Leibniz Gemeinschaft

Final Report
Leibniz Competition

Title: **Epigenetic regulation of ImmuneAging: Heterochromatic DNA methylation as a regulator of T cell senescence (EpImAge)**

Application number: K59/2017

Period under review:
01.01.2018 – 30.06.2022

Leibniz Institute in charge:
Deutsches Rheuma-Forschungszentrum, ein Institut der Leibniz-Gemeinschaft (DRFZ)

Project leader:
Prof. Dr. Julia Polansky-Biskup

Executive Summary

Please briefly summarize the most important points of the individual chapters of the final report here (max. 4500 characters).

The EpImAge project set out to determine the molecular mechanism of proliferation-induced heterochromatic DNA demethylation (PIHD) and its functional consequences in human T lymphocytes. Furthermore, we set out to assess the occurrence and possible contribution of PIHD to immune aging and chronic inflammatory diseases (CID). As possible future options for therapeutic intervention, we aimed to identify modes for the prevention or reversal of PIHD.

Within the project, we were able to identify the S-phase length as the molecular driver for PIHD. In addition, as a possible consequence, we discovered a correlation between the enhanced PIHD suffered in the history of memory T cells and the future potential of these populations to differentiate. These findings indicate that the accumulation of proliferation events leads to an epigenetic imprint in T lymphocytes, which is long lasting and might be used as a biomarker to determine their future functional properties and their contribution to inflammaging in the elderly. Furthermore, we identified a molecular inhibitor, which is capable of preventing PIHD to occur during T cell proliferation. It represents a promising candidate for the targeted interference with PIHD in situations of strong proliferation, such as expansion cultures of T cells for the use in therapeutic applications. Moreover, we were able to identify PIHD-characteristic DNA methylation profiles in the long-lived rodent 'naked mole rat' but could not detect differences in PIHD between young and elderly healthy people in naïve T cells. Both findings indicate that PIHD is a proliferation-driven, rather than an aging-mediated effect. The analyses of PIHD in T cells derived from CID patients could not be finished within the time-frame of EpImAge, as sample acquisition was delayed during the COVID19 pandemic and technical challenges could not be overcome in time. However, the control cells from healthy individuals could be successfully analyzed and will serve as reference data sets as soon as the appropriate number of patient samples is successfully collected and processed.

During the course of EpImAge, three manuscripts on closely related topics were published by the project partners, and three manuscripts presenting the main part of the results are currently being prepared. The successful cooperation within EpImAge led to further collaboration initiatives, some directly for the benefit of EpImAge, and some also for follow-up research efforts on related topics. The two PhD students financed by the project received a well-structured education as members of an established graduation program (ZIBI Graduate School Berlin), with one of them graduating within the EpImAge run-time. The project was concluded after a cost-neutral extension of 6 months. Substantial in-kind resources from several project partners were invested, which strongly contributed to the success of the project.

Taken together, the collaborative EpImAge project could be successfully executed and revealed interesting findings, which will now be evaluated for their clinical implication in the field of adoptive T cell therapy (new grant proposal submitted to DFG). The initiated collaborations sparked new project ideas and interactions between science institutions within and outside the Leibniz Association. For the project leader, EpImAge was one of the first larger collaboration initiatives under her guidance, with the experience gained being highly valuable for her future academic career.

Contents

1. Achievement of objectives and milestones

Please explain briefly the progress made in implementing your project's main objectives and milestones, as stated in the application, during the period under review. Also explain any objectives/milestones that have not been implemented, or only in part.

This project set out to determine the molecular mechanism of proliferation-induced heterochromatic DNA demethylation (PIHD) and its functional consequences in human T lymphocytes. Furthermore, the occurrence and possible contribution of PIHD to immune aging and chronic inflammatory diseases should be assessed. As possible future options for therapeutic intervention, modes for the prevention or reversal of PIHD should be identified.

In the first work-package (*WP1 – molecular mechanisms, cooperation Walter, UdS Saarbrücken*), we were able reach our aim and clarified the molecular mechanism leading to PIHD using a highly elaborate in vitro T cell culture system, which allowed to precisely enumerate cellular divisions of individual T cells and to isolate them according to their number of undergone division rounds. Using this system, we were able to dissect the molecular mechanisms leading to PIHD, which are usually masked by heterogeneous proliferation behavior of T cells in bulk cultures. We identified the cell cycle speed, more precisely, the speed of S-phase progression, as the driver of PIHD in proliferating T cells. This finding was confirmed when we identified a small molecule inhibitor (*WP5 – compound, cooperation Nazaré, FMP*), which was able to prevent PIHD during T cell proliferation. While prevention of PIHD was almost complete, reversing past PIHD was not successful, indicating that suffered PIHD imprints in the epigenome are stable, even if the environmental conditions for DNA methylation maintenance are improved later in the lifetime of a cell. With this result, WP5 could also be concluded successfully.

Concerning the molecular and cellular consequences of PIHD (*WP2 – PIHD consequences, cooperation Neri, FLI Jena*), we found a slightly increased level of RNA expression in genomic regions undergoing PIHD, indicating that the reduced level of DNA methylation might facilitate adverse expression of transcripts which are usually suppressed due to the high degree of DNA methylation. On the cellular level, we analyzed the differentiation potential of central memory T cells in vitro and found it to correlate with the preformed degree of DNA methylation in the heterochromatin. Whether this finding remains correlative with lower heterochromatic methylation levels marking a population of enhanced differentiation likelihood, or whether there rather is a causal role for DNA methylation levels in T cell differentiation, remains to be determined. We also found that PIHD is enhanced during T cell differentiation processes. Taken together, these data indicate that cells displaying increased PIHD are more likely to contribute to the known inflammaging phenotype in the elderly, in whom highly proliferated memory T cell populations accumulate with pro-inflammatory phenotypes.

Assessment of PIHD in elderly people (*WP3 – elderly healthy people and CID patients, cooperation Radbruch, Siegmund, Enghardt, Charité Berlin*) is complicated by the fact that their T cell repertoire is enriched with memory T cells with an unknown proliferation history. Therefore, we assessed the more homogenous naïve CD8+ T cell pool and could not observe any heterochromatic DNA methylation loss in cells from elderly *vs.* young individuals. This indicates that aging alone does not result in PIHD, but that proliferation is an essential driver. We could confirm this in CD4+ naïve T cells from young donors, which we could separate into more and less proliferated populations based on the surface marker CD31.

Similar studies were planned to be conducted in patients suffering from chronic inflammatory diseases (CID). This project part was severely hampered by the COVID19 pandemic, which made acquisition of patient samples difficult (due to overburdening of clinical staff, interruption of clinical studies, etc.). In addition, the cell numbers we could isolate from various tissues (e.g. cerebral fluid, urine, synovial fluid) of patients with different CIDs (e.g. multiple sclerosis, systemic lupus erythematodes (SLE), rheumatoid arthritis) were too low to be analyzed for DNA methylation. However, we successfully teamed up with Ahmed Hegazy (department Prof.

Siegmund at Charité Berlin) to isolate T cells migrating into the inflamed gut in patients suffering from inflammatory bowel disease. While these isolations are still ongoing, the cellular counterparts from blood of healthy donors could already be isolated and analyzed and can be used for differential analyses once the patient-derived samples have been processed.

Finally, we assessed whether PIHD occurs in the long-lived rodent 'naked mole rat' (*NMR – WP4, cooperation S. Holtze, IZW Berlin*). Indeed, we could confirm that NMRs show the characteristic reduced methylation patterns indicative of PIHD, indicating that cells from these animals also suffer DNA methylation loss from proliferation events, although NMRs are largely protected from classical aging-dependent diseases. The results of the WP were already presented in the mid-term report. Further analysis of the PIHD mechanism in NMR could not be concluded due to technical limitations (see mid-term report).

In summary, the project could be successfully launched and run, (with some pandemic-based restrictions) and the aims of the WPs could be reached fully (WP1+5) or at least partially (WP2+3+4).

2. Activities and obstacles

Please outline the work and activities of the project partners in the period under review. Please also explain any obstacles or failures you may have encountered that led to delays in the schedule, to deviations from the original proposal or to unmet goals.

The project partners worked together successfully, with each partner contributing their respective tasks as planned in the project proposal (see also 'Achievements of aims'):

- Polansky, DRFZ Berlin (project leader): all wet-lab experiments incl. isolation of ex vivo samples, integrated data analysis, interpretation of results, conceptualization of publications
- Walter, UdS Saarbrücken: DNA methylation analyses
- Neri, FLI Jena: contribution to RNA expression analyses
- Siegmund, Hegazy, Radbruch, Enghardt, Charité Berlin: clinical partners, providing access to human tissue samples and clinical knowledge
- Holtze, IZW Berlin: provision of NMR tissue samples
- Nazaré FMP Berlin: contribution to inhibitor identification

Challenges encountered:

- restrictions encountered during COVID19-pandemic: closure of labs for several months in 2020, difficulties to include patient material, restriction in experimental lab work due to space capacity limitations in the labs, strongly increased child-care duties by the project leader and one PhD student
- limited cell numbers in human tissue samples which did not allow DNA methylation analyses
- technical difficulties concerning DNA methylation analyses of NMR samples

3. Results and successes

Please present the key results and successes in the areas of research (publications, completed theses and dissertations, acquisition of third-party funds, scientific events, etc.) and transfer (consultancy, technology transfer, public relations work).

The main results of the study are described under point 1 'Achievement of aims'.

- 1 PhD thesis could be successfully concluded (Christopher Kressler, grade: magna cum laude), 2 PhD theses + 2 Master theses are still ongoing
- Data on the project were presented at various national and international conferences (e.g. IHEC Annual conference on Epigenetics 2022 in Estrel (Canada), Annual Congress of the DGfI 2022 in Hannover, 2022 IMB conference on Epigenetic of Aging in Mainz, 2022 Max Planck Epigenetics meeting in Freiburg)

- Submission of 3 publications with results from EpImAge are planned in 2023.
- Three manuscripts were published on closely related topics, although the work presented was not directly funded by the EpImAge Project
  1. Salhab A, Nordström K, Gasparoni G, Kattler K, Ebert P, Ramirez F, Arrigoni L, Müller F, **Polansky JK**, Cadenas C, G Hengstler J, Lengauer T, Manke T; DEEP Consortium, **Walter J.** (2018). A comprehensive analysis of 195 DNA methylomes reveals shared and cell-specific features of partially methylated domains. *Genome Biol*. Sep 28;19(1):150. doi: 10.1186/s13059-018-1510-5.
  2. Shebzukhov Y, **Holtze S**, Hirseland H, Schäfer H, Radbruch A, Hildebrandt T, Grützkau A. (2019). Identification of cross-reactive antibodies for the detection of lymphocytes, myeloid cells and haematopoietic precursors in the naked mole rat. *Eur J Immunol.* Nov;49(11):2103-2110. doi: 10.1002/eji.201948124.
  3. Ou K, Hamo D, Schulze A, Roemhild A, Kaiser D, Gasparoni G, Salhab A, Zarrinrad G, Amini L, Schlickeiser S, Streitz M, **Walter J**, Volk HD, Schmueck-Henneresse M, Reinke P and **Polansky JK** (2021). Strong Expansion of Human Regulatory T Cells for Adoptive Cell Therapy Results in Epigenetic Changes Which May Impact Their Survival and Function. *Front. Cell Dev. Biol.,* 18 November 2021, doi: 10.3389/fcell.2021.751590.

4. Equal opportunities

Please describe briefly your initiatives and measures to ensure equal opportunities, especially in staff development and recruitment.

Improving the working conditions and career options for female scientist, parents and underrepresented minorities is a topic of great importance to the project leader Julia Polansky. While the directly funded personnel on EpImAge happened to be two males from Germany, the Polansky lab overall is more diverse (i.e., 2 international PhD students, 2 international Postdocs, 1 international master student). As one of the EpImAge-funded staff members is a father of two, taking parental leave after the birth of his second child was encouraged and appreciated, restrictions due to child-care duties were respected and the work schedule was adjusted accordingly. In addition, Julia Polansky is actively involved in supporting young female scientist in their careers as one of the speakers of the Leibniz Mentoring Network. This activity includes mentoring younger female scientist and the organization of an annual meeting for all network members. She also serves as a mentor in the Leibniz mentoring program and founded the Women-in-science-Team (WiSe-Team) at her new affiliation, the Berlin Institute of Health Center for Regenerative Therapies (BCRT) at the Charité - Universitätsmedizin Berlin, which aims at improving working conditions and career prospects for females and underrepresented minorities at the BCRT. As a 'working mum' of two young kids, Julia Polansky also serves as a role model for younger female scientists.

5. Quality assurance

Please describe briefly your measures for quality assurance, in particular in terms of complying with good scientific practice and making your research results available (open access).

- All PhD Students on the EpImAge project were integrated in structured PhD programs, were good scientific practice is taught at the beginning of the program and throughout the course work
- Experimental lab work is documented in an electronic lab book (LabFolder) which allows full traceability of changes in the documentation and direct links to the data generated
- Planning and execution of experiments as well as data analysis and interpretation are discussed in detail during weekly lab meetings and monthly one-on-one meetings with the PhD supervisor
- All data generated in the EpImAge project shall be published in open access format

6. Additional in-kind resources

Please estimate the value of in-kind resources generated within the project at your institute, the participating Leibniz institutes and/or the university partners. Please differentiate between human resources (e.g. in person-months for scientific and non-scientific staff) and materials.

Personnel at Polansky lab
Technical assistant: generation of DNA methylation data (15% since 09/2019, equaling 4.8 person-months; 21,120 EUR)
Scientists: contribution to experimental lab work (30% working time for entire project duration, equaling 16.2 person-months; 104,490 EUR)
2 master students (6 months each; 12 person-months)

Personnel at Walter lab
Scientists: generation and analysis of DNA methylation data (2x 5% working time for project duration, equaling 5.4 person-months; 34,830 EUR)
Technical assistants: generation of sequencing data (5% for 1y, eq. 0.6 person-months; 2,640 EUR)

Personnel at Holtze lab
Scientist: isolation of NMR organs (20% for 4 weeks, eq. 0.19 person-months; 840 EUR)

Consumables at Polansky lab
Reagents for T cell isolation, cell culture and flow cytometry (more required than initially anticipated): 8,000 EUR/year

Consumables at Walter lab
Sequencing reagents (more required than initially anticipated): 4,000 EUR

Services by DRFZ for Polansky lab
Access to flow cytometry core unit: 10 EUR/h, 3h/week, 46weeks/y for 3 years (analysis) + 75 EUR/h, 10h/month, 11month/y for 3 years (totaling 4,140 EUR + 24,750 Euro = 28,890 EUR)

7. Structures and collaboration

Please describe the structure of existing collaborations in the period under review, incl. any obstacles and challenges. Where relevant, describe changes to the governance of your project and/or the addition of new partners or collaborative relationships. If new collaborative relationships were forged with institutional partners during the period under review, please briefly describe the scientific benefit for your project.
If the changes resulted in additional agreements or changes to the existing collaboration agreement, please attach copies to this report.

The collaborations between the project partners were installed as planned, with the project leader being in the centre of any interaction. Based on this foundation, additional cooperation could be initiated, either benefitting the EpImAge project directly, (such as Helena Radbruch, Ahmed Hegazy, Phillip Enghard, Jens Rückert and Timo Nazari-Shafti from Charité for access to human tissue samples), or pursuing follow-up investigations on the topic (such as Claudia Waskow at FLI Jena and Pascal Giehr at LMU Munich, formally UdS Saarbrücken).

8. Outlook

Please provide an outlook on the most important future research questions and new fields of study that arise from this work.

The results from the EpImAge study have important implications for clinical application in the field of adoptive T cell therapy, as during the manufacturing of therapeutic T cell product, cells also suffer from proliferation-induced changes of the epigenome (PIHD, own work: Ou et al, Front. Cell Dev. Biol., 2021). Therefore, the next step will be to assess in how far PIHD negatively influences the product quality and if our identified inhibitor can improve the product

manufacturing process. In addition, the molecular insights gained from EpImAge will be utilized to develop a protocol for the induction of T cell phenotypes from human induced pluripotent stem cells (hiPSCs) as a possible new cellular source for therapeutic T cell products (DFG grant application submitted).