Generation of Orthotopically Functional Salivary Glands from Embryonic Stem Cells

Research Question:

Does the addition of mesenchyme cells to the transplanted induced Salivary Glands (iSGs) promote faster maturation?

Cherron Griffith
UAlbany RNA Institute
Larson Lab: Developmental Biology
August 2, 2024

Background

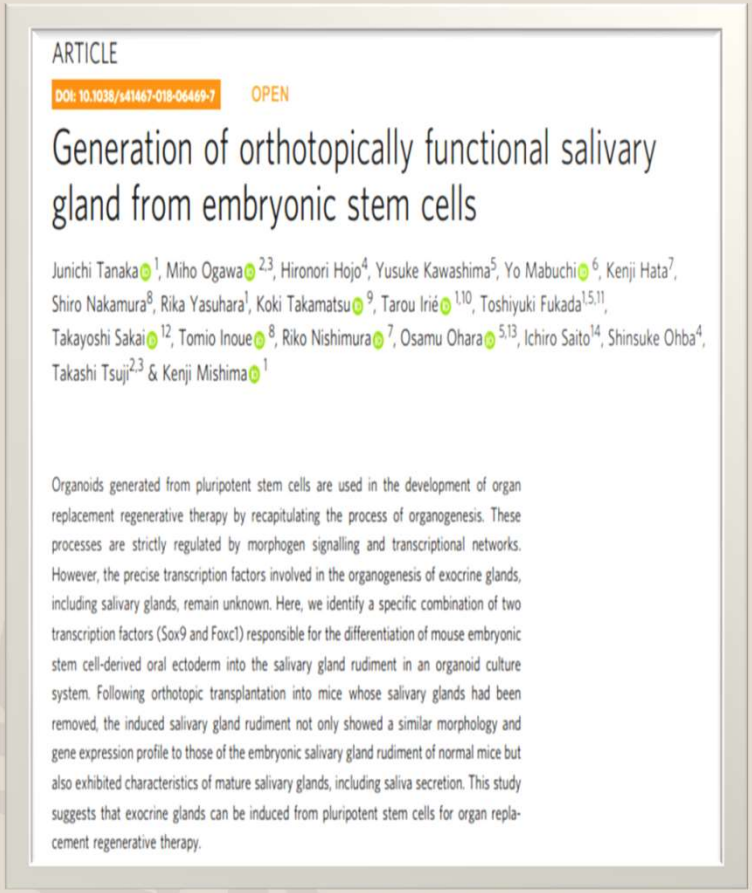
- Development of mouse embryonic stem cells into salivary glands
- Transplanted induced salivary glands (iSGs) with the transcription factors Sox2 and Foxc1

Research Question:

Does the addition of mesenchyme cells to the transplanted induced Salivary Glands (iSGs) promote faster maturation?

Project Overview:

- Differential Gene Analysis
- Gene Ontology Enrichment Analysis



ARTICLE

DOI: 10.1038/s41467-018-06469-7 OPEN

Generation of orthotopically functional salivary gland from embryonic stem cells

Junichi Tanaka[1], Miho Ogawa[2,3], Hironori Hojo[4], Yusuke Kawashima[5], Yo Mabuchi[6], Kenji Hata[7], Shiro Nakamura[8], Rika Yasuhara[1], Koki Takamatsu[9], Tarou Irié[1,10], Toshiyuki Fukada[1,5,11], Takayoshi Sakai[12], Tomio Inoue[8], Riko Nishimura[7], Osamu Ohara[5,13], Ichiro Saito[14], Shinsuke Ohba[4], Takashi Tsuji[2,3] & Kenji Mishima[1]

Organoids generated from pluripotent stem cells are used in the development of organ replacement regenerative therapy by recapitulating the process of organogenesis. These processes are strictly regulated by morphogen signalling and transcriptional networks. However, the precise transcription factors involved in the organogenesis of exocrine glands, including salivary glands, remain unknown. Here, we identify a specific combination of two transcription factors (Sox9 and Foxc1) responsible for the differentiation of mouse embryonic stem cell-derived oral ectoderm into the salivary gland rudiment in an organoid culture system. Following orthotopic transplantation into mice whose salivary glands had been removed, the induced salivary gland rudiment not only showed a similar morphology and gene expression profile to those of the embryonic salivary gland rudiment of normal mice but also exhibited characteristics of mature salivary glands, including saliva secretion. This study suggests that exocrine glands can be induced from pluripotent stem cells for organ replacement regenerative therapy.

Materials and Methods

HPCC
- Gathered ReadsPerGene files from the experimental data

RStudio
- Followed DGE analysis pipeline

RStudio
- DESeq2 program in RStudio to generate visual figures

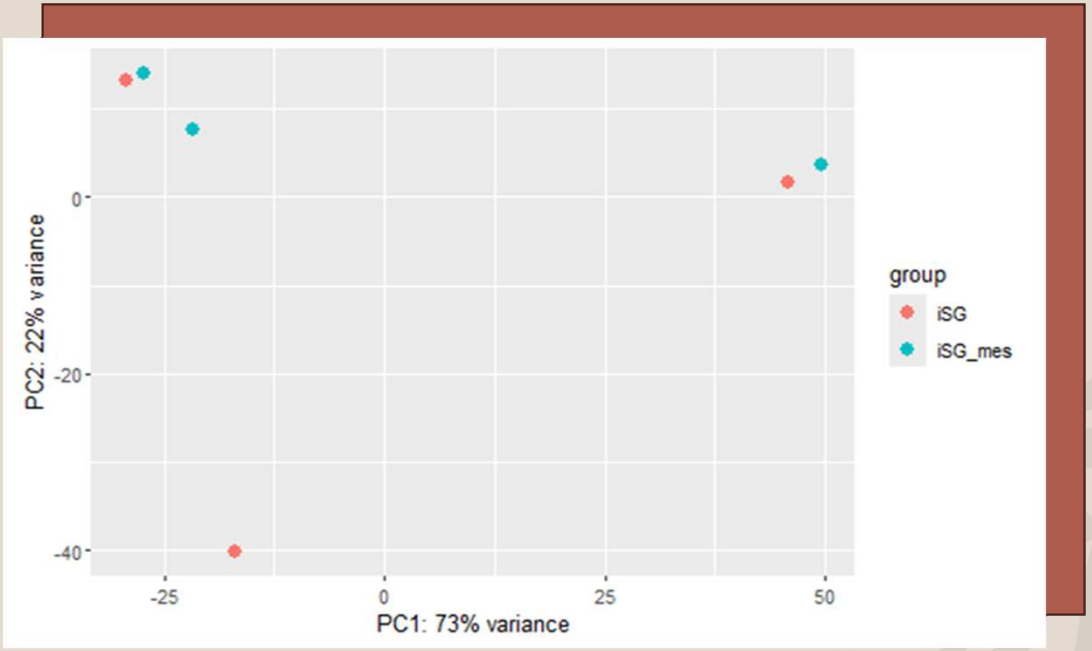
Metascape
- GO enrichment analysis

Differential Gene Expression (DGE) Analysis

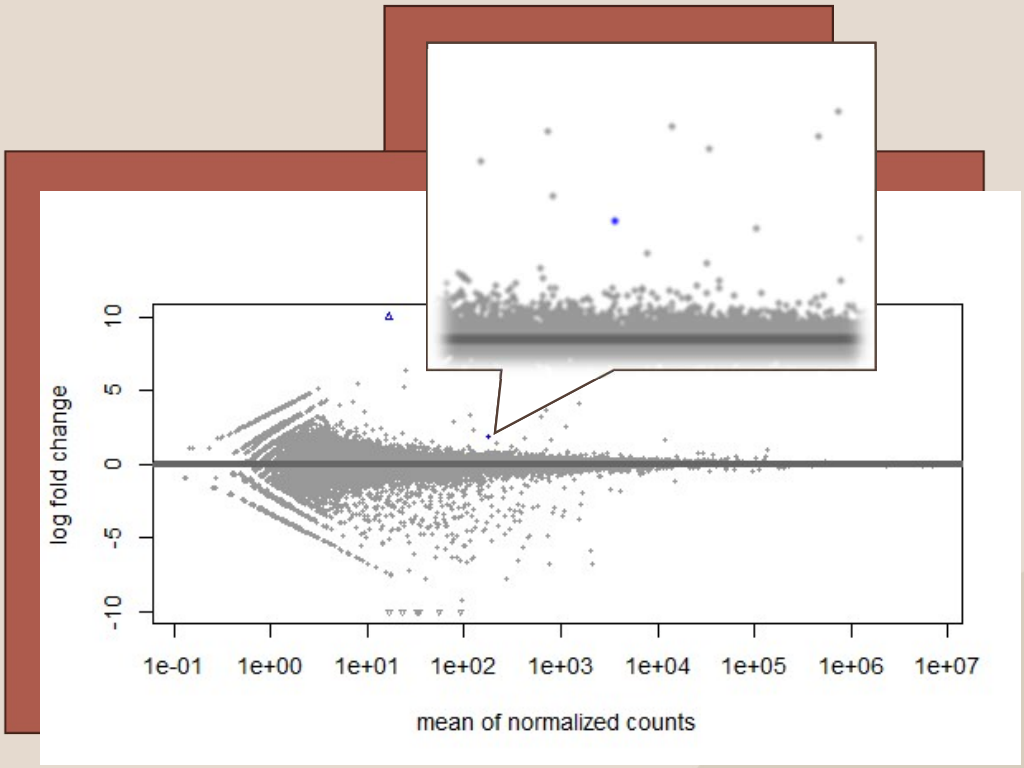
SEARCHING FOR DIFFERENTIALLY EXPRESSED GENES (DEGS) WITHIN THE EXPERIMENTAL DATA

Principal Component Analysis (PCA) Plot

- iSG without the mesenchyme cells (iSG) are in orange
- iSG with the mesenchyme cells (iSG_mes) are in green
- There is a small amount of variance between the iSG and iSG+mes samples
  - Do not cluster by condition
  - Cluster possibly by adjusted p-value



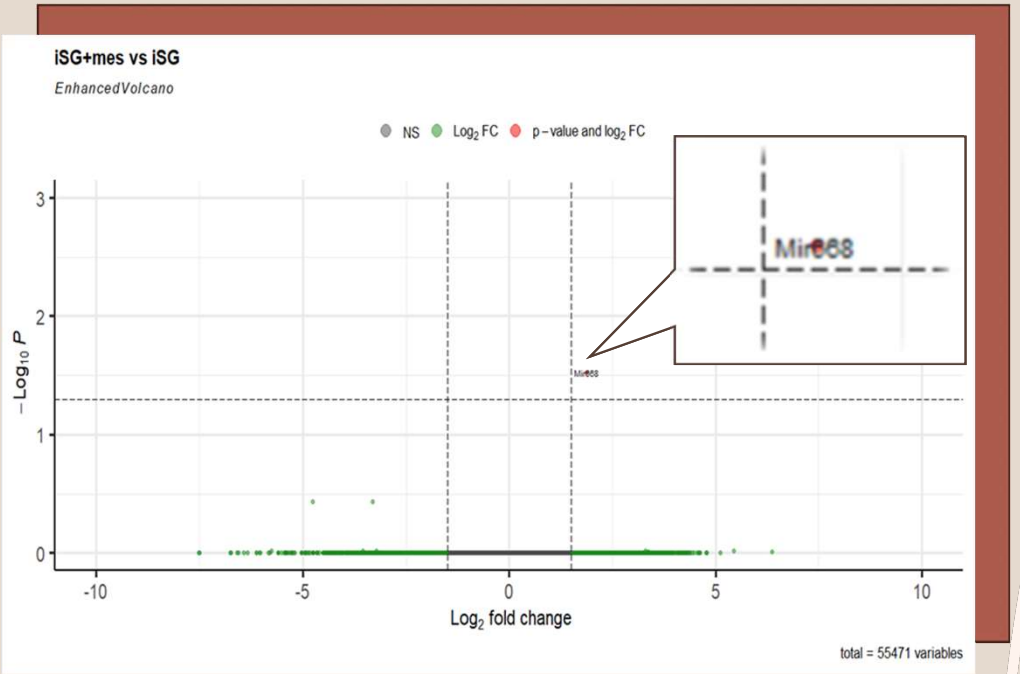
MA Plot



- Not a large amount of genes are differentially expressed
- Only one gene is significantly upregulated
  - indicated by the position of the single blue point in the positive Log2Fold range

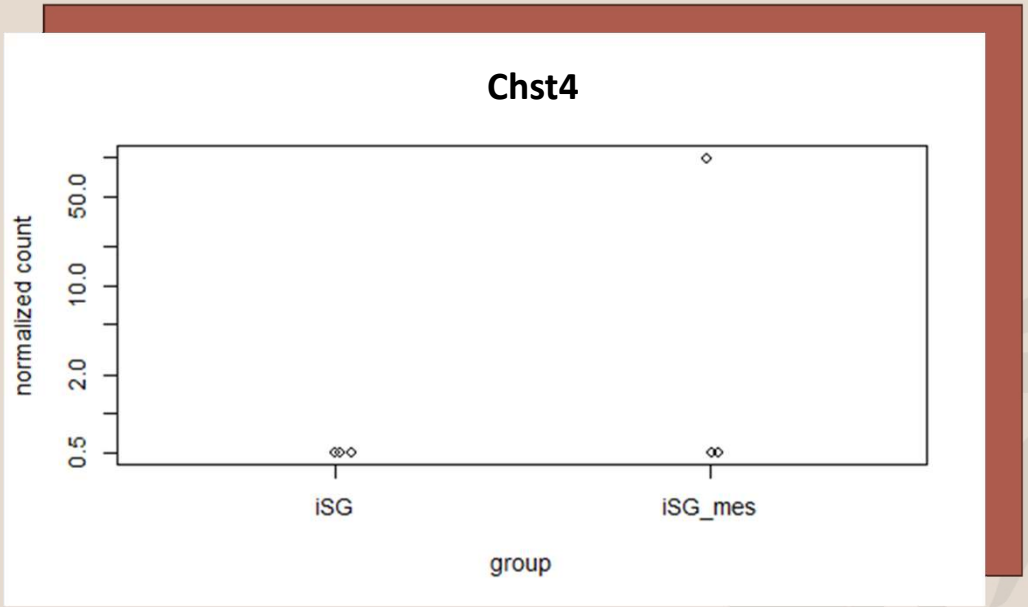
Volcano Plot

- Further supports that there are not many differentially expressed genes
- One point gives a positive Log2Fold change
  - Gene is Mir668
  - This gene is upregulated
- Corresponds with the MA plot

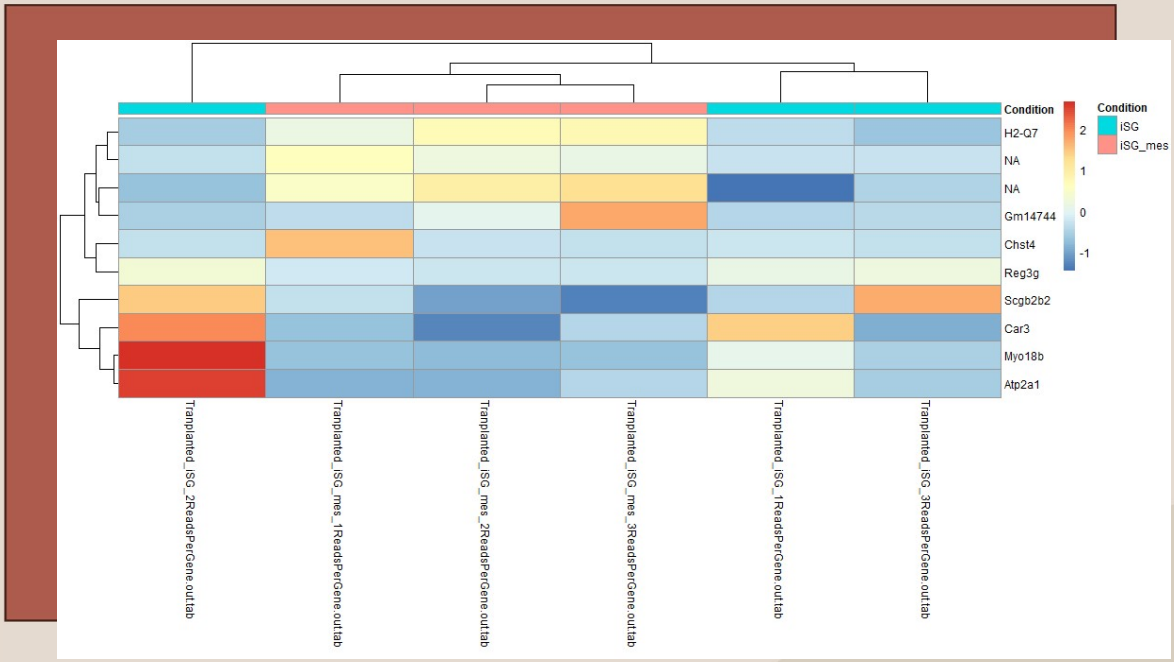


Normalized Count Plot

- Gene is plotted individually for each sample
  - Displays a significantly higher p-value in only one of the samples with the added mesenchyme
  - Correlates with what was seen in the PCA plot
  - Contradicts the MA and Volcano plots



Heatmap



- Some variance in expression between samples with the same condition
- Up Regulated Genes in iSG samples:

Reg3g Car3 Myo18b Atp2a1

- Up Regulated Genes in iSG+mes Samples:

Chst4 Gm14744 H2-Q7

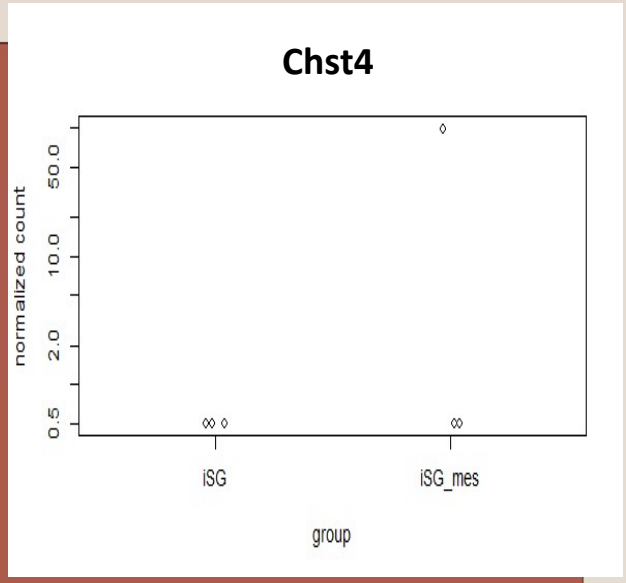
Gene Ontology (GO) Enrichment Analysis

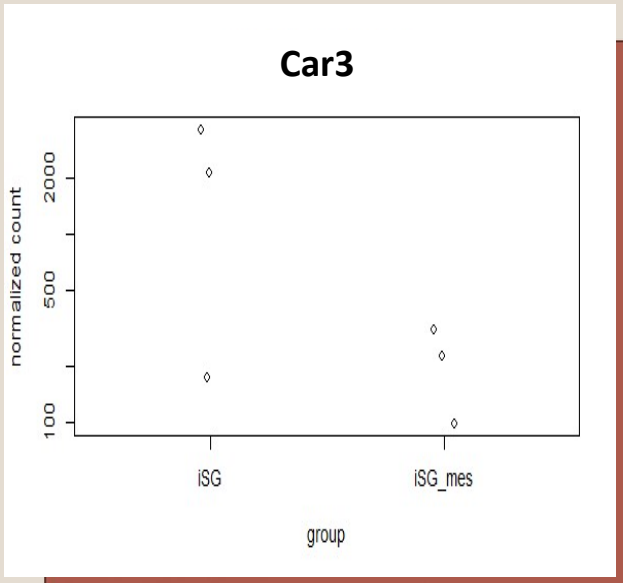
DESCRIBING THE BIOLOGICAL FUNCTION OF STATISTICALLY SIGNIFICANT GENES

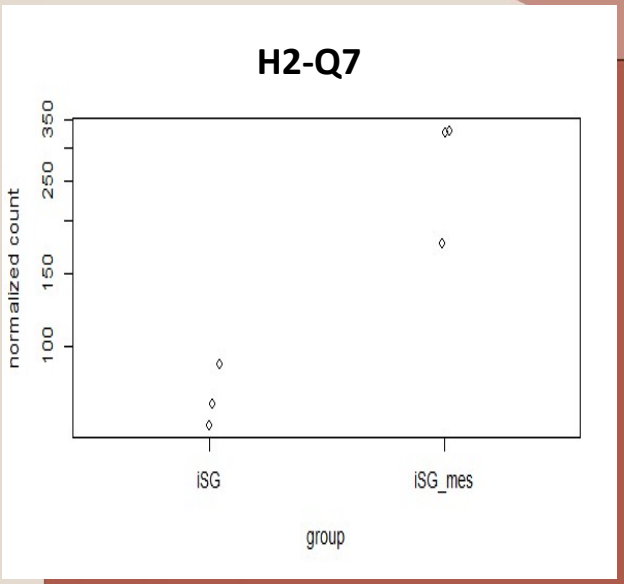
Metascape Gene Ontology Analysis Results

Gene Symbol	Description	Biological Process (GO)
Chst4	carbohydrate sulfotransferase 4	protein sulfation; positive regulation of leukocyte tethering or rolling; N-acetylglucosamine metabolic process
H2-Q7	histocompatibility 2, Q region locus 7	T cell mediated cytotoxicity directed against tumor cell target; T cell mediated immune response to tumor cell; antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-dependent
Myo18b	myosin XVIIIb	cardiac muscle cell development; cardiac cell development; vasculogenesis
Car3	carbonic anhydrase 3	one-carbon metabolic process; response to oxidative stress; response to bacterium
Reg3g	regenerating islet-derived 3 gamma	regulation of detection of glucose; positive regulation of detection of glucose; response to symbiont
Atp2a1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	maintenance of mitochondrion location; regulation of fast-twitch skeletal muscle fiber contraction; positive regulation of fast-twitch skeletal muscle fiber contraction
Gm14744	predicted gene 14744	Biological process
Mir668	microRNA 668	cellular response to hypoxia; miRNA-mediated post-transcriptional gene silencing; regulatory ncRNA-mediated post-transcriptional gene silencing

Metascape Gene Ontology Analysis Results (cont.)







Conclusions

- Promoted immune health in each of the three iSG+mesenchyme samples
- Up regulates the H2-Q7 gene associated with T-cell tumor targeted immune response
- Cannot confidently assume that mesenchyme cells promote better maturation

Future Directions

- Further study to see what cells/tissues directly developed from the mesenchyme cells, and then record their rate of maturation
- Experiment with cells/samples not induced with specific transcription factors
- Increase the amount of samples for more accurate results, and to see more trends within the data
- Test on other organs instead of salivary glands
- Test on samples exposed to factors that can test their immune response

Acknowledgements

Melinda Larson, Ph.D. – Project Mentor
Eric Jestel – Group Leader
Liv Ransiear – Group Leader
All contributors to the Scientific Article
Faculty and Staff of the Bioinformatics Program

Thank You!