HMGB1 as a Biomarker and Therapeutic Target in Triple-Negative Breast Cancer: Inhibitory Effects of Metformin on HMGB1/RAGE Signaling

Poster No
PJS_2507

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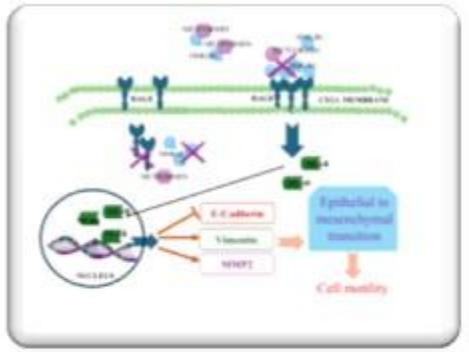
Keywords: EMT (epithelial-mesenchymal transition), HMGB1, RAGE, Breast cancer, metformin



Objective

High-mobility group box 1 (HMGB1) is a nuclear protein overexpressed in triple-negative breast cancer (TNBC), where it promotes tumor progression through interaction with the receptor for advanced glycation end products (RAGE). Metformin, an antidiabetic drug with emerging anticancer properties, can bind to HMGB1 and inhibit its pro-inflammatory functions. This study investigates the effects of metformin on HMGB1/RAGE signaling in TNBC cells by examining its impact on cell migration, HMGB1 and RAGE expression, NF-κB activity, and epithelial-to-mesenchymal transition (EMT), to explore its potential as a targeted therapy for tumors with elevated HMGB1/RAGE expression..

Materials and methods

The human triple-negative breast cancer cell lines MDA-MB-468 (ATCC USA) were treated with recombinant HMGB1 (800 ng/mL) and metformin (1 mM). The effects of metformin on HMGB1/RAGE signaling were evaluated using wound-healing assays to assess cell migration, immunofluorescence for protein localization, and Western blot analysis to examine the expression of RAGE, HMGB1, NF-κB, E-cadherin, vimentin, and β-catenin.

Results

Metformin prevents the stabilization of the RAGE receptor on the cell membrane and NF-kB signaling activation

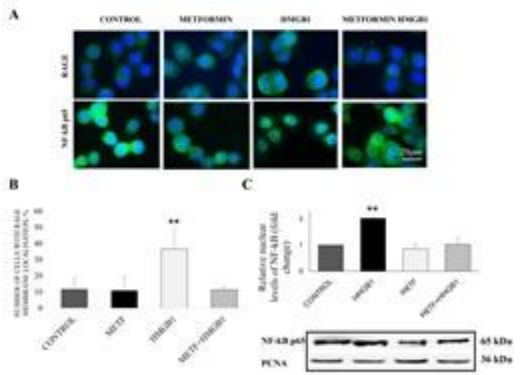


FIGURE 1. Effect of metformin on RAGE and NF-kB p65 protein localization in MDA-MB-468 cells. Representative immunofluorescence pictures of RAGE signal (green) in control cells as well as in treated cells with rHMGB1 or/and 1mM metformin (METF) (A) and graph from the analysis (B). Representative immunofluorescence pictures of NF-kB signal (green) in control cells as well as in cells treated with rHMGB1 or/and 1mM METF (A) and Western blotting results from the nuclear extracts (C). At least 100 cells per condition were counted. All data is given as means with SEM. Asterisks indicate a significant difference *p<0.05, **p<0.01, ***p<0.001 vs. control. Nuclei are stained with DAPI (blue).

Metformin Inhibits HMGB1-Induced Cell Motility

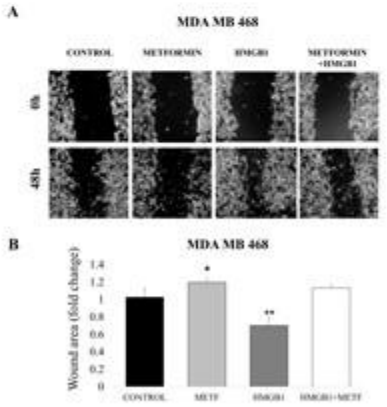


FIGURE 2. The effect of METF of HMGB1-induced migration of breast cancer cells. A: Representative pictures of wound closure in MDA-MB-468 cells treated with different concentrations HMGB1 and/or 1mM metformin at 0 h and 48 h. B: Quantification of wound healing. Data represents means ± SEM, *p ≤ 0.05; **p≤0.01 vs. control.

Metformin prevents HMGB1-Induced MMP-2 upregulation as well as EMT in TNBC

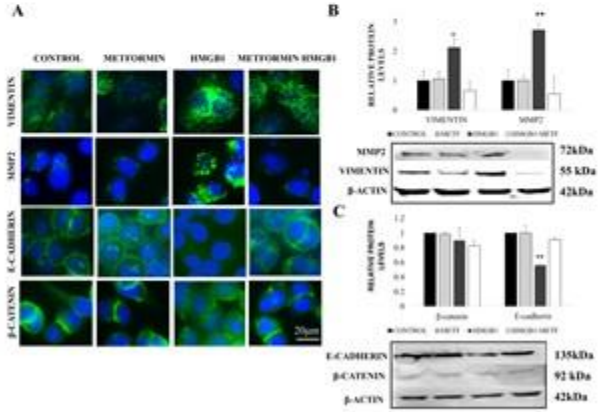


FIGURE 3. A: Immunolocalization of EMT markers E-cadherin, vimentin and β-catenin as well as MMP2 protein (green fluorescence). Nuclei are stained with DAPI (blue). B: Relative protein levels of MMP2 and vimentin in control MDA-MB-468 cells and cells treated with HMGB1 and/or metformin (METF). C: Relative protein levels of E-cadherin and β-catenin in control MDA-MB-468 cells and cells treated with HMGB1 and/or 1 mM metformin. Data represents mean ±SEM (n = 3), *p≤0.05, **p≤0.01, ***p≤0.001.

Metformin Decreases HMGB1 and RAGE Protein Levels in a Concentration-Dependent Manner

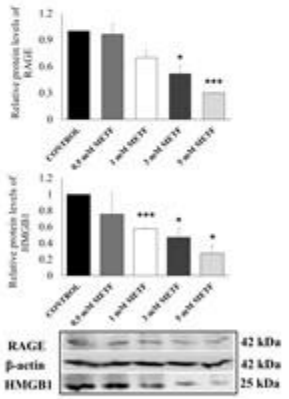


FIGURE 4. Protein expression levels of HMGB1 and RAGE in MDA-MB-468 cells treated with increasing concentration of metformin (METF). Data are given as mean and SEM. Asterisks indicate a significant difference *p≤0.05, **p≤0.01, ***p≤0.001 vs. control.

Conclusions

Our findings suggest that tumors with high HMGB1 and RAGE expression may be particularly responsive to metformin treatment, supporting the potential for biomarker-guided therapeutic strategies in TNBC.

Acknowledgement: This project is funded by Bulgarian National Scientific Fund, project №: КП-06-Н51/13

Integrated multi-omics analysis reveals key drivers of aggressiveness in Pancreatic adenocarcinoma (PAAD)

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Keywords: Multi-omics, Pancreatic cancer, Biomarkers, Solid tumors

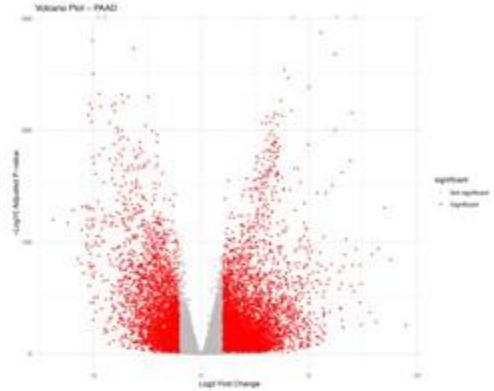
Objective: Objective: Pancreatic adenocarcinoma (PAAD) is one of the most lethal solid tumors, characterized by rapid progression, therapeutic resistance, and poor prognosis. The aim of this study was to identify molecular drivers of PAAD aggressiveness through an integrated multi-omics approach using TCGA data.

Methods: Differential gene expression was analyzed to detect significantly altered genes, followed by pathway and network analyses to identify affected molecular modules and oncogenic mechanisms. Principal component analysis (PCA) was performed to distinguish normal, primary, and metastatic tissues. Survival analyses, including Kaplan–Meier and multivariate Cox regression, were applied to evaluate the prognostic value of candidate genes. Gene–pathway interactions were visualized using network analysis.

Results: Differential expression analysis revealed a set of key upregulated oncogenes and downregulated tumor suppressors. PCA clearly separated tumor from normal samples and revealed a distinct metastatic signature. Survival analyses identified KRAS and TP53 as independent prognostic markers, with KRAS having the strongest impact on overall survival. Network visualization highlighted interconnected oncogenic pathways, suggesting shared mechanisms with other solid tumors.

Conclusion: The integrated multi-omics analysis identified KRAS and TP53 as major molecular drivers of PAAD aggressiveness and poor prognosis. These findings provide a comprehensive overview of the molecular alterations in PAAD and highlight potential biomarkers for improved risk stratification and novel therapeutic targets. Future steps will aim to integrate molecular profiling with clinical outcomes, advancing the development of precision oncology in PAAD.

Poster No PJS_2508



Differential Gene Expression Analysis in PAAD Using Volcano Plot

Volcano Plot visualization allows rapid identification of:

- Key genes with dramatically altered expression in pancreatic cancer.
- Potential biomarkers for early diagnosis or prognosis.
- Novel therapeutic targets for drug development.

Points on the plot represent individual genes and are categorized as follows:

- Significant – Upregulated (right): Genes strongly upregulated, potential oncogenes or disease markers.
- Significant – Downregulated (left): Genes strongly downregulated, potential tumor suppressors whose function is lost in disease.
- Not Significant: Genes showing no significant or statistically reliable changes; these form the majority and cluster around the center.

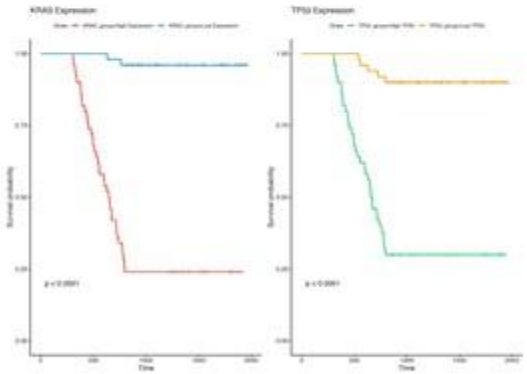
Principal Component Analysis (PCA) Distinguishes Normal, Primary, and Metastatic Tissues in PAAD

PC1 – Tumor Signal: Demonstrates fundamental differences in gene expression between tumor (primary and metastatic) and normal tissues.

PC2 – Metastasis Features: Although some overlap exists, metastatic tumors show distinct gene expression traits compared to primary tumors.

Tumor Confirmation: PCA confirms that PAAD tumors possess a unique molecular signature.

Metastatic Signature: Separate clustering of metastatic tissues suggests additional characteristic changes associated with metastasis.

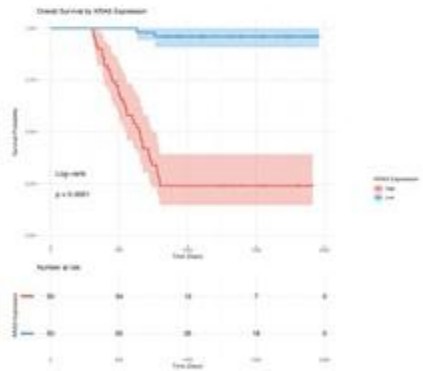


Kaplan–Meier Analysis: High KRAS and TP53 Expression Associated with Poor Overall Survival in PAAD

Patients were divided into high and low expression groups for each gene, and overall survival was tracked up to 2000 days.

KRAS Analysis: High KRAS expression is associated with sharply reduced survival.

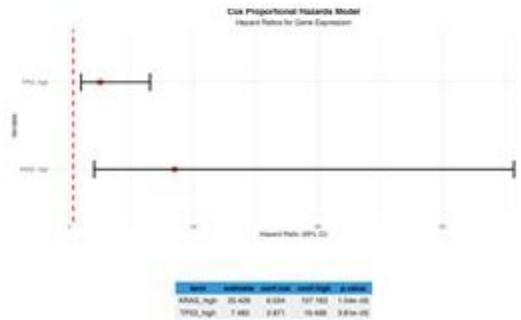
TP53 Analysis: High TP53 expression correlates with worse prognosis, indicating loss-of-function mutations.



Kaplan–Meier Analysis: High KRAS Expression Predicts Poor Survival in PAAD

High KRAS expression is strongly linked to worse clinical outcomes and shorter overall survival.

Conclusion: KRAS is not only a driver of PAAD development but also a robust prognostic biomarker for identifying high-risk patients.



Multivariate Cox Analysis Confirms KRAS and TP53 as Independent Prognostic Factors in PAAD

Both KRAS and TP53 independently influence survival, with high expression of each gene contributing to poor prognosis.

KRAS shows a stronger effect than TP53 (HR = 25.4 vs. HR = 7.5), making it a critical molecular prognostic marker.

Combined KRAS and TP53 evaluation allows risk stratification; patients with high expression of both genes have extremely poor prognosis and may benefit from aggressive or experimental therapies.

Network Visualization of Gene–Pathway Interactions in PAAD Reveals Key Regulatory Hubs

Central Pathways: The "Pancreatic cancer" pathway is the largest and most connected, confirming data specificity for PAAD. "Hepatocellular carcinoma" and "Breast cancer" pathways are also prominent, indicating overlap with other solid tumors.

Pathway Interconnectedness: Many genes are shared among multiple pathways, demonstrating common molecular mechanisms across cancers and potential for therapy repurposing.

This study was conducted using R and Bioconductor packages. The project uses realistic, synthetically generated transcriptomic data created based on the statistical properties of TCGA-PAAD RNA-seq files. The obtained results demonstrate the potential for developing multi-omics prognostic panels that can be applied in a future study of a Bulgarian patient cohort.

ACKNOWLEDGMENTS: The authors would like to thank Project 3.1.13 "Multi-Omics Approach for Molecular Profiling of Solid Tumors" and the scientific group "Oncogenetics and Genomics", Medical University – Sofia.

Distribution of Wild-Type Optineurin and Mutant p.Glu135Ter in MDCK-II Cells

Poster No PJS_2509

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Keywords: optineurin, mutant, ophthalmology, model cell line, transfection

Objective:

- To compare the subcellular distribution of wild-type optineurin (OPTN) and the truncating mutant OPTN-p.Glu135Ter in MDCK II epithelial cells.
- The focus was on localisation relative to the actin cytoskeleton, tight junctions and the Golgi apparatus.
- The aim was to assess whether the p.Glu135Ter mutation alters normal OPTN trafficking and cell-polarity-related localisation.

Materials and methods

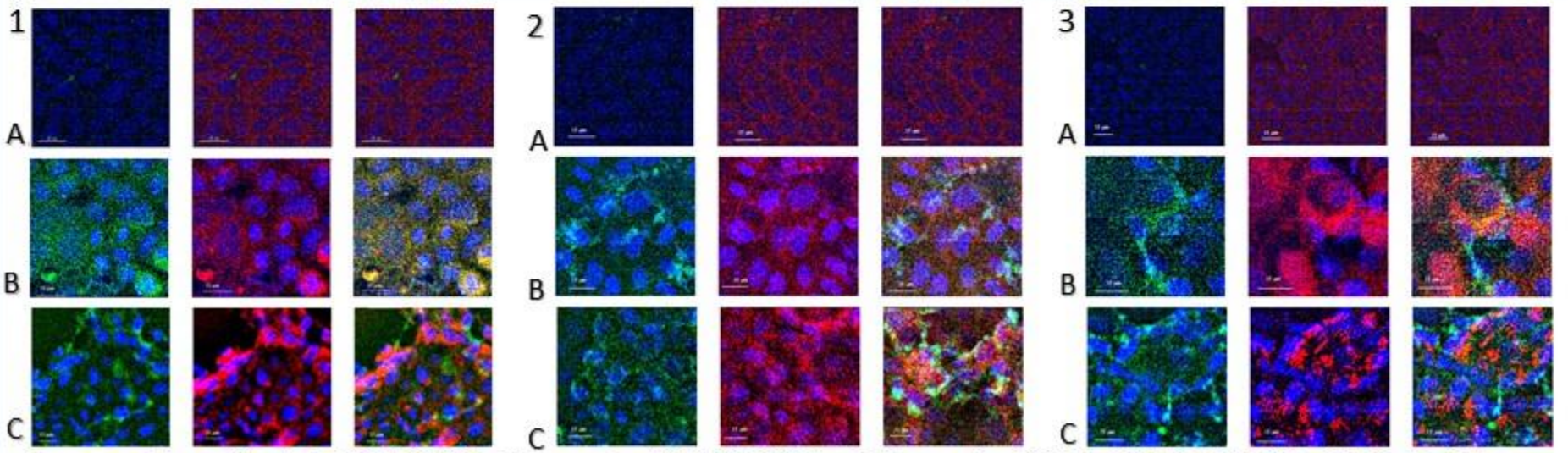
- CMV-driven eGFP-tagged constructs encoding wild-type OPTN and the c.403G>T (p.Glu135Ter) mutant were amplified in E. coli and purified by Maxiprep.
- A stable MDCK II line expressing wild-type OPTN-eGFP was generated by transfection and neomycin selection.
- The p.Glu135Ter-OPTN-eGFP construct was introduced by transient transfection.
- Control, OPTN-wt and p.Glu135Ter MDCK II cells were seeded on coverslips for fluorescence microscopy.
- Cells were fixed and stained with Phalloidin-TRITC for F-actin and DAPI for nuclei.
- Tight junctions were labelled with primary anti-ZO-1 antibody and the Golgi apparatus with anti-GM130, followed by Alexa Fluor 594 secondary antibody.

Results:

- OPTN-wt strongly co-localises with F-actin, indicating a close association with the actin cytoskeleton.
- In p.Glu135Ter-expressing cells, co-localisation with F-actin is reduced, suggesting weakened assosiation with the actin network.
- Compared with OPTN-wt, the p.Glu135Ter mutant shows increased accumulation at cell–cell contacts and more overlap with ZO-1, while ZO-1 integrity is preserved.
- OPTN-wt shows a compact perinuclear Golgi-associated pattern, whereas p.Glu135Ter displays a more dispersed signal, consistent with impaired targeting to Golgi-related compartments.

Conclusions:

- The data indicate that wild-type OPTN in MDCK II cells localises to the cytoplasm and perinuclear region, closely follows F-actin and shows partial association with the Golgi apparatus.
- The p.Glu135Ter truncation changes this pattern by reducing actin and Golgi co-localisation and increasing peripheral accumulation near tight junctions, without gross disruption of ZO-1 organisation.
- These findings suggest that OPTN-c.403G>T (p.Glu135Ter) is consistent with a loss-of-function variant at the level of intracellular localisation and compartment association.
- The MDCK II model appears well suited for further studies on how patient-derived OPTN mutations disturb vesicle trafficking and contribute to retinal cell pathology.



Fluorescence pictures of A: control MDCK II; B: MDCK II stably expressing wt OPTN; 1C: MDCK II transiently expressing p.Glu135Ter OPTN; left: OPTN-eGFP; middle: F-actin; right: merge; 2C: OPTN-eGFP; middle: ZO-1; right: merge; 3C: OPTN-eGFP, middle: GM130; right: merge.

ACKNOWLEDGMENTS: This project was funded by Ministry of Education and Science under contract DO1-361/2023, and the Scientific Research Fund at the Ministry of Education and Science under contract KP-06-N83/7 dated 05.12.2024 and contract 80-10-36 dated 23.05.2025 from the Research Fund of Sofia University.

Clearance of Indocyanine green as a new approach to liver function assessment in metabolic syndrome

PJS_2510

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Keywords: indocyanine green, clearance, metabolic syndrome, liver function

Objective

This study aims to evaluate the clearance of indocyanine green (ICG), a fluorescent dye completely excreted by the liver, as a marker for assessing liver function in rats with metabolic syndrome (MetS). MetS is an increasingly prevalent global health issue associated with an elevated risk of cardiovascular disease and type 2 diabetes. Liver function is most often affected as a result of the worsening glucose and lipid profile. The assessment of liver function includes measurement of the enzymatic activity of key liver enzymes, which are indicators of liver tissue damage.

Materials and Methods

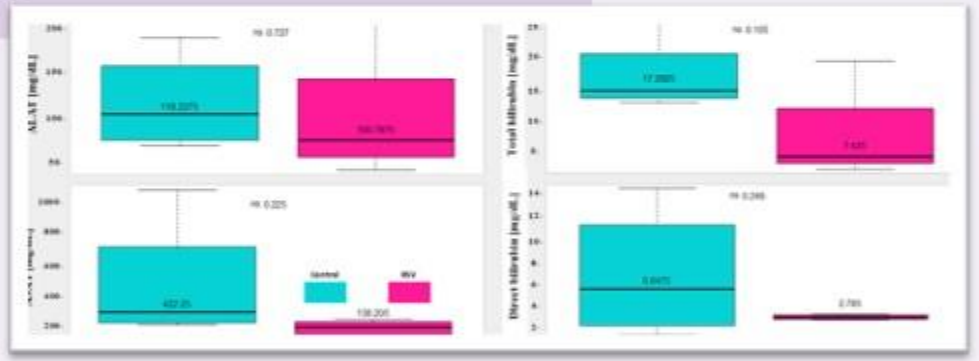
In this study, simultaneously with the standard protocol, the clearance of ICG was determined as a new approach to liver function assessment. MetS was induced in Wistar rats by a high-fructose diet for 12 weeks. Animals were divided into two groups: with untreated MetS and treated with resveratrol as a dietary supplement. Body weight, blood pressure, blood glucose, triglycerides, and cholesterol levels were monitored weekly. Daily intake of food and fructose solution was recorded. At the end of the experiment, animals were injected with ICG 30 μg/kg b.w. in the tail vein, and blood samples were collected at 5, 10, 15, 30, 45, 60 min, etc. until complete disappearance of the fluorescent signal. Terminal biochemical analyses include detailed lipid profile (HDL, LDL, cholesterol, triglycerides), liver enzymes activity (ALAT, ASAT), and bilirubin plasma level.

Results

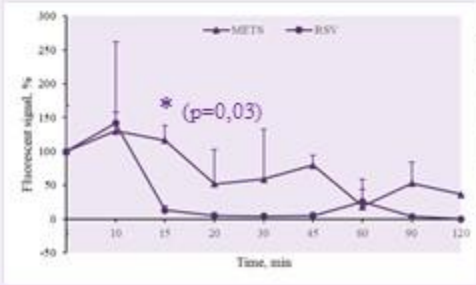
In the resveratrol-treated (RSV) group, terminal evaluations indicated preserved liver function with lower ASAT activity and decreased bilirubin plasma level compared with the untreated group, but the results are not statistically significant. The same trend of preserved liver function was observed in the ICG clearance assay. In the RSV group a 10-fold lower concentration of the dye was measured in the blood sample at 15th min, and in some animals, there was no fluorescent signal after 60 minutes. Whereas in the untreated group, the signal was noticed even 120 minutes after ICG application.

Conclusions

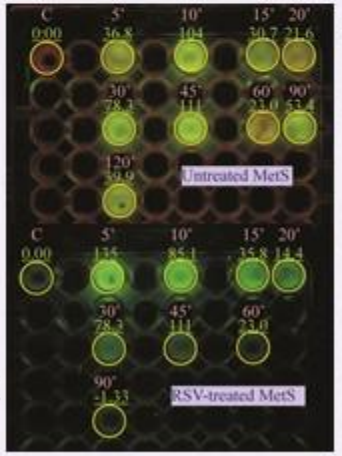
In conclusion, further studies are needed to verify this new approach to evaluate liver function. The method is a promising alternative to the established practice. It allows direct estimation of the preserved working liver capacity, rather than indirectly by the degree of liver tissue damage and accumulated bilirubin. These indicators may be symptoms of other diseases, and not be due to liver damage, such as increased hemolysis or muscle tissue damage.



Evaluation of liver function by standard biochemical tests.



Liver function assessment by ICG clearance. Summarized fluorescent signal was present as % of initial signal (5 min). The data is shown as mean±SEM (n=4).



Representative records of ICG clearance

ACKNOWLEDGMENTS: This research was funded by the Ministry of Education and Science of Bulgaria, Grant DO1-361/2023 "INFRAACT" of NRRI 2020–2027; EATRIS-Connect

Effect of mesenchymal stem cells on the proliferation and estradiol secretion of human granulosa cells under hypoxic and inflammatory conditions

Poster No **PJS_2511**

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Keywords: mesenchymal stem cells, granulosa cells, hypoxia, inflammation

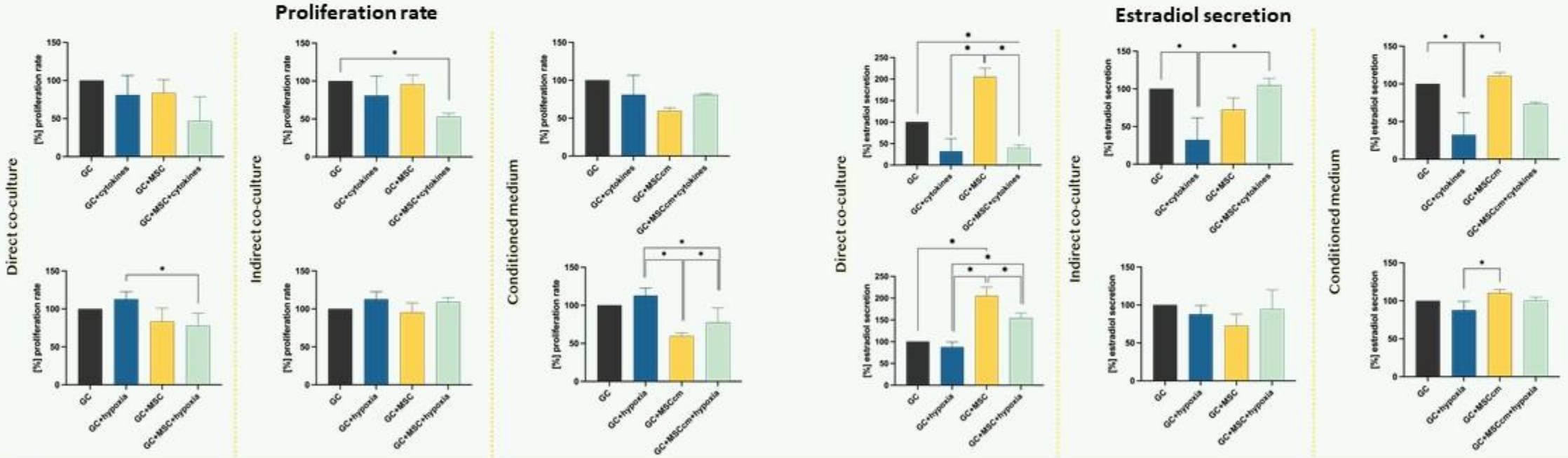
Objective

Given the regenerative and paracrine properties of mesenchymal stem cells (MSCs), this study aimed to examine their effects on human granulosa cell proliferation and estradiol secretion under inflammatory and hypoxic conditions.

Materials and methods

Three different experimental setups were set: classical co-cultivation of MSCs and granulosa cells; transwell co-cultivation system; cultivation of granulosa cells in the presence of conditioned medium obtained from MSCs.

Results



Conclusions

The data suggest that MSCs have regulatory effects on granulosa cell function and proliferation in the context of hypoxic and inflammatory conditions. Although further research is needed to standardize MSC production and assess their long-term safety and efficacy, MSCs are undoubtedly an innovative approach, providing an individualized and minimally invasive treatment for reproductive disorders.

ACKNOWLEDGMENTS **The project was funded by the National Research Fund, grant No. КП-06-Н51/2.**

Adipokine profiling and mtDNA epigenetic changes in adipose-derived MSCs following palmitic acid treatment

Poster No
PJS_2512

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Keywords: AdMSCs, preadipocytes, mtDNA, adipokines, epigenetic modifications

Objective

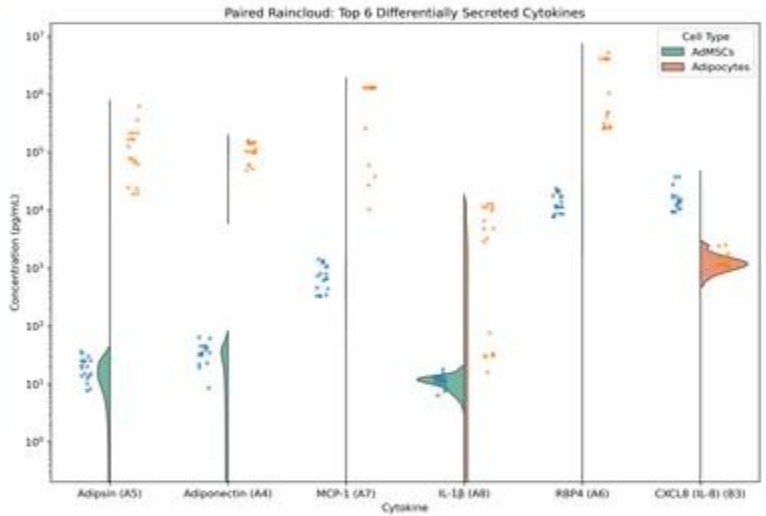
The aim of the study is to compare adipokine secretion patterns in adipose-derived mesenchymal stem cells (AdMSCs) and preadipocytes that exhibit different immunomodulatory properties. Long-read sequencing technology were used to investigate palmitic acid (PA) influence on epigenetic modifications in AdMSCs mitochondrial DNA (mtDNA) .

Materials and methods

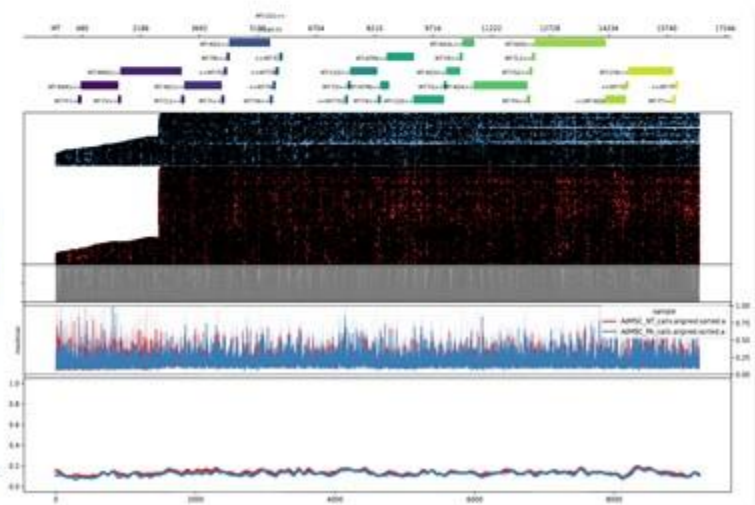
GridION Device for long-sequencing of native mtDNA

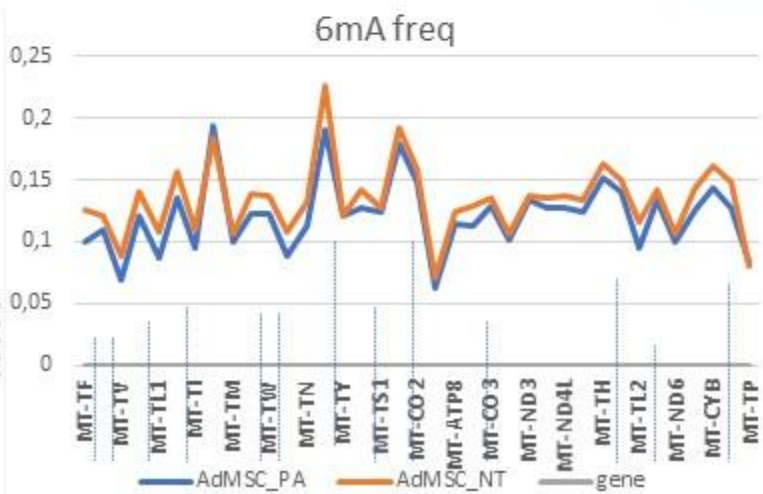
Sony MA900 Cell Sorter for 13plex Adipokine Detection with LEGENDplex Assay Kit

Results



High-concentration of Adipsin, Adiponectin, MCP-1 and RBP4 were found in preadipocytes, while CXCL8 (IL-8) predominate in AdMSCs.
Data underscore functional compartmentalization showed that AdMSCs are related to inflammatory sentinels, and preadipocytes to high-output metabolic regulators.





Genomic analysis plot reveals a modest shift in the methylation profile of methylated adenine (6mA), characterized by increased hypomethylation in PA-treated cells, compared to the non-treated in AdMSCs.

Conclusions

- After PA treatment, AdMSCs are early inflammatory sentinels, while adipocytes act as metabolic effectors.
- PA impacts mitochondrial epigenetic landscape of AdMSCs.
- These findings motivate single-molecule, heteroplasmy-aware epigenetic profiling as a sensitive readout of lipotoxic stress in metabolic cell models.

ACKNOWLEDGMENTS:
Supported by INFRAACT, Grant ДО1-361/2023, National Center of Biomedical Photonics (NCBMP DO1- 352), and BG-RRP-2.004-0003 “Research University: Medical University – Pleven”.

Long COVID - related endothelial injury: Evidence of ORF3a-induced mitochondrial dysfunction in SARS-CoV-2-infected endothelial cells

Poster No
PJS_2514

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Keywords: ORF3a, endothelial cells, mitochondrial dysfunction, ROS, Long COVID

Objective

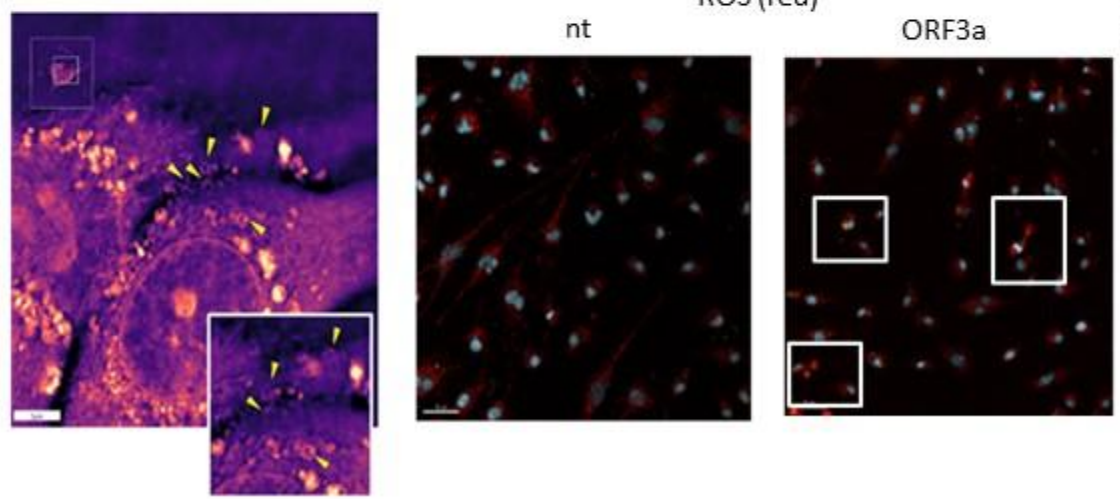
This study investigated how SARS-CoV-2 ORF3a expression affects mitochondrial function in human endothelial cells, aiming to reveal mechanisms underlying endothelial dysfunction and vascular complications associated with post-COVID conditions.

Materials and methods

Human endothelial HULEC-5a cells were cultured and transfected with 0.1 µg/mL ORF3a plasmid DNA. Mitochondrial morphology and structural integrity were assessed label-free using Nanolive 3D holotomography. Cellular oxidative metabolism was evaluated by analyzing reactive oxygen species after incubation with a redox-sensitive dye using Andor confocal microscopy. To quantify oxidative stress, cells were stained with a ROS-specific fluorescent dye.

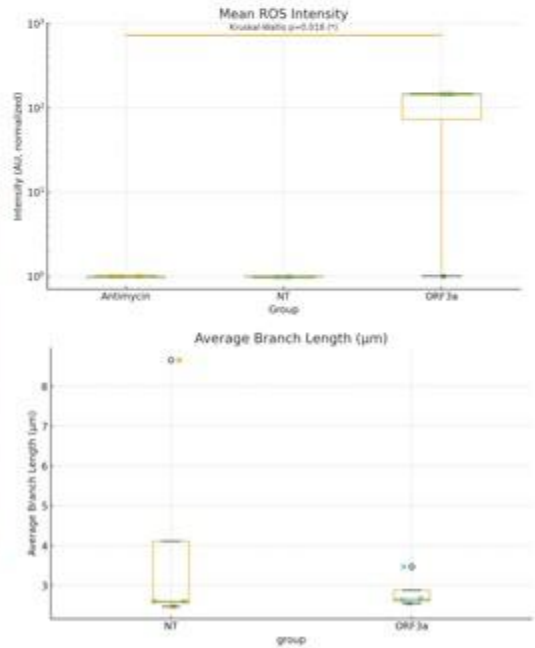
Results

ORF3a expression induced evident mitochondrial fragmentation and loss of structural integrity, accompanied by a significant increase in ROS fluorescence intensity compared to control cells as ROS were colocalized with ORF3a expression. These findings indicate that ORF3a-expressing endothelial cells undergo oxidative stress.



Conclusions

Our data demonstrate that SARS-CoV-2 ORF3a expression directly disrupts mitochondrial homeostasis. This mechanism may contribute to persistent vascular inflammation, microthrombosis, and autoimmunity observed in Long COVID.



ACKNOWLEDGMENTS: Research was partially funded by the Minsitry of Education and Science Grant DO-1 361/2024 INFRAACT and NSF of Bulgaria Grant No. КП-06-Д1-12

Differentiation of Primordial Germ Cell-like Cells from hESCs Using BMP-4 and hAFSC-4 Conditioned Medium

Poster No PJS_ 2516

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Keywords: PGCLCs; human embryonic stem cells; BMP4; amniotic fluid stem cells; conditioned medium

Objective:

To establish a simple, reproducible, and cost-effective adherent culture protocol for generating DDX4/VASA- and DAZL-positive primordial germ cell-like cells (PGCLCs) from human embryonic stem cells (hESCs) using BMP4 stimulation combined with conditioned medium (CM) derived from SSEA4-positive human amniotic fluid stem cells (hAFSC-4).

Results:

•By Day 9, small PGCLC groups appear as round, phase-bright cells.

•By Day 16, large protruding PGCLC clusters are clearly visible.

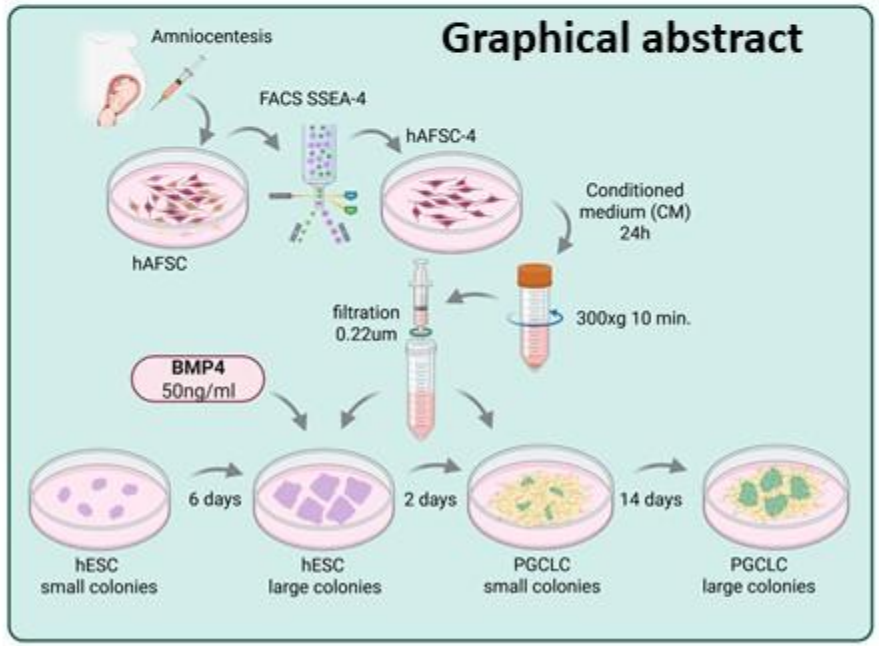
•40–60% of cells express DDX4/VASA and DAZL, confirming germline identity (**Fig.2** and **Fig.3**).

•hAFSC-4 CM improves survival, proliferation, and differentiation fidelity compared to BMP4 alone.

Conclusions:

This study demonstrates an effective, reproducible, and physiologically relevant method for generating PGCLCs from hESCs using BMP4 and hAFSC-4 CM in adherent cultures. The combined signaling environment enhances germ cell specification, survival, and expansion, providing a robust in vitro platform for studying early human germline development.

Graphical abstract



Materials and methods:

Cell Sources:

•hESC line B1 (feeder-free)

•SSEA4-enriched hAFSC (hAFSC-4) (**Fig 1.**)

PGCLC Differentiation:

•Day 0–2: hESCs treated with BMP4 (50 ng/mL) in hAFSC-4 CM

•Day 2–16: Culture continued in CM alone; medium changed every 2 days

•PGCLCs appear by Day 9 and form large clusters by Day 16

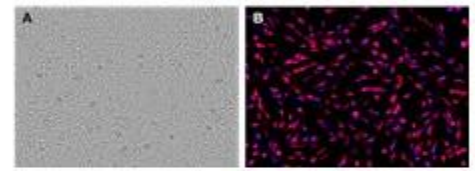


Figure 1. Human amniotic fluid stem cells. A) Image of hAFSCs. B) Double immunofluorescence with anti- SSEA-4 antibodies (red) and DAPI (blue.

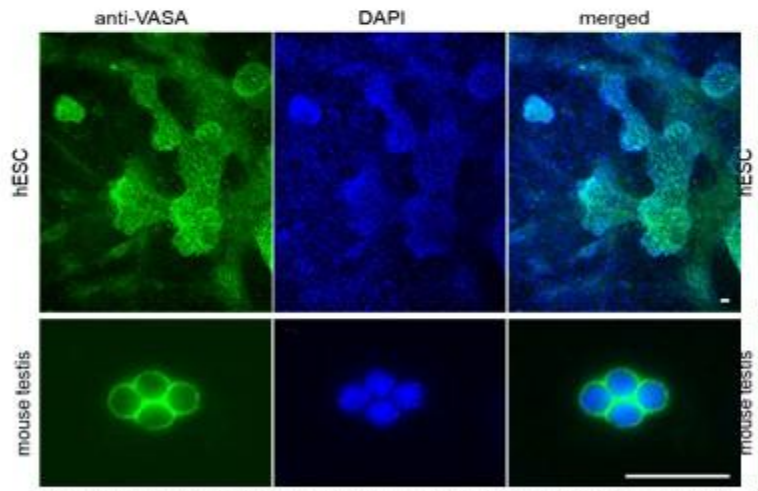


Figure 2. Expression of DDX4/VASA (anti-VASA) in the obtained primordial germ cell-like cells at day 16 after the start of differentiation (hESC). Primary cultured cells from mouse testis at day 4 were used as a positive control (mouse testis). Cell nuclei were visualized by DNA staining with DAPI. Scale bar: 50 µm.

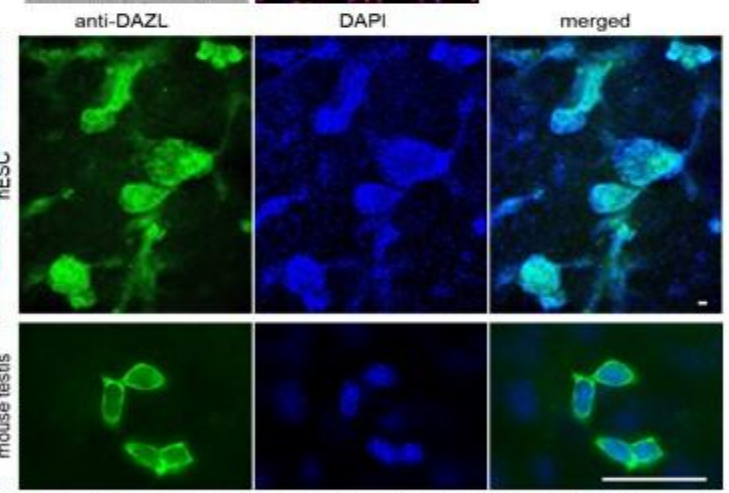


Figure 3. Expression of DAZL (anti-DAZL) in the obtained primordial germ cell-like cells at day 16 after the start of differentiation (hESC). Primary cultured cells from mouse testis at day 4 were used as a positive control (mouse testis). Cell nuclei were visualized by DNA staining with DAPI. Scale bar: 50 µm.

ACKNOWLEDGMENTS

This study was funded by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project SUMMIT BG-RRP-2.004-0008-C01 and DO1-361/2023.

The role of HMGB1/RAGE interaction in driving epithelial-to-mesenchymal transition and invasion in 2D and 3D breast cancer models

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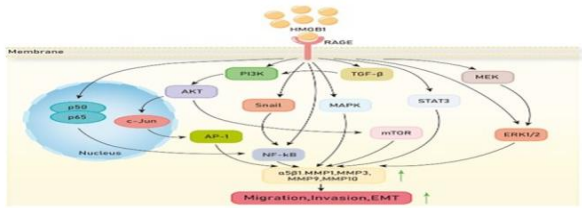
PJS_2517

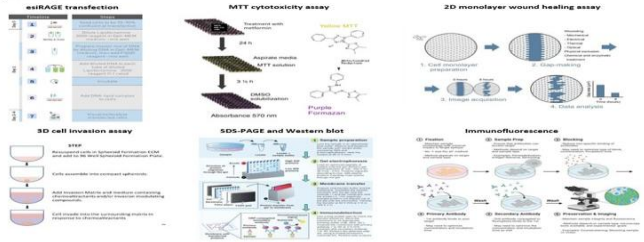
INTRODUCTION



High Mobility Group Box 1 (HMGB1) is a nuclear protein that, once secreted into the extracellular matrix, interacts with the transmembrane receptor for advanced glycation end products (RAGE). This interaction has been shown to be essential for inducing epithelial-mesenchymal transition (EMT) in triple-negative breast cancer (TNBC). When HMGB1 binds to RAGE, it activates downstream signaling cascades that inhibit epithelial markers while increasing mesenchymal marker expression, hence increasing TNBC cells' invasive and migratory ability. This mechanism promotes metastasis and resistance to treatments. Targeting this particular relationship may be a useful path for therapeutic approaches targeted at improving patient outcomes.

METHODS

This study employed various methodologies to explore the relationship between HMGB1 and the RAGE receptor in regulating the motility and invasion of MDA-MB-231 cells. Initially, MDA-MB-231 cells were transfected with esiRAGE using lipofectamine 3000 to validate the impact of this interaction on cell motility and invasion. Subsequently, metformin, a potential inhibitor of this process, was assessed. The IC50 concentrations of metformin in 2D and 3D MDA- MB-231 cell models were determined through MTT cytotoxicity assays.



Following this, wound healing and cell invasion assays were conducted to evaluate whether metformin at the IC50 concentration could effectively inhibit cell motility and invasion. Moreover, the study investigated the anti-EMT properties of metformin by analyzing the expression of EMT markers via western blot and immunofluorescence techniques.

CONCLUSION

Our study shows that the HMGB1/RAGE interaction drives cell motility and invasion in TNBC. Metformin, an anti-diabetic drug, effectively inhibits cell motility and invasion in both 2D and 3D TNBC models. Additionally, metformin reduces the expression of EMT markers linked to metastasis. These results highlight metformin's potential as a therapeutic agent for targeting the HMGB1/RAGE pathway and slowing TNBC metastasis, although further research is needed to fully understand metformin's therapeutic benefits in TNBC treatment.

RESULTS

In prior research conducted by our team, we demonstrated the direct interaction between HMGB1 and RAGE using co-immunoprecipitation. This study aims to explore the impact of this interaction on the motility and invasion capabilities of triple-negative breast cancer (TNBC) cells.

The investigation commenced by targeting RAGE and confirming its role in mediating the interaction with HMGB1. Through targeted gene silencing of RAGE, we established a platform to discern the direct impact of HMGB1/RAGE interaction on cellular behavior. Initial assessments focused on evaluating the consequences of this interaction on cell motility in 2D culture models and invasion in 3D culture models. Notably, the data revealed a significant augmentation in both cell motility and invasion upon exposure to exogenous HMGB1, affirming the functional relevance of the HMGB1/RAGE axis in driving these cellular processes.

Following this, we explored the therapeutic potential of metformin, a commonly used drug against type 2 diabetes, in mitigating EMT, a pivotal event implicated in cancer progression and metastasis. To determine the optimal dosage for subsequent experiments, we conducted MTT assays to assess cell viability and establish the half-maximal inhibitory concentration (IC50) values for both 2D and 3D TNBC cell models. Once the appropriate concentrations were identified, 2D and 3D cultures were treated with a combination of HMGB1 and IC50 metformin to investigate the potential reversal of HMGB1-induced cellular phenotypes.

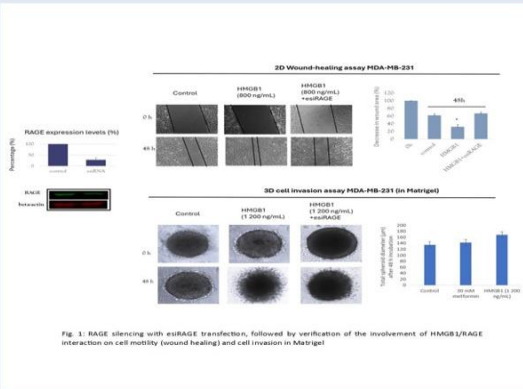


Fig. 1: RAGE silencing with esiRAGE transfection, followed by verification of the involvement of HMGB1/RAGE interaction on cell motility (wound healing) and cell invasion in Matrigel

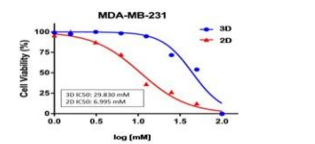


Fig. 2: MTT cytotoxicity analysis showing IC50 values of metformin in 2D and 3D MDA-MB-231 cells

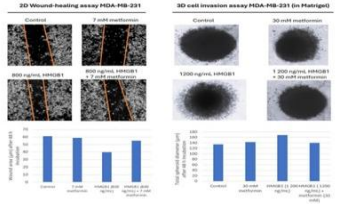


Fig. 3: Analysis of cell motility and invasion following treatment with exogenous HMGB1 and IC50 dose of metformin

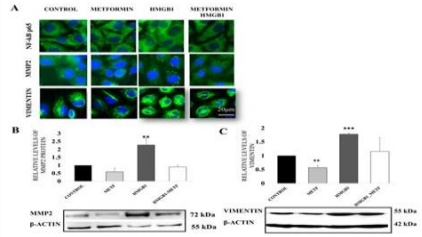


Fig.4 Immunolocalization of EMT markers NF-kB p65 and vimentin as well as MMP2 protein (green fluorescence). A: Representative pictures. Nuclei are stained with DAPI (blue). Relative protein levels of MMP2 (B) and vimentin (C) in control MDA-MB-231 cells and cells treated with HMGB1 and/or 3 mM metformin (METF). Data represents mean ± SD (n = 3), *p≤0.05, **p≤0.01, ***p≤0.001.

Our findings unveiled a remarkable attenuation of HMGB1-induced cell motility and invasion upon metformin treatment, indicative of its potent anti-EMT properties. To delve deeper into the underlying mechanisms, we employed immunofluorescence staining to visualize the expression of EMT markers within treated cell populations. Concurrently, Western blot analysis was utilized to validate the observed alterations in EMT marker expression at the protein level.

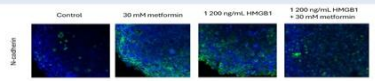


Fig. 5: Immunofluorescence staining of N-cadherin in 3D MDA-MB-231 spheroids. Green fluorescence depicts positive N-cadherin expression, whereas blue fluorescence is indicative of nuclei staining.

Collectively, these multifaceted analyses provided compelling evidence for the efficacy of metformin in counteracting the pro-invasive effects orchestrated by HMGB1, thus underscoring its potential as a therapeutic agent for impeding cancer metastasis through modulation of EMT processes.

BULGARIAN SCIENCE FUND

This research received funding from the Bulgarian National Scientific Fund under project number КП-06-Н51/13 on November 17, 2021.

Association studies of genes encoding receptors and ligands in patients with diabetes types 1

Poster No
PJS_2518

Dimitar Dimitrov[1,*], Veronika Krastanova[2], Radoslav Rangelov[3], Sylvia Pashkunova[4], Teodora Daneva[5]

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Keywords: keyword one, keyword two, keyword three, keyword four, keyword five

Introduction

Diabetes is one of the most dreaded diagnoses and unfortunately is still an incurable disease. Although the causes are not yet clear, association studies over the past two decades have shown that genetic imbalance between the killer immunoglobulin-like receptors (KIRs) on NK cells and their HLA class 1 ligands can enhance T-cell activation, which contributes to the pathogenesis of type 1 diabetes (T1D)

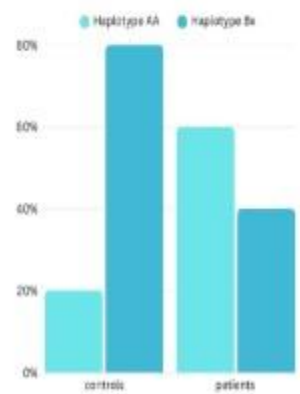
Objective

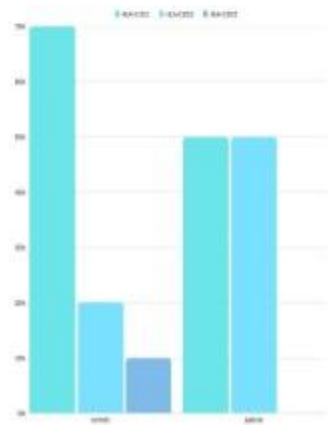
The main goal of this study is to assess the impact of genes, encoding receptors and their ligands and their interaction, on the etiology and risk of developing T1D and potentially identify genomic biomarkers

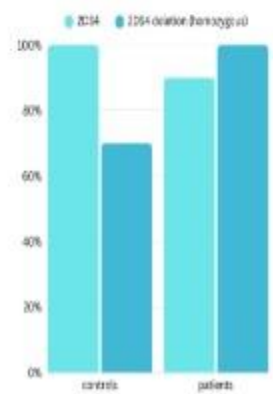
Materials and methods

Blood is collected from patients with T1D and control subjects. DNA from the blood samples is extracted and is genotyped for 16 KIRs, HLA-C allele groups and HLA-B27 allele using the PCR-SSP method.

Results







Conclusion

Various studies have examined the role of killer cell immunoglobulin-like receptors (KIRs), HLA class I ligands and their interactions on the pathogenesis of type 1 diabetes (T1D). Based on previous publications and research, we assume that there is a relationship between the differences in haplotype and genotype of patients diagnosed with T1D and that of healthy controls. The results might shed light on the genetic factors, contributing to the development of autoimmune diabetes and also identify key biomarkers, which can be used to determine genetic predisposition.

Immunological Profile, Inflammatory Biomarkers, and Autoimmune Features of Post-COVID-19 Syndrome: a Bulgarian observational prospective study

Poster No

Tsvetelina Velikova[1,*], Eleonora Stamenova[2], Antoaneta Mihova[3], Poli Dimcheva[3], Stanislava Zlateva[3], Rossen Mihaylov[3,4], Blagovesta Pencheva[3], Kosta Kostov[2,5]

[1]Medical Faculty, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria; [2] Department of Pulmonology, Clinic of Internal Medicine, University Hospital "St Anna", Dimitar Mollov Str. 1, 1701, Sofia, BULGARIA; [3] IMDL Ramus Ltd., Sofia, BULGARIA; [4]Medical College "Yordanka Filaretova", Sofia, BULGARIA; [5]Medical center "InSpiro", Sofia, BULGARIA

* Email: tsvelikova@medfac.mu-sofia.bg

Keywords: post-covid, antinuclear antibodies, autoantibodies, inflammation, symptoms

Objective

This study included 60 participants whose demographic and clinical characteristics were analyzed to explore post-COVID-19 symptom persistence and associated laboratory parameters and autoantibodies.

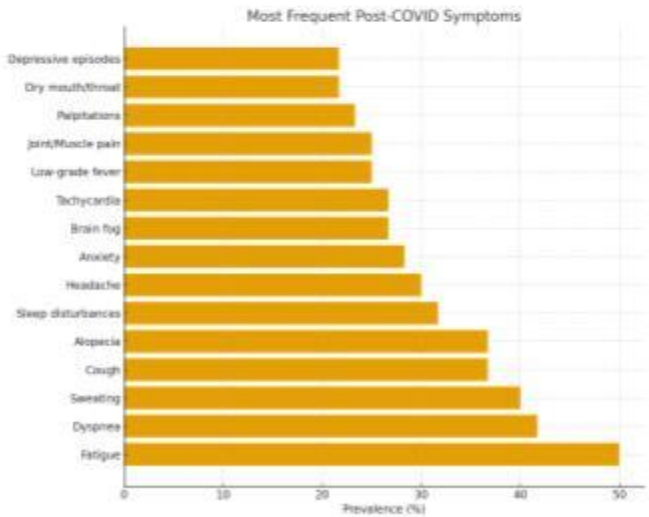
Materials and methods

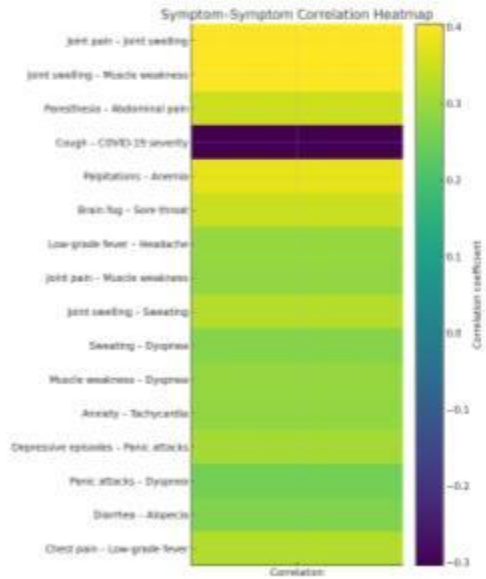
Antinuclear antibodies (ANA) were determined by indirect immunofluorescence (IIF), and specific autoantibodies were detected using the EUROLINE ANA Profile 23 (EUROIMMUN, Germany) in serum samples. The fluorescence pattern analysis was performed under a fluorescence microscope by an experienced (>15 years) operator and classified according to the international ICAP nomenclature.

Results

Thirty-one patients (51.7%) were men, mean age of 58.98 ± 15.45 years (22–82), and mean BMI 25.93 ± 3.62. Sixteen patients (26.7%) experienced severe disease.

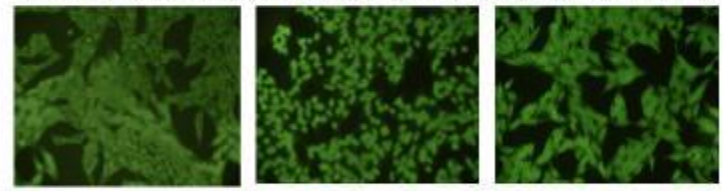
Twenty-seven patients (45%) tested positive for ANA as follows: 1:80 (11 individuals),1:160 (6),1:320 (4), 1:640 (3), and ≥1:1200 (3), and 55 individuals (91.6%) showed positive cytoplasmic fluorescence (1:80-1:640).





Among the ANA-positive patients, autoantibodies were detected against the following antigens: Mi2β [AC-4], PM-Scl75 [AC-8], RP11 [AC-10], RP155 [AC-10], dsDNA [AC-1], Ro-52, RNP/Sm [AC-5], Sm [AC-5], SS-B [AC-4], Ku [AC-4], PCNA [AC-13], Scl-70 [AC-29], gp210 [AC-11], DFS70 [AC-2].

IIF patterns: cytoplasmic, homogenous, cytoplasmic+ nuclear



Conclusions

Our findings suggest that post-COVID-19 symptoms are prolonged and multisystemic, with persistent fatigue and respiratory complaints being most prevalent. Mild elevations in inflammatory markers indicate a sustained low-grade inflammatory state, and high prevalence of autoantibodies, underscoring the need for long-term monitoring and rehabilitation strategies in post-COVID care.

ACKNOWLEDGMENTS. This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008.

The role of HMGB1/RAGE interaction in driving epithelial-to-mesenchymal transition and invasion in 2D and 3D breast cancer models

Poster No: PJS_2517

Desislava Vladimirova[1] Mihaela Aleksandrova[2], Shazie Myashkova[1], Iva Ugrinova[1], **Jordana Todorova**[1]

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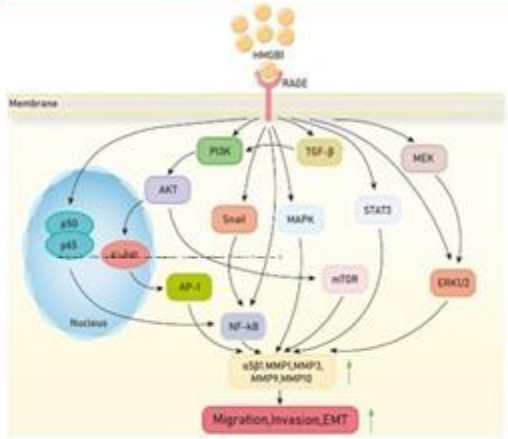
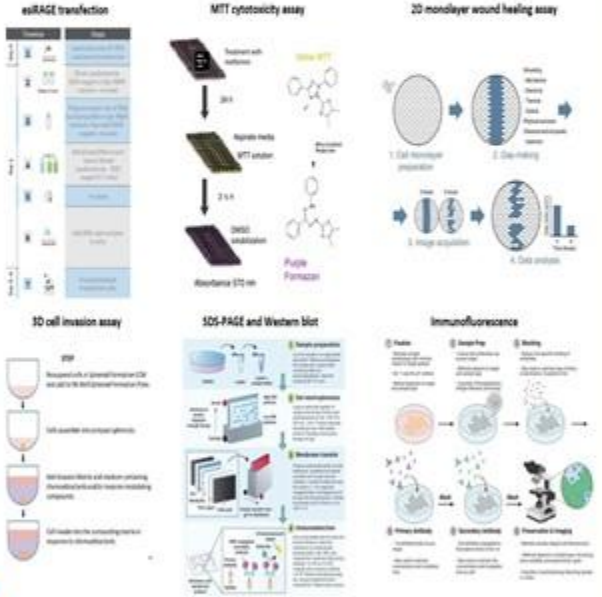
INTRODUCTION



High Mobility Group Box 1 (HMGB1) is a nuclear protein that, once secreted into the extracellular matrix, interacts with the transmembrane receptor for advanced glycation end products (RAGE). This interaction has been shown to be essential for inducing epithelial-mesenchymal transition (EMT) in triple-negative breast cancer (TNBC). When HMGB1 binds to RAGE, it activates downstream signaling cascades that inhibit epithelial markers while increasing mesenchymal marker expression, hence increasing TNBC cells' invasive and migratory ability. This mechanism promotes metastasis and resistance to treatments. Targeting this particular relationship may be a useful path for therapeutic approaches targeted at improving patient outcomes.

METHODS

This study employed various methodologies to explore the relationship between HMGB1 and the RAGE receptor in regulating the motility and invasion of MDA-MB-231 cells. Initially, MDA-MB-231 cells were transfected with esiRAGE using lipofectamine 3000 to validate the impact of this interaction on cell motility and invasion. Subsequently, metformin, a potential inhibitor of this process, was assessed. The IC50 concentrations of metformin in 2D and 3D MDA- MB-231 cell models were determined through MTT cytotoxicity assays.



Following this, wound healing and cell invasion assays were conducted to evaluate whether metformin at the IC50 concentration could effectively inhibit cell motility and invasion. Moreover, the study investigated the anti-EMT properties of metformin by analyzing the expression of EMT markers via western blot and immunofluorescence techniques.

CONCLUSION

Our study shows that the HMGB1/RAGE interaction drives cell motility and invasion in TNBC. Metformin, an anti-diabetic drug, effectively inhibits cell motility and invasion in both 2D and 3D TNBC models. Additionally, metformin reduces the expression of EMT markers linked to metastasis. These results highlight metformin's potential as a therapeutic agent for targeting the HMGB1/RAGE pathway and slowing TNBC metastasis, although further research is needed to fully understand metformin's therapeutic benefits in TNBC treatment.

BULGARIAN SCIENCE FUND

This research received funding from the Bulgarian National Scientific Fund under project number КП-06-Н51/13 on November 17, 2021.

RESULTS

Initial assessments focused on evaluating the consequences of HMGB1/RAGE interaction on cell motility in 2D culture models and invasion in 3D culture models. Notably, the data revealed a significant augmentation in both cell motility and invasion upon exposure to exogenous HMGB1, affirming the functional relevance of the HMGB1/RAGE axis in driving these cellular processes.

Following this, we explored the therapeutic potential of metformin, a commonly used drug against type 2 diabetes. To determine the optimal dosage for both 2D and 3D TNBC cell models for subsequent experiments, we conducted MTT assays. Once the appropriate concentrations were identified, 2D and 3D cultures were treated with a combination of HMGB1 and IC50 metformin to investigate the potential reversal of HMGB1-induced cellular phenotypes.

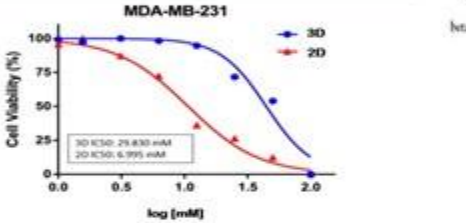


Fig. 2: MTT cytotoxicity analysis showing IC50 values of metformin in 2D and 3D MDA-MB-231 cells

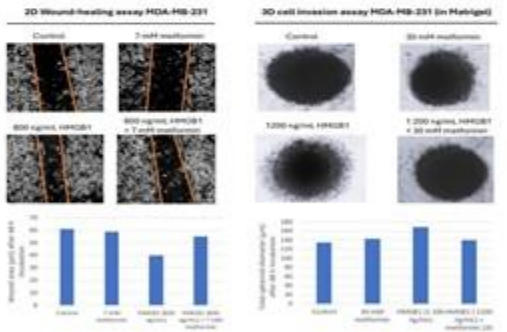


Fig. 3: Analysis of cell motility and invasion following treatment with exogenous HMGB1 and IC50 dose of metformin

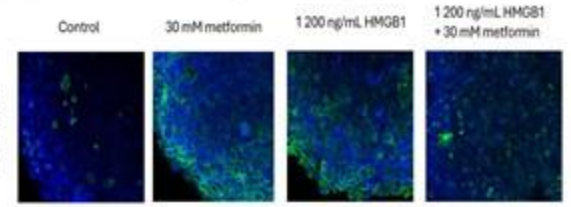
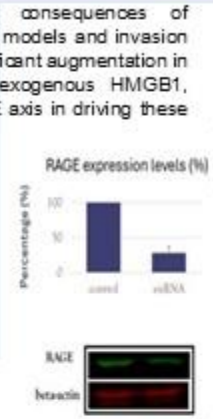
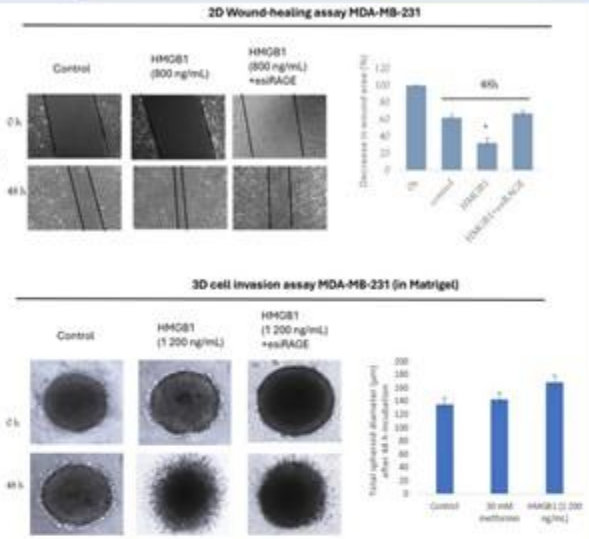
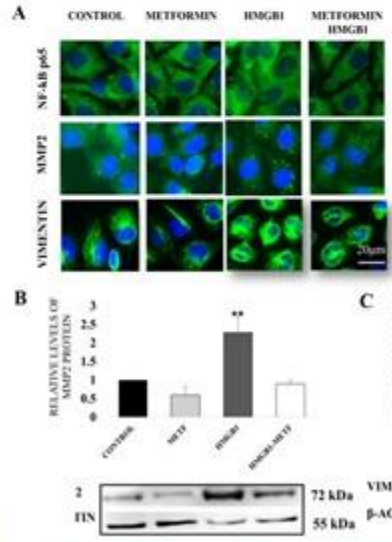


Fig. 5: Immunofluorescence staining of N-cadherin in 3D MDA-MB-231 spheroids. Green fluorescence depicts positive N-cadherin expression, whereas blue fluorescence is indicative of nuclei staining.







To delve deeper into the underlying mechanisms, we employed immunofluorescence staining to visualize the expression of EMT markers within treated cell populations. Concurrently, Western blot analysis was utilized to validate the observed alterations in EMT marker expression at the protein level.

Collectively, these multifaceted analyses provided compelling evidence for the efficacy of metformin in counteracting the pro-invasive effects orchestrated by HMGB1, thus underscoring its potential as a therapeutic agent for impeding cancer metastasis through modulation of EMT processes.

Съфинансирано от Европейския съюз

Healing and regenerating effect of snail mucus extract on hard to heal and necrotic wounds

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Poster No

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Objective

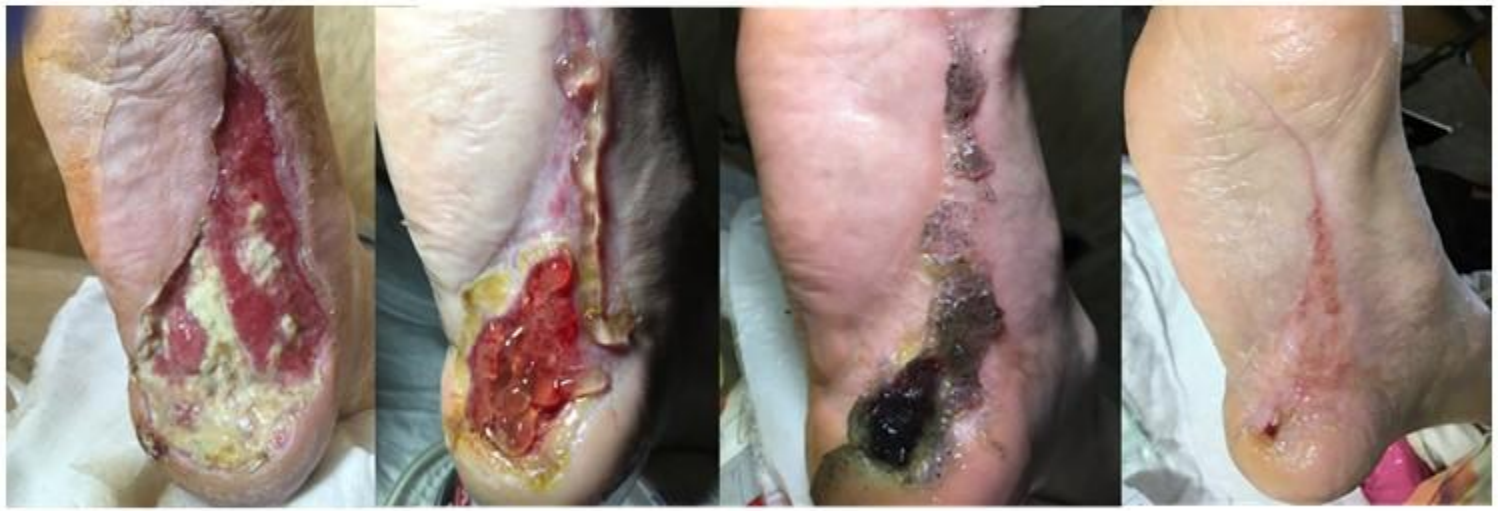
the aim of the presented study is to report the results of the *in vivo* application of a combined extract of the mucus of the garden snail *Cornu aspersum*, enriched with extracts of marigold (*Calendula officinalis*) and plantain (*Plantago major*) in acute, chronic, necrotic and difficult-to-heal wounds of large areas.

Materials and methods

A case of treatment of a mixed diabetic wound with a large area on the sole of the right foot of a 64-year-old man with insulin-dependent diabetes is presented. Treatment was carried out 3 times a week for 6 months with the developed combined extract. During the treatment, the levels of tissue regeneration, local inflammatory process, local pain on a visual analog scale (VAS), pH values and microbiological control of wound secretion samples were monitored.

Results

Complete tissue regeneration from the necrotic area with a large area to functional skin and subcutaneous tissue, complete epithelialization and fine cicatrix were established. It has been proven that the *C. aspersum* mucus extract created and studied by us in combination with herbal extracts is a natural, safe and effective alternative remedy for the treatment of difficult-to-heal wounds. A new protocol for the treatment of difficult-to-heal, chronic and necrotic wounds with a large area is also presented.

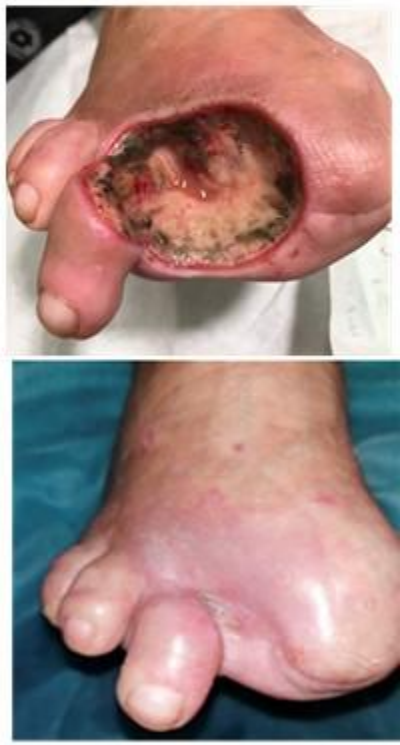


Diabetic foot - a difficult-to-heal wound after 4 years of treatment – various methods

In the process of wound healing, activated carbon gel was applied to absorb the toxins and clean the wound

Conclusions

The obtained results prove the effectiveness of the mucus of the garden snail *C. aspersum*, enriched with herbal extracts from C. *officinalis* and *P. major*, in the treatment of chronic diabetic wounds.



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