Draft Report
CERAD UMBRELLA 5 TRANSGENERATIONAL EFFECTS / EPIGENETICS MEETING, NMBU Campus Adamstuen, 25 August 2016.

Attendants:
Jorke Kamstra, Peter Aleström, Leif Lindeman, Jan Lyche Ludvig, Knut Erik Tollefsen, Jens Thaulow, Ian Mayer, Terje Christensen, Lena Sareisian, Selma Hurem, Godfrey Etokebe, Ann-Karin Hardie Olsen, Nur Duale, Leonardo Martin Martin, Erik Rasmussen, Hallvard Haanes, Hans Christian Teien, Dag Anders Brede, Dag Marcus Eide, Erica Maremonti, Christine Instanes, Brit Selbu, Deborah Oughton.

Goal of the CERAD Umbrella 5 epigenetics project meeting was to present progress regarding epigenetic analyses within CERAD selected model organisms and discuss further collaboration and future plans. With 23 participants, all CERAD partners involved in the subject area, except for representatives from the plants section, attended the meeting.

The meeting was opened by Peter Aleström, who stressed out the fact there are several collaboration axes established within UMB5, and the focus on science dedicated to this meeting. We should focus on the time up to the midterm evaluation and postpone strategies for the next 5 years until the evaluation outcome can no longer be pushed.

Presentations by NIVA, NIH, NMBU-IMB and NMBU-Vetbio

1. Jens Thaulow, NIVA: *Dahpnia Magna* epigenetics.

Jens presented results from exposures with two compounds that should affect global DNA methylation, 5-azacytidine (5AC, DNA methylation inhibitor) and nickel. Initially they found a relative huge amount of hmC regardless of exposures, but it could not be replicated in follow up experiments. The overall DNA methylation percentage was found around 0.1%.
Future experiments, regarding DNA methylation analyses will focus on optimization of the method, using the positive controls.
Histone modification enrichment was measured around promoter regions of genes responsible for DNA methylation, the DNA methyltransferases (Dnmts). Exposure effects on the histone modification H3K4me3 were associated with expressed genes. H3K4me3 was more enriched at Dnmt1 following exposures to 5AC compared to the promoter region of Dnmt3a, whereas Dnmt3b was unaffected.
The plan is to perform gamma radiation exposures in November 2016 at Figaro. Jens stressed out that their focus should be on transgenerational experiments.
NIVA has full access to the Daphnia genome sequence via Janna Asselman in Ghent.
During the discussion Anka remarked that high levels of hmC are found in *D. pulex* (Strepetkaitė et al 2016).
Brit noted that whole organism tissue analyses could hide tissue specific effects. Jens replied that it is hard to extract tissues from Daphnia's due to small size and low amounts of material.
Dag wondered about the significance for gene expression control of the low 5mC levels in Daphnias, which according to Jens could be the plasticity of the genome or different epigenetic mechanisms playing a more important role.

2. Anka Ann-Karin Hardie Olsen and Nur Duale, FHI: Epigenetics in radiation exposed mice.

FHI performed radiation experiments in mice with different additives (Selenium and Arsenic). There were adverse effects with different exposure setup, like genotoxicity, reproductive effects, behavioral effects and investigation is going on regarding colon cancer. A first paper about effects of radiation exposure in combination with SeIR + Se-diet with C57BL/6 WT and Ogg1 KO mice is accepted in *Scientific Reports*.

Regarding epigenetics analyses were performed on global methylation and locus specific methylation with pyrosequencing on maternally imprinted genes, transposable elements and paternally imprinted miRNAs. Albeit these analyses were based on solid rationales, no changes were found in any of the experiments. However, changes could very well be present outside the analyzed targets.
Future plans is to extend the DNA methylation analysis with SMRT sequencing or reduced representation bisulfite sequencing (RRBS). Additionally, changes in histone marks will be investigated.
Nur presented work on gene expression/miRNA patterns and showed differentially expression genes following radiation exposure in testes of Ogg-/- mice and WT mice. He argued for using qRT-PCR rather than more expensive RNA-seq. Only with Ogg-/- mice a clear separation between controls and exposed. Several pathways were affected in both lines, but there was very little overlap in genes affected by radiation exposures. A nice method for measuring a battery of miRNAs was presented and is still in development.

3. Dag Anders Brede, NMBU-IMB: Differential gene expression in Salmon.

Gene expression presented following exposure to radiation at increasing dose rates. The initial experiments on miSEQ at Campus Ås generated not enough reads. Follow-up with Nextseq analysis and 16 million reads. From the 1, 10 and 40 mGy/h dose rates, the 40 mGy/h showed the highest number of differentially expressed genes (DEGs), dose rate response with expressed genes. Dag showed many pathways affected that could be related to observed phenotypes, such as impaired vascularization, hemorrhages and eye development. He stressed out the importance of looking at splice variants with the example of p53 in salmon. As possible evidence for epigenetics mechanisms, there were several DEGs involved in chromatin organization, regulation of chromatin organization.
Future plans will focus on DEGs and histone marks in gastrula stage embryos, to be comparable with zebrafish data from NMBU-Vet.
Remark by Brit: We should try to develop a cross species exposure protocol in order to better compare the different effects over species. Dag suggests using zebrafish as the standard in this case and focus on gastrula stage embryos.

4. Jorke Kamstra, Leonardo Martin and Leif Lindeman, NMBU-Vet: Zebrafish epigenetics and transgenerational effects.

Jorke gave an overview of basic epigenetic mechanisms and the importance of epigenetics in AOPs and transgenerational non-genetic inheritance. He also emphasized the interplay between different epigenetic mechanisms and the resulting epigenetic "landscape". Three main methods for DNA methylation studies, together with their cost for different species (depending on genome size), were discussed: (i) Global methylation, (ii) genome-wide BIS-seq and (iii) locus specific BIS-seq. Results from recent RRBS analysis of 6 dpf zebrafish larvae exposed to MEHP, revealed many differentially methylated regions (DMRs). Pathway analysis (IPA) of DMRs show compound specific pathways affected. Ongoing analysis of whole genome BIS-seq (WGBS) datasets from zebrafish exposed to gamma radiation during gametogenesis reveal a vast number of DMRs in F1 embryos (5.5hpf), located predominantly at promoter regions. The results will be followed up with PCR in in F2 and F3 generations. Plan is a manuscript to be ready for publication by the end of 2016.
Leonardo presented the complexity of small RNAs in general and during zebrafish embryogenesis as part of his thesis work. For RNA-seq datasets generated from same experiment as the WGBS described by Jorke. In the ongoing bioinformatics analysis Leonardo focus on piRNA, miRNA and 5' tRNA halves, which have already shown to be sensitive following compound exposures in rodent studies (Schuster et al 2016), and results are to be expected within 2016.
Leif presented his results regarding post translational histone modifications. Genes affected during embryogenesis, *cebpa, hnf4a and vegf,* show radiation sensitive effects on histone modifications.

The most interesting finding is the non-monotonic dose rate response when corrected to histone H3 levels, indicating that maybe even lower dose rate exposures should be considered.

General discussion.

Deborah pointed out three focus area keywords:

- Biomarkers (gene expression and epigenetic marks)
- Transgenerational (epigenetic) effects
- The epigenetic landscape with interplay between DNA methylation, histone modifications and miRNAs correlated to gene expression and functional phenotypes.
- Comparative studies between the CERAD selected model species

Dag suggested zebrafish as a core animal model for these comparative studies. He believes it would be wise to use the zebrafish data to formulate hypotheses to test in other models rather than performing experiments as if we had no a priori knowledge.

The urgent need for bioinformatics resources was discussed:

- nodes and data backup
- training to include more partner scientists/students in more advanced analyses

Brit remarks that UMB3, 4 and 5 should discuss this and propose budget plans for bioinformatics. This needs to be followed up.
Peter noted that Jorke and Leonardo participate in the Norwegian Bioinformatics Research School NORBIS which is open for all.

Brit noted that field experimentation should be possible maybe with epigenetic markers found in the lab experiments. There are two professors from Kiev and Fukushima collaborating within the CERAD project and this might give opportunities to do epigenetics in human cohorts

A CERAD reproduction seminar is planned for in November (Ian Mayer). Details will be announced later.

Peter concluded with that we already are collaborating a lot in different axes on several levels and that it should be the short term priority to further strengthen those activities – and finally that we should aim at having more of these scientific presentations