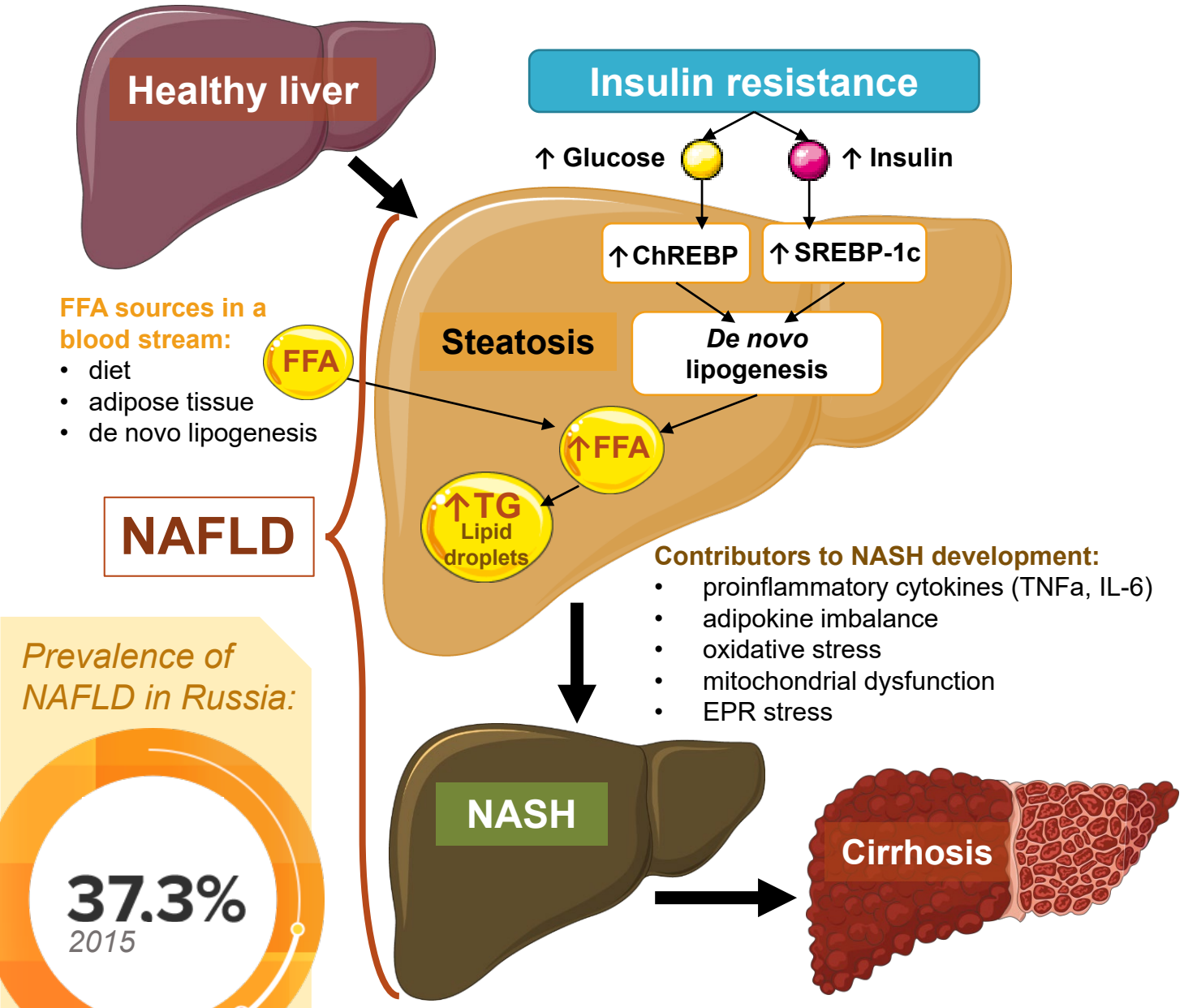
13th International Multiconference on
"Bioinformatics of Genome Regulation and Structure/Systems Biology"

MiRNA-dependent regulation of ERBB signaling pathway genes in NASH patients

Bograya M.*, Vulf M., Shunkina D., Karpeeva K., Komar A., and Litvinova L.
mbograya@mail.ru

Center for Immunology and Cellular Biotechnology, Immanuel Kant Baltic Federal University, Kaliningrad, Russia

Research objective



NASH – non-alcoholic steatohepatitis

NAFLD - non-alcoholic fatty liver disease

In recent years, there is increasing evidence for the involvement of miRNAs in the epigenetic regulation of NAFLD development.

The aim of this study was to **compare the miRNA profiles of steatosis and steatohepatitis** (two major stages of NAFLD) to gain insight into NAFLD development and predict metabolic pathways that undergo changes in miRNA-dependent regulation in the pathology.

The study is important for the development of pathophysiology because microRNAs are perspective diagnostic and therapeutic biomarkers for NAFLD.

Full article is free to access by link:
DOI: 10.3389/fcell.2021.736677
Link is down below in the comments!

Materials and methods

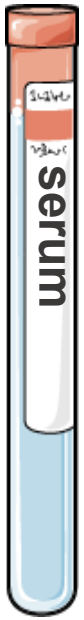
GSEA – gene set enrichment analysis

Characteristics of patients

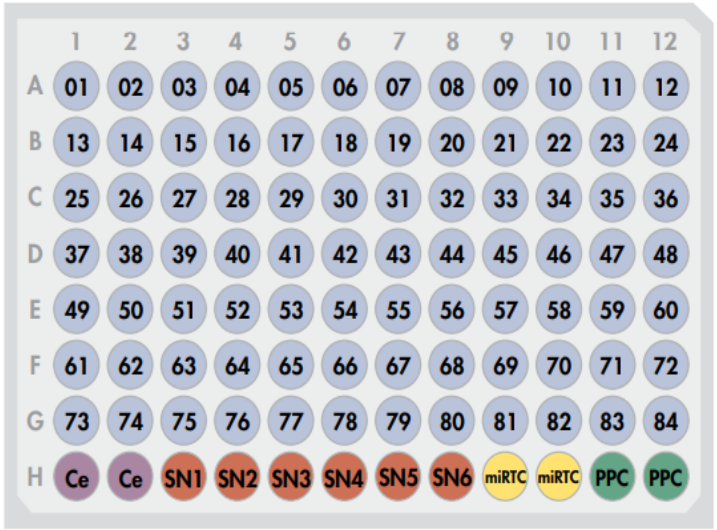
	Healthy donors, $n = 4$	Group of patients with steatosis, $n = 6$	Group of patients with NASH, $n = 6$
	1	2	3
Sex (female/male)	2/2	3/3	4/2
Age	32.50 ± 9.50	41.75 ± 9.91	43.50 ± 11.50
BMI, kg/m^2	21.88 ± 0.66	51.05 ± 10.04 p 1-2**	42.95 ± 4.67 p 1-3***

***p < 0.001, ***p < 0.0001; significance was determined using the t-test (mean ± SD).*

miRNA PCR Array Analysis



1. Sample collection and storage
2. Total RNA isolation (Thermo Fisher Scientific MagMAX mirVana Total RNA Isolation Kit)
3. Reverse transcription (Qiagen miScript II RT Kit)
4. PCR analysis (Qiagen miScript miRNA PCR kit)



miRNA PCR Array Human Serum & Plasma
MIHS-106Z, Qiagen, GmbH

Statistical Analysis of Experimental Data

PCR data were analyzed using **miScript miRNA PCR Array Data Analysis Tool software** (Qiagen, Hilden, Germany). All Cq values greater than 35 were excluded. The normal distribution was checked by Shapiro–Wilk test. If the sample fitted the normal distribution Student's t-test was used. Otherwise non-parametric Mann–Whitney test was applied. Differences were considered significant at $p<0.05$.

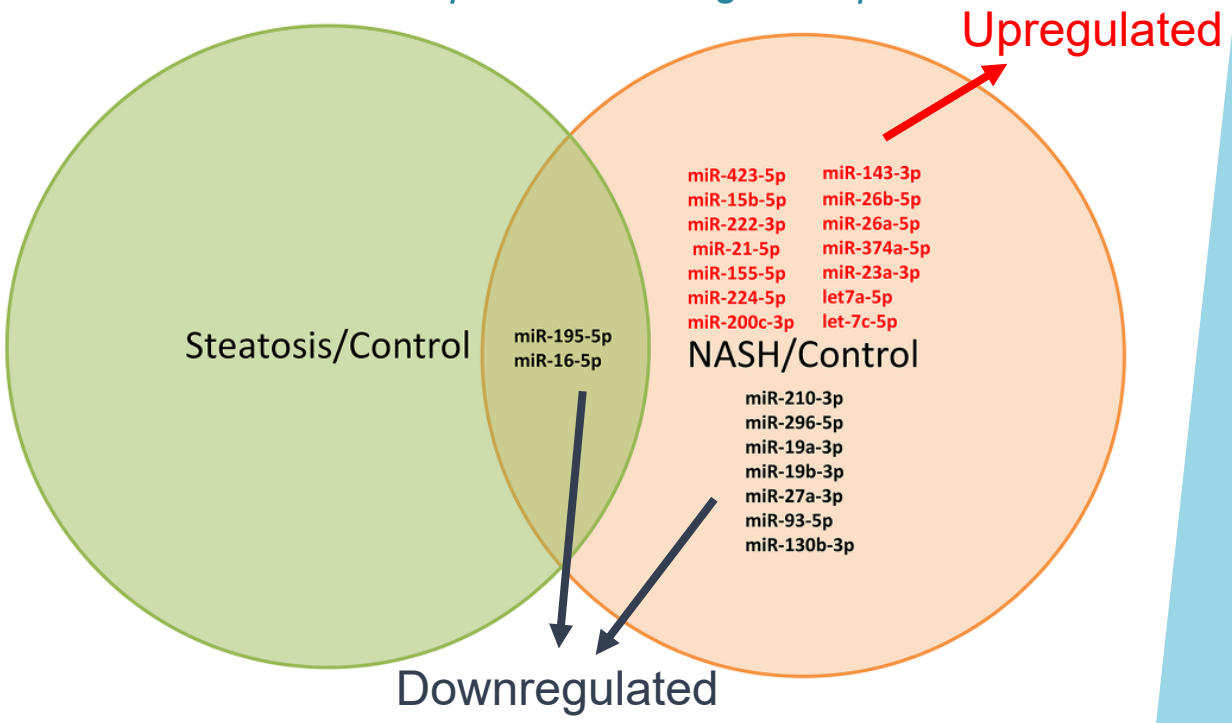
MiRNA Target Mining and GSEA

MiRNA targets were predicted by **miRWalk** with a binding probability threshold of 100%. The miRWalk structure includes the TargetScan (version 7.1), miRDB (version 5.0) and miRTarBase (version 7.0) datasets. **GSEA** (based on hypergeometric tests) was performed in miRWalk on the KEGG database to elucidate the specific biological functions of the predicted targets. Paths were sorted by p-values ($p<0.05$ was considered significantly enriched).

Results

1 Identification of DEMs in steatosis and NASH

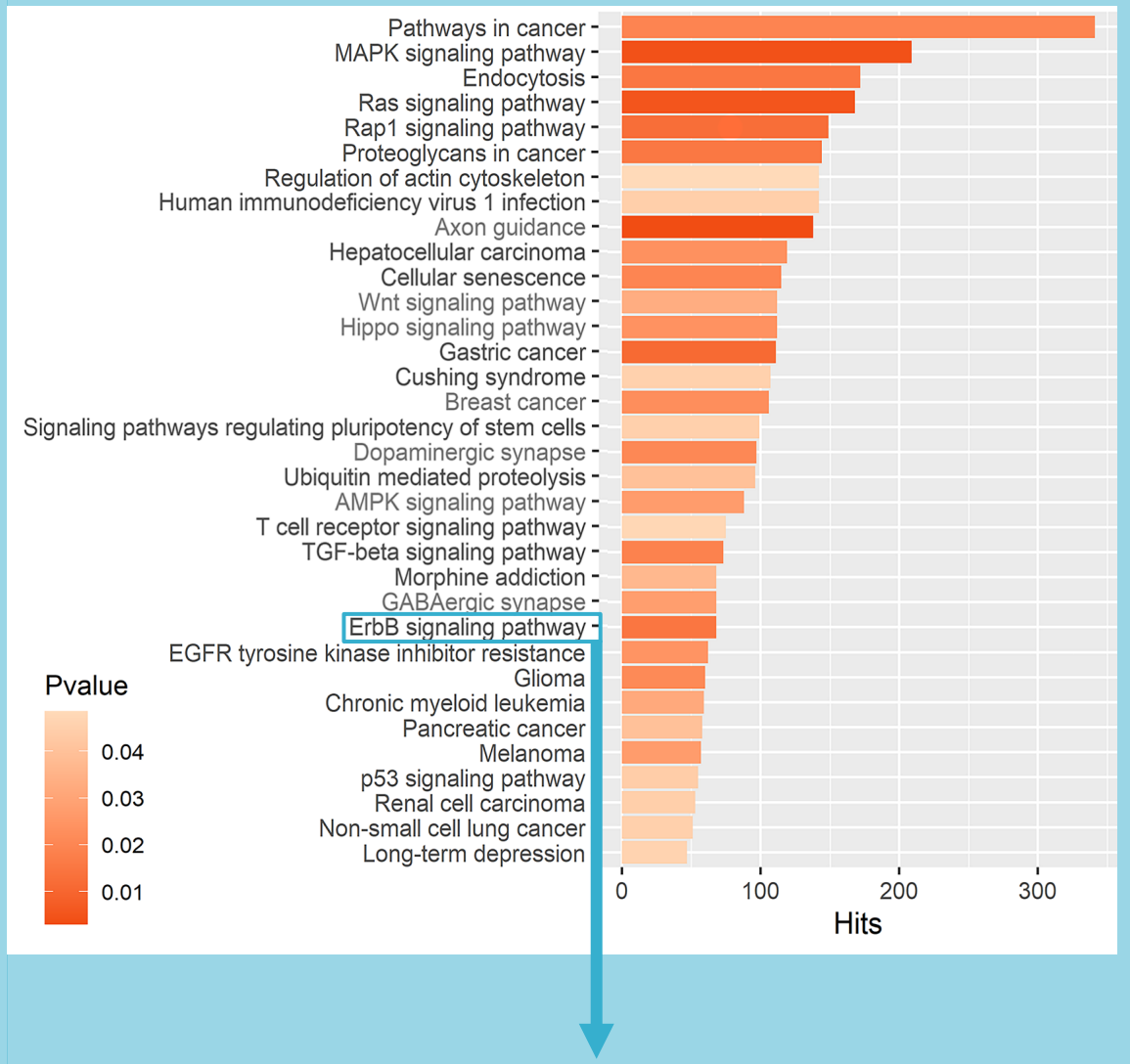
DEM: at least twofold expression change and p<0.05



2 Target mining for 21 NASH DEMs in MiRWalk

11,106 predicted targets

3 GSEA on KEGG database



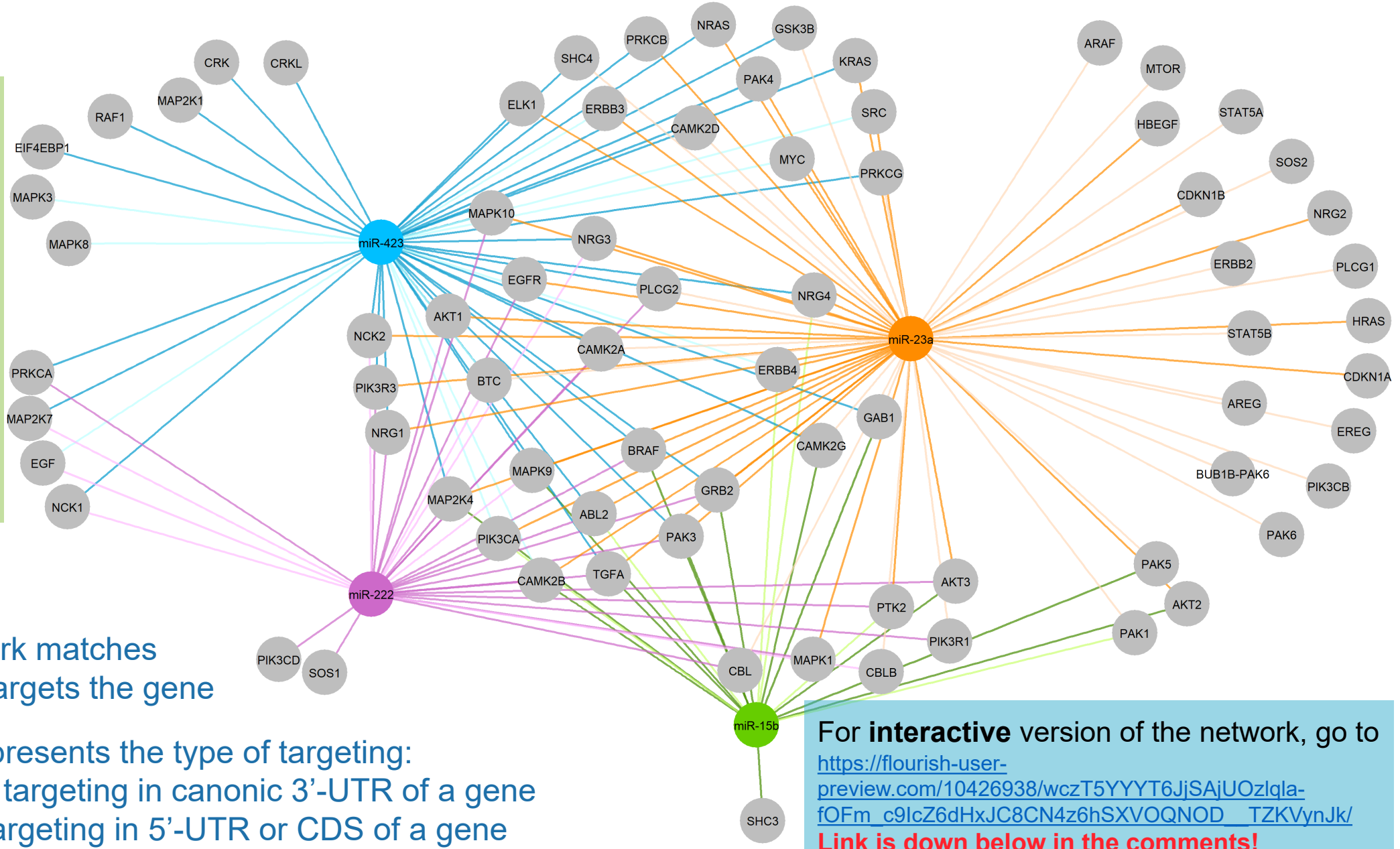
Started to investigate miRNA-dependent regulation of ERBB signaling in liver pathologies

DEM – differentially expressed miRNA

4 Choice of four main miRNAs responsible for ERBB signaling regulation

miR-15b-5p **miR-222-3p**
miR-23a-5p **miR-423-5p**

75 genes out of 85 genes in ERBB signaling (gene list is obtained from KEGG) is targeted by four upregulated miRNAs in NASH patients



Edge color in network matches the miRNA, which targets the gene

Edge brightness represents the type of targeting:
bright edge color – targeting in canonic 3'-UTR of a gene
faint edge color – targeting in 5'-UTR or CDS of a gene

For **interactive** version of the network, go to https://flourish-user-preview.com/10426938/wczT5YYYT6JjSAjUOzlqla-fOFm_c9IcZ6dHxJC8CN4z6hSXVOQNOD__TZKVynJk/
Link is down below in the comments!

Important to address!

Our bioinformatics analysis has a **limitation:** a correct GSEA requires the expression levels of differentially expressed genes (DEGs), not just a list of genes

Our goal now is to find and implement a **mathematical model that can convert the expression levels of DEM into those of target DEGs** with sufficient confidence

Conclusion and further prospects

The presented study is an example of how data from microRNA screening can be used to find new potential contributors to pathology. The literature search revealed that ERBB signaling in liver diseases is poorly understood. In the future, we plan to investigate the production of ERBB signaling genes and proteins in liver and how miR-222-3p, miR-423-5p, and miR-23a-5p act on these genes.

Funding

This research was funded by State Assignment Grant No. FZWM-2020-0010 and Grant of the President of the Russian Federation No. MK -2072.2022.3.