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

Project title

Identification and characterization of photoprotective properties of CYP11A1-derived vitamin D metabolites in keratinocytes

1.1. Project goals

The aim of the project is defying the protective role of 20(OH)D3 and other CYP11A1-derived vitamin D metabolites against UV radiation in the human epidermal keratinocytes.

1.2. Outline

The (OH)nD3 compounds containing C1α(OH) have been defined as agonists at the genomic site of the VDR, while (OH)nD3 lacking C1α(OH) act as "biased" agonists. The latter are characterized by poor activation of CYP24A1 and non-calcemic effects. The crystal structures of 20(OH)D3 and 1,20(OH)2D3 bound to the VDR have been obtained. Neither could bind to the non-genomic site. The anti-cancer activities of (OH)nD3 in epidermal cells were only partially dependent on a functional VDR and were further affected by the position of the hydroxyl group. Furthermore, we have shown that CYP11A1-derived (OH)nD3 compounds protect epidermal keratinocytes against UVB-induced damage by enhancing anti-oxidative responses and DNA repair mechanisms, inhibiting pro-inflammatory pathways, and promoting keratinocyte differentiation. These effects involve mechanisms such as Nrf2 and p53 translocation to the nucleus, induction of anti-oxidative enzymes, and inhibition of NFκB and IL-17 signaling. We have also demonstrated that CYP11A1-derived (OH)nD3 compounds are active in vivo. Additionally, the vitamin D metabolome has been established in human serum. Based on our previous work showing the radioprotective, DNA-repairing, and antioxidative properties of CYP11A1-derived vitamin D3 hydroxymetabolites—as well as their anti-cancer, anti-proliferative, and anti-inflammatory activities in both in vitro and in vivo models—it is proposed to test their efficacy against UV-induced pathology. The goal is to establish them as preventive and/or therapeutic agents against UVB-induced damage, including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). The proposed studies should support application of selected non-toxic D3-hydroxymetabolites, including 20,23(OH)2D3 and 20,25(OH)2D3, in photoprotection and provide a basis for planning clinical trials to validate their efficacy as therapeutics or adjuvants in the treatment or prevention of solar keratosis, SCC, or BCC in vulnerable populations. This is of particular importance because classical 1,25(OH)2D3 and its derivatives containing C1α(OH) are calcemic and toxic at high concentrations. In contrast, CYP11A1-derived compounds lacking C1α(OH) are non-toxic and non-calcemic, while those with C1α(OH) exhibit reduced calcemic effects.

1.3. Work plan

Year 1
-literature review
–testing of 2D and 3D models for UV exposure (primary keratinocytes, established cell cultures, in ovo models)
- optimization of assays for assessing changes in cell proliferation (measured by DNA synthesis, cell number and cell cycling by flow cytometry and colony formation in monolayer)
- optimization the protocols for measuring differentiation markers (at gene and protein levels) including keratins K1, K10 and K14, involucrin, loricrin, filaggrin and transglutaminase
-establishing the protocol for apoptosis evaluation: morphology, DNA fragmentation, TUNEL
-establishing the protocols for UV-induced DNA damages: level of thymine dimers (CPD and 6,4PP) and DNA fragmentation by comet assay
-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 abovementioned parameters after treatment keratinocytes with vitamin D metabolites and UV exposure
-preparation of review manuscript
Year 3
-the results analysis and evaluation of photoprotection of vitamin D derivatives
-visiting in prof. A. Slominski's lab, Dep. 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. Epub 2022 Jul 1.

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.