Investigation of the role of PARP2 in acne metabolomics – PD121138

The original hypothesis of the PD121138 research plan was that poly(ADP-ribose) polymerase (PARP)2 regulates the lipid homeostasis of the skin, most importantly that of the sebocytes of the sebaceous gland. Disturbances in cutaneous lipid homeostasis has been associated with the pathomechanism of several inflammatory skin diseases, such as acne and psoriasis. The role of PARP2 in skin physiology and pathophysiology is not characterized, therefore, our research plan provides novel basic research with translational potential as there are multiple, FDA- approved PARP inhibitors used in clinical practice.

During the realization of the project we had to face many technical challenges. Our very first task was to create PARP2 knock-down sebocytes from the widely used SZ95 human sebocyte cell line as acne is primarily characterized by dysfunctional, inflamed sebaceous glands. We decided to apply shRNA-mediated gene silencing of PARP2 as we had extensive experience with this technique. We succeeded in PARP2 knock-down in SZ95 cells and were able to start characterization of the cells with respect to potential alterations in lipid metabolism. We had good results suggesting that PARP2 indeed controls lipid synthesis of SZ95 cells. However, after a while the cells started to "erase" the shRNA from their genome, and we could not work with them anymore. Unfortunately, we lost several months trying to make these cells work, but we managed to figure a solution by ordering a specific CRISPR/Cas9 plasmid from Santa Cruz Biotechnology that delivered good results to our departmental colleagues who used those constructs before.

In the meanwhile, we did not waste our time and defined another project. Keratinocytes are the most abundant cell types in the skin, which are capable of the biosynthesis of a wide-range of lipids, including steroids. Psoriasis is an inflammatory skin disease featuring aberrant keratinocyte biology and is associated with impaired steroidogenesis in the skin. We decided to investigate if PARP2 may be involved in the pathomechanism of psoriasis, similarly, as we have previously demonstrated in the case of PARP1 (Kiss et al., 2019). We performed one of the most accepted animal model of psoriasis, the imiquimod (IMQ)-induced dermatitis in $PARP2^{+/+}$ and $PARP2^{-/-}$ mice. Visually, the developed dermatitis was significantly less severe in the case of $PARP2^{-/-}$ mice. We performed immunohistochemistry (IHC) on lesional skin sections of the mice that revealed slower proliferation and better differentiation of epidermal keratinocytes in $PARP2^{-/-}$ mice as determined by lower number of BrDU positive nuclei and stronger cytokeratin 10 immune reactivity in the epidermis of $PARP2^{-/-}$ mice. In addition, we analyzed skin sections of psoriasis patients, and we detected a marked increase in PARP2 expression in psoriatic lesions compared with healthy skin, that is in line with our results in mice. Taken together, these *in vivo* findings indeed suggested a role of PARP2 in psoriasis pathomechanism, and the genetic deletion of PARP2 rendered mice protected against the severity of IMQ-induced psoriasis-like skin inflammation. To find a possible molecular mechanism explaining our findings, we performed CYP11A1 IHC on mice skin sections. CYP11A1 catalyzes the first step in the synthesis of all types of steroid hormones, and IHC revealed increased expression, and possibly induced steroidogenesis, in the skin of $PARP2^{-/-}$ mice. We decided to repeat the IMQ-induced dermatitis model with the application of an inhibitor of CYP11A1, aminoglutethimide (AMG). However, at that point we did not have enough $PARP2^{+/+}$ and $PARP2^{-/-}$ mice. Until we have a new breed, we set out to *in vitro* characterize the effect of PARP2 in keratinocyte biology.

We started working with a widely used human keratinocyte cell line, the HaCaT. We performed shRNA-mediated knock-down of PARP2. However, again we had difficulties maintaining the manipulated cells. After about five passages the HaCaT cells carrying the PARP2 shRNA started changing morphology and behavior that ultimately led to the inability of the cells to attach to the surface of culture dishes. We were forced to ditch the HaCaT cells and look for alternative solutions. Our colleagues at the University of Szeged (Kornélia Szabó), helped us by providing a human epidermal keratinocyte cell line, the HPV-Ker

that they had been using for their studies successfully for many years. With the HPV-Ker cells we were successful in PARP2 knock-down using the lentiviral vector containing the specific PARP2 shRNA. First, we treated the cells with IMQ, which induced a marked increase in the expression of several pro-inflammatory cytokines (e.g. IL6, IL23A, IL1b) in the control cells, while its effect on shPARP2 cells was negligible. Next we applied treatment with a mixture of IL17A and TNFa to recapitulate a psoriasis-like pro-inflammatory environment for the keratinocytes. Similarly as in the case of IMQ, there was a significant induction in the expression of pro-inflammatory cytokines in sc cells, but not in the PARP2 depleted cells. We checked whether steroidogenesis may be induced in keratinocytes upon PARP2 depletion and whether this may be the reason of the anti-inflammatory phenotype of shPARP2 cells. We applied the steroid synthesis inhibitor AMG prior to treatment with IL17A+TNFa. We found that AMG could prevent the anti-inflammatory effect of PARP2 depletion in shPARP2 keratinocytes, suggesting that increased steroid production might underlie the effect. The most obvious explanation in this regard was that in shPARP2 cells the synthesis of glucocorticoids (chiefly cortisol) may be increased, and the produced glucocorticoids may exert anti-inflammatory effect on keratinocytes in an autocrine manner. Although we could not decisively confirm activation of the enzymes involved in cortisol synthesis, we nevertheless measured cortisol levels in cell supernatants by liquid chromatography-tandem mass spectrometry (LC/MS) analyses, but we could not detect significant alterations. We also performed IHC for glucocorticoid receptor in the skin sections of mice, but again without detectable differences between $PARP2^{+/+}$ and $PARP2^{-/-}$ mice. Therefore, we had to drop the theory of elevated glucocorticoid synthesis in keratinocytes upon deletion or depletion of PARP2.

Our next step in this project will be the RNA-seq analyses of sc and shPARP2 cells to determine the differentially expressed genes and pathways upon PARP2 depletion.

Meanwhile, the CRISPR/Cas9 plasmids finally arrived later in the year so we immediately started to prepare PARP2 knock-out SZ95 cells. After about two months the cells were ready and we started working with them. We confirmed increased total cholesterol and lipid levels in the PARP2 k.o. cells using a cholesterol determining kit and Nile Red and Adipored lipid dyes. We sent the control and PARP2 k.o. cells for lipidomics analyses and the results show a clear segregation between the cell lines in the major lipid species, but thorough analysis of the large lipidomics data is under progress. We also ordered RNA-seq analysis of the cells, and the results show massive differences between control and PARP2 k.o. sebocytes with the dysregulation of over 5000 genes.

In addition, we have started our clinical project aiming to determine possible fecal and cutaneous microbiome alterations in two inflammatory skin diseases, psoriasis and hidradenitis suppurativa. Our dermatologist colleagues at the Department of Dermatology (University of Debrecen) are collecting feces, blood and skin swabs from the patients. One half of skin swabs will be subjected to microbiological culturing and the other half will be analyzed by next-generation sequencing of the 16S ribosomal RNA gene hypervariable regions. Fecal samples will also be subjected to sequencing, and blood sera will be subjected to metabolomics analysis to reveal whether it is possible for gut microbiota metabolites to enter into circulation and reach distant organs, such as the skin.

Taken together, we have three major projects that have taken shape during the PD project, although the finalization of those projects will be realized in the following years. However, we already have strong results and clear routes to follow and now we have the necessary financial support in the form of an FK grant from NKFIH about to start on October 1, 2020. Therefore, we are absolutely positive that these projects will be published in high-impact papers in the following years.

Naturally, we managed to finalize several projects during the PD period and we wrote high-impact reviews, as well.

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