BABES-BOLYAI UNIVERSITY
FACULTY OF BIOLOGY AND GEOLOGY
DOCTORAL SCHOOL OF INTEGRATIVE BIOLOGY

DOCTORAL THESIS

Summary

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Advancing Melanoma Treatment: Targeted Therapeutic Strategies to Overcome Drug Resistance

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"Continuous effort, not strength or intelligence, is the key to unlocking our potential."

Liane Cordes

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OUTLINE OF THE THESIS

Keywords: drug resistance, extracellular vesicles, tumor microenvironment, Hypoxia inducible factor 1 (HIF-1), metabolic reprogramming, active targeting nanocarriers

The current thesis consisted of 5 chapters that were shortly presented below.

Chapter 1 provided an overview on the hallmarks of cancer, the involvement of the key players that modulate tumor development emphasizing the role of tumor-associated macrophages (TAMs) and extracellular vesicles as essential intercellular communication tools in the tumor microenvironment (TME) that promote and support all protumor processes. Moreover, the role of the transcription factor, hypoxia-inducible factor (HIF-1) in the tumor development and aggressiveness, as main regulator of tumor metabolic reprogramming and the acquisition of drug resistance in cancer cells was emphasized. Noteworthy, an overview on the normoxic regulatory actions of HIF-1 in cancer cells, that have constitutive expression of this protein, was also provided. In tight relation with the development of drug resistance in cancer cells, this thesis also presented treatment strategies aimed at overcoming tumor evasion, integrating them with the core scientific findings. Thus, the first two experimental chapters (**Chapter 2 and 3**) described how B16.F10 murine melanoma cells (cultured in 2D and 3D models) might adapt to the initial antitumor actions of different drugs (Simvastatin (**SIM**) and Doxorubicin (**DOX**)), leading to further development of drug tolerance via modulation of tumor metabolism and TME-coordinated processes. Based on these findings, **Chapter 4** investigated the antitumor efficacy of the combined administration of SIM and DOX via nanoformulations that have the ability to target actively both TAMs (as main cell type with protumor functions within TME) and melanoma cells *in vivo*. Building upon the success of IL-13-functionalized liposomal nanocarriers to specifically target TAMs, the second part of the **Chapter 4** assessed whether this therapeutic approach based on IL-13-conjugated liposomes might be extended to deliver an anti-inflammatory drug, prednisolone phosphate (**PLP**), to target TAMs-mediated inflammatory angiogenesis, and further reduce cancer aggressiveness.

The last chapter, **Chapter 5** provided a comprehensive summary of the key findings related to drug resistance mechanisms and the therapeutic strategies designed to counteract these processes in melanoma cells, with particular emphasis on treatment efficacy. Future research should

prioritize the validation of these findings in more complex biological models including studies using human cell lines or patient-derived cells, to enhance the translational relevance of the results and to further optimize the characteristics of the drug delivery systems.

The logical progression presented in this thesis, starting with the exploration of drug resistance mechanisms, to developing targeted therapies to overcome these mechanisms, ensures that each chapter connects to the previous one, and creates a meaningful scientific contribution to cancer research.

CHAPTER 1. Introduction

1.1 Molecular mechanisms involved in the hallmarks of cancer

Cancer is a group of diseases primarily characterized by uncontrolled cell division, resistance to death signals, and the ability to spread throughout the body. In healthy cells, all these processes are under strict control. The journey to understanding cancer's complexities began in 2000, when researchers Douglas Hanahan and Robert Weinberg identified and proposed a set of six essential and common traits/characteristics of these tumors known as the **Hallmarks of Cancer**, acquired during the progression of tumors (Hanahan & Weinberg, 2000). This framework played an essential role in understanding the complex biology of cancer and have been instrumental in guiding research and therapeutic strategies. Over the years, the concept of cancer hallmarks has evolved to include new traits and processes, reflecting the dynamic nature of cancer and its implications for therapy development (Hanahan, 2022; Hanahan & Weinberg, 2011).

1.2 The tumor microenvironment (TME) exploited by tumor cells

The tumor microenvironment (TME) is a highly heterogeneous area of tumors consisting of non-malignant cells, such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), adipocytes, tumor endothelial cells (TECs), tumor-educated platelets (TEPs), dendritic cells (DCs), T lymphocytes, natural killer (NK) cells, mast cells and mesenchymal stromal cells (MSCs) (**Figure 2**) (Hanahan & Coussens, 2012). Each of these cell types contribute to neoplastic progression (Fridman et al., 2017; Hanahan & Coussens, 2012; Jayatilaka & Phillip, 2019; Okamoto et al., 2019)

1.3 Hypoxia inducible factor 1 (HIF-1) as key modulator of tumor development under hypoxia as well as normoxia

1.2.1 HIF-1-induced effects on tumor development under hypoxia

In solid tumors, the TME often lacks oxygen due to abnormal blood vessels and high tumor cell activity. HIF-1α plays a central role in helping tumor cells adapt to hypoxia by directly upregulating VEGF, TGF-β, IL-10, and PD-L1, strengthening the tumor's ability to suppress the immune response (Logtenberg et al., 2020) (X. Meng et al., 2018). It also promotes immune escape by increasing immune checkpoint expression, inducing cytotoxic T cell apoptosis, and blocking phagocytosis (Shen et al., 2022)(Logtenberg et al., 2020).

1.2.2 HIF-1-induced effects on tumor development under normoxia

Cancer cells exhibit several adaptations under normoxic conditions to support their uncontrolled growth and survival, as demonstrated in human breast cancer cell (Li et al., 2018). While hypoxia has been extensively studied in cancer biology, emerging evidence suggests significant molecular changes occur even under normoxic conditions (J. Zhang et al., 2025). HIF-1's role in cancer metastasis under normoxia shows that it is not restricted to low-oxygen conditions. It is critical in all stages of cancer metastasis, including local migration, extracellular matrix remodeling, and EMT (Araos et al., 2018). Even under normoxic conditions.

1.4 Metabolic reprogramming in cancer is a hallmark of malignancy

Cancer cells rely on altered metabolic pathways to sustain their rapid growth, survival, and resistance to treatment. A key adaptation is their preference for glycolysis over oxidative phosphorylation, even in the presence of oxygen - a phenomenon known as the Warburg effect. This shift leads to increased lactate production, which fuels tumor proliferation. Additionally, cancer cells develop a strong dependency on glutamine to sustain the TCA cycle, making it an important therapeutic target. The overexpression of glucose transporters like GLUT1 further enhances glucose uptake, supporting cancer metabolism. Oncogenic pathways such as PI3K/Akt and c-Myc regulate these metabolic adaptations, giving tumors a survival advantage (Röhrig & Schulze, 2016; Tsouko et al., 2014; Yu, 2011; S. Zhang et al., 2024)

1.5 Drug resistance acquisition in cancer

Cancer resistance to different treatments is a result of a very complex interaction between the cancer cell's intrinsic (innate) and extrinsic (acquired) factors. Pre-existing genetic mutations, tumor heterogeneity, and the activation of intracellular defense pathways are examples of intrinsic factors that confer resistance by activating different oncogenic pathways, changing drug targets, desensitizing to therapies, improving DNA repair mechanisms, and activating survival pathways, which in turn enable cancer cells to enhance their resistance to the cytotoxic effects of treatments (Dzobo et al., 2018). Extrinsic factors, on the other hand, mostly consist of TME components that actively contribute to cancer cells capacity to avoid the cytotoxic effects of different anticancer treatments (Labrie et al., 2022).

1.6 Strategies to overcome drug resistance

The tolerance that cancer and TME cells develop to therapies not only limits the efficacy of current therapies but highlights the need for advanced strategies to overcome resistance and improve prognosis (Kurt Yilmaz & Schiffer, 2021). Together, these advances offer new ways to treat cancer and manage resistance (L. Meng et al., 2024; Sadida et al., 2024). Targeted treatments are intended to selectively target the molecules or pathways that are abnormally active in cancer cells, as opposed to standard chemotherapy, which damages both malignant and healthy cells. One of the main advantages of targeted therapy is that it can address drug resistance (Garg et al., 2024). Targeted cancer therapies include several classes of inhibitors that disrupt key cellular processes.

The Aim and the Objectives of the Thesis

General Aim

This thesis aimed to investigate the mechanisms underlying drug-induced early resistance in melanoma cells to further develop efficient therapeutic approaches for melanoma treatment.

General Objectives

1. Assessment of the mechanisms by which B16.F10 melanoma cells (cultured in 2D and 3D models) might develop tolerance to the antitumor actions of different drugs (Simvastatin (**SIM**) and Doxorubicin (**DOX**)).

Thus, **Chapter 2** aimed to explore how inhibition of the constitutive level of hypoxia-inducible factor 1 (HIF-1) by SIM affects cancer cell metabolism of B16.F10 murine melanoma cells grown in 2D culture, while **Chapter 3** focused on identification of the mechanisms linked to the early settlement of DOX resistance, in the same cancer cells grown as spheroids, following repeated exposure to a subinhibitory concentration (IC30) of the cytotoxic drug.

2. Development of active-targeting therapies based on nanoformulations/nanocarriers with improved antitumor activity and potential to prevent drug resistance development in B16.F10 melanoma tumors.

Thus, **Chapter 4.1** aimed to develop an improved targeted therapy for melanoma by optimizing and evaluating the efficacy of a sequential administration of nano-systems based on IL-13-conjugated long-circulating liposomes containing simvastatin (IL-13-LCL-SIM) to target tumor-associated macrophages (TAMs), and PEG-stabilized extracellular vesicles loaded with doxorubicin (PEG-EV-DOX) to target melanoma cells. **Chapter 4.2** was focused on the development of IL-13-conjugated long-circulating liposomes containing prednisolone phosphate (IL-13-LCL-PLP) in order to enhance drug specificity for TAMs-mediated angiogenesis and inflammation, ultimately reducing cancer aggressiveness in B16.F10 murine melanoma-bearing mice.

CHAPTER 2

Under Normoxia Simvastatin Shifts Lipid Metabolism of B16.F10 Melanoma Cells to Support Their Aggressiveness

Our previous studies demonstrated a tight connection between simvastatin (SIM) inhibition of B16.F10 murine melanoma cell proliferation *in vivo* and *in vitro* and strong suppression of the subunit α of hypoxia inducible factor 1 (HIF-1) (HIF-1 α) production in these aggressive cancer cells. This study investigates the impact of SIM on the metabolic reprogramming of B16.F10 murine melanoma cells under normoxic conditions. Previous studies have shown that SIM inhibits the expression of hypoxia-inducible factor 1 (HIF-1α), a key regulator of cancer metabolism, even in normoxia. Building on these findings, we aim to explore the molecular mechanisms by which SIM modulates melanoma cell metabolism, particularly focusing on lipid metabolism and energy regulation. To achieve this, RNA-seq analysis was conducted to examine gene expression changes in metabolism. Western blot analysis was used to assess protein expression levels of key metabolic regulators, including HIF-1α, glucose transporter 1 (GLUT1), fatty acid synthase (FASN), stearyl-CoA desaturase 1 (SCD1), and acyl-CoA oxidase (ACOX3), while enzymatic assays determined the catalytic activities of key enzymes such as phosphofructokinase (PFK) and lactate dehydrogenase (LDH). Our results showed that SIM treatment significantly modulated the expression of genes involved in cholesterol biosynthesis and fatty acid metabolism, inducing a shift toward unsaturated fatty acid production and enhanced isoprenoid synthesis. Furthermore, SIM administration led to a reduction in glycolysis, as evidenced by decreased catalytic activities of PFK and LDH, redirecting melanoma cells toward fatty acid metabolism. We also investigated the effect of SIM on the mevalonate pathway, noting that SIM reduced the expression of sterol regulatory element-binding proteins (SREBPs) and forkhead box M1 (FOXM1), key regulators of cholesterol biosynthesis. In contrast, several mevalonate pathway enzymes were upregulated, excluding HMG-CR. This shift towards enhanced lipid biosynthesis may contribute to melanoma cell survival and metastatic potential, supporting the idea that SIM treatment promotes lipid-derived signaling molecule production, such as isoprenoids and prostaglandins, in the normoxic tumor microenvironment. In summary, SIM reprogrammed melanoma cell metabolism by inhibiting glycolysis and promoting lipid metabolism, particularly unsaturated fatty acids and isoprenoids. These shifts likely enhanced melanoma cell survival and aggressiveness. Targeting

lipid biosynthesis, prostaglandin turnover, and glutamine metabolism could have disrupted the metabolic flexibility of melanoma cells, offering a potential strategy to overcome normoxic HIF-1α-driven adaptive mechanisms and improve treatment outcomes.

CHAPTER 3

Exploring Mechanisms of Early Acquired Resistance to Doxorubicin in Melanoma in 3D Model

Chemoresistance continues to remain a significant barrier to the effective treatment of various cancers, including melanoma. Previous studies, including our own, have demonstrated the limited efficacy of doxorubicin (DOX) against melanoma cells and tumors, both *in vitro* and *in vivo*, primarily due to acquired chemoresistance. This study explores early adaptive responses in B16.F10 melanoma spheroids after two sub-inhibitory DOX exposures (≈IC30), focusing on chemoresistance mechanisms in cell cycle regulation, DNA repair, apoptosis, angiogenesis, and invasiveness. Melanoma spheroids were cultured in ultra-low attachment plates and treated with DOX in two 48-hour phases separated by 48-hour recovery periods, followed by viability, morphology, gene/protein expression, and enzyme activity assessments using RNA-seq, RT-qPCR, Western blot, and gelatinase assay, respectively. Functional analysis confirmed downregulation of cell cycle pathways, suppression of stress response and survival pathways (e.g., TNFA_SIGNALING_VIA_NFKB and MTORC1_SIGNALING). Despite mRNA data showing cell cycle arrest (downregulated *CDK1, Ticrr, Cdc20*) and major DNA damage and impaired repair (downregulated *BRIP1, BARD1, Rad51, Rmi2*), after first exposure to DOX, cells probably bypassed cell cycle checkpoints by upregulating transcripts and proteins linked to pro-survival (*Tnfrsf19*, cIAP-2) and angiogenesis (VEGF, eotaxin-1), and by upregulating transcripts related to cell adhesion (*Pcdh beta* family members), and ECM remodeling (*Ecm1*, *Jam2*), suggesting early adaptation mechanisms to DOX. With a second DOX exposure, melanoma spheroids showed stronger chemoresistance, evidenced by enhanced angiogenesis (upregulated *Aqp1, VEGF,* and *Ackr3*), increased invasiveness (upregulated *MMP-2*, favored MMP-9 activity), and a 40% increase in pNF-kB levels, reinforcing drug tolerance and aggressiveness. This study provides valuable insights into the molecular drivers of chemoresistance, revealing that early DOX resistance in melanoma arises from adaptive mechanisms enhancing survival, angiogenesis, and invasiveness. Repeated exposure to DOX reinforces these traits, sustaining drug tolerance.

CHAPTER 4. Overcoming drug-induced resistance with active targeting therapeutic approaches

Section 4.1. Active Tumor-Targeting Nano-formulations Containing Simvastatin and Doxorubicin Inhibit Melanoma Growth and Angiogenesis

Primary melanoma aggressiveness is determined by rapid selection and growth of cellular clones resistant to conventional treatments, resulting in metastasis and recurrence. There is an urgent need to develop selective and specific drug delivery strategies for modulating the interaction between cancer cells and immune cells within the tumor microenvironment. This study proposes a novel combination therapy consisting of sequential administration of SIM incorporated in IL-13-functionalized long-circulating liposomes (IL-13-LCL-SIM) and DOX encapsulated into PEG-coated extracellular vesicles (PEG-EV-DOX) to selectively target both tumor-associated macrophages and melanoma cells. To this end, IL-13 was conjugated to LCL-SIM which was obtained via the lipid film hydration method. EVs enriched from melanoma cells were passively loaded with doxorubicin. Subsequently, the therapeutic agents were administered i.v in B16.F10 melanoma-bearing mice, and tumor size was monitored during treatment. The molecular mechanisms of antitumor activity were investigated using angiogenic and inflammatory protein arrays and western blot analysis of invasion (HIF-1) and apoptosis markers (Bcl-xL and Bax). Quantification of oxidative stress marker malondialdehyde (MDA) was determined by HPLC. Immunohistochemical staining of angiogenic markers CD31 and VEGF and of panmacrophage marker F4/80 was performed to validate our findings. The *in vitro* data showed that IL-13-functionalized LCL were preferentially taken up by tumor-associated macrophages and indicated that sequential administration of IL-13-LCL-SIM and PEG-EV-DOX had the strongest antiproliferative effect on tumor cells co-cultured with tumor associated macrophages (TAMs). Accordingly, strong inhibition of tumor growth in the group treated with the sequential combination therapy was reported *in vivo*. Our data suggested that the antitumor action of the combined treatment was exerted through strong inhibition of several pro-angiogenic factors (VEGF, bFGF, and CD31) and oxidative stress-induced upregulation of pro-apoptotic protein Bax. This novel drug delivery strategy based on combined active targeting of both cancer cells and immune cells was able to induce a potent antitumor effect by disruption of the reciprocal interactions between TAMs and melanoma cells.

Section 4.2. Targeting of M2 macrophages with IL-13-functionalised liposomal prednisolone inhibits melanoma angiogenesis *in vivo*

The intricate cooperation between cancer cells and nontumor stromal cells within melanoma microenvironment (MME) enables tumor progression and metastasis. We previously demonstrated that the interplay between tumor associated macrophages (TAMs) and melanoma cells can be disrupted by using long circulating liposomes (LCL) encapsulating prednisolone phosphate (PLP) (LCL-PLP) that inhibited tumor angiogenesis coordinated by TAMs. In the present study, our goal was to improve LCL specificity for protumor macrophages (M2-like (i.e., TAMs) macrophages) and to induce a more precise accumulation at tumor site by loading PLP into IL-13-conjugated liposomes (IL-13-LCL-PLP), since IL-13 receptor is overexpressed in this type of macrophages. The IL-13-LCL-PLP liposomal formulation was obtained by covalent attachment of thiolated IL-13 to maleimide-functionalized LCL-PLP. C57BL/6 mice bearing B16.F10 s.c melanoma tumors were used to investigate the antitumor action of LCL-PLP and IL-13-LCL-PLP. Our results showed that IL-13-LCL-PLP formulation remained stable in biological fluids after 24h and it was preferentially taken up by M2 polarized macrophages. IL-13-LCL-PLP induced strong tumor growth inhibition compared to nonfunctionalized LCL-PLP at the same dose, by altering TAMs-mediated angiogenesis and oxidative stress, limiting resistance to apoptosis and invasive features in MME. These findings suggest IL-13-LCL-PLP might become a promising delivery platform for chemotherapeutic agents in melanoma.

CHAPTER 5

General conclusions

Chapter 2 Our data showed that B16.F10 melanoma cells adapted to SIM treatment by undergoing significant metabolic changes, namely enhanced lipid metabolism. This led to the accumulation of unsaturated fatty acids and possibly the synthesis of isoprenoids and prostaglandins, supporting melanoma cell survival and aggressiveness.

Chapter 3 The study revealed that early resistance to DOX in B16.F10 melanoma cells grown as spheroids was induced by adaptive mechanisms that promoted cell survival, angiogenesis, and increased invasiveness. These findings highlighted the importance of identifying and targeting early drug-resistance biomarkers in order to overcome therapy evasion in melanoma.

Chapter 4.1 The novel sequential targeted therapy developed in this study effectively targeted the B16.F10 melanoma microenvironment, leading to almost total tumor growth suppression, mainly due to the anti-angiogenic effect. IL-13-functionalized LCL-SIM specifically targeted TAMs pro-angiogenic functions and sensitized the melanoma cells to PEG-EV-DOX-induced cytotoxicity.

Chapter 4.2 The therapeutic effect of IL-13-LCL-PLP resulted from its ability to alter TAMs-mediated inflammatory angiogenesis, reducing tumor-protective roles which normally lead to resistance to therapy.

Novelty of the studies

Chapter 2 offers a novel contribution by examining the effects of SIM on melanoma cell metabolism under normoxic conditions, a less understood aspect compared to the well-studied hypoxic responses.

Chapter 3 provides new insights into early-stage DOX resistance in B16.F10 melanoma cells grown as a 3D model, mimicking the tumor microenvironment's architectural, molecular, and microenvironmental heterogeneity, and highlighting biomarkers linked to angiogenesis, invasion, and apoptosis resistance, an approach not previously explored.

Chapter 4 novelty consists in the development of targeted therapeutic strategies that reduce melanoma tumor aggressiveness and related-resistance mechanisms, through simultaneous targeting of melanoma cells and TAMs-mediated protumoral processes within the TME.

Future perspectives

Chapter 2 To assess the clinical relevance of findings in 2D culture, further validation in more complex systems, such as spheroids, organoids with human cell lines would help confirm the translatability of our results. Moreover, our data highlight potential metabolic targets in melanoma cells that could be therapeutically exploited, opening avenues for novel treatment strategies.

Chapter 3 Future studies should explore the effects of DOX on melanoma cells in 3D culture with stromal cells to better understand TME cross-talk and heterogeneity in chemoresistance. Identifying biomarkers in patient-derived cells could help develop strategies to overcome DOX resistance and enable personalized therapies for improved outcomes.

Chapter 4.1 Future studies could optimize combination therapy to reprogram TAMs from an M2 to an M1 phenotype, enhancing antitumor immune responses. Combining this approach with

immune checkpoint inhibitors may improve therapeutic outcomes in melanoma and other aggressive cancers.

Chapter 4.2 To enhance the therapeutic effect, future studies should explore the simultaneous administration of the therapy proposed in this study with other therapeutic strategies.

In conclusion, this doctoral thesis provides valuable insights into melanoma drug resistance mechanisms and presents novel therapeutic strategies that hold significant promise for future clinical applications.

List of publications included in the thesis

Negrea, G., Rauca, V. F., Meszaros, M. S., Patras, L., Luput, L., Licarete, E., Toma, V. A., Porfire, A., Muntean, D., Sesarman, A., & Banciu, M. (2022). Active Tumor-Targeting Nano-formulations Containing Simvastatin and Doxorubicin Inhibit Melanoma Growth and Angiogenesis. Frontiers in pharmacology, 13, 870347. https://doi.org/10.3389/fphar.2022.870347.

Sesarman, A., Luput, L., Rauca, V., Patras, L., Licarete, E., Meszaros, S., Dume, B., **Negrea, G.,** Toma, V., Muntean, D., Porfire, A., Banciu, M. (2024). Targeting of M2 macrophages with IL-13-functionalized liposomal prednisolone inhibits melanoma angiogenesis in vivo. Journal of Liposome Research, 34(4), 535–546. https://doi.org/10.1080/08982104.2024.2315452

List of publications not included in the thesis

Patras L, Sesarman A, **Negrea G**, Dragan SM, Meszaros M-S, Licarete E, Rauca V, Luput L, Alupei M, Porfire A, Banciu M. Cancer therapeutic strategies to rewire tumor microenvironment .SEE J Immunol. 2025 Mar 27;8 (CITIM):030. https://doi.org/10.3889/seejim.2025.6091

Tóth, Z. R., Kiss, J., Todea, M., Kovács, G., Gyulavári, T., Sesarman, A., **Negrea, G.,** Vodnar, D. C., Szabó, A., Baia, L., & Magyari, K. (2022). Bioactive Properties of Composites Based on

Silicate Glasses and Different Silver and Gold Structures. Materials (Basel, Switzerland), 15(5), 1655. https://doi.org/10.3390/ma15051655.

Tóth, Z. R., Kiss, J., Todea, M., Kovács, G., Gyulavári, T., Sesarman, A., **Negrea, G.**, Vodnar, D. C., Szabó, A., Baia, L., & Magyari, K. (2022). Bioactive Properties of Composites Based on Silicate Glasses and Different Silver and Gold Structures. Materials (Basel, Switzerland), 15(5), 1655. https://doi.org/10.3390/ma15051655

Patras, L., Ionescu, A. E., Munteanu, C., Hajdu, R., Kosa, A., Porfire, A., Licarete, E., Rauca, V. F., Sesarman, A., Luput, L., Bulzu, P., Chiroi, P., Tranca, R. A., Meszaros, M., **Negrea, G.**, Barbu-Tudoran, L., Potara, M., Szedlacsek, S., & Banciu, M. (2022). Trojan horse treatment based on PEG-coated extracellular vesicles to deliver doxorubicin to melanoma in vitro and in vivo. Cancer biology & therapy, 23(1), 1–16. https://doi.org/10.1080/15384047.2021.2003656

List of participations at international conferences and symposiums

During my doctoral studies, I have actively attended over 10 conferences, with oral and poster presentation. For example:

Poster presentation at the **48th FEBS Congress, 29 June–3 July 2024, Milano, Italy** – "Giorgiana Negrea, Loredana Balacescu, Alina Sesarman, Manuela Banciu.Simvastatin Triggers a Metabolic Shift Towards Lipid Metabolism in B16.F10 Murine Melanoma Cells – "FEBD 2024 published FEBS Open Bio 14 (Supplement 2) (2024) 94–516 DOI: 10.1002/2211-5463.13837Ó, P-26-015, pp 259.

Poster presentation at the **EMBO Congress, 29-31 May 2024, Rimini, Italy** – "Giorgiana-"Gabriela Negrea, Szilvia Meszaros, Ștefan Drăgan, Vlad-Alexandru Toma, Bogdan-Răzvan Dume, Valentin-Florian Rauca, Laura Pătraș, Emilia Licărete, Manuela Banciu, Alina Sesărman. Unveiling the Complexity of Tumor Microenvironment Chemoresistance in Melanoma via Spheroid Mimicry (2024)" - EMBO WS „The many faces of cancer evolution", Rimini 2024 | Abstract Book pp. 73, P34.

Oral presentation at the **The Annual International Conference of Romanian Society of Biochemistry and Molecular Biology (RSBMB), September 2023, Cluj-Napoca, Romania** - Simvastatin – "Giorgiana G. Negrea, Loredana Bălăcescu, Alina Sesărman, Manuela Banciu. Induced Energy Metabolic Shift Towards Lipid Metabolism in B16.F10 Murine Melanoma Cells". Abstract published in STUDIA UNIVERSITATIS BABEŞ-BOLYAI, BIOLOGIA, 68, 2, 2023, ABSTRACT 58, pp. 34.

e-Poster presentation as Short 5 minutes talk at the **2nd Conference edition Connecting scientists and Physicians for Next Generation Cancer Management, Oncohub " 21 - 23 September 2022,** Po**iana Brașov, Romania –"**Giorgiana Negrea, Valentin-Florian Rauca, Marta Szilvia Meszaros, Laura Patras, Lavinia Luput, Emilia Licarete, Vlad- Alexandru Toma, Alina Porfire, Dana Muntean, Alina Sesarman and Manuela Banciu. Innovative Tumor-targeting Nanoformulations Containing Simvastatin and Doxorubicin Decrease Murine Melanoma Aggresiveness *in vivo"* **Oncohub conference** Poiana Brasov 2022 |Abstract Book**,** Pp. 32.

Oral presentation at the **International Conference and XXXIX Scientific Session of the Romanian Society for Cell Biology - October 21st-23rd, Cluj-Napoca, Romania RSCB 2022** – "Giorgiana G. Negrea, Loredana Bălăcescu, Alina Sesărman, Manuela Banciu. Modulatory Effects of Simvastatin on the metabolism of B16.F10. murine melanoma cells under normoxia in vitro." RSCB, October 2022, Cluj-Napoca.

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