ALCAZAR

Exploring Expression of Y-linked males specific genes during mouse gastrulation

Rosa Alcazar[1], Lindsay Hayes[2], Matt McCoy[2], and Frederick J Tan[5]

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine, and Carnegie Institution.

Abstract
The Y chromosome is required for male sexual development. We hypothesized that there are developmental roles precede sexual dimorphism. To identify Y-linked genes expressed in early stages of embryogenesis, we used a single-cell RNA sequencing (scRNAseq) mouse gastrulation dataset. We found 9 evolutionarily conserved mammalian Y-linked genes and checked for their expression patterns in mouse embryos stages from day 6.5 (E6.5) to day 8.5 (E8.5) during gastrulation. We found in all samples robust of Ddx3y, Eif2s3y, Rgp1, Uty, and Kdm5d genes; limited expression in Zfy1 and Uba1y; and no expression in SRY and Usp9y. This supports the hypothesis for development roles for Y-linked genes during embryogenesis

We would like to further identify the cell types and sequential expression patterns as well as check for the expression of target genes.

Introduction

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The evolution of the y chromosome is….
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We used Pijuan-Sala et al. mouse gastrulation dataset that contains the transcriptional profiles of 116,312 single cells from mouse embryos. These were collected 6.5 to 8.5 days post-fertilization

Genes expressed in the Y chromosomes
- Sex differentiation timeline versus expression of y-linked genes
- differential gene expression
- gastrulation in mouse
- conservation of genes linked to the Y

Gene list
MGD by stage

Find some picture about y-linked genes

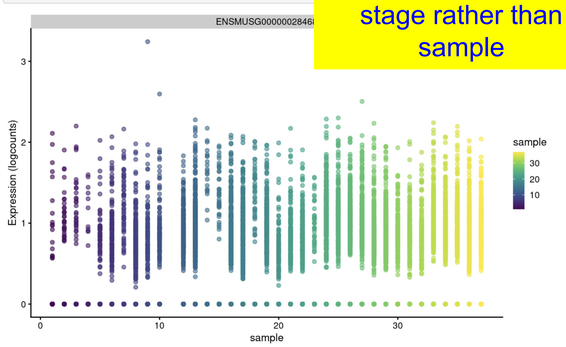
Methods

Which male specific genes are expressed during gastrulation?

Explore Rgp1 male specific marker in drosophila but on chromosome 4 in mouse

```
gene_name <- "Rgp1"
gene_id <- rowData(sce)[which(rowData(sce)[,2] %in% gene_name),]$
plotExpression( sce, gene_id, "sample", colour_by="sample" )
```

Remake x-axis as stage rather than sample



Explore Uba1y male specific marker

Results – STAGES

```
gene_name <- "Uba1y"
gene_id <- rowData(sce)[which(rowData(sce)[,2] %in% gene_name),]$ENSEMBL
plotExpression( sce, gene_id, "sample", colour_by="sample" )
```

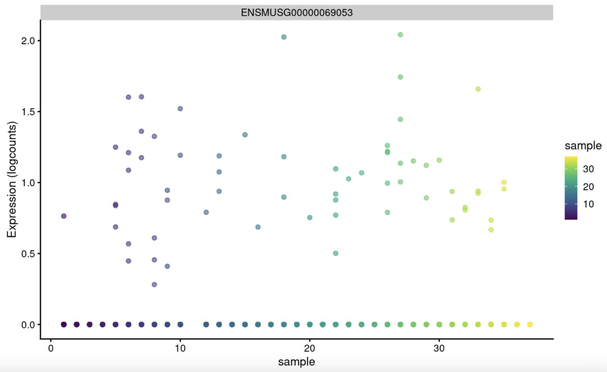


Figure 1. Expression of Xist and Y-linked genes during mouse gastrulation.

Explore SRY male specific marker

```
gene_name <- "Sry"
gene_id <- rowData(sce)[which(rowData(sce)[,2] %in% gene_name),]$ENSEMBL
plotExpression( sce, gene_id, "sample", colour_by="sample" )
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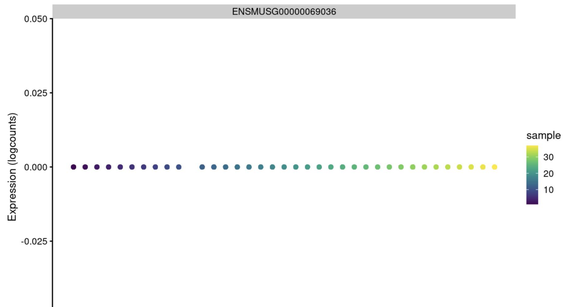
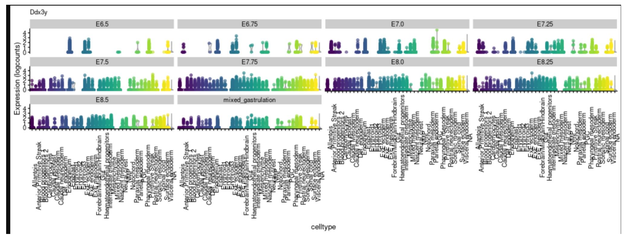
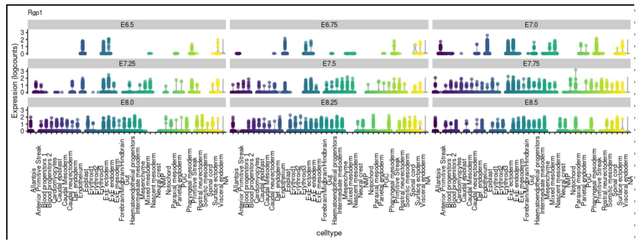


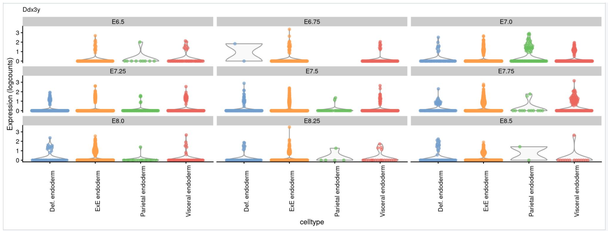
Results -CELL TYPES

In what stages and cell types (from E6.5 to E8.5) are Y-linked genes expressed?





The expression of Ddx3y in endoderm cells



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this paper used sexing of mouse embryos
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6139635/

Acknowldegements

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Scraps for Poster Exploring Expression of Y-linked males specific genes during mouse gastrulation

Rosa Alcazar[1], Lindsay Hayes[2], Matt McCoy[2], and Frederick Tan[5]

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine, Carnegie Science.

Abstract
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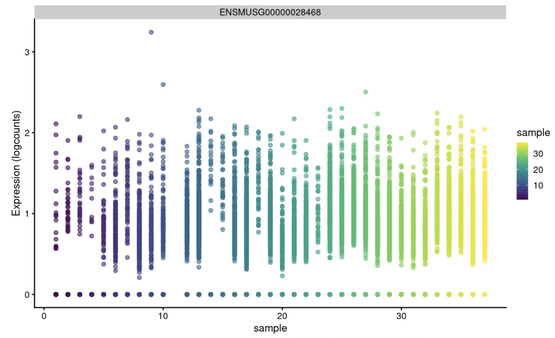
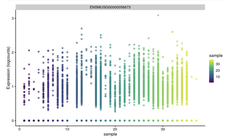
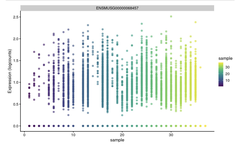


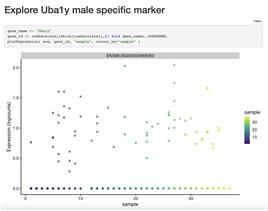
Explore kdm5d male specific marker



Explore Uty male specific marker



Explore Uba1y male specific marker



Explore Zfy1 male specific marker

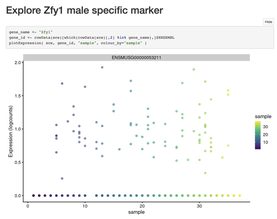
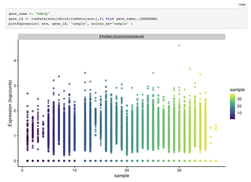


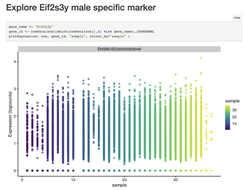
Figure 1. Expression of Xist and Y-linked genes during mouse gastrulation.

Results -Cell Types

In what stages between E6.5 and E8.5 are Y-linked genes expressed?

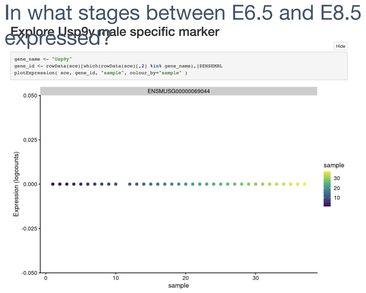


Explore Eif2s3y male specific marker

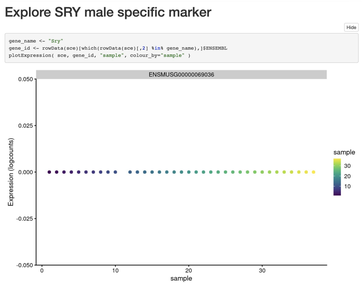


In what stages between E6.5 and E8.5 are Y-linked genes expressed?

Explore Usp9y male specific marker



Explore SRY male specific marker



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Sex typing embryos using expression values of Y-linked genes

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine, Carnegie Insitutions,

HAD TO ADJUST DIRECTION -IN CLASS DISCUSSION

~~Abstract~~
~~Y-linked genes are specifically expressed in Males. The MGD we are using does not distinguish embryos as male or female. We wanted to determine if the gene expression during gastrulation was sufficient to determine the sex of the embryos. we identified X genes that were Y-linked and we checked the expression from E6.5 to E8.5. We also check for expression by celltype. We found …….~~
~~This suggests…..~~
~~Some possible issues in identifying correctly the sex may be…..~~
~~We would like to know try….~~

Introduction

MGD by celltype
~~DNA is packed into structures called chromosome. Sex is determined by differences in gene expression. These difference can from the expression genes on the sex chromosomes but also of the downstream regulation of targets of genes on the Y chromosome. The Y chromosome is very interested as it has a fraction of the genes the X has and have the necessary instructions coded for sex differentiation.~~
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~~Genes expressed in the Y chromosomes~~
- ~~Sex differentiation timeline~~
- ~~differential gene expression~~
- ~~gastrulation in mouse~~
- ~~conservation of genes linked to the Y~~

~~Gene list~~
~~MGD by stage~~
~~MGD by celltype~~

Results

~~Can we sex embryos in the MGD?~~

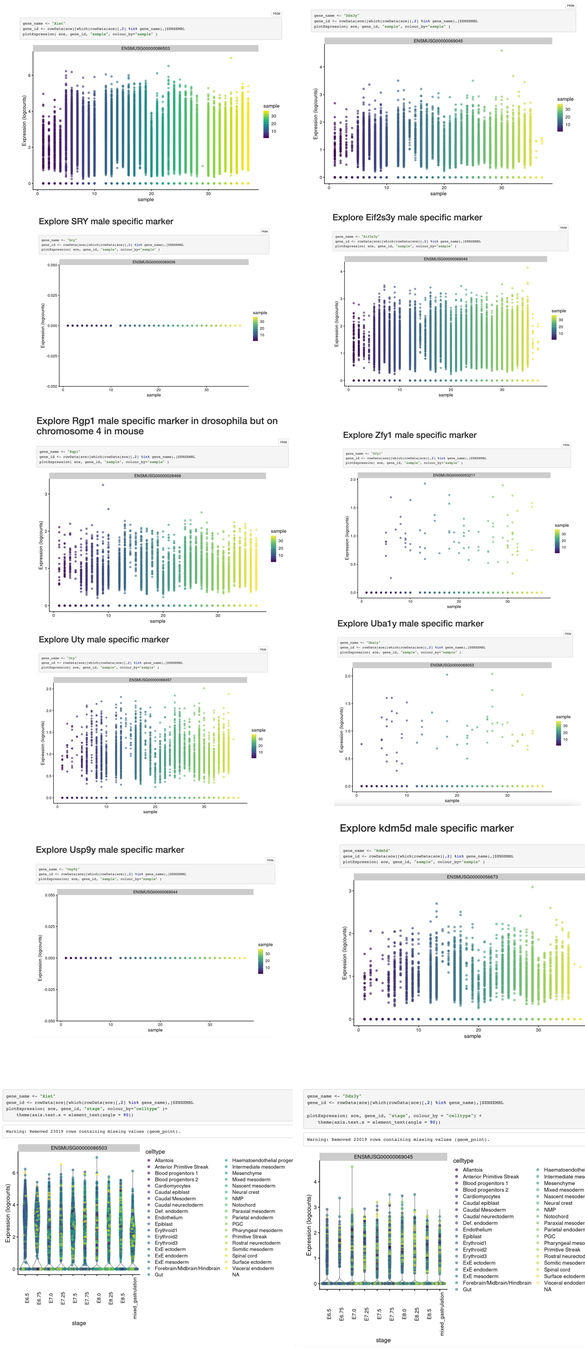

Figure 1. ~~Expression of Xist and Ddx3y in the embryos used for scRNAseq. A) We see expression of both the Xist and Ddx3y in all samples. B) We see expression of both Xist and Ddx3y in all stages.~~

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YANI

Exploring if Wnt7B is part of the Canonical Wnt Signaling Pathway

Yani Trevino[1], Matt McCoy[2], Lindsay Hayes[3], Caitlin Pozmanter[4], and Rosa Alcazar[51]

Clovis Community College, Stanford School of Medicine, and Johns Hopkins School of Medicine

Abstract

The scientific community has yet to fully understand the pathway and function of a multitude of genes. One of which being Wnt7b. It is important that the scientific community strives to uncover precise functions and pathways of genes like Wnt7b because in understanding basic biological systems more can be discovered about how genetic diseases and cancers work. Treatments for these can be made after understanding a gene's functions and pathways it's involved in. By looking for co-expression of genes closely linked to Wnt7b and genes known to be part of the Wnt canonical signaling pathway, I was able to find evidence that supports Wnt7b being part of the canonical signaling pathway. The most significant findings I revealed were a strong connection between beta catenin and Wnt7b suggesting dependency and co-expression of Wnt7b with genes known to be a part of canonical Wnt signaling pathway.

Introduction

The Wnt family of genes is one of the most essential families in the genome. The Wnt genes all play an important role in the Wnt signaling pathway. With proper regulation life will proceed in a healthy manner for multiple organisms: flies, mice, zebra fish, humans etc. The list goes on because the Wnt signaling pathway has been a crucial part of the evolution of multicellular organisms (Holstein, 2012). There are 19 known Wnt genes. The exact function and pathway of each one of the Wnt genes isn't fully known. Generally the Wnt genes play an important role in embryonic development (Ng, 2019), but there's limited information about specific family members function and role in Wnt signaling.

Understanding how a gene interacts with other genes and chemicals in a pathway gives major insights about how the gene functions. Wnt7b is one of the family member that lacks a distinct pathway. However, the scientific community has been able to identify a probable function for Wnt7b gene. Wnt7b is protein coding gene located on chromosome 15 (UCSC) responsible for cell death, differentiation, population proliferation; establishment of localization, immune system process, response to stimulus, signaling, and system development (MGI). Wnt7b's regulator gene is FoxB2 (Koch, 2021). It is well known that the Wnt genes can only signal through three pathways (Schubert, 2013), one canonical and two non-canonical. For clarification, there are two types of Wnt signaling pathways: canonical and non-canonical. In summary, the canonical pathway is beta catenin dependent and the non-canonical functions dependent of beta catenin (Ng, 2019). There is currently no conclusive knowledge or pathway mapped out showing Wnt7b's exact interactions and thus function. There are only 'maybes'. Using R we can make inferences about the pathway Wnt7b is involved in. This means that by looking at Wnt7b's interactions with other genes and it's levels of co-expression with genes confirmed to play well known roles in canonical Wnt signaling, Wnt7b can be confirmed to be part of the canonical Wnt signaling pathway.

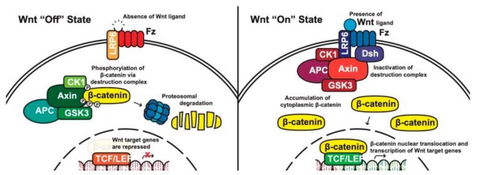


Fig 1A **Canonical**

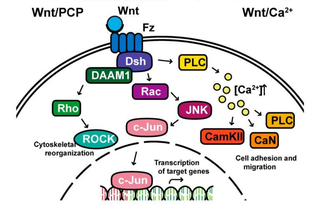
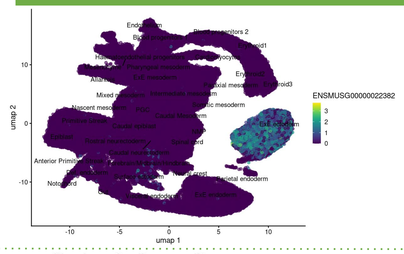


Fig 1b **Non-Canonical**

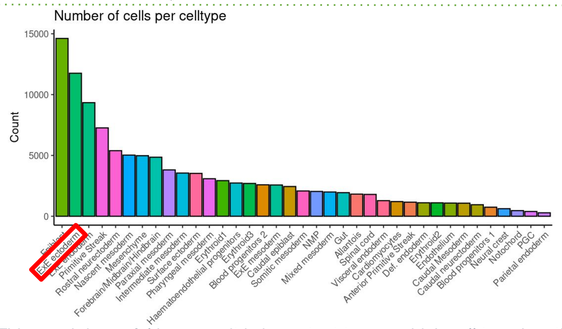
Methods

FlyBase and MGI were used to find a Wnt family gene of interest. Once found a literature search was conducted. Simultaneously, a mouse gastrulation dataset in RStudio was used to generate several graphs using R. Other databases like UCSC Genome Browser, KEGG Pathways, String Network, and CellXGene were used at various time points.

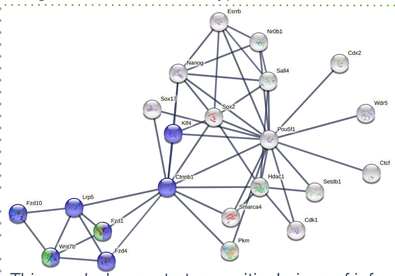
Evidence on why Wnt7B is part of the canonical pathway.



This graph show us the enrichment of Wnt7B in ExE ectoderm cell type. This is significant because ExE ectoderm cell types are important for embryo development.



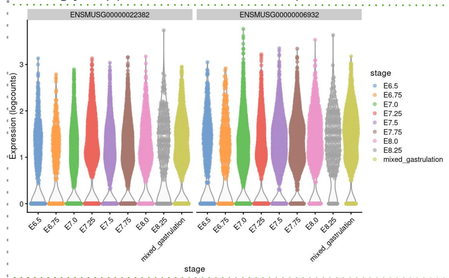
This graph is useful because it is important to see which cell type has the highest number of cells of all the various cell types in embryonic development. Knowing this is important because it can give important insights about which cell types are most crucial to embryonic development.



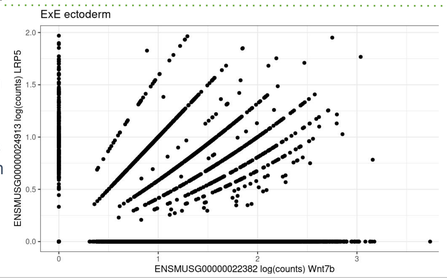
Blue-Canonical Wnt signaling pathway, this is significant because it suggests that Wnt7B is part of the canonical Wnt signaling pathway.

Green-Wnt signaling pathway, planar cell polarity pathway

This graph demonstrates a critical piece of information: a connection between Wnt7b and Ctnnb1, aka beta catenin. This is significant because it suggests that Wnt7b is dependent on beta catenin. If dependent on beta catenin, that'd strongly support that Wnt7b takes part in a canonical Wnt signaling pathway.

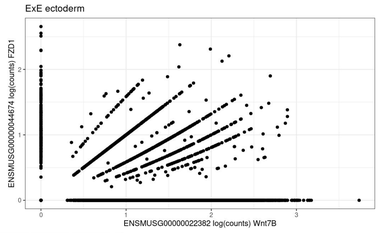


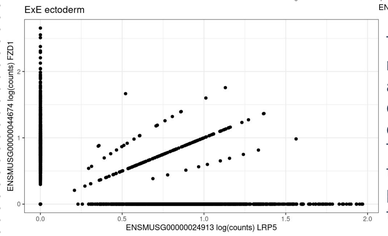
This graph shows relationship between Ctnnb1(beta catenin) and Wnt7B. Significant because shows high co-expression between both genes and might suggest that Wnt7b is dependent on beta catenin.



This 2D scatter plot shows an adequate relationship between LRP5 and Wnt7b. Important because may signify Wnt7b is working with LRP5 in the Wnt "On" state when the 2 genes bind along with an FZD gene.

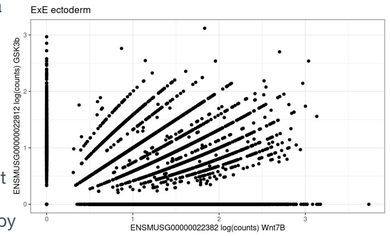
This 2D plot shows a decent relationship between FZD1 and Wnt7b. This is important because, as previously mentioned, it may serve as an indication that Wnt7b binds to LRP6 and an FZD gene.

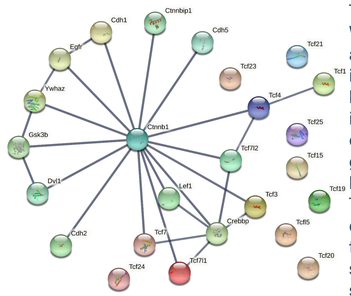




This 2D plot displays the relationship between FZD1 and LRP5. The plot demonstrates reasonable co-expression of the 2 genes. This is significant because these two genes can potentially act as a receptor to the signaling molecule, Wnt7b.

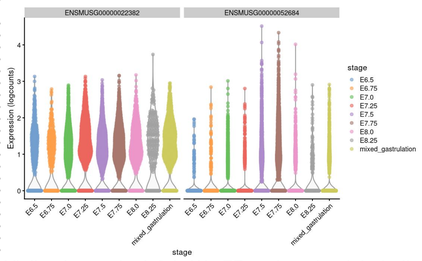
This 2D graph demonstrates a strong relationship between Wnt7b and GSK3b. GSK3b is part of the 'destruction complex' in Wnt canonical signaling that aims to phosphorylate beta catenin in the absence of the Wnt7B ligand. When Wnt7b is present and binded to the destruction complex, which is supported by this graph, Axin can inhibit GSK3b from phosphorylating beta catenin.





TCF that interacts closest with Wnt7B and Ctnnb1 is not specified anywhere in the literature. It's important that Ctnnb1 is examined because out of the 3 beta catenins it has the most similar count expression with Wnt7B. This string graph demonstrates connections between all 14 TCF's and Ctnnb1. There was no significant co-expression because TCF is typically upregulated until later stages that this data set doesn't show (MGI).

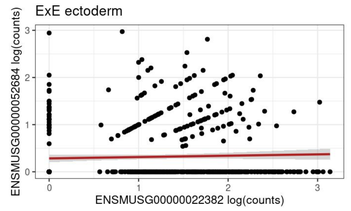
Evidence Wnt7b isn't part of the non-canonical pathway.



Violin plot on the left is Wnt7B and on the right is C-Jun. C-jun is a protein coding gene that is crucial to the non-canonical planar cell polarity pathway. In comparing these graphs it's notable that they don't demonstrate co-expression very well. This is significant because it supports the idea that Wnt7B is part of the canonical signaling pathway. If it was part of the non-canonical signaling pathway then, in theory it would have similar expression to c-jun.

gene1	gene2	rho
<chr>	<chr>	<dbl>
ENSMUSG00000052684	ENSMUSG00000022382	0.0287771

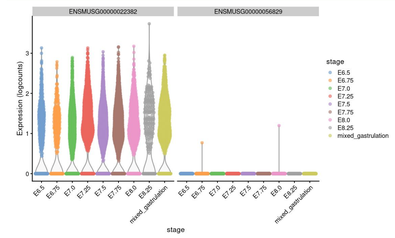
The correlation coefficient above and the 2D graph on the right don't demonstrate any significant relationship between the 2 genes. This supports the idea that Wnt7b isn't part of the canonical pathway because c-jun is essential to the non-canonical PCP pathway.



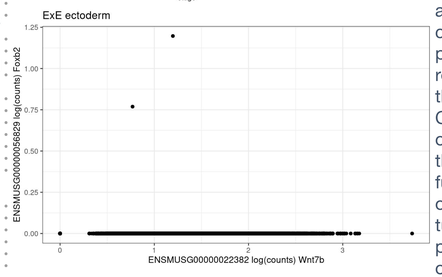
Conclusions

The purpose of this project was to find if there was evidence to support if Wnt7b is part of the canonical Wnt signaling pathway. One of the most significant findings was a potential dependency of beta catenin to Wnt7b. This piece of information was crucial because the biggest indicator of a type of pathway a gene is involved is it's catenin dependency. Another crucial finding was no novel link of Wnt7b to c-jun, a gene important in the non-canonical Wnt signaling pathway. C-jun is one of the most important genes to non-canonical signaling. It was important to hypothesis that there be no link between Wnt7b and c-jun.The biggest limitation this project had was determining a significant link between Wnt7b and Ctnnb1 with TCF genes. Access to another data set and a longer research time frame are two contributors that could've helped combat this problem. The findings of this project help to propose a trajectory for the role that Wnt7b may have in a canonical Wnt signaling pathway.

Future: Differential Expression of FoxB2 important for ""Off" Wnt Signaling State



These three figures are significant because they show differential expression between Wnt7b and FoxB2. One key detail about the information being presented is the correlation coefficient, it's negative. Insights about "Off" state of canonical signaling can potentially be seen if removing FoxB2 later in the organism's life. Changing the expression of FoxB2 may prevent the Wnt7b ligand from functioning. The absence of Wnt7b ligand would in turn lead to the proteasomal degradation of beta-catenin.



gene1	gene2	rho	p.value
<chr>	<chr>	<dbl>	<dbl>
ENSMUSG00000022382	ENSMUSG00000056829	-0.0008412458	0.9273255

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1.) Schubert M, Holland LZ. The Wnt Gene Family and the Evolutionary Conservation of Wnt Expression. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-2013. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6212/
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3.) Ng LF, Kaur P, Bunnag N, Suresh J, Sung ICH, Tan QH, Gruber J, Tolwinski NS. WNT Signaling in Disease. Cells. 2019 Aug 3;8(8):826. doi: 10.3390/cells8080826. PMID: 31382613; PMCID: PMC6721652.
4.) Mouse Genome Informatics (MGI). Available from: informatics.jax.org
5.) UCSC Genome Browser (UCSC). Available from: genome.ucsc.org

Acknowledgements

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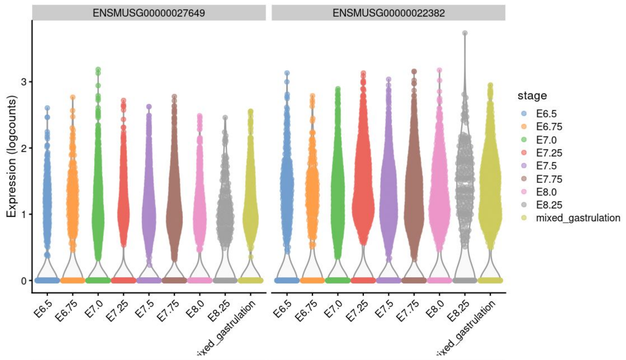
Wnt2 is the ortholog gene in flies, when mutants arise, these are the phenotypes.

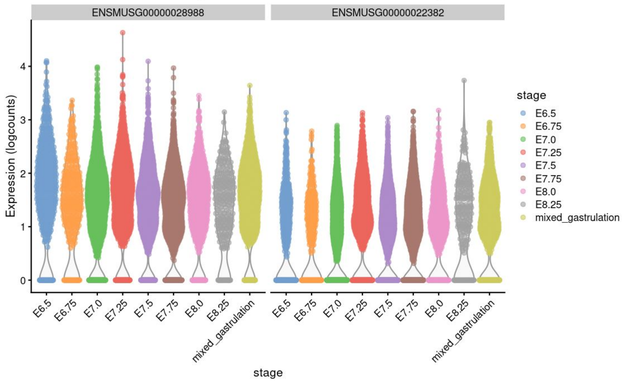
lethal, with Scer\GAL4$^{dPit-559.1}$	Wnt2$^{UAS.cDa}$
lethal | embryonic stage, with Scer\GAL4$^{ptc.PS}$	Wnt2$^{UAS.cDa}$
lethal | larval stage, with Scer\GAL4$^{dpp.PS}$	Wnt2$^{UAS.cDa}$
lethal - all die before end of pupal stage, with Scer\GAL4$^{sTubt48.PC}$	Wnt2$^{UAS.cDa}$
partially lethal	Wnt2^{unspecified}
viable	Wnt2^{4} Wnt2^{66} Wnt2^{80} Wnt2^{99} Wnt2^{E} Wnt2^{I} Wnt2^{K} Wnt2^{L} Wnt2^{O} Wnt2^{P} Wnt2^{RJ}
viable, with Dcr-2$^{UAS.cDa}$, Scer\GAL4$^{dHec.PLw}$	Wnt2^{MD0736}
viable, with Scer\GAL4$^{Mef2.PR}$	Wnt2^{MD0736}
viable, with Scer\GAL4$^{prr-MD037}$	Wnt2^{MD0736}
viable, with Scer\GAL4$^{Tin.GA}$	Wnt2^{MD0736}
Sterility	**Allele**
male semi-sterile (with Wnt2^{E})	Wnt2^{E}
male semi-sterile (with Wnt2^{O})	Wnt2^{L}
male sterile	Wnt2^{unspecified}
male sterile | recessive	Wnt2^{4} Wnt2^{66} Wnt2^{80} Wnt2^{99} Wnt2^{E} Wnt2^{I} Wnt2^{K} Wnt2^{L} Wnt2^{O} Wnt2^{P} Wnt2^{RJ}
Other Phenotypes	**Allele**
abnormal neuroanatomy | dominant	Wnt2^{O}
flightless | heat sensitive	Wnt2^{RJ}
some die during pupal stage, with Scer\GAL4$^{sTubt48.PC}$	Wnt2$^{UAS.cDa}$

However, there are Wnt genes that have been very well researched and any type of mutation can lead to death or disease of the organism. By comparing levels of co-expression, the number of Wnt genes by cell type in various stages, and interaction of Wnt genes, we can determine which genes might be genetic redundancies.

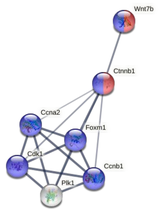
2D plot, or co-expressed genes actual number/ co-relation score

There are 2 other known beta-catenin genes present in gastrulation those are Ctnnbl1 and Ctnnbip1. The graphs below demonstrate the expression of Wnt7B versus Ctnnbl1 and Ctnnbip1, respectfully. Expression is pretty similar but not as comparable as Ctnnb1. These beta catenin's might work with Wnt7B at some stages but Ctnnb1 is working with Wnt7B more consistently.





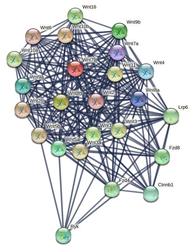
Red- canonical Wnt signaling pathway
Blue- Positive regulation of cell population proliferation



This image shows the level of expression of 6 out of the 19 Wnt genes in the ExE ectoderm cell type throughout the various embryonic stages

There was no significant co-expression between Ctnnb1 and and of the TCF's. Go back over graphs bc doesnt Beta catenin need to accumulate so it'd make sense that it has higher expression than TCF, bc there is no apparent co-expression. But in order for beta catenin to transcribe Wnt target genes, a dishevelled gene needs to bind to the destruction complex.

. the second string image is all 19 Wnt genes interacting together



look up c-jun target cells (refine search for stage and cell type))and look for co-expression with wnt

look at other data sets later on bc too early to tell w TCF might just be proteins

re does TCF turn on, after 8.5, maybe b/w 9 and 13

redundant for this process, maybe in gastrulation

Experiments

"Phenotypes of embryos heterozygous for deficiencies" might be an approach helpful for us. What the researchers did was cross male and female stocks with different deficiencies and looked at the phenotypes that arose as a result of those crossings early on in gastrulation versus later on. We could do this for the Wnt family genes to see how they might function and how might they work together. Might be a good hypothetical experiment to run for presentation. I'd look for instances where mutations don't affect offsprings, this might suggest genetic redundancies.

♀	ed-sz1		ed-dp		dp-h28		M24F-B		dp-h25		dp-h19		dp-h24		dp-cl-h3	
♂	vf	late	vf	late	vf	late	vf	late	vf	late	vf	late	vf	late	vf	late
ed-sz1	–	–														
sc19-8			++	+												
ed-dp			+++	+++											(+)	–
M24F-11			+++	+++												
dp-h28					+	+										
M24F-B							++	++								
dp-h25									++	+++*						
dp-h19			+++	+							+++	+++			(+)	–
dp-h24													–	–		
dp-cl-h3															(+)	–
sc19-10											++	++				
sc19-5			++	+							+++	++				
sc19-4			++	+												
wt			++	+							++	+				

Males (columns) and females (rows) from the indicated stocks were crossed to each other and embryos from the crosses were stained with antibodies against Twist and Even-skipped. They were evaluated independently by three observers for the defects in ventral furrow formation and later events in gastrulation as described in the text. Defects were scored as very strong (+++) to weak ((+)) or absent (−). If a field does not contain a value, the cross was not performed.
*Embryos from this stock show a strong general patterning defect.
doi:10.1371/journal.pone.0007437.t002

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MARK

Wnt6 and Wnt10a have the strongest correlation in the Wnt Family

Mark Sanchez[1], Lindsay Hayes[2], Matt McCoy[2], and Rosa Alcazar[4]

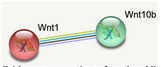
Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine, and Carnegie Institution.

Abstract

The **Wnt family** is a group of 19 genes which activate signal transduction pathways. Of the 19 genes in the Wnt family, only 6 appear on the same chromosome, those being Wnt1, Wnt10b, Wnt3, Wnt9b, Wnt6, and Wnt10a.

Functional Link

The STRING Database provides a score to measure why two genes may or may not have a functional link. Those scores are determined by a series criteria.

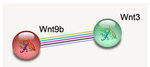


Evidence suggesting a functional link:

Neighborhood in the Genome:	none / insignificant.	
Gene Fusions:	none / insignificant	
Cooccurence Across Genomes:	none / insignificant	
Co-Expression:	none, but putative homologs are coexpressed in other organisms (score 0.064).	show
Experimental/Biochemical Data:	none / insignificant	
Association in Curated Databases:	yes (score 0.900).	show
Co-Mentioned in Pubmed Abstracts:	yes (score 0.737). In addition, putative homologs are mentioned together in other organisms (score 0.207).	show
Combined Score:	0.912	

Note: the two proteins have some sequence similarity (243.4 bits over 338 amino acids. click here to see the alignment).

72% sequence similarity

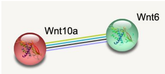


Evidence suggesting a functional link:

Neighborhood in the Genome:	none / insignificant.	
Gene Fusions:	none / insignificant	
Cooccurence Across Genomes:	none / insignificant	
Co-Expression:	none, but putative homologs are coexpressed in other organisms (score 0.067).	show
Experimental/Biochemical Data:	none, but putative homologs were found interacting in other organisms (score 0.092).	show
Association in Curated Databases:	yes (score 0.900).	show
Co-Mentioned in Pubmed Abstracts:	yes (score 0.717). In addition, putative homologs are mentioned together in other organisms (score 0.147).	show
Combined Score:	0.919	

Note: the two proteins have some sequence similarity (190.3 bits over 341 amino acids. click here to see the alignment).

56% sequence similarity



Evidence suggesting a functional link:

Neighborhood in the Genome:	none / insignificant.	
Gene Fusions:	none / insignificant	
Cooccurence Across Genomes:	none / insignificant	
Co-Expression:	yes (score 0.095). In addition, putative homologs are coexpressed in other organisms (score 0.068).	show
Experimental/Biochemical Data:	none / insignificant	
Association in Curated Databases:	yes (score 0.900).	show
Co-Mentioned in Pubmed Abstracts:	yes (score 0.856). In addition, putative homologs are mentioned together in other organisms (score 0.397).	show
Combined Score:	0.918	

Note: the two proteins have some sequence similarity (246.5 bits over 352 amino acids. click here to see the alignment).

70% sequence similarity

Chromosomes

The following figures are the Wnt family pairs were they are found on their respective mouse chromosomes in the UCSC genome browser. The number in brackets is the distance between the pairs in the chromosomes measured by **bp**, or **base pairs**.

Figure 1. The Wnt pairs displayed in the respective chromosomes

a.

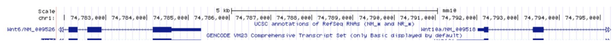
Chromosome 15
Wnt10b and Wnt1
←←← [11,707 bp] →→→

b.

Chromosome 11
Wnt9b and Wnt3
←←← [24,354 bp] →→→

c.

Chromosome 1
Wnt6 and Wnt10a
→→→ [6,700 bp] →→→



Considering that each chromosome found in mice have around 100,000,000 - 200,000,000 **base pairs**, the gap between the genes is minuscule when a chromosome is looked at in its entirety.

Cell type Expression

The following are **Umaps**, a method of visualization for data sets. They are displayed over a mice fetus and the areas highlighted the brightest have the most expression during **gastrulation**.

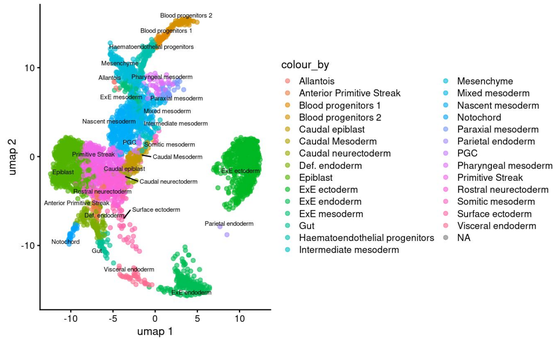
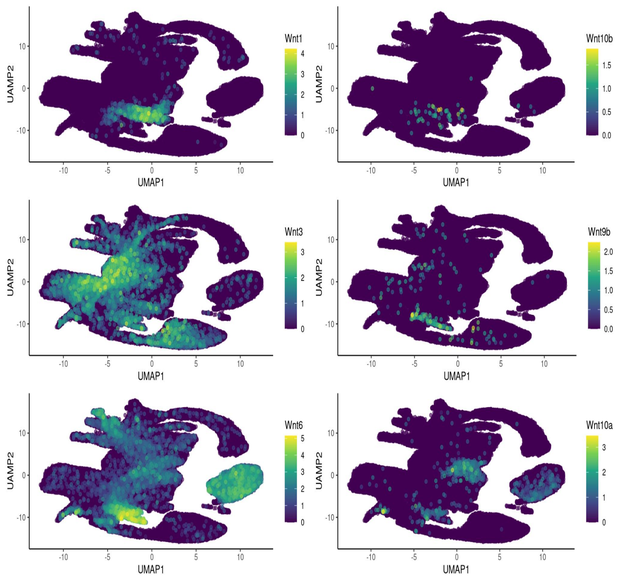


Figure 2. The expression of the Wnt pairs during gastrulation in mice



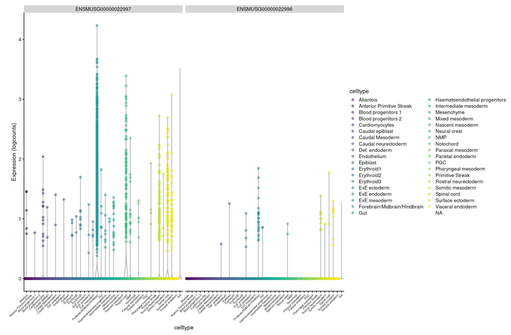
Wnt3 is expressed in nearly every area of gastrulation but its pair, Wnt9b, is concentrated in the Surface Ectoderm.

Wnt6 is expressed highly in the Surface Ectoderm and the Exe. Ectoderm. However, the expression of Wnt10a in those areas does not match with Wnt6.

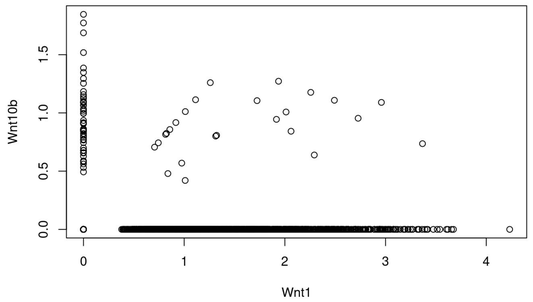
The only pair that appears to have any correlation are Wnt1 and Wnt10b, which seem to be expressed the most in the Surface Ectoderm.

Cells that express both genes

Expressed highly in the forebrain



When looking at pair Wnt1 and Wnt10b, most cells seem to align on the X and Y-axis, meaning that there isn't very much overlap.



Discussion

It appears that Wnt6 and Wnt10a, despite being so close to each other on the same chromosome, do not have a strong correlation. A possible field of research to look into is whether the direction of transcription determines whether a pair of genes have co-expression or not.

Acknowledgements

Miller JR. The Wnts. Genome Biol. 2002;3(1):REVIEWS3001. doi: 10.1186/gb-2001-3-1-reviews3001. Epub 2001 Dec 28. PMID: 11806834; PMCID: PMC150458.

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LULU

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721

The Role of Hox Genes the formation of Hematopoietic Stem Cells During Mouse Gastrulation

Leul Tigabu[5], Lindsay Hayes[2], Matt McCoy[2], and Rosa Alcazar[1]

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine, Carnegie Science.

Abstract

The Homeobox genes are a group of highly conserved genes that are found in all multicellular animals. As these genes are responsible for morphology they are all incredibly active during gastrulation and are important in the process of cellular specialization. In the case of blood cells it is known that HoxC4 and HOXB4 are integral to their specialization. Since some Hox genes are responsible for the formation of blood cells it would be useful to know how what other hox genes have an interplay with the earlier mentioned ones in the precursor germ layers of blood cells

Instroduction

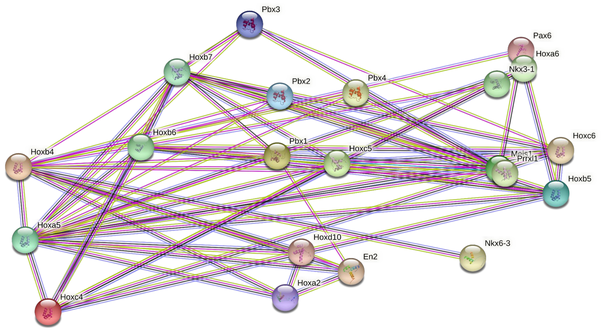
Hematopoietic Stem cells are a multipotent stem cells that can specialize into two lines of blood cells called the mylenoid and the lymphoid. They are the two lines of blood cells that are responsible for the formation of all blood cells in the body. As such it stands to reason that changes in the pathways of these genes can impact the formation of blood cells. As hox genes are a heavily conserved group of genes it would be prudent to contrast the expression of genes with the formation of hematopoietic stem cells. Understanding the way that the Hox genes influence blood formation may give insight into how blood has evolved and which of its traits have been conserved

Method

Since the formation of the hematopoietic stem cells is reliant on the germline of cells they belong to it would be prudent to find the germ layer that blood cells originate from and test it for activation of the HOXC4 and HOXB4 gene. Following that cooperating genes and pathways could be found with the Kegg database. After that the best way to determine the interplay between the Hox genes in the precursor Hematopoietic stem cells would be a differential expression chart to see which genes are most transcribed along with the two known to be integral to the process (Hoxc4 and Hoxb4)

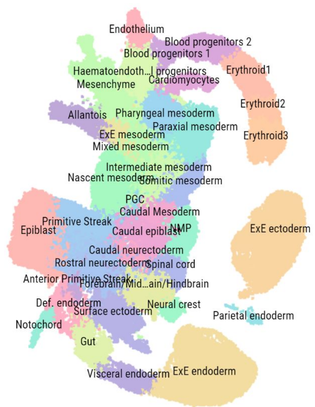
Results

Through kegg we can examine the pathways that allow the genes to interact. The most direct connection between Hoxb4 and Hoxc4 is the Hoxa5 gene which indicates they may work together in. the Hoxc5, Hoxb6, Hoxa6 and hoxb7 are also joined to Hoxa5 which may indicate they will have correlated expression in the germline responsible for the formation of Hematopoietic Stem Cells.



In order to find which hox genes are responsible for the formation of the Hematopoietic stem cells their precursor cells must be known. In the case of blood cells the mesoderm line is the one responsible for the formation of blood cells in the future. In the case of gastrulation there are multiple types of mesoderm that are responsible for developing into blood tissue. Those germ layers are the axial and paraxial mesoderm of the anterior truck along with the caudal regions of the mesoderm.

After the precursor germ layer is found a Umap can be used to see coexpression of strongly correlated hox genes in the same tissue. All of the hox genes show little to no activation in the paraxial mesoderm so are unlikely to have much influence in the are. However there is activation of all but the Hoxa6 gene in the caudal mesoderm indicating that hox genes may play some part in the blood cell lines that come from there.



Results

Fig 1. Umaps of hoxc4 and hoxb4

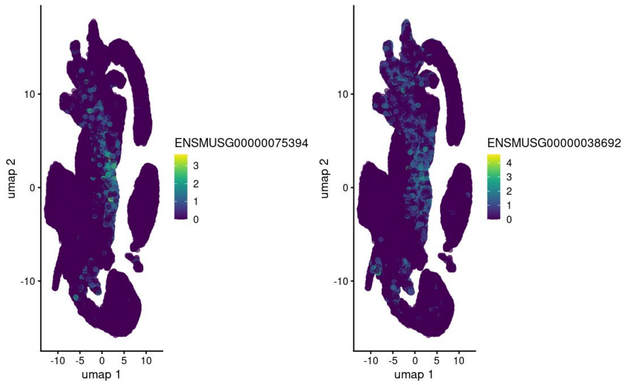


Fig 2 Umaps of hoxa6 and hoxb6

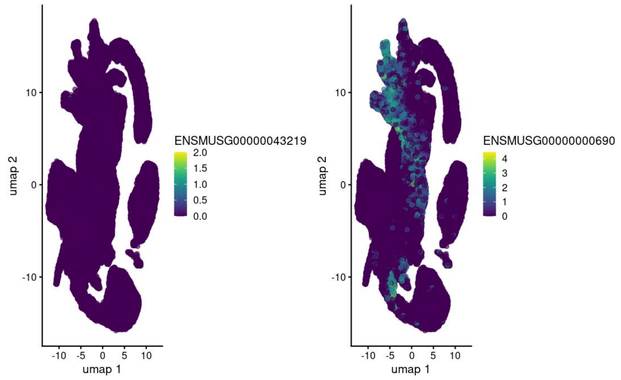
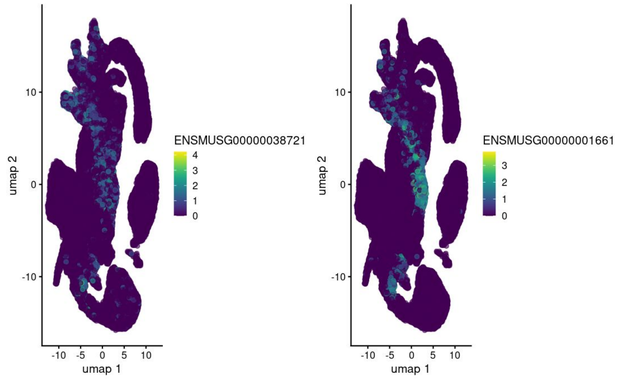
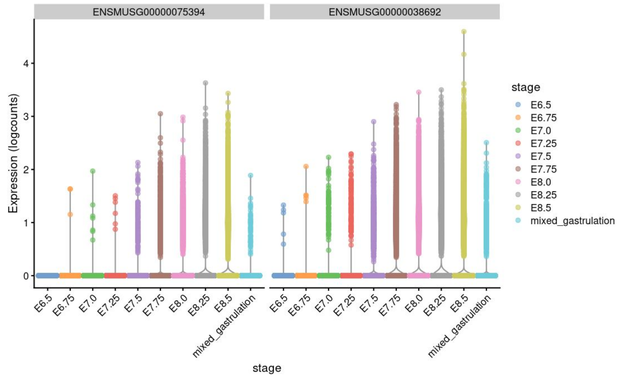


Fig3. Umaps of hoxc6 and hoxb7



Discussion

The data shows that other hox genes are present in the germ lines that are responsible for the formation of hematopoietic stem cells. This indicates that they have an impact in the way the cell lines that descend from them form. Unfortunately this is where the limits of the data set begin to show as while the differential expression charts can show the activation of the genes in certain tissue they can not be used to show what the roles of the genes are in the cells. As a result it is impossible to tell what the genes are doing in the tissue with this data set. A follow up study with a different data set could look into how the genes interact with each other and how they form the different tissues over time. It would also help to know the time period where the Hematopoietic cells are formed as it would allow for the comparison of the the time of highest transcription in the hox gene to the period of gastrulation where Hematopoietic cells begin to form. The expression of he Hoxb4 and Hoxc4 genes over the period of gastrulation



HoxC4 and Hoxb4 gastrulation over time

Aknowledgement

Lehmann R. (2012). Germline stem cells: origin and destiny. *Cell stem cell*, *10*(6), 729–739. https://doi.org/10.1016/j.stem.2012.05.016

Dzierzak, E., & Speck, N. A. (2008). Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nature immunology*, *9*(2), 129–136. https://doi.org/10.1038/ni1560

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Shamoun

The role of Pitx2 in early stage embryonic muscular development

Shamoun Divanyan[1], Lindsay Hayes[2], Matt McCoy[3]and Rosa Alcazar[4]

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine,

Abstract:

Skeletal muscles are heterogeneous tissues that makes up roughly 40% of a humans body mass, and is composed of a diverse number of individual muscle fibers that can vary in size, shape, and contractile output. In the embryo, the biological process of skeletal muscle formation—myogenisis— is highly complex, and well coordinated by a multitude of regulatory factors. The activation, proliferation, and differentiation of embryonic Myocytes, and later adult satelite cells, to form healthy contractible muscle tissues is heavily reliant on the collective regulation driven by genes such in the "Paired box Family", and "Myogenic regulatory factors". Pitx2 has emerged as a key player in early stage muscle development, and cell differentiation. While Pitx2 is generally expressed in almost every cell type it is most highly expressed in the Exe endoderm, Paraxial Mesoderm and endoderm, Mesenchyme, and Surface endoderm—most of which are prenatal precursors to Skeletal muscle and myocardium. While the first signs of muscle fiber development are detected at about Embryonic day (E11-E14), Pitx2 is expressed far earlier in gastrulation than previously thought; which begs the question—at what embryonic age are the genes involved in Myocyte differentiation and proliferation expressed?

Introduction

With the seemingly endless "craze" and obsession of some people to get as muscular, and strong, as possible; it comes to no surprise as to why some people may be eager to know the genetic factors and limitations to professional and recreational Power/Bodybuilding. Upon inspection of the genetics of 5 time "Ironman Pro" winner, Flex Wheeler, and later the 2017 "Worlds Strongest Man" winner, Eddie Hall, it became known that a certain mutation in a gene allowed it's inheritors to naturally gain an elevated amount of muscle mass, dubbing it the "Hercules Gene". Their mutation in the gene "Myostatin" —which regulates the proliferation of myogenic satellite cells and hypertrophy of adult muscle cells—effectively allows both Eddie Hall and Flex Wheeler to have a comparative advantage in their respective sports and competitions.

While myostatin plays a large role in the regulation of hypertrophy in later adult life (in both mice and humans) it plays a far lesser role in the proliferation and myogenesis of the muscular bodies in embryonic development. Which begs the question, what is responsible for the early stage differentiation of myocytes to form the major skeletal muscle bodies?

Pitx2 has emerged as a key player, upstream of myostatin, to activate the pathway for myocyte differentiation and hyperplasia in later life. Pitx2 is a bicoid–related homeobox gene that is asymmetrically expressed in the lateral plate mesoderm and is regulated by the Nodal/lefty pathway, and plays multiple roles for the development of the primary myogenesis, and is required for EOM precursor specification and survival, acting as an anti-apoptotic factor in the pre-myogenic mesoderm and subsequently activating the myogenic program in these cells through direct binding to *Myf5* and *Myod* promoters (Zacharias et al., 2011).

Results

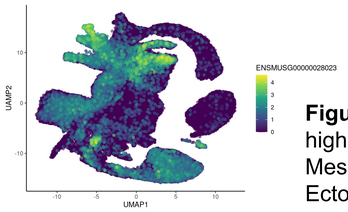


Figure 1: Pitx2 UMAP depicts high regional expression in the Mesoderm, Endoderm, and Ectoderm

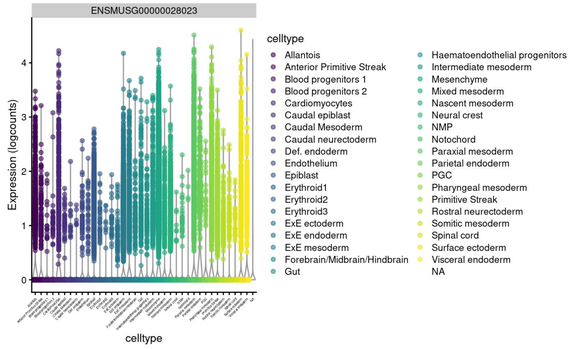


Figure 2A: General Regional Expression of Pitx2 throughout cell types

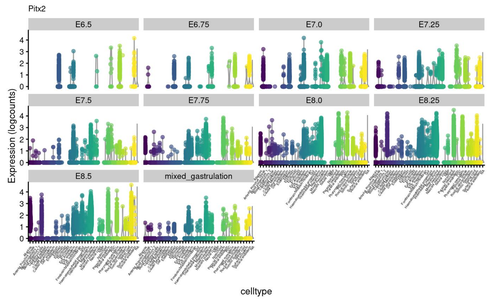


Figure 2B: Expression of Pitx2 over time (E6.5-E8.5, inc Mixed Gastrulation)

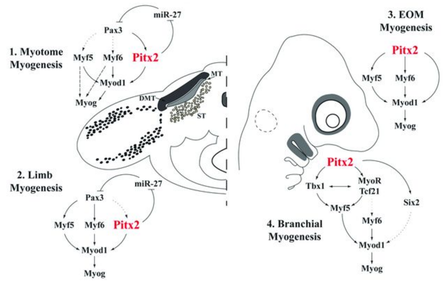


Figure 3: Pitx2 in Embryonic myogenesis ~E11

My data seems to suggest that Pax3, Pitx2, Six2, Mcs Tbx1 and Tcf21 are all expressed during the gastrulation period and may be beginning to interact with each other for the rudimentary steps of Myogenesis. Similarly due to the lack of Myf5 and Myf6 expression, and the relative high expression of Tbx1, Six2 , Mscand Tcf21 throughout gastrulation, it would seem as though Myogenesis of the Branchial cells may begin first (Striated musculature of the Head and neck.)

This logically makes some sense because it is around gastrulation stage E7-E9.5 where the "cranial head" begins to fold and give the cells the typical "embryo shape" with some features that can be easily distinguished as the head, the forelimb buds, and the trunk of the organism.

	Expression of genes in embryonic morphogenisis									
						No Expression	High Expression	Some Expression		
	Stages									
	Gene	E6.5	E6.75	E7.0	E7.25	E7.5	E7.75	E8.0	E8.25	E8.5	Mixed Gastrulation
	Pax3	Low	Low	High	High	High	High	High	High	High	Low
	Pitx2	High	High	High	High	High	High	High	High	High	High
	Myf6	None	None	Low	Low	None	None	None	Low	Low	None
	Myf5	Low	None	Low	Low	Low	Low	None	Low	Low	None
	Myod1	Low	None	Low	Low	Low	Low	Low	Low	Low	Low
	Myog	None	None	None	None	None	None	None	None	Low	None
HM: MyoR	Msc	Low	Low	High	High	Low	Low	None	None	High	Low
	Tbx1	None	None	Low	Low	High	High	High	High	High	Low
	Tcf21	None	None	None	None	High	High	High	High	High	High
	MSTN	None	None	None	None	None	None	None	Low	Low	None
	Six2	Low	Low	High	High	High	High	High	High	High	High

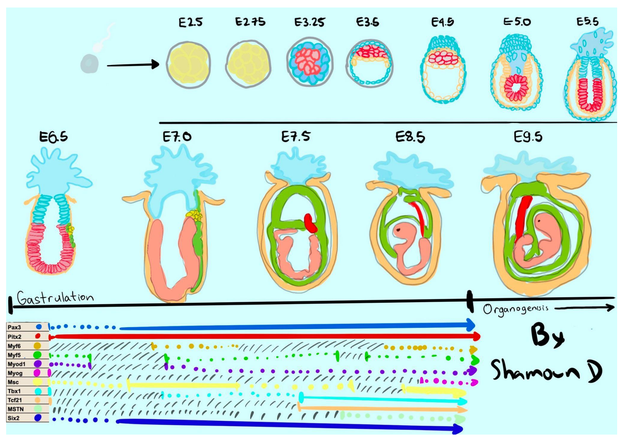


Figure 4A: A table of the expression genes involved in embryonic myogenesis during gastrulation E6.5—E8.5. Red=No expression , Yellow = Low expression , Green = High expression

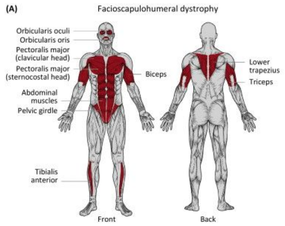
Figure 4B: Visual depiction of what the embryo looks like during the different stages of gastrulation and when the respective genes are expressed in a time line.

Methods

- Kegg Pathway:
  - Utilized to visualize the Protein-Protein interactions between genes involved in myogenisis
- R-Studio via Sciserver:
  - Utilized to generate qualitative data on regional, and stage expression from the Gastrulation Data Set.
    - Generated UMAPs, Violin plots depicted

Discussion

In light of the new "Hercules Gene" and how a Myostatin Defficiency can result in accelerated levels of hypertrophy, many different research teams—funded by major supplementation companies—have been looking into the potential supplementation of something that may inhibit high expression of the Myostatin gene in Muscle-with limited results at best. Which begs the question—could there also be a potential supplementation of Pitx2 to promote muscular hyperplasia ? From multiple medical research papers a deficiency in Pitx2 in the earlier stages of embryonic development (Pre E11-14) suggests embryo-lethality, whereas a deficiency post (E11-E14) would result in Facioscapulohumeral muscular dystrophy—a rare muscle wasting disorder that targets the facial musclature, the shoulders, upper back and arms.



Where to go next

Some areas in which research could be furthered is looking at the mutational effects of a hyperactive and hypoactive Pitx2 gene during gastrulation to see if there are any noticeable changes in the development of the musculature and organogenesis of the mice. Similarly due to its role in regulating hyperplasia, it is worth looking into its' implementation as a possible treatment for patients suffering from Muscular Atrophy and Dystrophy. I would also like to know the transcriptional variance between male and female subject to see whether or not a difference in the concentration of Pitx2 and Myostatin may influence the amount of muscle an individual may retain.

Acknowledgments

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- Zacharias AL, Lewandoski M, Rudnicki MA, Gage PJ. Pitx2 is an upstream activator of extraocular myogenesis and survival. Dev Biol. 2011 Jan 15;349(2):395-405. doi: 10.1016/j.ydbio.2010.10.028. Epub 2010 Oct 28. PMID: 21035439; PMCID: PMC3019256.
- Chang CN, Singh AJ, Gross MK, Kioussi C. Requirement of Pitx2 for skeletal muscle homeostasis. Dev Biol. 2019 Jan 1;445(1):90-102. doi: 10.1016/j.ydbio.2018.11.001. Epub 2018 Nov 8. PMID: 30414844; PMCID: PMC6289786.
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Kyle

The Relationship Between Genes in Maturity of Onset of Diabetes of the Young and Early Gastrulation

Kyle Ortiz[1], Lindsay Hayes[2], Matt McCoy[3]and Rosa Alcazar[4]

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine,

Abstract

Foxa2, Foxa3, and HNF1B all have one thing in common: they are present in the maturity of onset diabetes of the young. This specific case of diabetes differs from Type 1 and Type 2 since it's caused by a genetic mutation. Since it results from a mutation that'd be from birth, I was curious to find out if these same genes that play into the pathway would also be present in early gastrulation. To test this, I used a single-cell RNA sequencing mouse gastrulation dataset that spanned from days 6.5-8.5. What I learned was that, sure enough, these same genes were relatively expressed in the early gastrulation dataset and didn't seem to disappear after day 8.5. So, the evidence supports my hypothesis that genes in MODY5 also appear in early gastrulation.

Introduction

I decided to research Foxa2 because I found it's in so many species from mice, to humans, and fruit flies. It's also apart of so many different important life processes like DNA-binding, transcription, regulation of fat metabolism, and much more. When I searched up Foxa2 on Kegg pathways and found the MODY pathway, I was intrigued since I had never heard of these unique diabetes. More specifically, this type of diabetes is in its own category because it runs strongly in families, causes primary insulin secretion defects, is typically diagnosed below the age of 25, and can be caused by 14 different genetic mutations. One of these 14 mutations so happens to be HNF1B, specifically MODY5, and this gene is also involved in type 2 diabetes and pancreatic cancer.When this gene mutates and causes diabetes, insufficient levels of insulin result from abnormal pancreatic beta cells. Meanwhile, Foxa2 (which appears in early embryonic development, type 2 diabetes, and the negative regulation of detection of glucose) and Foxa3 (which is involved in cellular glucose homeostasis) are both a part of the forkhead box protein family.

Methods

- Kegg Pathway:https://www.kegg.jp/pathway/map04950
  - I used this to find genes of interest in MODY to then research
- I used a single-cell RNA sequencing mouse gastrulation dataset in Rstudio to create the UMAPS, Violin Plots, and the graph of expression over stages.

Results

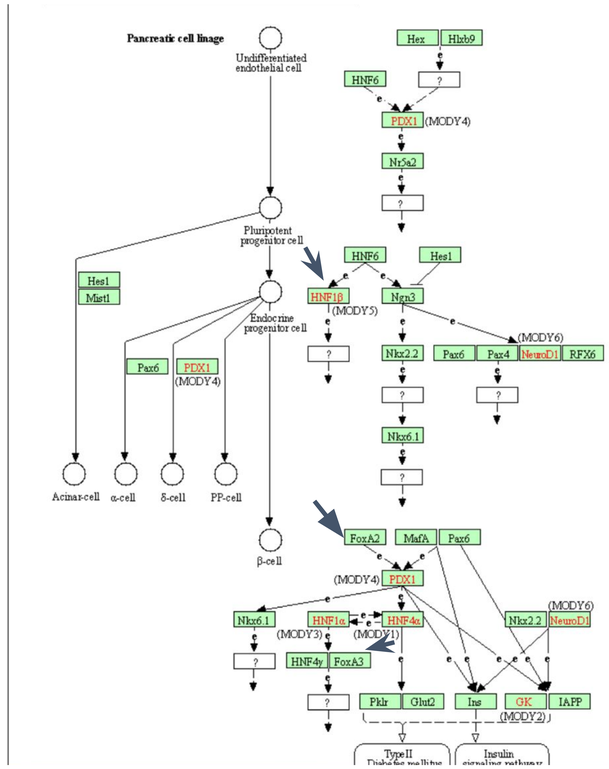

Figure 1: This is the human Kegg pathway for MODY which includes Foxa2, Foxa3, and HNF1B.

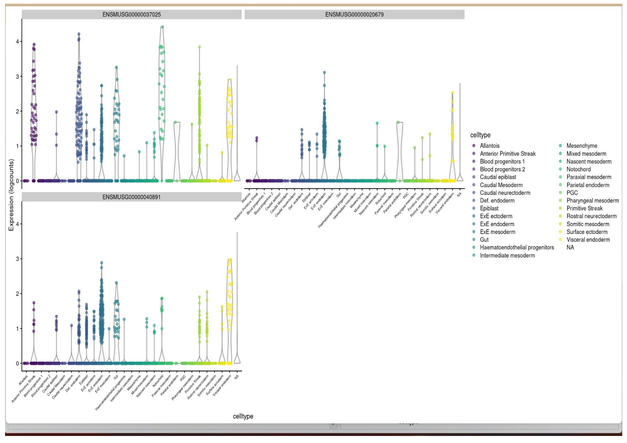

Figure 2: This is the expression of Foxa2 (top left), HNF1B (top right), and Foxa3 (bottom left) throughout all cell types in early gastrulation
***Major difference is Foxa2 has expression in APS while others don't and shared expression in ExE endoderm**

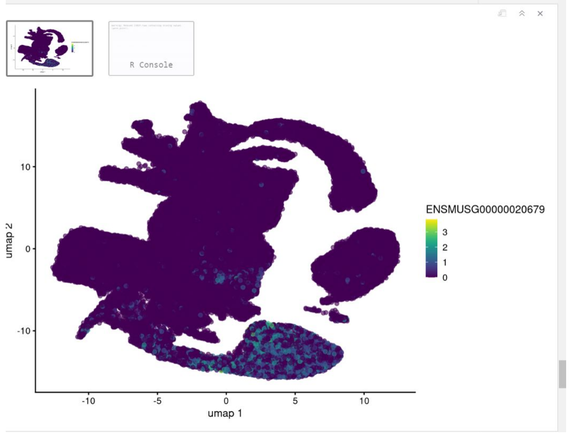

Figure 3A: HNF1B UMAP

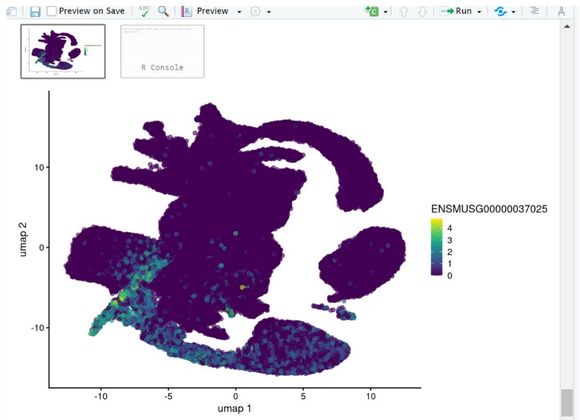

Figure 3B: Foxa2 UMAP

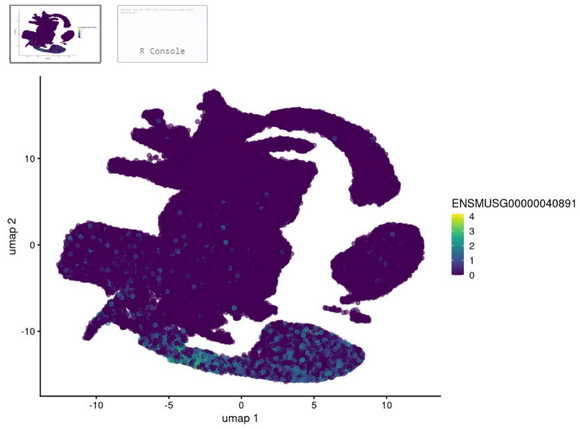

Figure 3C: Foxa3 UMAP

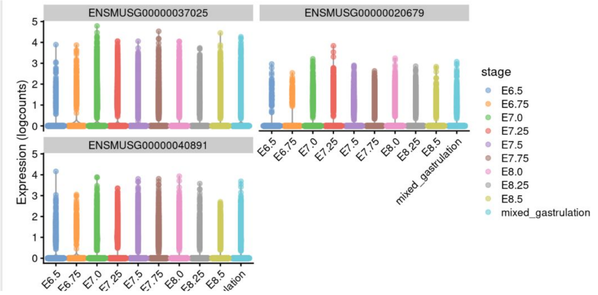

Figure 4: All 3 gene expression (Foxa2 top left, HNF1B top right, and Foxa3 bottom left) from day 6.5-8.5

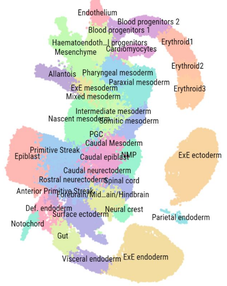

Figure 5: The cell type names in their respective regions.

Discussion

It can be seen that Foxa2, Foxa3, and HNF1B are prominent in gastrulation in both Figure 2, which shows the expression in different cell types, and then in Figure 4, which represents gene expression throughout the cell in a 2-day span. While the genes are similar in the abundant expression within the ExE endoderm, Foxa2 is more prominent all around with a lot of expression in the Anterior Primitive Streak. Then, Figure 3A-C just shows the respective gene expressions in their regions and how dense they are in that specific region. These findings support my research since it's clear that these genes that show up in Figure 1 (the pathway for MODY) are found way earlier in gastrulation. However, I expected this since MODY is a genetic mutation disease, and since genetic mutations happen at birth, the genes should also appear in early gastrulation. If I were to continue my research, I'd likely compare the HNF1B mutation variant to the other 13 mutations that also cause MODY.

Acknowledgements

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Yaneth

There is a specific point in gastrulation in which the lefty genes directly interact.

Yaneth Perez[1], Lindsay Hayes[2], Matt McCoy[3]and Rosa Alcazar[4]

Clovis Community College, Stanford School of Medicine, Johns Hopkins School of Medicine,

Move methods before results

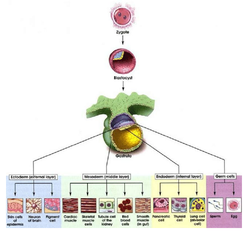
Past studies have established that the TGF-beta superfamily includes the lefty genes, Lefty1 and Lefty2, in both humans and mice. In the TGF-beta pathway, Lefty1 plays a critical role is left-right asymmetry determination of organs during early embryo development. This pathway does not show whether Lefty1 and Lefty2 directly interact, but rather Nodal and Smad2 served as a connection between them. In the TGF-beta signaling pathway, which regulates pluripotency of stem cells, we see Nodal and Smad2 control the transcription of Lefty2. In this pathway Lefty2 plays a role in embryonic stem cell signaling. In humans, Lefty1 is highly expressed in the pancreas and colon, which arises from the endoderm. Whereas Lefty2 is highly expressed in the female tissue (i.e cervix), which arises from the mesoderm and mesenchyme cell types. It is still unknown whether they interact, and if they do,

Hypothesis

The Lefty genes are present during gastrulation therefore lefty1 will show high expression levels in the endoderm cell types during the early stages of gastrulation in mice and lefty2 will show high levels of expression in the mesoderm cell types.

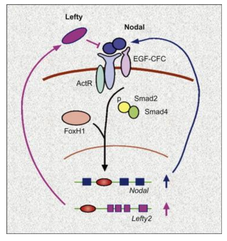
Introduction

Three layers of tissue are formed during early development. The endoderm is the inner layer, the mesoderm is the middle layer, and the ectoderm is the outer layer. From the endoderm arises organs involved in the digestive system like the pancreas and colon. From the mesoderm arises organs involved in the reproductive system like the cervix.



https://www.researchgate.net/figure/Differentiation-to-all-three-embryonic-germ-layers-and-subsequently-to-different-tissues_fig3_8551939

In the left-right axis determination pathway, Lefty1 acts as a ligand that represses nodal outside of the cell. The nodal and activin receptors in the membrane send a signal to smad2/3 which then binds to smad4. They then move into the nucleus and act as components to lefty2 transcription.



https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/left-right-determination-factor

Results
What cell types and stages are Lefty1 and Lefty2 expressed?

Figure 1:

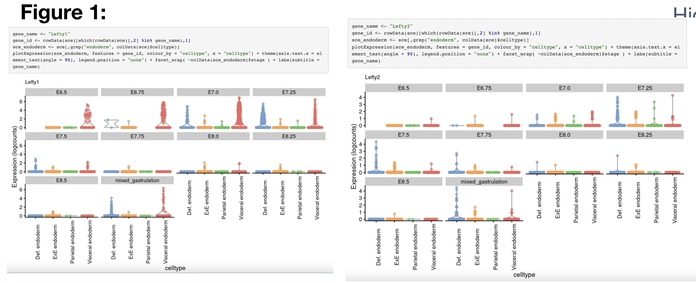
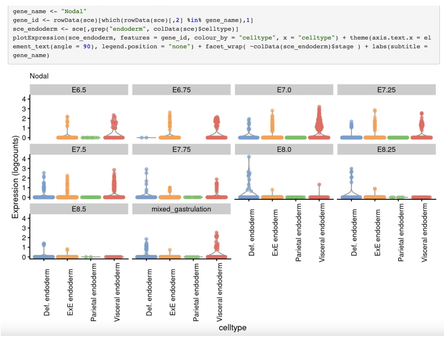


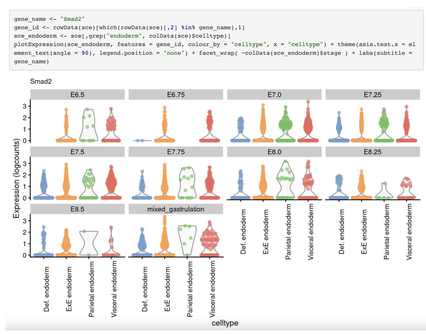
Figure 1. Lefty1 and Lefty2 are differentially expressed in endoderm except in definitive endoderm.

Lefty1-Endoderm Expression
We found Lefty1 is expressed in visceral endoderm through stag
E6.5 to E7.25. High levels of expression in the Def. endoderm fo
stages E7.0 and E7.25
Lefty2-Endoderm Expression
Relatively low levels of expression throughout the stages. With
exception to Def. endoderm and visceral endoderm expression i
E7.25.

Nodal-Endoderm Expression
High levels of expression in the ExE and Visceral endoderm from stages E6.5 to E7.5.

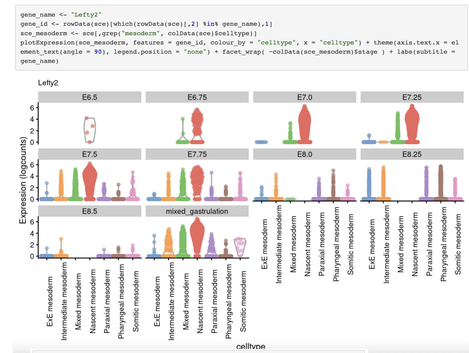


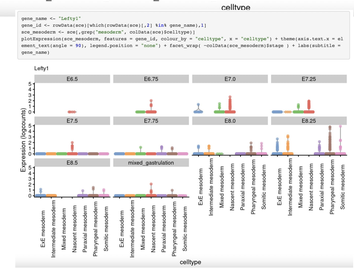
Smad2-Endoderm Expression
Highly expressed in all endoderm cell types from stages E7.0 to E8.5.



Results-Where do the genes related to the female reproductive tissue have expression in?

Lefty2-Mesoderm Expression
Highly expressed in nascent mesoderm from stages E6.5 to
.75.





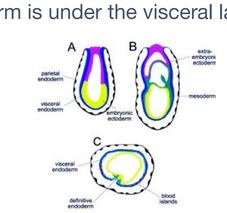
Group figures and write a figure legend fig 1; E7.25 def endoderm..what is repressing lefty1 to allow for lefty2 transcription?

Methods

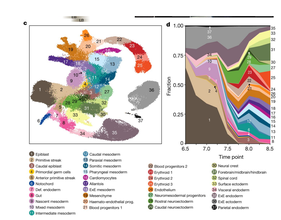
Kegg pathways:
TGF-beta https://www.genome.jp/pathway/map04350
stem cells https://www.genome.jp/pathway/map04550
To find genes that interact with the lefty genes.
Using a single-cell RNA sequencing (scRNAseq) mouse gastrulation dataset in RStudio, I was able to plot the cell type expression of each gene and the stages they're expressed in.

Discussion

Endoderm celltypes in relation to each other…
def endoderm is under the visceral layer and eventually replaces it



Why the nascent mesoderm disappears in stage E8.0…



Acknowledgements

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https://www.nature.com/articles/s41586-019-0933-9

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There May Be a Specific Point in Gastrulation when Lefty Genes Interact

Yaneth Perez[1], Lindsay Hayes[2], Matt McCoy[3], and Rosa Alcazar[1]

[1]Clovis Community College, [2]Johns Hopkins University School of Medicine, [3]Stanford University School of Medicine

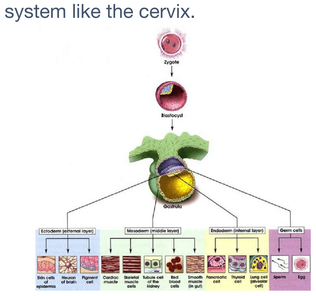
Past studies established that the TGF-beta superfamily includes the lefty genes, Lefty1 and Lefty2, in both humans and mice. In the TGF-beta pathway, Lefty1 plays a critical role is left-right asymmetry determination of organs during early embryo development. This pathway does not show whether Lefty1 and Lefty2 directly interact, but rather Nodal and Smad2 served as a connection between them. In the TGF-beta signaling pathway, which regulates pluripotency of stem cells, we see Nodal and Smad2 control the transcription of Lefty2. In this pathway Lefty2 plays a role in embryonic stem cell signaling. In humans, Lefty1 is highly expressed in the pancreas and colon, which arises from the endoderm. Whereas Lefty2 is highly expressed in the female tissue (i.e cervix), which arises from the mesoderm cell types. It is still unknown whether they interact, and if they do, where and when.

Hypothesis

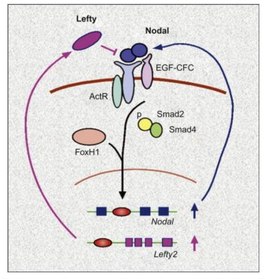
The Lefty genes are present during gastrulation therefore Lefty1 will show high expression levels in the endoderm cell types during the early stages of gastrulation in mice and Lefty2 will show high levels of expression in the mesoderm cell types.

Introduction

Three layers of tissue are formed during early development. The endoderm is the inner layer, the mesoderm is the middle layer, and the ectoderm is the outer layer. From the endoderm arises organs involved in the digestive system like the pancreas and colon. From the mesoderm arises organs involved in the reproductive system like the cervix.



In the left-right axis determination pathway, Lefty1 acts as a ligand that represses nodal outside of the cell. The Nodal and activin receptors in the membrane send a signal to Smad2/3 which then binds to Smad4. They then move into the nucleus and act as components to Lefty2 transcription.



Methods

Using a single-cell RNA sequencing (scRNAseq) mouse gastrulation dataset in RStudio, I was able to plot the cell type expression of each gene and the stages they are expressed in. Plot expression maps were created with the R package scram, scater, and tidyverse.

Results

Figure 1. Lefty1 and Lefty2 are both expressed in the stage E7.25 in Endoderm Cell types.

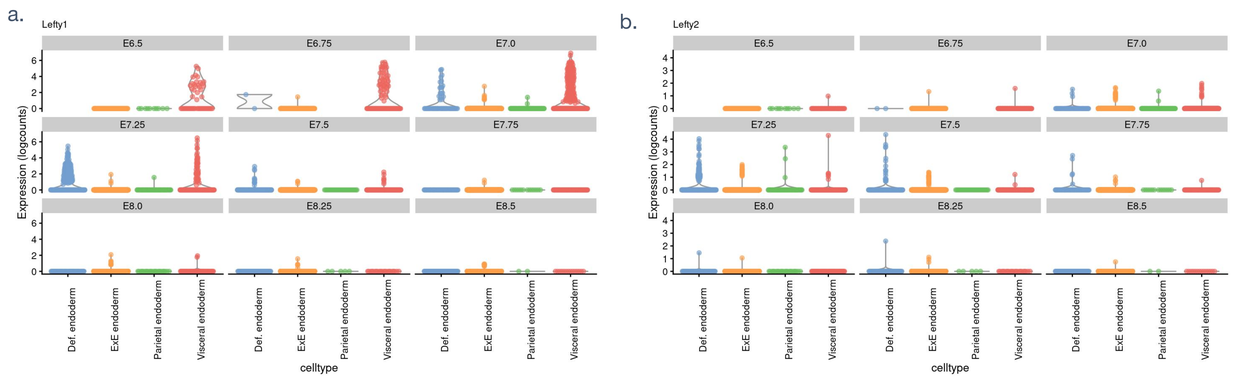


Figure 1: a) Lefty1 is highly expressed in the visceral endoderm in the first four stages (E6.5-E7.25). Its visceral endoderm expression shifts to the definitive endoderm in stage E7.25, then both expression levels diminish throughout the final stages.b) Lefty2 has relatively low levels of expression when compared to Lefty1 with the exception in stage E7.25. Here its expression spikes in the definitive endoderm and then also begins to diminish, much like Lefty1. There is also some expression in the visceral endoderm in stages E7.0 and E7.25.

Figure 2. Lefty2 is expressed in the nascent mesoderm.

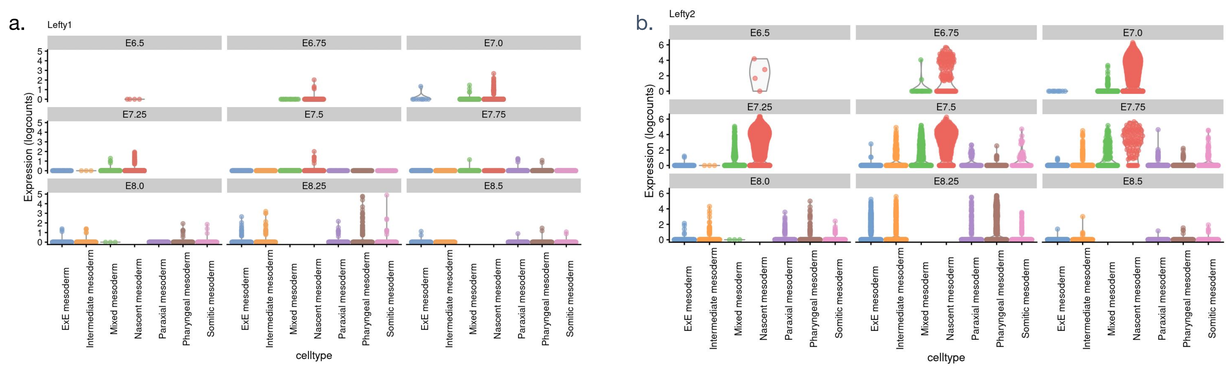


Figure 2: a) Lefty1 has very low levels of expression in the mesoderm cell types. It is first expressed in the nascent mesoderm and spikes in stage E7.0 before expression decreases in the later stages. b) Lefty2 has high levels of expression in the nascent mesoderm up until stage E.80. Similarly, its expression in the mixed mesoderm also disappears in this stage. All other mesoderm cell types then gain cell expression up until E8.25.

Discussion

Figure 3. Lefty1 and Lefty2 are tissue specific.

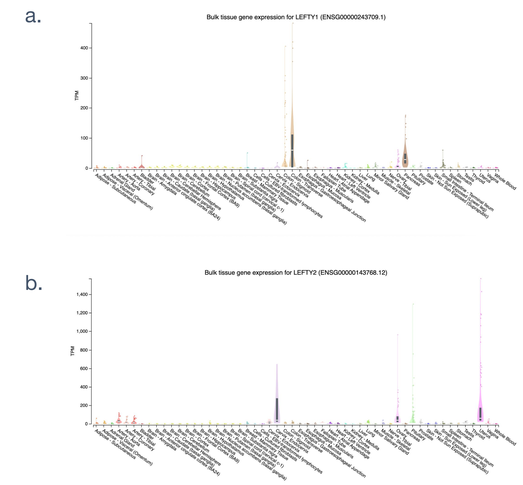
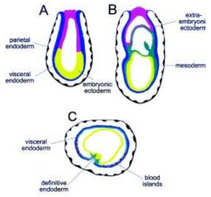
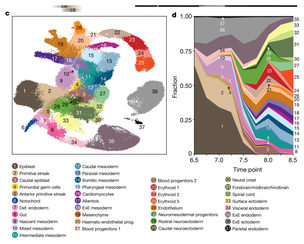


Figure 3: a) From GTEx Portal, Lefty1 is only expressed in the colon and pancreas, which arise from the endoderm. This explains why the plots for Lefty1 show much higher expression in endoderm cell types than mesoderm. b) Lefty2 is only expressed in the cervix, ovary, and uterus (female reproductive tissue). These arise from the mesoderm and explains why Lefty2 has high levels of expression in the mesoderm cell types.



The definitive endoderm is under the visceral endoderm and eventually replaces it. This can explain why in stage E7.0 and E7.25 gene expression shifts from the visceral endoderm to the def. endoderm in fig1a and fig1b.

In stage E8.0, cells in the nascent mesoderm, a pluripotent stem cell, migrate to the mesoderm and def. endoderm because the nascent mesoderm disappears and other cell types emerge in correlation with the germ layers and given rise to specific organs.



References and Acknowledgements

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Pijuan-Sala, B., Griffiths, J. A., Guibentif, C., Hiscock, T. W., Jawaid, W., Calero-Nieto, F. J., Mulas, C., Ibarra-Soria, X., Tyser, R. C. V., Ho, D. L. L., Reik, W., Srinivas, S., Simons, B. D., Nichols, J., Marioni, J. C., & Göttgens, B. (2019, February 20). *A single-cell molecular map of mouse gastrulation and early organogenesis*. Nature.

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There May Be a Specific Point in Gastrulation when Lefty Genes Interact

Yaneth Perez[1], Lindsay Hayes[2], Matt McCoy[3], and Rosa Alcazar[1]

[1]Clovis Community College, [2]Johns Hopkins University School of Medicine, [3]Stanford University School of Medicine

Past studies established that the TGF-beta superfamily includes the lefty genes, Lefty1 and Lefty2, in both humans and mice. In the TGF-beta pathway, Lefty1 plays a critical role is left-right asymmetry determination of organs dur[illegible] development. This pathway [illegible] Lefty1 and Lefty2 directly in[illegible] Smad2 served as a connect[illegible] TGF-beta signaling pathway [illegible] pluripotency of stem cells, we see Nodal and Smad2 control the transcription of Lefty2. In this pathway Lefty2 plays a role in embryonic stem cell signaling. In humans, Lefty1 is highly expressed in the pancreas and colon, which arises from the endoderm. Whereas Lefty2 is highly expressed in the female tissue (i.e cervix), which arises from the mesoderm cell types. It is still unknown whether they interact, and if they do, where and when.

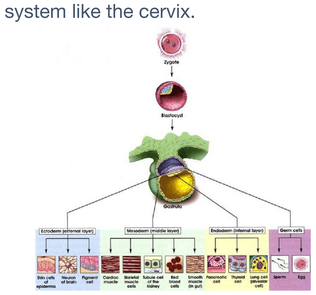
Discuss the gap in knowledge of Lefty interaction. Show the string assignment

Hypothesis

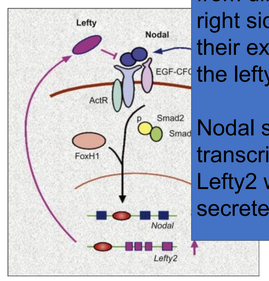
The Lefty genes are present during gastrulation therefore Lefty1 will show high expression levels in the endoderm cell types during the early stages of gastrulation in mice and Lefty2 will show high levels of expression in the mesoderm cell types.

Introduction

Three layers of tissue are formed during early development. The endoderm is the inner layer, the mesoderm is the middle layer, and the ectoderm is the outer layer. From the endoderm arises organs involved in the digestive system like the pancreas and colon. From the mesoderm arises organs involved in the reproductive system like the cervix.



In the left-right axis determination pathway, Lefty1 acts as a ligand that represses nodal outside of [illegible] activin receptors in the membrane sen[illegible] which then binds to Smad4. They then [illegible] and act as components to Lefty2 trans[illegible]



Lefty1 prevents Nodal and Lefty2 from diffusing to the right side (keeps their expression to the lefty side)

Nodal signals for transcription of Lefty2 which is then secreted

Methods

Using a single-cell RNA sequencing (scRNAseq) mouse gastrulation dataset in RStudio, I was able to plot the cell type expression of each gene and the stages they are expressed in. Plot expression maps were created with the R package scram, scater, and tidyverse.

Results

Figure 1. Lefty1 and Lefty2 are both expressed in the stage E7.25 in Endoderm Cell types.

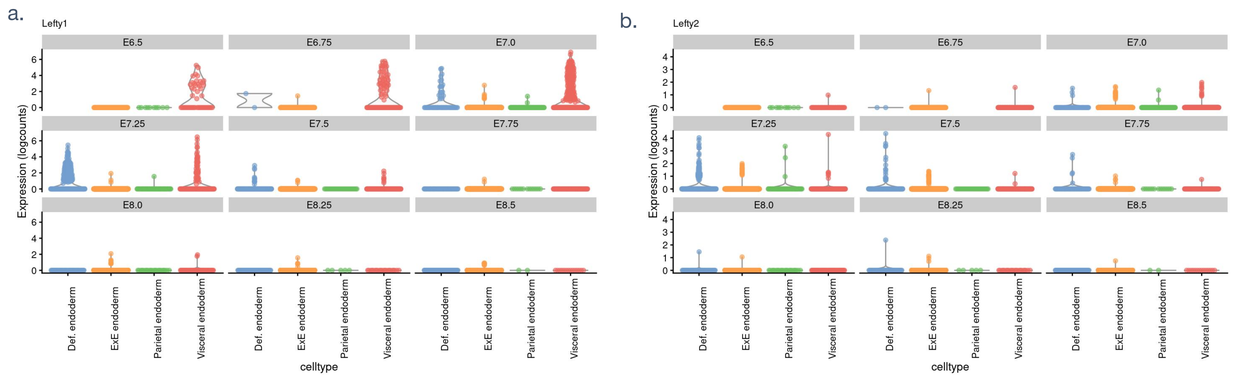


Figure 1: a) Lefty1 is highly expressed in the visceral endoderm in the first four stages (E6.5-E7.25). Its visceral endoderm expression shifts to the definitive endoderm in stage E7.25, then both expression levels diminish throughout the final stages.b) Lefty2 has relatively low levels of expression when compared to Lefty1 with the exception in stage E7.25. Here its expression spikes in the definitive endoderm and then also begins to diminish, much like Lefty1. There is also some expression in the visceral endoderm in stages E7.0 and E7.25.

Figure 2. Lefty2 is expressed in the nascent mesoderm.

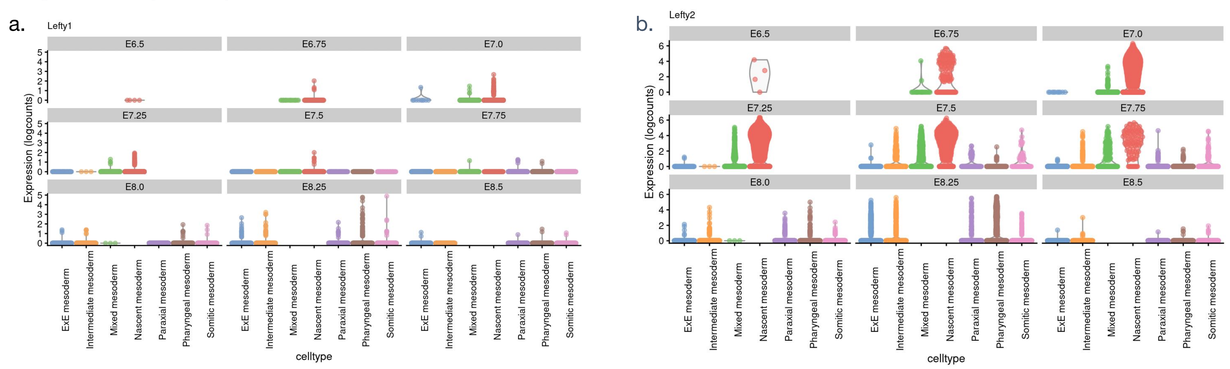
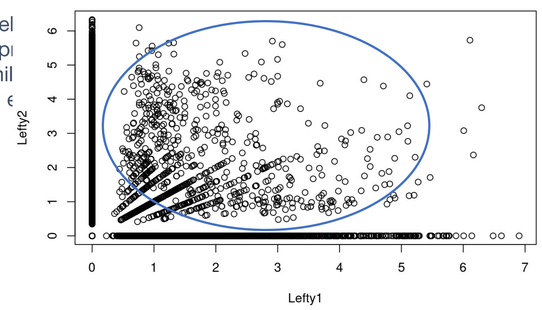


Figure 2: a) Lefty1 has very low level [illegible] and spikes in stage E7.0 before exp[illegible] mesoderm up until stage E.80. Simil[illegible] mesoderm cell types then gain cell [illegible]

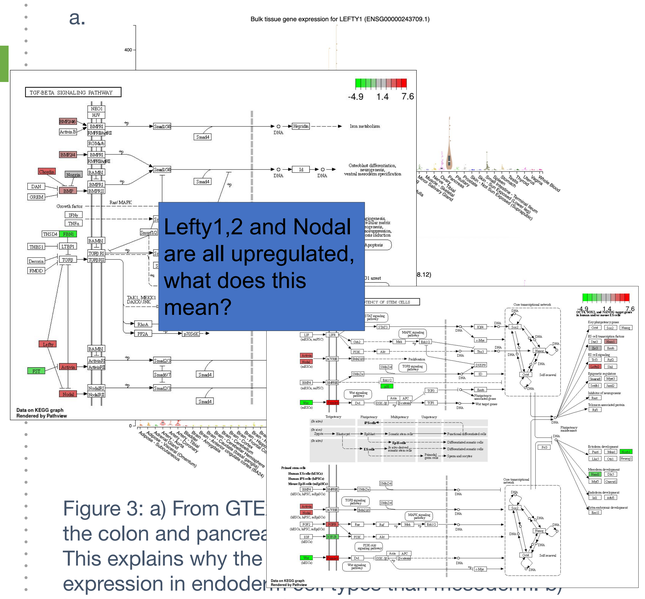


[illegible]sed in the nascent mesoderm [illegible]ls of expression in the nascent [illegible] in this stage. All other

Rstudio shows that there are cells that both Lefty genes are expressed in. What/where are these cells and what time are they both expressed in within these cells

Discussion

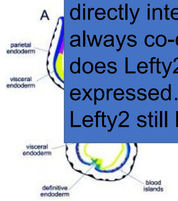
Figure 3. Lefty1 and Lefty2 are tissue specific.



Lefty1,2 and Nodal are all upregulated, what does this mean?

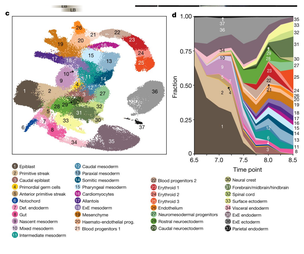
Figure 3: a) From GTE[illegible] the colon and pancrea[illegible] This explains why the [illegible] expression in endoder[illegible] Lefty2 is only expressed in the cervix, ovary, and uterus (female reproductive tissue). These arise from the mes[illegible] of expr[illegible]

Possible conclusion: Nodal (as a receptor) is a link between Lefty 1 & 2, so they never directly interact and therefore are not always co-expressed…future question: does Lefty2 need Lefty2, to be expressed… if Lefty1 is not expressed can Lefty2 still be expressed



[illegible]der the [illegible]ually [illegible]hy in [illegible]xpression [illegible]erm to the def. endoderm in fig1a and fig1b.

In stage E8.0, cells in the nascent mesoderm, a pluripotent stem cell, migrate to the mesoderm and def. endoderm because the nascent mesoderm disappears and other cell types emerge in correlation with the germ layers and given rise to specific organs.



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The Co-Expression of the Lefty Genes

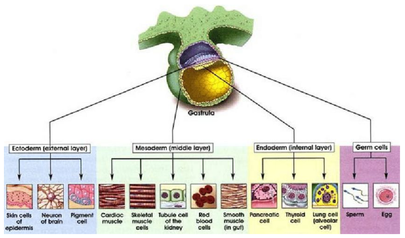
Yaneth Perez[1], Lindsay Hayes[2], Matt McCoy[3], and Rosa Alcazar[1]

[1]Clovis Community College, [2]Johns Hopkins University School of Medicine, [3]Stanford University School of Medicine

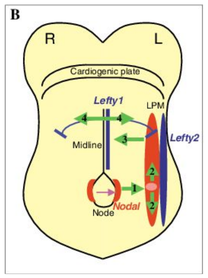
Past research has focused on defining the roles of genes involved in left-right asymmetry determination of organs during development. Three transforming growth factor (TGF-β) family members are involved in this determination; Lefty1, Lefty2, and Nodal. Most studies published around these genes have focused their research on how the Lefty genes regulate Nodal signaling. These publishications have confirmed that Lefty and Nodal are coexpressed, but have yet to confirm the coexpression of Lefty1 and Lefty2. The research shown on this poster further looked into their direct interaction during mouse gastrulation.

Introduction

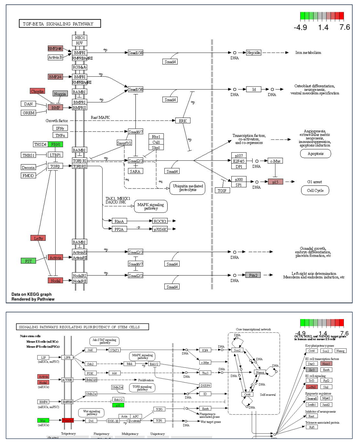
During gastrulation, pluripotency stem cells differentiate into the three primary germ layers. The endoderm being the innermost layer, mesoderm the middle layer, and ectoderm the outer layer. Cells in these germ layers later differentiate into tissue and organs. From the endoderm rises organs involved in the digestive system like the pancreas and colon, where Lefty1 is highly expressed. And the mesoderm gives rise to organs involved in the reproductive system like the cervix, where Lefty2 is highly expressed.

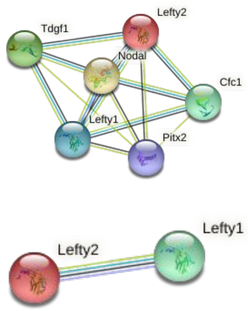


Left-right asymmetry determination involves Lefty1, Lefty2, and Nodal. The role of Lefty1 is to prevent Nodal and Lefty2 from diffusing to the right side. One study established, in mice, that Lefty1 functioned as a midline barrier that restricts Nodal signaling to the left lateral plate mesoderm (LPM). Nodal produced in this region induces Lefty2 expression (Shiratori et al., 2006). And therefore, Lefty1 also acts as a regulator to Lefty2 by regulating Nodal.



Kegg Pathways and String Network





Within the TGF-beta pathway and the pluripotency of stem cells pathway, Lefty1, Lefty2, and Nodal were all upregulated. This means that these three genes increase their expression and work together. Although these figures show that Lefty1 and Lefty2 are in separate pathways, what seems to connect them is Nodal. In String Network Analysis, all three genes were always shown as close interactors. However, Lefty1 and Lefty2 had a co-expression score (black line) of 0.163 on a scale of 0-1. This means that the level of confidence that these genes are associated is low. These genes also did not show co-occurrence. Although the Lefty genes are mentioned together throughout published papers, there doesn't seem to be a consistent or confident agreement about their interaction.

Celltype Expression

Figure 1. Lefty1 and Lefty2 are expressed in the Visceral endoderm and Nascent mesoderm.

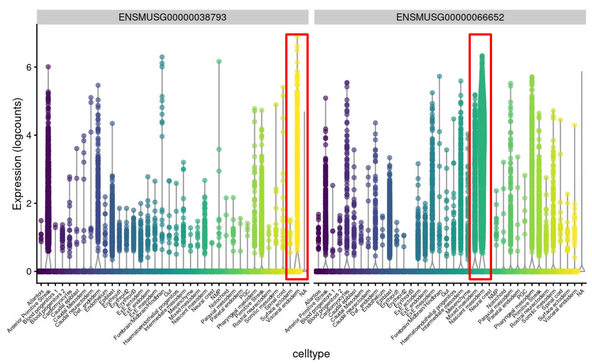
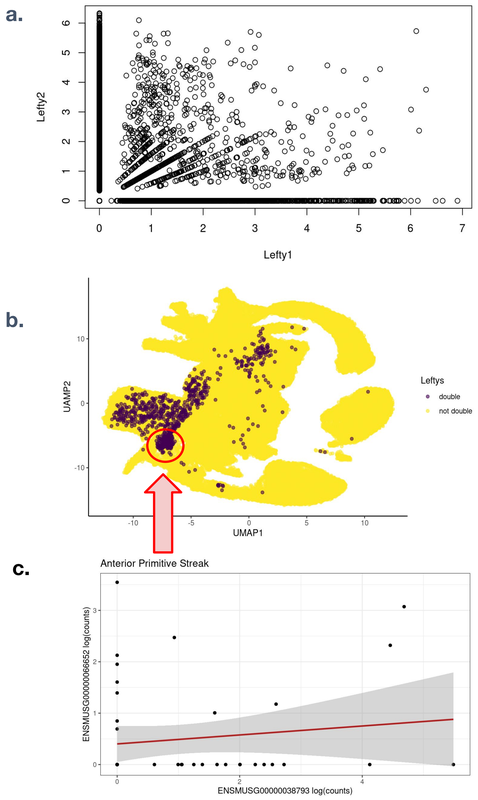


Figure 1 shows the differential cell type expression of Lefty1 and Lefty2. Lefty1 is more expressed in endodermal cell types where its highest expression is in the visceral endoderm (yellow). Whereas, Lefty2 is more expressed in the mesodermal cell types with its highest expression being in the nascent mesoderm (green).

Lefty1 and Lefty2 Co-Expression

Figure 2. The Lefty genes are co-expressed within some cells.



In figure 2a. the points on the y-axis only have Lefty2 expression and the points on the x-axis only have Lefty1 expression. All the points in the center have expression of both genes. The UMAP in figure 2b. shows the location of cells (in purple) that express both Lefty1 and Lefty2. They were co-expressed within 1078 cells. These cells were located within the Epiblast, Primitive Streak, and Anterior Primitive Streak (APS). Figure 2c. shows their co-expression in the APS. They had a rho value of 0.0357 and p.value of 0.0148 which means they are positive correlated but not significantly.

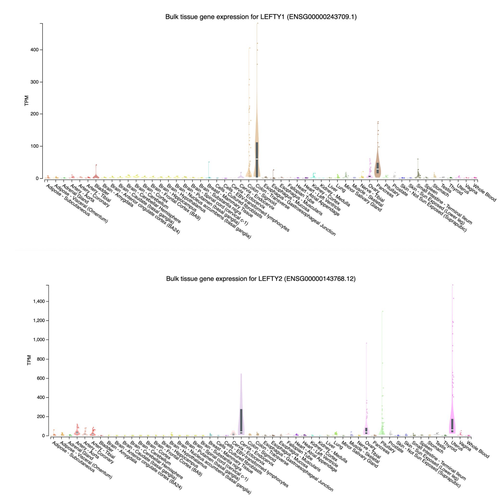
Methods

Using the RNA-seq. data from the mouse gastrulation dataset in Rstudio, I was able to create these plots showcasing gene expression through different cell types and stages. For further analysis I also utilized String Network and KEGG Pathway. To view more details on the code that created these plots, visit my R Notebook.

Discussion

In my previous research, I found that both Lefty1 and Lefty2 were expressed in the Def. endoderm, Visceral endoderm, and Nascent mesoderm during stage E7.25. After establishing their presence within the these cell types and in the same stage, I wondered if they directly interacted there. My results in Figure 2, show that Lefty1 and Lefty2 are co-expressed within a few cells. However, unlike my past results, these cells are in the some of the earliest cell types like the Epiblast and Anterior Primitive Streak (APS). The APS has a high intensity of Nodal signaling and is the source of the mesoderm and endoderm. Based of these results, it looks like Lefty1 and Lefty2 may be co-expressed in cells during the early stages of gastrulation but as germ layers arise, these genes diffuse into different cell types. This would explain why these genes have different tissue specificity. Further research should faith map the Lefty genes during gastrulation to track where they are first expressed and where they end up.

Figure 3. Lefty1 and Lefty2 are tissue specific.



The UCSC genome browser shows that Lefty1 has high expression in the colon and pancreas. Whereas Lefty2 has its highest expression in the cervix, uterus, and ovary (female reproductive tissue).

Acknowledgements

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Shiv

Investigating Mdh1's Involvement In Suppressing Cell Proliferation of Pancreatic Cancer In Mouse Gastrulation

Shiv Mehrotra-Varma[1], Lindsay Hayes[2], Matt McCoy[3], and Rosa Alcazar[1]

[1]Clovis Community College, [2]Johns Hopkins University School of Medicine, [3]Stanford University School of Medicine

C-MOOR

Studies have shown that the arginine methylation of MDH1 by CARM1 suppresses the cell proliferation of pancreatic cancer. MDH1, a gene crucial to metabolism and The Citric Acid Cycle, in part catalyzes an unconventional metabolic pathway that pancreatic cancer cells rely on for glutamine production, which is needed for cell proliferation. The arginine methylation of MDH1 by CARM1 inhibits MDH1 and leads to glutamine depletion, thus lowering the rate of pancreatic cancer cell proliferation.

This study delves to determine whether or not arginine methylation of MDH1 by CARM1, leading to glutamine depletion and suppressed cell proliferation, occurs as early as gastrulation. This was done by examining the gene expression of several genes of interest across gastrulation to find relationships between them. The expression of genes correlated to MDH1 inhibition, suppressed cell proliferation, and the arginine methylation of MDH1 were compared with the expression of MDH1 using violin plots and 2D scatter plots created with R. Certain relationships between the genes of interest and MDH1 would indicate that the arginine methylation of MDH1 is occurring as early as gastrulation.

This discovery could prove to be invaluable in the fight to treating pancreatic cancer patients, especially so in rare embryonic cases, and preventing future incidences of cancer in high-risk patients.

Introduction

Cancer continues to be a major cause of disease and death across the globe, resulting in nearly ten million deaths each year. In cancer patients malignant cells divide uncontrollably and metastasize locally and to remote organs and tissues. Many scientists view the progression of cancer as a microevolutionary process; a series of mutations in a cell causes it to proliferate more than its immediate neighbors, resulting in the formation of tumors. The rate of cancer cell proliferation, or how quickly a cancer cell copies its DNA and divides into two cells determines the speed of progression of disease. Part of the survival strategy of cancer stem cells may be manifested by alterations in cell metabolism, which is key to cell proliferation (Feitelson et al., 2015).

Alterations in cell metabolism may stimulate tumorigenesis, or the transformation of normal cells to malignant cells. However, studies have shown that investigating the genes involved in metabolism stimulating cell proliferation may provide insight into suppressing cancer cell proliferation.

Malate Dehydrogenase 1 (MDH1), an enzyme encoded by the MDH1 gene, is one of these genes critical to cell metabolism. MDH1 catalyzes the reversible oxidation of malate to oxaloacetate in the Citric Acid Cycle. Localized to the cytoplasm, MDH1 plays a crucial role in metabolic coordination between the cytosol and mitochondria.

Studies have shown that the arginine methylation of of MDH1 by protein arginine methyltransferase 4 (PRMT4/CARM1) represses mitochondrial respiration, inhibits glutamine utilization, reduces cellular NADPH level, and sensitizes cells to oxidative stress. PRMT4 has also been shown to inhibit MDH1 by disrupting its dimerization.

Pancreatic cancer cells rely on an unconventional metabolic pathway to rewire glutamine metabolism and produce NADPH. MDH1, along with aspartate aminotransferase and malic enzyme 1, catalyzes this pathway, meaning that the arginine methylation of MDH1 by CARM1 may inhibit glutamine metabolism and suppress pancreatic cancer (Wang et al., 2016).

The suppression of cell proliferation of pancreatic cancer in embryos has not been studied previously. Although cancer is rarely found in embryos, studying gene expression in mouse gastrulation may provide insight into MDH1 suppressing cancer cell proliferation later in life.

This project aims to discover whether or not the inhibition of MDH1, and thus, the suppression of pancreatic cancer, can be seen as early as gastrulation, the embryonic stage in which the embryo changes from a blastula (containing a single layer of cells) to a gastrula (containing multiple layers of cells). The expression of certain genes was investigated in various mouse embryos throughout gastrulation using R. Specifically, signs of MDH1 being inhibited and the suppression of cell proliferation were studied. If successful, this study could contribute to the effort of preventing cancer as early as gastrulation.

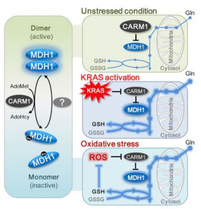


Fig. 1: The following diagram explains the process of the arginine methylation of MDH1 by CARM1 (https://pubmed.ncbi.nlm.nih.gov/27840030/).

Methods

Along with a thorough review of relevant literature, the String Database (string-db.org) was utilized to identify several key genes of interest related to the inhibition of MDH1. Nearly all genes including several proto-oncogenes were involved in the primary metabolic process. Eventually, the following genes of interest were identified:

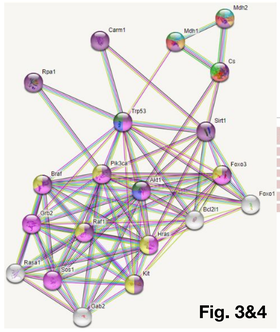
MDH1: an essential gene in metabolism that encodes an enzyme which catalyzes the NAD/NADH-dependent, reversible oxidation of malate to oxaloacetate in metabolic pathways like the citric acid cycle. In part catalyzes a metabolic pathway which pancreatic cancer cells depend on.

SIRT1: encodes the protein Sirtuin 1, which activates mitochondrial signals and pathways regulating the expression levels of genes (such as MDH1) crucial for proliferation and ATP generation (Hadar et al., 2017) - inhibited by the knockdown of MDH1.

PRMT4/CARM1: encodes the protein CARM1, which plays an important role in androgen receptors - methylates and inhibits MDH1 by disrupting its dimerization through arginine methylation.

KRAS2: a proto-oncogene that encodes the KRAS2 protein - activating mutations of KRAS2 are possibly the single most common genetic abnormality in pancreatic cancer, present in 90%–95% of cases (Maitra et al., 2008).

mTORC1: encodes the protein mTORC1, which activates the translation of proteins - inhibition of mTORC1 leads to glutamine deprivation, so it can be used as an indicator of suppressed cell proliferation.



description	count in network	strength	false discovery rate
Tricarboxylic acid cycle	3 of 25	2.12	0.00028
Proto-oncogene	6 of 98	1.83	1.89e-07
Malate metabolic process	2 of 8	2.44	0.0021
NADH metabolic process	2 of 27	1.91	0.0118
Primary metabolic process	13 of 6369	0.35	0.0260
Oxaloacetate metabolic process	3 of 7	2.67	6.17e-05
Endometrial cancer	9 of 58	2.23	3.40e-16
Solid cancer cell	2 of 2	3.04	0.00058

Fig. 3&4

Finding Evidence of MDH1 Knockdown:

The relationship and expression of these genes was studied by extracting information from the database using R. Certain patterns between the genes of interest serve as evidence that MDH1 is being inhibited. For example, as SIRT1 regulates the expression of MDH1, a direct relationship between the expression of these two genes would indicate that SIRT1 is being inhibited because of the knockdown of MDH1. Furthermore, CARM1 and MDH1 should have an inverse relationship, as CARM1 inhibits MDH1. When CARM1 expression increases, MDH1 expression should decrease. Various 2D scatter plots, UMAPs, and violin plots were generated in RStudio using data collected from embryos (E6.5-E8.5 days) in gastrulation. All of the genes of interest were very widely expressed as they are involved in metabolism, a process crucial to the survival of a cell, so the cell type was only slightly taken into consideration.

Finding Evidence of Suppressed Cell Proliferation:

As the database does not contain embryos with pancreatic cancer, resources are too limited to find direct evidence of cell proliferation. Thus, another method to look at signs of suppressed cell proliferation needed to be found.

Research has demonstrated that pancreatic cancer cells are sensitive to glutamine (Gln) deprivation (Guillaumond et al., 2013, Ying et al., 2012). Most cells utilize Gln to fuel the The Citric Acid Cycle, however, oncogenic activation of KRAS2 in PDAC cells repurposed Gln through a distinct pathway in which Gln-derived aspartate was transported into the cytoplasm. This pathway (discussed in the Introduction) is catalyzed by MDH1, and a decrease in MDH1 leads to glutamine deprivation. Glutamine deprivation is directly associated with suppressed cell proliferation, and thus can be used as an indicator for it.

Inhibition of the mTORC1 gene leads to glutamine depletion (Fan et al., 2020). The expression of mTORC1 was compared to the expression of the genes of interest in hopes of finding evidence of glutamine depletion and suppressed cell proliferation as a result of the arginine methylation of MDH1 by CARM1 in gastrulation.

Results

Evidence of MDH1 Knockdown:

Various different options were examined in R Studio to look for relationships between genes of interest over gastrulation. Originally, violin plots were chosen as the ideal method to compare gene expression. However, as many of the genes of interest were expressed widely across cell types, violin plots were difficult to analyze.

The violin plots collected are shown below (endodermal cell type), however, these plots were difficult to analyze. 2D scatter plots were then created with R in an attempt to display gene expression in a clearer format. These scatter plots showed the relationship between each gene in a much clearer fashion when compared to the violin plots.

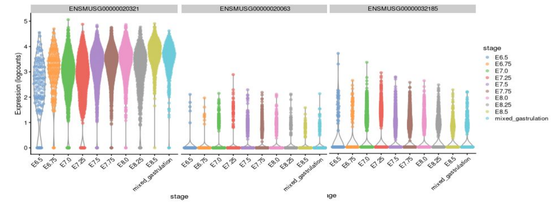


Fig. 5, 6, & 7: MDH1 on the left, SIRT1 in the middle, CARM1 on the right

After creating these violin plots, there was still insufficient evidence to effectively support a relationship between MDH1 and the genes of interest. MDH1 was virtually evenly expressed, so it was difficult to determine how gene expression changed throughout gastrulation. Furthermore, SIRT1 and CARM1 did not show a definitive pattern when changing geneexpression across gastrulation.

2D Scatter plots, shown below, demonstrated a relationship between the expression of genes in a much clearer fashion.

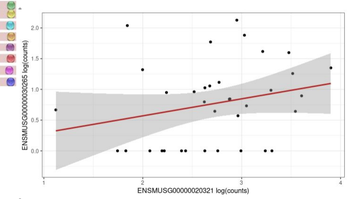


Fig. 8: MDH1 X-axis, SIRT1 Y-Axis

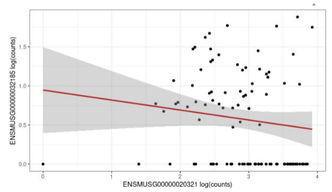


Fig. 9: MDH1 X-axis, CARM1 Y-Axis

Fig. 8: The best-fit line, along with the points showing gene expression, clearly show a direct relationship between MDH1 and SIRT1. The inhibition of MDH1 and the inhibition of SIRT1 should be directly correlated, and the graph supports this. As the expression of MDH1 increases, so does the expression of SIRT1. The graph clearly demonstrates a direct relationship between SIRT1 and MDH1, indicating that SIRT1 is acting as an indicator of MDH1 expression levels. However, the plot does not provide evidence to indicate that MDH1 knockdown is occurring.

Fig. 9: An inverse relationship between MDH1 and CARM1 is clearly demonstrated by the graph. The arginine methylation of MDH1 by CARM1 leads to the inhibition of MDH1, so a decrease in CARM1 expression should lead to an increase in MDH1 expression, and vice versa. As MDH1 levels increase the best-fit line clearly demonstrates a decrease in CARM1, indicating that CARM1 is causing the knockdown of MDH1.

Both of these 2D scatter plots serve as evidence that the knockdown of MDH1 is occurring. SIRT1 is being inhibited, which studies have shown to be directly correlated with MDH1 knockdown. Moreover, CARM1 and MDH1 expression counts have an inverse relationship, demonstrating that the expression of CARM1 is causing MDH1 inhibition.

Evidence of Suppressed Cell Proliferation:

As it is currently not possible to look at pancreatic cancer cells in gastrulation with the dataset, the mTORC1 gene was used as an indicator of cell proliferation. Inhibition of mTORC1 is directly associated with glutamine deprivation, which is directly associated with suppressed cell proliferation. Thus, a direct correlation between the expression of mTORC1 and MDH1 would serve as evidence of suppressed cell proliferation.

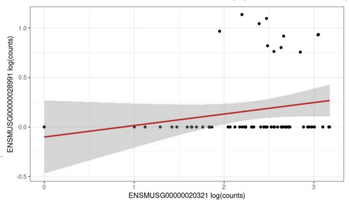


Fig. 10: MDH1 X-axis, mTORC1 Y-Axis

Fig. 10: Not much coexpression is displayed between mTORC1 and MDH1, however, the best fit line does seem to show a slight correlation (direct relationship) between MDH1 and mTORC1. As the expression of mTORC1 increases, so does the expression of MDH1, indicating that mTORC1 may be indicating increased cell proliferation because of increased MDH1 expression.

Since the 2D plots show that CARM1 is inhibiting MDH1 and that MDH1 is directly correlated to cell proliferation, evidence suggests that the process of suppressed cell proliferation *is* occurring as early as gastrulation.

Discussion

Examining the violin and scatter plots from RStudio showed that:

- The expression of SIRT1 is directly correlated with higher MDH1 levels, suggesting that low levels of SIRT1 does serve as an accurate indicator of MDH1 knockdown.
- The expression of CARM1 is associated with lower MDH1 levels, suggesting that the arginine methylation of MDH1 by CARM1 is occurring in gastrulation.
- mTORC1 and MDH1 are directly related, suggesting that MDH1 levels are directly responsible for cell proliferation rates, which are being lowered by the presence of CARM1.

Analyzing the plots provided favorable results, however, a limitation of the project was that the correlations between each gene may have been affected by unknown factors. Although evidence suggests the relationships discovered between the expressions of these genes is a direct result of the arginine methylation of MDH1 by CARM1, it is unknown whether or not this process is the sole reason for what was found. Furthermore, all plots of gene expression were made using the endothelial cell type - results may vary when expression is looked at in different cell types.

Other than proving the hypothesis, results solidified claims about many of the genes of interest already discussed in relevant literature (I.E. SIRT1 regulates the expression levels of genes). The effect of this process in suppressing pancreatic cancer in children or embryos is unable to be studied with current resources, however, literature has shown the process of MDH1 knockdown to suppress pancreatic cancer cell proliferation in adult patients.

Conclusion

The research conducted suggests that CARM1 is critical to regulating the metabolism of pancreatic cancer via MDH1 methylation, affirming claims of this theory already found in scientific literature. More importantly, evidence shows that cell proliferation is already occurring as early as gastrulation. This discovery could prove to be invaluable in the fight to treating pancreatic cancer patients, especially so in rare embryonic cases. Currently, development of selective and potent CARM1 activators to fight cancer is under intensive study (Zeng et al., 2013). Moreover, more research conducted on this process specifically in embryos could potentially provide insight into how to prevent future cases of pancreatic cancer.

In the future, gene expression could be studied in a small cohort of embryos with cancer. Furthermore, the specific phases of gastrulation, or in what phase of gastrulation does MDH1 knockdown most occur, could be studied. Current literature and this study only look into suppressing cell proliferation of specific age groups - a wider demographic should be studied, and results should also be compared across various demographics.

References

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