Decoding the transcriptional response to ischemic stroke in young and aged mouse brain

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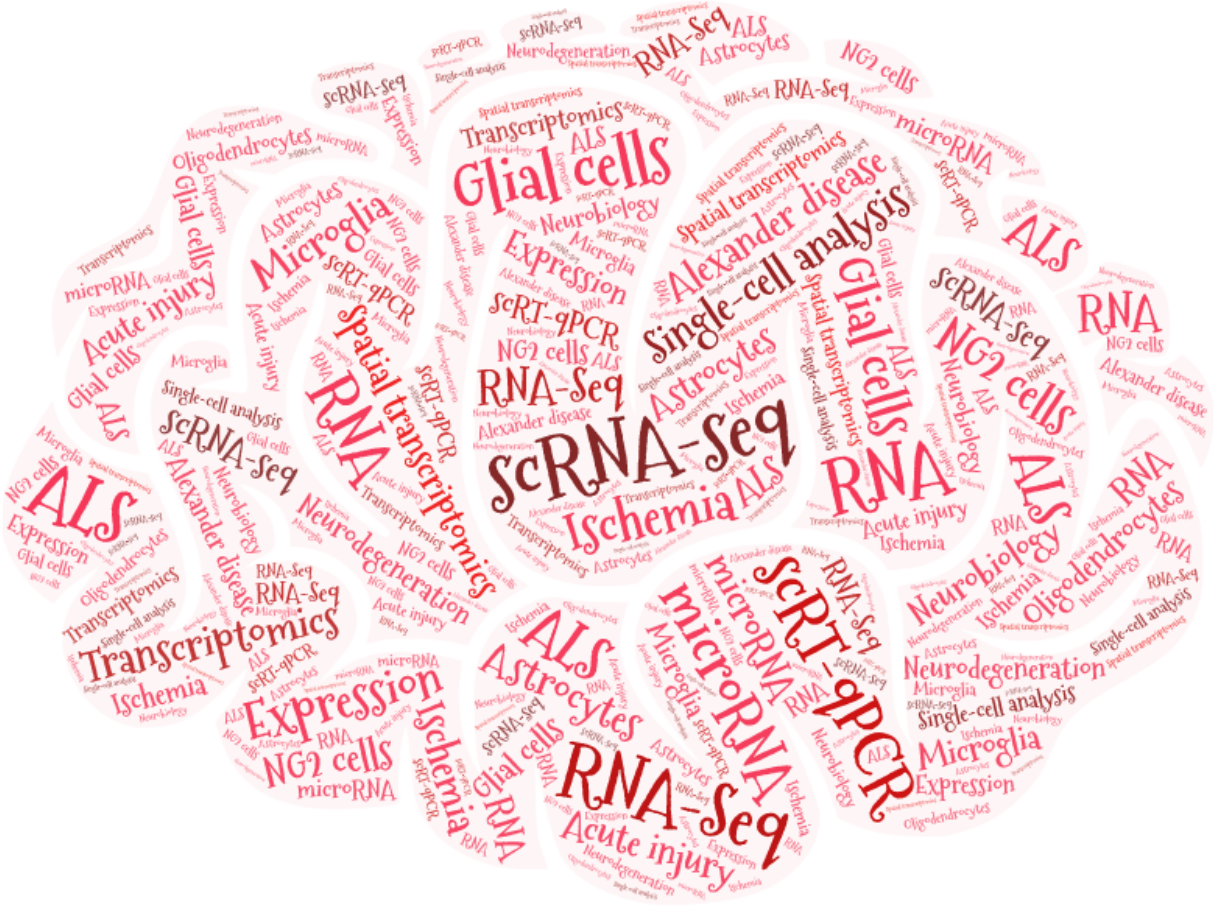
Research interest

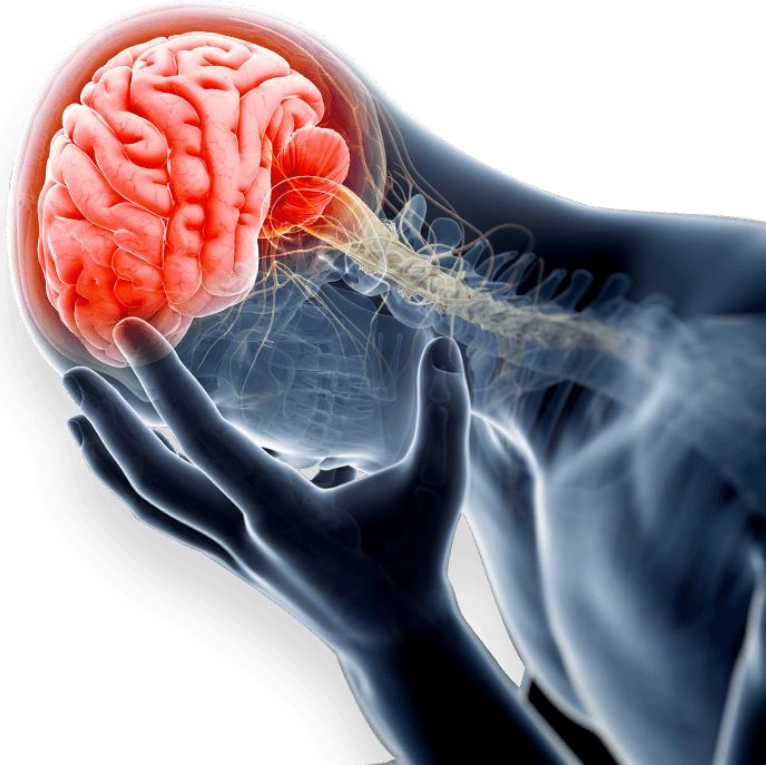
- Gene expression analysis
  - Bulk RNA-seq
  - Single-cell RNA-seq
  - Spatial transcriptomics
- Method development and QC
- Glial cell biology
  - Acute injury
  - Neurodegeneration



Ischemic stroke

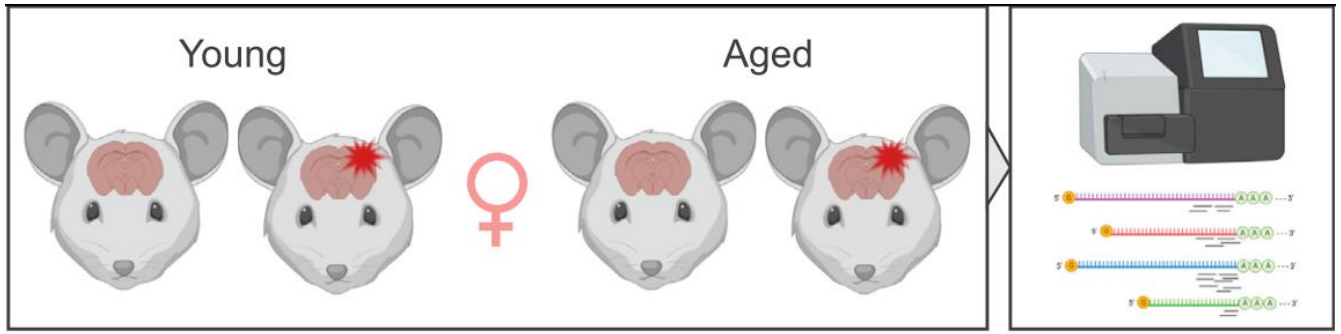
- Disease of aging
  - Most strokes in people > 65 years
  - Elderly shows higher mortality and poorer quality of life after stroke compared to young
- Sexually dimorphic disease (age modifies the influence of sex)

- These factors are often ignored in preclinical research
  - Most preclinical studies have used only young, male animals
- Complex factors underlying worsened stroke outcome in the elderly thus remain poorly understood, particularly in females

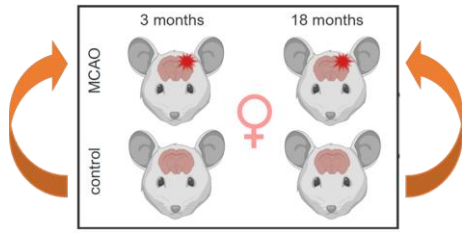


Experimental design

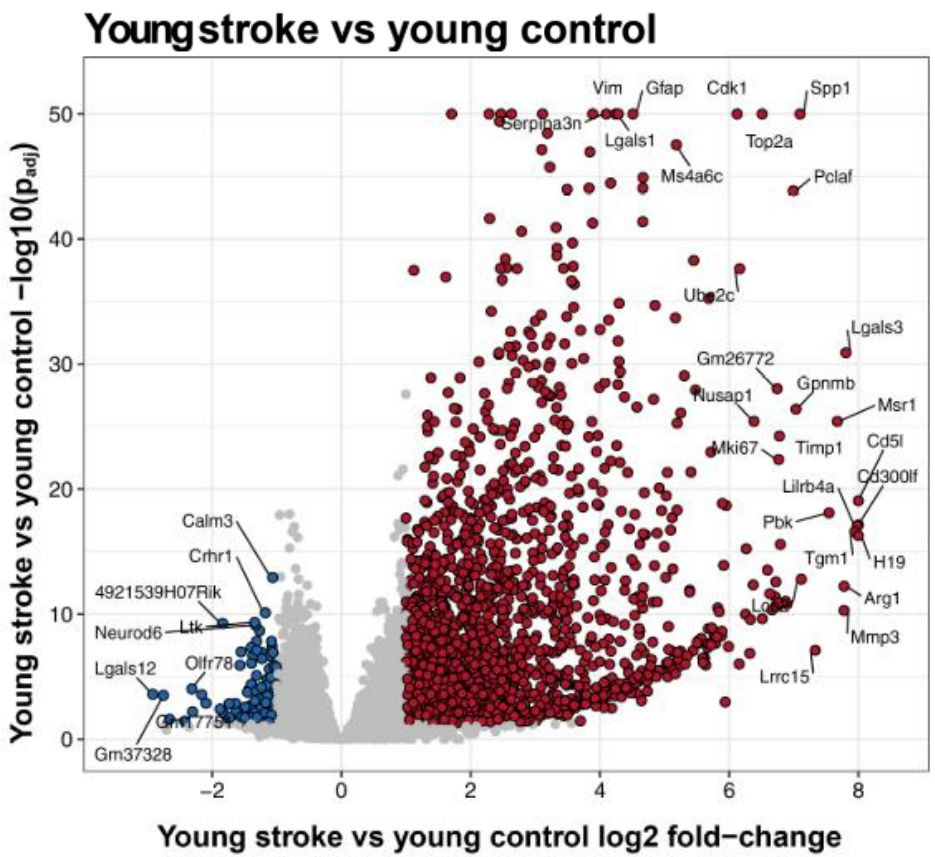
- Female young (3m) and aged (18m) mice
- Permanent middle cerebral artery occlusion (MCAO) to model ischemic stroke (STAIR recommendation)
- Brains (penumbral cortex) collected at 3 days after MCAO and analyzed by RNA-Seq

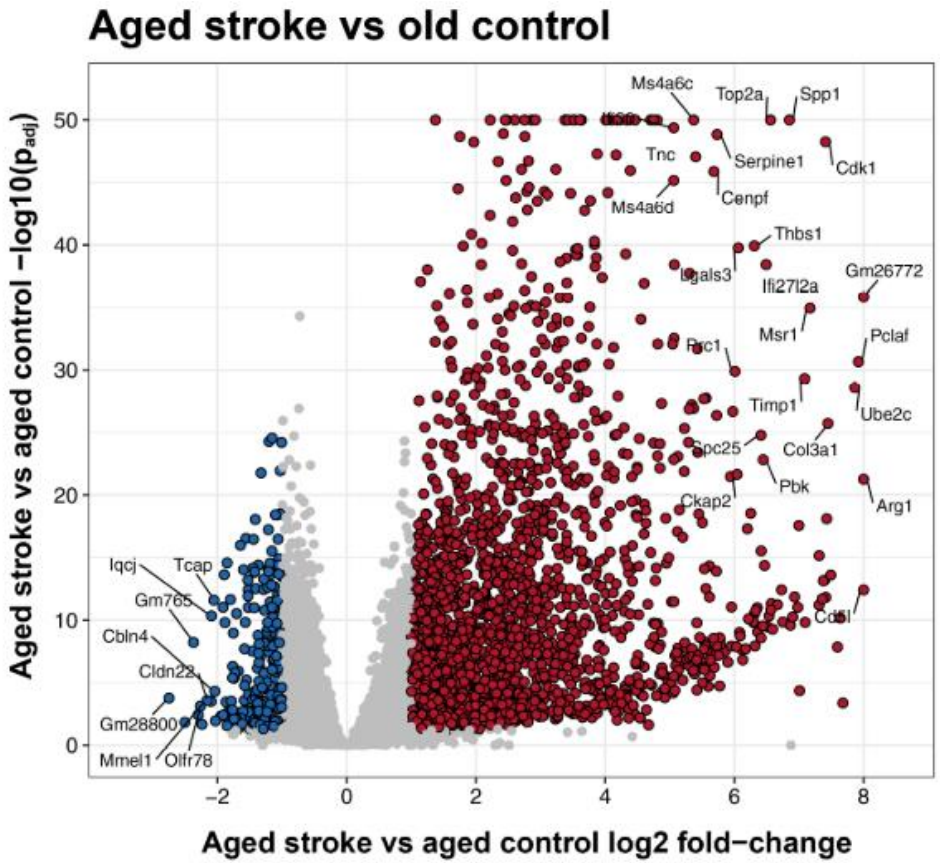


Aging alters the magnitude of the transcriptional response to ischemic stroke



Different response to ischemic impact





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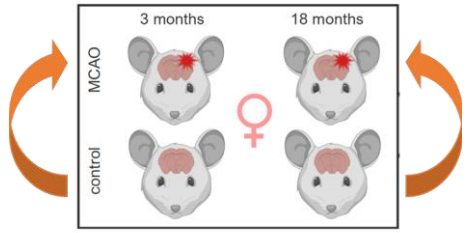
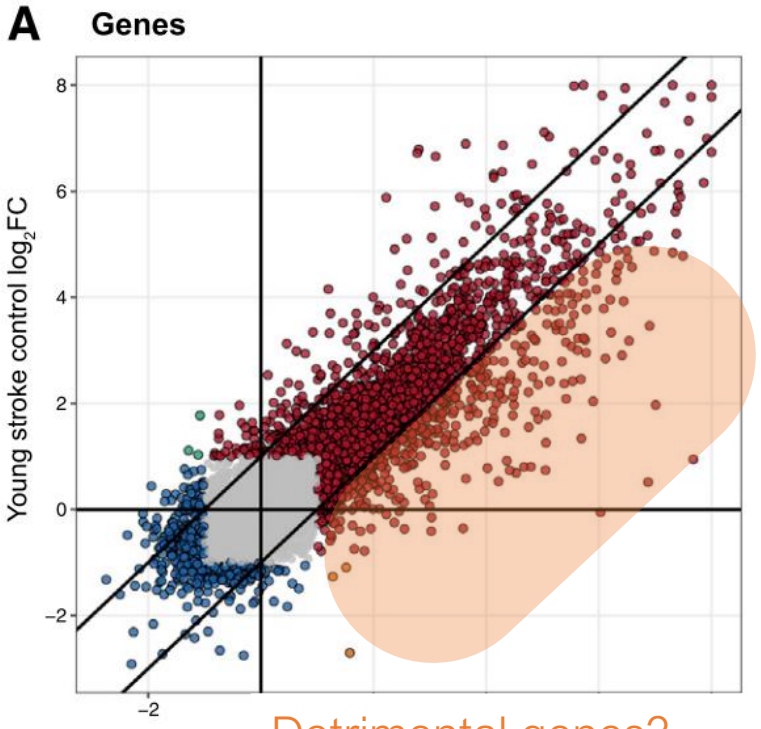
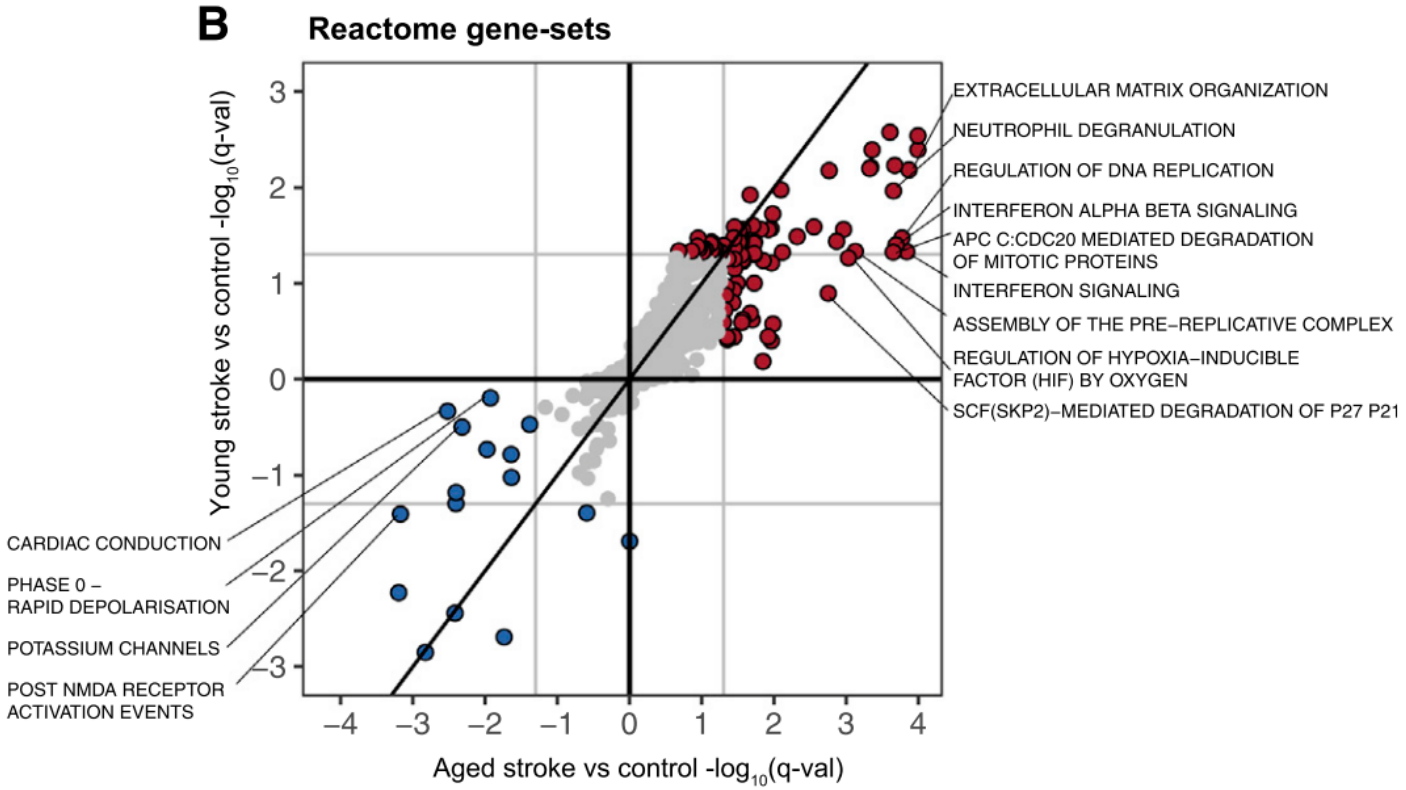


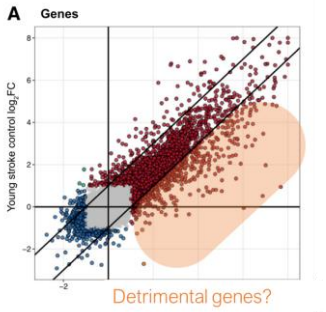
Different response to ischemic impact

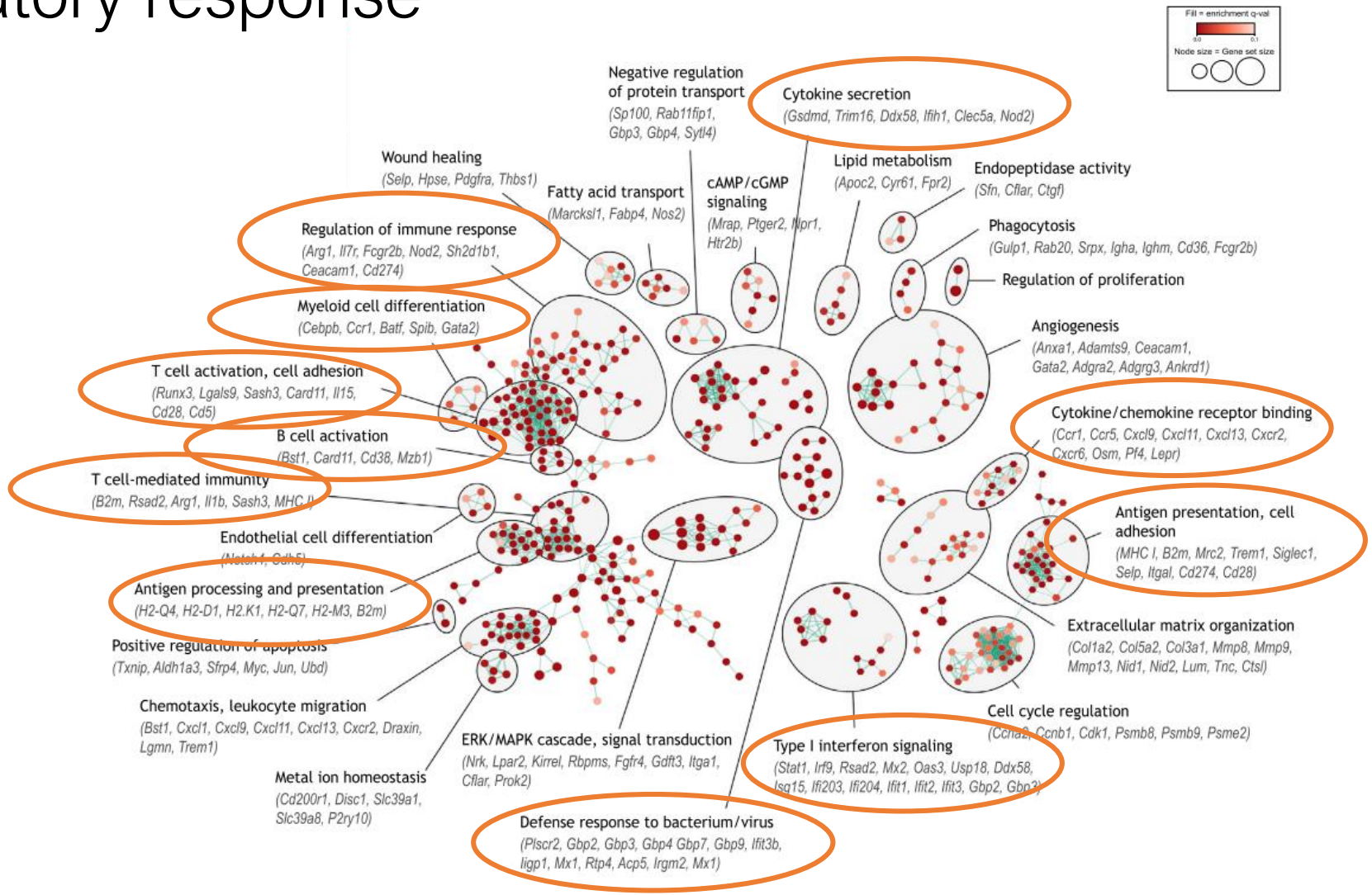


Detrimental genes?



Combination of aging and stroke leads to massive activation of type I interferon signaling and aggravated inflammatory response





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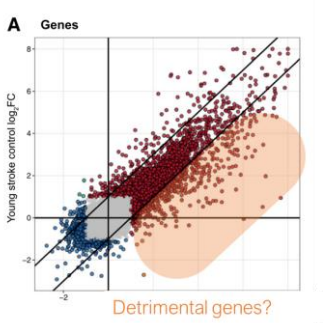
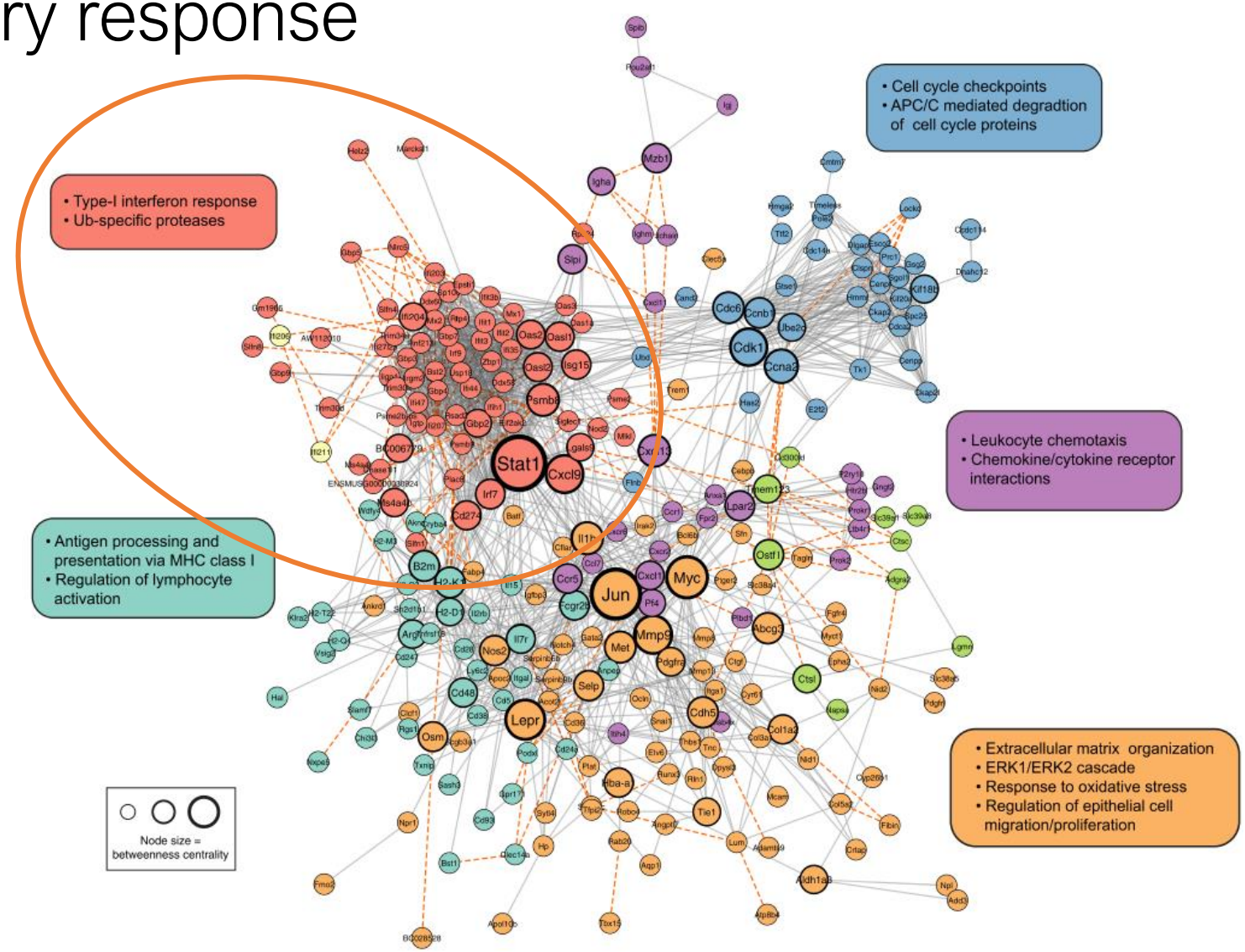
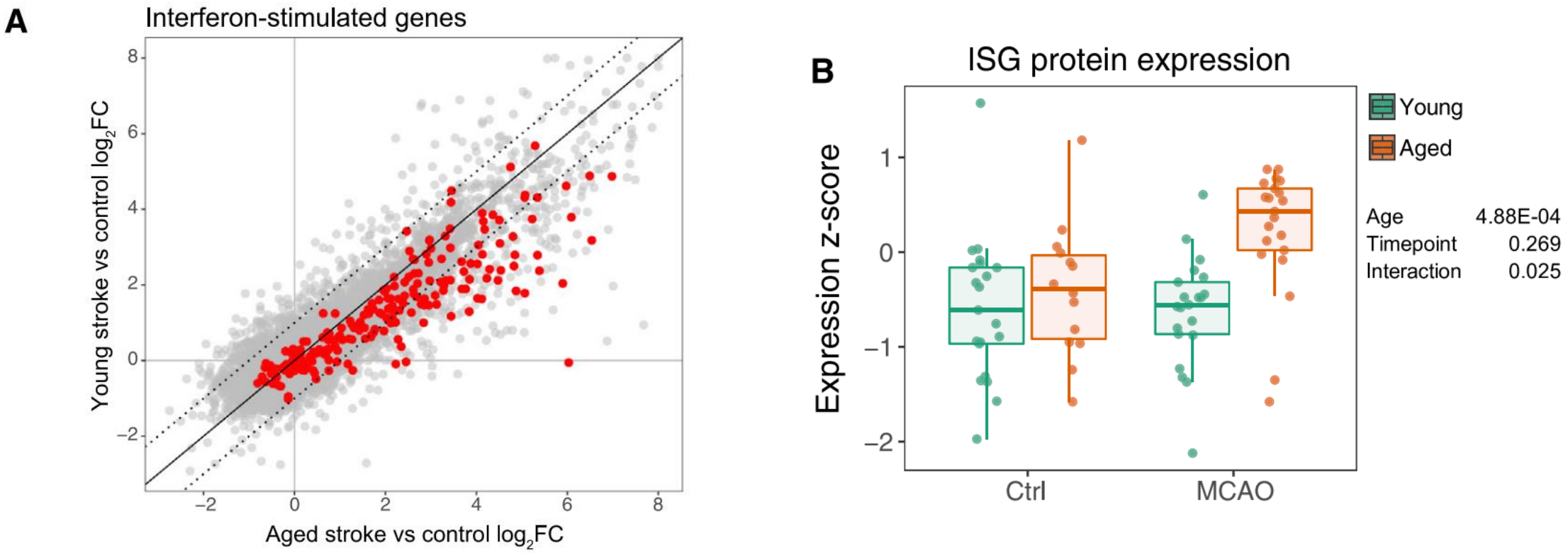
Combination of aging and stroke leads to massive activation of type I interferon signaling and aggravated inflammatory response

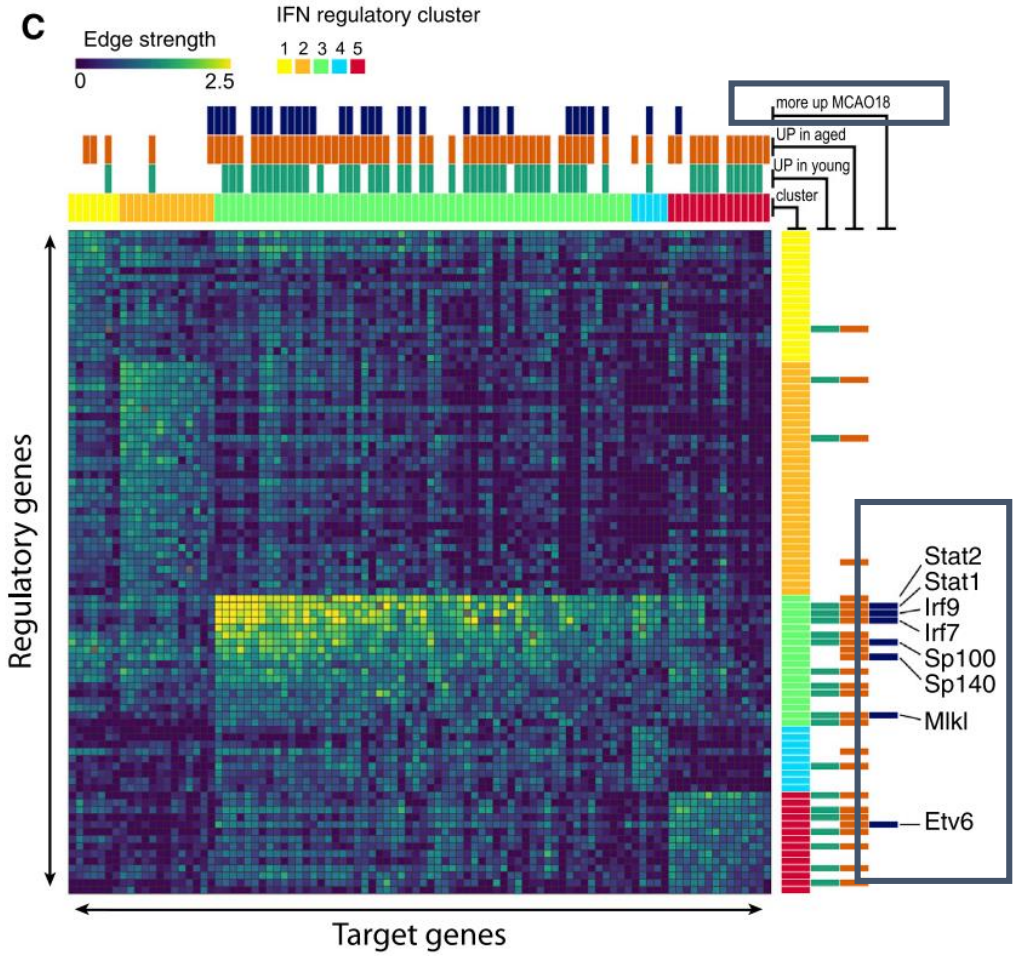




Age-dependent activation of type I IFN regulatory modules after stroke



Age-dependent activation of type I IFN regulatory modules after stroke



Cell
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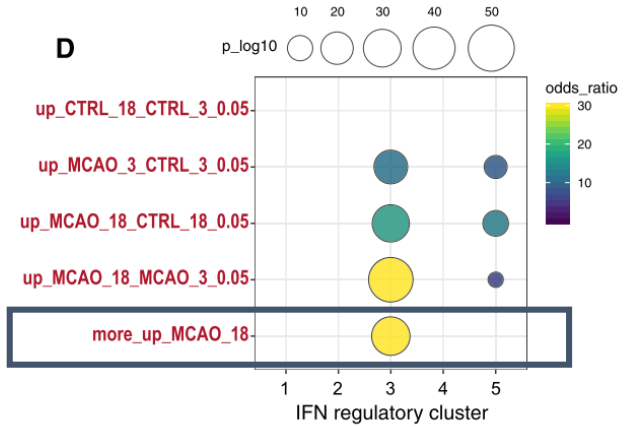
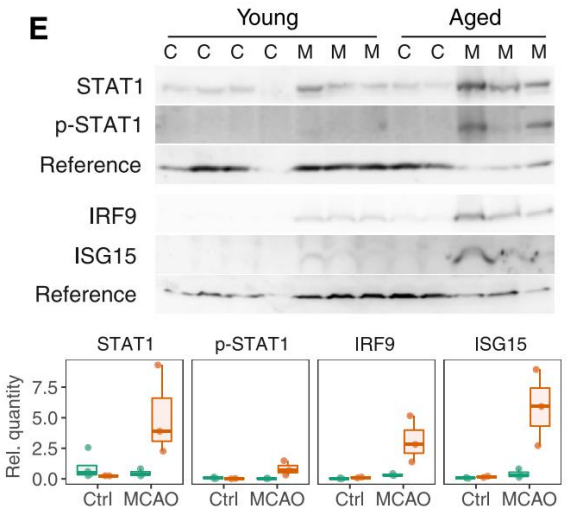
Resource

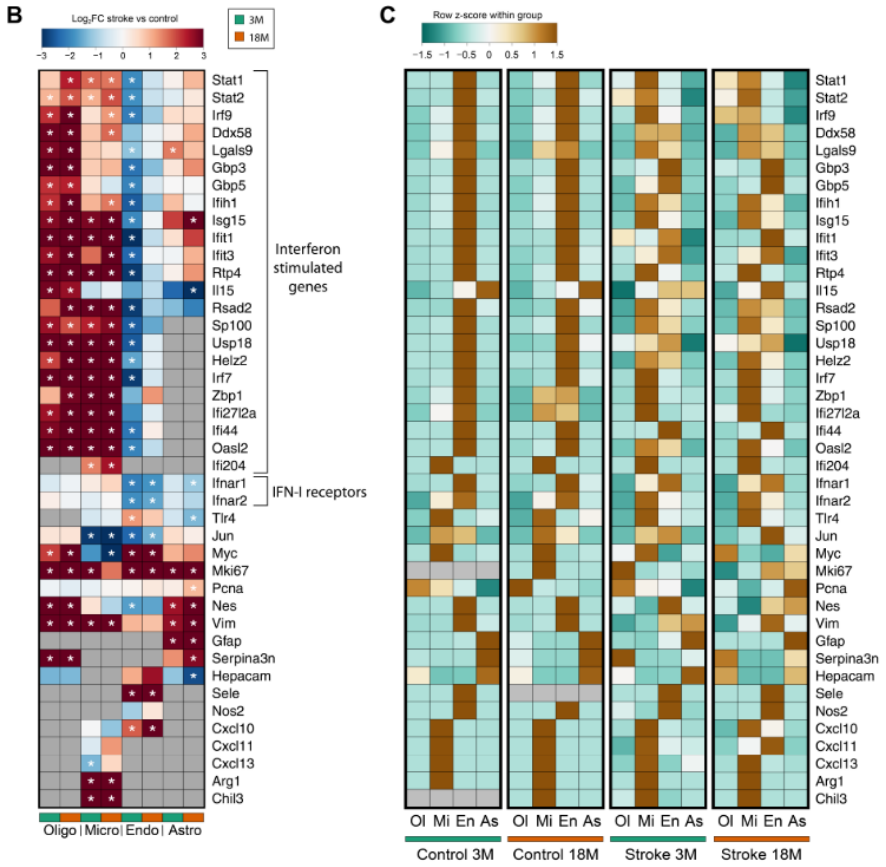
Parsing the Interferon Transcriptional Network and Its Disease Associations

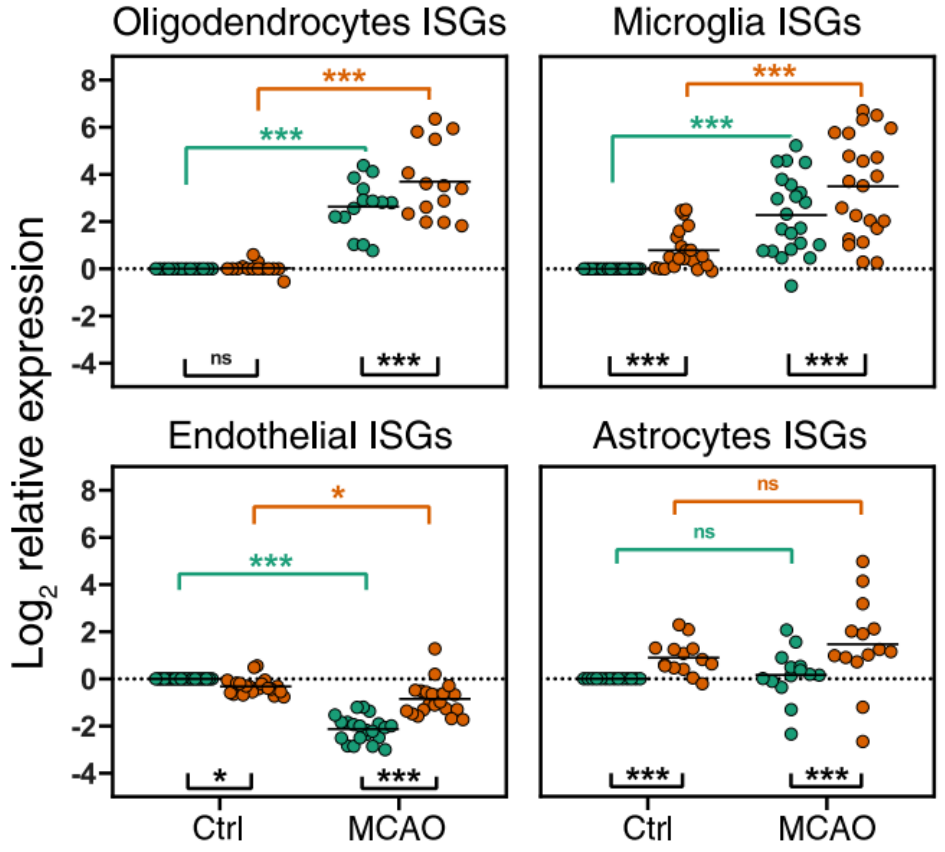
Sara Mostafavi [1, 2, 8], Hideyuki Yoshida [1, 8], Devapregasan Moodley [1], Hugo LeBoité [1], Katherine Rothamel [1], Towfique Raj [3, 5], Chun Jimmie Ye [3], Nicolas Chevrier [4], Shen-Ying Zhang [6], Ting Feng [1], Mark Lee [3], Jean-Laurent Casanova [6], James D. Clark [7], Martin Hegen [7], Jean-Baptiste Telliez [7], Nir Hacohen [3], Philip L. De Jager [3, 5], Aviv Regev [3] ... Christophe Benoist [1]





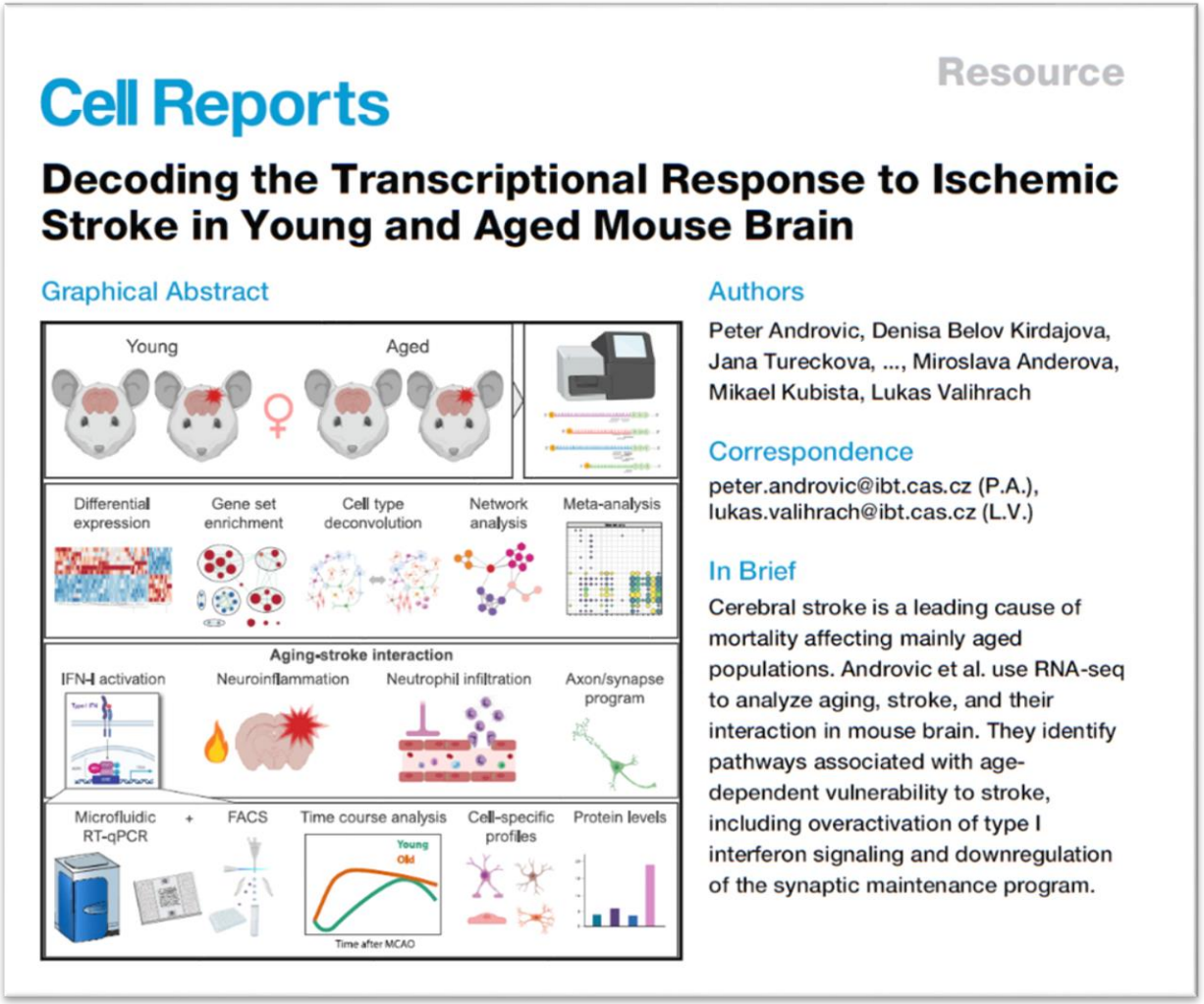
Cell-specific analysis of IFN-I signaling in young and aged mice after stroke





Summary

- Response to stroke in young and aged brain is similar, but differs in magnitude
- Aged ischemic brain is characterized by upregulation of type-I interferon signaling
- Glial cells main contributors



Acknowledgements

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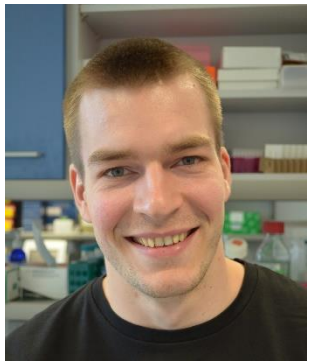

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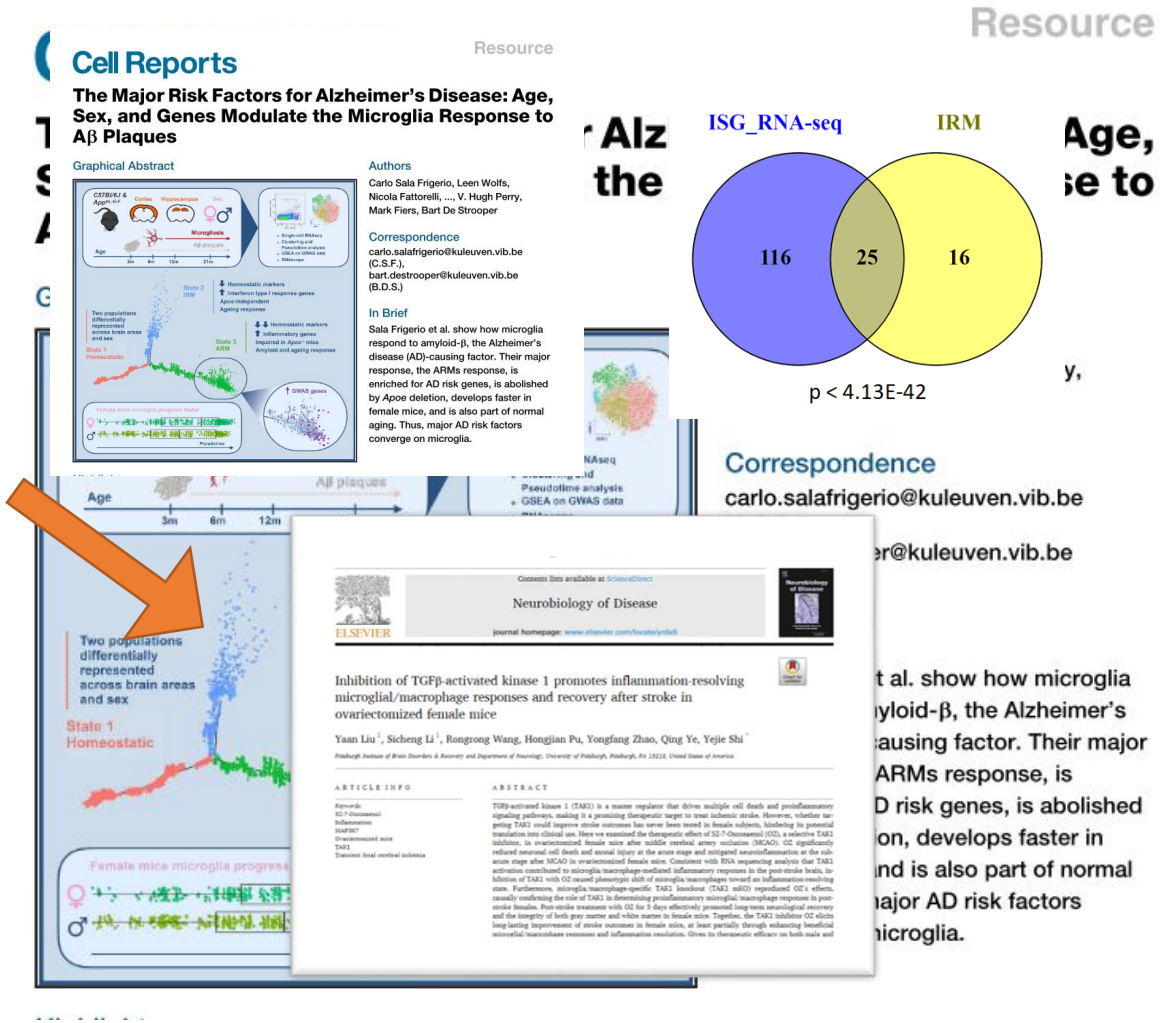
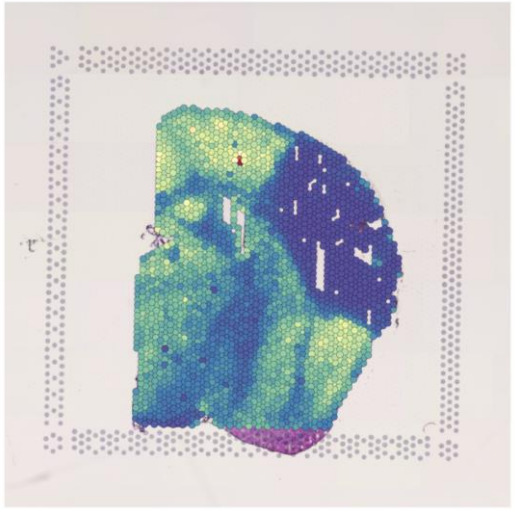
Follow-up work

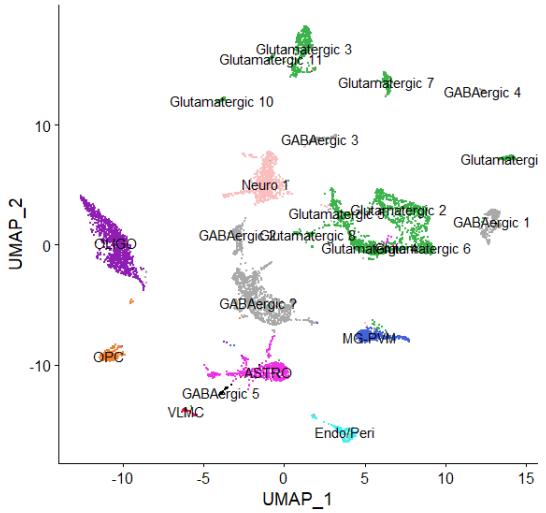


- Stroke-induced interferon response showed strong overlap with markers of interferon response microglia (IRM)
- Manipulation of inflammatory response governed by microglia improves regeneration after transient MCAO

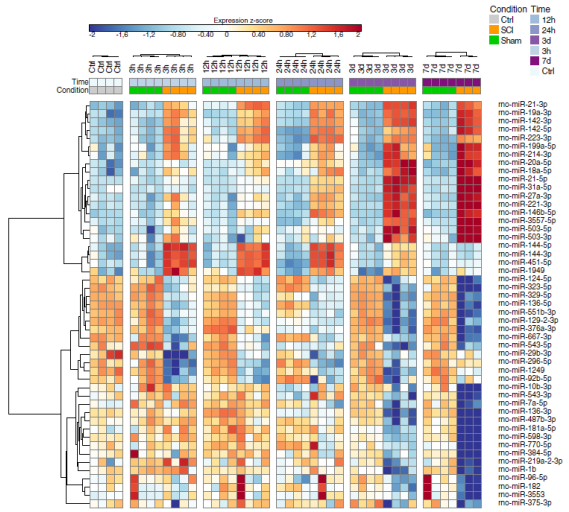
Follow-up work



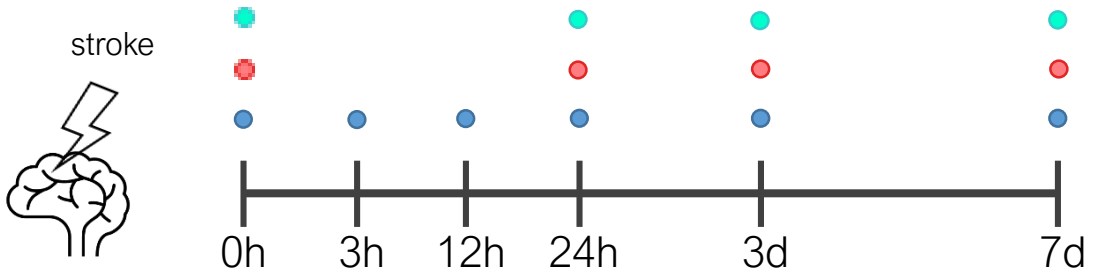
Spatial Transcriptomics (Visium)

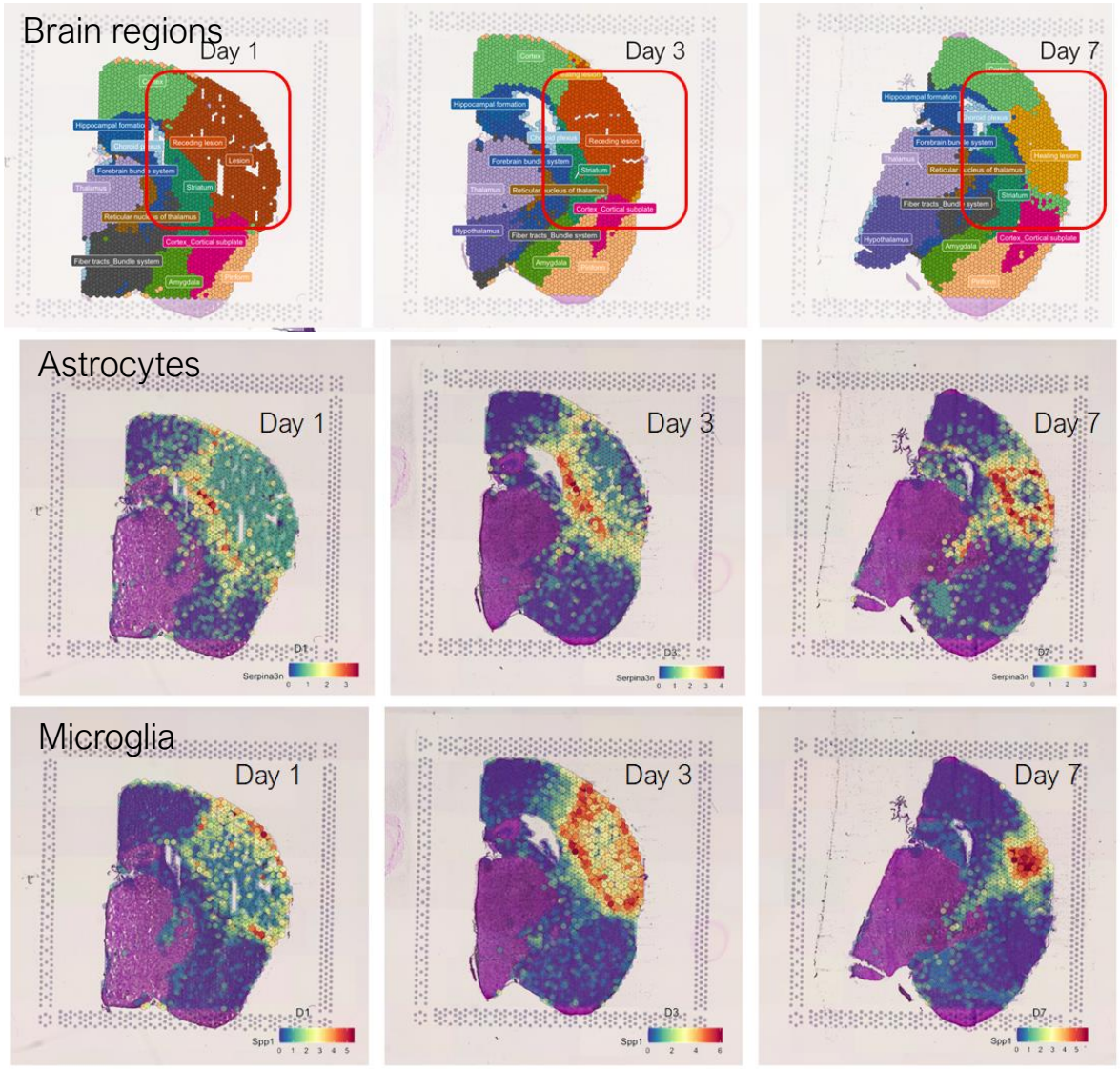


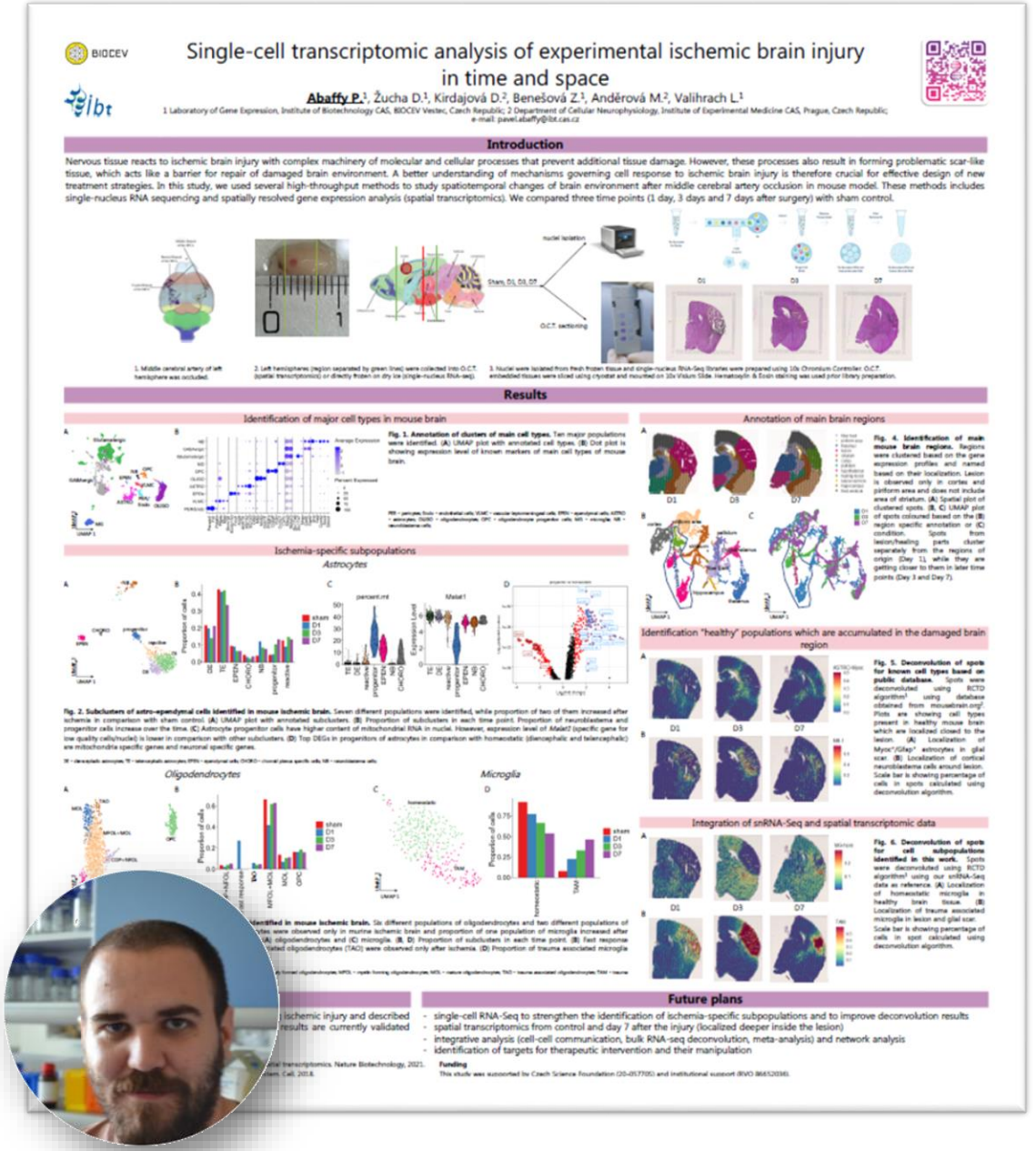
Single-nucleus RNA-Seq



Bulk RNA-Seq







Single-cell transcriptomic analysis of experimental ischemic brain injury in time and space

Abaffy P.[1], Žucha D.[1], Kirdajová D.[2], Benešová Z.[1], Anděrová M.[2], Valihrach L.[1]

1 Laboratory of Gene Expression, Institute of Biotechnology CAS, BIOCEV Vestec, Czech Republic; 2 Department of Cellular Neurophysiology, Institute of Experimental Medicine CAS, Prague, Czech Republic;

Introduction

Nervous tissue reacts to ischemic brain injury with complex machinery of molecular and cellular processes that prevent additional tissue damage. However, these processes also result in forming problematic scar-like tissue, which acts like a barrier for repair of damaged brain environment. A better understanding of mechanisms governing cell response to ischemic brain injury is therefore crucial for effective design of new treatment strategies. In this study, we used several high-throughput methods to study spatiotemporal changes of brain environment after middle cerebral artery occlusion in mouse model. These methods includes single-nucleus RNA sequencing and spatially resolved gene expression analysis (spatial transcriptomics). We compared three time points (1 day, 3 days and 7 days after surgery) with sham control.

Results

Identification of major cell types in mouse brain

Annotation of main brain regions

Ischemia-specific subpopulations

Astrocytes

Identification "healthy" populations which are accumulated in the damaged brain region

Oligodendrocytes

Microglia

Integration of snRNA-Seq and spatial transcriptomic data

Future plans

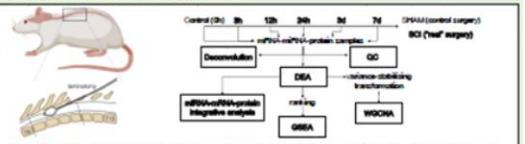
- single-cell RNA-Seq to strengthen the identification of ischemia-specific subpopulations and to improve deconvolution results
- spatial transcriptomics from control and day 7 after the injury (localized deeper inside the lesion)
- integrative analysis (cell-cell communication, bulk RNA-seq deconvolution, meta-analysis) and network analysis
- identification of targets for therapeutic intervention and their manipulation

Multimodal expression analysis of spinal cord injury in rat model

Ruslan Klassen [1,2], Daniel Zucha [1,2], Barka Benesova [1,2], Eva Rohlova [1,2], Peter Androvic [1,4], Pavel Abaffy [1], Lucia Urdzikova-Machova [5], Natalie Romanyuk [5], Lukas Valihrach [1]

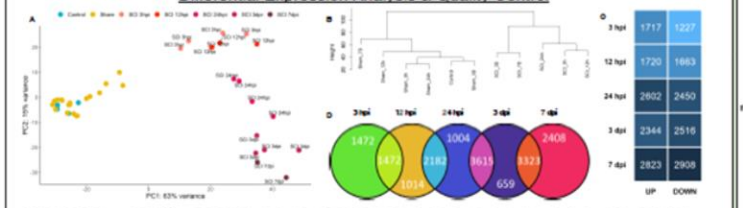
Background & Experimental design

Spinal cord injury (SCI) is a severe and devastating neurological disease with no effective treatment strategy. SCI involves complex interaction of wide spectrum of molecules governing biochemical and physiological processes during the progression of the injury. Although, some progress has been made in characterization of molecular networks underlying pathophysiological changes in SCI, a deeper understanding of these processes is still missing. Here, we performed complex multiomics analysis of experimental contusion model of SCI. We collected **RNA-seq, smallRNA-seq** and **proteomics** data on *Rattus norvegicus* with experimental SCI at different time intervals (**3, 12, 24 hours after injury** and **3, 7 days after injury**) and compared them with SHAM surgery controls. To analyze genome-wide expression changes, we applied various methods, such as **DEA, WGCNA, GSEA, multimodal network analysis**, as well as analysis of the cell type proportion.



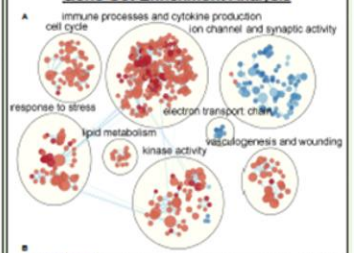
For each time point, 4 samples were taken from each group (SHAM and SCI). Qualitative data analysis and hierarchical clustering of samples were made. After excluding detected samples, a quantitative analysis was carried out where many unique genes with differential expression were recognized (log2fold change) ≥ 0.58; adjusted P-value ≤0.05). Computer deconvolution was also performed to display the relative changes in cell types at different time points after SCI. Finally, the analysis of the correlation between miRNA and mRNA expression levels was performed.

Differential Expression Analysis & Quality Control



PCA plot (A) obtained as a result of quantitative analysis of data before DEA and hierarchical clustering (B) of samples. These two plots show how the samples from the early time points (3, 12, 24 hours) separate from the samples from the later time points (3, 7 days). The number of differentially expressed genes (D) and their distribution between time points (D) are shown in the following figures. These data reflect an increase in the total number of DE genes over the time post injury, but there is also a trend towards an increase in the number of genes shared between subsequent time points, with a decrease in the number of unique genes for a specific time point, except for 7 dpi.

Gene Set Enrichment Analysis



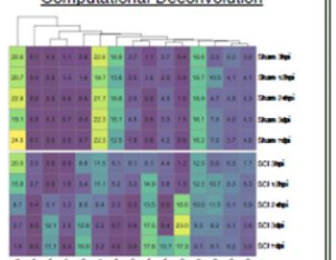
Gene set enrichment analysis reveals biological processes altered upon spinal cord injury in rats. Enriched GO terms are visualized as network (A), with nodes representing enriched GO terms, and lines between nodes representing overlapping gene sets. Most upregulated cellular processes include metabolic pathways, extracellular matrix remodeling, activation of cell cycle and process associated with immune response such as cytokine production. Most downregulated genes are associated with transmembrane ion channel transport, synaptic activity and electron transport channel. The processes with the highest significance values are displayed in the figure (B), where the color of the process on the x-axis symbolized the direction of the changes (red - up-regulated, blue - down-regulated, black - in both).

miRNA-mRNA-proteomic Integrative Analysis

The results of the integration of miRNA-mRNA (A) and miRNA-proteins (B) with each other based on predictions of miRNA targets obtained in miRWalk. The results of DEA were used for integration. The threshold parameters for miRNA-mRNA correlation analysis were set as: adjusted P-value ≤0.05 and r ≤-0.85. The threshold for miRNA-protein correlation was lower (adjusted P-value ≤0.05; r -negative). The negative correlation value means that an increase in the amount of miRNA leads to a decrease in its mRNA and/or protein targets. In both figures, miRNAs that are common to both miRNA and proteins are shown in yellow.

Up & Down Regulated Genes

Computational Deconvolution



Estimated proportion changes for major cell types between sample groups. Computational deconvolution was performed using CibersortX algorithm to estimate the proportion changes for major cell types based on transcriptomic data from Mitch et al., 2021. Plot shows between-group comparisons. Spinal cord injury is accompanied by mostly proliferation of microglia and dividing myeloid cells along with neuronal and oligodendrocytes death.

Conclusion and next steps

- DCI is manifested by massive changes in gene expression, resulting in over 9000 differentially expressed genes.
- Transcriptome response is divided into early response (up to 24 hours after injury) and late response (3 and 7 days after injury).
- The early response is associated with cell death, cell migration, angiogenesis. Activation of immune cells begins 12 hours after injury and reaches its peak in 3-7 days. The processes associated with synaptic activity are suppressed up to 3 days after the injury. Starting from 3 days from injury, there is a change in the organism response, which is often referred as a sub-acute stage of injury in the literature (James et al., 2011).
- Transcriptomic changes are partly explained by decrease of oligodendrocytes, astrocytes, and neuronal cells. After injury in the late phase, the level of fibroblasts, macrophages, monocytes, microglia and dividing myeloid cells increases.
- Further, we plan to to find, with the help of other databases and available literature, the key miRNAs playing the role in SCI, their potential targets and characterized processes involved.

References

[1] Klassen, R., Svoboda, M. [2] Methodical Guide to Spinal Cord Injury Treatment and Functional Changes from Acute to Chronic Stage [3] Mitch, W. et al., 2021. Plot shows between-group [4] Li, Q., Cao, L., Tian, Y. et al. Single-cell analysis of the ischemic injury in the spinal cord and intervention in the hyperbaric oxygen [5] Ma, Y., Wang, X., Jia, T. et al. Neuroinflammation and [6] Ma, Y., Wang, X., Jia, T. et al. Neuroinflammation and

Contact

Transcriptional profiling of human cerebral organoids harbouring an Alexander disease-causing GFAP mutation

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INTRODUCTION

Alexander disease (AxD) belongs to rare severe neurodegenerative disorders. It is caused by mutations in an intermediate filament protein GFAP, an important component of cytoskeleton expressed primarily by astrocytes[1]. Effects of these mutations can be effectively studied using human cerebral organoids differentiated from patient-derived induced pluripotent stem cells (iPSCs), as they allow modeling diseases' phenotype on the human genetic background[2].
AxD patient-derived brain organoids exhibited an aberrant phenotype in comparison with their isogenic controls already in early stages of development. In this study, we performed bulk transcriptomic analysis on these young organoids in order to identify changes of gene expression preceding and accompanying the observed developmental impairment. To better identify genes and pathways potentially linked with the AxD, we compared our data also with a previously published dataset[3].

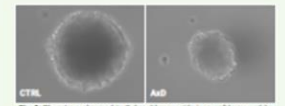


Fig. 1: Phenotype observed in 8-day-old organoids is possibly caused by mutation in GFAP. Isogenic control samples (CTRL) maintain round and regular shape whereas diseased organoids appear small, asymetrical and struggling with development. Organoids above are derived from 825 cell line and captured under 20x magnification.

RESULTS

Differential Expression Analysis

- ✓ GFAP is expressed at a very low level
- ✓ dysregulated genes are present since day 3
- ✓ cell lines share these genes with one another and also with the reference dataset

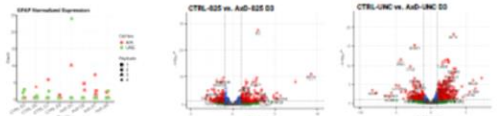


Fig. 2: Very low expression of GFAP was detected in our samples. Nevertheless, a mutation in this protein seems to effect the early development of brain organoids.

Fig. 3: Volcano plots are showing a high number of differentially expressed genes (DEGs) in AxD organoids compared to isogenic controls already at day 3 in both studied cell lines (825, UNC). Genes with (log2FC) > 1 and padj < 0.1 were considered significantly up- or downregulated and are highlighted by red colour.

Fig. 4: AxD cell lines 825 and UNC share multiple genes in each time point. The amount of common upregulated genes steadily increases in time. Downregulated genes follow a similar trend, although at lower numbers. Several upregulated genes in both cell lines were also found in the dataset published by Li et al. 2018[3], where patient-derived induced astrocytes (iA) and post mortem brain samples (PMB) were investigated. At day 9, three genes are present in all four compared gene sets. Fewer common downregulated genes were identified and none of them is shared across the four gene sets.

Gene Set Enrichment Analysis (GSEA) Using Gene Ontology Database

- ✓ terms related to plasma membrane, adhesion, migration and immune response are upregulated in AxD samples
- ✓ terms linked to mitochondrial and ribosomal functions are downregulated in 825 cell line

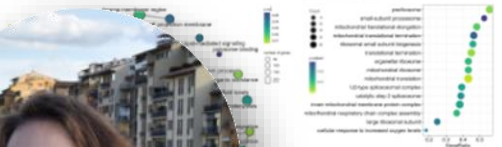
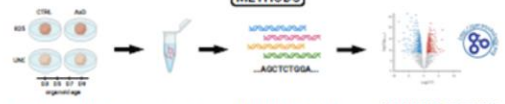


Fig. 5: GSEA revealed a high number of dysregulated Gene Ontology (GO)[4] terms in our samples. Padj < 0.1 was considered as the significance threshold and only top 50 (15 resp.) terms are visualized. At day 9 the upregulated terms in UNC line shown here were mostly related to plasma membrane, transmembrane transport, cell adhesion, migration, but also immune-response activation. These terms were also reported previously in iA cultures and post mortem brain samples[5]. Downregulated GO terms in the 825 line, where the phenotype seems to be more pronounced, include mitochondrial and ribosomal processes.

METHODS



SAMPLE PREPARATION
- organoids differentiated from 2 patient-derived iPSC lines (AxD-825, AxD-UNC)
- organoids differentiated from isogenic control iPSC lines with corrected mutation (CTRL-825, CTRL-UNC)
- samples harvested at 4 timepoints (day 3, 5, 7 and 9)

LIBRARY PREPARATION AND SEQUENCING
- sequencing library prepared using QuantiSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina
- samples sequenced on Illumina NovaSeq Platform

DIFFERENTIAL EXPRESSION AND ENRICHMENT ANALYSES
- sequencing data preprocessed using UMI-tools, Trimmomatic, SortMeRNA and STAR
- data analyzed using R packages DESeq2[6] (differential expression) and clusterProfiler[7,8] (GSEA)

META ANALYSIS

CONCLUSIONS

- The two studied iPSC lines differ in transcriptomic changes and the timepoint of their onset during the early organoid development.
- Very low expression of GFAP was detected at the mRNA level. However, the effect of its mutation appears to be substantial. Follow-up experiments will attempt to further explain this phenomenon.
- A number of genes differentially expressed between CTRL and AxD was detected already at day 3 of organoid development.
- Gene Ontology terms related to cell adhesion, migration and development are dysregulated and are to some extent shared by both cell lines.
- Mutation in GFAP might also result in dysregulation of mitochondrial and ribosomal functions, as they naturally interact with cytoskeleton.
- Common DEGs and GO terms were found in comparison with a published dataset[3].
- This experiment is currently validated by RT-qPCR and immunohistochemistry and provides a baseline for follow-up experiments including single-cell RNA sequencing.

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Getting more out of bulk RNA-Seq with digital cytometry

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Introduction

Bulk RNA-Seq data provide insightful look onto the overall state of transcriptome in the researched tissue. Bulk RNA-Seq is however being overshadowed by single-cell RNA sequencing, which allows for detailed identification of processes involved in damage and disease. Here, we discuss the use of deconvolution algorithm CibersortX, developed by Newman et al (2019), which **estimates cell type abundancies in bulk tissue** based on a single-cell reference. We have applied CibersortX to describe the dynamics of tissue composition in bulk specimen of two distinct models of acute brain injury, **succesfully identifying the differences down to rare cellular subtypes.**

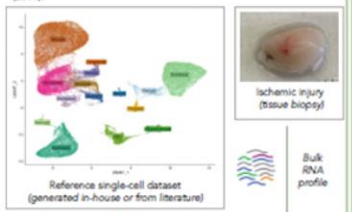
Conclusions

Digital cytometry (= deconvolution) enables:

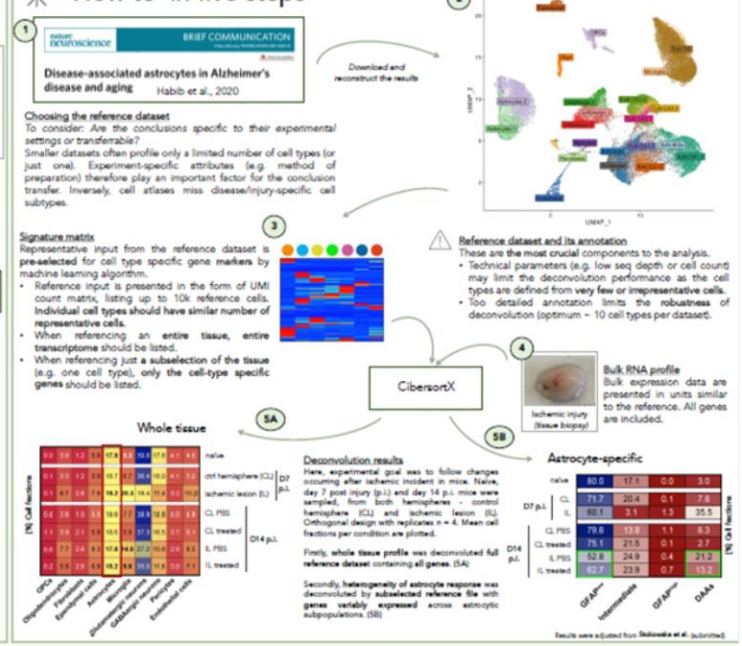
- To dissect **cell type composition** in bulk samples, even for **rare** (~1%) populations
- Identification of unconventional cell type markers
- To estimate the **cell type-specific expression** for robust markers

Framework

Framework of digital cytometry (deconvolution by Newman et al (2019):



'How to' in five steps



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Small RNA-sequencing for Analysis of Circulating miRNAs: Benchmark Study

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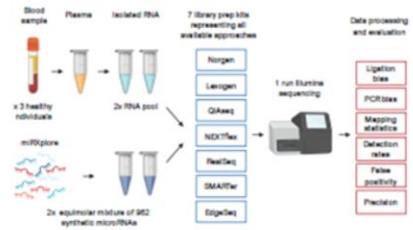
Background

The application of **small RNA sequencing** (small RNA-seq) for profiling of **circulating microRNAs** (miRNAs), novel **promising biomarkers**, is increasing. However, the accuracy of the method is **limited by bias** introduced by ligation, PCR or polyadenylation. Therefore recently, **new approaches** were developed to prevent the biases. Here, we present **comparison of all small RNA-Seq library preparation approaches** that are commercially available for quantification of miRNAs in biofluids.

Experimental design



Conclusions

- There is **no single protocol outperforming others** across all metrics.
- **Protocol-specific biases** give rise to limited overlap of measured miRNAs profiles between methods and cause their low correlation.
- The correlation can be improved by **normalization** approach utilizing bias ratios **learnt from miRXplore** sample.
- The application of UMIs has only marginal effect on overall quantification bias.
- Traditional two-steps ligation methods introduce large bias.
- Protocols performing well in majority of metrics use capture probes (EdgeSeq) or randomized adaptors (NEXTflex).

Funding

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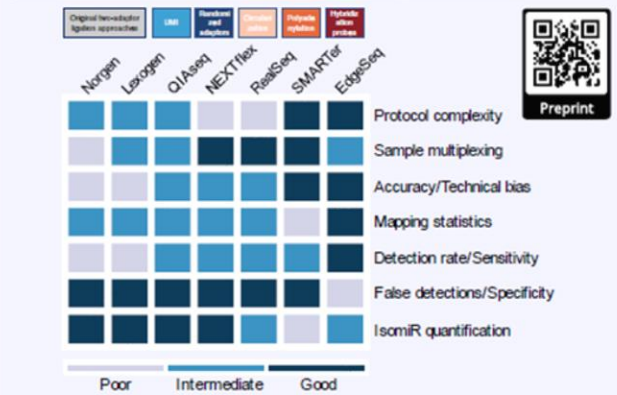
Selection of suitable protocol for small RNA-seq

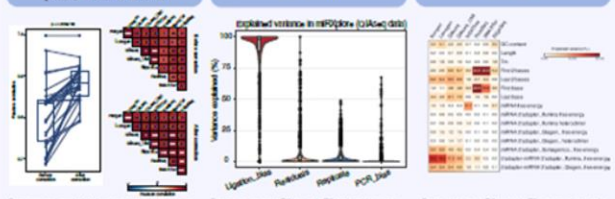


Preprint

Normalization of data with factors learnt from equimolar pool leads to higher between protocol correlations

Ligation is the main contributor of the overall bias in the small RNA-seq

First two bases of miRNA affects ligation of adaptor in RealSeq



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