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

Project title

CYP11A1-derived secosteroids as therapeutic agents in UVB induced skin cancer

1.1. Project goals

The aim of the project is defying the define mechanism of action of CYP11A1-derived D3-hydroxyderivatives against epidermal skin cancers.

1.2. Outline

Ultraviolet radiation (UVR) depending on its wavelength, penetrates the different layers of the skin, thus affecting DNA integrity, cell, and tissue homeostasis, inducing mutations, and affecting the expression of a plethora of genes including oncogenes and tumor suppressor genes. It can also modify the expression and activity of growth factors and their receptors, as well as having local and systemic immunosuppressive effects. UVB after absorption by the DNA induces covalent bond formation between adjacent pyrimidines leading to the production of mutagenic photoproducts. UVB is the etiological factor of skin cancers. Non-melanoma skin cancers (NMSCs), encompassing squamous and basal cell carcinomas (SCC and BCC) are the most common malignancies in humans, having an enormous economic burden on the health system of the World. Over 80% of NMSCs occur in sun-exposed sites, head and neck and back of hands, attesting to a crucial role for UVR in carcinogenesis. UVB, absorbed by a B ring of 7-dehydrocholesterol, generates previtamin D3, which undergoes temperature dependent isomerization to vitamin D3, prohormone, which to exert biological activity has to be activated by hydroxylations in positions C25 and C1, to produce 1,25(OH)2D3 in the canonical pathway. 1,25(OH)2D3, in addition to regulating calcium homeostasis, has important pleiotropic activities that include stimulation of differentiation and inhibition of proliferation of cells of different lineage, anti-cancerogenic effects and stimulation of innate and inhibition of adaptive immunity and of inflammation. In the skin, it plays a significant role in the formation of the epidermal barrier, it regulates functions of adnexal structures and has a wide variety of ameliorating effects on skin cancer and on proliferative and inflammatory cutaneous diseases. Due to its calcemic (toxic) effect, chronic therapeutic use of 1,25(OH)2D3 at pharmacological doses is severely limited. We have discovered novel metabolic pathways of vitamin D3 activation initiated by the action of a rate limiting enzyme of steroidogenesis, CYP11A: D3→20(OH)D3 + 22(OH)D3→(OH)nD3. In adrenals and placenta, 20(OH)D3 is the main metabolite being more abundant than the classical 25(OH)D3. Different hydroxyforms of vitamin D3 after activation by CYP enzymes would attenuate oxidative stress, DNA damage, and suppress cutaneous cancerogenesis as well as the tumor progression. These are consistent with

reported anticancer properties of 1,25(OH)2D3 and its protection against DNA damage on systemic level are also recognized. But its usefulness in cancer treatment is more promising due to less toxic action.

1.3. Work plan

Year 1
-literature review
- optimization of cell culture conditions for BCC ASZ001 mouse line, human UW-BCC1 BCC and human SCC A431 and SCC13 lines
- optimization models for testing vitamin D derivatives: 2D (monolayer), 3D in vitro (spheroids) and 3D in vivo (in ovo)
- establishing the protocol for proliferation and differentiation evaluation: MTT/XTT test, SRB test, markers of differentiation (keratins, involucrine, loricrin, filaggrin)
-establishing the UV exposure experimental set-up, sample size and replicas necessary for proper statistics as well as experimental design including shame-treated samples
Year 2
-testing of effects of CYP11A1-derivatives on established models in vitro
- preparation of review manuscript
Year 3
-testing of effects of CYP11A1-derivatives on established models in vivo
-the results analysis
-visiting in prof. A. Slominski's lab, Department of Dermatology, Cancer Chemoprevention Program, Comprehensive Cancer Center, University of Alabama at Birmingham, AL, USA
Year 4
-manuscripts preparation
-PhD thesis writing.

1.4. Literature *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

1. Biological Effects of CYP11A1-Derived Vitamin D and Lumisterol Metabolites in the Skin. Slominski AT, Kim TK, Janjetovic Z, Slominski RM, Li W, Jetten AM, Indra AK, Mason RS, Tuckey RC. J Invest Dermatol. 2024 Oct;144(10):2145-2161. doi: 10.1016/j.jid.2024.04.022.
2. Novel Vitamin D3 Hydroxymetabolites Require Involvement of the Vitamin D Receptor or Retinoic Acid-Related Orphan Receptors for Their Antifibrogenic Activities in Human Fibroblasts. Janjetovic Z, Qayyum S, Reddy SB, Podgorska E, Scott SG, Szpotan J, Mobley AA, Li W, Boda VK, Ravichandran S, Tuckey RC, Jetten AM, Slominski AT. Cells. 2024 Jan 26;13(3):239. doi: 10.3390/cells13030239.
3. CYP11A1-derived vitamin D hydroxyderivatives as candidates for therapy of basal and

squamous cell carcinomas. Slominski AT, Brożyna AA, Kim TK, Elsayed MM, Janjetovic Z, Qayyum S, Slominski RM, Oak ASW, Li C, Podgorska E, Li W, Jetten AM, Tuckey RC, Tang EKY, Elmets C, Athar M. Int J Oncol. 2022 Aug;61(2):96. doi: 10.3892/ijo.2022.5386.

4. Chemical synthesis, biological activities and action on nuclear receptors of 20S(OH)D3, 20S,25(OH)2D3, 20S,23S(OH)2D3 and 20S,23R(OH)2D3. Brzeminski P, Fabisiak A, Slominski RM, Kim TK, Janjetovic Z, Podgorska E, Song Y, Saleem M, Reddy SB, Qayyum S, Song Y, Tuckey RC, Atigadda V, Jetten AM, Sicinski RR, Raman C, Slominski AT. Bioorg Chem. 2022 Apr;121:105660. doi: 10.1016/j.bioorg.2022.105660. Epub 2022 Feb 8.
5. Evidence for Involvement of Nonclassical Pathways in the Protection From UV-Induced DNA Damage by Vitamin D-Related Compounds. De Silva WGM, Han JZR, Yang C, Tongkao-On W, McCarthy BY, Ince FA, Holland AJA, Tuckey RC, Slominski AT, Abboud M, Dixon KM, Rybchyn MS, Mason RS. JBMR Plus. 2021 Sep 29;5(12):e10555. doi: 10.1002/jbm4.10555. eCollection 2021 Dec.
6. Hydroxylumisterols, Photoproducts of Pre-Vitamin D3, Protect Human Keratinocytes against UVB-Induced Damage. Chaiprasongsuk A, Janjetovic Z, Kim TK, Schwartz CJ, Tuckey RC, Tang EKY, Raman C, Panich U, Slominski AT. Int J Mol Sci. 2020 Dec 9;21(24):9374. doi: 10.3390/ijms21249374.
7. The Role of Classical and Novel Forms of Vitamin D in the Pathogenesis and Progression of Nonmelanoma Skin Cancers. Slominski AT, Brożyna AA, Zmijewski MA, Janjetovic Z, Kim TK, Slominski RM, Tuckey RC, Mason RS, Jetten AM, Guroji P, Reichrath J, Elmets C, Athar M. Adv Exp Med Biol. 2020;1268:257-283. doi: 10.1007/978-3-030-46227-7_13.

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 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.