“Babeş-Bolyai” University

Cluj-Napoca, România

MANUELA BANCIU, Ph.D.

Liposomal targeting of anticancer agents to inhibit tumor microenvironment-mediated processes

HABILITATION THESIS

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ABSTRACT

The author of the present habilitation thesis, Manuela Banciu has developed the research and academic activity in an interdisciplinary manner by combining several scientific areas in the field of Biological Sciences (Biochemistry, Immunology, and Molecular Biology), Bionanotechnology, as well as Biomedical Sciences (Oncobiology, Cancer Immunology). Thus, this thesis entitled **"Liposomal targeting of anticancer agents to inhibit tumor microenvironment-mediated processes"** aimed to highlight the main findings of the author's research after the completion of Ph.D. thesis (December 2007) until present (2018). Noteworthy that this scientific activity is a result of the implementation of the therapeutic bionanotehnology knowledge aquired by the author during her PhD training at the Utrecht University, The Netherlands. Based on this theoretical and practical expertise, the main scientific achievements of the author have been focused on the development of the tumor-targeted therapies that suppress protumor processes coordinated by tumor microenvironment. The value of these findings is proved by the articles published in peer-reviewed international journals in the following research areas according to the Web of Science Thomson Reuters: Biochemistry and Molecular Biology, Oncology, Pharmacology, and Pharmacy. The research presented within the actual thesis was funded mainly by three national projects (PN II – RU 387/2010, contract number 145/2010; PN-II-PTPCCA-2011-3-2-1060, contract nr.95/2012; and PN-II-RU-TE-2014-4-1191, contract nr. 235/01.10.2015).

The main part of this habilitation thesis consists of the chapter 2 that describes the most relevant scientific achivements which were focused on three principal directions:

1) the assessment of the interplay between tumor-associated macrophages (TAMs) and tumor cells in the modulation of supportive processes for tumor growth;

2) the elucidation of the main molecular mechanisms of the cytotoxicity of small molecule anticancer agents that can be exploited for future tumor-targeted therapies;

3) the development of tumor-targeted therapies by using different TAMs re-education strategies.

Previous oncological studies including PhD thesis of the author have shown that TAMs are principal tumor microenvironmental cells in the coordination of key processes for promoting and supporting tumor growth (such as angiogenesis, inflammation, oxidative stress, and metastatic potential of cancer cells). In this view, the main findings of the research conducted by the author proved:

- the role of TAMs-driven oxidative stress in the development of two types of solid tumors: B16.F10 murine melanoma and C26 murine colon carcinoma;
- the link between TAMs-mediated oxidative stress and tumor microenvironment angiogenic capacity .

These data obtained as main author were published in peer-reviewed journals as:

- Alupei MC, Licarete E, Patras L, **Banciu M.** (2015). Liposomal simvastatin inhibits tumor growth via targeting tumor-associated macrophages-mediated oxidative stress. *Cancer Lett* 356 (2): 946-952.
- Luput L, Licarete E, Sesarman A, Patras L, Alupei MC, **Banciu M.** (2017). Tumor-associated macrophages favor C26 murine colon carcinoma cell proliferation in an oxidative stress-dependent manner. *Oncol Rep* 37(4): 2472-2480.

One of the major goals of the research was the investigation into the main molecular mechanisms of the antitumor activities of the nonconventional anticancer drugs that can be exploited for future tumor-targeted therapies. In this view, different types of statins that are hypocholesterolemic agents, were tested. Nevertheless, statins can possess antitumor actions at concentrations 100- to 500-fold higher than those needed for reaching cholesterol lowering activity.

The main findings of our research are presented below as follows:

- Only lipophilic statins exerted strong cytotoxic effects on B16.F10 murine melanoma cells.
- The strong inhibition of the subunit α of the hypoxia inducible factor 1 (HIF-1 α) expression is the principal cause for the cytotoxicity induced by lipophilic statins in B16.F10 murine melanoma cells *in vitro.* Thus, preferential overexpression of HIF-1α in melanoma cells compared with healthy melanocytes might offer specificity for future *in vivo* anticancer therapeutic approaches based on lipophilic statins.

Moreover, as the mechanisms of the cytotoxicity of different conventional cytotoxic drugs could be influenced by tumor microenvironment, we assessed whether TAMs could influence cancer cells response to 5-fluorouracil (5-FU), a known cytotoxic drug for colorectal cancer treatment.

The main findings regarding the 5-FU cytotoxicity on C26 murine colon carcinoma cells in the presence of TAMs were presented below.

- The chemosensitivity of these cancer cells to 5-FU treatment is enhanced in the presence of TAMs via mediating an overall strong reduction of inflammatory and angiogenic proteins production in tumor microenvironment.

- Nevertheless, TAMs protect cancer cells against pro-oxidant effect of 5-FU by maintaining ROS levels in the physiological range of C26 cell oxidative stress.

Altogether, our results suggest that further therapeutic strategies of colorectal cancer should employ combined administration of 5-FU with pharmacological agents that prevent TAMs to maintain physiological range of tumor oxidative stress.

The main results of the studies shown above were published as main author in the following research articles:

- Alupei MC, Licarete E, Cristian FB, **Banciu M** (2014). Cytotoxicity of lipophilic statins depends on their combined actions on HIF-1α expression and redox status in B16.F10 murine melanoma cells. *Anticancer Drugs* 25(4):393-405.
- Licarete E, Sesarman A, Rauca VF, Luput L, Patras L, **Banciu M**.(2017). HIF-1α acts as a molecular target for simvastatin cytotoxicity in B16.F10 melanoma cells cultured under chemically induced hypoxia. *Oncol Lett* 13(5):3942-3950.
- Patras L, Sesarman A, Licarete E, Luca L, Alupei MC, Rakosy-Tican E, **Banciu M.** (2016) Dual role of macrophages in the response of C26 colon carcinoma cells to 5-fluorouracil administration. *Oncol Lett* 12(2):1183-1191.

The elucidation of molecular mechanisms of the cytotoxicity of statins as well as the data regarding the role of tumor microenvironment in the modulation of cancer cells response to cytotoxic drugs helped us to gain evidence for the development of future tumor-targeted therapies based on TAMs re-education strategies. In other words, these therapeutic approaches might „re-educate" TAMs to treat cancer after their conversion into antitumor macrophages. Thus, the research group led by the author tested different types of anticancer therapies based on long-circulating liposomes (LCL) for passive tumor targeting of the pharmacological agents. As previous studies have proved the natural tropism of LCL for TAMs, the hypothesis of the studies presented within this habilitation thesis was that all processes favorable for tumor development coordinated by TAMs might be significantly affected by properly designed LCL-encapsulated drugs. Thus, the antitumor efficacy of single liposomal drug therapy based on tumor- specific delivery of the lipophilic statin-simvastatin (SIM) using small-sized liposomes coated with polyethylene glycol (PEG) (LCL-SIM) in two cancer experimental models: B16.F10 murine melanoma as well as C26 colon carcinoma was assessed. Furthermore, the antitumor efficacy of the combined liposomal therapy based on simultaneous targeting of TAMs using long-circulating liposomal prednisolone disodium phosphate (LCL-PLP) and cancer cells using long-circulating liposomal 5-fluorouracil (LCL-5-FU) in C26 murine colon carcinoma- bearing mice was also tested.

With regard to antitumor activities of both therapeutic strategies tested, our main findings are presented below.

- Encapsulation of SIM in LCL offers an increased antitumor activity of this lipophilic statin in B16.F10 melanoma and C26 colon carcinoma tumor models.

- The mechanism of the antitumor activity of LCL-SIM was dependent on tumor type. Thus, the principal cause of antitumor activity of LCL-SIM in B16.F10 melanoma-bearing mice is the strong inhibition of TAMs-driven oxidative stress and the metastatic potential of this aggressive type of cancer while the antitumor activity of the same liposomal statin formulation in C26 colon carcinoma was mainly based on suppression of tumor angiogenesis and inflammation as well as direct cytotoxic effects on C26 colon carcinoma cells.

- The antitumor efficacy of the combined therapy based on the simultaneous administration of LCL-PLP and LCL-5-FU in C26 murine colon carcinoma-bearing mice was superior to that determined by single administration of each liposomal formulation in the same tumor model.

- The antitumor activity of the combined therapy based on LCL-PLP and LCL-5-FU was based on the inhibition of tumor angiogenesis and inflammation.

- The anti-angiogenic and anti-inflammatory actions of the combined liposomal drug therapy was favored by the controlling effect of the treatment on oxidative stress in C26 colon carcinoma microenvironment that was polarized toward an antineoplastic phenotype.

These results were published in:

- Alupei MC, Licarete E, Patras L, **Banciu M**. (2015). Liposomal simvastatin inhibits tumor growth via targeting tumor-associated macrophages-mediated oxidative stress. *Cancer Lett* 356 (2):946-952 (as main author);

- Porfire A, Tomuta I, Muntean D, Luca L, Licarete E, Alupei MC, Achim M, Vlase L, **Banciu M**. (2015). Optimizing long-circulating liposomes for delivery of simvastatin to C26 colon carcinoma cells. *J Liposome Res* 25(4):261-269 (as main author);

- Luput L, Licarete E, Drotar DM, Nagy AL, Sesarman A, Patras L, Rauca VF, Porfire A, Muntean D, Achim M, Tomuta I, Vlase L, Catoi C, Dragos N, **Banciu M**. (2017). *In Vivo* Double Targeting of C26 Colon Carcinoma Cells and Microenvironmental Protumor Processes Using Liposomal Simvastatin. *J Cancer* 9(2):440-449 (as main author);

- Achim M, Tomuta I, Muntean D Porfire A, Tefas LR, Patras L, Licarete E, Alupei MC, Vlase L, **Banciu M** (2017) Optimization and *in vitro* evaluation of 5-fluorouracil - loaded long - circulating liposomes, *FARMACIA* 65 (1): 82-91 (as main author);

- Patras L, Sylvester B, Luput L, Sesarman A, Licarete E, Porfire A, Muntean D, Drotar DM, Rusu AD, Nagy AL, Catoi C, Tomuta I, Vlase L, **Banciu M**, Achim M (2017) Liposomal prednisolone phosphate potentiates the antitumor activity of liposomal 5-fluorouracil in C26 murine colon carcinoma *in vivo. Cancer Biol Ther 18(8):* 616-626 (as main author, corresponding author);
- Sylvester B, Porfire A, Muntean DM, Vlase L, Lupuţ L, Licarete E, Sesarman A, Alupei MC, **Banciu M**, Achim M, Tomuţă I. (2018). Optimization of prednisolone-loaded long-circulating liposomes via application of Quality by Design (QbD) approach. *J Liposome Res 28(1)*: 49-61 (as co-author).

Moreover, in tight connection with the main topic of the habilitation thesis, the author published a review on the current status of antitumor actions of statins and tumor-targeted delivery systems used for these drugs, as shown below:

- Licarete E, Sesarman A, **Banciu M**. (2015) Exploitation of pleiotropic actions of statins by using tumor-targeted delivery systems, *J Microencapsul 32(7)*: 619-631.

Last but not least, the topics of the research presented above afforded the author to develop several collaborations with different national and international research groups in the field of Biomedical Sciences but also in different subdomains of the Biological Sciences. Within these scientific collaborations the author conducted studies on the following topics: elucidation of the main molecular mechanisms of action of different chemical compounds with antitumor potential; development of drug delivery systems for theranostics of chronic inflammation- related diseases, and the assessment of the oxidative stress involvement in the adaptative mechanisms of different organisms.

Regarding teaching activity, at present, the author has led several undergraduate courses and laboratories including General Biochemistry (Structural and Metabolism Biochemistry), Enzimology, as well as Master Courses such as Modern Biochemistry and Biophysics Methods, Bionanotechnology, *etc*. It is worth mentioning that the author's teaching competences are strongly supported by the theoretical knowledge and practical skills acquired during her scientific career in the field of Biochemistry (enzymatic assays, HPLC, protein purification by low pressure chromatography, gradient centrifugation, gel electrophoresis, characterization of proteins by spectrophotometry, spectrofluorimetry, membrane vesicles preparation); Bionanotechnology (liposome and polymeric nanoparticle preparation and characterization); Immunology (ELISA,

immunoblot, immunofluorescence staining and microscopy, antibody purification – affinity chromatography and precipitation, immunohistochemistry), Proteomics (protein array).

The final part of the habilitation thesis was dedicated to scientific and professional development plans of the author. Thus, as future perspectives of the research the author has planned to develop tumor-targeted therapies by exploiting tumor intercellular communication tools such as cancer cell regulation of TAMs and extracellular vesicle-based tumor intercellular interactions. These treatment approaches will aim to re-activate immune system cells to fight against cancer and extracellular vesicles being produced by the malignant cells themselves will be used as “trojan horses” to deliver cytotoxic drugs inside the tumor. In this view, in 2017 the author has gained a national research project entitled “Tumor intercellular communication tools- inspiration for future tumor-targeted therapies” (Project code PN-III-P4-ID-PCE-2016-0342, contract 91/2017). Importantly, the author’s plans for future scientific development will also be focused on the consolidation of the research group aiming to explore tumor microenvironment intercommunication tools by using an interdisciplinary approach (biochemical, molecular, bioinformatics, experimental methods).

As academic development plans, the author intends to improve the courses of Biochemistry (Structural and Metabolism Biochemistry), Bionanotechnology, Enzimology, Modern Biochemistry and Biophysics Methods with new scientific and applicative aspects. The author together with her colleagues have planned to write a book on Biochemistry and Molecular Biology methods that will include her own research protocols suitable for laboratory classes attended by undergraduate and master students. The author intended to lead a course for PhD students entitled ”Oxidative stress in biomedical and biological research” that will be focused on oxidative stress involvement in pathologies but also in physiological processes (such as aging, defence mechanisms of the organisms, adaptation to social conditions).