ROMANIAN ACADEMY

INSTITUTE OF BIOLOGY AND PATHOLOGY
„NICOLAE SIMIONESCU"

PhD THESIS

Epigenetic mechanisms involved in the onset of oxidative and inflammatory stress in atherosclerosis

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CONTENTS

Introduction and objectives

Cardiovascular diseases are the leading cause of death in developed countries, including Romania (60%). Atherosclerosis is a complex, multifactorial and multigenic disease characterized by thickening and stiffening of the arterial walls - causing progressive narrowing of the vascular lumen. This is a determinant of coronary and cerebrovascular disease. Atherosclerosis primarily affects: a) elastic arteries (aorta, carotid, iliac) b) medium and large muscular arteries (coronary, cerebral, popliteal). The most common risk factors for atherosclerosis are - uninfluential like advanced age, male gender, genetic predisposition and - influential as: dyslipidemia, increased LDL (low density lipoprotein), reduced HDL (high density lipoprotein), smoking, high blood pressure, diabetes, hypertension, obesity, sedentary lifestyle, psychosocial factors (Libby, 2002). Although the entire surface of the blood vessels is exposed to risk factors, however, atherosclerotic lesions occur only in certain particular areas of the vascular tree: the inner wall of the vascular bifurcations, the arterial curves and the aortic valves. (Simionescu et al, 1986).

Primary therapy for atherosclerosis includes drugs that lower endogenous cholesterol biosynthesis, inhibitors of 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (statins), but, nevertheless, approximately 50% of patients develop atherosclerotic lesions in the absence of hypercholesterolemia. The use of statins prevents complications of acute coronary heart disease by stabilizing atherosclerotic plaque but not by reducing its size (Aikawa and Libby, 2004). To date, therapeutic approaches are limited to risk factors, primarily targeting hypercholesterolemia and hypertension, but the maximum effectiveness of these strategies in clinical trials has been 30-40% (Soto et al, 2012). Therefore, there is a need to design specific therapies to target the pathological mechanisms of atheroma formation and development.

Macrophages (Macs) play an important role in the formation of atherosclerotic plaques. These are derived from monocytes and are versatile cells that modify their phenotype in response to factors existing in the microenvironment of the plaque. Macs come from the hematogenous bone marrow and go through different stages, from the pluripotent stem cell to monoblast-promonocyte-circulating monocyte. Endothelial dysfunction caused by transcribed and accumulated LDL particles in the subendothelium causes the expression of MCP-1 which will lead to the diapedesis of monocytes through the junctions of endothelial cells. They will differentiate into activated Macs, accumulate lipids and turn into foam cells. The increased inflammatory response will be maintained by the secretion of pro-inflammatory molecules and by the production of reactive oxygen species (ORS). Also, apoptotic macs are responsible for the formation of the necrotic center and the progression of the disease. Due to the important role that macrophages play in the different stages of atherosclerotic plaque formation, they represent attractive targets for the development of therapies.

Recent studies point-out the crucial role of epigenetic mechanisms in modulating the Mac phenotype in atherosclerosis. HAT enzymes catalyze the acetylation of lysine residues in the amino-terminal region of nucleosomal histones. Following this process, chromatin decondensation takes place, which facilitates the access of transcription factors to their consensus elements in the DNA sequence and implicitly the activation of gene expression. Histone acetylation is associated with myocyte hypertrophy, apoptosis, oxidative stress, heart remodeling, arrhythmia, heart failure, hypertension and inflammation.

The aim of this doctoral thesis is to identify new molecular mechanisms of epigenetic nature in macrophages, in order to develop new anti-inflammatory and anti-oxidant therapeutic approaches in atherosclerosis.

The hypothesis that determined this study is that the imbalance of epigenetic mechanisms involved in histone acetylation causes altered expression of genes with an important role in modulating the function of pro-inflammatory (type M1) and anti-inflammatory (type M2) macrophages in atherosclerosis.

The first part of the thesis will discuss current issues related to the inflammatory process associated with atherosclerosis, the involvement of inflammation and Mac in all stages of development of atheromas and epigenetic mechanisms, especially in terms of acetylation and

deacetylation, mechanisms involved in the process of atherosclerotic plaque formation. The original part of the thesis focuses on:

(1) Analysis of the impact of atherogenic conditions on HAT and HDAC histone acetylation / deacetylation system;

(2) Investigating the involvement of HAT and HDAC in the installation of oxidative and inflammatory stress in polarized macrophages in atherosclerosis;

(3) Establishing the ability of pharmacological compounds directed to HAT / HDAC in order to reduce the formation of atherosclerotic plaques in ApoE -/- mice.

Several studies suggest that in addition to genetic factors, epigenetic mechanisms that translate the effects of gene- (micro) environment interactions contribute to atherosclerosis by regulating the expression of very important genes associated with inflammation, extracellular matrix synthesis, cell proliferation and differentiation (Khyzha et al, 2017; Zhang et al, 2018). Oxidative stress is an important regulator of many pathological processes that lead to the formation of atheroma plaques. However, the involvement of epigenetic mechanisms in mediating overexpression of ORS and oxidative stress in atherosclerosis is not yet defined (Kietzmann et al, 2017). In this study we focused on the HAT-dependent mechanisms involved in modulating Nox5 expression, a member of the Nox family identified and characterized in human Mon and Mac that contributes to overexpression of ORS and oxidative stress in atherosclerosis. (Manea et al, 2015).

Recent studies indicate the role of ORS produced by Nox5 in the etiology of cardiovascular disease (CVD), but the precise mechanisms governing Nox5 induction are not fully known (Jha et al, 2017). Therefore, understanding the molecular pathways of Nox5 gene regulation is a necessary step for the development of advanced anti-oxidative stress therapies aimed to significantly reduce cardiovascular events associated with destabilizing vulnerable atherosclerotic plaque (eg stroke, myocardial infarction, sudden death). Thus, we hypothesized that elevated Nox5 levels may be partially mediated by changes in chromatin topology. To address this problem, an experimental design was performed using non- and atherosclerotic human samples obtained as residual materials from patients who underwent endarterectomy and human Mac surgery in culture.

The original results obtained in this thesis are the following:

1. The corresponding protein levels p300, HAT1, H3K27ac and Nox5 are induced in human atherosclerotic carotid arteries and are abundant in the area of the fibrous head and atherosclerotic lipid core expressing pan-Mac markers.

2. Stimulation of human LPS with LPS induces epigenetic changes observed by increased HAT1 levels and histone acetylation (H3K27ac, H3K9ac).

3. Pharmacological inhibition of HAT reduces the gene and protein expression of Nox1, Nox2 and Nox4 in LPS-stimulated Mac; overexpression of p300 or HAT1 induces activation of Nox5 gene transcription.

4. Recruitment of p300 and HAT1 proteins is increased in the Nox5 gene promoter in LPS-treated human Mac.

5. HDAC class I (HDAC1, HDAC2, HDAC3), class IIa (HDAC4), class IIb (HDAC6) and HDAC IV (HDAC11) are increased in human atherosclerotic carotid arteries and in the hypercholesterolemic aorta of ApoE-/- mice.

6. Pharmacological inhibition of HDAC by SAHA significantly reduces the development of atherosclerosis and markers of immune cell infiltration in the aorta of ApoE-/- mice.

7. HDAC-dependent signaling pathways mediate the induction of Nox1 and Nox4 expression in the aorta of ApoE - / - mice.

8. SAHA treatment induces a decrease in NOS2 and MMP9 proteins in the aorta, major contributors to vascular inflammation and remodeling in atherosclerosis.

9. HDAC class I, IIa, IIb and IV subtypes as well as Nox1, Nox2 and Nox4 isoforms are significantly elevated in pro-inflammatory M1 mouse mac in vitro.

10. Pharmacological inhibition of HDAC (by SAHA) reduces the expression of Nox1, Nox2, Nox4 and TNFα levels in M1 mouse Mac culture.

The data of this doctoral thesis reinforce the belief that p300 has a role in mediating the induction of Nox5 expression in Mac, in inflammatory conditions and provides new data that create a link between epigenetically modified mechanisms and Reactive-Oxygen-Species overproduction caused by Nox5 in atherosclerosis.

Given that oxidative stress is an incriminating factor in atherosclerosis, pharmacological targeting of the epigenetic mechanisms of the pathways that control Nox5 expression may be an effective therapeutic strategy in the treatment of this disease.

Interestingly, our results provide evidence that in the context of inducing different HDAC isoforms, the pan-HDAC SAHA inhibitor has similar anti-atherosclerotic effects in mice, comparable to previous studies that showed that systemic or specific ablation of a single HDAC isoform slows atherosclerosis. Therefore, the existence of compensatory or redundant regulatory mechanisms in the HDAC system, as well as a complex network between HDAC subtypes and gene-specific cell / transcription factors remains an open issue. In this context, the use of pan- or HDAC-specific inhibitors in atherosclerosis is still disputed and requires in-depth studies. Collectively, the main findings of this study indicate that in atherosclerosis the pharmacological inhibition of HDAC growth reduces the increased expression of Nox subtypes in the aorta and also oxidative stress, leading to decreased inflammatory markers and the formation of atherosclerotic lesions in mice. Because multiple HDAC subtypes are also elevated in human atherosclerosis, HDAC-targeted pharmacological interventions could be a new effective therapeutic strategy in atherosclerosis.

Overall, the new results of this study indicate that in atherosclerosis the pharmacological inhibition of HDAC growth reduces the increased expression of Nox subtypes in the aorta and also oxidative stress, leading to decreased inflammatory markers and the formation of atherosclerotic lesions in mice. Because multiple HDAC subtypes are also elevated in human atherosclerosis, HDAC-targeted pharmacological interventions could be a new effective therapeutic strategy in atherosclerosis.

Bibliography

1. Aikawa M, Libby P (2004) Atherosclerotic plaque inflammation: The final frontier?. *Canadian Journal of Cardiology* 20: 631-4.
2. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2007) Molecular biology of the cell. Garland Science.
3. Chen F, Li X, Aquadro E, Haigh S, Zhou J, Stepp DW, Weintraub NL, Barman SA, Fulton DJR (2016) Inhibition of histone deacetylase reduces transcription of NADPH oxidases and ROS production and ameliorates pulmonary arterial hypertension. *Free Radical Biology and Medicine* 99:167-178.
4. Chen X, Barozzi I, Termanini A, Prosperini E, Recchiuti A, Dalli J, Mietton F, Matteoli G, Hiebert S, Natoli G (2012) Requirement for the histone deacetylase Hdac3for the inflammatory gene expression program in macrophages. *Proc. Natl. Acad.Sci. U. S. A*. 109: E2865–E2874.
5. Chinetti-Gbaguidi G, Sophie C, Staels B (2015) Macrophage subsets in atherosclerosis. *Nature Reviews Cardiology* 12:10–17.
6. Hassle M, Egger G (2012) Epigenomics of cancer - emerging new concepts. Biochimie 10: 1-12.
7. Kietzmann T, Petry A, Shvetsova A, Gerhold JM, Görlach A (2017) The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. British Journal of Pharmacology 174: 1533-1554.
8. Khyzha N, Alizada A, Wilson MD, Fish JE (2017) Epigenetics of atherosclerosis: emerging mechanisms and methods. Trends in Molecular Medicine 23: 332-347.
9. Kouzarides T (2007) Chromatin Modifications and Their Function. Cell 128: 693-705.
10. Kurdistani SK, Tavazoie S, Grunstein M (2004) Mapping Global Histone Acetylation Patterns to Gene Expression. Cell 11: 721–733.
11. Libby P (2002) Inflammation in atherosclerosis. Nature 420: 19-26.
12. Libby P, Ridker PM (2006) Inflammation and Atherothrombosis: From Population Biology and Bench Research to Clinical Practice. Journal of the American College of Cardiology 48: A33-46.
13. Manea A, Manea SA, Gafencu AV, Raicu M, Simionescu M (2008) AP-1-dependenttranscriptional regulation of NADPH oxidase in human aortic smooth muscle cells:role of p22phox subunit. *Arterioscler. Thromb. Vasc. Biol*. 28: 878–885.

14. Manea A (2010) NADPH oxidase-derived reactive oxygen species: involvement in vascular physiology and pathology. *Cell and Tissue Research* 342: 325-339.
15. Manea A, Tanase LI, Raicu M, Simionescu M (2010) Jak/STAT signaling pathwayregulates nox1 and nox4-based NADPH oxidase in human aortic smooth musclecells. *Arterioscler. Thromb. Vasc. Biol.* 30: 105–112.
16. Manea A, Tanase LI, Raicu M, Simionescu M (2010) Transcriptional regulation ofNADPH oxidase isoforms, Nox1 and Nox4, by nuclear factor-kappaB in humanaortic smooth muscle cells. *Biochem. Biophys. Res. Commun*. 396: 901–907.
17. Manea A, Simionescu M (2012) Nox enzymes and oxidative stress in atherosclerosis. Frontiers in Bioscience (Scholar Edition) 4: 651-670.
18. Manea A, Manea SA, Florea IC, Luca CM, Raicu M (2012) Positive regulation of NADPH oxidase 5 by proinflammatory-related mechanisms in human aortic smooth muscle cells. *Free Radical Biology and Medicine* 52: 1497-1507.
19. Manea SA, Todirita A, Raicu M, Manea A (2014) C/EBP transcription factors regulate NADPH oxidase in human aortic smooth muscle cells. Journal of Cellular and Molecular Medicine 18: 1467-1477.
20. Manea A, Manea SA, Gan AM, Constantin A, Fenyo IM, Raicu M, Muresian H, Simionescu M (2015) Human monocytes and macrophages express NADPH oxidase 5; a potential source of reactive oxygen species in atherosclerosis. Biochem Biophys Res Commun 461:172-9.
21. Manea SA, Constantin A, Manda G, Sasson S, Manea A (2015) Regulation of Nox enzymes expression in vascular pathophysiology: Focusing on transcription factors and epigenetic mechanisms. Redox Biology 5: 358-366.
22. Manea SA, Fenyo IM, Manea A (2016) c-Src tyrosine kinase mediates high glucose-induced endothelin-1 expression. International Journal of Biochemistry & Cell Biology 75: 123-130.
23. Manea SA, Antonescu ML, Fenyo IM, Raicu M, Simionescu M, Manea A (2018) Epigenetic regulation of vascular NADPH oxidase expression and reactive oxygen species production by histone deacetylase-dependent mechanisms in experimental diabetes. Redox Biology 16: 332-343.
24. Schiano C, Vietri MT, Grimaldi V, Picascia A, De Pascale MR, Napoli C (2015) Epigenetic-related therapeutic challenges in cardiovascular disease. *Trends in Pharmacological Sciences* 36: 226-235.
25. Selemidis S, Sobey CG, Wingler K, Schmidt HH, Drummond GR (2008) NADPH oxidases in the vasculature: molecular features, roles in disease and pharmacological inhibition. *Pharmacol Ther* 120:254-91.
26. Sima AV, Stancu CS, Simionescu M (2008) Vacular endothelium in atherosclerosis. *Cell and Tissue Research* 335: 191.

27. Simionescu M (2007) Implications of Early-Structural-Functional Changes in the Endothelium for Vascular Diseases. *Atherosclerosis, Trombosis and Vascular Biology* 2: 266-74.
28. Simionescu N, Vasile E, Lupu F, Popescu G, Simionescu M (1986) Prelesional events in atherogenesis. Accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit. The American Journal of Pathology 123: 109-125.
29. Soto Y, Acosta E, Delgaro L, Pérez A, Falcón V, Bécquer MA, Fraga Á, Brito V, Álvarez I, Griñán T, Fernández-Marrero Y, López-Requena A, Noa M, Fernández E, Vázquez AM (2012) Antiatherosclerotic effect of an antibody that binds to extracellular matrix glycosaminoglycans. Arterioscler Thromb Vasc Biol 32(3): 595-604.
30. Sumida A, Canugovi C, Lozhkin A, Hayami T, Madamanchi NR, Runge MS (2019) NOXA1-dependent NADPH oxidase regulates redox signaling andphenotype of vascular smooth muscle cell during atherogenesis. *Redox Biol.* 21: 101063.
31. Wang Y, Miao X, Liu Y, Li F, Liu Q, Sun J, Cai L (2014) Dysregulation of Histone Acetyltransferases and Deacetylases in Cardiovascular Diseases. *Oxidative Medicine and Cellular Longetivity* 10: 641968-61979.
32. Wang ZY, Qin W, Yi F (2015) Targeting histone deacetylases: perspectives for epige-netic-based therapy in cardio-cerebrovascular disease. *J. Geriatr. Cardiol.* 12: 153–164.
33. Zhong Y, Li J, Wang JJ, Chen C, Tran JT, Saadi A, Yu Q, Le YZ, Mandal MN, Anderson RE, Zhang SX (2012) X-box binding protein 1 is essential for the anti-oxidantdefense and cell survival in the retinal pigment epithelium. *PLoS One* 7: e38616.

List of author's publications

Papers published in ISI-listed international journals

1. Manea SA, **Vlad ML**, Fenyo IM, Lazar AG, Raicu M, Muresian H, Simionescu M, Manea A (2020) Pharmacological inhibition of histone deacetylase reduces NADPH oxidase expression, oxidative stress and the progression of atherosclerotic lesions in hypercholesterolemic apolipoprotein E-deficient mice; potential implications for human atherosclerosis. *Redox Biology* 101338 ***Factor de impact 9.99***

2. **Antonescu ML**, Manea SA, Raicu M, Muresian H, Simionescu M, Manea A (2019) Histone acetyltransferase-dependent pathways mediate NADPH oxidase 5 up-regulation in human macrophages under inflammatory conditions: a potential mechanism of reactive oxygen species overproduction in atherosclerosis. *Oxid Med Cell Longev* 2:3201062. ***Factor de impact: 4,87***

3. Manea SA, **Antonescu ML**, Fenyo IM, Raicu M, Simionescu M, Manea A (2018) Epigenetic regulation of vascular NADPH oxidase expression and reactive oxygen species production by histone deacetylase-dependent mechanisms in experimental diabetes. *Redox Biology* 16: 332-343. ***Factor de impact 7,8***

4. Stanica L, Rosu-Hamzescu M, Gheorghiu M, Stan M, **Antonescu ML**, Polonschii C, Gheorghiu E (2017) Electro-optical sensing of cellular effects under hypoxic conditions and carbonic anhydrase inhibition. *Hindawi Limited* 10: 1155. ***Factor de impact 1.34***

Articles in preparation

1. **Vlad ML**, Lazar AG, Fenyo IM, Manea SA, Manea A (2020) Inhibition of miR-155-5p reduces NADPH oxidase expression and oxidative stress in the aorta of hypercholesterolemic ApoE-deficient mice; potential implication in human atherosclerosis. *Cells.* **Factor de impact: 5.08**.

2. Manea SA, **Vlad ML**, Rebleanu D, Lazar AG, Calin M, Simionescu M, Manea A (2020) High resolution near-infrared fluorescence imaging of reactive oxygen species overproduction associated with atherosclerosis in hypercholesterolemic apolipoprotein E-deficient mice. *Antioxidants*. **Factor de impact: 5.01.**

Oral presentations

1. Lazar A, Cosac MT, **Vlad ML**, Manea A, Manea SA. Cross-communication between histone acetyltransferase and histone deacetylase epigenetic enzymes augments oxidative stress and fibrosis in the kidney of diabetic mice. "European Atherosclerosis Society Congress 2019 – 87th EAS Congress". Science at a Glance Section, Netherlands, 2019.
2. **Antonescu ML**, Manea SA, Fenyo IM, Constantin A, Simionescu M, Manea A. Epigenetic mechanisms of oxidative stress in atherosclerosis., EAS advanced course on AIL, March 22-25 2016, Amsterdam, Netherlands.

List of posters presented at scientific events

1. **Vlad ML**, Manea SA, Lazar AG, Raicu M, Muresian H, Simionescu M, Manea A. Epigenetic regulation of NADPH oxidase 5 expression by histone acetyltransferase-activated mechanisms in human macrophages exposed to inflammatory conditions; potential role in atherosclerosis. "ICBP Nicolae Simionescu - 40 years, Anniversary symposium", 2019
2. Lazar AG, Cosac MT, **Vlad ML**, Raicu M, Manea A, Manea SA. Activation of p300 histone acetyltransferase-dependent signaling pathways induces NADPH oxidase

expression and oxidative stress in the kidney of diabetic mice. "ICBP Nicolae Simionescu - 40 years, Anniversary symposium", 2019

3. **Vlad ML**, Manea SA, Lazar AG, Cosac MT, Raicu M, Muresian H, Simionescu M, Manea A. Activation of histone acetyltransferase-dependent signaling pathways induces macrophage polarization towards a pro-inflammatory M1-like phenotype in vitro; potential implication in human atherosclerosis. "The 11th National Congress with International Participation and the 37th Annual Scientific Session of the Romanian Society of Cell Biology", 20-23 iunie 2019, Constanta, Romania
4. Lazar AG, Cosac MT, **Vlad ML**, Raicu M, Manea A, Manea SA Activation of P300 histone acetyltransferase dependent signaling pathways induces NADPH oxidase expression and oxidative stress in the kidney of diabetic mice. "The 11th National Congress with International Participation and the 37th Annual Scientific Session of the Romanian Society of Cell Biology", 20-23 iunie 2019, Constanta, Romania
5. Cosac MT, **Vlad ML**, Manea SA, Lazar AG, Raicu M, Simionescu M, Manea A. Pharmacological inhibition of histone lysine demethylase JARID1b down-regulates the expression of pro-inflammatory molecules in cultured M1-polarized human macrophages. "The 11th National Congress with International Participation and the 37th Annual Scientific Session of the Romanian Society of Cell Biology", 20-23 iunie 2019, Constanta, Romania
6. Manea A, Manea SA, **Vlad ML**, Lazar AG, Cosac MT, Simionescu M. Histone deacetylase subtypes are part of positive feedback mechanisms controlling their own expression in the atherosclerotic aorta of hypercholesterolemic ApoE-/- mice. "29th European Meeting on Hypertension and Cardiovascular Protection", 2019
7. Manea SA, **Vlad ML**, Lazar AG, Fenyo IM, Cosac MT, Manea A. Identification of novel microRNAs associated with atherosclerotic lesion formation in the aorta of hypercholesterolemic ApoE-/- mice; potential implications for human atherosclerosis. "29th European Meeting on Hypertension and Cardiovascular Protection", 2019
8. **Vlad ML**, Manea SA, Lazar AG, Raicu M, Muresian H, Simionescu M, Manea A. NADPH oxidase – derived reactive oxygen species augment inflammatory macrophage responses via redox-sensitive histone deacetylase-dependent epigenetic mechanisms in experimental atherosclerosis. "European Atherosclerosis Society Congress 2019 – 87th EAS Congress" 26-29 Mai 2019, Maastricht, Olanda
9. Lazar AG, Cosac MT, **Vlad ML**, Manea A, Manea SA Cross communication between histone acetyltransferase and histone deacetylase epigenetic enzymes auguments oxidative stress and fibrosis in the kidney of diabetic mice. European Atherosclerosis Society Congress 2019 – 87th EAS Congress" 26-29 Mai 2019, Maastricht, Olanda
10. Manea SA, **Vlad ML**, Lazar AG, Cosac MT, Muresian H, Simionescu M, Manea A. Novel microRNAs associated with advanced human atherosclerotic lesions - potential biomarkers and therapeutic targets. "5th ESPT Congress - Precision Medicine and Personalised Health", 2019
11. Manea A, Manea SA, **Vlad ML**, Lazar AG, Cosac MT, Simionescu M. P300/CBP-histone acetyltransferase mediates the up-regulation of NADPH oxidase expression and oxidative

stress in the aorta of diabetic mice. "7th World Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension", 2019

12. Manea SA, Lazar AG, **Vlad ML**, Cosac MT, Manea A. Induction of histone deacetylase signaling pathways augments vascular inflammation and remodeling in diabetic mice. "7th World Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension", 2019
13. Manea SA, **Antonescu ML**, Rebleanu D, Lazar AG, Calin M, Manea A Ultrasound-based imaging of reactive oxygen species overproduction associated with atherosclerosis in hypercholesterolemic apolipoprotein E-deficient mice. The 43rd FEBS Congress 2018, Czech Republic
14. Manea A, Manea SA, **Antonescu ML**, Lazar AG, Muresian H, Simionescu M. Epigenetic regulation of inflammatory macrophage polarization by histone deacetylase-dependent mechanisms in experimental atherosclerosis. The 43rd FEBS Congress 2018, Czech Republic
15. **Antonescu ML**, Manea SA, Lazar AG, Raicu M, Muresian H, Simionescu M, Manea A. Epigenetic control of macrophage polarization by histone acetylation/deacetylation enzymes in experimental atherosclerosis. "The 36th Annual Scientific Session of the Romanian Society for Cell Biology and the 10th National Congress with International participation", 2018, Craiova, Romania
16. Manea SA, **Antonescu ML**, Rebleanu D, Lazar AG, Raicu M, Calin M, Manea A. High resolution near-infrared fluorescence imaging of reactive oxygen species overproduction associated with atherosclerosis in hypercholesterolemic apolipoprotein E-deficient mice. "The 36th Annual Scientific Session of the Romanian Society for Cell Biology and the 10th National Congress with International participation", 2018, Craiova, Romania
17. Lazar AG, **Antonescu ML**, Fenyo IM, Manea A, Manea SA. Histone acetyltransferase-dependent signaling pathways mediate endothelin-1 up-regulation and markers of vascular dysfunction in experimental diabetes. "1st Olympiad in Cardiovascular Medicine", International Symposium on Experimental & Clinical Cardiology, 17-19 Mai 2018, Athens, Greece
18. **Antonescu ML**, Lazar AG, Manea SA, Raicu M, Muresian H, Simionescu M, Manea A. Pharmacological inhibition of NADPH oxidase down-regulates the expression of pro-inflammatory markers in classically-activated macrophages in vitro: potential implication in human atherosclerosis. "1st Olympiad in Cardiovascular Medicine", International Symposium on Experimental & Clinical Cardiology, 17-19 Mai 2018, Athens, Greece
19. **Antonescu ML**, Lazar AG, Manea SA, Raicu M, Muresian H, Manea A, Simionescu M. Up-regulation of macrophage NADPH oxidase 5 expression and reactive oxygen species production by histone acetyltransferase-dependent mechanisms in atherosclerosis. "Protecting the Code: Epigenetic Impacts on Genome Stability", 28 octombrie – 02 noiembrie 2017, Berlin, Germania
20. Manea SA, **Antonescu ML**, Lazar A, Fenyo IM, Manea A. Pharmacological inhibition of histone acetyltransferase reduces endothelin-1 expression and mitigates markers of

vascular dysfunction in diabetes. "European Society for Pharmacogenomics and Personalized Therapy, 4th Conference", 2017

21. **Antonescu ML**, Manea SA, Simionescu M, Manea A. NADPH oxidase 5 expression are is regulated by histone acetyltransferase 1 and P300 – dependent in human macrophages. "85th EAS Congress", 23-26 aprilie 2017, Praga
22. Manea A, **Antonescu ML**, Fenyo IM, Manea M. In vivo silencing of histone deacetylase 1 displays anti-atherosclerotic effects in hypercholesterolemic apolipoprotein E deficient mice. "Heart Failure 2017 - 4th World Congress on Acute Heart Failure", 28 aprilie - 04 mai 2017, Paris, Franta
23. Manea A, Manea SA, **Antonescu ML**, Fenyo IM, Raicu M, Simionescu M. Protein expression profiling of histone acetyltransferases and histone deacetylases in human and experimental atherosclerosis. "Heart Failure 2017 - 4th World Congress on Acute Heart Failure", 28 aprilie - 04 mai 2017, Paris, Franta
24. **Antonescu ML**, Manea SA, Muresian H, Simionescu M, Manea A. Histone acetyltransferases control macrophage-type NADPH oxidase 5 up-regulation and reactive oxygen species formation in atherosclerosis. "9th National Congress with International Participation and 35th Annual Scientific Session of the Romanian Society for Cell Biology", 06 - 12 iunie 2017, Iasi, Romania
25. Manea SA, Fenyo IM, **Antonescu ML**, Preda B, Raicu M, Muresian H, Manea A. Histone deacetylase-dependent epigenetic pathways mediate oxidative stress and inflammation in experimental atherosclerosis. "9th National Congress with International Participation and 35th Annual Scientific Session of the Romanian Society for Cell Biology", 06 - 12 iunie 2017, Iasi, Romania
26. Manea SA, **Antonescu ML**, Lazar A, Fenyo IM, Manea A. Pharmacological inhibition of histone acetyltransferase reduces endothelin-1 expression and mitigates markers of vascular dysfunction in diabetes. "European Society for Pharmacogenomics and Personalized Therapy, 4th Conference", 2017
27. Manea SA, **Antonescu ML**, Lazar AG, Fenyo IM, Manea A. Pharmacological inhibition of histone acetyltransferase reduces endothelin-1 expression and mitigates markers of vascular dysfunction in diabetes. "European Society for Pharmacogenomics and Personalized Therapy, 4th Conference", 03 - 08 octombrie 2017, Catania, Italy
28. **Antonescu ML**, Manea SA, Simionescu M, Manea A. Epigenetic regulation of NADPH oxidase 5 expression by p300/histone acetyltransferase in human macrophages. 8th National Congress with International Participation and 34rd Annual Scientific Session of the RSCB, 8-12 iunie 2016, Oradea, Bulletin of Romanian Society for Cell Biology, No. 44, June 2016, pg. 74
29. Manea A, Manea SA, **Antonescu ML**, Fenyo IM, Raicu M, Simionescu M. Pharmacological inhibition of histone deacetylase reduces vascular NADPH oxidase expression and reactive oxygen species formation in experimental diabetes. 8th National Congress with International Participation and 34rd Annual Scientific Session of the RSCB,

8-12 iunie 2016, Oradea, Bulletin of Romanian Society for Cell Biology, No. 44, June 2016, pg 80

30. Manea A, Manea SA, Fenyo IM, **Antonescu ML**, Raicu M, Simionescu M. Pharmacological inhibition of histone deacetylase reduces oxidative stress and inflammation in the aorta of diabetic mice. The 4th International Symposium on Adipobiology and Adipopharmacology (ISAA), 28-31 octombrie 2015, Bucuresti, Romanian Journal of Diabetes, Nutrition and Metabolic Diseases, Volumul 22 (2015)/Supplement 2, pg. 29

Awards

UEFISCDI awards

1. **Antonescu ML**, Manea SA, Raicu M, Muresian H, Simionescu M, Manea A (2019) Histone acetyltransferase-dependent pathways mediate NADPH oxidase 5 up-regulation in human macrophages under inflammatory conditions: a potential mechanism of reactive oxygen species overproduction in atherosclerosis. *Oxid Med Cell Longev* 2:3201062. ***Factor de impact: 5.08***
2. Manea SA, **Antonescu ML**, Fenyo IM, Raicu M, Simionescu M, Manea A (2018) Epigenetic regulation of vascular NADPH oxidase expression and reactive oxygen species production by histone deacetylase-dependent mechanisms in experimental diabetes. *Redox Biology* 16: 332-343. ***Factor de impact 7,8***

Poster awards

1. **Antonescu ML**, Manea SA, Lazar AG, Raicu M, Muresian H, Simionescu M, Manea A. Epigenetic control of macrophage polarization by histone acetylation/deacetylation enzymes in experimental atherosclerosis. "The 36th Annual Scientific Session of the Romanian Society for Cell Biology and the 10th National Congress with International participation", 2018, Craiova, Romania
2. Lazar AG, Cosac MT, **Vlad ML**, Raicu M, Manea A, Manea SA Activation of P300 histone acetyltransferase dependent signaling pathways induces NADPH oxidase expression and oxidative stress in the kidney of diabetic mice. "The 11th National Congress with International Participation and the 37th Annual Scientific Session of the Romanian Society of Cell Biology", 20-23 iunie 2019, Constanta, Romania

Grants

1. PNCDI III, P2 - Increasing the competitiveness of the Romanian economy through CDI

Project type: Demonstration experimental project (PED), Contract no. 342/2020

Project title: Advanced teranostic strategy in atherosclerosis that integrates pharmaco-epigenomic interventions and biomimetic microbubbles for targeted administration of drugs using ultrasound

Period: August 2020 - July 2022

Project director: Dr. Adrian Manea

2. PNCDI III, P2 - Increasing the competitiveness of the Romanian economy through CDI

Project type: Demonstration experimental project (PED), Contract no. 265/2020

Project title: Innovative pharmacological strategy based on triterpenes for the treatment of micro- and macrovascular complications associated with diabetes - preclinical study

Period: August 2020 - July 2022

Project director: Dr. Simona-Adriana Manea

3. PNCDI III, Program 4 - Fundamental and frontier research

Project type: Complex frontier research projects, Contract no. 5/2018

Project name: Targeting the mechanisms of innate immunity for better risk stratification and identification of new therapeutic options in myocardial infarction

Timeframe: July 2018 - June 2022

Project Executive: Acad. Maya Simionescu

4. Competitiveness Operational Program, Priority Axis 1: Research, technological development and innovation (RDI) in support of economic competitiveness and business development

Type or project: Attracting staff with advanced skills from abroad to strengthen R&D capacity, Contract no.115/2016

Project name: Targeted therapies for aortic valve disease in diabetes (THERAVALDIS)

Timeframe: September 2016 - December 2020

Project Manajer: Dr. Agneta Simionescu

5. PNCDI III, Program 1 - Development of the national research and development system

Type of project: Research projects to stimulate young independent teams, Contract no. 51/2018

Project name: Preclinical strategy for reducing vascular inflammation and oxidative stress in atherosclerosis by modulating new molecular mechanisms dependent on non-coding RNA

Timeframe: May 2018 - July 2020
Project Manager: Dr. Adrian Manea

6. PNCDI III, Program 4 - Fundamental and frontier research
Type of project: Exploratory research projects, Contract no. 69/2017
Project name: New epigenetic mechanisms involved in the activation of anti-inflammatory macrophages - potential therapeutic targets in atherosclerosis
Timeframe: July 2017 - December 2019
Project manager: Acad. Maya Simionescu

7. PNCDI III, Program 2 - Increasing the competitiveness of the Romanian economy through research, development and innovation
Type of project: Experimental – demonstrative project, Contract no. 137/2017
Project name: Non-invasive nanotechnology-based method for molecular imaging of oxidative stress in cardiovascular disease
Timeframe: January 2017– September 2018
Project Manager: Dr. Adrian Manea

8. PNII IDEI, Contract no. 107/2011
Project name: Molecular mechanisms involved in the regulation of oxidative stress in atherosclerosis: the development of innovative functional nanocomplexes for antioxidant therapy
Time frame: January 2012– December 2016.
Project Manager: Acad. Maya Simionescu