

REVIEW

By Prof. Ivan Georgiev Ivanov

of the thesis of prof. Iva Ugrinova Zlatkova "*Common target, different agents: Fundamental vulnerabilities of the tumor cell as a basis for selective antitumor action*", submitted for awarding the scientific degree "Doctor of Science" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional direction 4.3. Biological Sciences, scientific specialty: Molecular Biology

1. Biographical information

Prof. Dr. Iva Ugrinova was born on 14.03.1968 in Sofia. She is a graduate of the Faculty of Biology, Sofia University "St. Kl. Ohridski", graduating in 1992 with a MS degree in "Biotechnology", specialization "Genetic and Cellular Engineering". After graduation, she worked as a biologist and since 1998 she has been a PhD student at the Institute of Molecular Biology "Acad. R. Tsanev" (IMB). After obtaining her PhD degree (2002), she held the positions of Senior Assistant, Associate Professor and Full Professor (2019). During 2014-2022 she was a Director and after her second term, she was appointed a Deputy Director. Since 2013, she has headed the Department of Chromatin Structure and Function. During her career development, Dr. Ugrinova has been a post-doctor (2004-2005 and 2007) at the Ecole Normale Supérieure de Lyon - Lyon, France, and in 2008 a Visiting Professor at the same institute. She is the author of 92 scientific articles, of which 87 in IF or Impact Rank Factor journals. Her papers have been cited 2349 times so far.

2. Overview of the thesis and the defense procedure

The documents related to the defense procedure have been prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for its implementation. The dissertation was discussed and approved for defense at an extended scientific meeting of the Department of Chromatin Structure and Function held on 13.07.2026.

Prof. Ugrinova's thesis covers 281 pages, includes 158 literary sources and is based on 10 scientific articles published in the period 2021-2026. It is structured in an unconventional style for Bulgaria but very common for many advanced countries. Following the two sections Literature Review and Goals & Objectives, the 10 authentic scientific articles are placed, after which the thesis achievements are discussed. The last chapter is dedicated to the author's future research plans. The investigations related to Prof. Ugrinova's thesis are supported financially by 7 research projects.

3. Evaluation of the thesis achievements

Prof. Ugrinova's dissertation is dedicated to the search for new antitumor agents, as well as to the clarification of their molecular mechanisms of action. In planning her research, she has followed an original and unconventional approach. Unlike most pharmacological studies, where the chemical structure of the active substances plays a leading role, Dr. Ugrinova puts biological targets first. The logic is as follows: The tumor cell survives thanks to three basic biological phenomena - a balanced redox potential, genomic stability and controlled cell death. In her thesis the three are considered as targets for affecting cellular homeostasis, whose disbalance could lead to cell death. From this point of view, chemical compounds with different structures could interact with the same target thus expressing similar biological effects. In this sense, the tumoricidal activity of the compounds is determined not so much by their chemical structure, but by their ability to interact effectively with the relevant biological target.

In her thesis, Prof. Ugrinova uses both synthetic and natural compounds, which are divided into three thematic groups: a) ferrocene-containing sulfonamides; b) heterocy-

clic-annulled naphthalimides; c) natural products and nanosystems based on biocompatible polymers.

The literature review is written in a understandable scientific style and is based on literature sources published during the last 1-2 decades. It starts with an updated concepts of the molecular and cellular mechanisms of action of the antitumor substances, most of which interact with the tumor suppressor p53 and the NF- κ B regulator of apoptosis and therapeutic resistance. For a better understanding of the thesis results, Dr. Ugrinova presents the molecular mechanisms of cell cycle, as well as its “checkpoints”, the mechanisms of DNA repair, replication stress, mechanisms of tumor cell adaptation, etc. After describing the foundations of tumor cell biology, she continues with an overview of the antitumor agents, with special attention paid to the ferrocene-containing compounds, heterocyclic-annulated naphthalimides, as well as the plant, fungal and animal antitumor proteins. Finally, some delivery systems for antitumor drugs are also discussed.

The in-depth analysis of the literature has allowed Dr. Ugrinova to find her research niche in an extremely competitive environment and to formulate the **Goals and Objectives** of her dissertation. As already mentioned, the thesis goal is to identify and characterize new antitumor agents of synthetic and natural origin capable of acting with one or more of the three biological targets discussed above.

Methodology. Various methods have been applied to develop Dr. Ugrinova’s thesis, including cell biology methods (cultivation of stable tumor cell lines to test the action of anti-tumor agents and study the mechanism of their action), molecular biology and biochemical methods (for studying proteins and nucleic acids as markers for the action of antitumor substances), spectral, physicochemical, microscopic methods, etc.

Results. The thesis results are described in details in each of the ten original articles related to the three thematic groups: ferrocene-containing sulfonamides (2 articles); heterocyclic-annulled naphthalimides (2 articles); natural products and nanosystems based on biocompatible polymers (6 articles).

a) Ferrocene-camphor sulfonamides

In order to improve the antitumor effect as well as to reduce the toxicity of the antitumor agents, in recent years pharmacists have turned to the creation of hybrid compounds combining the beneficial properties of two or more pharmacophores in one molecule. This is the case with the ferrocene-containing sulfonamides studied by Prof. Ugrinova. They combine a ferrocene fragment (bearing redox-active properties), a sulfonamide group (with bactericidal action) and a chiral camphor fragment, thus expecting the hybrid molecules to induce oxidative stress, to interact with basic cellular enzymes and therefore to be able to interact (at least) with one of the biological targets responsible for the tumor cell survival. These compounds are expected to change the activity of key cellular regulators such as p53 and NF- κ B (considered as markers for the efficiency of action of the new anti-tumor compounds).

Ferrocene-camphor-sulfonamide compounds were synthesized in collaboration with chemists and pharmacists and after preliminary screening, two of them (DK-164 and CC78) were selected for extensive study on a large panel of diverse tumor cell lines. The results obtained showed that DK-164 exhibits antitumor activity and high selectivity (unlike CC78) against breast cancer and non-small cell lung carcinoma. Studies also show that the antitumor effect of DK-164 is associated with the induction of oxidative stress, activation of p53-dependent signaling pathways, modulation of NF- κ B signaling, cell arrest and induction of apoptosis. Since the compound DK-164 has low water solubility, Dr. Ugrinova attempted to improve its bioavailability by incorporating it into biodegradable polymer micelles and found that this did not lead to a loss of its biological activity.

b) Heterocyclic naphthalimides

Heterocyclic naphthalimides interact directly with DNA and induce replicative stress, thereby overloading the repair system, disturbing cell cycle control, and activating apoptosis

(the third important axis for cell death). Many tumors exist in a state of chronic pro-apoptotic pressure and rely on a narrow set of survival mechanisms; therefore, a relatively weak additional pro-apoptotic effect is often sufficient to tip the scales towards irreversible cell death. Main coordinator of the three axes for cell survival is p53. This is also one of the reasons why Dr. Ugrinova included mutant cell lines with different TP53 status for studying the mechanism of action of the new benzodioxin naphthalimides. In them, cytotoxic activity is manifested at lower doses, and tumor selectivity is lower compared to normal cells. The obtained results show that some derivatives (benzodioxin- and benzofuran-containing naphthalimides) induce replication stress, suppress DNA synthesis and activate apoptosis. In general, the cellular response to these compounds depends on TP53 status. p53-competent cells respond predominantly through G1 arrest, and p53-deficient cells through cell cycle arrest in the G2/M phase.

c) Natural products and polymer nanosystems for biodelivery

This is the most voluminous section of Dr. Ugrinova's thesis, including 6 original articles dedicated to the antitumor activity of various products of animal, plant and fungal origin. Animal products are represented by the hemolymph of the Black Sea snail *Rapana venosa*, whose antitumor activity has been studied on models of breast cancer and lung carcinoma. Its hemolymph is rich in copper-containing hemocyanins, which are known for their immunostimulating and antitumor activity. Literature data indicate that both holoproteins and their structural subunits exhibit different biological activities. In collaboration with other colleagues, Prof. Ugrinova studied individual *Rapana venosa* hemolymph fractions and more specifically the HRv 50–100 kDa fraction. The current study shows that individual subfractions exhibit selective antiproliferative activity and potentiate the effect of cisplatin in breast cancer cell models. It is proven also that the antitumor effect of this fraction was due to induced oxidative stress, autophagy and p53 activation.

Natural products of plant origin are some of the oldest anti-tumor agents. According to Dr. Ugrinova's search, 50% of the newly approved anti-cancer drugs for the period 1981–2019 were natural products. She is personally interested in cannabidiol (CBD), extracts of Bulgarian mushrooms, including *Amanita muscaria*, and extracts from the tropical plant *Croton lechleri*.

Prof. Ugrinova proved that CBD exhibits antitumor activity in a cell model of lung carcinoma by inducing stress and cell death, with the type of cell death (apoptosis or necrosis) depending on the p53 status. The effect of mushroom extracts also depends on the cell type, but in general they have a low antiproliferative activity, and their effect is associated with influencing the tumor cell proliferation. Alcoholic extracts of *Croton lechleri* exhibit selective cytotoxicity against melanoma cells and induce apoptosis with minimal effect on healthy cells. She also investigated the activity of water-alcoholic extracts of *Amanita muscaria* on lung cell lines and found that they are cytotoxic against tumor cell models, including p53-deficient H1299 cells. Here, the active substances remain unidentified.

Considering the main pharmacological shortcomings of the antitumor agents (low water solubility, insufficient stability, short half-life and non-specific toxicity), Prof. Ugrinova, in collaboration with other colleagues in the field of chemistry and pharmacy, developed an original system to deliver drugs to tumor cells. She turned to self-assembling micelles composed of amphiphilic block copolymers consisting of a hydrophobic core and a hydrophilic crown. Thus, PEO-b-P(CyCL-co CL)-b-PEO triblock copolymer micelles were created for encapsulation of the ferrocene compound DK-164. It demonstrated high encapsulation efficiency (~98%), controlled release and preserved biological activity compared to the free compound. Another study describes the development of novel pH-sensitive nanocarriers by modifying conventional niosomes with hexadecyl-poly(acrylic acid)_n copolymers (HD-PAA_n). These systems were used for entrapment of curcumin (83%) and subsequent

pH-dependent drug release. Improved cytotoxic and apoptogenic activity was achieved compared to free curcumin.

4. Conclusions and contributions

Prof. I. Ugrinova's research is characterized with both fundamental and applied achievements. The obtained results confirm her working hypothesis that compounds of different origin and chemical structure can exhibit antitumor activity by affecting a small number of fundamental cellular vulnerabilities associated with cellular oxidation-reduction homeostasis, genomic stability and cell survival mechanisms. The results also show that the antitumor activity of many substances occurs even in cells with impaired p53 function, which indicates the existence of alternative p53-independent mechanisms for inducing cell death. Dr. Ugrinova proves that tumor selectivity can serve as an indicator of vulnerability-directed action of a substance. She and her team investigated for the first time the antitumor activity of heterocyclic benzodioxin and benzofuran naphthalimides and showed that it is associated with the induction of replication stress, activation of DDR signaling pathway and subsequent apoptosis. She proved for the first time the existence of antitumor activity of hemolymph fractions from *Rapana venosa* and established their chemosensitizing activity against cisplatin. New data were also obtained on the activity of natural products of plant and fungal origin, including cannabidiol, extracts of *Croton lechleri* and *Amanita muscaria*. Their activities are associated with the induction of oxidative stress and programmed cell death. An applied achievement is the creation and characterization of new biodegradable polymer nanosystems for delivery of antitumor substances to tumor cells.

5. Publications

Then articles have been published in relation with Prof. I. Ugrinova's thesis, in 9 of which she is the lead author. Of them 8 are in quartile Q1, and the rest in Q3 and Q4, respectively. The total IF of the ten papers is 48.9, and the number citations is 87. The thesis results were also reported at 20 national and international scientific forums.

6. Personal contribution to the thesis results

The fact that Prof. Ugrinova is the lead author in 9 out of 10 scientific publications, and also in most of the projects indicates that the overwhelming majority of the thesis achievements are due to her personal contribution, although their realization would not have been possible without the cooperation of her partners from IMB and other scientific organizations.

7. Summary assessment

The summary assessment according to the LDASRB is presented in the table below.

Indicator	Merit points (minimum required)	Merit points (Real)
A	50	50
B	100	100
C	100	227
D	100	174
Total	350	551

As seen from the table, Prof. Ugrinova's merit points significantly exceed those officially required by the LDASRB for acquisition of the scientific degree "Doctor of Sciences" in the field of Natural Sciences.

CONCLUSION

The thesis of Prof. Iva Ugrinova Zlatkova entitled “*Common target, different agents: Fundamental vulnerabilities of the tumor cell as a basis for selective antitumor action*” is dedicated to the search for new antitumor substances and the elucidation of their mechanisms of action. To this end, she explored an original approach based on the assumption that antitumor substances affect key systems for tumor cell survival, such as redox system, genomic stability and apoptosis. The concept that chemical compounds with different structures could express similar biological effects gives her reason to carry out a broad screening for such substances among both synthetic and natural biologically active compounds. As a result, she discovered new molecules with a pronounced antitumor activity among ferrocene-camphor sulfonamides, hybrid heterocyclic-annulled naphthalimides, hemolymph of the sea snail *Rapana venosa*, plants (*Croton lechleri*) and mushrooms (*Amanita muscaria*). Thus she enriched the worldwide database of candidate therapeutic agents for oncology and proved the correctness of her working hypothesis. She also developed an original drug delivery nanosystem based on self-organizing biodegradable polymers.

In connection with the thesis, 10 scientific articles were published (8 in journals with Q1, and the 10 works are from the last 5 years) in 9 of which she is the lead author. The thesis results have also been reported at 22 national and international scientific forums. As seen from the Table, the scientific indicators of Prof. Ugrinova significantly exceed those that are officially required by the LDASRB for acquisition of the scientific degree "Doctor of Sciences" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional direction 4.3. Biological Sciences, scientific specialty Molecular Biology. This gives me reason to confidently recommend to the esteemed Scientific Jury to award it to her.

10.07.2026 г.

Рецензент:

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