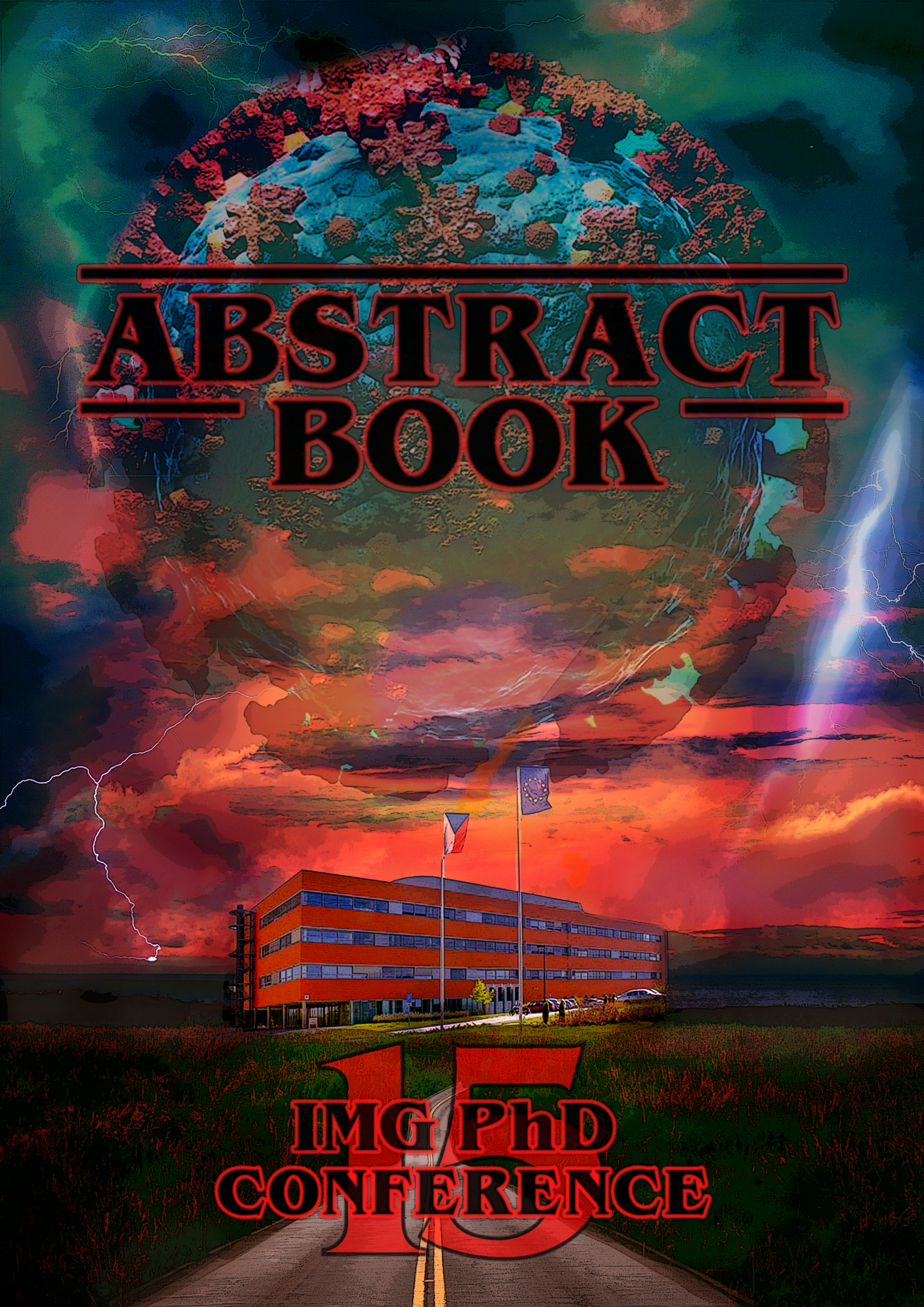
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FUNCTIONAL CHARACTERIZATION OF UNCONVENTIONAL NAIVE CD8+ 4-1BB+ T CELLS USING A NOVEL INDUCIBLE MONOCLONAL TCR MOUSE MODEL

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Single-cell RNA sequencing has challenged the traditional view of cell types, revealing their unexpected inner diversity. An exemplary uniform cell type is naive $CD8^+$ T cells, which stay inactive until they encounter their cognate antigen. After antigenic stimulation, naive cells give rise to potent short-lived effectors as well as different kinds of memory cells. However, it remains unclear whether the cell fate is determined by intrinsic properties of naive cells, TCR specificity, or the environmental stimuli.

In this study, we performed scRNAseq and scTCRseq on more than 50 000 murine $CD8^+$ T cells. Alongside established cell subsets, we revealed an unconventional population of naive $CD8^+$ T cells (T^{PAM}), characterized by the expression of 4-1BB (*Tnfrsf9*) and upregulation of the polyamine metabolism (*Odc1, Srm, Eif5a*). To study T^{PAM} cells *in vivo*, we developed an inducible OT-I TCR mouse model (TCR-switch). In TCR-switch mice, T cells develop a polyclonal TCR repertoire but are forced to express OVA-reactive TCR after switching. We show that upon infection with Lm-OVA or LCMV-OVA, TCR-switch T^{PAM} cells develop into unique long-lived $CX3CR1^+$ memory T cells. Targeting the axis establishing long-lived memory T cells might be beneficial in diverse therapeutic approaches, such as anti-cancer therapies or T-cell vaccination.

ROLE OF MECHANOTRANSDUCTION IN HEPATIC STELLATE CELLS (HSCs) ACTIVATION

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Fibroproliferative diseases have been shown to have caused about 45 percent of deaths in the modern world. In association with chronic inflammation, these diseases might ultimately result in cancer and metastasize to further the number of deaths. In the liver, the major contribution to fibrosis development comes from star-shaped cells called hepatic stellate cells (HSCs). Any insult to the liver, regardless of etiology, results in increased tissue stiffness, thereby activating mechanosignaling in HSCs and facilitating their activation. The activation results in deposition of extracellular matrix (ECM), which further drives stiffening of the liver thus creating a vicious feedback loop of activation and deposition.

Here, we tried to intercept fibrosis progression by targeting the mechanosignaling capacity of HSCs. For this, we have targeted plectin, a cytolinker protein known to be involved in mechanosensory pathways, using mouse HSCs-specific KO model. When compared to wild-type counterparts, we observed in newly generated KO mice significantly reduced ECM deposits upon toxic, carbon tetrachloride-induced insult to the liver. In addition, KO mice also displayed faster recovery and resolution of ECM deposits after removal of the toxic agent. To complement our *in vivo* studies, we prepared plectin-deficient human HSC cell lines using CRISPR/Cas9 technology. Analysis of these cells further confirms reduced capacity of targeted HSCs to deposit ECM. In conclusion, we have established targeting strategy for reducing fibrotic deposits in the liver, which can be useful in a number of liver diseases.

INDIVIDUAL BMP LIGANDS SECRETED BY VILLUS MESENCHYMAL CELLS INITIATE DISTINCT DIFFERENTIATION PROGRAMS IN ENTEROCYTES

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The main functions of mammalian intestine are absorption of nutrients and protection of the body from the potentially harmful intestinal content. Those functions are mainly performed by a single layer of differentiated epithelial cells – enterocytes, which continuously arise from the intestinal epithelial stem cells (IESCs), respectively from their progeny (transit amplifying cells). The identity of enterocytes is determined by sub-epithelial mesenchymal cells producing distinct Bmp ligands along the villus. In our work we show that individual Bmp ligands drive distinct differentiation programs during the migration of enterocytes up to villus tip. We probed the role of individual Bmp ligands as main initiators of enterocytes differentiation. We performed short-term cultivation of freshly isolated murine intestinal crypts treated with individual Bmp ligands followed by deep expression profiling, cell type characterization and proliferation analysis. This setup revealed distinct functions of individual Bmp ligands. Our data indicate that individual Bmp ligands uniquely influence the differentiation fate of intestinal epithelial cells. Bmp ligands strikingly differ in the ability to influence the intestinal epithelial cells in terms of regulating differentiation, proliferation rate and the regulation of gene expression. Furthermore, our results suggest that the places of action of individual Bmps are spatially separated along crypt-villus axis.

EMERGENCY GRANULOPOIESIS INDUCES A LYMPHOID TO MYELOID TRANSCRIPTIONAL SWITCH IN A SUBSET OF HEMATOPOIETIC STEM CELLS MARKED BY CD201 EXPRESSION

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Emergency granulopoiesis (EG) is the enhanced and accelerated production of granulocytes that occurs during infection. The process is known to be supported mostly by myeloid progenitors which enhance their proliferation and accelerate granulocytic differentiation upon pathogen sensing. Remarkably, hematopoietic stem cells (HSCs) can also sense pathogens, nevertheless whether and how HSCs contribute to EG remain elusive. To investigate that, we performed scRNA-seq analysis of sorted murine HSCs 4h after *in vivo* LPS treatment. Strikingly, we observed radical transcriptional changes between PBS control and LPS treated mice, mostly marked by alterations in HSC lineage bias. We identified a steady state lymphoid biased (ly-bias) subpopulation of HSCs, marked by expression of *Procr* (CD201). Following LPS stimulation, the CD201 expression is lost and this population is transcriptionally rewired to a myeloid-biased (my-bias) HSC population. We confirmed the loss of CD201 expression in HSCs by flow cytometry in mice after LPS and G-CSF treatment. When cultivated under myeloid differentiation conditions, the CD201- HSCs gave rise to mature granulocytes, whereas CD201+ HSCs remained rather immature. Next, we transplanted lethally irradiated mice with sorted CD201+ or CD201- HSCs. While CD201+ HSCs showed increased engraftment and preferential bias to produce lymphoid cells, CD201- HSCs showed decreased engraftment ability and a bias towards myeloid production. Surprisingly, LPS challenge of BM chimeras transplanted with ly-biased CD201+ HSCs led to EG response marked by rapid loss of ly-biased multipotent progenitors (MPPs) and the expansion of my-biased MPPs. In conclusion, we observed that specific subpopulations of HSCs support the early phases of EG by switching from ly-bias to my-bias HSCs, thus establishing alternative paths to supply elevated numbers of granulocytes.

EFFECTS OF CHRONIC INFLAMMATION ON HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSCs) are specific cells hence they can give rise to all blood types including myeloid and lymphoid lineages through a process called hematopoiesis. Destiny of HSCs is affected by different intrinsic and extrinsic factors and a poorly investigated extrinsic factor is chronic inflammation. Chronic inflammation represents long-term activation of immune system by enhancing proliferation and recruitment of mononuclear leukocytes. To study the effect of chronic inflammation on HSC fate we employed CMO (chronic multifocal osteomyelitis) mice, which develop bone and skin inflammation, increased levels of pro-inflammatory cytokines and extramedullary hematopoiesis. Limiting dilution assay showed increased bone marrow cellularity on expense of functionality in CMO mice. The aim of this project is to analyze transcriptome changes in HSCs during chronic inflammation and to check whether chronic inflammation promotes development of leukemia. To study alterations in inflammatory conditions we performed ATAC-seq and RNA-seq. Transcriptomic analysis revealed that CMO HSC upregulate expression of inflammatory genes and present a myeloid bias profile. Several loci exhibited open regulatory regions in chronic inflammatory conditions such as *ELANE, MMP9* and *Ltf*. Next step was to assimilate two data sets from ATAC-seq and RNA-seq. Mapping of transcripts was done using ENSEMBL database. Integration of data sets presented open chromatin accessibility and increased gene expression of *Ccl6, S100a8* and *S100a9* in HSCs from CMO mice. Next aim was to find link between chronic inflammation and leukemia. We employed *in vitro* and *in vivo* experiments using MLL-AF9 cell line. Results showed that chronic inflammation promotes pro-inflammatory profile in HSCs and fast expansion of MLL-AF9 induced leukemia.

TAKING A CLOSER LOOK AT THE ROLE OF SPLICING FACTOR MUTATIONS IN RETINITIS PIGMENTOSA USING A RETINAL ORGANOID MODEL

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Retinitis pigmentosa (RP) is one of the most common inherited retinopathies worldwide. It is characterized by a progressive loss of photoreceptors and shows remarkable clinical and genetic heterogeneity. While most mutations causing RP have been found in proteins essential for photoreceptor function such as rhodopsin some mutations are surprisingly affecting ubiquitously required splicing factors such as Prpf8. The mechanism of how these mutations exclusively cause RP in these patients remains elusive. Here we present the development of a human retinal organoid model to shed light on the underlying disease mechanism. Using CRISPR-Cas9 gene editing we introduced an RP-linked mutation into the regulatory Jab1/MPN domain of Prpf8 in human induced pluripotent stem cells (hiPSC) and differentiated them into three-dimensional retinal organoids. Our results show that retinal organoids differentiated from mutated hiPSC look-alike organoids from wild-type hiPSC containing both rod and cone photoreceptors as well as Müller and amacrine cells. However, transcriptome analysis revealed differential splicing of neural and retinal disease-associated genes as well as changes in expression of circular RNAs, while differential gene expression was only slightly affected. These data indicate that perturbation in splicing of specific genes and changes in expression of circular RNAs is causing the RP phenotype.

ENLIGHTENING THE QUIETNESS - THE SEARCH FOR EARLY SILENCED PROVIRUSES

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Integration of retroviral DNA into the genome of an infected cell is a hallmark of retroviral infection. Genomic and epigenomic features at the site of integration may determine the expression of retroviral genes. While some proviruses (integrated retroviral genome) are stably expressed for several months, other proviruses are silenced shortly after integration. In the case of HIV infection, provirus silencing is the first step leading to the establishment of the latent reservoir *in vivo*, the main hurdle in curing HIV infection. Thus, understanding the mechanisms involved in early proviral silencing is crucial for the functional cure of HIV. With the current methodology, it is possible to distinguish between transcriptionally active and inactive proviruses after 2-3 days post-transduction. However, whether the proviruses identified as transcriptionally silenced were silenced immediately after, or several hours post-integration remains to be unknown.

To discriminate between the early and immediately silenced populations of proviruses, we developed a novel approach. First, the sensor cells are generated using transposon-mediated insertion of a detection cassette. Later, a replication-defective retroviral vector transducing a recombinase gene is used as a detector. If the recombination is detected while concurrently not expressing the retroviral marker, the provirus was transcriptionally active after integration but was silenced shortly afterward. A population of cells with early silenced proviruses could then be obtained and further studied.

Our data from initial experiments demonstrate that the sensor cells can be prepared within 2-3 weeks using the *piggyBac* transposon system. Three days after transduction, a population of cells possessing early silenced proviruses is observed. Our data suggest that most human embryonic kidney cells (293T) transduced by avian sarcoma leukosis virus (ASLV) contain proviruses that were transiently active after integration. On the contrary, our results obtained by using previously established methods indicated that most of the ASLV proviruses undergo rapid silencing after the transduction of mammalian cells.

Our approach can be easily applied to virtually any model of retroviral infection. Acquired data will help to elucidate conditions under which a temporal proviral expression is observed as well as processes occurring shortly after proviral integration.

STRATEGIES FOR ACTIVATING CANONICAL RNAi IN HUMAN CELLS

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RNA interference (RNAi), sequence-specific mRNA degradation induced by double-stranded RNA (dsRNA), is the main antiviral innate immunity pathway in plants and invertebrates. In mammals, however, this pathway has been replaced by the interferon response, which relies on a set of protein sensors triggered by pathogen-associated molecular patterns. Endogenous full-length Dicer does not support RNAi well because it poorly processes dsRNA. In this project, we aim to boost RNAi in human cells by producing truncated Dicer variants which would have enhanced dsRNA-processing activity. For this purpose, we produced and tested truncated Dicer variants carrying deletions in the N-terminal helicase domain, which consists of HEL1, HEL2i and HEL2 subdomains. Up to this point, a combined △HEL1△HEL2 mutant is the most efficient RNA-inducing variant. Additionally, we tested Morpholino antisense oligos to induce exon skipping and produce a shorter Dicer variant from endogenously expressed Dicer. We report that it is possible to modify endogenous Dicer to enhance RNAi activity in human cells by altering splicing of endogenous nascent transcripts.

COMPUTATIONAL DETECTION OF FULL-LENGTH RETROTRANSPOSON INSERTIONS IN GENOME ASSEMBLY OF GOLDEN HAMSTER

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Retrotransposons are repetitive genetic elements that spread through the genome utilizing the "copy-and-paste" mechanism: insertions are first transcribed into RNA, then reverse-transcribed into cDNA and finally re-integrated into the genome at another locus. Retrotransposon insertions contribute to the significant portion of the mammalian genome content. For example, in the current assemblies of human and mouse genomes, approximately 42% and 39% of all genome sequences are annotated as retrotransposon-derived, respectively. In order to survive in the genome that is hostile towards their expression and ensure their dissemination, it is advantageous for the retrotransposons to express and transpose in the germline or during early development. Epigenetic reprogramming which happens during early stages of development necessary for the totipotency of the zygote provides an opportunity for their expansion.

By inserting themselves stochastically into chromosomes, retrotransposons are a threat to the genome stability and host genomes employ various tactics to silence them. Control of the retrotransposon expression is especially tight in the germline and in the early embryos. PIWI-interacting RNAs (piRNAs) is a germline-specific silencing mechanism targeting young retrotransposons and safeguarding "immortal" germline genome. Ribonucleoprotein complex composed of small RNA (called piRNA) and protein from PIWI subfamily of Argonaute nucleases forms the active component of the pathway. It recognizes retrotransposon targets based on the specificity provided by piRNA and silences it through PIWI protein functions – both by cleaving retrotransposon transcript in the cytoplasm and by directing repressive chromatin modifiers to its genomic origin.

With aim to assess abundance of retrotransposon-targeting piRNAs, we developed algorithm which identifies potentially active, full-length intact insertions of retrotransposon subfamilies in the genome assembly of golden hamster. Algorithm found 110 intact insertions of rodent-specific LTR retrotransposons called intracisternal A particle (IAP) as well as 110 insertions of intact non-LTR LINE1 elements from the Lx5/6 subfamily. Beside quantifying piRNAs antisense to intact retrotransposon insertions, this annotation allowed us to study their methylation status utilizing whole-genome bisulfite sequencing.

HEMATOPOIESIS IN SEA LAMPREY: HUMAN SWEAT AND TEARS AND LAMPREY BLOOD

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Hematopoiesis is a process specific for vertebrates during which terminally differentiated blood cells with the specific functions arise from a hematopoietic stem cells (HSC). In our project we are focused on the evolution of hematopoiesis. In common model organisms, the hematopoietic scheme is quite conserved so if we want to study it's evolution we have to reach for a phylogenetically older taxon of the vertebrates. Therefore we decided to study hematopoiesis of a sea lamprey as a member of a jawless taxon which happens to be phylogenetically the oldest taxon with still living representatives up to date. Does the lamprey hematopoiesis follow the common vertebrate scheme? Is the hematopoiesis in the lamprey established during an embryonic development the same way as we see in a classical models? To find out we studied lamprey blood cells with classical microscopic and histochemistry methods to map the lamprey blood cell specter. We implemented the method of an in situ hybridization to visualize the expression. We implemented a state-of-the-art sequencing experiments both on bulk and single cell level with a complex bioinformatics tools to get insight how the hematopoiesis in a sea lamprey looks on the genetic level. Does this bloody adventure have an end? Is it a happy end?

SPO11 KNOCK-OUT IN CHICKEN AS A WAY TOWARD THE GENETIC STERILITY AND EFFICIENT TRANSGENESIS

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Chicken is a valuable model organism, which however offers only limited possibilities for genetic manipulation and reverse genetics. Successful derivation and culture of primordial germ cells (PGCs) opened the way toward genome editing in chickens. The current protocols rely on the PGCs derivation from early embryos, their genetic modification *ex vivo*, and their subsequent reintroduction into embryos. This method was further improved by developing the orthotopic transplantation of PGCs into the testes of adult recipient roosters. The principal advantage of this approach is the gain of genetically manipulated heterozygotes instead of chimeras in the F1 generation. On the other hand, the recipient roosters have to be irradiated before transplantation which requires a long period of convalescence.

In this study, we present the generation of genetically sterile roosters by knock-out of the *Spo11* gene. Spo11 is an endonuclease introducing double-strand-breaks of DNA in early meiotic prophase I and its knock-out leads to genetic sterility in mice. We successfully produced spermatozoa with Spo11 frameshifting mutation after transplantation of Spo11 +/- PGCs into irradiated recipients. Although the F2 generation roosters Spo11 -/- were producing spermatids, their sterility was confirmed via intravaginal and intramagnal insemination. It was confirmed that Spo11 -/- roosters had morphologically normal testes with empty tubules before puberty and filled with spermatids after puberty. We detected a nucleotide sequence of a fluorescence marker in spermatozoa of Spo11 -/- roosters transplanted via PGCs. Analysis of the morphology and ploidy of Spo11 -/- spermatozoa will be shown. Currently, we are waiting for their offspring to hatch to confirm that Spo11 -/- roosters are suitable recipients of genetically manipulated PGCs and are able to produce viable offspring. This will further facilitate a new era in the technology of genetic modification in chickens.