Leibniz Gemeinschaft

Abschließender Sachstandsbericht
Leibniz-Wettbewerb

Postdoc-Network on aging-induced impairments in regeneration and stem cell functionality - RegenerAging
Antragsnummer: SAW-2015-FLI-2

Berichtszeitraum: 01.04.2015 – 31.03.2019

Federführendes Leibniz-Institut: Leibniz Institut für Alternsforschung – Fritz-Lipmann-Institut (FLI)

Projektleiter/in:

Helen Morrison

Table of content

1. Achievement of objectives and implementation of milestones

Our objective was to build a structured, interdisciplinary postdoc-network to foster career development of early career postdocs in biomedical sciences on aging and regeneration. The scientific goal was to identify targets for future therapies and intervention strategies to ameliorate aging-associated organ dysfunction and disease. To accompany the research topic we aimed to implement innovative educational measures to strengthen and accelerate postdoctoral education. Broadly these included (i) interdisciplinarity and translation of research (ii) career development of female scientists, (iii) international connectivity (iv) educational support activities. We achieved nearly all of our objectives:

(1) The scientific focus – all postdocs worked on highly interconnected research topics addressing organ homeostasis, regeneration and stem cell functionality in aging across different organ systems with genetic and innovative animal models. Postdoc selection in 2015 - according to our criteria of early career, international and gender equality we organised a recruitment campaign including international and national advertisement – two mini symposia for candidate selection were organized with a structured selection procedure through an experienced selection committee. This recruitment outreach attracted a large pool of excellent international and national candidates fitting to our criteria. We employed 5 postdocs: 4 female and 1 male (British, Indian, Taiwanese, and two German).

(2) The implementation of innovative education measures: Structural measures were achieved as follows (a) Interdisciplinarity - the postdocs were co-supervised by 2 experts from different areas of basic science and / or academic medicine through co-supervisor of different disciplines (b) Translational potential was achieved by clinical co-supervisors/advisors. (c) Internationalization was achieved with international experts as advisors including potential international experts hosting the postdoc for a 2-4 week period in the last year. (d) Assurance of gender equality was addressed by including women mentors and the provision of childcare. (e) Accomplishment of structured supporting activities including: Progress reports and internal networking was installed at the beginning of the "RegenerAging postdoc-network" and included external advisors. Networking and career planning considered an essential part of successful science was implemented where Postdocs were connected with high-ranking scientists and invited them to give lectures at FLI. One example - a symposia was organized by the postdocs which complemented the Jena Aging Meeting (JAM2018) and included high profile international academics and industry professionals focused on Aging.

Courses on fundamental questions of science, scientific writing (including grant writing) and scientific presentation were implemented. Courses and workshops on state-of-the-art scientific methodology were offered. Implementation of mandatory courses on good scientific practice was achieved. Leadership and management qualification programs were offered (internal and external courses). The postdocs participated in international and national conferences with posters and scientific presentations. Postdocs participated in a teaching course offered by the Graduate academy Jena to learn didactics and methodology of university teaching.

2. Activities and obstacles

The RegenerAging postdoc network has persistently focused on aging induced impairments of pathways important for organ maintenance and regeneration, involving different stem cells and tissue types and a spectrum of genetic and innovative animal models.

However during these research activities one issue of concern created an obstacle pertaining to the *Causa 'Mouse Husbandry'* 2016 – the impact of this issue impacted all projects using *in vivo* systems – animal experimentation was stopped and was followed by a period of structural reorganization addressing all concerns. All projects from the RegenerAging were interrupted and affected by this issue. To overcome this significant interruption, we swiftly reorganized the projects to continue the research in alternative systems. Also during this period (2016-2018) we progressively regained the allowance of animal experimentation.

Overall the original application goals were not affected. Only the scientific focus and the specific aims in individual scientific projects were modified along with potential changes in co-advisor changes to fit the altered project (in some cases co-supervisors were not identified). Despite this all projects remained as 5 interconnected research topics that still addressed the central focus 'on aging induced impairments of pathways important for organ maintenance and regeneration'. Many of the RegenerAging postdocs, despite the interruption were able to publish their research activities. Also there are a number of manuscripts from the RegenerAging postdocs that are in preparation with the aim to publish as soon as possible.

Our original aim was to fast track early career academics into independent group leaders. In retrospect it turned out too ambitious to recruit 5 high potentials during the brief selection period, which are able to develop into independent junior PIs. Nevertheless, we are fully convinced about the strategic efficacy of the postdoc framework and will continue its use for the benefit of future postdocs. Furthermore, we see the successful outcome with the past recruits leading to several publications and positive career developments. Despite the fact that the generation of results and the structural education offer was an achievement, we were unable to identify at this point in time an independent young academic that could successfully compete for Junior PI positions. On analysis three years is too short for internationally competitive group leader positions/ or ERC startups. To overcome this obstacle our aim for early career scientists should rather focus on selected postdoc candidates showing great potential that could be mentored into an application for DFG Emmy Noether program. Educational structural activities were successfully implemented as described above – these activities were not affected by the *Causa 'Mouse Husbandry'* 2016.

3. Results and achievements

The Morrison postdoc contributed to the project on **aging associated impairments on the long-term maintenance and regeneration of adult neurons in the peripheral nervous system (PNS).** The postdoc contributed to the discovery that the PNS regeneration shows an age-dependent regenerative decline, with 'repair Schwann cells (SC) exhibiting alterations in pathways that limit Schwann cell 'repair-aiding' responses. Conducting omics screens on intact and regenerating peripheral nerves, the postdoc identified several candidate inflammatory pathway alterations. This included the discovery of macrophage engagement during peripheral nerve regeneration. Upon injury, macrophage recruitment was altered with a persistent hyper-inflammatory state impairing nerve regeneration. Moreover, a low-grade, chronic upregulation of specific pro-inflammatory cytokines (CCL2, CCL11) in the intact uninjured nerve during aging was discovered affecting nerve maintenance and impairing regeneration. CCL11 affected young SC behavior both *in vitro* and *in vivo*, implicating CCL11 as a nerve-aging factor (published).

Furthermore, the postdoc leads the ongoing investigation **LZTR1 – Critical regulator of mitochondrial homeostasis** on LZTR1 as a candidate involved in Schwannomatosis, with important implications for maintenance of sensory fibers involved in neuropathic pain. LZTR1 was initially suggested to be a transcriptional regulator and later associated with the Golgi apparatus. However, the postdoc obtained experimental evidence that localizes LZTR1 exclusively to mitochondria and demonstrates expression within the PNS solely to sensory SC fibers. The evidence suggests that LZTR1 is involved in mitochondria homeostasis, and loss of LZTR1 in SCs leads to dysfunctional mitochondria, and loss of adaptive responses to cope with altered mitochondria. With additional institutional funding, the postdoc is currently finalizing the manuscript and aims to stay in basic research focused on aging. Because LZTR1 is expressed in many organ systems, and we hypothesize that loss of LZTR1 will lead to organism-wide alterations in metabolic stress responses. We expect that the protective, adaptive stress pathways responding to the oxidative stress might be maintained in young animals but may lead to accelerated loss of metabolic stress responses and metabolic failure during advanced aging. The postdoc initiated collaborative work with the Rudolph postdoc to test additional aspects of LZTR1 functions including metabolic aging of hematopoietic stem cells (HSCs). Transfer activities: Participation in the development of a potential protein replacement therapy to prevent schwannoma formation: human recombinant soluble

rhNRGβ1 replaced the lost axonal NRGβ1 type III, a SC differentiation signal preventing schwannoma development *in vivo* (patented). Also, the postdoc contributed to the identification of sterile chronic inflammation on old nerves. Showing benefits of an anti-inflammatory treatment *in vivo*. The aim is to develop molecular inhibitors targeting the inflammation affecting regeneration in the elderly.

The v. Maltzahn and Kaether postdoc focuses **on systemic hormonal alterations during aging, e.g., the Klotho protein expressed in the hypothalamus and in the pituitary gland**. Several major hypothalamic-pituitary-peripheral organ axes are changed in Klotho-deficient premature aging mice, implicating systemic Klotho in the maintenance and regeneration of multiple organ systems. The postdoc used the Klotho hypermorphic mouse line and regeneration experiments were performed. The results show that satellite cell function is severely impaired upon loss of Klotho expression, in culture and during regeneration *in vivo*. Addition of recombinant Klotho protein inhibits aberrant excessive Wnt signaling in aged satellite cells thereby restoring their functionality. In conclusion, the anti-aging hormone Klotho counteracts aberrant canonical Wnt signaling in satellite cells and might be one of the naturally occurring inhibitors of canonical Wnt signaling in skeletal muscle (published). Furthermore, the postdoc published another paper, on injection of self-delivering siRNAs in regenerating skeletal muscle (JoVE, accepted). The postdoc currently continues a research project at the FLI and was promoted to a managerial position as Head of the FLI animal house. Transfer activities in a collaborative project with the von Maltzahn group pertaining to ameliorating cachexia by targeting Wnt signaling: Demonstrating that cachexia dependent muscle loss can be reversed by application Wnt7a.

Wang postdoc focused **on the role of canonical and non-canonical DNA damage response (DDR) pathways, regulated by NBS1, in neurodegenerative processes**. Dysregulation of this molecular key mediator of DDR pathway leads to chromosomal instability syndromes, which are accompanied by a neurological defect, called Nijmegen breakage syndrome. Previous projects in the Wang lab demonstrated that loss of NBS1 in neuronal cells, by using conditional Nestin-Cre knockout system, causes microcephaly and greatly impaired neurogenesis. Now the postdoc studies the microenvironment and how the microglial cells contribute to the neuronal degeneration. An important project on how dysfunctional DNA damage responses affect intercellular communication within the brain. The postdoc discovered several essential physiological functions of microglial cells that are altered. Beside the production of inflammatory mediators, like IL-6, and the migratory capacity, the metabolism of microglia isolated from mutant brains is altered. The working hypothesis is that a knockout of NBS1 in neuronal cells leads to alteration in the intercellular communication in the brain. NBS1 damaged neurons triggers a damage response in microglia leading to the activation of microglia releasing inflammatory mediators contributing to the brain phenotype (manuscript in preparation). The postdoc is currently still employed and intends to finalise the manuscript and intends to stay in an academic track in aging research.

The Englert postdoc project was to assess **the cellular and molecular pathways during kidney and tail fin regeneration in two different fish species, namely zebrafish and the short-lived killifish *Nothobranchius furzeri***. During the course of this project the postdoc made a surprising observation that there are significant numbers of immune cells that appeared to be tightly associated with kidney tubules. The postdoc followed up on this discovery and extensively characterized these cells in both fish species. The largest proportion of those immune cells turned out to be macrophages (manuscript in preparation for submission to *Cells*). This postdoc moved to the UK and took a research coordination career change at Surrey University. A second postdoc form the Englert group (included as the 6th member of the postdoc network - female) this postdoc introduced a project on heart regeneration. This project studies **the regenerative capacity of the heart in the two fish species**. Preliminary data suggest that while zebrafish can efficiently regenerate its heart after damage, killifish cannot. Comparable to the situation in the kidney, finding a number of immune cells within the heart that seemed to be closely connected to cells of the myocardium. Prompted by this discovery of specific immune cells in the kidney and in the heart the postdoc is currently analyzing the role of those cells for the regenerative process,

e.g. by modulating the immune system during the regenerative response. This postdoc is still currently employed and intends to stay in an academic research career focused on aging.

The postdoc in the Rudolph lab contributed to the study of the influence of **systemic interventions, e.g., dietary restriction (DR) on stem and progenitor cells in aging organisms**. Dietary restriction is the best-documented intervention that induces metabolic adaptation and a slowdown of aging. However, the postdoc contributed to the discovery that aging profoundly abrogates metabolic plasticity of hematopoietic stem and progenitor cells in response to DR. When exposed to *ad libitum* (AL) diet, hematopoietic stem and progenitor cells (HSPCs) from young mice employ both glycolysis and mitochondrial metabolism, which in response to DR shifts to oxidative metabolism leading to enhanced metabolic efficiency. HSPCs from aged AL-fed mice, in contrast, exhibited chronic mitochondria dysfunction, a subsequent stronger dependency on glycolysis, and a complete failure to activate mitochondrial metabolism in response to DR. The postdoc focused on the analysis of changes in the proteome of aging hematopoietic stem cells (HSCs). The work showed that alterations in the stoichiometry of ribosomal proteins in aging HSCs associate with alteration in proteasome activity. Moreover, the work revealed evidence for changes in the expression of members of the spliceosome – a protein complex involved in RNA splicing and genes expression. Transfer activities: Interestingly mutations in spliceosome encoding genes occur in human aging in the hematopoietic system, but it is currently not known, how mutations in these genes are selected in the aging hematopoietic system. The work provides some evidence that the loss of stoichiometry in the spliceosome may contribute to this process of mutation selection. As mutations in the aging hematopoietic system increase the risk of cardiovascular diseases in aging, the observed changes in the spliceosome of aging HSCs may not only contribute to the selection of mutant HSC clones but also to disease development in aging. Based on these results and the important translational aspects, the postdoc proceeded now with postdoctoral studies on cardiovascular disease development in the clinical department of Prof. Schulze at University Hospital in Jena (UKJ).

4. Equal opportunities

One important aspect of the postdoc network was the career development of female scientists. The recruitment was done via national and international online channels, which led to the selection of four female and one male postdoc for the network. Furthermore, three postdocs were recruited internationally from Great Britain, Taiwan, and India, and two postdocs were from Germany. One postdoc had to interrupt the project work for maternity and parental leave, and was reintegrated right afterwards, currently finishing the network-project in the laboratory. The postdoc-network gave us the opportunity to develop and implement postdoc guidelines – the intention was to offer all postdocs in the institute to benefit from the established 'educational and career development structural measures' implemented as a result of the network. To specifically enter the RegenerAging postdoc network we stipulated that an application has to be submitted outlining the motivation to join the 'interdisciplinary postdoc-network for early career development in biomedical sciences on aging and regeneration' – as a result an additional 6th German female postdoc successfully joined the network. The funding of the project was based on independent funding agency.

5. Quality assurance

We consider research integrity to be a core aspect in science, which should involve researchers at any level of their careers. We implemented a range of quality assurance mechanisms directly involving the enrolled postdocs thus benefitting their development and research results. To begin with, postdocs attended courses for further education on good scientific practice, image analysis and processing, biostatistics, publishing and peer review, and various aspects of animal experimentation (e.g. FELASA courses). Furthermore, post-docs of RegenerAging were part of the FLI postdoc club (currently about 50 postdocs at FLI), which during their monthly meetings addresses multiple scientific aspects including quality assurance. The continuous exchange amongst postdocs supports a flow of knowledge in between them, thus feeding back into practical research integrity. An add-on towards the

very end of the funding period was the introduction of a new electronic laboratory book for recording and archiving research data, thus allowing a brief experience of this novel tool.

In addition to quality assurance measures within the FLI, we implemented further unbiased external expertise. All projects within RegenerAging were co-supervised by external experts. Furthermore, as another means for quality assurance prior to submission for publication, the data is collected at FLI and scrutinized externally for statistics, figure, and text quality. In total, the work within RegenerAging was successful and has led so far to nine publications in international peer-reviewed journals, with six of them being open access.

6. Additional own resources

With the postdoc-network RegenerAging we applied for personnel funding for postdocs from the Leibniz association and contributed on several levels from our institutional budget. We covered the expenses of research consumables for the five postdocs, totaling at 225.000 Euro. The networking- and conference-associated travel costs, and the training measures in form of courses summed up to 61.200 Euro. Furthermore, we extended the contracts of two postdocs for another eight months beyond the SAW funding period, which requires another 101.000 Euro. In total the FLI contributed an additional 387.200 Euro in direct costs for the realization of the project. On top we estimate the following in-kind contributions for technical assistance in the laboratory (one person-month per postdoc per year) as well as administrative support for all matters of human resources, relocation to Jena, organization of the courses and travel, consultation on grant writing (also one person-month per postdoc per year), which together accumulates to about 153.000 Euro. In summary the Leibniz Association provided 1,29 Mio Euro for RegenerAging, which were completed with another 0,54 Mio Euro by the FLI. The course program was continuously developed under RegenerAging, and was mostly open to other postdocs and PhD students as well and received very positive feedback. Due to this favorable experience the FLI is keeping up the course program for the next generations of postdocs and PhD students.

7. Structures and cooperation

A clear cooperative structure has developed during the RegenerAging postdoc network period with the University Hospital Jena (UKJ) and their activities on ‘healthy aging’. From the basis of the RegenerAging postdoc network the FLI has jointly organized the training of clinician scientists within the DFG-funded ‘OrganAge’ activity (coordinated by O. Witte, Neurology). Additionally, there is interaction with the EKFS-funded postdoc network ‘AntiAge’ at UKJ. We have shared common seminars and symposia between the networks – supporting scientific exchange and career development opportunities. Implementing and intensifying these scientific, clinically oriented career development cooperation with UKJ are an important component of the FLI research and postdoc network strategy to contribute to ‘healthy aging’.

8. Outlook

We will continue to offer the structured course program. We consider the RegenerAging postdoc network as the blueprint to support postdoc education and to give them opportunities to accelerate scientific careers in aging research. We have to sharpen the co-supervision and further promote internationalization. The research focus on organ homeostasis and regeneration is regulated by cell-intrinsic, local and systemic factors, all subject to aging-associated changes and still remains a focus. We are still committed to uncovering new age-dependent mechanisms leading to organ dysfunction and regenerative decline. Our collaborative research strategy across all disciplines will prove indispensable and will continue in the future, the integration of the UKJ will strengthen our transfer activities. Also, integration of experienced system biologists will support new discoveries from complex omics data sets. The research focus on microbiota and aging investigating mechanisms that promote aging associated changes in the microbiome and the consequences on organ maintenance and regeneration is a clear future direction to be integrated in the postdoc network.