Abstracts of the published papers authored by Georgi Nikolaev Georgiev

B4.1 **G Georgiev**, R Dimitrov, P Todorov, Y Dimitrov, R Konakchieva. Melatonin receptors in human spermatozoa-new findings and relevance to assisted reproduction. (2012) Journal of Reproductive Immunology 1 (94), 105, https://doi.org/10.1016/j.jri.2012.03.439; IF 4.054, Q1;

Increased cytochrome P450 aromatase activity, elevated 17-beta estradiol (E2) and low testosterone (T)/E2 index have been associated with impairment of male gametes competence. Although aromatase-inhibitory activity of melatonin (MEL) in breast cancer patients has been suggested in many studies, the mechanism of this interaction is far from understood. Despite the intensive research on its antioxidant properties, the principal role and cellular targets of the evolutionary conserved melatonin remains elusive in regard to human reproduction. To clarify the meaning of melatonin signal present in human reproductive tract in the present study we aimed to demonstrate expression and localization of specific membrane melatonin receptor type 1 (MT1) in human competent spermatozoa. We also studied the MTNR1A gene transcription in semen samples processed to eliminate semen plasma influence. Our results demonstrated in-situ the presence of membrane melatonin receptor in competent human male gametes. The observed localization of the receptor protein suggested role for melatonin in capacitation events, which may have several important implications to assisted reproductive techniques. The negative relation between the expression of cytochrome P450 aromatase and the MT1 receptor in semen samples of healthy donors and infertile patients points out at the significance of steroid inhibitory action of melatonin in male reproductive tract. Dissected functional responses and transduction pathways linked to specific receptor activation by melatonin in human reproductive cells have further to be elucidated.

B4.2 **Georgiev, G.N.**, Marinova, E., Konakchieva, R., Todorov, P. Melatonin selectively influences the transcription of pluripotency and differentiation markers in human non-cancer cells (2019) Biotechnology and Biotechnological Equipment, 33 (1), pp. 286-293. https://doi.org/10.1080/13102818.2019.1571440 - IF 1.632, Q3;

Melatonin (MEL) may influence the efficiency of reprogramming both by somatic cell nuclear transfer and by direct induction of pluripotent stem cells (iPSC) through a yet unidentified mechanism. Transcription factors linked to cell reprogramming and cell signalling may be differentially expressed according to cell differentiation status. To address the effect of MEL on the expression of transcription factors linked to reprogramming, we used two distinct in vitro models of cellular plasticity: human foreskin fibroblasts (HFF) and primary human granulosa-lutein cells (GLC). Realtime quantitative polymerase chain reaction (qRT-PCR) analysis revealed amplification of transcripts for KLF4, MYC and NANOG in both cell types. In GLC, treatment with 10 nmol/L of MEL provoked significant up-regulation of the expression of MYC and NANOG compared to controls. KLF4 expression was not altered in GLC but was significantly down-regulated in MEL-treated HFF cells. Alterations in the expression of ERK1/2 and pERK1/2 in GLC as analyzed by Western blot were not observed regardless of the MEL treatment. On the contrary, HFF cells responded to MEL treatment with 1.6-fold higher levels of pERK1/2 compared to the non-treated controls. Our data suggest that the activation of MT1 melatonin receptor is probably related to phosphorylation of ERK1/2 at least in expanding HFF, which subsequently may act to alter gene expression and regulate cell fate. In conclusion, we demonstrated for the first time, the selective effect of MEL in vitro at physiological

concentration on transcription factors regulating pluripotency and differentiation in human non-cancer cells according to cell differentiation status.

B4.3 **Georgiev, G.N.**, Mourdjeva, M., Oreshkova, T., Pankov, R., Konakchieva, R. MT1 and MT2 melatonin receptor expression and in vitro melatonin effect on the pha-dependent activation of human PBMC (2019) Comptes Rendus de L'Academie Bulgare des Sciences, 72 (11), pp. 1500-1506. DOI:10.7546/CRABS.2019.11.07 - IF 0.378, Q2;

Combination of advanced fluorescence technologies with cell-based assays prove to become indispensable tool to study in depth mechanisms in a biological context and meets the challenges of performing high throughput cell screening for biomedical applications. To explore the immunomodulatory activity of the pineal hormone melatonin on human PBMC activation and cell cycle progression we have used the automated CytellTM Cell Imaging System (GE Healthcare) which combines the functions of a digital microscope, an image cytometer, and a cell counter in a single instrument. We detected quantitative alterations of the cell cycle entry and progression inflicted by melatonin. Immune fluorescence showed characteristic pattern of both types melatonin receptors MT1 and MT2 as well as modification of actin rearrangement and CD25+ expression during PBMC activation.

B4.4 Nikolov, G., **Georgiev, G.N.**, Marinova, E., Mourdjeva, M., Konakchieva, R. UP-Regulation of MT1 and MT2 receptors by in vitro melatonin and modulation of alpha-tubulin and aromatase P450 expression in human granulosa-lutein cells (2020) Comptes Rendus de L'Academie Bulgare des Sciences, 73 (3), pp. 348-354. DOI: 10.7546/CRABS.2020.03.07 – IF 0.378, Q2;

Ovarian granulosa-lutein cells (GLCs) prove to be useful model to study receptors and signalling mechanism involved in steroidogenesis and differentiation plasticity linked to follicular growth and oocyte quality. Reports of high levels of melatonin in pre-ovulatory follicular fluids together with demonstration of specific melatonin receptors in follicular cells let us investigate possible targets of the hormone in GLC aspirated from follicular fluid. We demonstrated by confocal immunofluorescence specific expression of both MT1 and MT2 receptors (MR) which was up-regulated by melatonin treatment and confirmed also by Western blotting. By dual immunofluorescence labelling we were able to show colocalization of the signal for alpha-tubulin and MT1 in the perinuclear cytoplasmic area of GLC, while the MT2 signal appeared weaker around the nuclei and turned diffuse cytoplasmic after 48 h of melatonin treatment. Up-regulation of MR was observed along with stimulation of P450 aromatase protein expression which points out at the significance of melatonin in processes regulating follicular development.

В4.5 **Nikolaev, G.**; Robeva, R.; Konakchieva, R. Membrane Melatonin Receptors Activated Cell Signaling in Physiology and Disease. *Int. J. Mol. Sci.* **2022**, *23*, 471. https://doi.org/10.3390/ijms23010471 – IF 5.924, Q1;

The pineal hormone melatonin has attracted great scientific interest since its discovery in 1958. Despite the enormous number of basic and clinical studies the exact role of melatonin in respect to human physiology remains elusive. In humans, two high-affinity receptors for melatonin, MT1 and MT2, belonging to the family of G protein-coupled receptors (GPCRs) have been cloned and identified. The two receptor types activate Gi proteins and MT2 couples additionally to Gq proteins to modulate intracellular events. The individual effects of MT1 and MT2 receptor activation in a variety of cells are complemented by their ability to form homo- and heterodimers, the functional relevance of which is yet to be confirmed. Recently, several melatonin receptor genetic polymorphisms were discovered and implicated in pathology—for instance in type 2 diabetes, autoimmune disease, and cancer. The circadian patterns of melatonin secretion, its pleiotropic effects depending on cell type and condition, and the already demonstrated cross-talks of melatonin receptors with other signal transduction pathways further contribute to the perplexity of research on the role of the pineal hormone in humans. In this review we try to summarize the current knowledge on the membrane melatonin receptor activated cell signaling in physiology and pathology and their relevance to certain disease conditions including cancer.

Г7.1. Pastuschek, J., Bus, T., Poetzsch, J., Raabe, M., Winkler, S., Fritzsche, A., Schleussner, E., Markert, U., **Georgiev, G.**, COV434 granulosa cell line: take it or leave it? (2014) Journal of Reproductive Immunology, 101–102, https://doi.org/10.1016/j.jri.2013.12.102; IF 4.054, Q1;

The granulosa cell line COV434 is widely used in ovarian research and in the investigation of human granulosa cells. Considering the convenience provided by cell lines in general and the absence of an efficient isolation method to avoid contaminations with other cell types, our aim was to identify novel features of COV434 and use it as a new and accessible model for broader applications. Using different culture conditions we examined the 17β-estradiol synthesis and the expression of four stem cell markers. Additionally, we focused on the role of the IL6-type cytokines LIF and OSM in the JAK/STAT pathway. In order to use COV434 as an alternative to primary human granulosa cells, we aimed to study in more detail its characteristics and compare it with primary cells. Summarizing all the features analyzed, we were able to demonstrate similarities, but also some differences between the two cell types. Furthermore, our results indicate novel findings and open new research topics in relation to human granulosa cells.

Г7.2. Pastuschek, J., Poetzsch, J., Morales-Prieto, D.M., Schleußner, E., Markert, U.R., **Georgiev, G.** Stimulation of the JAK/STAT pathway by LIF and OSM in the human granulosa cell line COV434 (2015) Journal of Reproductive Immunology, 108, pp. 48-55. https://doi.org/10.1016/j.jri.2015.03.002 - IF 4.054, Q1;

The development of the follicle and competent oocyte is highly coordinated, requiring inter-play among several systems. These implicate endocrine, immune, and metabolic signals, intrafollicular paracrine factors from theca, mural, and cumulus granulosa cells, and the oocyte itself. Granulosa cells play a key role in their interaction. COV434 is one of the few human granulosa cell lines that can be used as an in vitro model for ovarian research. We aimed to evaluate the possible activation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway by IL-6-type cytokines leukemia inhibitory factor (LIF) and oncostatin M (OSM) in COV434 cells. Expression of GP130 (glycoprotein 130), STAT3 (signal transducer and activators of transcription 3), PIAS3 (protein inhibitor of activated STAT 3), and SOCS3 (suppressor of cytokine signaling 3) genes after stimulation with LIF or OSM was assessed using RT-qPCR (real-time PCR). GP130 transcripts were significantly upregulated after incubation with LIF or OSM for 24 h. Expression of the STAT3gene was stimulated only after incubation with LIF, but not OSM. SOCS3 showed significant upregulation for all time periods and the levels of PIAS3 were initially down- and after24 h upregulated. Furthermore, the major signaling components of the JAK/STAT pathway, GP130 and STAT3, and the kinase activation patterns of STAT3, were examined at protein level. We found constitutive protein expression for GP130, STAT3, pSTAT3(ser727) and upregulation of pSTAT3(tyr705) by LIF and OSM. Our results demonstrate the activation of the JAK/STAT pathway by LIF and OSM in human granulosa cells.

Г7.3. Petrova, V.Y., Kujumdzieva, A.V., Tomova, A.A., **Georgiev, G.**, Stefanova, N., Pankov, R.G. Superoxide dismutase and catalase participate in the regulation of quiescent state of human fibroblasts: In silico and biochemical analysis (2016) Comptes Rendus de L'Academie Bulgare des Sciences, 69 (4), pp. 467-474. IF 0.378, Q2;

The quiescent state is typical for cells in multicellular organism, but although widespread, yet little is known about the mechanisms that lead to its establishment and maintenance. In this article we explore the relationship between quiescence and cells oxidative status by examining the behaviour of the antioxidant enzymes superoxide dismutase and catalase in two different cell lines – mouse and human fibroblasts, brought to quiescence by two different approaches – contact inhibition and growth in three-dimensional environment. By using bioinformatic analysis and biochemical studies we demonstrated similarity in the types SODs and catalases expressed in both cell lines and common behaviour, resulting in a tenfold activation of both types of enzymes at quiescent state. The results support the notion for existence of a common program, activated at cell quiescence which is associated with reduction of reactive oxygen species.

Г7.4. Uzunova, V., Tzoneva, R., Stoyanova, T., Pankov, R., Skrobanska, R., **Georgiev, G.**, Maslenkova, L., Tsonchev, Z., Momchilova, A. Dimethylsphingosine and miltefosine induce apoptosis in lung adenocarcinoma A549 cells in a synergistic manner (2019) Chemico-Biological Interactions, 310. https://doi.org/10.1016/j.cbi.2019.108731 – IF 5.192, Q2;

Lung cancer is one of the most common and lethal types of oncological diseases. Despite the advanced therapeutic approaches, the prognosis for lung cancer still remains poor. Apparently, there is an imperative need for more efficient therapeutic strategies. In this work we report that concurrent treatment of human adenocarcinoma A549 cells with specific concentrations of two antitumor agents, the sphingosine kinase 1 inhibitor N, N dimethylsphingosine (DMS) and the alkylphosphocholine miltefosine, induced synergistic cytotoxic effect, which was confirmed by calculation of the combination index. The simultaneous action of these agents induced significant decrease of A549 cell number, as well as pronounced morphological alterations. Combined drugs caused substantial apoptotic events, and significant reduction of the pro-survival marker sphingosine- 1-phosphate (S1P), when compared to the individual treatments with each of the anticancer drugs alone.
Miltefosine is known to affect the synthesis of choline-containing phospholipids, including sphingomyelin, but we report for the first time that it also reduces S1P. Here we suggest a putative mechanism underlying the effect of miltefosine on sphingosine kinase 1, involving miltefosine-induced inhibition of protein kinase C.
In conclusion, our findings provide a possibility for treatment of lung cancer cells with lower concentrations of the two antitumor drugs, DMS and miltefosine, which is favorable, regarding their potential cytotoxicity to normal cells.

Г7.5. Momchilova, A., Staneva, G., Tzoneva, R., Scrobanska, R., **Georgiev, G.**, Hadzhilazova, M., Maslenkova, L., Pankov, R. Resveratrol affects sphingomyelin and cholesterol in three-dimensional fibroblast cultures (2019) Comptes Rendus de L'Academie Bulgare des Sciences, 72 (4), pp. 479-484. DOI: 10.7546/CRABS.2019.04.07; IF 0.378, Q2;

The effect of resveratrol on the lipid compositions and sphingolipid metabolism has been investigated in fibroblast three-dimensional cultures. The obtained results demonstrated that almost all tested membrane lipids remained unchanged except sphingomyelin which was augmented, and phosphatidylcholine, which was reduced as a result of resveratrol treatment. In addition, the membrane level of cholesterol was markedly decreased. Since cholesterol oxidation is a pathogenic factor in various physiological processes, studies were performed for determination of the susceptibility of plasma membrane cholesterol to oxidative attack. The data showed that resveratrol treatment induced a decrease of the percentage of oxidized cholesterol, compared to controls. This is important from biological point of view, because cholesterol, together with sphingomyelin, form functionally active raft domains which participate in various cellular processes including cell signalling. Thus, alterations in the relative proportions of the raft lipid components would alter signal transductions and could induce lateral re-organization of the membrane structure. In conclusion, the present results clearly show that treatment of fibroblasts with resveratrol induces changes in the major raft lipid components and in the susceptibility of cholesterol to oxidative attack.

Г7.6. Peruhova, M., Peshevska-Sekulovska, M., Krastev, B., Panayotova, G., Georgieva, V., Konakchieva, R., **Nikolaev, G.**, Velikova, T.V. What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis? (2020) World Journal of Gastroenterology, 26 (42), pp. 6556-6571. doi:10.3748/wjg.v26.i42.6556 – IF 5.742, Q1;

In the last two decades, the vision of a unique carcinogenesis model for colorectal carcinoma (CRC) has completely changed. In addition to the adenoma to carcinoma transition, colorectal carcinogenesis can also occur *via* the serrated pathway. Small non-coding RNA, known as microRNAs (miRNAs), were also shown to be involved in progression towards malignancy. Furthermore, increased expression of certain miRNAs in premalignant sessile serrated lesions (SSLs) was found, emphasizing their role in the serrated pathway progression towards colon cancer. Since miRNAs function as post-transcriptional gene regulators, they have enormous potential to be used as useful biomarkers for CRC and screening in patients with SSLs particularly. In this review, we have summarized the most relevant information about the specific role of miRNAs and their relevant signaling pathways among different serrated lesions and polyps as well as in serrated adenocarcinoma. Additional focus is put on the correlation between gut immunity and miRNA expression in the serrated pathway, which remains unstudied.

Г7.7. **Georgiev, G.**, Konakchieva, R., Momchilova, A., Pankov, R. Quiescent primary fibroblasts sequester activated ERK1/2 into the lipid rafts (2020) Comptes Rendus de L'Academie Bulgare des Sciences, 73 (3), pp. 363-370. DOI: 10.7546/CRABS.2020.03.09; IF 0.378, Q2;

Cell quiescence is a widespread state, typical for majority of somatic and adult stem cells in the multicellular organisms. Its determination as a separate period (G0), different from the phases of the cell cycle, raised the fundamental question of clarifying the mechanisms that cause its establishment and maintenance. With the present work, we tested the hypothesis that entry into the G0 is related to the confinement of activated extracellular-signal regulated kinase (ERK/2) in the membrane raft domains thus preventing its nuclear localization and subsequent activation of the proliferation program. Using primary human fibroblasts and their ability to become contact inhibited we developed a protocol for enrichment of cell populations with quiescent fibroblasts. Comparing proliferating and quiescent populations, we demonstrated that lipid rafts, isolated from G0 populations are enriched in total ERK1/2 and unlike rafts from proliferating cells, contain activated ERK1/2. These results suggest that cells may use sequestration of activated ERK1/2 into the lipid raft domains as a general mechanism for suppressing proliferation.

Г7.8. Velikova, T., Krastev, B., Lozenov, S., Gencheva, R., Peshevska-Sekulovska, M., **Nikolaev, G.**, Peruhova, M. Antibiotic-related changes in microbiome: The hidden villain behind colorectal carcinoma immunotherapy failure (2021) International Journal of Molecular Sciences, 22 (4), art. no. 1754, pp. 1-11. https://doi.org/10.3390/ijms22041754 – IF 5.924, Q1;

The interplay between drugs and microbiota is critical for successful treatment. An accumulating amount of evidence has identified the significant impact of intestinal microbiota composition on cancer treatment response, particularly immunotherapy. The possible molecular pathways of the interaction between immune checkpoint inhibitors (ICIs) and the microbiome can be used to reverse immunotherapy tolerance in cancer by using various kinds of interventions on the intestinal bacteria. This paper aimed to review the data available on how the antibiotic-related changes in human microbiota during colorectal cancer (CRC) treatment can affect and determine ICI treatment outcomes. We also covered the data that support the potential intimate mechanisms of both local and systemic immune responses induced by changes in the intestinal microbiota. However, further better-powered studies are needed to thoroughly assess the clinical significance of antibiotic-induced alteration of the gut microbiota and its impact on CRC treatment by direct observations of patients receiving antibiotic treatment.

Г7.9. Videv, P., Mladenov, N., Andreeva, T., Mladenova, K., Moskova-Doumanova, V., **Nikolaev, G.**, Petrova, S.D., Doumanov, J.A. Condensing effect of cholesterol on hBest1/POPC and hBest1/SM langmuir monolayers (2021) Membranes, 11 (1), art. no. 52, pp. 1-8. https://doi.org/10.3390/membranes11010052; IF 4.106, Q2;

Human bestrophin-1 protein (hBest1) is a transmembrane channel associated with the calcium-dependent transport of chloride ions in the retinal pigment epithelium as well as with the transport of glutamate and GABA in nerve cells. Interactions between hBest1, sphingomyelins, phosphatidylcholines and cholesterol are crucial for hBest1 association with cell membrane domains and its biological functions. As cholesterol plays a key role in the formation of lipid rafts, motional ordering of lipids and modeling/remodeling of the lateral membrane structure, we examined the effect of different cholesterol concentrations on the surface tension of hBest1/POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) and hBest1/SM Langmuir monolayers in the presence/absence of Ca2+ ions using surface pressure measurements and Brewster angle microscopy studies. Here, we report that cholesterol: (1) has negligible condensing effect on pure hBest1 monolayers detected mainly in the presence of Ca2+ ions, and; (2) induces a condensing effect on composite hBest1/POPC and hBest1/SM monolayers. These results offer evidence for the significance of intermolecular protein–lipid interactions for the conformational dynamics of hBest1 and its biological functions as multimeric ion channel.

Г7.10. Evangelatov, A., Naidenova, D., **Georgiev, G.**, Momchilova, A., Pankov, R. Effects of hyperglycemia on wound healing in three-dimensional cell culture (2021) Comptes Rendus de L'Academie Bulgare des Sciences, 74 (6), pp. 861-867. IF 0.378, Q2;

Hyperglycemia is the most deteriorating factor causing pathological and functional changes in patients with Diabetes mellitus including non-healing wounds, damaging feet and legs. Despite the intensive studies, the molecular mechanisms of action of high glucose concentrations on cells are poorly defined. Here, we used an in vitro model system, based on experimentally wounded three-dimensional fibroblast cell culture, in order to study wound healing under normoglycemic and hyperglycemic conditions. Following the wound closure for thirteen days by phase-contrast microscopy demonstrated that high glucose delayed the healing process. This was due to decreased fibroblast transdifferentiation into myofibroblasts as judged by reduced expression of the myofibroblasts marker α-smooth muscle actin. Probing the activity of TGF-β signalling pathway, which is responsible for fibroblast activation, showed deficiency in Smad2/3 phosphorylation even after addition of exogenous TGF- β1. These results indicate that high glucose concentrations are able to attenuate TGF- β signal transduction pathway and this effect should be considered an important contributing factor when therapies for diabetic wound healing are designed.

Г7.11. Momchilova, A., Markovska, T., **Georgiev, G.**, Pankov, S., Staneva, G., Petkova, D., Krastev, P., Pinkas, A., Pankov, R. Quercetin affects membrane lipids and apoptosis in three-dimensional fibroblast cultures (2021) Biotechnology and Biotechnological Equipment, 35 (1), pp. 943-952. https://doi.org/10.1080/13102818.2021.1939785; IF 1.632, Q3;

The polyphenol quercetin is associated with numerous beneficial health effects in various pathologies such as oxidative stress, neurodegenerative disorders, inflammation, neoplastic processes, age-related diseases, and so on. However, the molecular mechanisms underlying the effects of quercetin and its implication in the pharmaceutical practice require additional clarification. In this study we analyzed the biochemical mechanisms of quercetin effect on membrane lipids of fibroblasts cultured as three-dimensional (3D) cultures, which resemble living tissues more adequately compared to cellular monolayers. Quercetin treatment of 3D fibroblasts induced alterations of the plasma membrane phospholipid and fatty acid composition as well as in the phospholipid metabolizing enzymes sphingomyelinase and phospholipase A2. The level of sphingomyelin (SM), which acts as endogenous membrane antioxidant, was elevated due to quercetin action. Incubation of 3D fibroblasts with quercetin induced augmentation in the saturated fatty acids and reduction of all polyunsaturated fatty acids in the membrane phospholipid molecules of the plasma membranes, indicating an additional increase of the resistance to oxidative damage. Quercetin treatment reduced cholesterol susceptibility to oxidation, a phenomenon which is probably related to the observed increase in the level of SM. In addition, Western blot analysis showed augmented phosphorylation of Akt and expression of Bcl-2 in quercetin-treated cell, indicating that apoptosis was suppressed by quercetin. In conclusion, the reported results contribute to a better understanding of the beneficial effects of quercetin on the structure and functions of plasma membranes of 3D cell cultures, which are a more adequate model of living tissues.

Г8.1. **Georgi Georgiev**, Jana Pastuschek, Stefan Neubeck, Udo R. Markert (2013) Part I: Substances Secreted by the Preimplantation Human Embryo. In Immunology of Pregnancy 2013, Bentham Science, **doi:** 10.2174/978160805733711301002O

https://www.eurekaselect.com/117505/chapter/part-i%3A-substances-secreted-by-the-preimplantation-human-embry

Human preimplantation embryos secrete a number of soluble factors into their environment, be it in vivo or in vitro. In vivo, these signals are fundamental for survival and implantation of the blastocysts. In vitro, during an assisted reproduction treatment, embryo-derived signals may be detected in the conditioned culture media and might serve for the estimation of embryo quality and its capacity to implant. Furthermore, the same fundamental soluble factors may be added to preimplantation embryo culture media or instilled into the uterus during embryo transfer. Several molecules have been detected in conditioned embryo media. The available literature about the most prominent factors, HLA-G, interleukins, hCG, PAF, leptin, SP-1, EPF, and Wnt beta catenin, are reviewed in this article. Several published results are contradictory or are based on doubtful analyses, which detect protein concentrations in the media which exceed the weight of the entire blastocyst. This review discloses the littleness of the current knowledge about the human embryo secretome, the difficulties of analyses and the need of further investigation.