Lec 4 Gastrulation of mamallian

Fgf4 partial expression may prestage the fate of Epiblast cell before the other expression markers

Grb2 downstream of Fgf4. Fgf4 mutant epiblast cells can not transition to later stage.

Oct4 and Nanog Pluripotency TFs Nanog goes down when cell goes into later (prime) stage

NANOG and GATA6 mutual inhibit each other

EPI: FGF4 NANOG

PrE: FGF4 GATA6 (More receptor, more signaling, less negative feedback loop, GATA6 is activated)

In future: combine ESC,TSC and Xen cells to reconstitute embryo formation

FGF Inhibitor prevent PrE, promote EPI. generate a lot of naive ES cells

TS Cell: need FGF4 (promote proliferation) + TGF beta

MEF-CM makes TGF beta. Feeder medium (MEF)

XEN Cells: primitive endorderm.

single factor can convert ES cell to TSC and XEN cell

activin and Fgf4 will differentiate ESC to EpilCs.

ESC can not differenbtiate to germcell without going through EpiLCs

NANOG high in FGF4 mutant (bag of ES cells)

GASTRULATION
Important the survival of organism.
Formation of AP axis is important for the process of gastrulation. So it happens between Implantation and Gastrulation
T-box TF : a marker of primitive streak(posteria) Hesx1 anterior
Distal is later converted to anterior
Nodal and Wnt inhibitors express in anterior
nodal, wnt and BMP4 are important for primitive streak formation.

Gastrulation (continued)

Brachyury marker of primitive streak

Wnt 3 important for streak formation. KO BMP4 not gastrulate

loss of function is the essence test.

fgf recptor single no phenotype double gives phenotype

Brachyury mutaant does not affect gastrulation.

beauty of single cell transcriptomic is to look for unknown genes important for gastrulation.

unbiased and give gene information never known before.

Two kinds of Epigenetics: Imprinting and X-Inactivation

Dosage compensation (X-Inactivation)

Tsix express on X that escapes inactivation(repress Xist)

Lecture on TGF Beta

TGF Beta tumor suppressor or pro-metastatic?

What is the pathway that can be interpreted so many ways?

Hypothesis: TGF Beta binds to Type II, then recruits type I. Therefore the Hybrid has labeled Type I receptor in the blot.

Biochemical studies find out Type II phosphorylates Type I, type I then phosphoryltes downstream factors

ligand is cleaved and wrapped by ECM ———- Latent ligand

ligand is released from latent, bind to receptor transphosphorylate , smad activate, into nucleus.

CDK8/9 phosphorylation (recruit transcriptional cofactor YAP) prime for third (GSK3) phosphorylation, later for degradation.

tgf BETA AND bmP recurit different smad binding partners, activate different transcriptional program

partner transcriptional factor is context dependant (In addition, the epigenetic nature of target genes)

Tgf Beta important for immune cell differentiation, Tgf Beta plays a role in inhibiting immune function.

Role of TGF Beta in Cancer

Duality: 1. Early on TGF Beta is tumor suppressive 2. Later on TGF-BETA is pro metastic

TGF Beta stop CDK2 (Cell Cycle arrest)

loss TGF Beta is not initiator, is enabler (RAS tumor)

Add TGF Beta to KP Mouse organoid, apoptosis and EMT. (With Smad4)

Tumor progression of TGF-BETA

TGF Beta cause cancer cells to go to quiescence, to evade immune cell attack.

metastasis is the confrontation of regenerative cancer stem cell and immune system (innate immune system)

Review Session
Mosaic: fate is determined. cell is determined to be this fate
Regulative: cell is determined by the context.
mosaic vs regulative: whether they require input to develop.
Instructive: concentration of this signal directs the development of this cell type. (direct instruction) Permissive: environment directs the fate. (not by a direct exogenous source)
Take ES Cells from Epiblast Embronic Stem cells. —- Manipulate (e.g. Crisper/Cas9) —- Insert into early blastocyst —-Make Chimera
nanog Gata6 first express then seperate into Epiblast and PrE
direct ES cells to other fates
all rossetes revolving together to cause embryo movement

HH Signaling

SHH regulates axon guidance in GLI independent manner

GLI3 mainly repressor / GLI2 mainly activator

The Organizer activity of notochord is from SHH

border can ensure gradiant of SHH, generating different cell types.

Gli2 ventral (activate), Gli3 (repressor) dorsal. Therefore generating different neuron tissue.

loss of Gli2 leads to loss of ventral cell types

mostly GLI3 Repressor and GLI2 Activator are only required for proper cell types in the spinal cord.

GLI2 and GLI3 requirements are different in different tissues(limb structure vs spinal cord)

Notch Signaling

Most mutants are found in sex link, because there is only one copy. The phenotype is more noticeable.

Notch loss one copy, has wing defects phenotypes —— Happloinsufficiency (low is genomes)

neurogenic mutants – too much nervous system. too many neural cells. —— Notch is one of the mutant, notch tells cell not to be neural cell.

Core Notch signaling pathway components:

DL/+ Wing thickning of veins

N/+ and Dl/+ double fix each other ——It will only makes sense they are receptor/ligand pair

N -/- always neural, cell autonomous (RECEPTOR) DI -/- Some cells Notch function, non autonomous (Ligand)

Notch signal cut the receptor