Universitat Autònoma de Barcelona

Research Project

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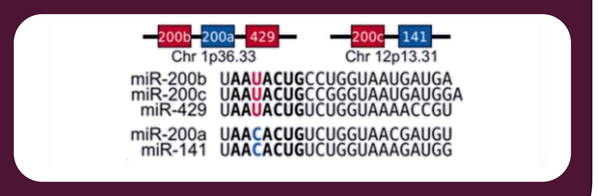
Degree in Biochemistry

miR-200 Family Regulation and its Role in Epithelial-Mesenchymal Transition

MicroRNAs (miRNAs) are non-coding RNAs, 18-25 nucleotides in length that regulate gene expression post-transcriptionally. miRNA action is performed through the inhibition mRNA transcription into proteins.
The participation of miRNA in cancer development has been discovered recently. It has been seen that miRNAs can prevent or enhance cancer depending on which genes are repressing.
miR-200 family is a group of microRNAs organized in two clusters involved in the maintenance of epithelial characteristics and in the inhibition of epithelial-mesenchymal transition (EMT). During EMT cells change their characteristics from epithelial to mesenchymal and this enables them to detach from the primary tumor and produce metastasis.



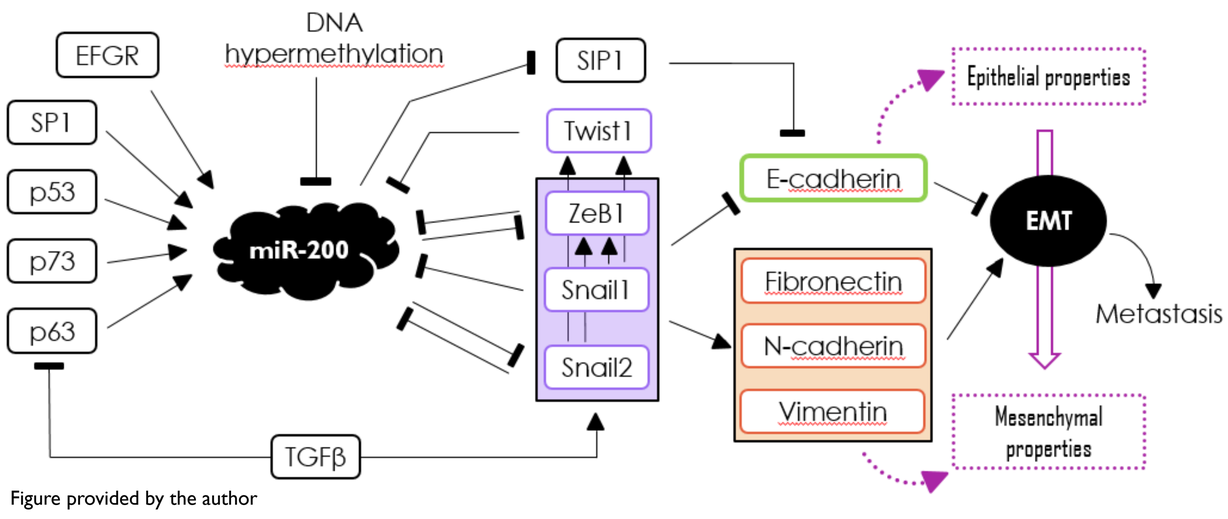


Figure provided by the author

Objectives

1 . Identification of negative and positive regulatory elements located in the promoter.

- Functional promoter analysis using luciferase reporter assay.

2 . Identification of cis-regulatory elements (TF binding sites, promoter):

- Bioinformatically: Databases for DNase, FAIRE, ChIP, histone modifications, Transcription factor motif, compare between species.
- Experimentally: ChIP-seq method against the TFs predicted bioinformatically.

3 . Identification of large regulatory elements (enhancers, insulators).

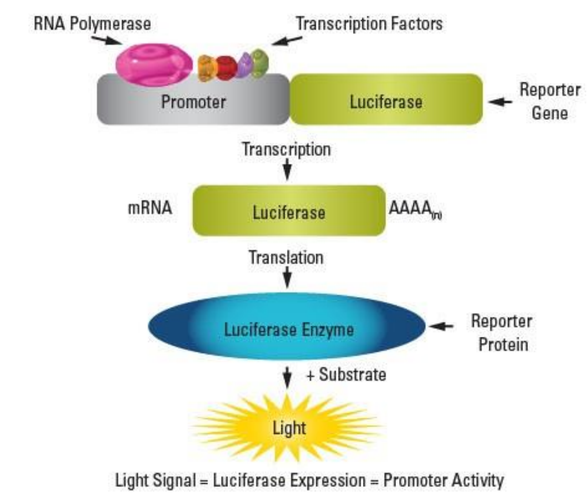
- ChIA-PET

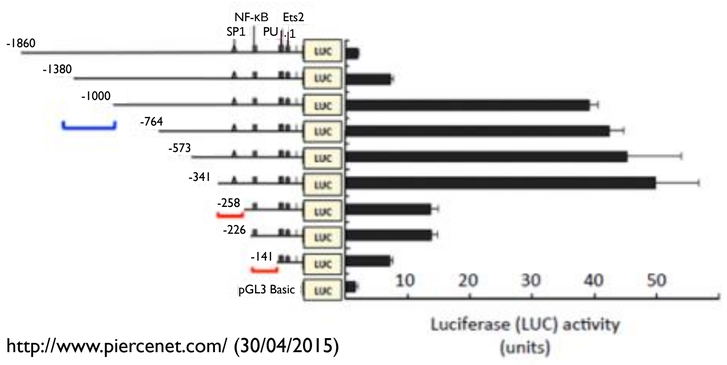
Hypothesis

Are microRNAs truly regulated as normal genes?
Find cis-regulatory elements (promoter, transcription factor binding sites) and large regulatory elements (enhancers, repressors, insulators) for the two different clusters of miR-200 family, and compare them.

Materials & Methods

1. Luciferase reporter assay

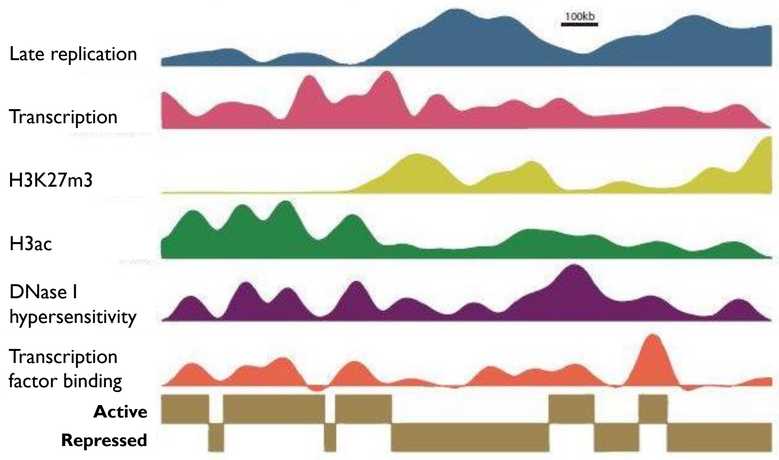




http://www.piercenet.com/ (30/04/2015)

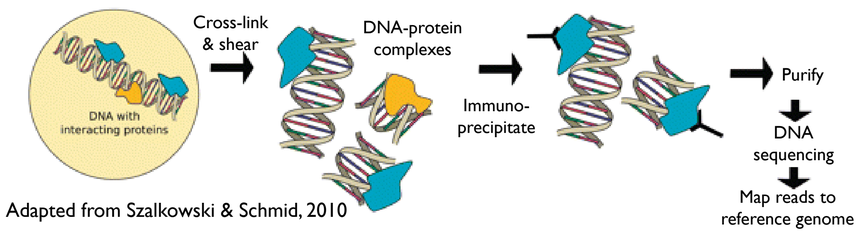
2.a. Bioinformatics

Hypothetical chromatin marks used to identify active and repressed genomic regions.



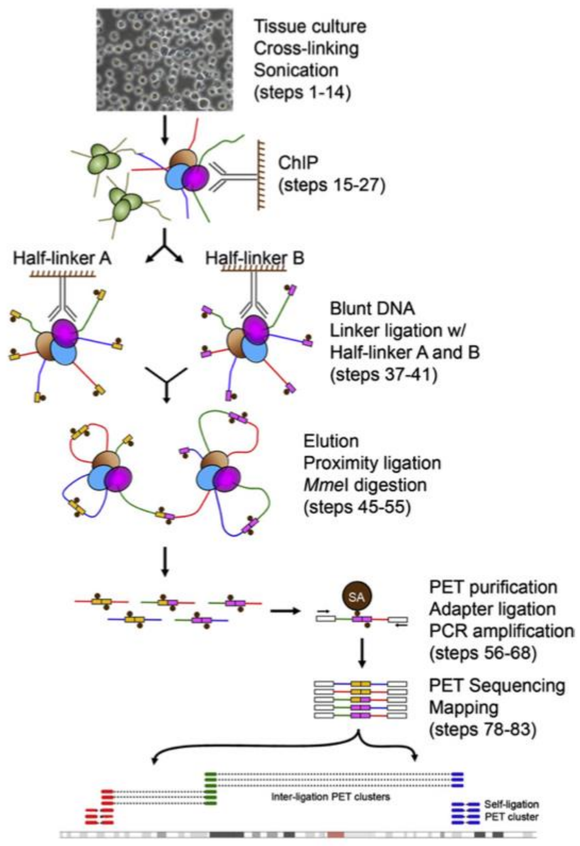
Carlberg & Molnar, 2014

2.b. ChIP-seq



Adapted from Szalkowski & Schmid, 2010

3. ChIA-PET



Zhang et al., 2012

Conclusions

I expect to identify different regulatory elements in the promoter regions of the two miR-200 clusters. Among the different transcription factors binding miR-200, I expect to find the ones already known (verifying results) and new ones (discoveries). Both clusters should be similarly regulated and any difference on them could mean that they have different functions, different targets and/or different signaling pathways involucrate.
Future approaches could be to identify and characterize every molecule involved in the regulation of miR-200. Once knowing its regulation, it could be possible to design drugs or techniques to enhance its expression and inhibit EMT in cancer. Repressing metastasis in cancer patients will highly decrease mortality rate.

References

Only relevant references are cited below:

- Adam R. Karpf (2013) Epigenetics Alterations in Oncigenesis. Springer. Chapter II: 139-140
- Antonio Díaz-López et al. (2014) Zeb1 and Snail1 engage miR-200f transcriptional and epigenetic regulation during EMT. Int. J. Cancer. 136, E62-E73
- Ester Sanchez-Tilló, Yongqing Liu, Oriol de Barrios, et al. (2012) EMT-activating transcription factors in cancer: beyond EMT and tumor invasiveness. Springer.
- Fullwood, M.J., Han, Y., Wei, C.L., Ruan, X. and Ruan, Y. Chromatin interaction analysis using paired-end tag sequencing. Curr Protoc Mol Biol, Chapter 21. Unit 21 15 21-25.