MINI SYMPOSIUM

FRONTIERS IN SINGLE CELL AND FUNCTIONAL GENOMICS

Long Non-coding RNAs:
From Immune and Disease Biology to Therapeutic Opportunities

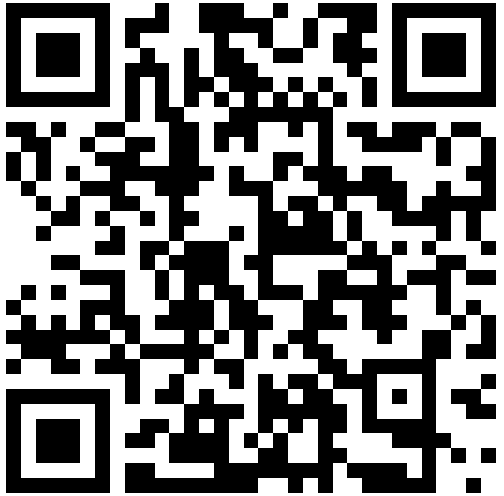
Jordan RAMILOWSKI
Yokohama City University/RIKEN IMS

Mahidol University, 2026-02-23

Today's Menu

- **Introduction to lncRNAs**:
  Biogenesis, classification and function
- **FANTOM Consortium**:
  Worldwide effort to study lncRNAs
- **Our lncRNA Research overview:**
  Hereditary kidney cancer,
  Parkinson's Disease
  Dendritic cell differentiation and infection
- **Potential of lncRNAs to drug discovery**

⚠ Scan the QR



Feel free to check the (49) slides later!

YCU Bioinformatics Lab

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Assistant Professor

Main Research Themes

- long non-coding RNAs (lncRNAs) in immunity and cancers
- Cell-cell communication
- Software, databases & webtools development

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Check our website and get in touch: https://www-user.yokohama-cu.ac.jp/~bioinfo/hp/

Central dogma of life

- DNA provides the blueprint, but **RNA and proteins define cell behavior**
- **Gene expression patterns explain differences across cell types and what drives disease**

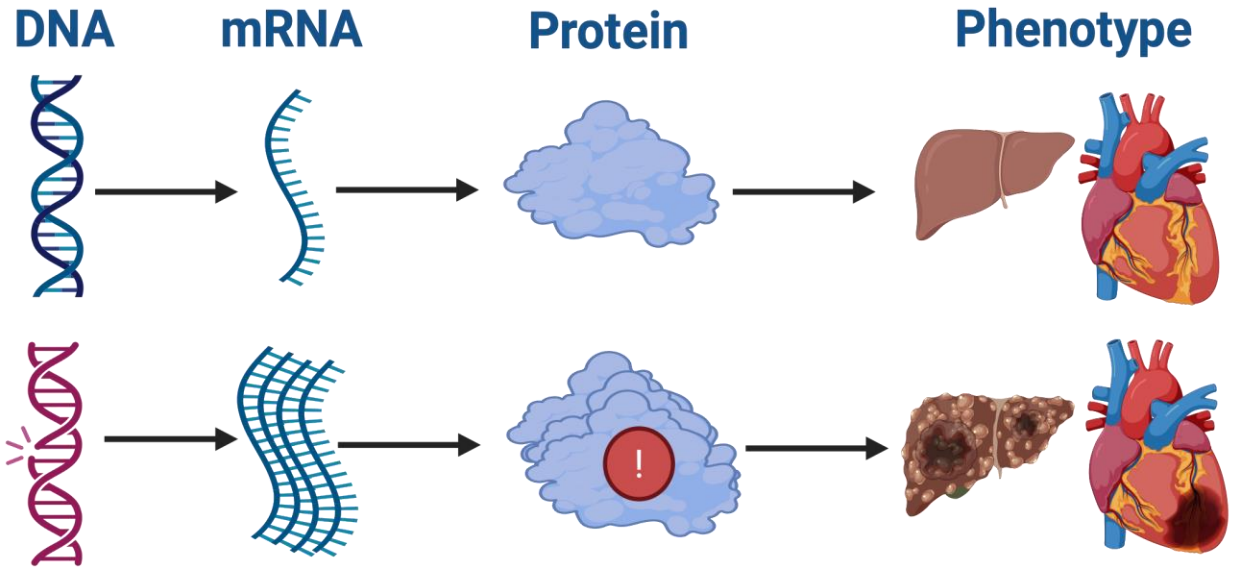
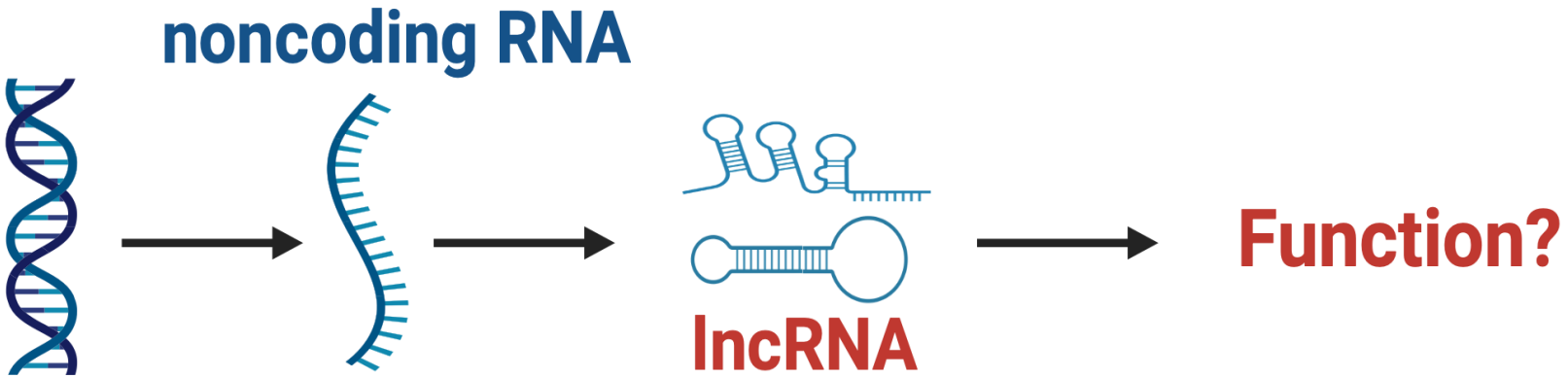


Image created with Biorender

Beyond the central dogma of life…

There exists noncoding RNAs that can be potentially functional!



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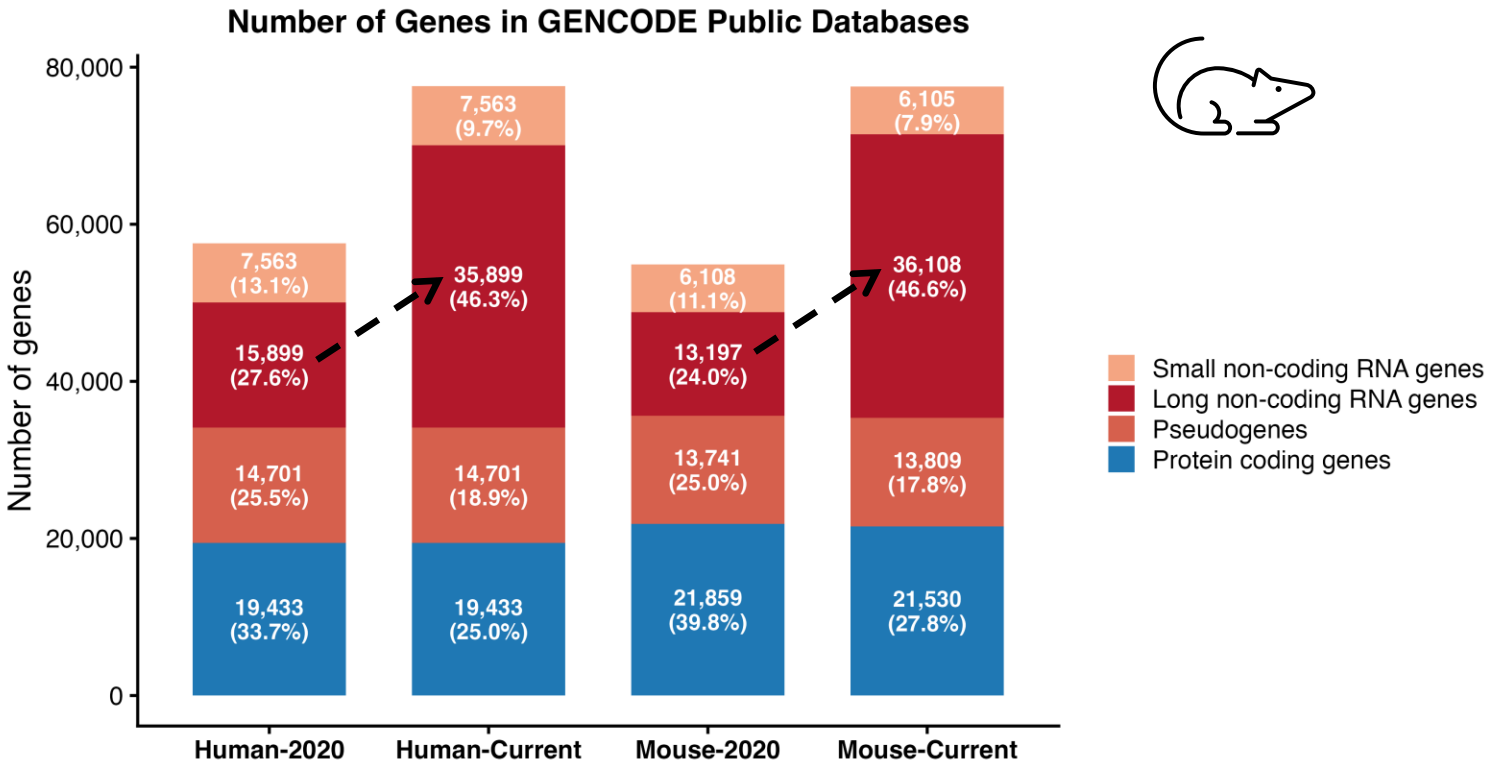
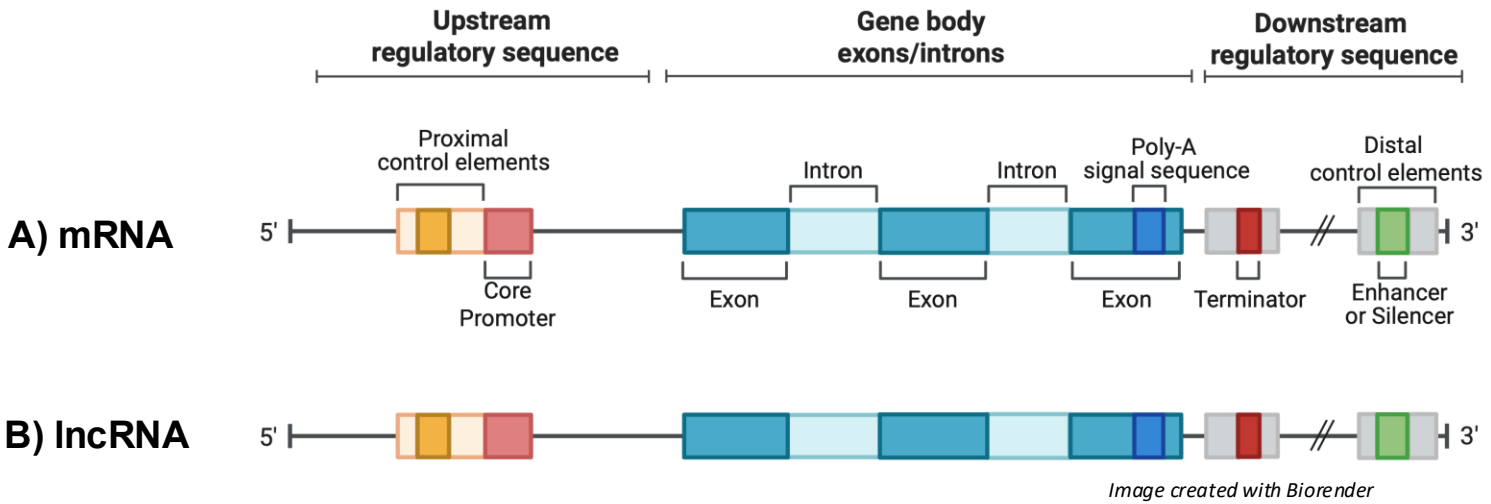
Most mammalian genes are non-coding



The number of known lncRNA genes has largely increased since 2020!

How do lncRNA genes look like?



Can you spot differences between A and B?

Many lncRNA resemble mRNAs but lack CDS

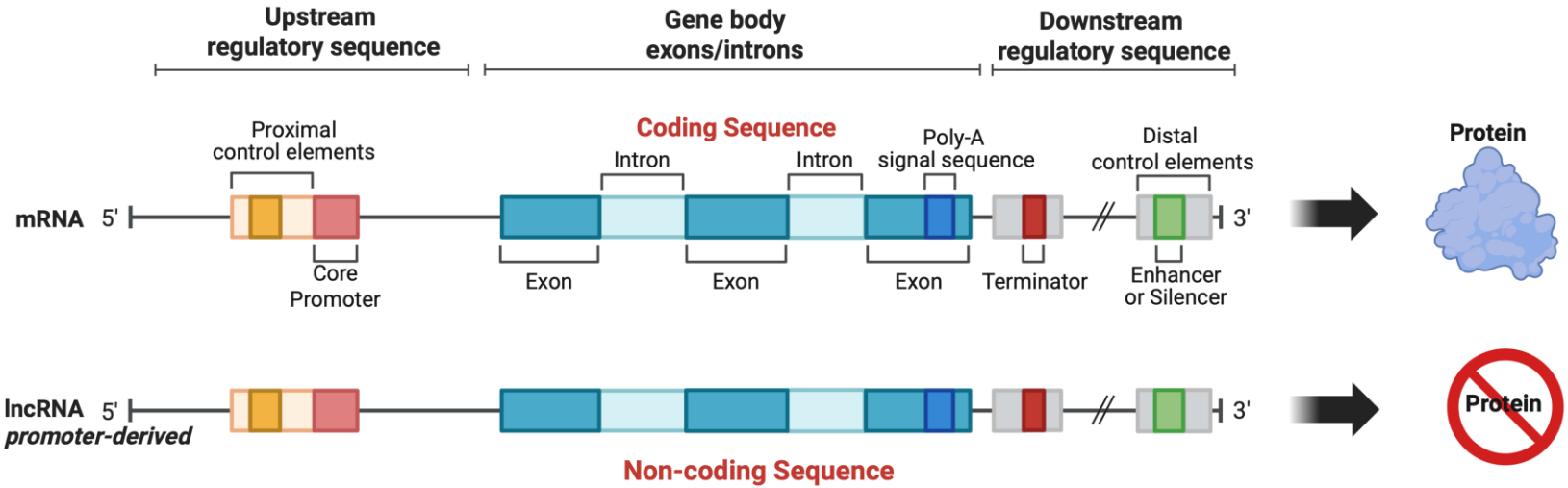
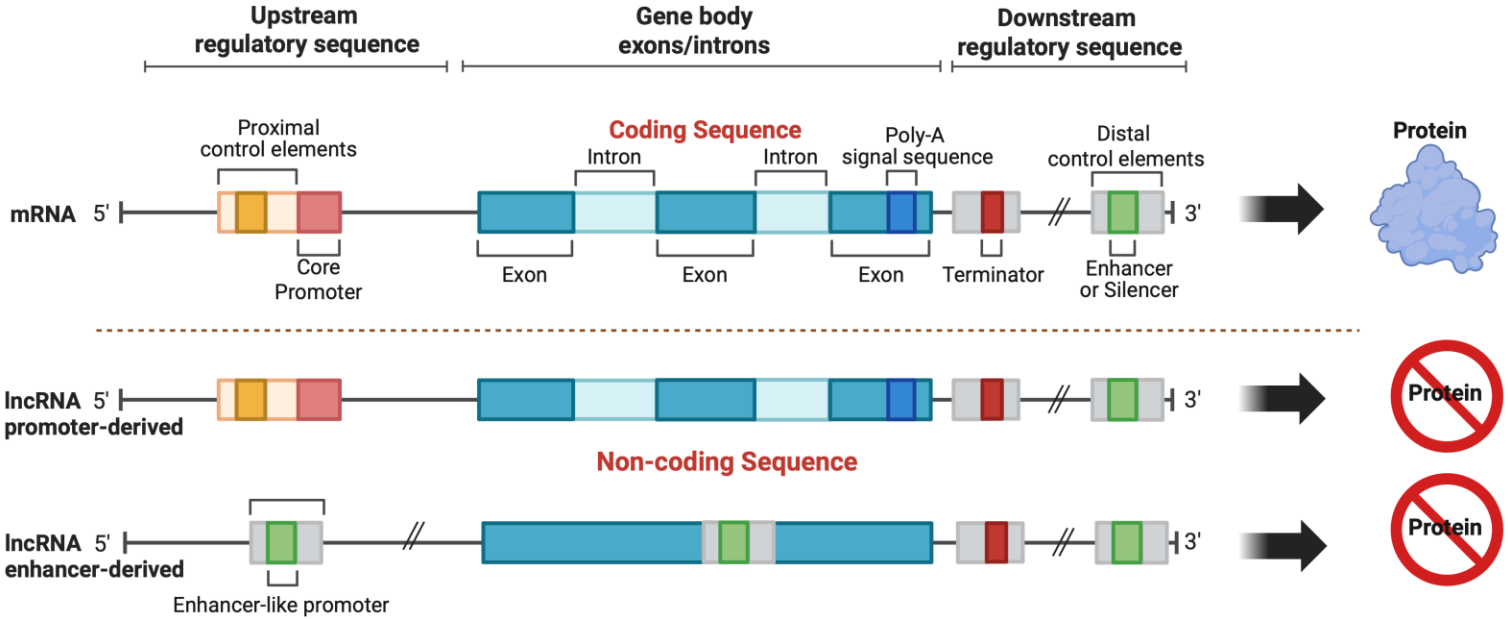


Image created with Biorender

Similarity to mRNAs

- transcribed mostly by RNA polymerase II
- originate from promoters
- often capped, spliced and polyadenylated

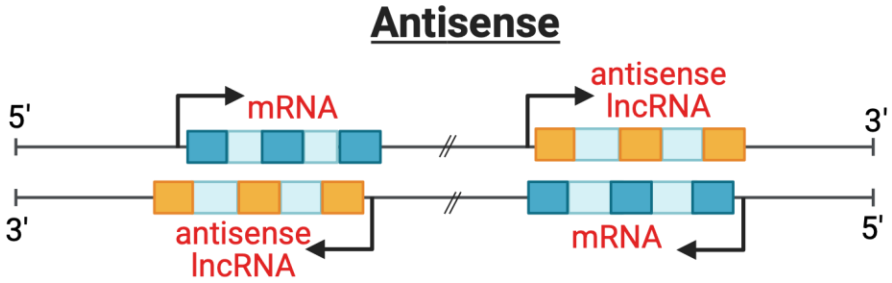
Other lncRNAs might be more unique



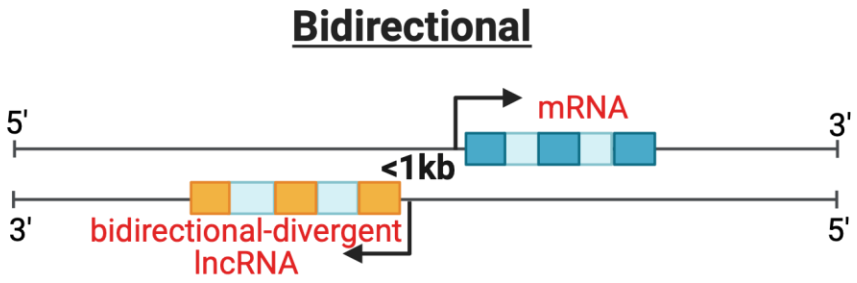
Differences with mRNAs

- lack functional open reading frames
- some originate from enhancers
- less spliced and many lack Pol-A tails

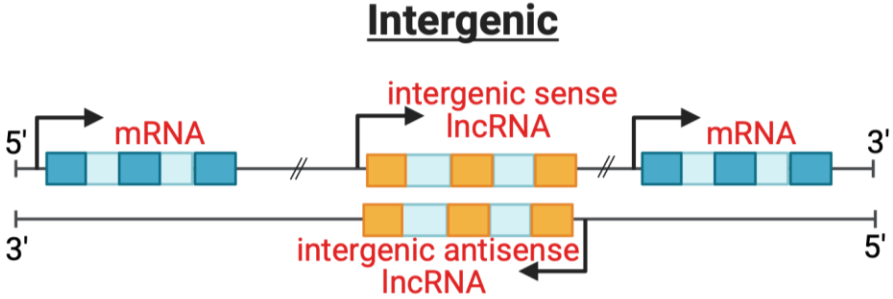
lncRNA can be classified based on genomic locations



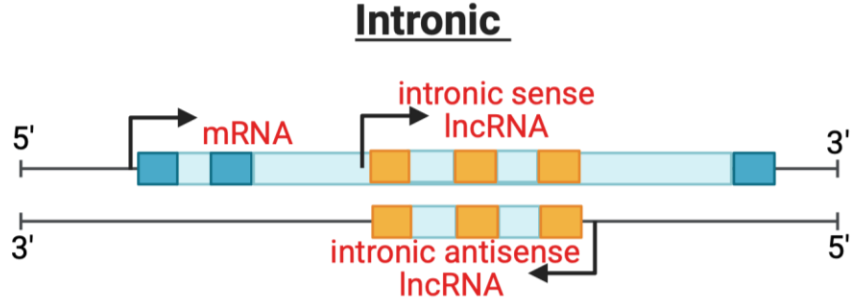
Antisense: overlaps a protein-coding gene on the opposite strand



Bidirectional: transcribed in opposite direction from a nearby gene's promoter

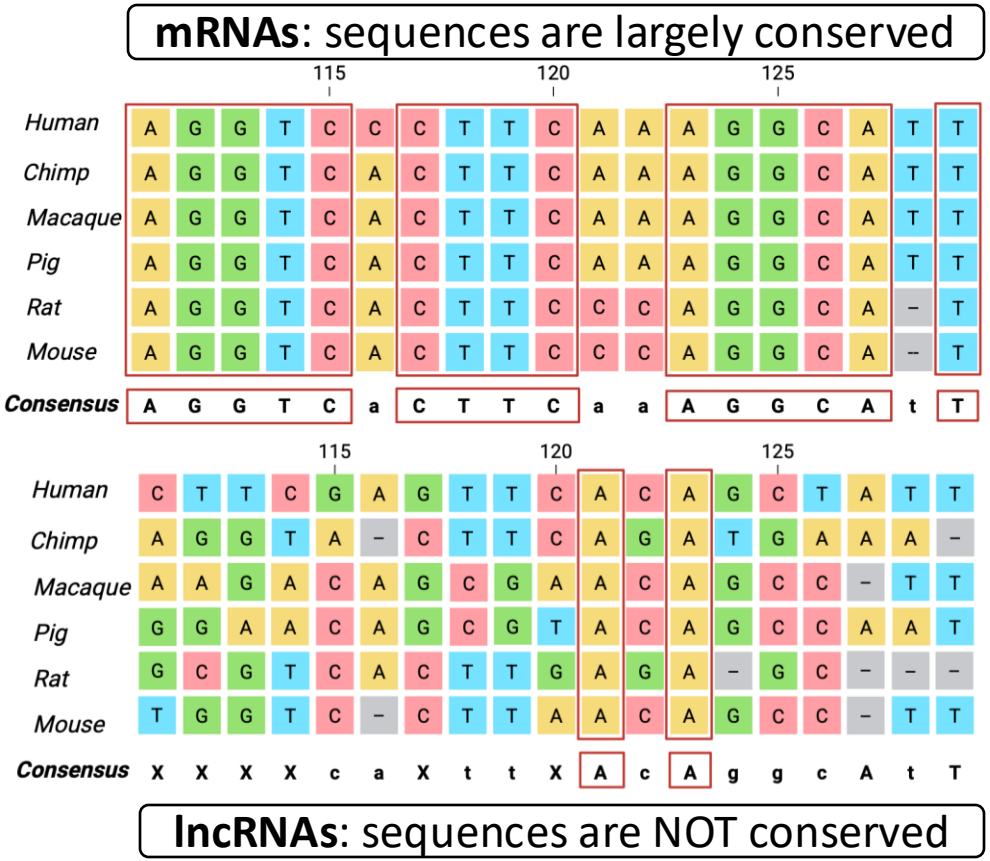


Intergenic: located between protein-coding genes



Intronic: entirely within an intron of a coding gene

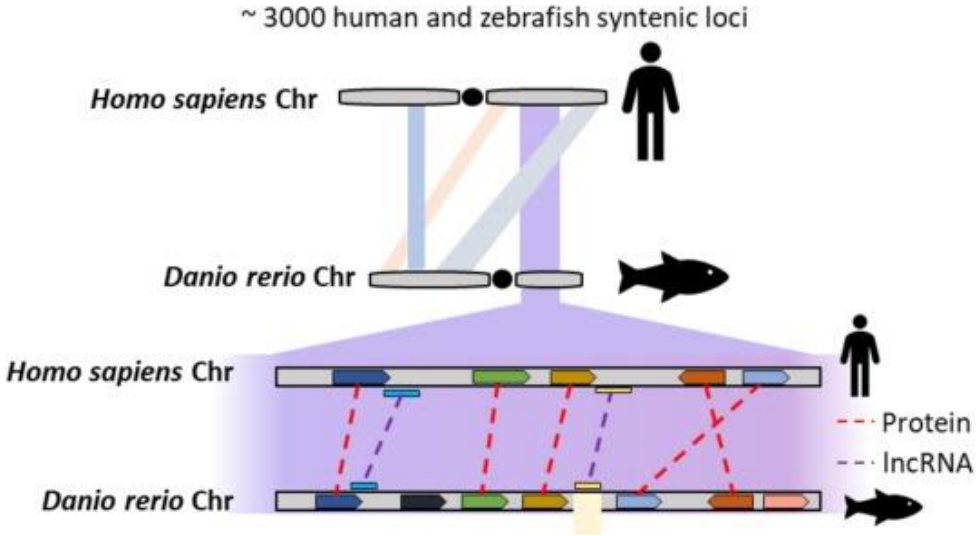
lncRNA sequences are not very well conserved



- Conservation in mRNAs coding regions is high as they produce **specific functional protein domains**
- lncRNA sequences generally show low conservation across species

Schematic generated with BioRender to illustrate the idea; not an exact depiction

lncRNA sequences are not conserved but their location is



Synteny (Conserved Gene Order)

- when genes are in the same order on chromosomes in different species
- helps scientists understand evolutionary relationships, showing which parts of the genome have been preserved over time

Image from Ranjan et. al., 2025

Those lncRNAs in proximity of mRNAs important to a given biological system are of our main interest!

lncRNAs show restricted expression patterns

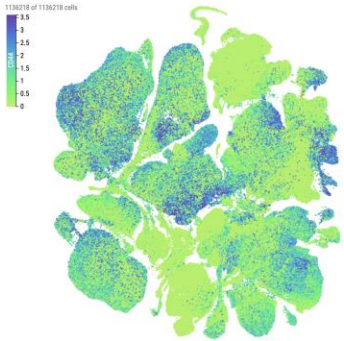
mRNAs: more highly and broadly expressed

lncRNAs: generally cell-type specific

Average mRNA

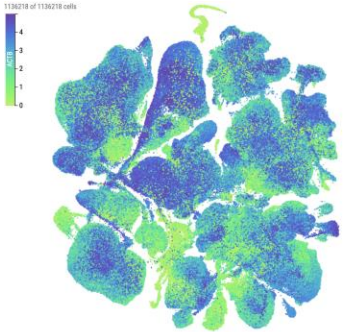
CD44

(broadly expressed)



Housekeeping mRNA

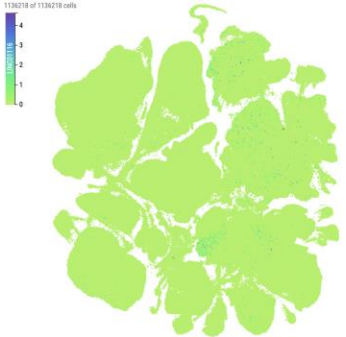
ACTB



Average lncRNA

LINC01116

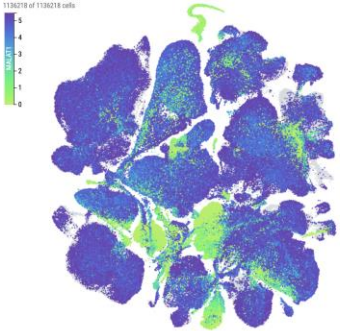
(restricted expression)



Housekeeping lncRNA

MALAT1

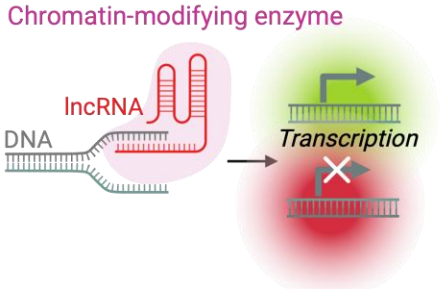
(a famous lncRNA)



[Data: CZ CellxGene Tabula Sapiens (75 tissues)]

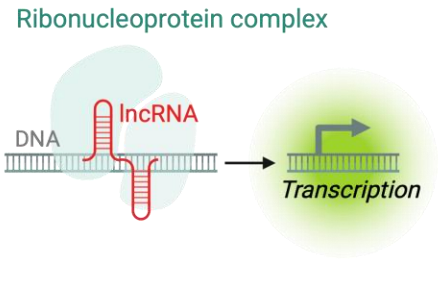
Major Mechanism by which lncRNAs regulate gene expression

1. Chromatin Modification Recruitment



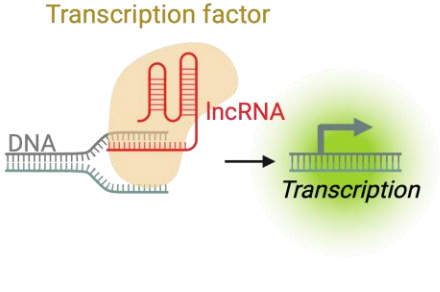
- **lncRNAs recruit chromatin-modifying enzymes** (e.g., methyltransferases, acetyltransferases) to specific DNA regions, altering chromatin structure to activate or repress gene expression.

2. Macromolecular Complex Formation



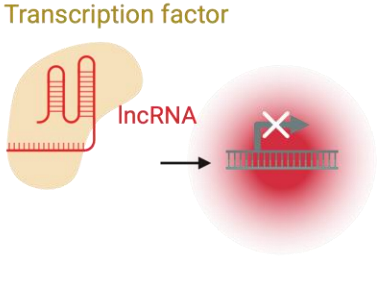
- **lncRNAs serve as scaffolds helping to assemble proteins into complexes** that regulate transcription or chromatin remodeling, enabling precise gene expression and activity control.

3. Molecular Signaling in Response to Cues



- **lncRNAs act as signals, activating transcription factors** or pathways in response to developmental or environmental cues, modulating gene expression based on cell needs.

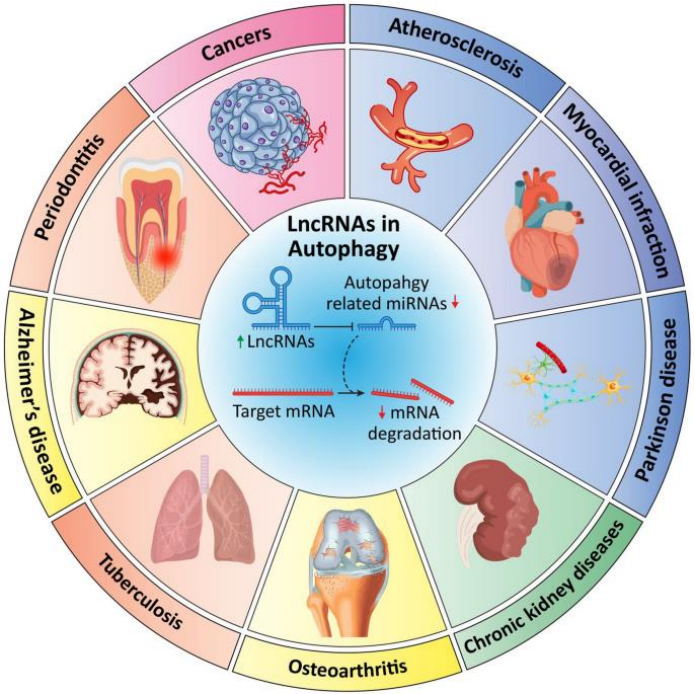
4. Gene Suppression Mechanisms



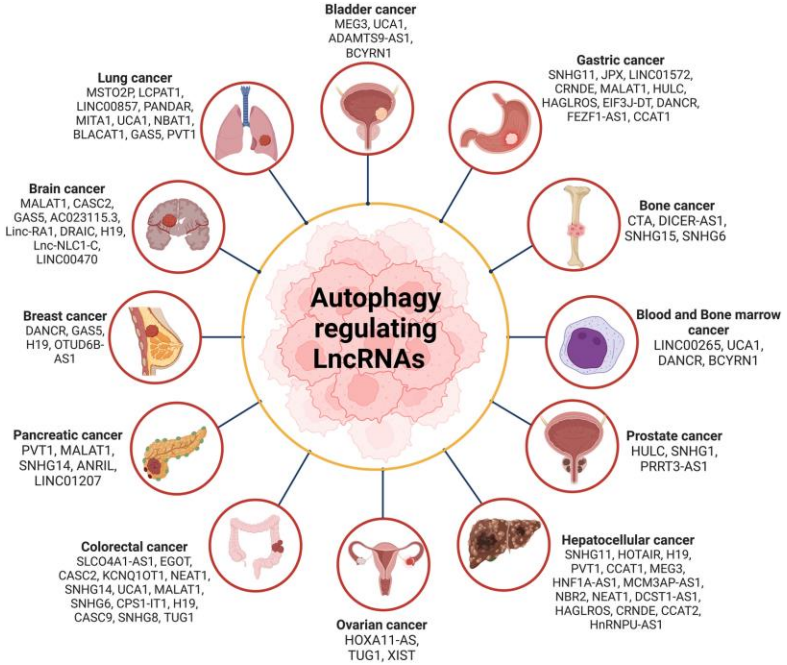
- **lncRNAs bind to transcription factors, RNA-binding proteins, or microRNAs**, preventing their action on target genes, effectively suppressing expression.

Selected lncRNAs are known to regulate various diseases & cancers

lncRNAs in various diseases



lncRNAs in multiple cancers



Many lncRNAs still remain functionally uncharacterized.

Images from Kumar *et al., Journal of Biomedical Science* (2024)

FANTOM: Functional Annotation of Mammalian Genomes

Worldwide Genomic Consortium led by RIKEN since 2000

Annotation of 60,770 full-length mouse cDNAs

Okazaki *et al.*
Nature 420,(2002)

©Nature 2002

Transcriptional regulatory network

Suzuki *et al.*
Nat. Gen. 41,(2009)

©Nature 2009

The atlas of promoters & Enhancers

Forrest *et al.*
Nature 507,(2014)

©Nature 2014

FANTOM 1 Mouse full-length cDNA

FANTOM 2 cDNA Clone bank Libraries

FANTOM 3 Promoter Analysis Database

FANTOM 4 Basin Network analysis

FANTOM 5 Atlas of

FANTOM 6 lncRNAs

Developed functional gene annot.

Kawai *et al.*
Nature 409,(2001)

©Nature 2001

Discovery of new transcriptional landscape

Carninci *et al.*
Science 309,(2005)

©AAAS 2005

Atlas of long non-coding RNAs

Hon, Ramilowski *et al.*
Nature 543,(2017)

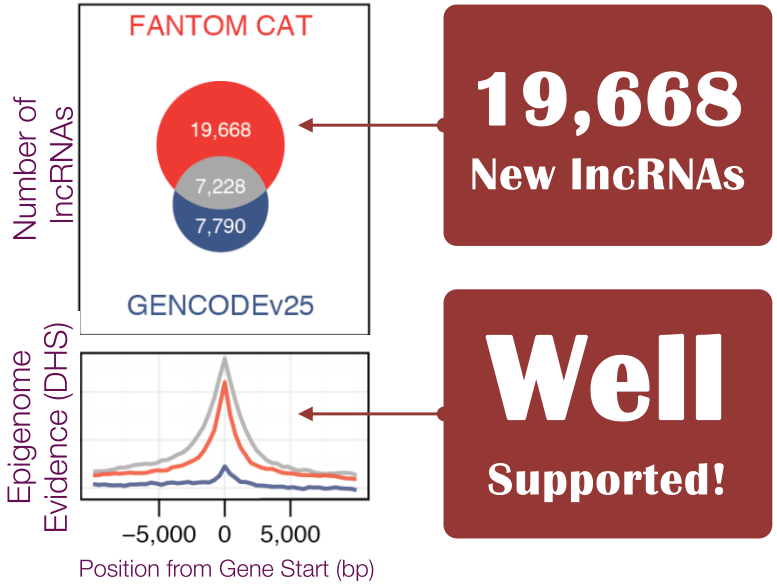
©Nature 2017

Selected landmark papers

FANTOM Consortium 2017 Summer Meeting:
"Cracking mysteries of human lncRNAs"

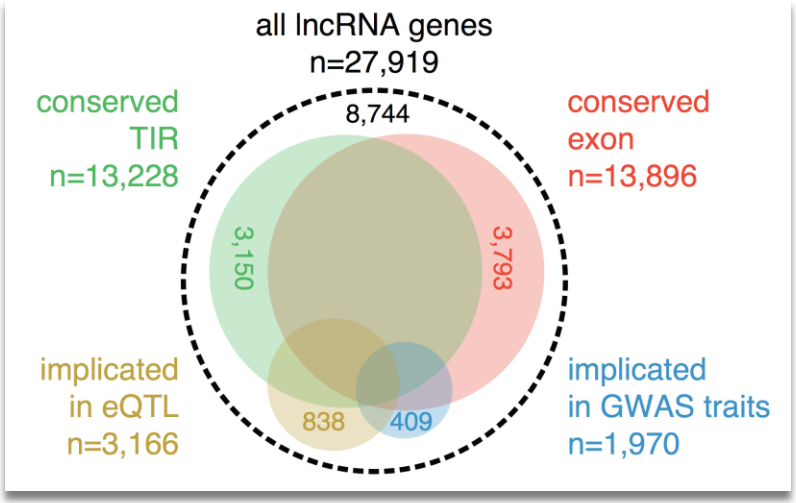
FANTOM 5: Computational Atlas of lncRNA Functions (2017)

FANTOM CAT (CAGE Associate Transcriptome) of 27,919 human lncRNAs:
Expression profiles (10,000+) + Epigenome Data + Annotations



19,668
New lncRNAs

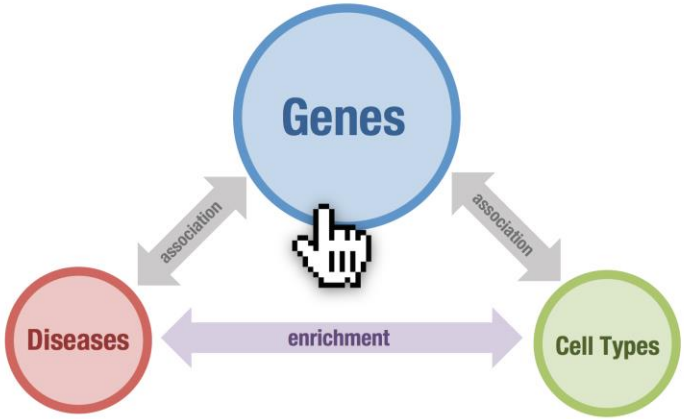
Well
Supported!



19,175 (67%) lncRNAs implicated in a variety of functional traits.

Hon, et al. ***Nature*** 543, 2017

Online Interactive Resources
http://fantom.gsc.riken.jp/cat/



Published: 01 March 2017

An atlas of human long non-coding RNAs with accurate 5′ ends

Chung-Chau Hon, Jordan A. Ramilowski, [...] Alistair R. R. Forrest ✉

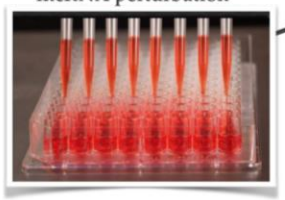
Nature **543**, 199–204(2017) | Cite this article

FANTOM 6: Experimental Functions of lncRNAs

FANTOM 6 Pilot: 285 lncRNA targets in Human Dermal Fibroblasts

Targeted 285 lncRNAs
(5 ASOs / lncRNA)
+ positive controls
+ negative controls (NCs)

Large-scale
lncRNA perturbation



Large-scale
Real time imaging

Measured Morphology & Growth for all lncRNA targets
(5,000 wells)

Large-scale
RNA Extraction & QPCR

Selected ASOs with KD eff >50%
340 ASOs/
154 lncRNAs

CAGE Production

Made ~1,000 CAGE libraries

CAGE sequencing

Assessed Molecular Functions
DEGs, Pathways,
TF-binding motifs

Bioinformatics

Automated Platform + Extensive Data Analysis

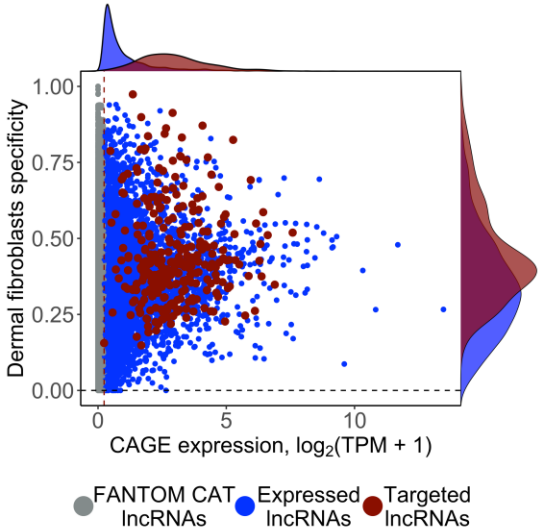
FANTOM 6: Unbiased selection of lncRNAs!

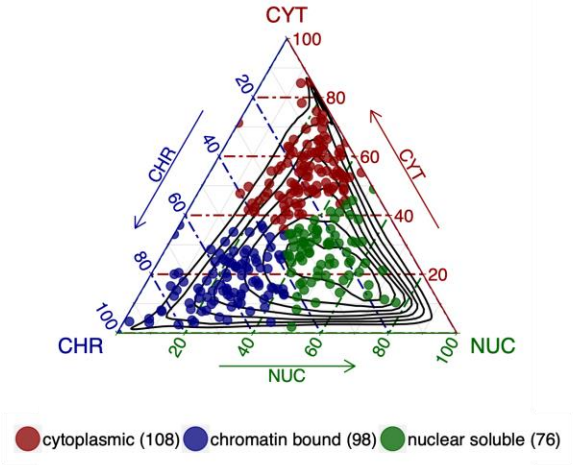
VARIABLE EXPRESSION LEVELS

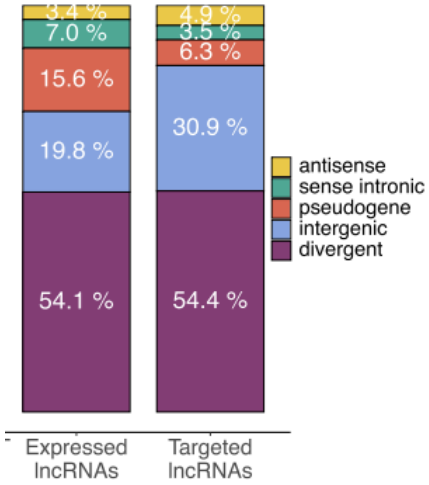
LOW+HIGH EXPRESSION

ALL CELLULAR LOCALIZATIONS ALL CLASSES OF LNCRNAS

1/3 NUC+1/3 CHROM BOUND+1/3 CYT







Ramilowski, Yip, et al. **Genome Res**. 30:1060, 2020

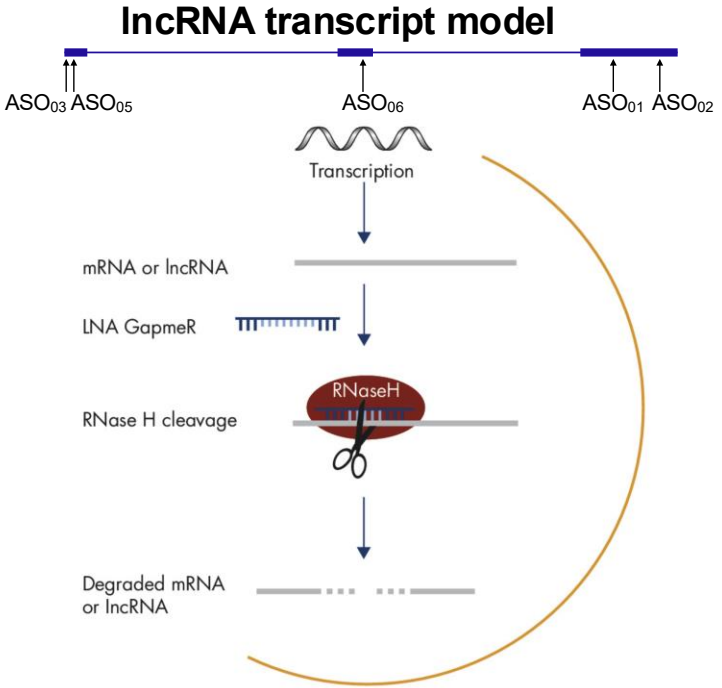
FANTOM 6: *Loss-of-Functions Experiments*

If lncRNA KD caused the phenotype/gene expressions change assign the "function" to the lncRNA (e.g. Growth modification, cell-cycle)

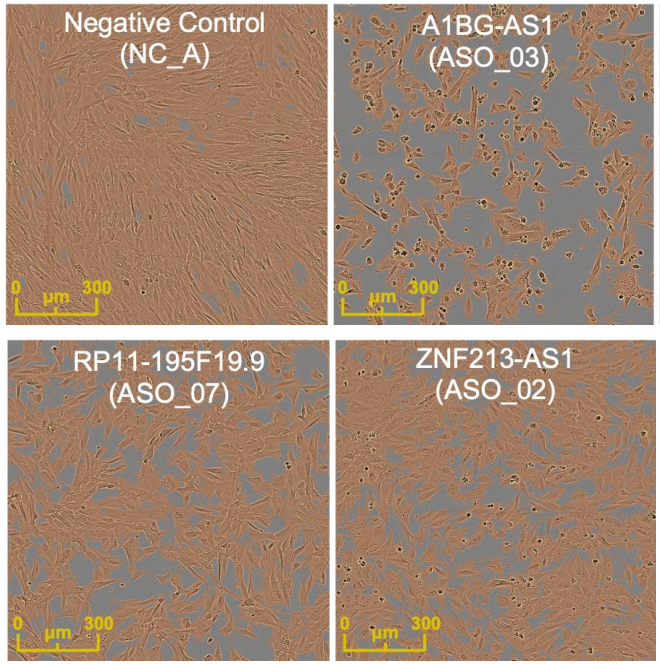
1. **Negative Control cells**: measure a selected cellular phenotype (e.g. growth) and gene expression values
2. **KD cells**: knock-down each lncRNA and measure the phenotype/gene expression values again
3. **Compare** the changes between **KD and Negative Control cells**

Antisense Oligo (ASO) knockdown mechanism:

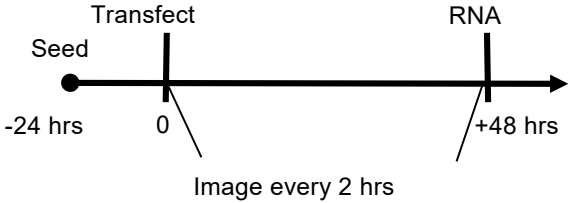
- ASO is ~12-18 nt long DNA sequence
- binds to a selected RNA transcripts



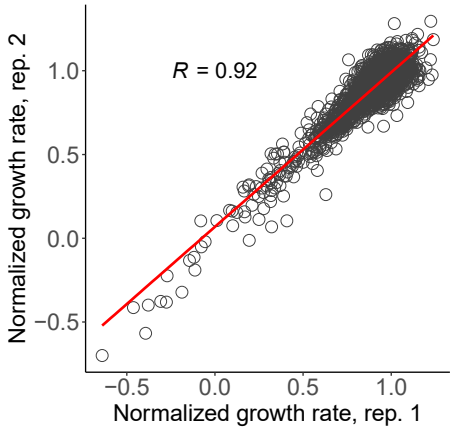
15-20% of lncRNAs affect growth!



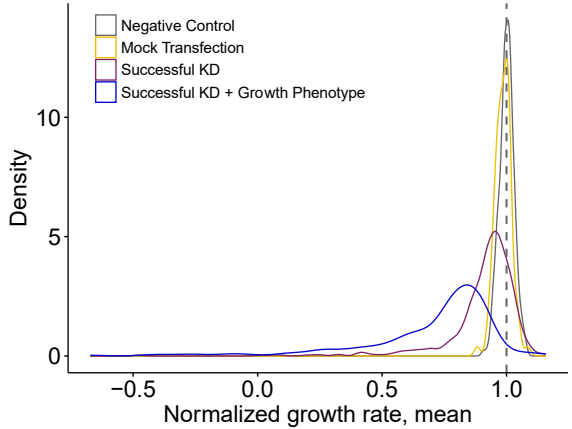
Incucyte Images



Incucyte platform was used to measure morphology changes of NCs and KD cells.



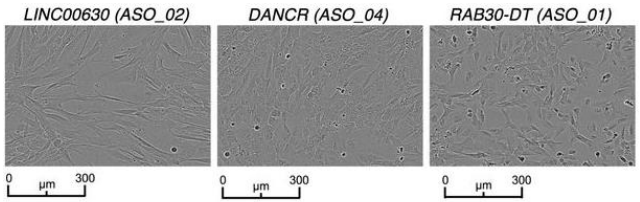
Normalized growth rate correlates well across reps (each point is NC or KD cell)



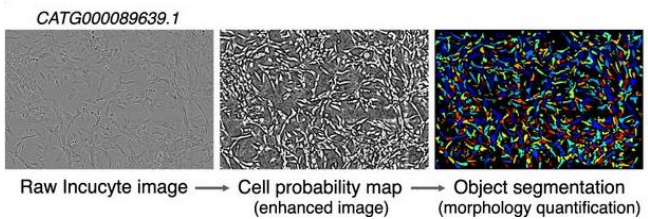
~25% of ASOs with KD_eff >50% affected cell growth.
(each small bar is NC or KD cell)

~30% lncRNAs regulate Cell Growth & Morphology

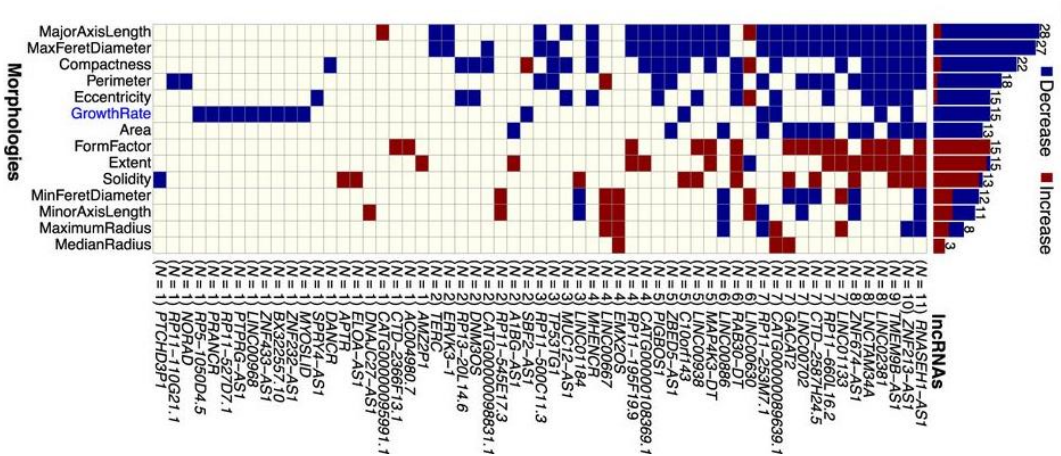
1. Distinct cell morphologies changes
(example: three selected lncRNAs)



2. Morphology imaging processing using AI
(example: one novel lncRNA target)



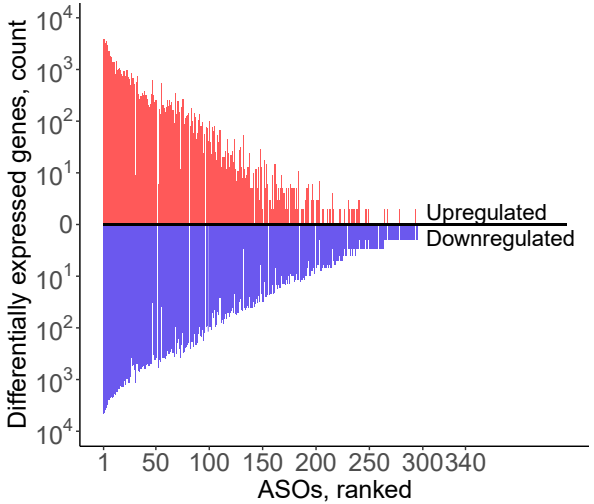
3. lncRNAs can regulate multiple morphologies
(all lncRNA in HDF data)

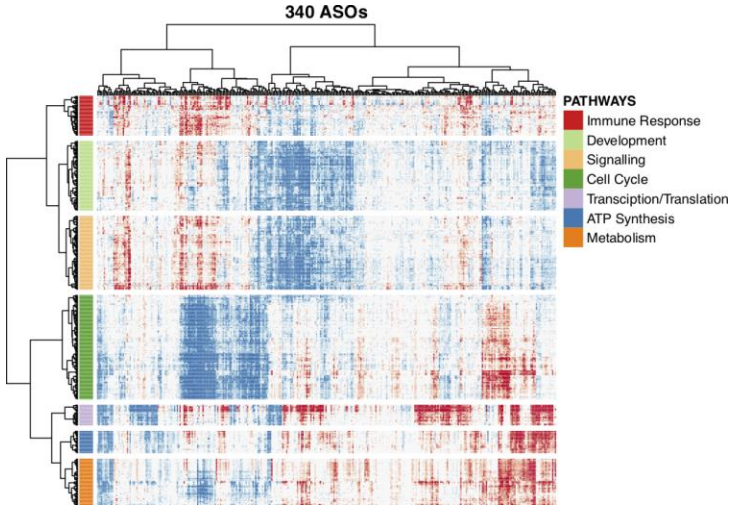


Ramilowski, Yip, et al. **Genome Res**. 30:1060, 2020

lncRNAs show diverse molecular signatures of functions

Molecular functions of **lncRNAs, were inferred by comparing transcriptome after each ASO KD targeting one lncRNA (KD_eff > 50%) with the transcriptome of matching NCs.**



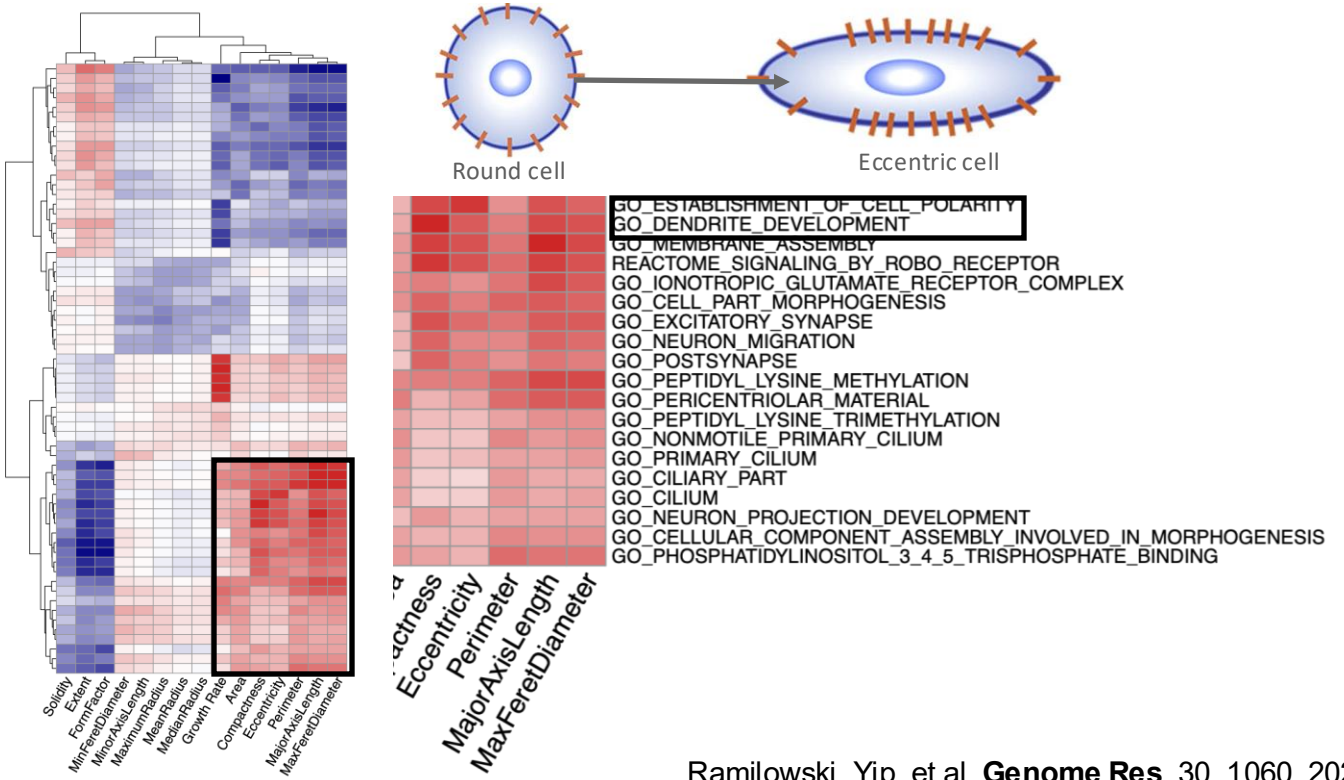


ASO KDs showed a wide ranged of DEGs (left) & dysregulated biological pathways (right). Overall ~20% lncRNAs show changes in biological function signatures.

Ramilowski, Yip, et al. **Genome Res**. 30, 1060, 2020

Molecular phenotype can predicting cellular phenotypes

Comparing other morphological changes (e.g., "*cell eccentricity*") was also positively correlated with relevant molecular pathways (e.g., "*establishment of cell polarity*", "*dendrite development*").



Ramilowski, Yip, et al. **Genome Res**. 30, 1060, 2020

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Tsukasa Kouno	Shohei Noma	Shintaro Aoki	Akiko Minoda	Hideya Kawaji	**Jessica Severin**	Lusy Handoko	Machiko Kashiwagi
Joachim Luginbuehl	Naoko Suzuki	Hiroo Inaba	Lusy Handoko	Michihira Tagami	Kosuke Hashimoto	Jen-Chien Chang	Nobuyuki Takeda
Fernando Lopez	Fumi Hori	Michihira Tagami	Haruka Yabukami	Atushi Kondo	Marina Lizio	Kyoko Hamada	Teruaki Kitakura
Chinatsu Yamamoto		Chitose Takahashi	Masaaki Furuno	Fumi Hori	Bogumil Kaczkowski	Riccardo Borroni	Juha Kere
Youtaro Shibayama		Nozomi Moritsugu	Kazuhide Watanabe		Andrew Kwon		Tom Gingeras
Suzy Szumowski		Naoto Kondo	**Jen Chien**		**Saumya Agrawal**		

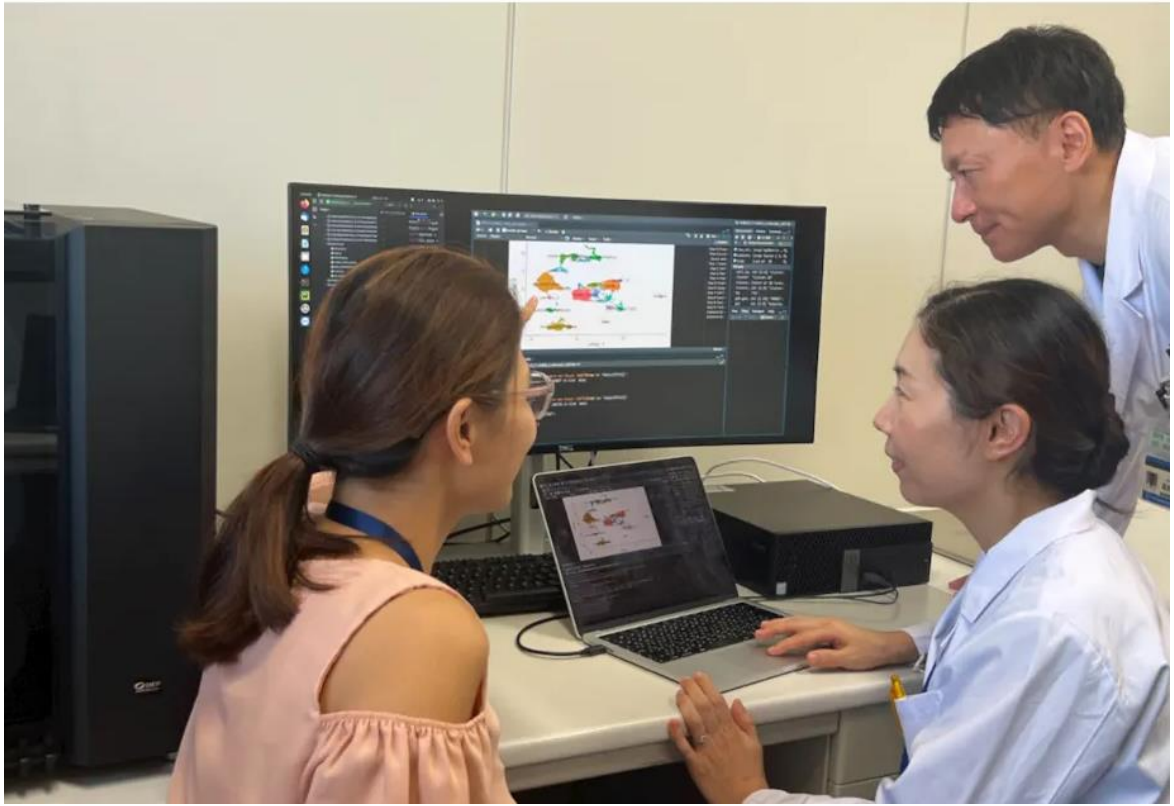
AND HUNDREDS OF RESEARCHERS FROM UNIVERSITIES ACROSS THE GLOBE.

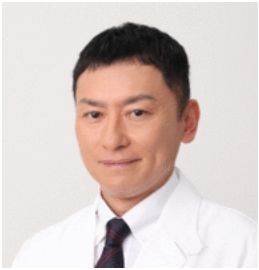
Our Current lncRNA Research

Funtion & mechanism of lncRNAs using multi-omics data

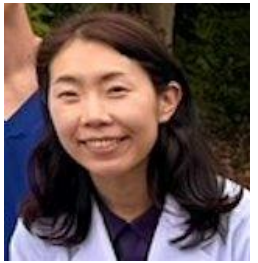
Note: *Our lab has only started in its current form just over one year ago. Most projects are collaborative and very much ongoing….*

Kidney cancer: collaboration with YCU Urology Department





Prof. Hisashi HASUMI
Yokohama City University
Hospital Urology Dept.



Sachi KAWAURA, M.D.
Ph.D. Student
(co-supervised)

lncRNA in Hereditary Leiomyomatosis Renal Cell Cancer

HLRCC: 遺伝性平滑筋腫症・腎細胞癌症候群

- Autosomal dominant disorder from **germline FH (Fumarate Hydratase) gene mutations**
- **FH loss** causes fumarate accumulation, promoting tumorigenesis and altering DNA and histones

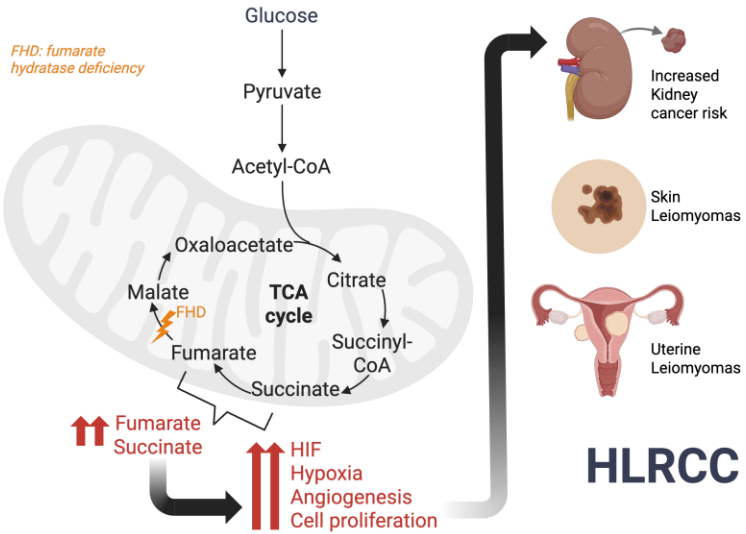
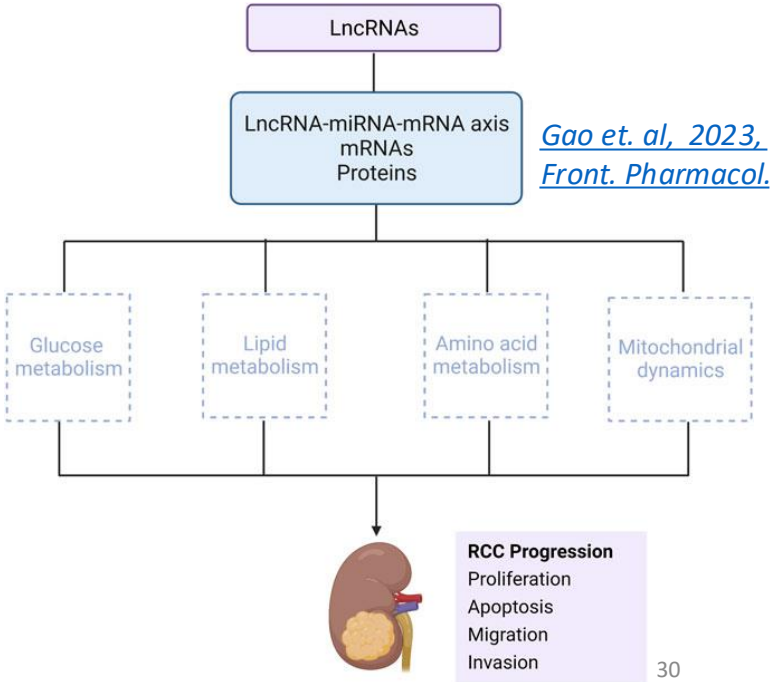


Image based on D'Indinosante et. al., 2025

lncRNAs were shown to be implicated in RCC. Are lncRNAs potentially involved in HLRCC?



Gao et. al, 2023, Front. Pharmacol.

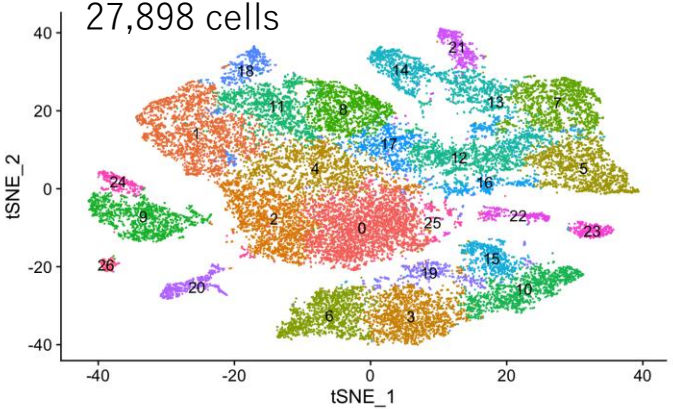
The study uses 4 scRNA samples and focuses on non-blood cells

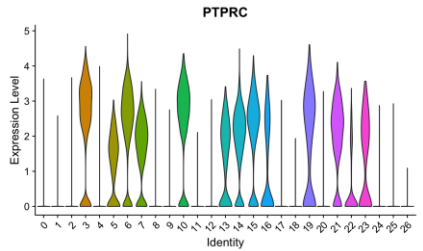
1. HLRCC tumor
2. HLRCC lymphnode
3. Normal Sample-1
4. Normal Sample-2

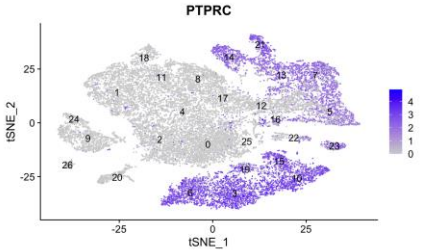
Seurat Analysis

- Data Integration
- Quality Control
- Clustering & Annot

24,414 genes
27,898 cells

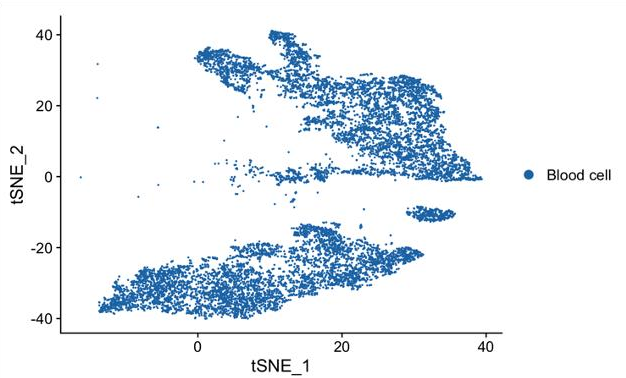






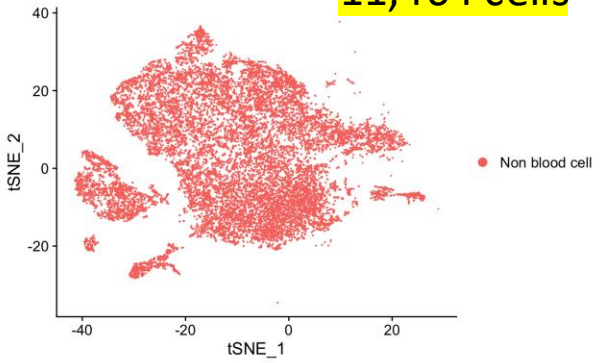
Divided to non-blood cell
and blood cell using PTPRC (CD45)

Blood cell



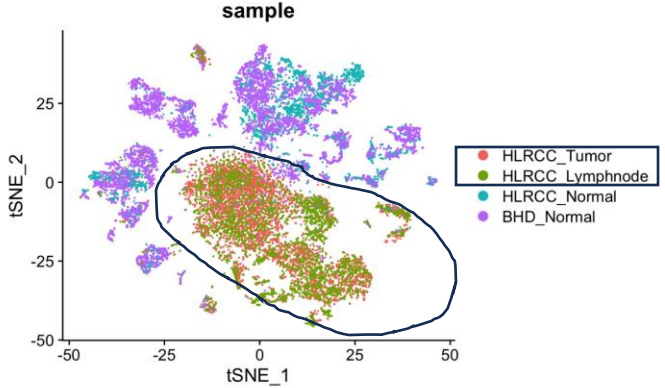
Non-blood cell

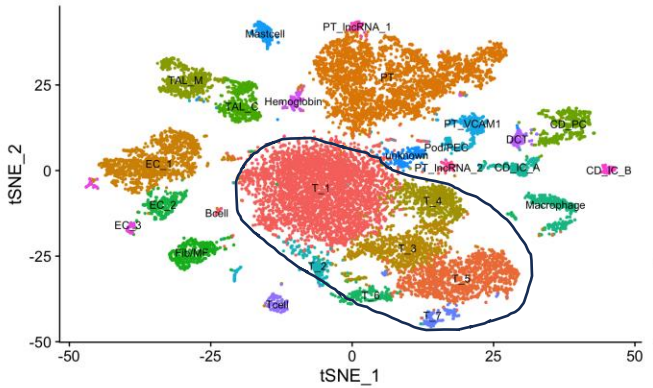
11,464 cells

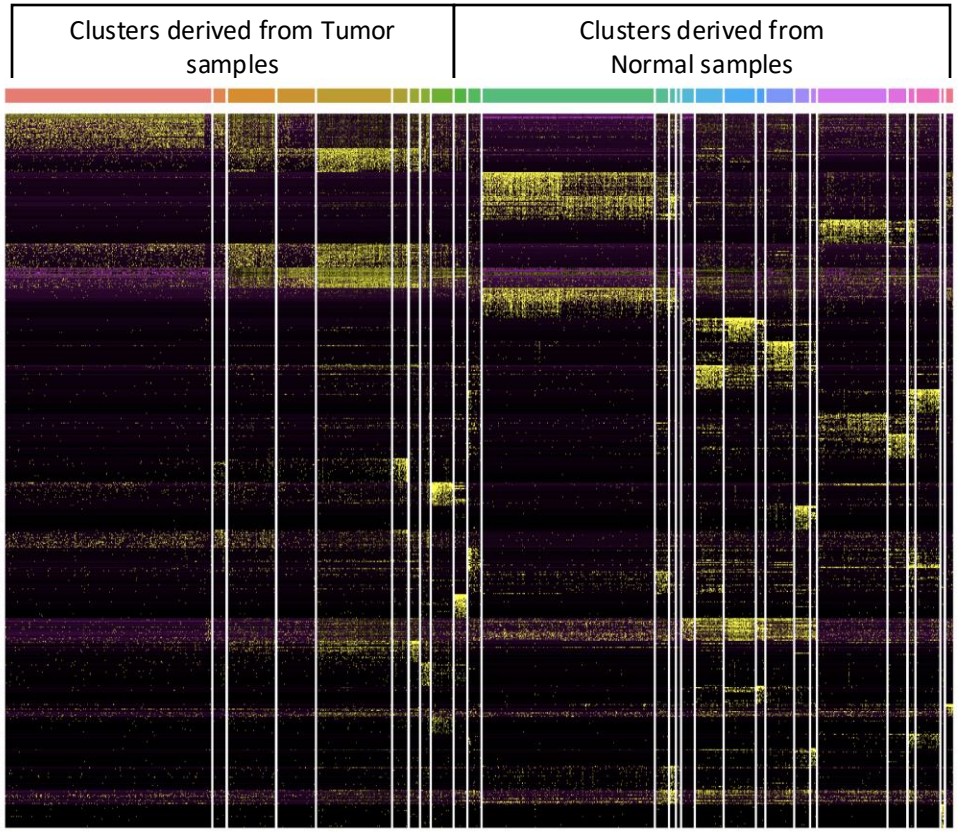


Unpublished data

There are 7 tumor clusters distinctive from normal samples







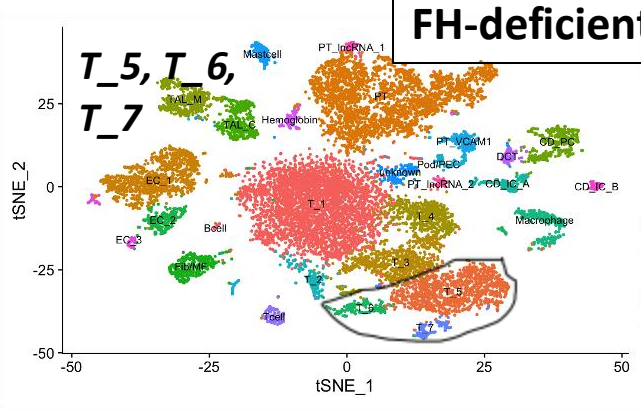
Unpublished data

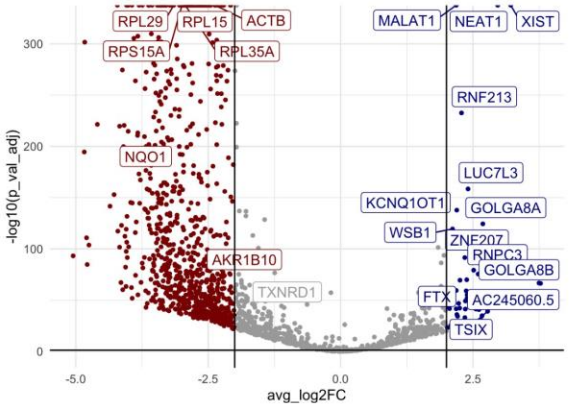
32

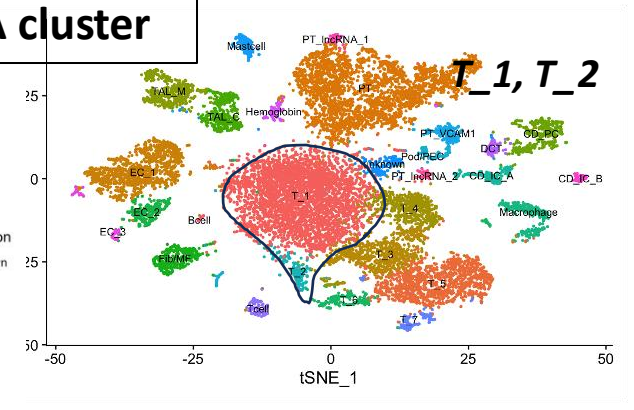
Tumor clusters contain the FH-deficient & lncRNA clusters

FH-deficient cluster

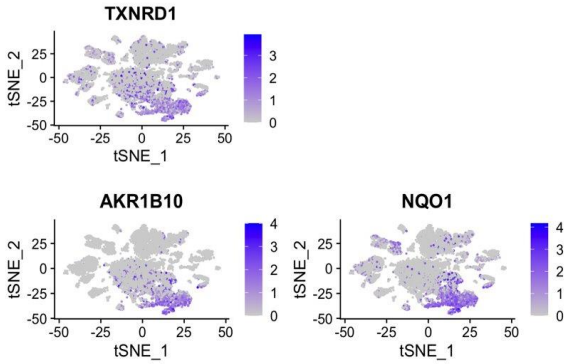
lncRNA cluster

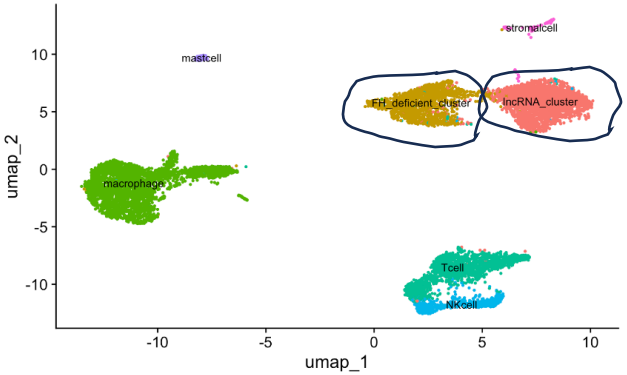




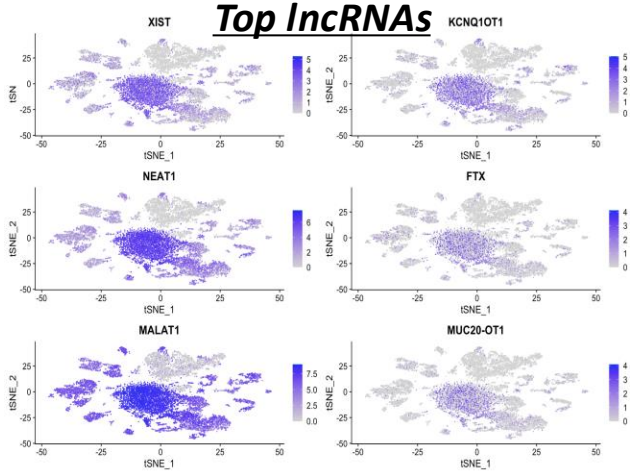


FH Target genes



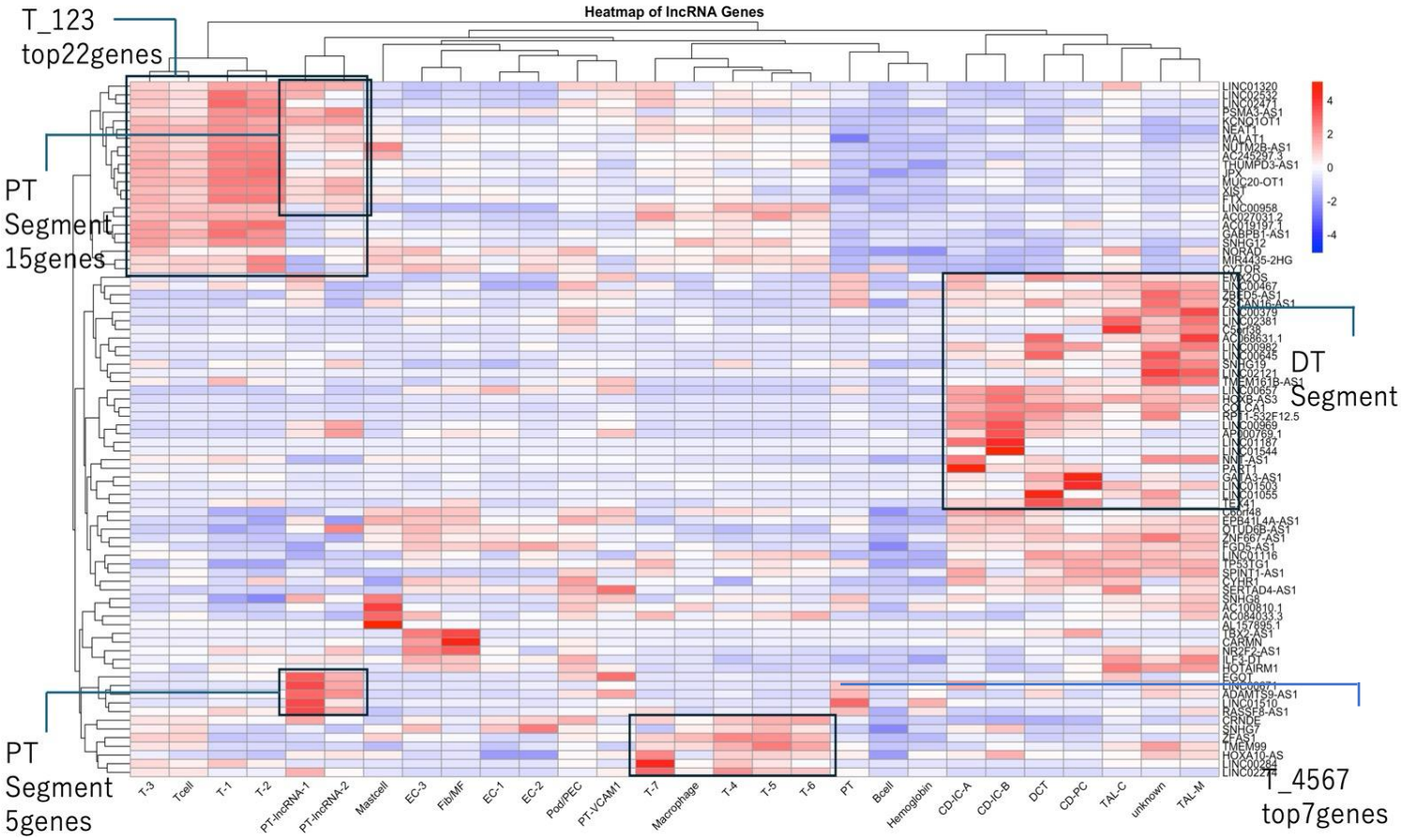


Top lncRNAs



Unpublished data

81 lncRNAs are identified - some are specific to each cell type



Unpublished data

On-going research

・Elucidate the role of the lncRNA cluster in HLRCC, such as whether it exhibits characteristics of cancer stem cells

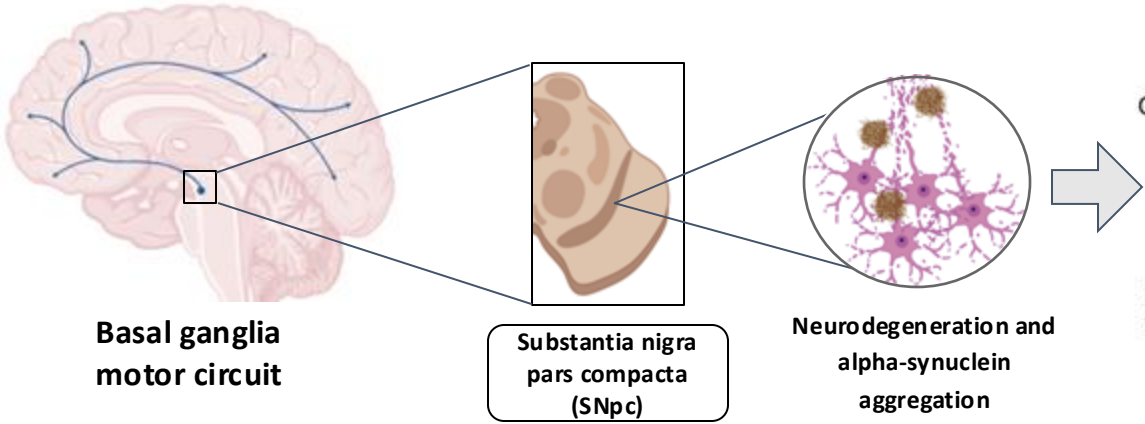
・Investigate whether the lncRNA cluster is specific to HLRCC by validating against bulk RNA-seq data from other renal cancers

- And more...

Parkinson's Disease affects millions worldwide

Parkinson's Disease Overview:

- ***Basal ganglia motor circuit*** (multiple brain regions) and the ***Substantia Nigra pars compacta (SNpc)*** are responsible for regulating *voluntary movement*
- PD starts with the **progressive degeneration of dopaminergic neurons** located **in SNpc** and by affecting voluntary movement the disease causes various symptoms
- **2nd most prevalent neurodegenerative disease** - over 10 million people live with PD
- **Costs of hundreds of billions of JPY**, driven by healthcare expenses, caregiving needs, and lost productivity



Common PD symptoms

DOI: 10.1016/S0140-6736(21)00218-X

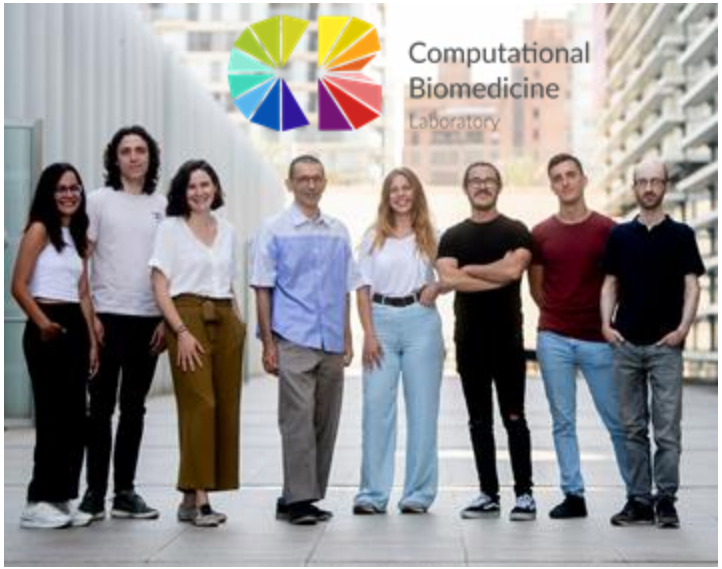
lncRNA characterization in Parkinson's Disease (PD)

Collaboration with Universities and Research Centers in Valencia, Spain

Fernando GORDILLO-GONZÁLEZ
PhD Student
YCU intern student Aug-Nov, 2025

Francisco GARCÍA-GARCÍA
Professor & Group Leader

CIPF VALENCIA BIOMEDICAL RESEARCH FOUNDATION
CENTRO DE INVESTIGACIÓN PRÍNCIPE FELIPE



Laboratory Website

GENERALITAT VALENCIANA
Conselleria d'Hisenda i Model Econòmic

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

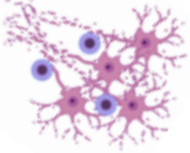
Project supported by a competitive Spanish CIBEFP grant (ref: 2024/125)

lncRNAs are known to play roles in PD and similar disorders

Abnormal protein aggregation

MALAT1, HOTAIR, SNHG14, UCA1

Autophagy and apoptosis

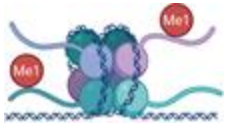


NEAT1, HAGLROS, NORAD, GAS5

Mitochondrial dysfunction

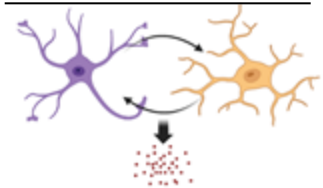
naPINK1, lncRNA-p21

Epigenetic dysregulation



MAPT-AS1

Neuroinflammation



lincRNA-p21, SNHG1

Pitfalls of the above studies:

- Evidence: expression correlation → no precise mechanism of action
- Few omics-related studies with a holistic view of the lncRNA landscape in PD
- No cell-type specificity for lncRNAs (bulk studies)

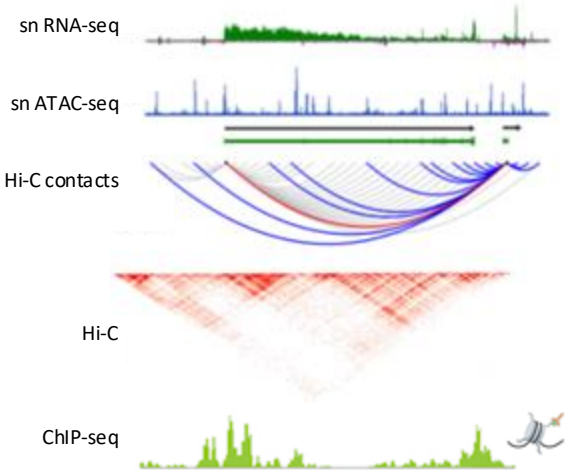
See more:10.1007/s11064-021-03230-3

Multi-omic characterization of lncRNAs roles in PD

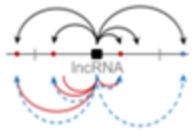
Reanalysis of multi-omics Public Data | 14 post-mortem SNpc brain samples (GSE148434) [*Lee AJ et al. 2023*]

We just began re-processing the above public multi-omics datasets with a focus on lncRNAs.

We aim to identify all lncRNAs involved in PD and to unravel their regulatory networks in the disease.



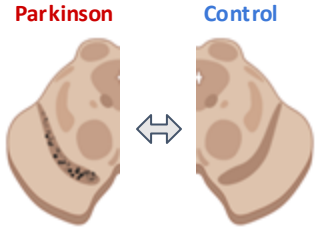
GENCODE 35.899 lncRNAs



lncRNA regulation

Cell type specificity

Regulatory networks



Contextualization in Parkinson's disease

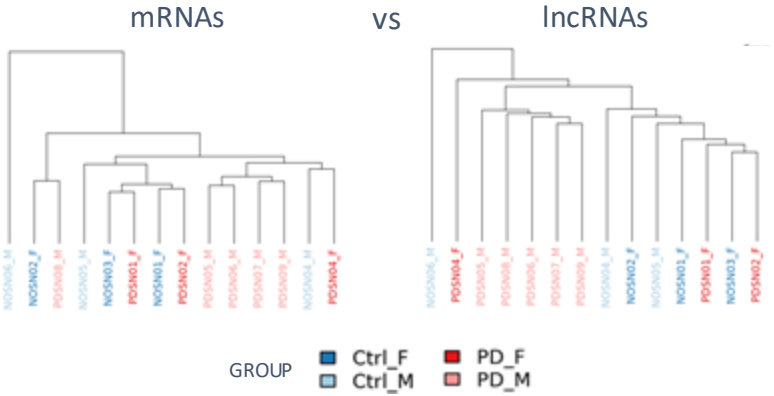
Preliminary results: bulk RNA-seq

Most comprehensive lncRNA identification in PD

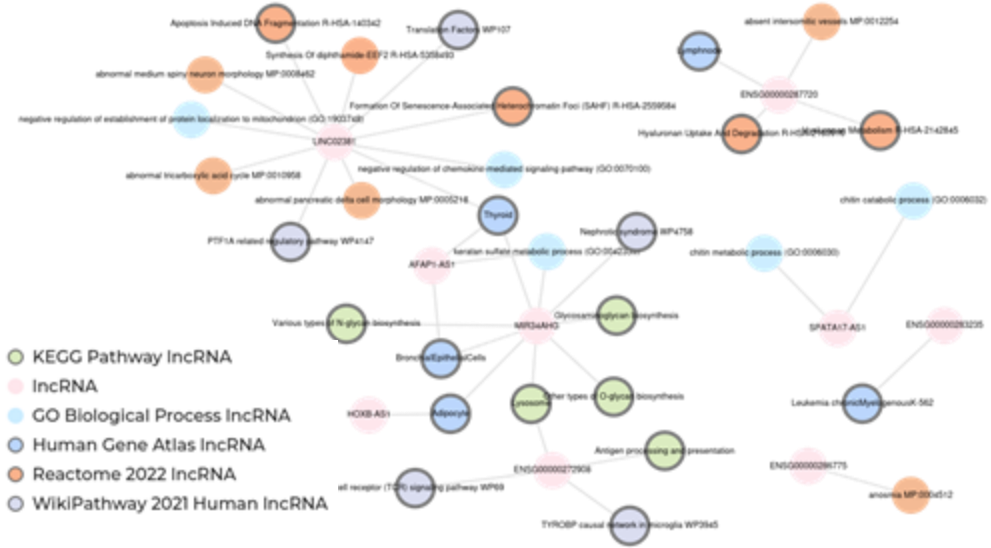
Lee AJ, et al.
44
lncRNAs
sufficiently expressed

Our analysis
12.325
lncRNAs
sufficiently expressed

Better clustering when using lncRNAs



Identified **48 lncRNAs differentially expressed**, related with important PD functions such as chemokine-mediated pathway (GO:0070100) or apoptosis DNA fragmentation (R-HSA-140342)

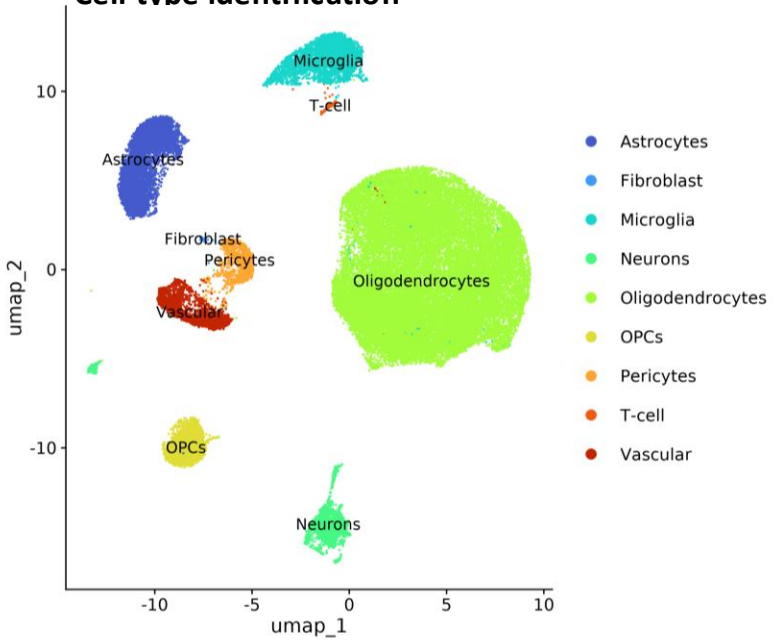


Unpublished data

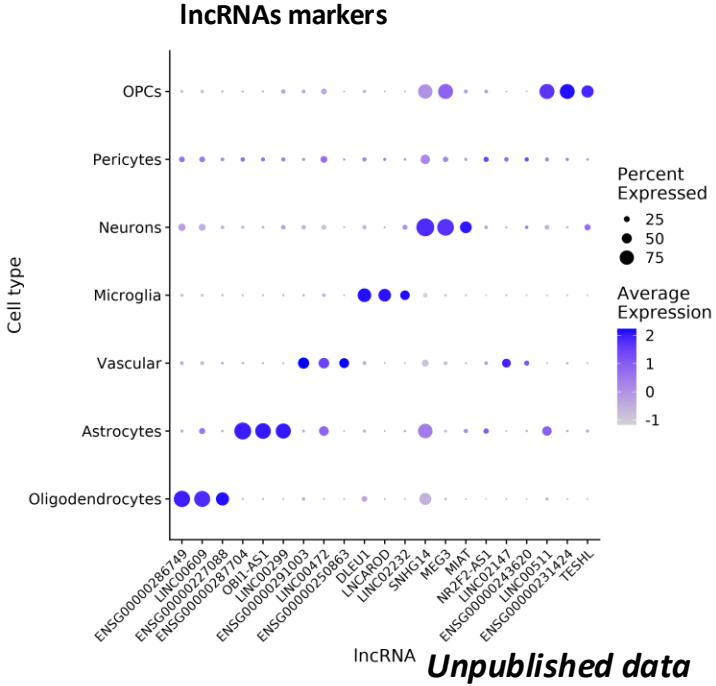
Preliminary results: single nuclei RNA-seq

- Reprocessing the single-cell data with an updated reference annotation, resulting in ~45,000 cells accurately classified into major brain cell types (left UMAP).
- lncRNAs with cell type–specific expression (right dotplot) were also identified, which may serve as novel markers for distinguishing cell types and subtypes.

Cell type identification



lncRNAs markers



Unpublished data

lncRNAs in Immune Responses

Prof. Daisuke Kurotaki

Kumamoto University
JAPAN

Team Leader Chung Chau Hon

Dr. Ramzan Umarov

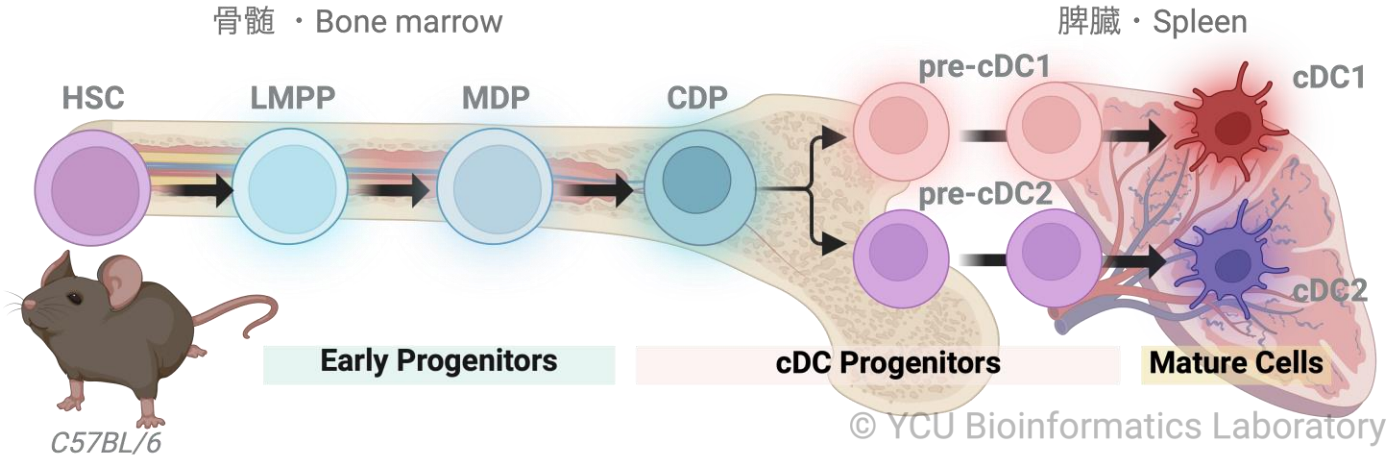
RIKEN IMS
JAPAN

Unpublished data; **KAKENHI Grant-in-Aid for Scientific Research (B) Project**

Dendritic Cells: Function & Differentiation

DCs: are professional Antigen Presenting Cells (APCs) priming regulatory and cytotoxic T-cells to orchestrate a variety of adaptive immune responses upon infection and in cancers.

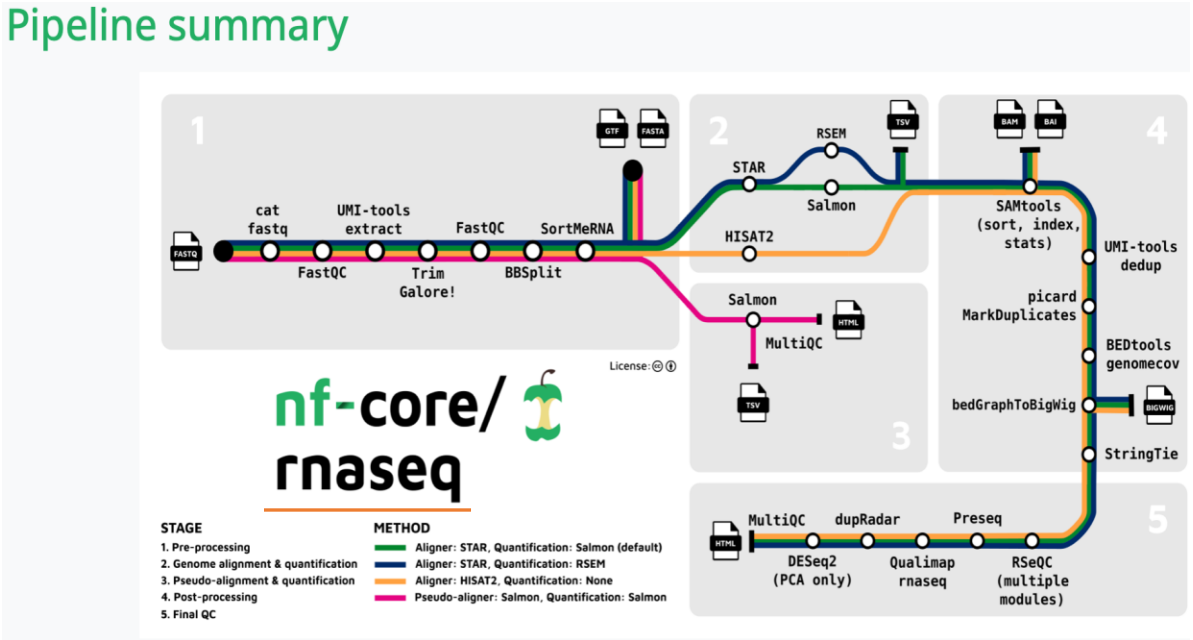
DCs differentiate mainly in bone marrow and mature in spleen in an IRF8-dependent manner.



1. **LMPP**: Lymphoid-Myeloid Primed Progenitors
2. **MDP:** Monocyte Dendritic Cell Progenitors
3. **CDP:** Common Dendritic Cell Progenitors
4. **pre-cDC1/pre-cDC2:** pre-Dendritic Cells
5. **cDC1/cDC2:** classical Dendritic Cells

lncRNA & enhancer atlas in cDC1 differentiation and responses

***de-novo* assembly:** applying public & own bioinformatics pipelines to hundreds of RNA-seq in-vivo myeloid cell and progenitor libraries we found ~6,000 novel lncRNAs in cDC differentiation time-course.

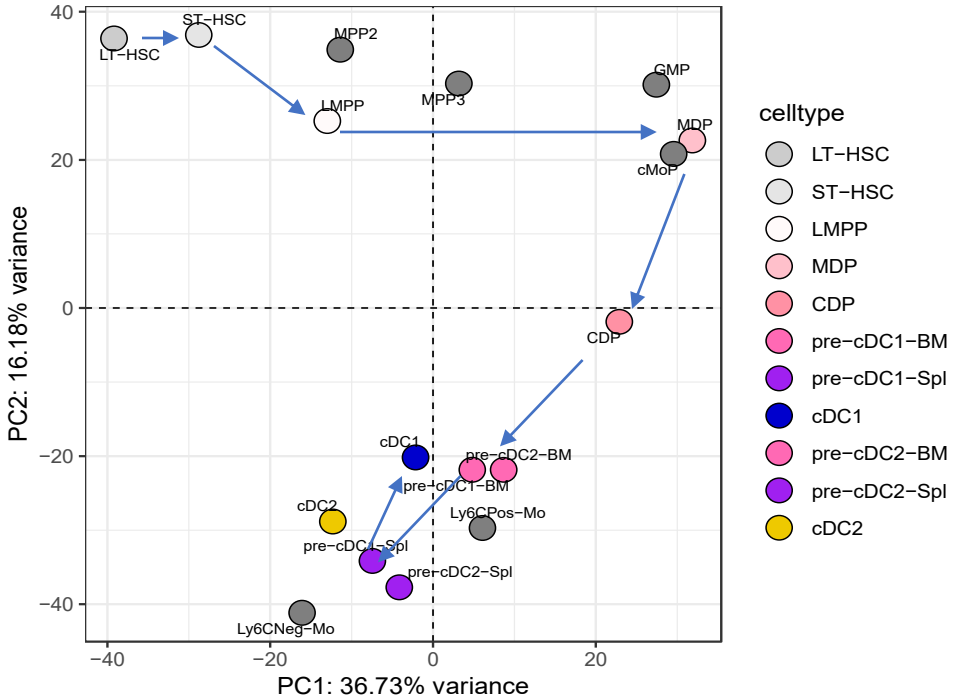


https://nf-co.re

Serverx2: 4TB RAM/240CPU cores

We found thousands of lncRNA specific to DC differentiation

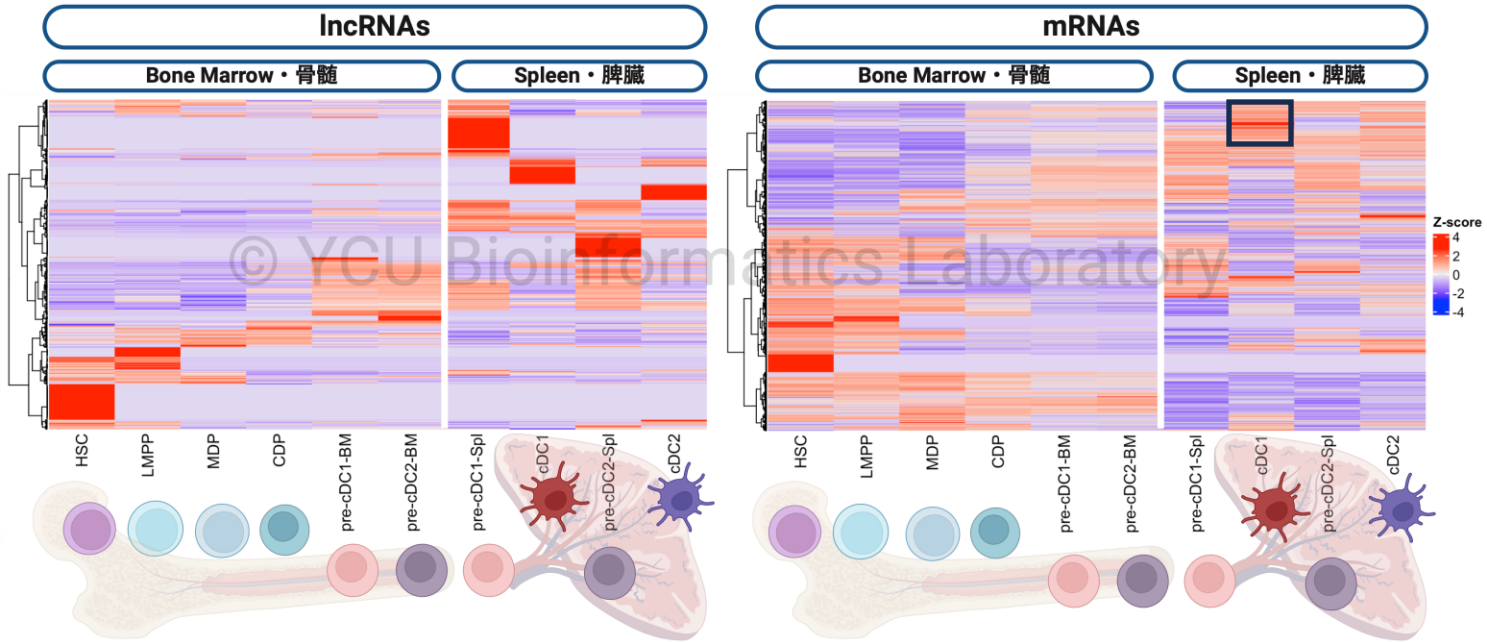
***de-novo* assembly:** applying nf-core & own bioinformatics pipelines to RNA-seq in-vivo data; We also found ~6,000 novel lncRNAs in cDC differentiation time-course.



Unpublished data

lncRNAs are much more cell type specific than mRNAs

Plotting expression scaled across cell types (Z-score) for **lncRNA (left) & mRNAs (right) across differentiation data reveals much more restricted patterns of lncRNA expression.**

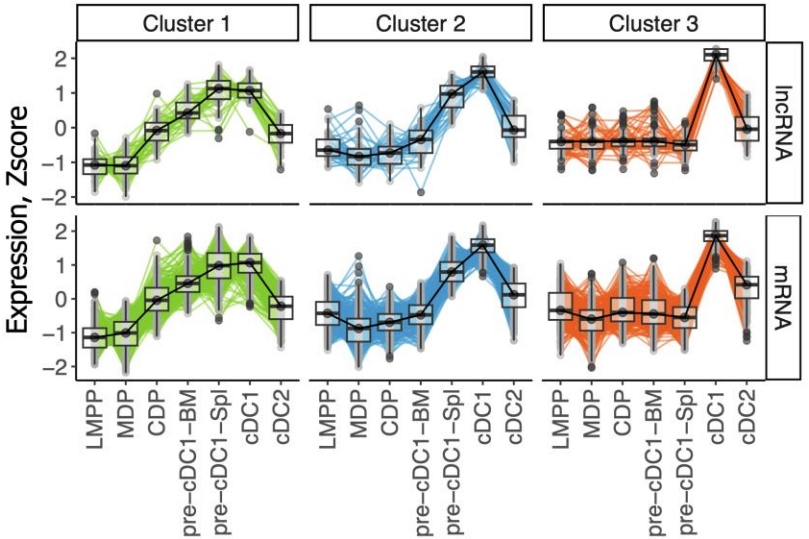


Let's focus on DC1 specific mRNAs.

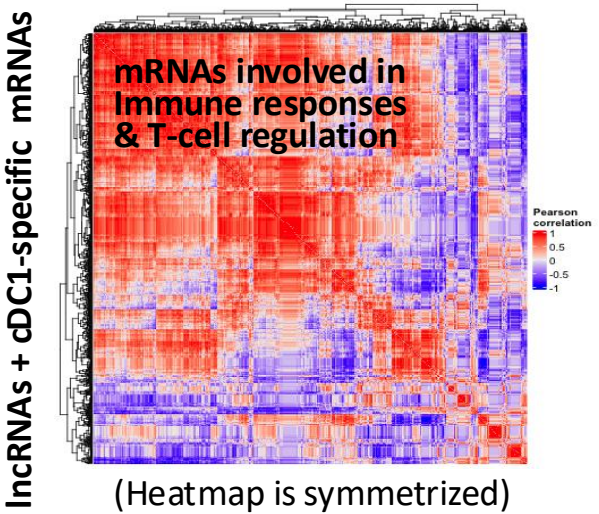
Unpublished data

cDC1-specific mRNAs & lncRNAs form a co-regulatory network

Using Hi-C data we identified lncRNA in proximity of cDC1-specific mRNAs.



lncRNAs + cDC1-specific mRNAs



(Heatmap is symmetrized)

Many **cDC1-specific lncRNA & mRNAs form a highly-correlated expression network** in cDC1 differentiation. **Usually, such *quilt by association* suggests that genes have commonly regulated.**

Unpublished data

Ongoing efforts

- Define functional roles of lncRNAs co-expressed with mRNAs during **cDC1 differentiation**
- Characterize immune enhancer–derived lncRNAs (e-lncRNAs) and their regulatory mechanisms
- Predict and classify lncRNA motifs and secondary structures to infer functional domains

- Dissect lncRNA dynamics in **emergency myelopoiesis** using 5’ scRNA-seq – generating data for IL-12–infected mice (bone marrow & spleen; D0, D1, D3, D7).

Why lncRNAs Are Good Drug Targets and Why Pharma Cares

Biological rationale

- Cell- and disease-specific expression → precision & safety
- Regulatory hubs (chromatin, transcription, RNA processing)
- Often nuclear → ASO-accessible
- Allele / isoform specificity feasible

Therapeutic advantages

- No enzymatic active site required
- ASOs / siRNAs enable direct RNA degradation
- Perturbation can mimic genetic loss-of-function

Strategic opportunity

- Large, largely untapped target space
- Strong potential in cancer, immune, and rare diseases

Industry signal: real money on lncRNAs

Pharma	Investment $$$	Disease Targets
Eli lily with Haya Therapeutics	**$1 billion**	Obesity and related metabolic conditions
Bayer with NextRNA Therapuetics	**$547 million**	Cancer

Among others
and many more in
the non-coding space...