RESEARCH PROJECT PROPOSAL

Improving microRNA target prediction:

A novel integrative approach using dynamic transcriptomic profiling data

Carlos José Gallardo Dodd, Bachelor in Genetics (2018-2019), Universitat Autònoma de Barcelona (UAB)
Supervised by **Antonio Barbadilla Prados**, Institut de Biotecnologia i Biomedicina (IBB)

BACKGROUND

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNA molecules that extend to about 19-24 nucleotides in length and are thought to be one of the most predominant built-in features regulating gene expression. Their dysregulation has been found influential in a great number of disease conditions and to adequately characterize miRNA is a matter of great therapeutic importance. Several efforts have been made attempting to generate effective computational models to **predict miRNA-mRNA interactions**, but the performance of current prediction algorithms is still far from optimal due to a high false positive rate that results in heavily increased costs when validating the results [1].

Recent advances have allowed to characterize miRNA while considering transcriptome dynamics and they have even been linked to transcriptome changes within disease context. To this end, the ability to **identify** differentially expressed genes (**DEGs**) and differentially expressed miRNAs (**DEmiRNAs**) between different time points becomes of great importance.

Within this project proposal, a novel integrative procedure for large-scale miRNA identification and target prediction is developed for further testing. The aim is to reduce the false discovery rate (FDR) in target prediction by performing it over **clustered dynamical transcriptome and miRNome profiles** upon a given experimental condition.

PROJECT DEVELOPMENT

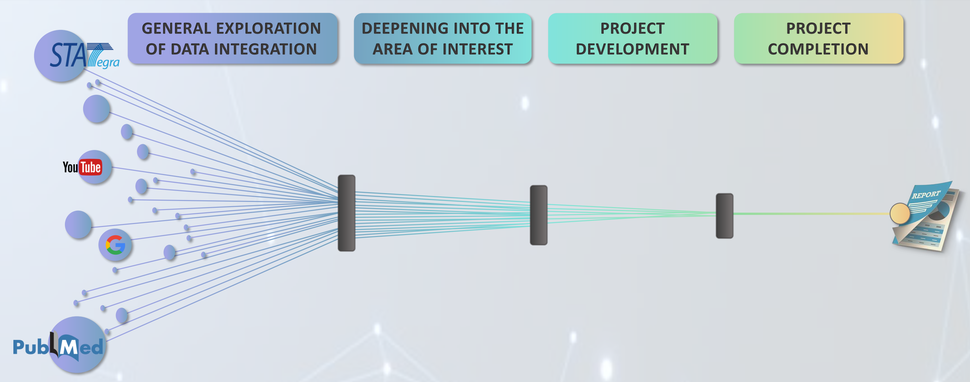

Figure 1. Scheme representing the different steps completed throughout the project.

HYPOTHESIS AND OBJECTIVES

miRNA-target associations can be established with higher precision by **integrating time course expression data**

OBJECTIVE 1

To improve the detection rate of specific miRNA-target associations incorporating dynamic transcriptomic profiling data

OBJECTIVE 2

To apply this novel methodology in the characterization of human keratinocyte response upon UVB-exposure

EXPERIMENTAL DESIGN

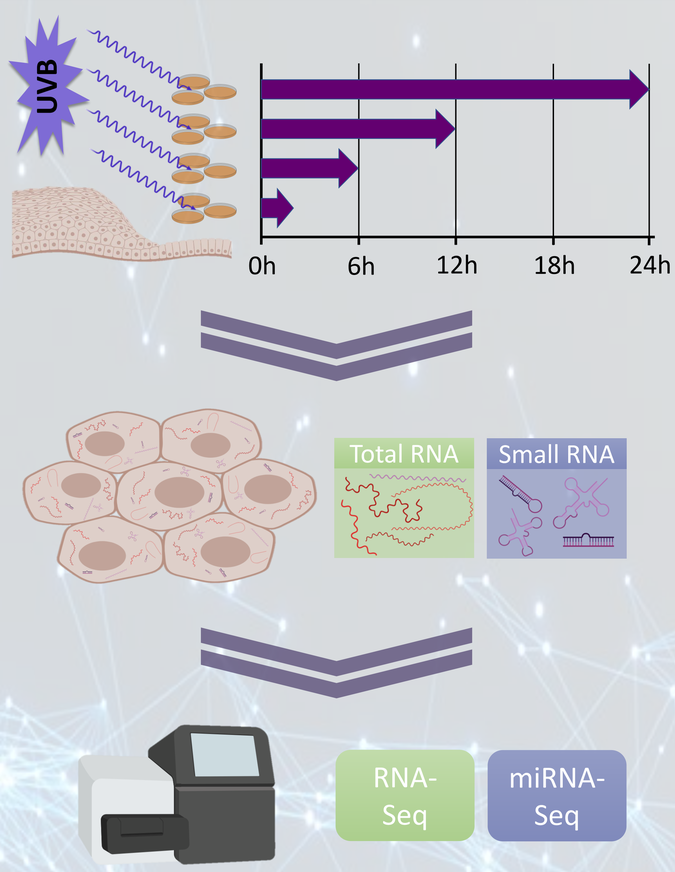

Figure 2. Different experimental steps of the project.

I. Culturing conditions and UVB irradiation

- Human epidermal keratinocyte (**HEK**) cell cultures will be exposed to 60 mJ/cm^2 of **UVB radiation**
- Three biological replicates per condition

II. RNA isolation

- Isolation at **different time points** post-UVB exposure (0, 6, 12 and 24h)
- Independent isolation of **total RNA** and **small RNA** fractions

III. Next-generation sequencing

Illumina **HiSeq3000** sequencing platform

- Total RNA libraries ➞ **pair-end** sequencing (2x125bp)
- Small RNA libraries ➞ **single-end** sequencing (1x50bp)

METHODS AND EXPECTED RESULTS

1 Differential expression analysis

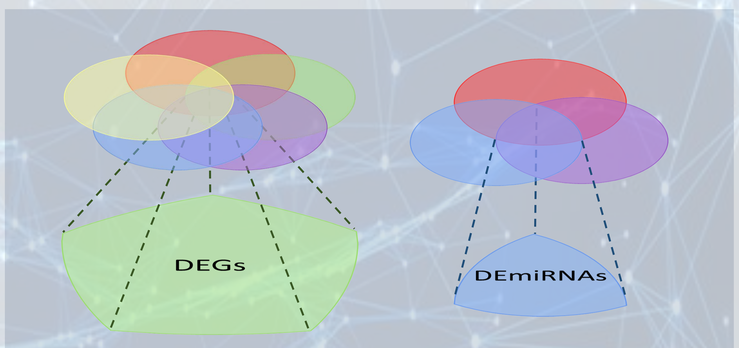

Figure 3. Differentially expressed molecules identified by integrating the results of different identification methods. DESeq2, NOISeq, limma-voom, baySeq and edgeR are used for detecting DEGs. The list of DEmiRNAs is obtained as the intersected outcome of DESeq2, NOISeq and limma-voom [2].

2 Integrative expression profiling

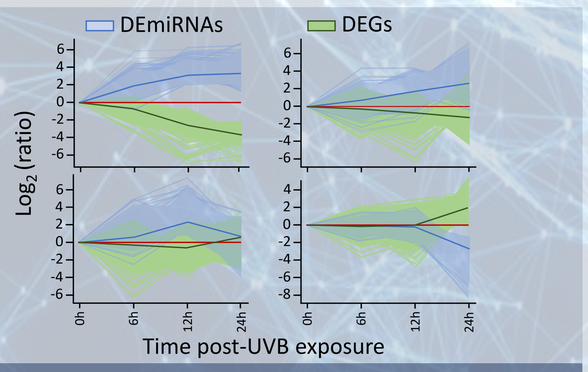

Figure 4. Representation of different trajectory-based clusters. Linear Mixed Model Spline (LMMS) framework is used for modelling and performing hierarchical clustering (HC) over molecule trajectories [3]. 0 log_2-ratio baseline (red) and averaged trajectories (blue/green) are shown.

3 miRNA target prediction

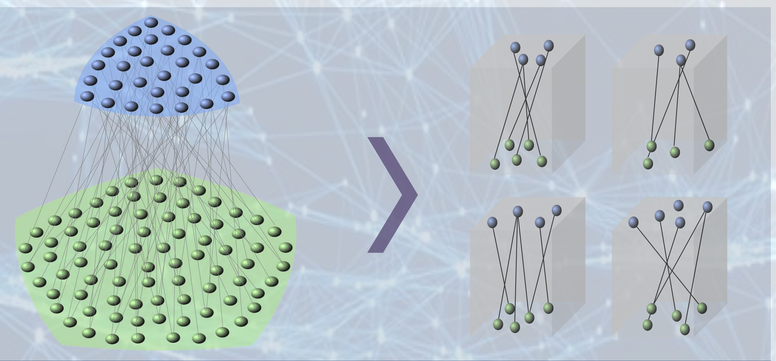

Figure 5. Representation of predicted miRNA-target associations. MirTarget v4.0 is used for identifying the whole set of interactions between DEmiRNA space (blue) and the space of detected DEGs (green). Initial interaction set is filtered to those that are cluster-specific. Molecules (spheres) and interactions (grey lines) are shown.

4 Validation with miRNA knockdown data

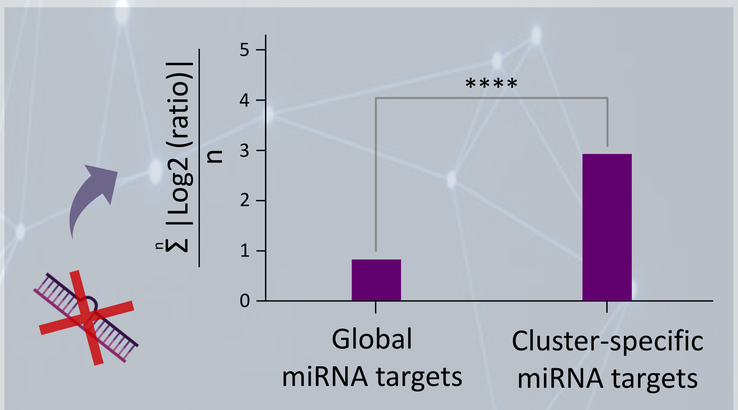

Figure 6. Averaged expression changes for predicted targets in knockdown experiments. Differential expression analysis allows the identification of DEGs and log_2-ratio calculation. Averaged values are shown for the whole set of potential targets and the constrained cluster-specific interaction list.

5 Pathway analysis

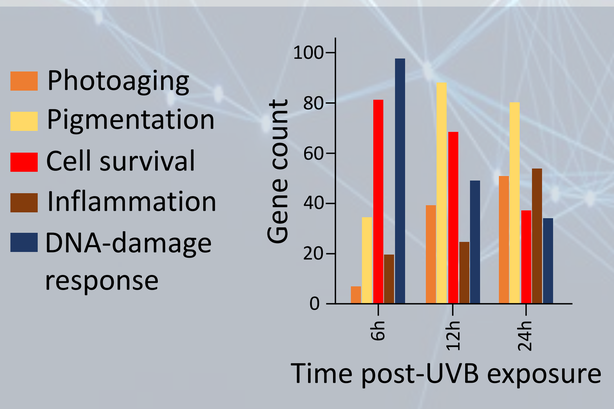

Figure 7. Pathway gene enrichment analysis at different time points post-UVB exposure. DAVID bioinformatics resources is used to categorize DEGs into relevant cellular processes. Gene enrichment for five different processes is depicted in their respective colours.

DISCUSSION

Some miRNA-target associations can be overlooked with this approach

REDUCED SENSITIVITY

I. **Complex regulations** may lead to non-complementary expression patterns
II. Target expression may just be affected at the **protein level**

BIBLIOGRAPHY

1. Liu W, Wang X. Prediction of functional microRNA targets by integrative modeling of microRNA binding and target expression data. Genome Biology. 2019Jan22;20(1).
2. Costa-Silva J, Domingues D, Lopes FM. RNA-Seq differential expression analysis: An extended review and a software tool. Plos One. 2017Dec21;12(12).
3. Spies D, Renz PF, Beyer TA, Ciaudo C. Comparative analysis of differential gene expression tools for RNA sequencing time course data. Briefings in Bioinformatics. 2017Oct6;20(1).

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