ROMANIAN ACADEMY
School of Advanced Studies of the Romanian Academy
Institute of Cell Biology and Patology „Nicolae Simionescu”

DOCTORAL THESIS SUMMARY

Innovative nanotherapies for aortic valve disease in diabetes

DOCTORAL SUPERVISOR:
Acad. MAYA SIMIONESCU

DOCTORAL STUDENT:
GEANINA VOICU

2022

Table of contents

DOCTORAL THESIS SUMMARY

Keywords: calcific aortic valve disease, valvular interstitial cells, osteodifferentiation, transcription factor Runx2, diabetes, nanotherapy, polyplexes, targeted lipopolyplexes, collagen IV, vascular cells adhesion molecule VCAM-1, osteogenic molecules, calcium deposits

Total number of pages – 219

Total number of figures in Part I – 12

Total number of tables in Part I – 2

Total number of figures in Part II – 43

Total number of tables in Part II – 8

Bibliographical references – 462

Papers published in ISI quoted journals during the doctoral internship – 7 (2 as principal author)

Papers in preparation – 2

Posters presented at international scientific conferences – 3 (as principal author)

Oral presentations at international scientific conferences – 1 (as principal author)

Patents – 1

Scholarships obtained during the doctoral program – 1 (scholarship from Romanian Academy)

Participation in national research projects related to the topic of the doctoral thesis – 1

Introduction and objectives

Calcific aortic valve disease is a degenerative disease that affects 5% of the population over 65 years old, Romania being one of the countries with the highest mortality ratio in Europe (http://www.romedic.ro/stenoza-aortica). Early diagnosis and treatment management are of paramount importance in the treatment of aortic valve disease, as there is no treatment to block its progression. In patients with severe symptoms, valve replacement surgery remains the only approach (Kanwar et al., 2018; Iung et al., 2003), the severe prognosis of the disease is not influenced by the current medication used to treat the symptoms. The progression of valve calcification and the necessity of a surgical intervention vary considerably from one patient to another, the total obstruction of the valve taking place within 2-5 years of the onset of calcification (Owens et al., 2010).

The aortic valve is formed by three layers of valvular interstitial cells (VIC) that maintain the valve homeostasis, separated from heart cavities by a layer of valvular endothelial cells (VEC) (Rutkovskiy et al., 2017). The most important pathological process in calcific aortic valve disease is osteoblastic differentiation of VIC, mastered by Runt-related transcription factor 2 (Runx2)/Core-binding factor subunit alpha-1 (Cbfa1). Runx2 determines the overexpression of osteodifferentiated VIC specific molecules: alkaline phosphatase, osteocalcin, osteopontin, bone sialoprotein and contributes to the active deposition of calcium in the valve (Hortells et al., 2018).

Although altered lipid metabolism and inflammation are the first processes that occur in calcific aortic valve disease, the active deposition of calcium is the most important mechanism in disease progression (Pawade et al., 2015). Diabetes is both a risk factor and a predictor of aortic valve degeneration, contributing to the progression of calcification by inducing valvular endothelial cell dysfunction, extracellular matrix remodeling, inflammation, and ectopic calcification (calcium deposition in tissues other than bone) (Larrew et al., 2013).

At the moment there are no particular targets or specific therapies for aortic valve disease treatment in association with diabetes. Nanotherapy for aortic valve disease is an area of research that is rapidly developing, and its effects include improved living conditions, increased life expectancy, and decreased morbidity and mortality.

The aim of this doctoral thesis was to identify the mechanisms and molecules involved in the osteodifferentiation of valvular interstitial cells, the main process in calcific aortic valve disease in association with diabetes mellitus, and the development and application of targeted nanotherapeutic strategies to block this process in order to reduce or block early aortic valve calcification..

The following **specific objectives** were proposed and achieved in the doctoral thesis:

1. Study of the mechanisms involved in VIC osteodifferentiation and identification of potential therapeutic targets. This evaluation described early and advanced changes induced, *in vitro*, by increased glucose concentrations and osteogenic factors in VIC.
2. Design, production and characterization of targeted nanoparticles to block VIC osteodifferentiation and validating the therapeutic effect of these nanotherapies using *in vitro* experimental models. Polyplexes consisting of C60-PEI fullerene complexed with shRNA plasmids and specifically targeted lipopolyplexes, formed by encapsulating the mentioned polyplexes in liposomes, were obtained and characterized. The therapeutic effect of nanoparticles was established on two- and three-dimensional cell culture systems.
3. Preclinical validation of nanotherapies developed using the *in vivo* experimental model of hyperlipemic and diabetic ApoE-deficient mouse. In this study, the accumulation of the previously obtained and characterized *in vitro* targeted lipopolyplexes in the dysfunctional valve and the therapeutic effect of these nanoparticles in the aortic valve of hyperlipemic and diabetic ApoE-deficient mice were demonstrated.

Doctoral thesis structure

The doctoral thesis is structured in two principal parts, divided into eight chapters.

The first part, composed of three chapters, comprises theoretical aspects of aortic valve biology and pathology, and, also, of principal therapies for aortic valve disease. **The first chapter** describes the anatomy, physiology, ontogeny and pathology of the aortic valve, emphasizing the main cellular mechanisms and molecules involved in calcific aortic valve disease in diabetes. **The second chapter** refers to the current state of therapeutic approaches in calcific aortic valve disease, and **the third chapter** details the different types of interfering RNA or drugs-carrying nanoparticles used to treat cardiovascular disease, their major transport pathways to the aortic valve, and the mechanism of action of interfering RNA in calcified aortic valve therapy. This chapter also refers to the overexpressed molecules in the affected aortic valve, which can be used to target nanoparticles (collagen IV, vascular cells adhesion molecule 1).

The second part of the thesis contains the original contributions, structured in five chapters. **The first chapter** describes the *in vitro* human aortic VIC (oVIC) osteodifferentiation protocol using an HGMO culture medium that contains high glucose concentrations and osteogenic factors (ascorbic acid, dexamethasone and β-glycerophosphate), appropriate in both two-dimensional (2D) and three-dimensional culture (3D). In the early stages of calcific aortic valve disease, after VEC layer permeabilization, lipids and immune cells reach the subendothelial layer. Cytokines released by the immune cells initiate the proinflammatory process, inducing the extracellular matrix remodeling and phenotypic transition of VIC into myofibroblasts (aVIC), characterized by an overexpression of α smooth muscle actin and the presence of stress fibers. In the advanced stages, in response to pro-osteogenic stimulation (inflammation, mechanical stress), VIC osteodifferentiation takes place, the main process involved in the ectopic calcification of valve leaflets. Diabetes is known to accelerate the active deposition of calcium in the aortic valve (Bossé et al., 2009).

The main results obtained:

- HGMO medium that contains high glucose concentrations and osteogenic factors induces VIC osteodifferentiation (oVIC) by overexpression of osteogenic molecules (alkaline phosphatase, osteopontin, bone sialoprotein, bone morphogenic protein) and
- Abnormal increase in alkaline phosphatase enzymatic activity and accumulation of calcium in oVIC.

The results of this study were published in the journal Pharmaceutics (impact factor 6.321) (Voicu et al., 2020).

Chapter 2 presents the polyplexes formation protocol by complexation of C60-polyethyleneimine fullerene conjugate (C60-PEI) with plasmids containing specific short hairpin RNA (shRNA) sequences for the Runx2 transcription factor involved in VIC osteodifferentiation, characterization and therapeutic effect induced by Runx silencing in oVIC by cells incubation with these polyplexes.

The main results obtained:

- According to physicochemical characterization, polyplexes have a dimension of about 250 nm at an N/P ratio bigger than 25, and the Zeta potential increases depending on the N/P ratio, being +15 mV at an N/P ratio of 25.
- Polyplexes are cytocompatible at all investigated N/P ratios (10, 15, 20, 25).
- Polyplexes are taken up efficiently by VIC, as evidenced by the intracellular presence of red dots representing the fluorescent plasmid Cy3.
- Polyplexes are effective transfection vectors at an N/P ratio of 25.
- Functional tests showed that polyplexes that contain shRNA plasmid sequences coded as sh_1 and sh_2 (C60-PEI/sh_1, C60-PEI/sh_2) decrease the protein expression of transcription factor Runx2, and other osteogenic molecules involved in VIC activation and osteodifferentiation (αSMA, Smad2/3, pSmad2/3, alkaline phosphatase, osteopontin, bone sialoprotein, bone morphogenic protein 4).
- Polyplexes C60-PEI/sh_1, C60-PEI/sh_2 decrease the alkaline phosphatase activity after a single or a double transfection of oVIC. Decreased alkaline phosphatase activity is maintained more than a week after the last transfection (up to 21 days).

The results of this study were published in the journal Pharmaceutics (impact factor 6.321) (Voicu et al., 2020).

Chapter 3 presents the lipopolyplexes formation protocol by encapsulation of C60-PEI/sh_1 preformed polyplexes at an N/P ratio of 25 in anionic liposomes stabilized with polyethyleneglycol and their specific targeting to overexpressed molecules in oVIC: collagen IV, vascular cells adhesion molecule 1 (VCAM-1). These nanoparticles are also characterized by the evaluation of their potential to block the osteoblastic differentiation of VIC cultured in 2D and 3D systems.

Despite the high efficacy of *in vitro* transfection, the use of polyplexes *in vivo* has not had significant therapeutic effects due to the positive charges that cause nonspecific interaction with plasma proteins and the accumulation of these polyplexes in the liver and spleen (Fischer et al., 2004). To increase the stability of the polyplexes in the blood circulation and to improve the transfection rate, while decreasing the toxicity, the polyplexes can be encapsulated in sterically stabilized liposomes with polyethyleneglycol (Ko și Bickel, 2012), which also allows the functionalization of liposomes for a targeted transport to the affected site (Calin și Manduteanu, 2017).

The main results obtained:

- oVIC present an overexpression of collagen IV and VCAM-1, molecules that could be used for lipopolyplexes targeting.
- Lipopolyplexes were obtained by encapsulating C60-PEI/sh_1 polyplexes in anionic liposomes, using the reverse phase evaporation technique.
- Physicochemical characterization of lipopolyplexes indicated a mean dimension of about 200 nm and a negative Zeta potential, of -30 mV, the lipopolyplexes being colloidal and electrolytically stable aqueous dispersion.

- Lipopolyplexes ColIV-LPP/shControl and V-LPP/shControl are cyto- and hemocompatible and are efficiently taken up by oVIC.
- Lipopolyplexes ColIV-LPP/shRunx2 and V-LPP/shRunx2 induce the significant reduction of Runx2 gene and protein level in oVIC, both in 2D and 3D systems, which also has the effect of reducing the levels of the other osteogenic molecules involved in VIC osteodifferentiation (osteopontin, bone sialoprotein, bone morphogenic protein 2).
- Functional tests showed that oVIC transfection with ColIV-LPP/shRunx2 and V-LPP/shRunx2 decreases the alkaline phosphatase activity and blocks calcium deposit formation through a mechanism that involves lowering the level of Runx2.

The results of this study were published in the International Journal of Molecular Sciences (impact factor 6.009) (Voicu et al., 2022).

The fourth chapter comprises results obtained *in vivo*, on a diabetic and hyperlipidemic ApoE-deficient animal model intravenously injected with double-targeted lipopolyplexes to collagen IV and VCAM-1 (ColIV/V-LPP/shControl). The biodistribution of these lipopolyplexes in the murine organs, and especially in the aortic valve, was investigated using the IVIS technique, the lipopolyplexes being labeled with rhodamine-DSPE that permits the evaluation of the lipopolyplexes accumulation in organs *ex vivo*. Due to its photostability and visible spectrum emission, rhodamine is used successfully in fluorescence imaging and microscopy studies (de Almeida et al., 2007).

The main results obtained:

- Aortic valves of diabetic and hyperlipidemic ApoE-deficient mice present an increased expression of collagen IV and VCAM-1.
- ColIV/V-LPP/shControl lipopolyplexes are located specifically in the dysfunctional aortic valve, the biodistribution being correlated with the presence of high collagen IV and VCAM-1 expression in the valve.
- Fluorescent expression of YFP protein in the aortic valves is maintained for up to 48 hours after ColIV/V-LPP/pEYFP injection.

Results presented in **chapter 5** demonstrate the therapeutic effect of double-targeted lipopolyplexes containing shRNA-Runx2 sequences in a diabetic and hyperlipidemic ApoE-deficient animal model.

The main results obtained:

- ColIV/V-LPP/shRunx2 lipopolyplexes induce the significant reduction of Runx2 gene and protein level in the aortic valve, also reducing the gene expression of osteopontin, osteocalcin and alkaline phosphatase. ColIV/V-LPP/shRunx2 determine the reduction of Runx2 gene level in other organs too (lungs, kidneys, liver), but without a statistical significance.
- Reducing Runx2 levels blocks lipid deposition and decreases alkaline phosphatase activity in the aortic valve.

The results obtained in the present doctoral thesis led to the following **original conclusions**:

- High glucose concentrations and osteogenic factors (ascorbic acid, dexamethasone, β-glycerophosphate) determine a time-dependent increase of osteogenic molecules' protein expression involved in VIC activation (α smooth muscle actin) and osteodifferentiation (Runx2, alkaline phosphatase, osteopontin, bone sialoprotein, bone morphogenic protein). The increase in the protein level of osteogenic molecules

is associated with the high activity of alkaline phosphatase enzyme and the increase in the concentration of calcium in osteodifferentiated VIC, with the increase being directly proportional to the period of cells' exposure to osteogenic factors.

- Nanopolyplexes formed by (C60)-PEI fullerene complexation with shRNA-Runx2 plasmids are cytocompatible and efficiently transport intracellularly shRNA-Runx2 plasmids, determining the reduction of protein level of osteogenic molecules Runx2, α smooth muscle actin, Smad2/3, alkaline phosphatase, osteopontin, bone sialoprotein, bone morphogenic protein 4, as well as reducing alkaline phosphatase activity.
- Lipopolyplexes formed by C60-PEI/ARNsh-Runx2 polyplexes encapsulation in liposomes functionalized with peptides that specifically recognize collagen IV and VCAM-1 (ColIV-LPP/ARNsh-Runx2 and V-LPP/ARNsh-Runx2), molecules overexpressed in the dysfunctional aortic valve, are adequate nanocarriers for targeted transport of shRNA plasmids *in vivo*. These targeted lipopolyplexes determine the significant reduction of gene and protein expression of osteogenic molecules in osteodifferentiated VIC cultivated in two culture systems, two-dimensional in monolayer and three-dimensional in hydrogel constructs obtained from porcine aortic root. Moreover, the alkaline phosphatase activity and calcium concentration are reduced after VIC transfection with ColIV-LPP/ARNsh-Runx2 and V-LPP/ARNsh-Runx2 lipopolyplexes.
- *In vivo*, in an animal model of diabetic and hyperlipidemic ApoE-deficient mice, collagen IV and VCAM-1 double-targeted lipopolyplexes selectively accumulate in the dysfunctional aortic valve. Repeated administration of lipopolyplexes reduces atherosclerotic lesions by decreasing the expression of osteogenic factors Runx2, osteopontin, osteocalcin, alkaline phosphatase involved in calcific aortic valve disease.

This doctoral thesis comes up with the following **original contributions**:

► establishing an *in vitro* model of osteodifferentiation of valvular interstitial cells.

► silencing the Runx2 transcription factor may be a new therapeutic strategy effective in blocking VIC osteodifferentiation and aortic valve disease progression.

► Runx2-specific shRNA nanocarriers targeted to the affected aortic valve are suitable for *in vivo* administration. These nanocarriers are lipopolyplexes, namely liposomes that encapsulate C60-PEI/shRNA-Runx2 polyplexes, functionalized with collagen IV and vascular cell adhesion molecule 1 (VCAM-1) recognition peptides. Lipopolyplexes block osteoblastic differentiation of valvular interstitial cells *in vitro* and calcification of the aortic valve *in vivo* by reducing the level of the transcription factor Runx2 and other osteogenic proteins (osteopontin, bone sialoprotein, alkaline phosphatase, osteocalcin and bone protein).

RESULTS CAPITALIZATION

The results obtained during the doctoral program within the Institute of Biology and Cell Pathology "Nicolae Simionescu" of the Romanian Academy were capitalized as follows:

- **7 publications** in **ISI quoted journals**: **2 as first-author** and **5 as co-author**
- **one oral presentation** and **3 posters** at **international conferences**
- **one patent application at OSIM.**

One of the articles published as a first-author was selected to illustrate the cover of the „International Journal of Molecular Sciences" in April 2022 issue, "VCAM-1 targeted nanocarriers of shRNA-Runx2 for calcific valve disease treatment". The research results were awarded by UEFISCDI with 4 prizes.

WORKS PUBLISHED IN ISI QUOTED INTERNATIONAL JOURNALS (7 articles)

First-author (2):

1. **Geanina Voicu**, Daniela Rebleanu, Cristina Ana Mocanu, Gabriela Tanko, Ionel Droc, Cristina Mariana Uritu, Mariana Pinteala, Ileana Manduteanu, Maya Simionescu, Manuela Calin, VCAM-1 Targeted Lipopolyplexes as Vehicles for Efficient Delivery of shRNA-Runx2 to Osteoblast-Differentiated Valvular Interstitial Cells; Implications in Calcific Valve Disease Treatment, International Journal of Molecular Sciences, 2022, 23, 3824; **IF** in 2022 6.009
2. **Geanina Voicu**, Daniela Rebleanu, Cristina Ana Constantinescu, Elena Valeria Fuior, Letitia Ciortan, Ionel Droc, Cristina Mariana Uritu, Mariana Pinteala, Ileana Manduteanu, Maya Simionescu, Manuela Calin, Nano-Polyplexes Mediated Transfection of Runx2-shRNA Mitigates the Osteodifferentiation of Human Valvular Interstitial Cells, Pharmaceutics, 2020, 12, 507; **IF** in 2020 6.321

Co-author (5):

1. Cristina Ana Mocanu, Elena Valeria Fuior, **Geanina Voicu**, Daniela Rebleanu, Florentina Safciuc, Mariana Deleanu, Ioana Madalina Fenyo, Virginie Escriou, Ileana Manduteanu, Maya Simionescu, Manuela Calin, P-selectin targeted RAGE-shRNA lipoplexes alleviate atherosclerosis-associated inflammation, Journal of Controlled Release, 2021, 338, pp. 754–772; **IF** in 2021 10.61
2. Elena Valeria Fuior, Cristina Ana Mocanu, Mariana Deleanu, **Geanina Voicu**, Maria Anghelache, Daniela Rebleanu, Maya Simionescu, Manuela Calin, Evaluation of VCAM-1 Targeted Naringenin/Indocyanine Green-Loaded Lipid Nanoemulsions as Theranostic Nanoplatforms in Inflammation, Pharmaceutics, 2020, 12(11), pp. 1–21, 1066; **IF** in 2020 6.321
3. Monica Tucureanu, Alexandru Filippi, Nicoleta Alexandru, Cristina Ana Constantinescu, Letitia Ciortan, Razvan Macarie, Mihaela Vadana, **Geanina Voicu**, Sabina Frunza, Dan Nistor, Agneta Simionescu, Dan Teodor Simionescu, Adriana

Georgescu, Ileana Manduteanu, Diabetes-induced early molecular and functional changes in aortic heart valves in a murine model of atherosclerosis, Diabetes and Vascular Disease Research, 2019, 16(6), 562-576; **IF** in 2019 2.707

4. Elena Valeria Fuior, Mariana Deleanu, Cristina Ana Constantinescu, Daniela Rebleanu, **Geanina Voicu**, Maya Simionescu, Manuela Calin, Functional Role of VCAM-1 Targeted Flavonoid-Loaded Lipid Nanoemulsions in Reducing Endothelium Inflammation, Pharmaceutics, 2019, 11(8), 391; **IF** in 2019 4.773
5. Cristina Ana Constantinescu, Elena Valeria Fuior, Daniela Rebleanu, Mariana Deleanu, Viorel Simion, **Geanina Voicu**, Virginie Escriou, Ileana Manduteanu, Maya Simionescu, Manuela Calin, Targeted Transfection Using PEGylated Cationic Liposomes Directed Towards P-Selectin Increases siRNA Delivery into Activated Endothelial Cells, Pharmaceutics, 2019, 11(1), 47; **IF** in 2019 4.773

PATENTS

1. OSIM application no. A/00811, inventors Calin Manuela, Rebleanu Daniela, Constantinescu Cristina Ana, **Voicu Geanina**, Deleanu Mariana, Manduteanu Ileana: „Method for obtaining nanocarriers used for RNA interference vectorization and targeted delivery to aortic valve cells"

WORKS PRESENTED AT INTERNATIONAL SCIENTIFIC EVENTS:

Oral presentations (1):

1. **Geanina Voicu**, Daniela Rebleanu, Elena Valeria Fuior, Cristina Ana Mocanu, Maria Anghelache, Mihaela Turtoi, Florentina Safciuc, Ileana Manduteanu, Maya Simionescu, Manuela Calin, VCAM-1 targeted lipopolyplexes carrying Runx2-shRNA mitigate the osteodifferentiation of 3D-cultured human valvular interstitial cells, The 42nd Anniversary Symposium of the Institute of Cell Biology and Pathology „Nicolae Simionescu" and The 38th Annual Scientific Session of the Romanian Society of Cell Biology with international participation, Bucharest, 2021.

Posters (3):

1. **Geanina Voicu**, Cristina Ana Mocanu, Daniela Rebleanu, Agneta Simionescu, Maya Simionescu, Ileana Manduteanu, Manuela Calin, Down-regulation of Runx2 by C60-

PEI/shRNA-Runx2 polyplexes reduces the osteodifferentattion of valvular interstitial cells, Workshop Theravaldis, Bucharest, 2020

2. **Geanina Voicu**, Cristina Ana Constantinescu, Daniela Rebleanu, Agneta Simionescu, Maya Simionescu, Ileana Manduteanu, Manuela Calin, Polyplexes carrying a shRNA plasmid downregulate the transcription factor Runx2 in valvular interstitial cells exposed to high glucose concentrations and osteogenic factors, Prague, 2019
3. **Geanina Voicu**, Cristina Ana Constantinescu, Daniela Rebleanu, Agneta Simionescu, Maya Simionescu, Ileana Manduteanu, Manuela Calin, High glucose concentrations potentiate the induction of osteoblast phenotype of valvular interstitial cells exposed to osteogenic factors, The 11th National congress with international participation and The 37th Annual Scientific session of Romanian Society of Cell Biology (under Romanian Academy), Constanța, 2019

AWARDS

Awarding UEFISCDI research results:

1. Cristina Ana Mocanu, Elena Valeria Fuior, **Geanina Voicu**, Daniela Rebleanu, Florentina Safciuc, Mariana Deleanu, Ioana Madalina Fenyo, Virginie Escriou, Ileana Manduteanu, Maya Simionescu, Manuela Calin, Journal of Controlled Release, 2021, 338, pp. 754–772; **IF** in 2021 10.61
2. **Geanina Voicu**, Daniela Rebleanu, Cristina Ana Constantinescu, Elena Valeria Fuior, Letitia Ciortan, Ionel Droc, Cristina Mariana Uritu, Mariana Pinteala, Ileana Manduteanu, Maya Simionescu, Manuela Calin, Nano-Polyplexes Mediated Transfection of Runx2-shRNA Mitigates the Osteodifferentiation of Human Valvular Interstitial Cells, Pharmaceutics 2020, 12, 507; **IF** in 2020 6.321
3. Elena Valeria Fuior, Cristina Ana Mocanu, Mariana Deleanu, **Geanina Voicu**, Maria Anghelache, Daniela Rebleanu, Maya Simionescu, Manuela Calin, Evaluation of VCAM-1 Targeted Naringenin/Indocyanine Green-Loaded Lipid Nanoemulsions as Theranostic Nanoplatforms in Inflammation, Pharmaceutics, 2020, 12(11), pp. 1–21, 1066; **IF** in 2020 6.321
4. Elena Valeria Fuior, Mariana Deleanu, Cristina Ana Constantinescu, Daniela Rebleanu, **Geanina Voicu**, Maya Simionescu, Manuela Calin, Functional Role of

VCAM-1 Targeted Flavonoid-Loaded Lipid Nanoemulsions in Reducing Endothelium Inflammation, Pharmaceutics, 2019, 11(8), 391; **IF** in 2019 4.773

SCHOLARSHIP OBTAINED DURING THE DOCTORAL PROGRAM

Doctoral scholarship: Romanian Academy (SCOSAAR): 2017-2020

RESEARCH FUNDING (GRANTS):

1. **PN-II-RU-TE-2014-4-1837**

Research project to stimulate the establishment of young independent research teams

Project title: Endothelial-targeted nanotherapies designed to silence receptor for advanced glycation products (RAGE) in atherosclerosis (NANORAGE)

Period: 2015-2017

Project director: Dr. Manuela Călin

2. **POC ID P_37_298; 115/13.09.2016**

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

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

Period: 2016-2020

Project director: Dr. Agneta Simionescu

3. **13PCCDI/2018**

Complex projects carried out in consortia Research Development Innovation

Project title: Smart therapies for non-communicable diseases based on the controlled release of pharmacological compounds from encapsulated cells after genetic manipulation or vectored bionanoparticles (INTERA)

Period: 2018-2020

Project director: Acad. Maya Simionescu

4. **PN-III-P4-ID-PCCF-2016-0050**

Complex frontier research projects

Project title: Mimicking the mechanisms of life through supramolecular chemistry approaches, in five dimensions

Period: 2018-2022

Project director: Dr. Aatto Laaksonen

5. **PN-II-P4-ID-PCE-2020-2465**

Exploratory research projects

Project title: Targeted therapy based on biomimetic nanocarriers for the resolution of inflammation in atherosclerosis (NANORES)

Period: 2021-2023

Project director: Dr. Manuela Călin.

References:

1. Bossé, Y.; Miqdad, A.; Fournier, D.; Pépin, A.; Pibarot, P.; Mathieu, P., 2009. Refining Molecular Pathways Leading to Calcific Aortic Valve Stenosis by Studying Gene Expression Profile of Normal and Calcified Stenotic Human Aortic Valves, Circulation: Cardiovascular Genetics, 2(5), pp. 489–498
2. Calin, M.; Manduteanu, I., 2017. Emerging Nanocarriers-based Approaches to Diagnose and Reduce Vascular Inflammation in Atherosclerosis, Current Medicinal Chemistry, 24(6), pp. 550–567
3. de Almeida, R. F. M.; Borst, J.; Fedorov, A.; Prieto, M.; Visser, A. J. W. G., 2007. Complexity of Lipid Domains and Rafts in Giant Unilamellar Vesicles Revealed by Combining Imaging and Microscopic and Macroscopic Time-Resolved Fluorescence, Biophysical Journal, 93(2), pp. 539–553
4. Fischer, D.; Osburg, B.; Petersen, H.; Kissel, T.; Bickel, U., 2004. Effect of poly(ethylene imine) molecular weight and pegylation on organ distribution and pharmacokinetics of polyplexes with oligodeoxynucleotides in mice, Drug Metabolism and Disposition, 32(9), pp. 983–992
5. Hortells, L.; Sur, S.; st. Hilaire, C., 2018. Cell Phenotype Transitions in Cardiovascular Calcification, Frontiers in Cardiovascular Medicine, 5
6. http://www.romedic.ro/stenoza-aortica
7. Iung, B., 2003. A prospective survey of patients with valvular heart disease in Europe: The Euro Heart Survey on Valvular Heart Disease, European Heart Journal, 24(13), pp. 1231–1243
8. Kanwar, A.; Thaden, J. J.; Nkomo, V. T., 2018. Management of Patients With Aortic Valve Stenosis, Mayo Clinic Proceedings, 93(4), pp. 488–508
9. Ko, Y. T.; Bickel, U., 2012. Liposome-Encapsulated Polyethylenimine/Oligonucleotide Polyplexes Prepared by Reverse-Phase Evaporation Technique, AAPS PharmSciTech, 13(2), pp. 373–378

10. Larrew, T.; Chow, J.; Schulte, J.; Simionescu, D.; Simionescu, A., 2013. The roles of various metabolic enzymes in diabetic cardiomyopathy—in vivo and in vitro approach, Cardiovascular Pathology, 22(3), pp. e48
11. Owens, D. S.; Katz, R.; Takasu, J.; Kronmal, R.; Budoff, M. J.; O'Brien, K. D., 2010. Incidence and Progression of Aortic Valve Calcium in the Multi-Ethnic Study of Atherosclerosis (MESA), The American Journal of Cardiology, 105(5), pp. 701–708
12. Pawade, T. A.; Newby, D. E.; Dweck, M. R., 2015. Calcification in Aortic Stenosis, Journal of the American College of Cardiology, 66(5), pp. 561–577
13. Rutkovskiy, A.; Malashicheva, A.; Sullivan, G.; Bogdanova, M.; Kostareva, A.; Stensløkken, K.; Fiane, A.; Vaage, J., 2017. Valve Interstitial Cells: The Key to Understanding the Pathophysiology of Heart Valve Calcification, Journal of the American Heart Association, 6(9)
14. Simionescu, N.; Vasile, E.; Lupu, F.; Popescu, G., 1986. Prelesional events in atherogenesis: Accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit, American Journal of Pathology, 123(1)
15. Voicu, G.; Rebleanu, D.; Constantinescu, C. A.; Fuior, E. V.; Ciortan, L.; Droc, I.; Uritu, C. M.; Pinteala, M.; Manduteanu, I.; Simionescu, M.; Calin, M., 2020. Nano-Polyplexes Mediated Transfection of Runx2-shRNA Mitigates the Osteodifferentiation of Human Valvular Interstitial Cells, Pharmaceutics, 12(6), pp. 507
16. Voicu, G.; Rebleanu, D.; Mocanu, C. A.; Tanko, G.; Droc, I.; Uritu, C. M.; Pinteala, M.; Manduteanu, I.; Simionescu, M.; Calin, M., 2022. VCAM-1 Targeted Lipopolyplexes as Vehicles for Efficient Delivery of shRNA-Runx2 to Osteoblast-Differentiated Valvular Interstitial Cells; Implications in Calcific Valve Disease Treatment, International Journal of Molecular Sciences, 23, pp. 3824-3847