Session 5: Transgenerational and epigenetic effects of chemicals

37 | Mechanisms underlying epigenetic effects of endocrine disruptive chemicals

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Epigenetic mechanisms drive critical processes during early development. Interference with these processes can permanently alter gene expression patterns, and thus affect cell and tissue functions. This, in turn, can lead to altered phenotypes in later life and increase susceptibility for diseases such as obesity, cancer, and psychiatric disorders. Growing evidence from human and animal studies suggest that endocrine disruptive chemicals (EDCs) induce such epigenetic changes. However, as yet little is known about the molecular mechanisms underlying EDC-induced epigenetic changes and their link to human health.

We have found that the EDC target estrogen receptor beta is involved in regulating DNA methylation at specific genomic regions by interacting with thymine DNA glycosylase, an enzyme involved in DNA demethylation. We also have indication that this interaction is disturbed by EDCs, thus providing a mechanism how these chemicals can induce DNA methylation changes. One goal is now to develop *in vitro* assays to monitor this interaction and test how it is affected by EDCs. Another goal is to link epigenetic changes induced by EDCs in cell models to neurodevelopmental and psychiatric disorders in humans. Understanding these mechanisms and connections is of great importance for risk assessment of EDCs and for developing sensitive methods to screen these chemicals for their potential to induce epigenetic changes.

38 | Exposure to copper during embryogenesis caused increased tolerance in subsequent generations, in the three-spined stickleback

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Aquatic environments worldwide are impacted by chemical pollution. The sustainability of fish populations within these polluted ecosystems is critically dependent on their ability to adapt to change via genetic and/or epigenetic mechanisms. We conducted a series of copper exposures in the stickleback to explore if prior exposure can result in altered susceptibility in later life or in subsequent generations. Stickleback embryos were exposed to 0.015mg/L copper during early life (0-9dpf), a concentration causing ~1.2% mortality, ensuring that selection for a tolerant genotype did not occur. Fish were then kept under control conditions until sexual maturation, when they showed differential responses to copper compared to a control population. Under control conditions, adult fish who had been pre exposed to copper during embryogenesis maintained a higher copper concentration in liver and gill tissue. In addition, fish pre-exposed to copper were able to uptake more copper when exposed to 0.01 or 0.02mg/L Cu in adult life, compared to the control population. Mortality curves on F1 embryos revealed that the embryos originating from parents who were exposed to copper during embryogenesis were significantly more tolerant to copper when compared to the control population kept in parallel. We also carried out mortality curves on F2 embryos in order to establish if this effect can be inherited across generations, and confirmed that similarly to that observed for the F1 generation, F2 embryos were also significantly more tolerant to copper compared to the control population. In addition, a greater proportion of these embryos were able to hatch under copper exposure when compared to the control population assessed in parallel. Our data supports the hypothesis that exposure to low levels of copper during early life has the potential to reduce the susceptibility of a vertebrate model in later life and across generations. In the future, we plan to explore the molecular mechanisms responsible for the differential susceptibility observed in this study.

39 | DNA methylation in the model organism Daphnia magna

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Daphnia, recently recognised as a model organism by the National Institute of Health, is gaining interest as a model for epigenetic research. The advantage of Daphnia is attributed to its cyclic parthenogenetic reproduction, allowing the separation of genetic and non-genetic effects. Additionally, its short generation time, ease of maintenance and increasing availability of genomic resources adds further value to this model. Therefore our aim was to generate comprehensive genome-wide methylome maps for Daphnia magna. To identify regulators of DNA methylation, we searched for homologs of the DNA methyltransferases in the Daphnia genome. DNMT1, DNMT2 and DNMT3 showed a low similarity to the human homologs. However, the conserved domain structure was very similar to human and to honey bee proteins. The expression levels of DNMTs were analysed with qPCR (at days 1, 5, 12, 21, 28). A dynamic change in expression was identified with age. DNMT1 expression increases after day 12 and it is maintained, while DNMT3 decreases after day 5, increasing again after day 28. The global methylation level was measured using LC-MS. Although the overall DNA methylation level is very low (0.14%) in Daphnia compared to humans, it is similar to other invertebrates. The methylome of D. magna was generated using Whole Genome Bisulfite Sequencing. Methylation almost exclusively occurs in a CpG context and is sparsely distributed, especially present along gene bodies. Moreover, DNA methylation profiles in Daphnia was affected by the chemical 5-azacytidine, a demethylating agent. Single-base resolution analysis revealed 3676 CpG positions differentially methylated after treatment with 5-azacytidine (3.7 mg L-1). These results demonstrate that despite the overall lower level of DNA methylation in Daphnia, its epigenome is responsive to stressors and thus has the potential to be used for epigenetic research.

40 | Differences between generations in the response to gamma irradiation in Arabidopsis thaliana

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Gamma radiation is an increasing contaminant of the environment due to nuclear medicine, nuclear energy production, milling and mining activities and the phosphate industry. To study the impact of gamma radiation on the environment it is important to study the long-term effects on plants and to reveal underlying mechanisms at a cellular and epigenetic level. Here, 7-days old Arabidopsis thaliana plants were exposed for 14 days to four different gamma dose rates: 20 mGy/h, 40 mGy/h, 85 mGy/h and 460 mGy/h. Three different plant groups were used: plants that were not exposed to gamma radiation before (P0) and plants that received the aforementioned gamma treatment during one (S1) or two (S2) previous generations. Analysis focused on phenotypic changes in the plants, antioxidative stress mechanisms and methylation of cytosine and transcription of genes in the methylation pathway as epigenetic markers. These latter endpoints were studied in three week old plants i.e. immediately after irradiation. Phenotypic analysis revealed that stem length and amount of seed buds generally increased with increasing dose rates. Differences between generations and treatments were also observed for seed weight: a decrease in seed weight with increasing dose rates in the P0 generation, which is in contrast with a dose rate dependent increase in the S2 generation. When looking at the antioxidative defence, an increase in the S1 generation was detected for catalase activity in the roots and for several peroxidases involved in cell wall modifications. The

increased activity of these peroxidases in the second generation can be taken as an indication that the increased formation of cell wall stiffening components is part of a plant's response to gamma irradiation. The overall percentage of the most prevalent epigenetic modification, 5mC, was analyzed as well. Results show a dose-rate dependent trend in all generations, with significant increases for the highest dose rates in the second (S1) and third generation (S2). Transcription of genes involved in methylation pathways generally followed similar patterns, with a dose rate dependent inducing trend in the P1, a decreasing trend in the S2 and significant differences for the third and fourth dose rate in the S1. In conclusion, results obtained so far suggest that plants adapt to consecutive gamma exposures as there are differences between generations for several phenotypic parameters, antioxidative mechanisms and at the level of methylation.

41 | The role of DNA methylation in AHR-mediated toxicity of PAHs in chicken embryo

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DNA methylation is an epigenetic mark that plays an important role in regulating gene expression. As with other epigenetic marks, patterns of DNA methylation are sensitive to environmental stressors (e.g. contaminants) and have the potential to be heritable (i.e. faithfully copied as cells divide, persisting beyond initial exposure). Here, we assess the role of DNA methylation in aryl hydrocarbon receptor (AHR) mediated toxicity of polycyclic aromatic hydrocarbons (PAHs) in the developing chicken embryo. Graded concentrations of the PAH, benzo[k]fluoranthene (BkF), were injected into the air cell of fertilized unincubated chicken eggs on embryonic day 0 (ED0). Liver tissue was sampled at several timepoints throughout development – ED7, ED10, ED19, and two days post-hatch (D2) – in order to assess BkF-associated patterns of cytochrome P4501A (CYP1A) mRNA expression and DNA methylation. Induction of CYP1A mRNA isoforms CYP1A4 (23-fold) and CYP1A5 (15-fold) was first observed starting on ED10, several days after the start of organogenesis. The transcriptional response was transient. By ED19, the signal was no longer present, a finding that corresponds well with previously published data suggesting that the developing chicken embryo rapidly metabolizes PAHs. In contrast, BkF-associated increases in DNA methylation at key regulatory sequences within the CYP1A4/5 shared promoter persisted through ED19 and were also detected in the hatched chick. We investigate how these persistent epigenetic marks may affect subsequent exposures to AHR ligands using a chicken embryo hepatocyte model. This work begins to explore linkages between early-life experiences in the embryo and later-life responses to PAHs.

42 | Intergenerational metabolic reprogramming in flies

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Given the rise in obesity and the parallel increase in comorbidities like diabetes, cardiovascular disease, stroke and cancer, it is exceedingly important to begin a systematic examination into heritable effects of diet and other environmental factors. The limitations of transgenerational rodent studies have made systematic and comprehensive examination of mechanisms underlying intergenerational reprogramming in mammals challenging. I will present a *Drosophila* model of paternal-diet-induced *I*nter-*G*enerational *M*etabolic *R*eprogramming (IGMR). Intriguingly, as little as two days of dietary intervention in fathers can stably reprogram offspring physiology, lifelong, confirming the responsiveness of mature sperm to physiological cues. Paternal sugar leads to H3K9/K27me3 dependent alterations that affect expression of metabolic genes in two distinct germline and zygotic windows. Novel findings provide evidence towards the involvement of the piRNA pathway in IGMR giving direction to the most difficult question challenging the field: What is the nature of the intergenerational signal? Critically, I will also provide evidence that a similar system regulating obesity-susceptibility exists in mice and humans.

The findings provide evidence that small changes in paternal life style can lead to drastic effects in offspring physiology. They also identify specific chromatin regulators and small RNA pathway members to be involved in either the generation of the signal and/or the establishment of the phenotype in the offspring, providing novel targets for therapeutic intervention.

43 | Effects of low dose gamma irradiation on histone modifications during zebrafish embryogenesis

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Epigenetic mechanisms allow gene regulation in a developmental context and as a response to environmental stimuli. The best studied epigenetic mechanisms are methylation on cytosine and post translational modifications (PTMs) on histone proteins. Chromatin immunoprecipitation (ChIP) is the standard assay of choice for analyzing the genomic localization of histone PTMs. The aim of this study was to assess epigenetic changes in zebrafish embryos after exposure to low doses of external 60Co gamma radiation. Early blastula AB wild type zebrafish embryos were irradiated for 3h at dose rates ranging from 0.5 to 40 mGy/h. Samples were collected at the early gastrula stage, 5.5 hours post fertilization (hpf), and ChIP analysis carried out on pools of 100 embryos. RNA-seq data showed a clear dose response relationship between the number of differentially regulated genes compared to controls, which increased with the gamma radiation dose. Analyzis of the gene expression data sets using Ingenuity Pathway Analysis (IPA) showed significant changes in gene networks around hnf4α (hepatocyte nuclear factor 4α) and cebpa (CCAAT/enhancer binding protein), whose promoters were used as candidate loci for histone PTMs inspection. After exposure to the lowest radiation dose, histone H3 revealed a significant enrichment of nucleosomes at all investigated loci. This suggests a global compactness and rearranging of the genome as a response to external stress. ChIP of H3K4me3, H3K27me3, H3K9me3 and H3K9ac did not correlate well with transcriptional status after irradiation. Interestingly, normalizing histone PTM enrichment against nucleosome density revealed a PTM and loci dependent dose response enrichment as function of gamma radiation dosage. At bactin2, which is not differentially regulated, there is a decline in enrichment of H3K4me3 and H3K9ac at the lowest gamma radiation exposure compared to the control, and this is followed by successive higher enrichment at the higher radiation dose rates. In conclusion, zebrafish appears as a sensitive model for low dose exposure experiments and ChIP is a sensitive method for detecting epigenetic changes at histone PTMs.

44 | Differential DNA Methylation in F2 Generation Testicular Cells Caused by Embryonic Exposure to Bisphenol A at F0 Generation

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Bisphenol A (BPA) is a compound used primarily to manufacture polycarbonate plastics and epoxy resins. It is also an additive in other products. Due to extensive use of BPA in commercial products, the threat to human and wildlife health posed by BPA-containing waste in the environment is a potential concern. We have previously observed transgenerational reproductive defects in unexposed F2 and F3 offspring of Japanese medaka (Oryzias latipes) caused by embryonic exposure of F0 offspring to BPA (Bhandari et al., 2015, Scientific Reports 4: 903). Here we report molecular

changes, specifically transcript abundance and DNA methylation of select promoters, in the testis of F0 fish (directly exposed as embryos) as well as in the F2 and F3 fish that had transgenerational phenotypes of impaired fertilization capacity and increased embryo mortality. BPA induced subtle changes in DNA methyltransferase enzyme expression in germ cells of the F0 adults exposed during embryonic development, and expression of Dnmt genes (Dnmt1, Dnmt3aa, Dnmt3bb) increased 2- to 10-fold in germ cells of the F2 males, accompanied by 2.5-fold increase in global DNA methylation. DNA methylation of the estrogen receptor promoter was significantly reduced in germ cells of F0 but was not altered in F2 germ cells. Elevated DNA methylation levels were maintained at the CpG island of androgen receptor (AR) core promoter of both testicular germ cells and somatic cells in the F2 generation. As expected, the expression of the AR gene in testicular somatic cells was significantly decreased, which confirms an inverse relationship between DNA methylation and gene expression. Together, these findings provide insights into transgenerational inheritance of BPA-induced epigenetic marks by germ cells and soma at the F2 generation.