РЕЗЮМЕТА на публикациите на гл. ас. д-р Мария Петрова на английски език

Резюметата на публикациите са представени според годината на публикуване във възходящ ред. Номерацията съответства на тази от Списъка с публикации, с които кандидата участва в конкурса.

1. **Г7.** Ugrinova, I., **Petrova, M.**, Chalabi-Dchar, M., Bouvet, P. (**2018**). Multifaceted nucleolin protein and its molecular partners in oncogenesis. *Advances in protein chemistry and structural biology*, *111*, 133-164. **JCR-IF**$_{2018}$ **3.78, SJR**$_{2018}$ **1.17, Q2**$_{2018}$ https://doi.org/10.1016/bs.apcsb.2017.08.001.

ABSTRACT

Discovered in 1973, nucleolin is one of the most abundant phosphoproteins of the nucleolus. The ability of nucleolin to be involved in many cellular processes is probably related to its structural organization and its capability to form many different interactions with other proteins. Many functions of nucleolin affect cellular processes involved in oncogenesis—for instance: in ribosome biogenesis; in DNA repair, remodeling, and genome stability; in cell division and cell survival; in chemokine and growth factor signaling pathways; in angiogenesis and lymphangiogenesis; in epithelial–mesenchymal transition; and in stemness. In this review, we will describe the different functions of nucleolin in oncogenesis through its interaction with other proteins.

2. **Г7.** Haladjova, E., Halacheva, S., Momekova, D., Moskova-Doumanova, V., Topouzova-Hristova, T., Mladenova, K., Doumanov, J., **Petrova, M.**, Rangelov, S. (**2018**). Polyplex Particles Based on Comb-Like Polyethylenimine/Poly (2-ethyl-2-oxazoline) Copolymers: Relating Biological Performance with Morphology and Structure. *Macromolecular bioscience*, *18*(4), 1700349. **JCR-IF**$_{2018}$ **2.89, SJR**$_{2018}$ **0.83, Q1**$_{2018}$ https://doi.org/10.1002/mabi.201700349.

ABSTRACT

The present contribution is focused on feasibility of using comb-like copolymers of polyethylenimine with poly(2-ethyl-2-oxazoline) (LPEI-comb-PEtOx) with varying grafting densities and degrees of polymerization of PEI and PEtOx to deliver DNA molecules into cells. The copolymers form small and well-defined particles at elevated temperatures, which are used as platforms for binding and condensing DNA. The electrostatic interactions between particles and DNA result in formation of sub-100 nm

polyplex particles of narrow size distribution and different morphology and structure. The investigated gene delivery systems exhibit transfection efficiency dependent on the copolymer chain topology, shape of the polyplex particles, and internalization pathway. Flow cytometry shows enhanced transfection efficiency of the polyplexes with elongated and ellipsoidal morphology. The preliminary biocompatibility study on a panel of human cell lines shows that pure copolymers and polyplexes thereof are practically devoid of cytotoxicity.

3. **Г7. Petrova, M.**, Schroeder, M., Pasheva, E., Todorova, J., Ugrinova, I. (**2019**). Generation of Stable Cell Lines Expressing GFP-HMGB1 Fused Protein for Studying Autophagy. *Comptes rendus de l academie bulgare des sciences*, *72*(9), 1221-1226. **JCR-IF$_{2019}$ 0.34, SJR$_{2019}$ 0.22, Q2$_{2019}$** DOI: 10.7546/CRABS.2019.09.09.

ABSTRACT

Genetically encoded biosensors allow the noninvasive imaging of specific biochemical or biorecognition processes with the preservation of subcellular spatial and temporal information. Fused fluorescent proteins have revolutionized the ability of researchers to study protein localization and dynamics in live cells. Recently HMGB1 was discovered as one of the key regulators on the crosstalk of autophagy and apoptosis and the role of the protein in both processes is connected with its translocation from the nucleus to the cytoplasm. To investigate the role of HMGB1 in autophagic flux we engineered human A549 – lung adenocarcinoma (expressing wild type p53) and H1299 – non-small cell lung carcinoma (p53 negative) cells to express HMGB1 fused at the C-terminus of the green fluorescent protein (GFP). Coupled to fluorescence microscope for automated image acquisition of living cells this HMGB1-GFP-based biosensor is appropriate tool for monitoring the dynamic of HMGB1 translocation from the nucleus to the cytoplasm which is considered a hallmark for autophagy.

4. **B4.** Schröder, M., Yusein-Myashkova, S., **Petrova, M.**, Dobrikov, G., Kamenova-Nacheva, M., Todorova, J., Pasheva, E., Ugrinova, I. (**2019**). The effect of a ferrocene containing camphor sulfonamide DK-164 on breast cancer cell lines. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *19*(15), 1874-1886. **JCR-IF$_{2019}$ 2.05, SJR$_{2019}$ 0.54, Q3$_{2019}$** https://doi.org/10.2174/1871520619666190724094334.

ABSTRACT

Background: Drug resistance is a major cause of cancer treatment failure. Most cancer therapies involve multiple agents, to overcome it. Compounds that exhibit strong anti-tumor effect without damaging normal cells are more and more in the focus of research. Chemotherapeutic drugs, combining different moieties and functional groups in one molecule, can modulate different regulatory pathways in the cell and thus reach the higher efficacy than the agents, which affect only one cellular process.
Methods: We tested the effect of recently synthesized ferrocene-containing camphor sulfonamide DK-164 on two breast cancer and one breast non-cancer cell lines. The cytotoxic effects were evaluated using the standard MTT-dye reduction and clonogenic assays. The apoptotic or autophagic effects were evaluated by Annexin V binding or LC3 puncta formation assays, respectively. Cell cycle arrest was determined using flow cytometry. Western blot and immunofluorescent analyses were used to estimate the localization and cellular distribution of key regulatory factors NFκB and p53.
Results: Compound DK-164 has well pronounced cytotoxicity greater to cancer cells (MDA-MB-231 and MCF-7) compared to non-cancerous (MCF-10A). The IC50 value of the substance caused a cell cycle arrest in G1 phase and induced apoptosis up to 24 hours in both tumor cells, although being more pronounced in MCF-7, a functional p53 cell line. Treatment with IC50 concentration of the compound provoked autophagy in both tumor lines but is better pronounced in the more aggressive cancer line (MDA-MB-231).
Conclusion: The tested compound DK-164 showed promising properties as a potential therapeutic agent.

5. **Г7.** Pasheva, E., **Petrova, M.**, Yusein-Myashkova, S., Todorova, J., & Ugrinova, I. (**2020**). The cellular translocation of NF-kappa B in breast cancer cell lines is affected by HMGB1 protein but not by its truncated form. *Comptes rendus de l academie bulgare des sciences*, 73(8), 1086-1091. **JCR-IF$_{2020}$ 0.38, SJR$_{2020}$ 0.24, Q2$_{2020}$** DOI: 10.7546/CRABS.2020.08.06.

ABSTRACT

Nuclear Factor kappa B (NF-κB) is a well-known transcriptional factor that controls a variety of target genes important in many biological processes as inflammation, immunity, including cancer and chronic inflammatory disease. NF-κB can be activated by many extracellular factors that lead to increased basal activity of NF-κB characteristic for various types of human cancer, especially in breast cancer. That is why NF-κB signalling provokes intensive studies and has long been targeted for cancer therapy.

There are well established stimuli as tumour necrosis factor α (TNFα) and interleukin (IL)-1β that cause the nuclear translocation of NF-κB to nucleus and the expression of target genes. Another molecule that was reported as a putative regulator of the tumourigenesis, expansion, and invasion of cancer cells was High mobility group box-1 (HMGB1) protein. HMGB1 can be secreted outside the cell and was supposed to act as a cytokine causing tumourigenesis, inflammation and increased metastatic potential of tumour cells. We focused on NF-κB activity in the breast cancer cell lines (MCF7-noninvasive, MDA-MB-231-invasive) as a putative downstream target of HMGB1 signalling. We found that only full length HMGB1 increases cell motility and induces cytosolic to nuclear translocation of NF-κB in both breast cancer cell lines. The truncated form has no functional effect.

6. **Г7.** Dushkov, A., **Petrova, M.**, Todorova, J., Gospodinov, A., Ugrinova, I. (**2021**). Natural medicine: an evaluation of the *in vitro* cytotoxic effect of several Bulgarian fungal species on two panels of cancer cell lines. *Bulgarian chemical communications*, 53(A), 035-041. **JCR-IF$_{2017}$ 0.24, SJR$_{2021}$ 0.17, Q4$_{2021}$ – 12 т.** DOI: 10.34049/bcc.53.A.0005.

ABSTRACT

The medicinal potential of Bulgaria's wild-growing fungi has, until recently, largely gone unexplored by the native scientific community. While many of the region's mushrooms have long been prized by residents as a valuable food source, they have generally not been thought of as having the medicinal value of certain herbs, for example. However, the growing body of scientific literature confirming the diverse beneficial effects of many Asian mushrooms (long held in high regard by ancient medicinal traditions of their respective regions), as well as the somewhat surprising discovery that some of these same mushrooms can be found in Bulgaria, has sparked our interest in exploring the effects they may have on different types of cancer, with the goal of either complementing existing treatments or, perhaps, uncovering new treatments based on compounds isolated from fungal extracts. We have prepared different ethanol and water extracts from the mushrooms *Trametes versicolor, Lenzites betulina, Fomes fomentarius, Fomitopsis betulina* and *Amanita muscaria* and have examined the primary cytotoxic effect of these crude extracts on a panel of human skin and lung cancer cells *in vitro*, with the goal of establishing their IC50 values via MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, comparing them, and gaining perspective for

future research. Our results show that all extracts exhibit varying degrees of cytotoxicity even at low concentrations, warranting further inquiries into their anti-tumor potential.

7. **Г7.** Todorova, J., Lazarov, L. I., **Petrova, M.**, Tzintzarov, A., Ugrinova, I. (**2021**). The antitumor activity of cannabidiol on lung cancer cell lines A549 and H1299: the role of apoptosis. *Biotechnology & Biotechnological Equipment*, *35*(1), 873-879. **JCR-IF$_{2021}$ 1.76, SJR$_{2021}$ 0.38, Q3$_{2021}$** https://doi.org/10.1080/13102818.2021.1915870.

ABSTRACT

In the recent years, the application of new antitumor drugs has focused on the replacement of conventional chemotherapeutics with compounds derived from natural products. Cannabidiol (CBD) is one of the 113 cannabinoids derived from the plant *Cannabis sativa* and is characterized with complex and not entirely understood biological function. Unlike the other most abundant cannabinoid in *Cannabis sativa* – tetrahydrocannabinol, cannabidiol has low affinity to the endocannabinoid receptors and the manifestation of its activity does not appear to rely on the endocannabinoid system. Cannabidiol is used in the treatment of many diseases including some types of cancer. The aim of our study was to evaluate the cytotoxic activity of cannabidiol and its effect on the process of programmed cell death. This process is directly involved in the antitumor effect of many drugs. Two lung cancer cell lines, A549 expressing p53 and H1299-p53 negative, were used. Apoptosis was monitored by Annexin V assay and the activation of Caspase 3/7. We found that CBD treatment led to a dose-dependent apoptosis increase in p53 positive A549 cells. The level of apoptosis in p53 negative H1299 cells was much lower and did not seem to be dose dependent. However, the total cell viability was similar for the two cell lines, which was due to the increased necrosis observed in H1299 cells.

8. **Г7.** Haladjova, E., Chrysostomou, V., **Petrova, M.**, Ugrinova, I., Pispas, S., Rangelov, S. (**2021**). Physicochemical properties and biological performance of polymethacrylate based gene delivery vector systems: Influence of amino functionalities. *Macromolecular Bioscience*, *21*(2), 2000352. **JCR-IF$_{2021}$ 5.86, SJR$_{2021}$ 0.89, Q1$_{2021}$** https://doi.org/10.1002/mabi.202000352.

ABSTRACT

Physicochemical characteristics and biological performance of polyplexes based on two identical copolymers bearing tertiary amino or quaternary ammonium groups are

evaluated and compared. Poly(2-(dimethylamino) ethyl methacrylate)-b-poly(oligo(ethylene glycol) methyl ether methacrylate) block copolymer (PDMAEMA-b-POEGMA) is synthesized by reversible addition fragmentation chain transfer polymerization. The tertiary amines of PDMAEMA are converted to quaternary ammonium groups by quaternization with methyl iodide. The two copolymers spontaneously formed well-defined polyplexes with DNA. The size, zeta potential, molar mass, aggregation number, and morphology of the polyplex particles are determined. The parent PDMAEMA-b-POEGMA exhibits larger buffering capacity, whereas the corresponding quaternized copolymer (QPDMAEMA-b-POEGMA) displays stronger binding affinity to DNA, yielding invariably larger in size and molar mass particles bearing greater number of DNA molecules per particle. Experiments revealed that QPDMAEMA-b-POEGMA is more effective in transfecting pEGFP-N1 than the parent copolymer, attributed to the larger size, molar mass, and DNA cargo, as well as to the effective cellular traffic, which dominated over the enhanced ability for endo-lysosomal escape of PDMAEMA-b-POEGMA.

9. **Г7.** Kalinova, R., Valchanova, M., Dimitrov, I., Turmanova, S., Ugrinova, I., **Petrova, M.**, Vlahova, Z., Rangelov, S. (**2021**). Functional polyglycidol-based block copolymers for DNA complexation. *International Journal of Molecular Sciences*, 22(17), 9606. **JCR-IF$_{2021}$ 6.21, SJR$_{2021}$ 1.18, Q1$_{2021}$** https://doi.org/10.3390/ijms22179606.

ABSTRACT

Gene therapy is an attractive therapeutic method for the treatment of genetic disorders for which the efficient delivery of nucleic acids into a target cell is critical. The present study is aimed at evaluating the potential of copolymers based on linear polyglycidol to act as carriers of nucleic acids. Functional copolymers with linear polyglycidol as a non-ionic hydrophilic block and a second block bearing amine hydrochloride pendant groups were prepared using previously synthesized poly(allyl glycidyl ether)-b-polyglycidol block copolymers as precursors. The amine functionalities were introduced via highly efficient radical addition of 2-aminoethanethiol hydrochloride to the alkene side groups. The modified copolymers formed loose aggregates with strongly positive surface charge in aqueous media, stabilized by the presence of dodecyl residues at the end of the copolymer structures and the hydrogen-bonding interactions in polyglycidol segments. The copolymer aggregates were able to condense DNA into stable and compact

nanosized polyplex particles through electrostatic interactions. The copolymers and the corresponding polyplexes showed low to moderate cytotoxicity on a panel of human cancer cell lines. The cell internalization evaluation demonstrated the capability of the polyplexes to successfully deliver DNA into the cancer cells.

10. **Г7.** Todorova, J., **Petrova, M.**, Pasheva, E., Ugrinova, I. (**2022**). The Effect of HMGB1 Protein and Its Truncated Form on the Expression of RAGE Variants in Breast Cancer Cells. *Comptes rendus de l academie bulgare des sciences*, 75(10), 1462-1468. **JCR-IF$_{2022}$ 0.3, SJR$_{2022}$ 0.18, Q3$_{2022}$** https://doi.org/10.7546/CRABS.2022.10.08.

ABSTRACT

HMGB1 protein is a DNA binding nuclear protein. Its properties to bind different non-B DNA conformations and also to bend linear DNA implicate the protein in many essential cellular processes as DNA replication, repair, transcription, remodeling, etc. HMGB1 plays important role outside the cell as it is passively released from necrotic cells and actively from apoptotic ones and binds its specific receptor for advanced glycation end products RAGE. HMGB1/RAGE interaction is implicated in various diseases including cancer. Different soluble RAGE forms were reported whose functional role is to serve as a decoy for the ligands and in this way to block the signaling pathway. How the ligands regulate the production of RAGE variants is a subject of great scientific interest. We studied the effect of HMGB1 and its truncated form lacking the C-terminus on the expression of full-length (flRAGE) and soluble RAGE (sRAGE) in breast cancer cell lines: MCF7 represents a hormone dependent cancer with better prognosis and MDA-MB-231 – hormone independent with substantial invasive capacity. HMGB1 stimulates the total RAGE expression in both breast cancer cells but in MCF7 the ratio changes and is in favor of the membrane fraction. The absence of the C-tail of HMGB1 provokes comparable changes in RAGE production in MCF7 cells as the whole HMGB1 molecule. In MDA-MB-231 cell line the total amount of RAGE was slightly affected but it is entirely represented by the membrane form and the soluble one is in negligible amounts.

11. **Г7. Petrova, M.**, Ugrinova, I., Pasheva, E. (**2022**). The Expression Level of RAGE Forms in Different Cancer Cell Lines. *Comptes rendus de l academie bulgare des sciences*, 75(5), 694-699. **JCR-IF$_{2022}$ 0.3, SJR$_{2022}$ 0.18, Q3$_{2022}$** https://doi.org/10.7546/CRABS.2022.05.09.

ABSTRACT

The receptor for advanced glycation end products (RAGE) is a cell surface multiligand receptor of the immunoglobulin superfamily. The interest of the researchers to RAGE was enhanced by the fact that it is expressed at low levels under normal physiology, but is upregulated in various pathological processes such as neuronal development, diabetes, inflammation, neurodegenerative disorders, cancer progression, cardiovascular disease (CVD), etc. The receptor can exist as full-length membrane-bound and soluble form and it is considered that the balance between the amount of soluble and full-length RAGE might be a key factor for RAGE induced dysfunction. Different human cancer cell lines with various invasive potential were studied for the expression level of RAGE forms. Our finding shows that in less invasive cancers with better prognosis as MCF7 breast cancer and A549 (NSCLC – non small cell lung cancer) the membrane form of RAGE is the minor one and in A549 is even absent. Cancerous cells with great invasive potential and bad prognosis as MDA-MB-231, H1299 and HeLA expressed predominantly full-length receptor and sRAGE is poorly represented.

12. **Г7.** Todorova, J., **Petrova, M.**, Pasheva, E., Ugrinova, I. (**2022**). The Ratio of RAGE Isoforms Is Affected by HMGB1 and Its Truncated Form Only in A549 but not in H1299 Lung Cancer Cell Lines. *Comptes rendus de l academie bulgare des sciences*, 75(6), 827-834. **JCR-IF$_{2022}$ 0.3, SJR$_{2022}$ 0.18, Q3$_{2022}$** https://doi.org/10.7546/CRABS.2022.06.06.

ABSTRACT

The Receptor of Advanced Glycated End products (RAGE) could exist in two forms, a membrane bound and a soluble one. RAGE is highly expressed during embryonic development and decreases in all tissues except the lung in adult organisms. Lung RAGE mediates lung cancer, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, etc. The lung cancers, among the most invasive of tumours, are reported to express low levels of RAGE. A specific RAGE ligand is the nuclear protein HMGB1. We examined the effect of HMGB1 and its truncated form lacking the C-tail (HMGB1ΔC) on RAGE expression in NSCLC cancer cell lines with different invasive capacity: A549 with better prognosis and H1299 with negative outcome. In A549 upon addition of ligands two important results are observed: (i) total RAGE expression is augmented 1.5 times and 1.7 times for HMGB1 and HMGB1ΔC, respectively, and (ii), a full-length membrane variant (flRAGE) is observed and in the presence of HMGB1ΔC its expression is comparable with the soluble one. In control H1299 lung cancer cells the

dominant form of RAGE was the flRAGE while sRAGE represents one third of the whole amount of the receptor. HMGB1 and HMGB1ΔC do not affect the total RAGE amount and the ratio between flRAGE and sRAGE. In the case of lung cancer, the higher amount of RAGE is related to better prognoses and less metastatic ability which means that the stimulation of RAGE expression by HMGB1 and HMGB1ΔC in A549 is considered a positive tendency. In the case of the more aggressive lung cancer H1299 both ligands do not change the RAGE behavior and in this way do not affect the invasive potential of the cancer cells.

13. **B4.** Schröder, M., **Petrova, M.**, Vlahova, Z., Dobrikov, G. M., Slavchev, I., Pasheva, E., Ugrinova, I. (**2022**). *In Vitro* Anticancer Activity of Two Ferrocene-Containing Camphor Sulfonamides as Promising Agents against Lung Cancer Cells. *Biomedicines*, *10*(6), 1353. **JCR-IF2022 4.7, SJR2022 0.9, Q12022** https://doi.org/10.3390/biomedicines10061353.

ABSTRACT

The successful design of antitumour drugs often combines in one molecule different biologically active subunits that can affect various regulatory pathways in the cell and thus achieve higher efficacy. Two ferrocene derivatives, DK-164 and CC-78, with different residues were tested for cytotoxic potential on non-small lung cancer cell lines, A549 and H1299, and non-cancerous MRC5. DK-164 demonstrated remarkable selectivity toward cancer cells and more pronounced cytotoxicity against A549. The cytotoxicity of CC-78 toward H1299 was even higher than that of the well-established anticancer drugs cisplatin and tamoxifen, but it did not reveal any noticeable selective effect. DK-164 showed predominantly pro-apoptotic activity in non-small cell lung carcinoma (NSCLC) cells, while CC-78 caused accidental cell death with features characteristic of necrosis. The level of induced autophagy was similar for both substances in cancer cells. DK-164 treatment of A549, H1299, and MRC5 cells for 48 h significantly increased the fluorescence signal of the NFkB (nuclear factor 'kappa-light-chain-enhancer' of activated B-cells) protein in the nucleus in all three cell lines, while CC-78 did not provoke NFkB translocation in any of the tested cell lines. Both compounds caused a significant transfer of the p53 protein in the nucleus of A549 cells but not in non-cancerous MRC5 cells. In A549, DK-164 generated oxidative stress close to the positive control after 48 h, while CC-78 had a moderate effect on the cellular redox status. In the non-cancerous cells, MRC5, both compounds produced ROS similar to the positive control for the same incubation

period. The different results related to the cytotoxic potential of DK-164 and CC-78 associated with the examined cellular mechanisms induced in lung cancer cells might be used to conclude the specific functions of the various functional groups in the ferrocene compounds, which can offer new perspectives for the design of antitumour drugs.

14. **Г7.** Haladjova, E., **Petrova, M.**, Ugrinova, I., Forys, A., Trzebicka, B., Rangelov, S. (**2022**). Hollow spherical nucleic acid structures based on polymer-coated phospholipid vesicles. *Soft Matter*, *18*(29), 5426-5434. **JCR-IF$_{2022}$ 3.4, SJR$_{2022}$ 0.86, Q1$_{2022}$** https://doi.org/10.1039/D2SM00355D.

ABSTRACT

A feasible one pot synthesis of hollow spherical nucleic acids (SNAs) using phospholipid liposomes is reported. These constructs are synthesized in a chemically straightforward process involving formation of unilamellar liposomes, coating the liposomes with a thin cross-linked polymeric layer, and grafting the latter with short (about 20 bases) DNA oligonucleotide strands. They consist of vesicular cores, composed of readily available phospholipid (1,2-dipalmitoyl-sn-glycero-phosphocholine), whereas the strands are deliberately arranged on the surface of the vesicular entities. The initial vesicular structure and morphology are preserved during the coating and grafting reactions. The novel hollow/vesicular SNAs are characterized with a hydrodynamic radius and radius of gyration of 78.3 and 88.5 nm, respectively, and moderately negative (14.2 mV) z potential. They carry thousands (5868) of oligonucleotide strands per vesicle, which are not strongly radially oriented and adopt an unextended conformation as anticipated from the smaller value of the grafting density compared to the critical grafting density at the transition to brush conformation. The constructs are practically devoid of toxicity and exhibit high binding affinity to complementary nucleic acids. Unlike any other nucleic acid structural motif, they cross the cell membrane and enter cells without the need of transfection agents.

15. **В4.** **Petrova, M.**, Vlahova, Z., Schröder, M., Todorova, J., Tzintzarov, A., Gospodinov, A., Velkova, L., Kaynarov, D., Dolashki, A., Dolashka, P., Ugrinova, I. (**2023**). Antitumor Activity of Bioactive Compounds from *Rapana venosa* against Human Breast Cell Lines. *Pharmaceuticals*, *16*(2), 181. **JCR-IF$_{2022}$ 4.6, SJR$_{2022}$ 0.8, Q1$_{2022}$** https://doi.org/10.3390/ph16020181.

ABSTRACT

This study is the first report describing the promising antitumor activity of biologically active compounds isolated from the hemolymph of marine snail *Rapana venosa*—a fraction with Mw between 50 and 100 kDa and two structural subunits (RvH1 and RvH2), tested on a panel of human breast cell lines—six lines of different molecular subtypes of breast cancer MDA-MB-231, MDA-MB-468, BT-474, BT-549, SK-BR-3, and MCF-7 and the non-cancerous MCF-10A. The fraction with Mw 50–100 kDa (HRv 50–100) showed good antitumor activity manifested by a significant decrease in cell viability, altered morphology, autophagy, and p53 activation in treated cancer cells. An apparent synergistic effect was observed for the combination of HRv 50–100 with cis-platin for all tested cell lines. The combination of HRv 50–100 with cisplatin and/or tamoxifen is three times more effective compared to treatment with classical chemotherapeutics alone. The main proteins in the active fraction, with Mw at ~50 kDa, ~65 kDa, ~100 kDa, were identified by MALDI-MS, MS/MS analyses, and bioinformatics. Homology was established with known proteins with antitumor potential detected in different mollusc species: peroxidase-like protein, glycoproteins Aplysianin A, L-amino acid oxidase (LAAO), and the functional unit with Mw 50 kDa of RvH. Our study reveals new perspectives for application of HRv 50–100 as an antitumor agent used alone or as a booster in combination with different chemotherapies.

16. **B4.** Schröder, M., **Petrova, M.**, Dobrikov, G. M., Grancharov, G., Momekova, D., Petrov, P. D., Ugrinova, I. (**2023**). Micellar Form of a Ferrocene-Containing Camphor Sulfonamide with Improved Aqueous Solubility and Tumor Curing Potential. *Pharmaceutics*, *15*(3), 791. **JCR-IF$_{2022}$ 4.6, SJR$_{2022}$ 0.8, Q1$_{2022}$** https://doi.org/10.3390/pharmaceutics15030791.

ABSTRACT

The discovery of new anticancer drugs with a higher, more specific activity and diminished side effects than the conventional chemotherapeutic agents is a tremendous challenge to contemporary medical research and development. To achieve a pronounced efficacy, the design of antitumor agents can combine various biologically active subunits in one molecule, which can affect different regulatory pathways in cancer cells. We recently demonstrated that a newly synthesized organometallic compound, a ferrocene-containing camphor sulfonamide (DK164), possesses promising antiproliferative activity against breast and lung cancer cells. However, it still encounters the problem of solubility in biological fluids. In this work, we describe a novel micellar form of DK164 with

significantly improved solubility in aqueous medium. DK164 was embedded in biodegradable micelles based on a poly(ethylene oxide)-b-poly(α-cinnamyl-ε-caprolactone-co-ε-caprolactone)-b-poly(ethylene oxide) triblock copolymer (PEO113-b-P(CyCL3 -co-CL46)-b-PEO113), and the physicochemical parameters (size, size distribution, zeta potential, encapsulation efficiency) and biological activity of the obtained system were studied. We used cytotoxicity assays and flow cytometry to determine the type of cell death, as well as immunocytochemistry to assess the influence of the encapsulated drug on the dynamics of cellular key proteins (p53 and NFkB) and the process of autophagy. According to our results, the micellar form of the organometallic ferrocene derivate (DK164-NP) exhibited several advantages compared to the free substance, such as higher metabolic stability, better cellular uptake, improved bioavailability, and long-term activity, maintaining nearly the same biological activity and anticancer properties of the drug.

17. **B4. Petrova, M.**, Vlahova, Z., Schröder, M., Tzintzarov, A., Velkova, L., Kaynarov, D., Dolashki, A., Dolashka, P., Ugrinova, I. (**2023**). Antitumor activity of bioactive compounds isolated from the hemolymph and mucus of the garden snail *Helix aspersa* against a panel of human cancer cell lines. *Comptes rendus de l academie bulgare des sciences*, 76(9), 1350-1359. **JCR-IF**$_{2022}$ **0.3, SJR**$_{2022}$ **0.18, Q3**$_{2022}$ https://doi.org/10.7546/CRABS.2023.09.05.

ABSTRACT

Cancer remains a significant global health concern, necessitating the search for new effective and safe anti-cancer agents. Natural products derived from plants and animals, including mollusks like snails, have gained attention as potential sources of novel anti-cancer compounds. Haemocyanins (Hcs), large copper-containing glycoproteins involved in oxygen transport in mollusks, have shown promise as anti-cancer agents. This study focuses on evaluating the *in vitro* anti-tumour efficacy of bioactive substances obtained from the hemolymph of the garden snail *Helix aspersa* on various human cancer cell lines representing different types of cancer. The results demonstrate that certain hemocyanin subunits from *H. aspersa* exhibit cytotoxic activity comparable to cisplatin, a widely used chemotherapy drug. Additional assays confirm the cytotoxic effects of the tested substances on cancer cells. The study underscores the potential of natural compounds from *H. aspersa* as alternative therapeutic agents for cancer treatment, while highlighting the need for further investigation. The identification of specific proteins

responsible for the observed anti-proliferative effects in the mucus of *H. aspersa* provides insights for the development of novel cancer therapies.