Unravelling the structural-functional complexity of a conserved long non-coding RNA

Liubov Makarova[1,2], Karina B. Sabalette[1,3], Isabel Chillon[4], Caroline Surcis[4], Robert Feil[4], Marco Marcia[1,2,3,*]

[1]EMBL Grenoble, France, [2]Uppsala University, Sweden, [3]Istituto Italiano di Tecnologia, Italy, [4]Institute of Molecular Genetics of Montpellier, France.
*E-mail: marco.marcia@icm.uu.se

Long non-coding RNAs (lncRNAs) are transcripts of more than 500 bases. They participate in vital cellular pathways by regulating gene expression and by scaffolding subcellular compartments. Consequently, lncRNAs are associated with human pathologies.

To establish sequence-structure-function correlations and explain the molecular mechanism of lncRNAs, secondary structure mapping by chemical (SHAPE) or enzymatic probing, remain conventional approaches. Importantly, emerging evidence acquired by low-resolution single-particle imaging methods, such as AFM, or by hydrodynamic techniques, such as SAXS and AUC, has identified lncRNAs whose 3D structure is important for their functions. However, no lncRNA 3D structures are currently resolved by conventional high-resolution methods. Structure prediction algorithms also perform poorly for lncRNAs, due to the length of these transcripts, to the low number of available RNA structures, and to the fact that it is still difficult to accurately analyse RNA evolutionary conservation. As a result, lncRNAs are still not exploited in human medicine.

To establish a robust approach to characterize lncRNA-mediated molecular mechanisms, here we focus on the maternally expressed gene 3 (MEG3) lncRNA, because this lncRNA has cellular, developmental and molecular roles. Indeed, MEG3 is a tumour suppressor, which acts *in trans* on the p53 pathway. It also contributes *in cis* to the imprinted gene expression at the *Dlk1-Dio3* locus, which is crucial for neural development and behaviour[1].

By integrating SHAPE, AFM and cell-based functional assays, our lab previously established that the MEG3 secondary and tertiary structures are essential for p53 regulation *in trans* [2,3]. Here, we determined the structure of mouse MEG3 to establish evolutionary sequence-structure correlations. Based on our results, we engineered mouse embryonic stem cells using the CRISPR/Cas9 technology, to mutagenize conserved structural motifs that are important for tumour suppression in human cells, and will now establish the role(s) of these motifs in imprinted gene regulation *in cis* during embryogenesis.

Our work will provide unprecedented mechanistic, nucleotide-resolution information about the roles of MEG3 in cancer and neurodevelopment, and will establish a paradigm for characterizing other medically-relevant human lncRNAs.

[1]Farhadova et al, Nucl Acids Res, 2024, doi: 10.1093/nar/gkae247
[2]Uroda et al, Mol Cell, 2019, doi: 10.1016/j.molcel.2019.07.025
[3]Uroda et al, Nat Prot, 2020, doi: 10.1038/s41596-020-0323-7