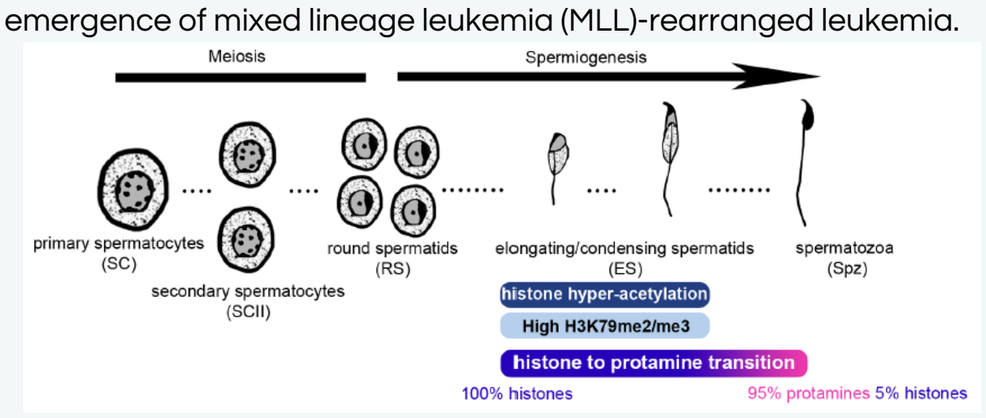
DEVELOPMENT OF A CELL MODEL TO INVESTIGATE THE IMPACT OF DOT1L ON MITOCHONDRIAL METABOLISM

What is the role of Dot1l?

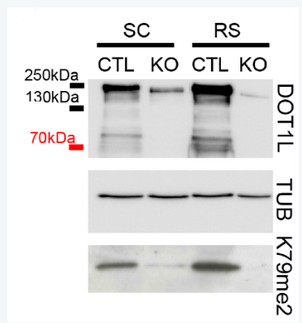
Dot1l is the only H3K79 methyltransferase. Postnatally, it is highly expressed in male germ cells, in particular during the postmeiotic phase called spermiogenesis. During that phase, histones are hyperacetylated, which facilitates the opening of the chromatin, and then progressively substituted by protamines to allow the high condensation of the genome in the spermatozoa. At the end of the process, only 5% of histones are maintained in mature spermatozoa.

Using a mouse model, in which Dot1l gene is specifically knocked-out (KO) in postnatal germ cells, the Cocquets's lab has found that DOT1L and H3K79 methylation are essential for the replacement of histones by protamines (Blanco et al., submitted).
Surprisingly, they also found that, in addition to its impact on chromatin remodelling, Dot1l KO deregulates mitochondrial metabolism and that Dot1l can interact with PDHE1, a protein of the pyruvate dehydrogenase complex (PDC) in the mitochondria. PDC in the mitochondrial matrix is involved in the conversion of pyruvate in Acetyl-CoA that will be used in the TCA cycle. Importantly, some PDC enzymes have been found to be nuclear, and Acetyl-CoA is the precursor of histone acetylation.

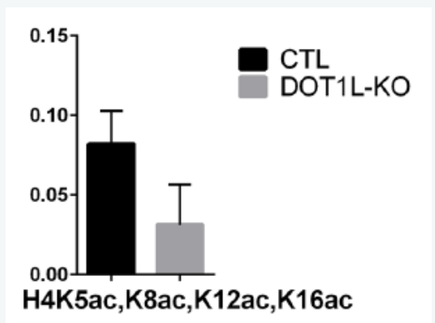
The relevance of Dot1l is due to its pleiotropic effects and its mutations, depending on the cell type and the developmental stage, can have various outcomes: from infertility and embryonic failure to cardiac misfunctioning. It is also involved in the emergence of mixed lineage leukemia (MLL)-rearranged leukemia.



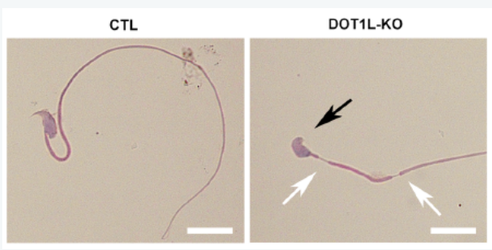
Previous results



Western Blot detection in germ cells protein extracts [1]



Mass Spectrometry of whole testis extract [1]

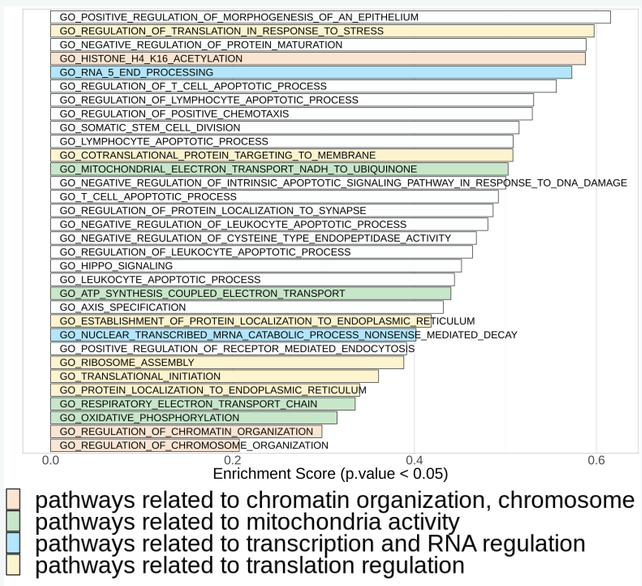


Representative pictures of Dot1l-KO and CTL spermatozoa [1]

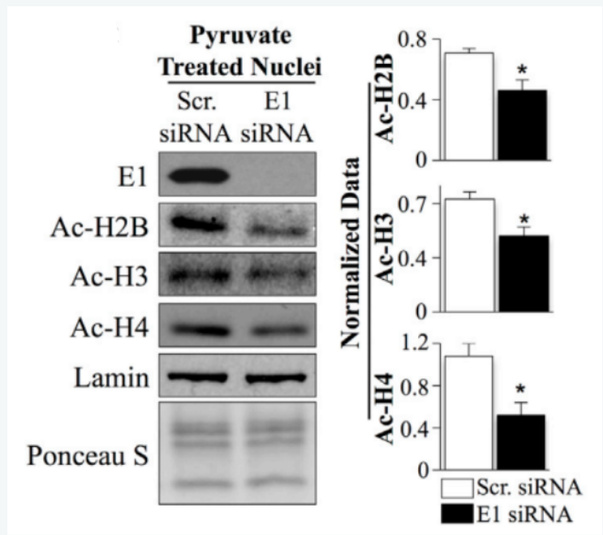
Number of peptides

Protein	DOT1L CO-IP (ab64077)				DOT1 CO-IP (CST D4O2T)				IgG CO-IP			
DOT1L	14	14	14	18	21	13	17	20			-	
AF10	8	8	14	14	14	16	14	16			-	
PDH E1α (testis)	32	31	28	31	3	3	-	1	4	2	-	3
PDH E2	20	20	22	23	4	1	2	2	1	2	-	-
PDH E1β	19	21	32	31	1	1	-	1	6	1	-	3
PDH X (E3BP)	11	12	16	18	-	-	-	-	-	-	-	-
PDH E1α	4	6	16	20	-	-	-	-	-	-	-	-
PDK3	4	3	2	2	-	-	-	-	-	-	1	-

Mass spectrometry analysis of Dot1l Co-Immunoprecipitation on protein lysate from WT testis [1]



GSEA analysis of Dot1l -KO vs CTL RNAseq [1]



Western Blot detection of acetylation on Pyruvate Treated Nuclei [2]

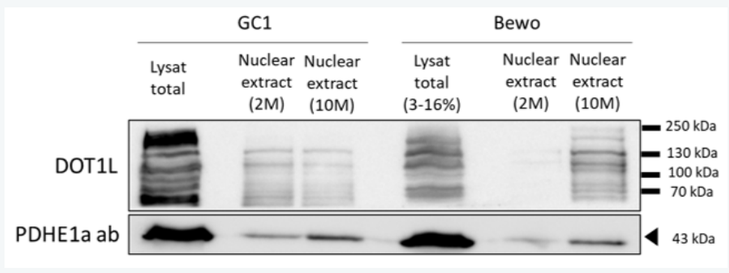
Project

Previous results suggest that there is a link between the histone methyltransferase Dot1l, histone acetylation, and mitochondrial metabolism, but the underlying mechanism remains unclear. To further investigate this link, it is necessary to move to a different model: cell lines, as metabolic analyses are complicated to perform "in vivo".
As it is impossible to culture meiotic and postmeiotic male germ cells, 2 different cell lines will be used, that have already shown to express both Dot1l and PDHE1 in the nucleus. Preliminary analyses indicate that transfected siRNA can induce an efficient knockdown of Dot1l.

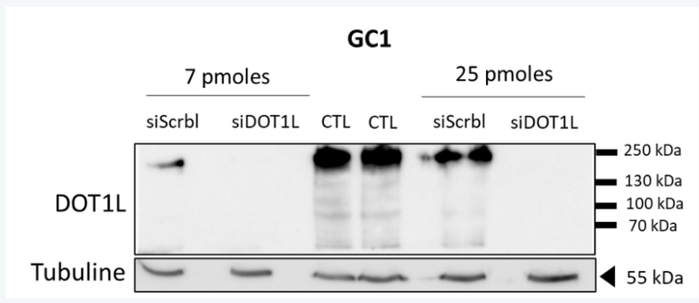
- GC-1: immortalized mouse spermatogonia cells;
- BEWO: human placental cell line.

Questions to answer:

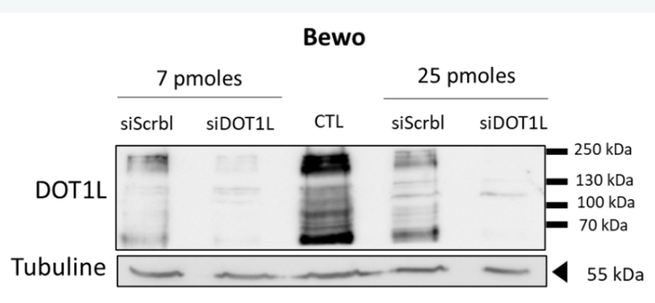
1. Does Dot1l knockdown have the same impact on gene expression in these cell lines than in male germ cells?
   - RT-PCR: for selected genes (found deregulated in previous RNAseq study);
   - RNA-seq: to have a complete view of differentially expressed genes;
2. Does Dot1l knockdown in these cell lines deregulate chromatin?
   - Quantify histone acetylation by Western Blot and Mass Spec;
3. Does Dot1l knockdown in these cell lines deregulate mitochondrial metabolism?
   - Metabolic analysis with seahorse analyzer and Mass Spec;
4. Is this effect mediated via its methyl transferase catalytic domain or not?
   - Comparison with the use of inhibitor (EPZ004777).



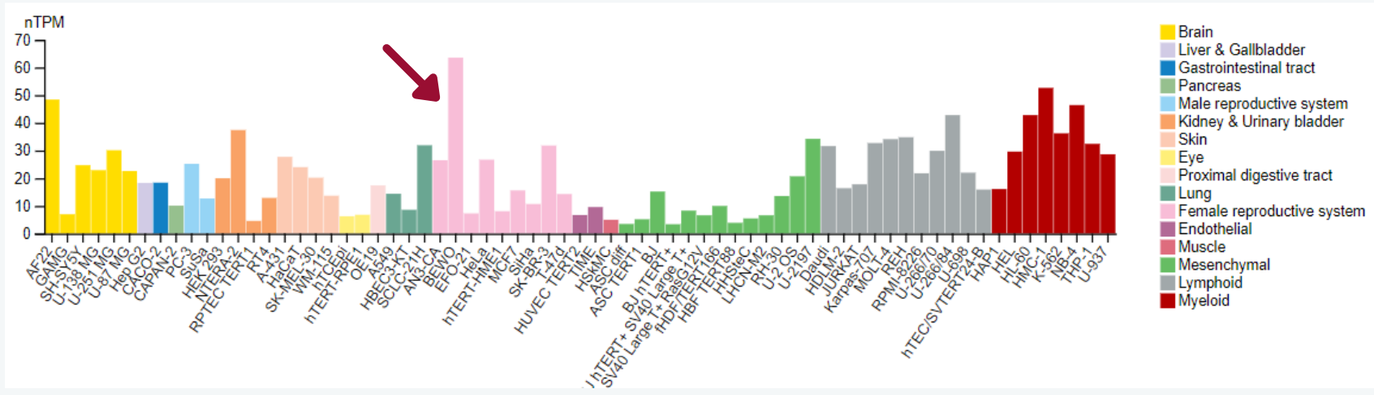
Western Blot detection in nuclear extracts [preliminary results]



Dot1l knockdown in GC1 cell lines [preliminary results]



Dot1l knockdown in BEWO cell lines [preliminary results]



Dot1l expression in different cell lines [proteinatlas.org]

[1]Blanco et al. The histone methyl transferase DOT1L regulates chromatin reorganization and mitochondrial activity, and interacts with the Pyruvate Dehydrogenase Complex in differentiating male germ cells (submitted)
[2]Sutendra G, Kinnaird A, Dromparis P, et al. A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation.

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