The role of m6A mRNA modification during endothelial-to-mesenchymal transition

Benedetta Zuliani, Loïc Ongena, Margaux Claes, Jonathan Bruyr, Franck Dequiedt

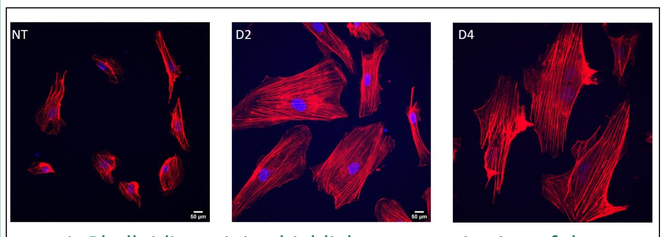
Laboratory of Gene Expression and Cancer, GIGA-Research Institute, University of Liège (Belgium)

Introduction

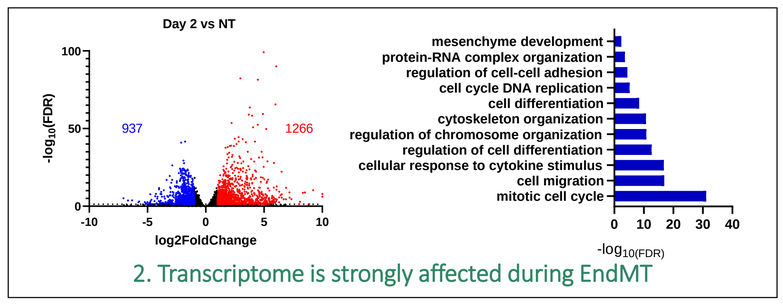
In response to environmental and mechanical stimuli, fully differentiated endothelial cells (ECs) can dedifferentiate into mesenchymal cells. This process is termed endothelial-to-mesenchymal transition (EndMT). The tumor microenvironnement is characterized by a pro-inflammatory context, hemodynamic abnormalities and hypoxia, all of which are known to promote EndMT. Recently, several studies have suggested that EndMT might be controlled by epigenetic mechanisms. N6-methyladenosine (m6A) is the most common mRNA internal modification and has been one of the main focuses in the field of epitranscriptomics over recent years. The EndMT m6A epitranscriptome has not been investigated yet. Our objectives are: 1) investigate the role of the m6A epitranscriptomic machinery in EndMT, 2) Identify transcripts whose m6A content is specifically affected during EndMT and 3) SNAI1 might be a primary target for EndMT-associated m6A reprogrammation..

Results

1. Characterization of EndMT in HUVEC cells

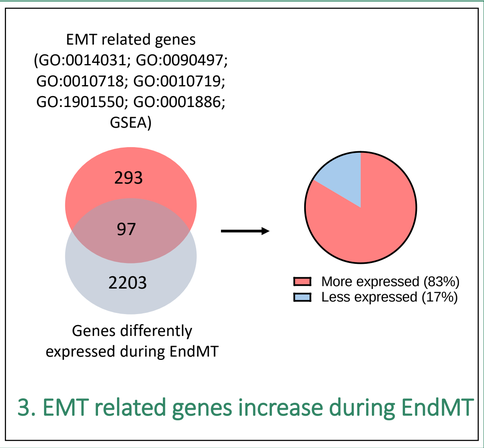


1. Phalloidin staining highlights reorganization of the cytoskeleton in HUVEC cells during EndMT



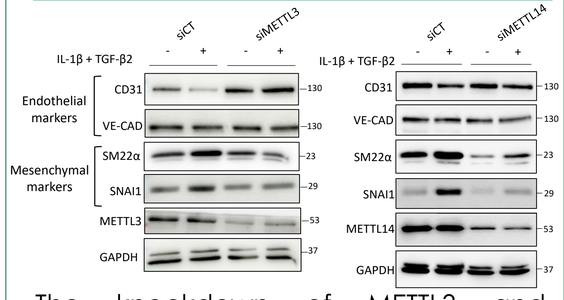
2. Transcriptome is strongly affected during EndMT

After the treatment of HUVEC cells with EndMT-promoting stimuli TGF-β2 and IL-1β, we verified the dedifferentiation of ECs by phalloidin staining and RNA-seq.



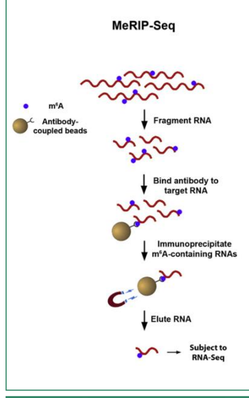
3. EMT related genes increase during EndMT

2. Unravelling the role of m6A during EndMT



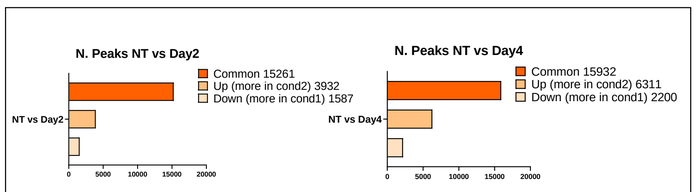
The knockdown of METTL3 and METTL14, members of the enzymatic complex responsible for m6A deposition, inhibits IL-1β and TGF-β2-induced EndMT. This suggests a crucial role for the m6A machinery in the promotion of EndMT.

3. Defining the EndMT-dependent m6A epitranscriptome

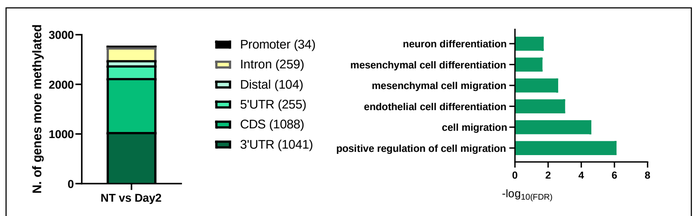


Conditions	Motifs	p value
CT	AUGGAC	1-e164
DAY 2	ACGGAC	1-e187
DAY 4	AUGGAC	1-e187

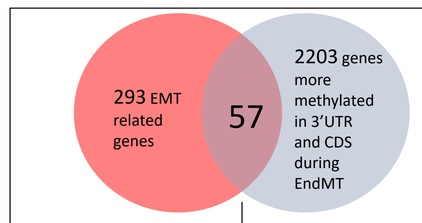
1. The m6A consensus motif is highly enriched in meRIP peaks for all conditions



2. We identified 20,000 called peaks, around 25% of which are decreased (down) or increased (up) during EndMT



3. The majority of the differentially methylated sites localize in 3'UTR and CDS of the transcripts

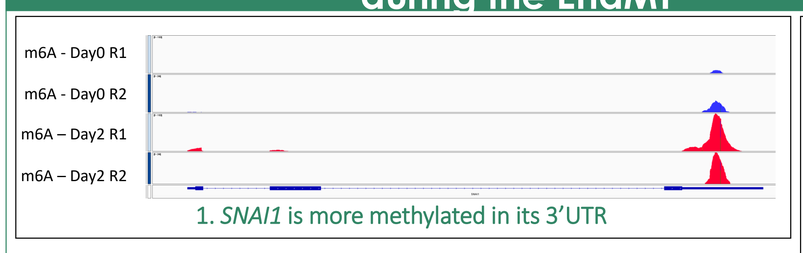


3 EndMT-related TFs: - **SNAI1**
- SNAI2
- MSX1

4. 20% of all EMT-related genes are hypermethylated in their 3'UTR and CDS at day 2 during EndMT

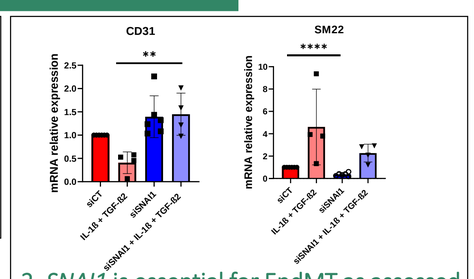
To gain a more complete understanding of EndMT-associated changes in m6A (i.e. EndMT m6A epitranscriptome), we performed methylated RNA immunoprecipitation sequencing (m6A-seq2, as described in Schwartz et al. Nature Methods 2021) at Day 0, 2 and 4 following either EndMT-inducing or no treatment of HUVEC cells.

4. m6A deposition on the 3'UTR of *SNAI1* changes during the EndMT



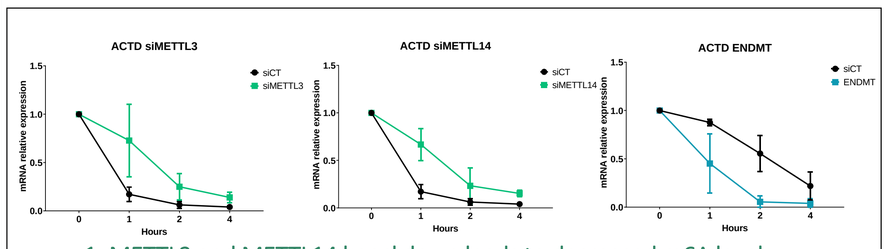
1. *SNAI1* is more methylated in its 3'UTR

The *SNAI1* transcript, which encodes a transcription factor involved in the early step of EndMT promotion, is more methylated in its 3'UTR after two days of treatment



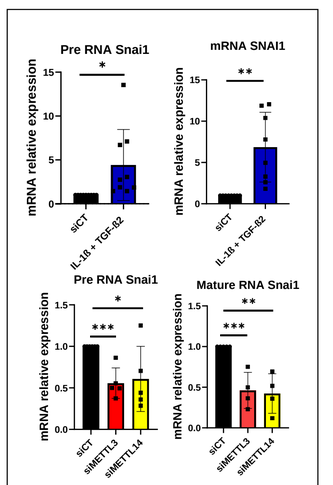
2. *SNAI1* is essential for EndMT as assessed by qPCR analysis of mesenchymal and endothelial markers

5. Differential methylation of *SNAI1* affects stability and expression of the transcript



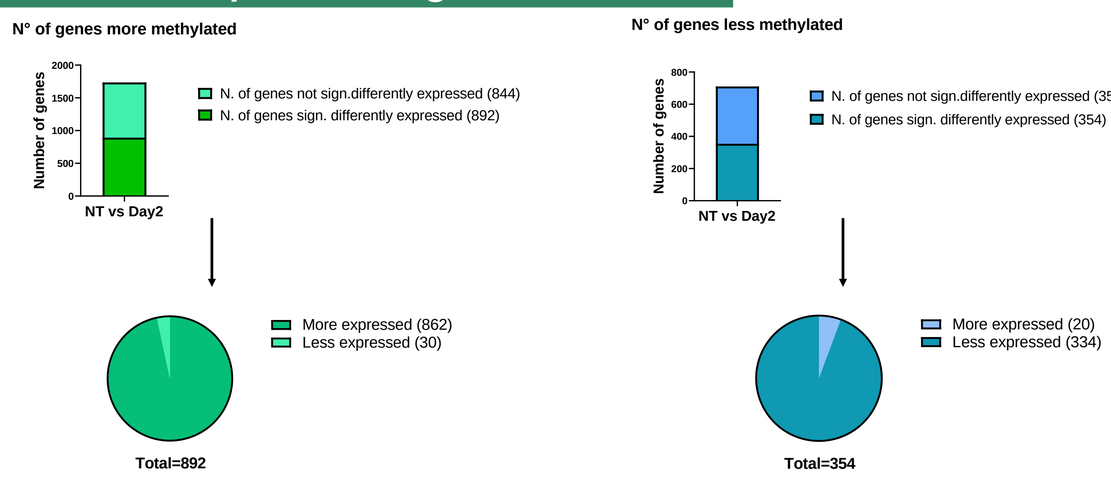
1. METTL3 and METTL14 knockdown leads to decreased m6A levels which promotes the stability of the transcript of *SNAI1*

m6A has an impact on the transcription of *SNAI1*

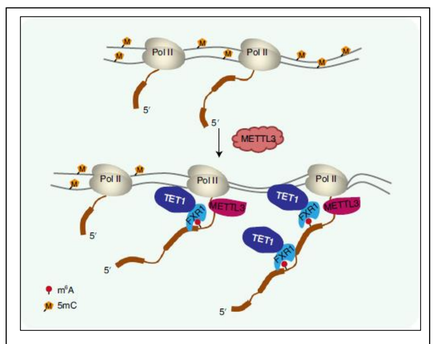


2. Knockdown of METTL3 OR METTL14 reduces synthesis of SNAI1 pre-mRNA

6. General correlation between m6A and transcription during EndMT

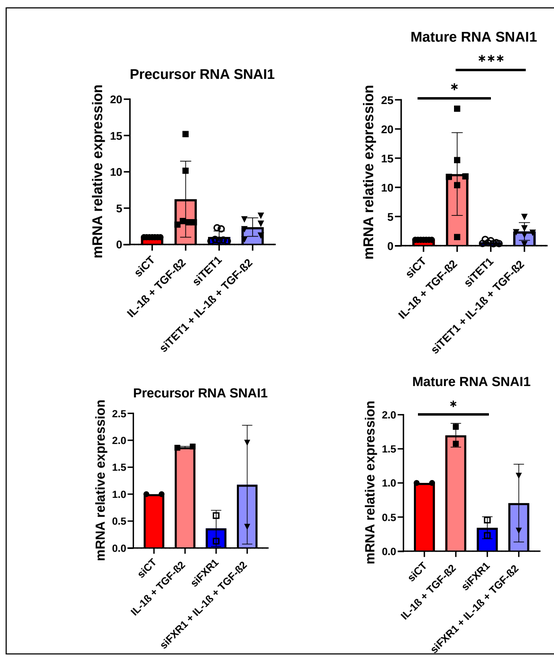


7. Possible correlation between m6A and 5mC



RNA m6A regulates transcription via DNA demethylation and chromatin accessibility (Deng et al., Nature Genetics 2022)

1. TET1 is recruited by the m6A reader FXR1 and this has an impact on the accessibility of chromatin



2. Knockdown of TET1 or FXR1 prevents the induction of *SNAI1* by EndMT

Conclusion & Perspectives

We established that METTL3 and METTL14 are necessary for EndMT induced by IL-1β and TGF-β2, demonstrating the role of the m6A machinery in the promotion of the EndMT. By performing m6A-seq analysis, we highlighted thousands of transcripts whose m6A profile is modified during EndMT. We further decided to focus on a specific m6A deposition on the 3'UTR of *SNAI1*, a transcription factor involved in the early steps of EndMT, and demonstrated that differential methylation of *SNAI1* transcript affects its stability. However, methylation of *SNAI1* 3'UTR reduces its stability and therefore cannot explain EndMT-induced increase in *SNAI1* expression. In addition to this, we also established that m6A has an impact on the transcription of *SNAI1*, probably via an interplay between the m6A and the DNA modification 5mC machineries, that we are currently investigating. All in all, our work provides support for the importance of the m6A epitranscriptome during EndMT differentiation.

télévie
fnrs LA LIBERTÉ DE CHERCHER
Fondation Léon Fredericq
LIÈGE université GIGA Molecular and Computational Biology

benedetta.zuliani@uliege.be