Innovative investigations in the host-microbiota crosstalk in dysbiosis

Small RNAs and mitochondria

Background and Project Idea

This project aims to shed light on a newly proposed crosstalk mechanism, by investigating the role of small RNAs and mitochondria in the host-microbiota interface in dysbiosis.

Dysbiosis, defined as the perturbation of the ecology of the gastrointestinal microbiome, is often accompanied by pathological alterations to the gut epithelial barrier. The weakening of the tight-junctions that confer the gut barrier its impermeability opens the path to the translocation of pathogens and toxins, impairing the overall animal health.

Mitochondrial activity is crucial for the stability of epithelial tight-junctions[1]. Research has shown that preserving the functional integrity of mitochondria can reduce the damages to the epithelial barrier as well as bacterial translocation[2]. Several studies have highlighted the possibility of an existing crosstalk between mitochondria and microbiota, mostly based on metabolites production, especially volatile fatty acids[3,4].

We propose that the crosstalk is not only metabolite-mediated, but also RNA-mediated. Small RNAs (sRNAs) in eukaryotes function as post-transcriptional regulators and communication molecules. Although their function in prokaryotes has not been fully elucidated yet, they might be produced and released as signalling molecules to trigger group behaviours or reactions from other prokaryotic cells as well as to potentially affect gene expression. Small bacterial RNA expression has been demonstrated to be altered in dysbiosis in parallel with the microbiome[5].

Therefore, the hypothesis of this project is that alterations to the regular patterns of sRNA expression and exchange of information between the microbiome and the host might result in an impaired function of the gut epithelial barrier.

The idea behind the experiment is that sRNAs of prokaryotic origin can translocate into the host epithelial cells and reach the mitochondria, where they induce damages to the organelles, in turn causing a disruption of the gut epithelial barrier. In addition, it is possible that these nucleic acids produced by the microbiome reach the bloodstream and affect other organs beyond the gut[6].

Experimental setup

The experiments were planned to identify differentially expressed small bacterial RNAs in the colon, liver and visceral fat of mice with chemically induced dysbiosis and impaired epithelial barrier, through the optimization of a bioinformatics pipeline combining small RNA-seq and metagenomics.

Microbiome composition and epithelial barrier integrity will be assessed. Differential sRNA expression in the colon content, liver and visceral fat will be correlated with parameters of mitochondrial dysfunction, to identify potential candidates responsible for the observed alterations and therefore of the host-microbiome crosstalk.

The evaluation of several mitochondrial integrity and regulation markers will allow to elucidate whether the alterations of these organelles in the gut might be induced also in the adipose tissue and the liver. The gene expression of cytochrome b (CYTb), ATP synthase membrane subunit 6 (ATP6), and ribosomal protein L24 (RPL24), among others, will be evaluated to assess mitochondrial activity. The

integrity of the epithelial barrier will be assessed by analysing the gene expression of tight-junction proteins (claudin-2 (CLDN2) and occludin (OCLN)). Furthermore, histological and immunohistochemical evaluations will be performed to assess the morphology and alterations of the epithelium after colitis induction, the number, size and morphology of the mitochondria as well as the expression of tight-junctions (OCLN, CLDN) and mitochondrial metabolism (collaboration with the University of Camerino, Italy).

Genomic DNA/mitochondrial DNA ratio will be calculated using digital PCR in collaboration with VetCore (Core Facility for Research of the University of Veterinary Medicine Vienna).

After some crosstalk candidates are found, we will test for the presence of the molecules in the cells in sections of the colon, including the visceral adipose tissue, and the liver, using fluorescence *in-situ* hybridization (FISH). The combination of RNA FISH and immunofluorescence staining of the mitochondria will allow us to co-localize the RNA strands and their distribution within the cells.

Summary of the Project's Results

The experimental setup was effective in inducing colitis, based on daily clinical observations of the animals and weight loss.

The fecal microbiota composition was evaluated with 16S rRNA gene sequencing. The microbiota was comparably similar in the control and treatment groups before the chemical induction of colitis. Shifts in microbial composition in the treatment group after colitis induction confirmed the onset of dysbiosis.

While the protocols and PCR conditions are being optimized per each sample type and primer pair, preliminary results for gene expression indicate an impaired mitochondrial function in the colon.

The genomic DNA/mitochondrial DNA ratio preliminary results also confirm the reduced number of mitochondria in the colon tissue.

Small RNA sequencing has been performed and bioinformatic analyses are ongoing.

In-situ hybridization protocols will depend on the results of the bioinformatics and statistical analyses.

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