1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: The role of melanogenesis modulation in regulation of melanoma behavior

1.1. Project goals

Since melanogenesis has the potential to regulate melanoma behavior and its sensitivity to anticancer treatment, the goals of the project is:

A- testing of effects of melanogenesis stimulation and inhibition on melanoma cell-cells and cell-matrix adhesion, cytoskeletal alternations and melanoma cells invasiveness

B- testing of melatonin, vitamin D and its derivatives on abovemantioned features in non-pigmented, moderately and strongly pigmented melanoma cells

C-identification of potential molecular targets involved in adhesion and cytoskeleton for anticancer treatment

D-analysis of these targets expression in clinical samples.

1.2. Outline

Malignant melanoma is the most rapidly increasing malignancy in the white population and its mortality rate is surpassed only by lung cancer. Unfortunately, since many years there has been no significant progress in efficiency of advanced melanoma therapies and survival rate of melanoma patients. Melanoma is a type of skin tumor that arises from melanocytes, the cells producing melanin. Its major role is protection against the harmful actions of solar radiation. However, under pathological conditions (e.g., melanoma), melanogenesis can induce genotoxic and mutagenic effects, thus contributing to tumor progression. The antioxidative properties of melanin, under physiological conditions protects skin against environmental insults, but under pathological conditions can attenuate radio- photo- and chemotherapy, with net undesirable clinical effect. Our previous research showed significant differences in biology of amelanotic and pigmented melanomas. We found that melanogenesis shortens survival in patients with metastatic melanoma. Patients with pigmented melanomas treated with radiotherapy showed significantly shorter survival when compared to amelanotic once. In addition amelanotic but not pigmented melanoma cells were sensitive to chemotherapeutic action of several drugs and natural compounds and ionizing radiation.

Melatonin is highly evolutionary conserved molecule with multifunctional activities, including regulation of circadian rhythms and cellular responses necessary for cell survival and restoration of homeostasis. It can modulate the cytoskeletal structure organization in normal and cancer cells and is able to switch microfilament phenotypes in mammary

cancer cells from invasive migratory cells to dormant microfilament phenotypes that occur in non- migratory cells. It has been found that melatonin inhibits cancer cell invasion and metastasis formation via Rho-associated protein kinase (ROCK)-regulated microfilament and microtubule organization that converge in a migration/anchorage switch. Melatonin decreases melanogenesis in melanoma cells. Vitamin D can lead to actin depolymerization in cancer cells, decreasing cells survival. On the other hand in some models melanin changed the cell elasticity making the melanoma cells less capable to spread than cells without the pigment. Thus the aim of this project is to test the hypothesis that natural compounds as melatonin and vitamin D can modulate adhesion, cytoskeleton and aggressiveness of melanoma cells acting on melanogenesis apparatus.

1.3. Work plan

Year 1–testing of:
-basic melanogenesis in SkMel-188, C32 and SkMel-1 cell lines (melanin level, EPR, Seahorse Flux)
-melanogenesis after treatment with melanogenesis regulators (as tyrosine, D-penicillamine, kojic acid)
-adhesion and cytoskeleton molecules expression (as kadherins, integrins, actins; ICC, PCR, FCM)
-migration potential (scratch tests, transwell migration) in relation to pigmentation
Year 2-testing of abovementioned parameters after treatment with melatonin and vitamin D
Year 3-the results analysis and indicating the molecules with changed expression; testing its inhibition in selected melanoma cell culture models
– testing of the expression of molecules indicated in Goal C in clinical samples (IHC).
Year 4-manuscripts preparation
-PhD thesis writing.

1.4. Literature

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- Benítez-King G, Soto-Vega E, Ramírez-Rodriguez G. Melatonin modulates microfilament phenotypes in epithelial cells: implications for adhesion and inhibition of cancer cell migration. Histol Histopathol. 2009 Jun;24(6):789-99. doi: 10.14670/HH-24.789.

- Brożyna A.A., Jóźwicki W., Roszkowski K., Filipiak J, Slominski A.T. Melanin content in melanoma metastases affects the outcome of radiotherapy, Oncotarget. 2016 Apr 5;7(14):17844-53. doi: 10.18632/oncotarget.7528
- Brożyna A.A., Jozwicki W.,A. Carlson, and A.T. Slominski. Melanogenesis affects overall survival and diseases free survival in American Joint Committee on Cancer stage III and IV melanoma patients; Hum Pathol. 2013 Oct;44(10):2071-4. doi: 10.1016/j.humpath.2013.02.022. Epub 2013 Jun 19
- Brożyna A., L. VanMiddlesworth, A. Slominski. Inhibition of melanogenesis as a radiation sensitizer for melanoma therapy, Int J Cancer. 2008 Sep 15;123(6):1448-56. doi: 10.1002/ijc.23664.
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- Ortíz-López L, Morales-Mulia S, Ramírez-Rodríguez G, Benítez-King G. ROCK-regulated cytoskeletal dynamics participate in the inhibitory effect of melatonin on cancer cell migration. J Pineal Res. 2009 Jan;46(1):15-21. doi: 10.1111/j.1600-079X.2008.00600.x. Epub 2008 May 12.
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- Zeng N, Salker MS, Zhang S, Singh Y, Shi B, Stournaras C, Lang F. 1α,25(OH)2D3 Induces Actin Depolymerization in Endometrial Carcinoma Cells by Targeting RAC1 and PAK1. Cell Physiol Biochem. 2016;40(6):1455-1464. doi: 10.1159/000453197. Epub 2016 Dec 20.

1.5. Required initial knowledge and skills of the PhD candidate

A degree (MSc or equivalent) in natural sciences (Biology, Microbiology, Molecular Biology, Biochemistry or closely related fields); desirable methodological skills: basic background in cell culture, molecular biology, biochemistry, cell biology, immunology, hands-on basic knowledge of analytical methods; the ability to work creatively and independently towards developing your own research project; English communication

skills, both written and spoken; a collaborative personality.

1.6. Expected development of the PhD candidate's knowledge and skills

Research skills: PhD candidate exhibits knowledge of advances and developments in their field; exhibits the new methodological skills-cytotoxicity tests, flow cytometry, electron paramagnetic resonance (EPR) spectroscopy, electron microscopy, quantification of mitochondrial metabolism in single cells; exhibits the new statistical and analytical skills; demonstrates knowledge of research in related fields and disciplines; critically analyses and synthesizes new and complex information from diverse sources, knows have a broad awareness and knowledge of key relevant funding sources and grant application procedures.

Communication skills: PhD candidate demonstrates effective writing and publishing skill, communicates and explains research to diverse audiences, including both specialist and non-specialist.

Team-working and leadership: PhD candidate develops and maintains effective relationships with colleagues, works in a collaborative environment, awareness of their own working style, that of others, and how they interact, understands leadership in team environments, recognizing the strengths of team members and work effectively to achieve mutual goal.