Strategies for the selection of miRNA candidates for the treatment of Sarcopenia using network-based analysis and differential expression scoring

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Sarcopenia is a progressive muscle wasting and it is a natural consequence of aging. There is not a cure for muscle loss. Target identification and validation is a pressing challenge, with many targets failing in clinical trials or showing poor association with the disease. This project aims to create model(s) of microRNA:target interactions for more efficient in silico selection of potentially therapeutic targets for sarcopenia.

Authors: Karen Guerrero Vazquez[1], Pilib Ó Broin[1], Katarzyna Goljanek-Whysall[2]

1 School of Mathematical & Statistical Sciences, National University of Ireland Galway.

2 School of Medicine, National University of Ireland Galway.

MiRNAs and their potential use as therapeutics

MicroRNAs (miRNAs) are small single-stranded RNAs that play an important role in the regulation of gene expression. Their simultaneous regulation of multiple genes and pathways and ability to deliver miRNAs into most tissues without inducing systemic immune response, makes them attractive therapeutic candidates.

Identifying miRNAs underlying a particular disease is still a challenge since many miRNAs are often ubiquitously expressed, have overlapping functions and can regulate multiple genes. Also, not all miRNAs are functionally relevant in a given cell type.

The analysis of the miRNAs one by one is time-consuming

Overlapping functions and can regulate multiple genes

Bias towards more studied miRNAs

Hard to undestand complex cause-effect relationships

Sarcopenia; a matter of time

Sarcopenia is progressive muscle wasting due to aging. Identification and validation of therapeutic targets is a pressing challenge, with many candidates showing poor association with the disease.

Computational prediction of therapeutictargets could decrease the attrition rates in the drug discovery pipeline by reducing the initial search phase.

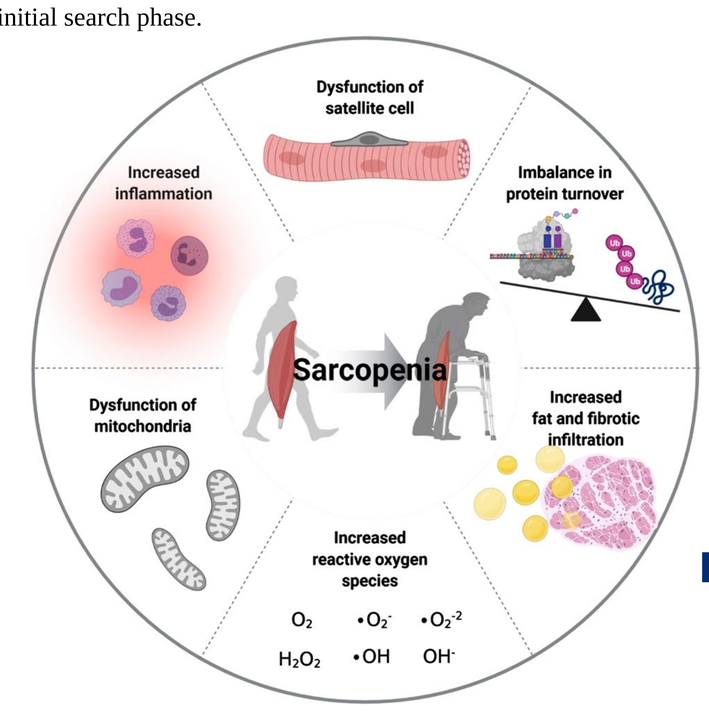


Figure 1: Factors causing sarcopenia, from Kim et al. (2021).

What can we do?

There are more than 160 different tools, apps, and databases dedicated to the study of microRNAs. miRBase, miRWalk, and miRNet are among the most popular.

miRBase is a state-of-the-art database of microRNA sequence annotations.

miRNet integrates user data with existing knowledge via network-based visual analytics.

miRWalk is an online resource for the prediction of microRNA (miRNA) target interactions and integrates results from TargetScan, miRDB, miRanda, and other popular databases.

Here, we present miRKat, a tool to identify miRNAs specifically involved in sarcopenia.

miRNA selection process

We collect an initial set of genes from RNAseq and microarray data from five published studies, with 246 samples of skeletal muscle from healthy participants with ages ranging from 19 to 85 years old., as you can see in Table 1.

Table 1: Studies considered in the selection of initial set of genes

GEO number	Samples	Min Age	Max Age	Reference
GSE152558	5	41	85	Tharakan, R., Ubaida-Mohien, C., Piao, Y., Gorospe, M., & Ferrucci, L. (2021).
GSE164471	53	34	80	Tumasian III, R. A., Harish, A., Kundu, G., Yang, J. H., Ubaida-Mohien, C., Gonzalez-Freire, M., ... & Ferrucci, L. (2021).
GSE157585	136	19	>65	Kulkarni, A. S., Peck, B. D., Walton, R. G., Kern, P. A., Mar, J. C., Windham, S. T., ... & Peterson, C. A. (2020).
GSE87105	16	30	78	Mercken, E. M., Capri, M., Carboneau, B. A., Conte, M., Heidler, J., Santoro, A., ... & de Cabo, R. (2017)..
GSE23527	36	29	76	Drummond, M. J., McCarthy, J. J., Sinha, M., Spratt, H. M., Volpi, E., Esser, K. A., & Rasmussen, B. B. (2011)

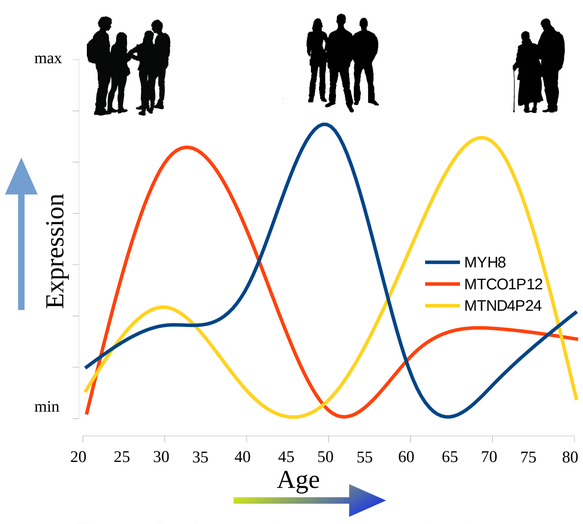


Figure 2: change of gene expression by age

We analyzed young, middle age, and older adults and determined differentially expressed genes among the age groups.

GeneMania and IntAct were used to find interactions among the differentially expressed genes, while also adding additional genes known to interact with them. miRNAs that target those genes were added frommirWalk, miRDB and mirTarbase. With this information we generated a graph which edges were the gene-gene interactions or miRNA-target relationship.

Finally, we calculate the relevance of the nodes using the closeness and pageRank centralities, two commonly used metrics that take in acount how close a node is to all other nodes and the importance of the node in the network respectibly. We select the top 5% nodes with the highest centrality. This was done using miRKat, our own miRNA selection tool.

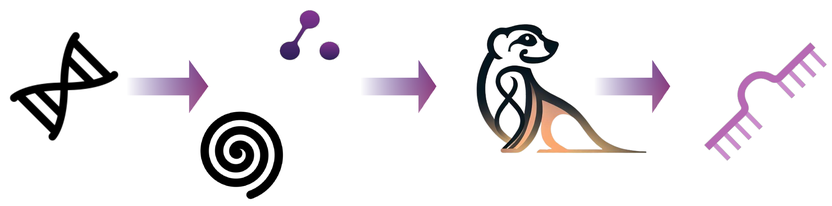


Figure 3: pipeline of tools for miRNA selection

Results I

We got a total of 245 differentially expressed genes distributed in the 5 experiments, the 6 combinations of ages, and up and down-regulated genes.

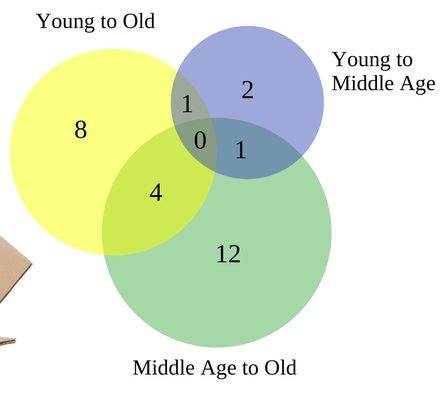


Figure 4: ven Diagram of common genes up and down regulated with age

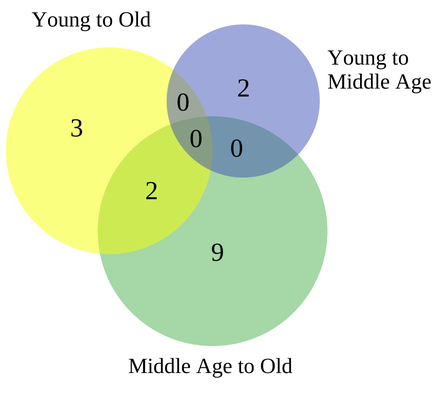


Figure 5: ven Diagram of common genes up regulated with age

For the purpose of this poster, we are going to focus on up-regulated genes in old (>65) compared to middle age (>35).

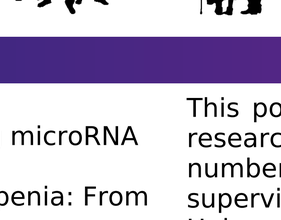


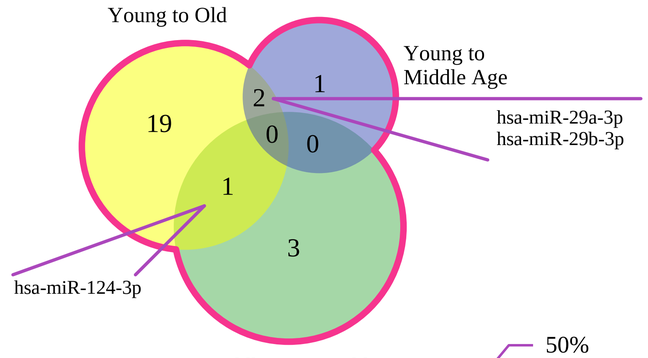
Figure 6: **A)** Networks generated based on the differentially expressed genes from Middle Age compared to Old and their interactions. **B)** miRNet output using the minimum network option with the same genes. **C)** network generated using miRKat closeness centrality. **D)** network generated using miRKat pageRank centrality.

Results III

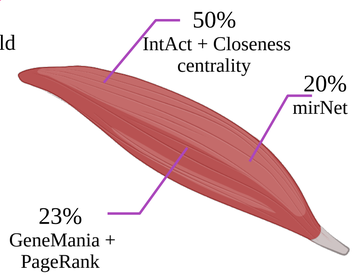
We identified more than 60 different miRNAs across all techniques.

Using Closeness centrality and IntAct we see 29 miRNAs, 3 of which appear in more than one stage of life.

MiRNAs selected



The models within miRKat lack access to information on miRNAs expression in tissues or enriched pathways, however, 50% and 23% or the miRNAs selected with closeness centrality and pageRank respectevly are found in muscle.

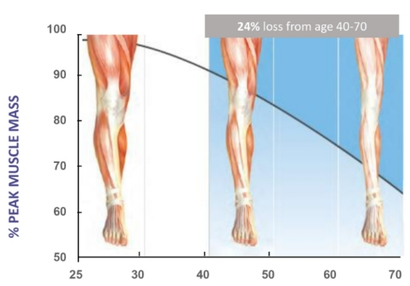


For miRNet this number was 20% although it is based on empirical sampling for enrichment analysis.

Conclusion and future work

miRNAs are important regulators of gene expression and potential therapeutic targets. Their selection for specific conditions such as sarcopenia, however, remains a challenge due to their complex functions and interactions.

Additional work is required to design objective scoring mechanisms to evaluate the quality of the selected miRNAs to ensure their relevance to sarcopenia. Most of the current evaluation methods imply a bias towards known miRNAs and genes.



Source: https://waytowellness.co.za/

Once the final selection of miRNAs and genes is established, the changes in the levels of the relevant miRNAs, genes, and their interaction over time will be considered in order to create a model of the progression of the interaction as a function of time.

Development of miRKat is ongoing, but it is publicly available at https://github.com/GuerreroVazquez

References

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Github

Lab site