Lima RM et al. J Cardiol Catheter 2019(1): 29-36.
https://doi.org/10.33513/cardiology/CACA-04

OCIMUM

Journal of Cardiology and Catheterization

Original Research

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

Raisa Melo Lima[1], Lucas Nojosa Oliveira[1], Marielle Garcia Silva[1], Lívia do Carmo Silva[1], Juliana Santana de Curcio[1], Leandro do Prado Assunção[1], Thaynara Gonzaga Santos[1], Isabele Pereira Tannous[1], Andreia Marcelino Barbosa[2], Kátia Karina Verolli de Oliveira Moura[2] and Kleber Santiago Freitas e Silva[1]

[1]Biological Sciences Institute, Federal University of Goiás, Brazil
[2]Replicon, Pontifical Catholic University of Goiás, Brazil

Received: 24 December 2018
Accepted: 26 January 2019
Version of Record Online: 06 February 2019

Citation

Lima RM, Oliveira LN, Silva MG, do Carmo Silva L, de Curcio JS et al. (2019) In silico Modulation of the Interaction Between VEGF and eNOS Proteins in Atherosclerosis as a Future Diagnostic and Therapeutic Approach. J Cardiol Catheter 2019(1): 29-36.

Correspondence should be addressed to
Kleber Santiago Freitas e Silva, Brazil
E-mail: smallbinho@hotmail.com

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 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.

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.

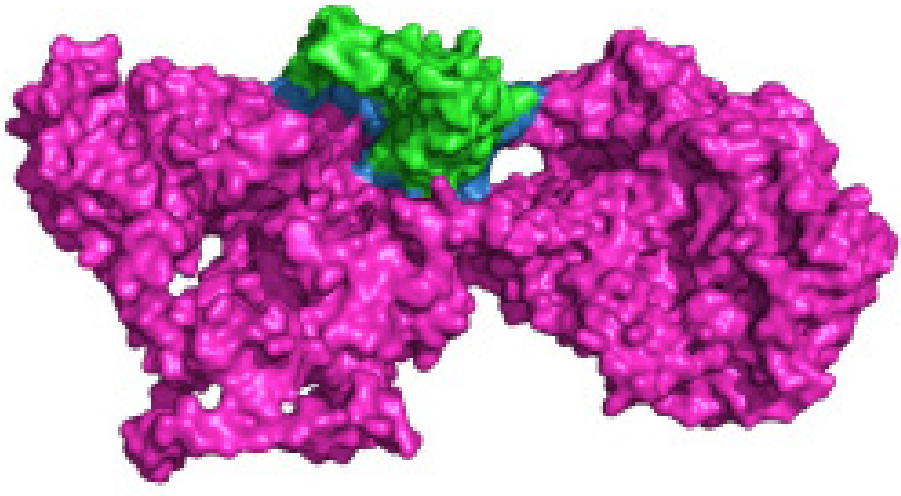

Figure 1: The interaction between VEGF and eNOS proteins.

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].

Hot Spot	Residue number	Score 1*	Score 2*	SNP
ARG	98	0.47	0.05	CYS, HIS
ARG	107	1.19	0.18	GLN, TRP,
ARG	140	0.64	0.14	-
ARG	187	1.76	0.36	GLN, GLY, TRP
TRP	190	1.40	0.27	nonsense
LEU	32	1.02	0.23	-
VAL	33	0.96	0.07	MET
PHE	36	0.09	0.03	-
GLU	38	1.03	0.10	-
TYR	39	1.62	0.38	synonymous
GLU	42	1.87	0.23	-
ILE	43	1.25	0.03	PHE
TYR	45	0.71	0.23	PHE
ARG	56	0.59	0.22	GLN
HIS	99	0.97	0.26	-

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.

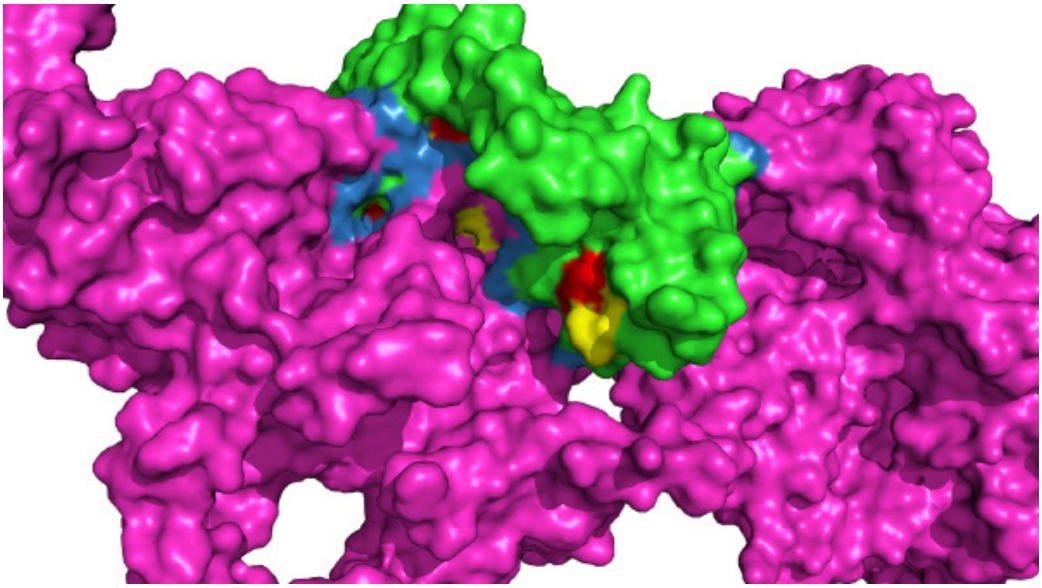

Figure 2: The polymorphic hot spots in the interface of interaction between VEGF and eNOS.

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].

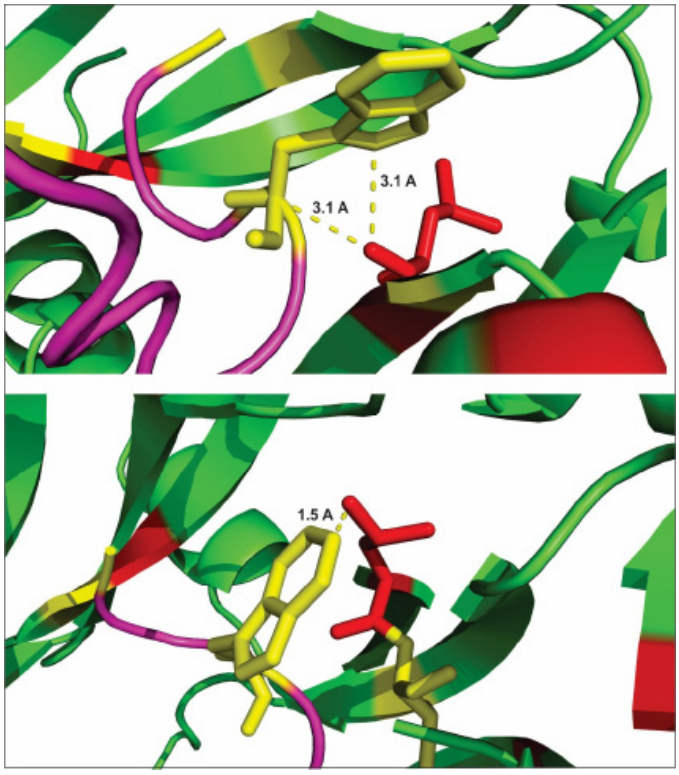

Figure 3: Wild type and polymorphic VEGF-eNOS complex.

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.

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 atherosclerosis. We designed four optimized peptides that could modulate the interaction

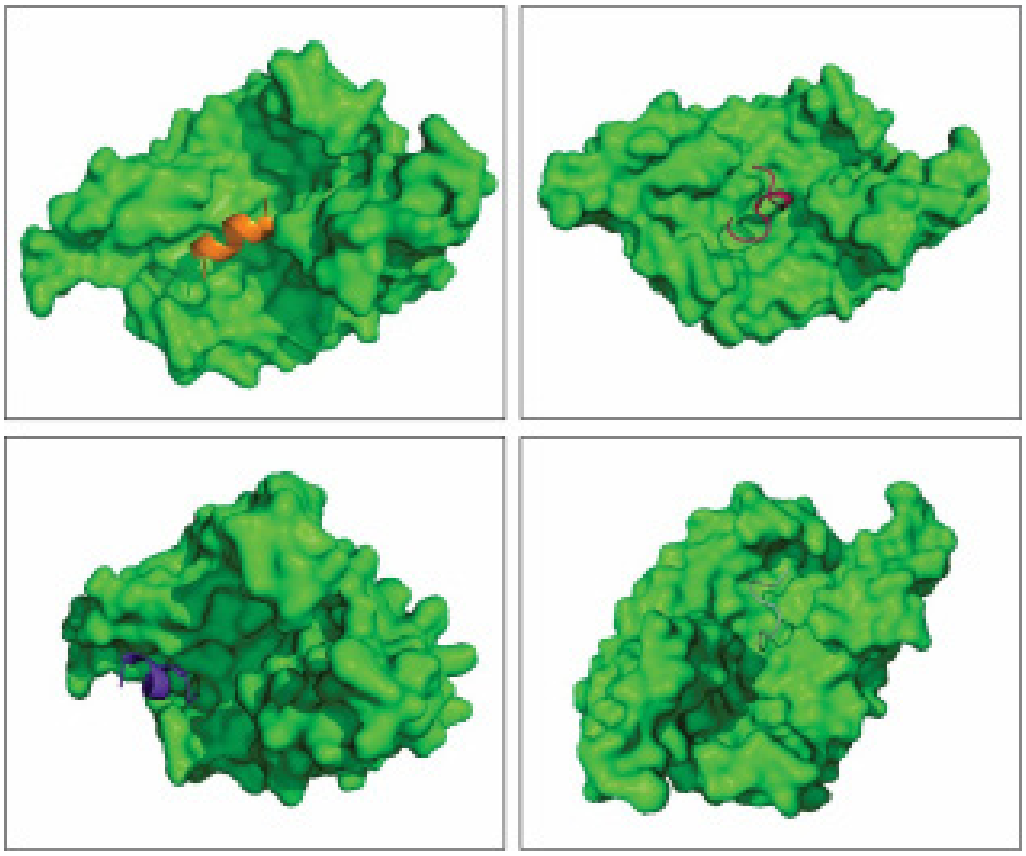

Figure 4: Modulating peptides of the interaction between VEGF and eNOS.

of VEGF and eNOS as a perspective of future treatment and better prognostic for atherosclerotic patients.

References

1. Vargas-Alarcon G, Perez-Mendez O, Herrera-Maya G, Posadas-Romero C, Posadas-Sanchez R, et al. (2018) The rs1805193, rs5361, and rs5355 single nucleotide polymorphisms in the E-selectin gene (SEL-E) are associated with subclinical atherosclerosis: The Genetics of Atherosclerotic Disease (GEA) Mexican study. Immunobiology 2018: S0171-2985(18)30191-S0171-2985(18)30198.
2. Rahman MS, Woollard K (2017) Atherosclerosis. Adv Exp Med Biol 1003: 121-144.
3. Kasahara K, Tanoue T, Yamashita T, Yodoi K, Matsumoto T, et al. (2017) Commensal bacteria at the crossroad between cholesterol homeostasis and chronic inflammation in atherosclerosis. J Lipid Res 58: 519-528.
4. Králová A, Králová Lesná I, Poledne R (2014) Immunological aspects of atherosclerosis. Physiol Res 63: 335-342.
5. Nanni L, Romualdi C, Maseri A, Lanfranchi G (2006) Differential gene expression profiling in genetic and multifactorial cardiovascular diseases. J Mol Cell Cardiol 41: 934-948.
6. Fredman G, Tabas I (2017) Boosting Inflammation Resolution in Atherosclerosis: The Next Frontier for Therapy. Am J Pathol 187: 1211-1221.
7. Kozarov E, Huber K, Wojta J (2015) Infection-associated biomarkers of inflammation in atherosclerosis. Curr Pharm Des 21: 1776-1782.
8. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL (2017) Oxidative Stress in Atherosclerosis. Curr Atheroscler Rep 19: 42.
9. Li H, Horke S, Förstermann U (2014) Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis 237: 208-219.
10. Campbell LA, Rosenfeld ME (2015) Infection and Atherosclerosis Development. Arch Med Res 46: 339-350.
11. Hurtubise J, McLellan K, Durr K, Onasanya O, Nwabuko D, et al. (2016) The Different Facets of Dyslipidemia and Hypertension in Atherosclerosis. Curr Atheroscler Rep 18: 82.
12. Leong X-F, Ng C-Y, Jaarin K (2015) Animal Models in Cardiovascular Research: Hypertension and Atherosclerosis. Biomed Res Int 2015: 528757.
13. Siasos G, Tsigkou V, Kokkou E, Oikonomou E, Vavuranakis M, et al. (2014) Smoking and atherosclerosis: mechanisms of disease and new therapeutic approaches. Curr Med Chem 21: 3936-3948.
14. de Ronde MWJ, Kok MGM, Moerland PD, Van den Bossche J, Neele AE, et al. (2017) High miR-124-3p expression identifies smoking individuals susceptible to atherosclerosis. Atherosclerosis 263: 377-384.
15. Su H, Pei Y, Tian C, Zhang Q, Liu L, et al. (2018) Relationship between high-sensitivity C-reactive protein and subclinical carotid atherosclerosis stratified by glucose metabolic status in Chinese adults. Clin Cardiol 42: 39-46.
16. Jun S, Datta S, Wang L, Pegany R, Cano M, et al. (2018) The impact of lipids, lipid oxidation, and inflammation on AMD, and the potential role of miRNAs on lipid metabolism in the RPE. Exp Eye Res 0014- 2018: S0014-4835(18)30398- S0014-4835(18)30401.
17. Stamenkovic A, Pierce GN, Ravandi A (2018) Oxidized Lipids: Not just another brick in the wall. Can J Physiol Pharmacol DOI: 10.1139/cjpp-2018-0490.
18. Döring Y, Soehnlein O, Weber C (2017) Neutrophil Extracellular Traps in Atherosclerosis and Atherothrombosis. Circ Res 120: 736-743.
19. Wang W, Tang Y, Wang Y, Tascau L, Balcerek J, et al. (2016) LNK/SH2B3 Loss of Function Promotes Atherosclerosis and Thrombosis. Circ Res 119: 91-103.
20. Horie N, Tateishi Y, Morikawa M, Morofuji Y, Hayashi K, et al. (2016) Acute stroke with major intracranial vessel occlusion: Characteristics of cardioembolism and atherosclerosis-related in situ stenosis/occlusion. J Clin Neurosci 32: 24-29.
21. Zhou L, Yao M, Peng B, Zhu Y, Ni J, et al. (2018) Atherosclerosis Might Be Responsible for Branch Artery Disease: Evidence From White Matter Hyperintensity Burden in Acute Isolated Pontine Infarction. Front Neurol 9: 840.
22. Kianoush S, Al Rifai M, Cainzos-Achirica M, Al-Mallah MH, Tison GH, et al. (2017) Thoracic extra-coronary calcification for the prediction of stroke: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 267: 61-67.
23. Nanmoku K, Watarai Y, Narumi S, Goto N, Yamamoto T, et al. (2017) Surgical Techniques and Procedures for Kidney Transplant Recipients With Severe Atherosclerosis. Exp Clin Transplant 15: 594-601.
24. Naftolin F, Mehr H, Fadiel A (2016) Sex Steroids Block the Initiation of Atherosclerosis. Reprod Sci 23: 1620-1625.
25. Gimbrone MA, García-Cardeña G (2018) Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circ Res 118: 620-636.
26. Tabas I, Bornfeldt KE (2016) Macrophage Phenotype and Function in Different Stages of Atherosclerosis. Circ Res 118: 653-667.
27. Couchie D, Vaisman B, Abderrazak A, Mahmood DFD, Hamza MM, et al. (2017) Human Plasma Thioredoxin-80 Increases With Age and in ApoE-/- Mice Induces Inflammation, Angiogenesis, and Atherosclerosis. Circulation 136: 464-475.
28. Novák J, Olejníčková V, Tkáčová N, Santulli G (2015) Mechanistic Role of MicroRNAs in Coupling Lipid Metabolism and Atherosclerosis. Adv Exp Med Biol 887: 79-100.
29. Dellamea BS, Leitão CB, Friedman R, Canani LH (2014)

Nitric oxide system and diabetic nephropathy. Diabetol Metab Syndr 6: 17.

30. Nakanishi K, Koshiyama T, Iba S, Ohba M (2015) Lipophilic ruthenium salen complexes: incorporation into liposome bilayers and photoinduced release of nitric oxide. Dalton Trans 44: 14200-14203.

31. Khaddaj Mallat R, Mathew John C, Kendrick DJ, Braun AP (2017) The vascular endothelium: A regulator of arterial tone and interface for the immune system. Crit Rev Clin Lab Sci 54: 458-470.

32. Zhao Y, Vanhoutte PM, Leung SW (2015) Vascular nitric oxide: Beyond eNOS. J Pharmacol Sci 129: 83-94.

33. Tiwari M, Prasad S, Pandey AN, Premkumar KV, Tripathi A, et al. (2017) Nitric oxide signaling during meiotic cell cycle regulation in mammalian oocytes. Front Biosci (Schol Ed) 9: 307-318.

34. Zhu Y, Liao W, Niu L, Wang M, Ma Z (2016) Nitric oxide is involved in hydrogen gas-induced cell cycle activation during adventitious root formation in cucumber. BMC Plant Biol 16: 146.

35. Gutsaeva DR, Montero-Huerta P, Parkerson JB, Yerigenahally SD, Ikuta T, et al. (2014) Molecular mechanisms underlying synergistic adhesion of sickle red blood cells by hypoxia and low nitric oxide bioavailability. Blood 123: 1917-1926.

36. Smyth E, Solomon A, Birrell MA, Smallwood MJ, Winyard PG, et al. (2017) Influence of inflammation and nitric oxide upon platelet aggregation following deposition of diesel exhaust particles in the airways. Br J Pharmacol 174: 2130-2139.

37. Radziwon-Balicka A, Lesyk G, Back V, Fong T, Loredo-Calderon EL, et al. (2017) Differential eNOS-signalling by platelet subpopulations regulates adhesion and aggregation. Cardiovasc Res 113: 1719-1731.

38. Sung JH, Lee BE, Kim JO, Jeon YJ, Kim SH, et al. (2015) Association between eNOS polymorphisms and risk of coronary artery disease in a Korean population: a meta-analysis. Genet Mol Res 14: 16508-16520.

39. Wang H, Qiu L, Ma Y, Zhang L, Chen L, et al. (2017) Naoxintong inhibits myocardial infarction injury by VEGF/eNOS signaling-mediated neovascularization. J Ethnopharmacol 209: 13-23.

40. Wang L, Li X, Zhang Y, Huang Y, Zhang Y, et al. (2018) Oxymatrine ameliorates diabetes-induced aortic endothelial dysfunction via the regulation of eNOS and NOX4. J Cell Biochem DOI: 10.1002/jcb.28006.

41. Chang CC, Hsu YH, Chou HC, Lee YG, Juan SH (2017) 3-Methylcholanthrene/Aryl-Hydrocarbon Receptor-Mediated Hypertension Through eNOS Inactivation. J Cell Physiol 232: 1020-1029.

42. Barbosa AM, Silva KSF, Lagares MH, Rodrigues DA, da Costa IR, et al. (2017) Atherosclerosis: analysis of the eNOS (T786C) gene polymorphism. Genet Mol Res 16: 1-9.

43. Wojtukiewicz MZ, Sierko E, Hempel D, Tucker SC, Honn KV (2017) Platelets and cancer angiogenesis nexus. Cancer Metastasis Rev 36: 249-262.

44. Pufe T, Petersen WJ, Mentlein R, Tillmann BN (2005) The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. Scand J Med Sci Sports 15: 211-222.

45. Johnson T, Zhao L, Manuel G, Taylor H, Liu D (2019) Approaches to therapeutic angiogenesis for ischemic heart disease. J Mol Med 97: 141-151.

46. Jaipersad AS, Lip GY, Silverman S, Shantsila E (2014) The role of monocytes in angiogenesis and atherosclerosis. J Am Coll Cardiol 63: 1-11.

47. Cheung CY (1997) Vascular endothelial growth factor: possible role in fetal development and placental function. J Soc Gynecol Investig 4: 169-177.

48. Lu HY, Zhao GL, Fu MF (2016) Polymorphisms in the vascular endothelial growth factor (VEGF) gene associated with asthma. Genet Mol Res 15: 1-8.

49. Abdelsaid M, Coucha M, Hafez S, Yasir A, Johnson MH, et al. (2017) Enhanced VEGF signalling mediates cerebral neovascularisation via downregulation of guidance protein ROBO4 in a rat model of diabetes. Diabetologia 60: 740-750.

50. Zhang B, Wang D, Ji TF, Shi L, Yu JL (2017) Overexpression of lncRNA ANRIL up-regulates VEGF expression and promotes angiogenesis of diabetes mellitus combined with cerebral infarction by activating NF- κB signaling pathway in a rat model. Oncotarget 8: 17347-17359.

51. Frezzetti D, Gallo M, Maiello MR, D'Alessio A, Esposito C, et al. (2017) VEGF as a potential target in lung cancer. Expert Opin Ther Targets 21: 959-966.

52. Taher M, Nakao S, Zandi S, Melhorn MI, Hayes KC, et al. (2016) Phenotypic transformation of intimal and adventitial lymphatics in atherosclerosis: a regulatory role for soluble VEGF receptor 2. FASEB J 30: 2490-2499.

53. Zhu X, Xie H, Liang X, Li X, Duan J, et al. (2017) Bilayered Nanoparticles with Sequential Release of VEGF Gene and Paclitaxel for Restenosis Inhibition in Atherosclerosis. ACS Appl Mater Interfaces 9: 27522-27532.

54. Ghoorah AW, Devignes MD, Smaïl-Tabbone M, Ritchie DW (2016) Classification and Exploration of 3D Protein Domain Interactions Using Kbdock. Methods Mol Biol 1415: 91-105.

55. Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, et al. (2017) The ClusPro web server for protein-protein docking. Nat Protoc 12: 255-278.

56. Darnell SJ, Page D, Mitchell JC (2007) An automated decision-tree approach to predicting protein interaction hot spots. Proteins 68: 813-823.

57. de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, et al. (1992) The fms-like tyrosine kinase, a receptor for vascular

endothelial growth factor. Science 255: 989-991.

58. Shizukuda Y, Tang S, Yokota R, Ware JA (1999) Vascular endothelial growth factor-induced endothelial cell migration and proliferation depend on a nitric oxide-mediated decrease in protein kinase Cdelta activity. Circ Res 85: 247-256.

59. Alexov E, Sternberg M (2013) Understanding molecular effects of naturally occurring genetic differences. J Mol Biol 425: 3911-3913.

60. Schaefer C, Rost B (2012) Predict impact of single amino acid change upon protein structure. BMC Genomics 13: S4. DOI: 10.1186/1471-2164-13-S4-S4.

61. Dimayuga PC, Zhao X, Yano J, Lio WM, Zhou J, et al. (2017) Identification of apoB-100 Peptide- Specific CD8+T Cells in Atherosclerosis. J Am Heart Assoc 6: 005318.

62. Silva RA, Giordano RJ, Gutierrez PS, Rocha VZ, Rudnicki M, et al. (2016) CTHRSSVVC Peptide as a Possible Early Molecular Imaging Target for Atherosclerosis. Int J Mol Sci 17: 1383.

63. Paulin N, Döring Y, Kooijman S, Blanchet X, Viola JR, et al. (2017) Human Neutrophil Peptide 1 Limits Hypercholesterolemia-induced Atherosclerosis by Increasing Hepatic LDL Clearance. EBioMedicine 16: 204-211.