotide Polymorphisms (SNPs) or mutations in these residues may increase the susceptibility to CAD.

The interaction between eNOS and calmodulin 1

Calmodulin 1 belongs to the calcium-modulated protein family. Their localization is mainly in the cytosol or attached to membranes and they are regulated the levels of calcium in cells and tissues, taking part in processes such as cell cycle progression, growth, movement, and signal pathways. Calmodulin 1 also regulates eNOS activity in endothelial cells in the presence of cellular stressors. In addition, eNOS is more expressed if calcium is available, then the protein dissociates from membranes and is phosphorylated in the cytosol [51], leading to local vasodilatation [52]. The interaction between eNOS and calmodulin can be modulated by polypeptides, drugs and other small molecules [53]. This is important regarding CAD therapeutics and prognostic, once alterations in the binding energy between those proteins can increase susceptibility to atherosclerosis [54], for example.

Figure 2 shows the interaction between eNOS and calmodulin 1 binding motif. This interaction plays an important role in regulating eNOS activity as a response to cellular stress. Anomalies may rise when alterations within the coding sequence of any of the mentioned genes and proteins leading to onset of vascular diseases. We found seven hot spot residues (Table 3) in the interaction interface of eNOS and calmodulin. Many of those residues are polymorphic and susceptible to mutation; on the other hand, several of those possible SNPs (single nucleotide polymorphisms) are synonymous and have no effect in the coding sequence of eNOS or calmodulin 1. Figure 2 also shows a couple of hot spot residues on the interaction interface of eNOS and calmodulin. The design of modulator peptides is an alternative for treatment of CAD and the development of molecular markers to identify susceptible patients at earlier stages of the disease.

Table 2: Hot spots predicted for the interaction between eNOS and NOSTRIN.

Protein	Residue	Score A[a]	Score B[b]	SNP
eNOS	ARG 70	0.93	0.10	LEU, HYS, CYS
eNOS	LEU 340	0.85	0.27	MET
eNOS	ARG 474	0.52	0.03	LEU, HYS, CYS
NOSTRIN	TYR 14	0.40	0.10	Synonymous
NOSTRIN	PHE 16	0.37	0.22	-
NOSTRIN	GLU 39	0.77	0.11	-
NOSTRIN	TRP 42	1.33	0.31	ASP
NOSTRIN	TYR 58	0.94	0.30	-

[a]Hot spot scores according to structure characteristics
[b]Hot spot scores according to chemical characteristics of interacting residues

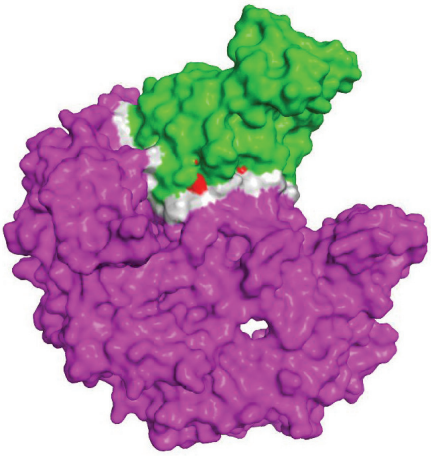

Figure 2: The interaction between eNOS and calmodulin 1. The eNOS protein is represented by pink while the camodulin 1 is represented by green. The interaction interface is represented by the color gray and the red regions are predicted hot spots. Calmodulin 1 regulates eNOS activity in endothelial cells as a response to cellular stress. The eNOS protein is more expressed when calcium is released, the protein detaches from membranes and is transported to cytosol leading to local vasodilatation.

The interaction between eNOS and cdc37

The protein cdc37 is a chaperone with a role in signal transduction; it interacts with protein partners in order to stabilize their structure so they can perform their activities properly. This is essential for eNOS to be active and disruption or reduction of the interaction efficiency may lead to several diseases, including CAD [55,56], diabetes [57] and cancer [58]. Chaperones and cdc family proteins have been

implicated in diseases related to inflammation (atheromatous plaques, for example) and immune response [59]. Genes coding for proteins related to inflammatory processes, such as eNOS and cdc37, are potentially candidates for biomarkers in order to assess the risk of CADs onset and progression. In addition, interactions of such genes could be modulated by synthetic or natural small molecules for therapeutics purposes [60-62].

We found ten hot spot residues (Table 4) on the interaction interface of eNOS and cdc37 (Figure 3) and among those residues, four are likely to carry SNPs and influence the susceptibility to CADs. The cdc37 protein regulates eNOS activity and prevent promiscuous eNOS activity [63]. Table 4 highlights the predicted hot spots and SNPs that may take place within this region, such alterations in either proteins, could lead to irregular binding or no binding at all and increase the predisposition to vascular diseases.

CONCLUDING REMARKS

CAD is costly and a complex public health problem around the globe. It is the disease with the highest rates of death worldwide. Family history of CADs is an indication for genetic counseling and genetic testing. Several genes such eNOS, NOSTRIN, cdc37 and calmodulin are potential candidates as genetic markers of vascular diseases. Knowledge on SNPs that increase susceptibility to CADs may shed new light on methods of prevention, diagnosis, treatment, genetic counseling and prognosis of CADs. PPIs are present in every compartment of a cell and they are responsible for a proper function of proteins, regulating cell cycle, cell homeostasis and disease onset and progression. Here, we hypothesize that PPIs could be one of the explanations for CAD susceptibility. We have shown an *in silico* approach of interaction between eNOS and the CAD-related proteins NOSTRIN, cdc37 and calmodulin 1 through the identification of hot spots within the interface of interaction between eNOS and target proteins. We also pointed that several SNPs identified in clinical practice and deposited on dbSNP were related to the predicted hot spots and could increase susceptibility to CAD significantly due to alteration of eNOS and partner proteins conformation or loss of function due to impairment of the PPIs they perform. Further studies need to be performed and through the hot spots presented here, eNOS, NOSTRIN, cdc37 and calmodulin 1 could be used as molecular markers and peptides PPI modulator could be design and tested as alternative therapies against CAD progression.

Table 3: Hot spots predicted for the interaction between eNOS and calmodulin 1.

Protein	Residue	Score A[a]	Score B[b]	SNP
eNOS	ARG 70	1.66	0.35	LEU, HYS, CYS
eNOS	LEU 100	0.22	0.01	-
eNOS	LEU 340	0.11	0.06	MET
Calmodulin 1	TYR 99	0.63	0.03	Synonymous
Calmodulin 1	ASN 137	1.41	0.09	Synonymous
Calmodulin 1	TYR 138	1.33	0.35	Synonymous
Calmodulin 1	GLU 139	1.23	0.05	VAL

[a]Hot spot scores according to structure characteristics
[b]Hot spot scores according to chemical characteristics of interacting residues

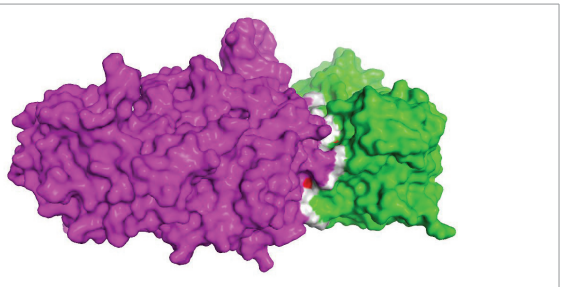

Figure 3: The interaction between eNOS and cdc37. The eNOS protein is represented by pink while cdc37 is represented by green. The interaction interface is represented by the color gray and the red regions are predicted hot spots. Reduction of the interaction efficiency between eNOS and cdc37 may lead to several diseases, including CAD, diabetes and cancer.

Table 4: Hot spots predicted for the interaction between eNOS and cdc37.

Protein	Residue	Score A[a]	Score B[b]	SNP
eNOS	ARG 202	1.44	0.23	LYS, SER
eNOS	PHE 208	0.61	0.32	LEU
eNOS	LEU 302	0.95	0.21	-
eNOS	PHE 303	1.07	0.37	-
eNOS	LEU 304	1.19	0.11	PHE
cdc37	ARG 60	1.06	0.25	-
cdc37	LEU 70	0.47	0.10	ARG
cdc37	LEU 76	1.17	0.24	-
cdc37	TYR 216	0.63	0.29	-
cdc37	PHE 238	1.11	0.29	-

[a]Hot spot scores according to structure characteristics
[b]Hot spot scores according to chemical characteristics of interacting residues.

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