FUND

WEEK 6
cancer, stem cells, cell signaling pathways

HOW I SPENT MY BREAK

THIS WEEK
- AT Intro to Cancer Biology
- AT Cell Communication Signaling
- AT Gprotein Coupled Receptors
- AT PI3 kinase signaling
- AT Nuclear Receptors
- AT Stem Cells Developmental Biology

CANCER BIOLOGY

uncontrolled cell growth & proliferation
a clonal process

The 10 Hallmarks of Cancer

1. sustained prolonged signaling
2. evading growth suppressors
3. inducing angiogenesis
4. avoiding immune destruction
5. enabling replicative immortality
6. activating invasion + metastasis
7. genome instability
8. resisting cell death
9. deregulating cellular energetics
10. tumor promoting inflammation

STEM CELLS & CANCER: what's the connection?

stem cell model of cancer = cancer is a neoplastic transformation of a stem cell and/or progenitor. If the source of cancer resides in quiescent stem cell, chemo agents that selectively target rapidly dividing cells WON'T WORK
no 100% eradication

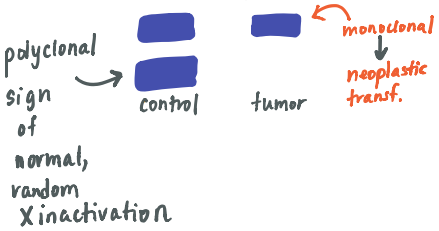
cancer is a clonal process = evidence of MONOCLONAL = identical genotype among pop'n
population of cells indicates one cell eenily out of control w/ cell prolif → likely neoplasm

Analysis of Clonality

sign that cancerous cell is growing OUT OF CONTROL

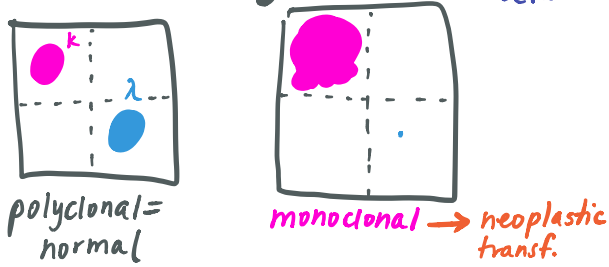
normally polyclonal

1) G6PD iso enzyme gel electrophoresis analysis



2) cytogenetics
look for somatic mutations, translocations
leukemic bone marrow: $5q^-$

3) κ or λ chain analysis in lymphoid progenitor cells



1 oncogenes

mutation, over expression, or translocation (amplification)
results in neophastic transformation
acts in a dominant — one mutated allele sufficient for phenotype

VS

2 tumor suppressor genes

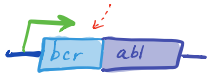
fxn: prevent cellular transformation and/or progression of established tumors (e.g. regulators of cell cycle)

2 = both copies must be inactivated in order for cancer to occur

OHSU

dx: CHRONIC MYELOGENOUS LEUKEMIA

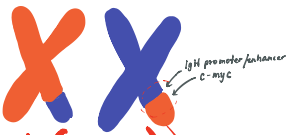
t(9;22)



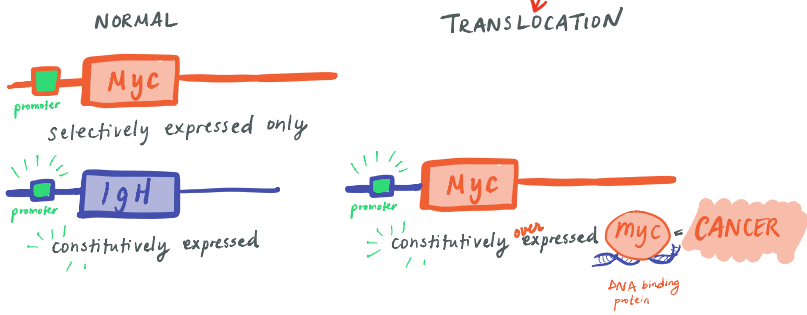
tx: tyrosine kinase inhibitors **IMATINIB** (competitively inhibits ATP aka Gleevec, a TKI. for binding site on fusion BCR/abl protein

dx: BURKITT'S LYMPHOMA

malignant lymphocyte cancer



t(8:14)



common human ONCOGENES

proto-onco gene	mutation type	cancers
RAS	pt mutations	broadly expressed! AML, ALL, multiple myeloma, lung cancer, colon cancer, bladder cancer, pancreatic cancer
BRAF	pt mutations, translocations	melanoma, colon cancer, thyroid cancer, lung cancer, prostate cancer
EGFR (nonsmokers, female, pts w/ chinese, Hong Kong, Indian, Filipinoh, Thai, Taiwanese, Viet ancestry)	pt mutations, translocations, insertions/deletions	lung cancer
KIT	pt mutations, translocations, insertions/deletions	GI stromal tumor, AML, germ cell tumors, melanoma, mast cell tumors
PIK3CA	pt mutations	breast cancer, colon cancer, endometrial cancer
ALK (younger, male, nonsmokers)	translocations	lung cancer
HER2	amplification, pt mutations	breast cancer, lung cancer, gastric cancer

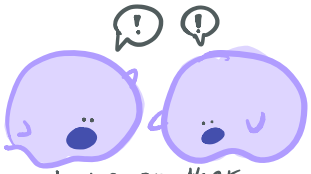
(normal gene before mutation = "proto-oncogene")

? 32 Y.O female comes in w/ shortness of breath, and coughing up blood. She has no history of smoking. She appears fatigued. The most likely genetic mutation belongs to which class of receptors?

a) GPCR
b) RTK
c) nuclear receptor
d) ligand-gated ion channel

? when designing drug targets what are some things to consider to make the most effective drug? Think about how to max
- specificity (how not to kill healthy cells)
- potency (where in pathway will be most consequential)

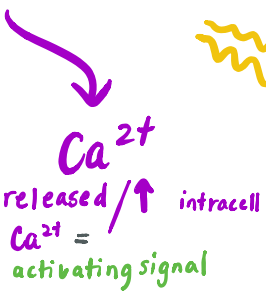
CELL-CELL SIGNALING

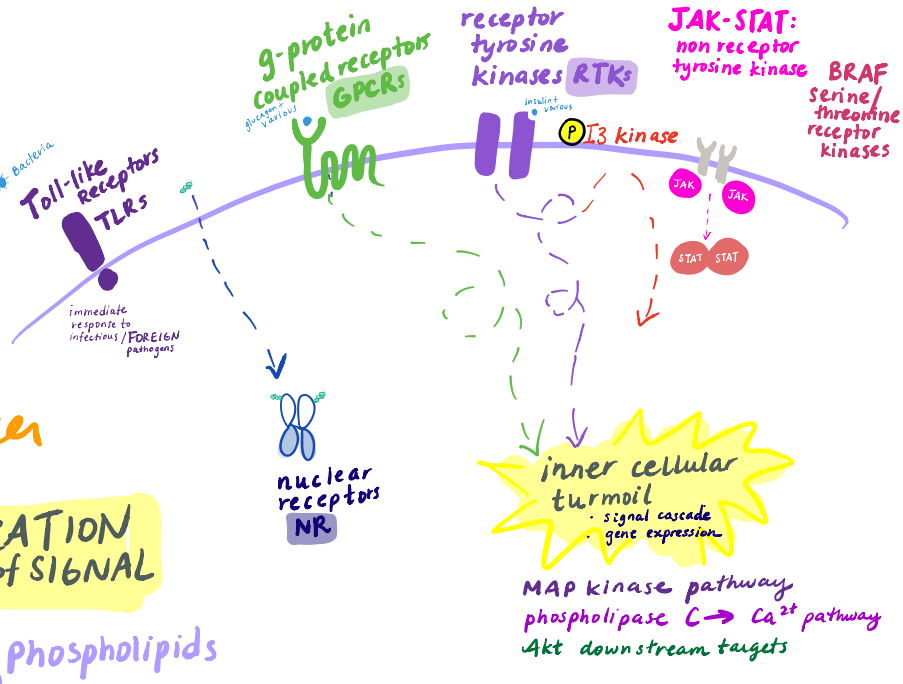


how cells communicate w/ one another

1. ligand-receptor binding
   INITIATION OF SIGNAL

2. downstream **2nd messenger SYSTEMS**
   PROPAGATION and/or AMPLIFICATION of SIGNAL

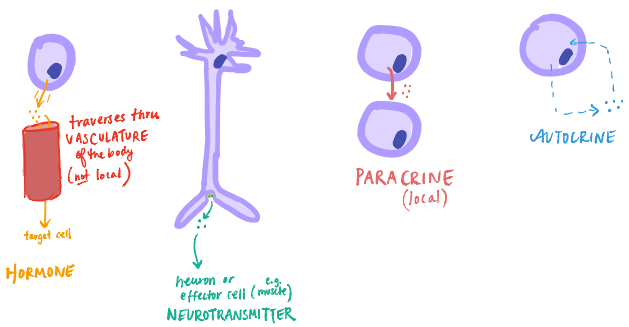




ligands

the thing that binds

FUNCTIONALLY classified



STRUCTURALLY CLASSIFIED

can be secreted (soluble) or membrane bound

- hormones
  - insulin
  - growth hormone
- growth factors
  - M-CSF
  - TGF-β
- cytokines
  - interleukins
  - interferons

- ephrins
- Notch ligands
- TNF ligands

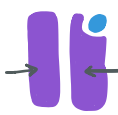
* release once cleaved

RECEPTOR

many classes have a defined (but complex) pathway
THIS CAN HELP US IDENTIFY DISTINCT, POTENT **drug therapies!**

RECEPTOR TYROSINE KINASES

EGF
IGF
PDGF
VEGF
HGF
FGF



1. ligand binding
2. dimerization & activated RTKs
3. autophosphorylation
4. recruitment of downstream signaling targets

G protein like Ras → Raf (effector) → MEK → MAPK

G PROTEIN-COUPLED Receptors

- ✓ 7-pass transmembrane receptor
- ✓ coupled to heterotrimeric G protein → ion channel regulation, PI3K pathway, gene expression regulation
- ✓ ligand = hormones, amines, light, Ca^{2+}, oderants
- ✓ extremely high yield in understanding cell-cell communication (>50% of drugs in development target GPCRs)

Gα: inherent GTP (active) to GDP (inactive) activity

classes of G_α subunits

- $G_{s\alpha}$ activates adenylyl cyclase + cAMP, PKA
- $G_{i\alpha}$ inhibits adenylyl cyclase; regulates ion channel by release of βγ
- $G_{q\alpha}$ activates phospholipase C (PLC) IP_3, DAG, ↑ Ca^{2+}, PKC
- $G_{12/13\alpha}$ activates rho GTPases → cytoskeleton remodeling, cancer
- G_t activates PDE rhodopsin/vision

Pathophysiology of some bacteria target G protein activity

dx: CHOLERA

bacterium: *Vibrio cholerae*

M.O.A: targets G_s subunit → to be locked in ON position

toxin from Vibrio causes ADP ribosylation of G_s (post-transl mod) → inhibited GTPase activity

constitutively ACTIVE $G_{s\alpha}$ subunit → sustained cAMP production

→ sustained opening of CFTR Cl^- channels

→ efflux of ions & water from GI tract

→ watery diarrhea

dx: WHOOPING COUGH (pertussis)

bacterium: *Bordetella pertussis*

M.O.A.: targets G_i subunit → to be locked in OFF position

toxin from Bordetella causes ADP ribosylation of G_i (post-transl mod) → inhibited GDP release

constitutively INACTIVE $G_{i\alpha}$ subunit → sustained cAMP production

cannot inactivate adenylyl cyclase nor open potassium channels

WAYS TO REGULATE ATTENUATE GPCR PATHWAY

1. RGS proteins speed up rate of GTP (active) to GDP (inactive) hydrolysis in Gα
2. GRK kinase phosphorylates GPCR, recruits arrestin binding → GPCR removed from P.M destined for degradation or recycling

NUCLEAR RECEPTORS

ligand = steroids ……> can traverse plasma membrane → regulate gene expression

relatively slower, long-lasting effect (vs. signaling cascade – ≠ fast & amplified!)

TYPE I

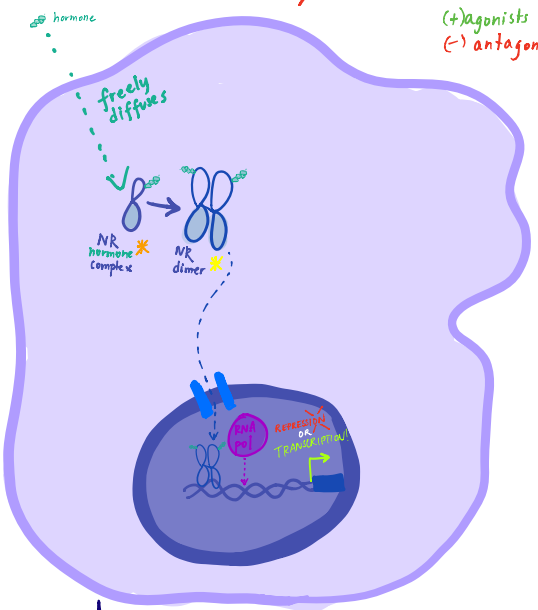
- estrogen receptor …… estradiol
  - sexual development, regulation of reproductive cycles, anti-inflamm, cognition & behavior
- gluco corticoid receptor …. cortisol
  - inhibit immune system, metabolic effects, development & cognition, fluid homeostasis
- mineral corticoid receptor .. aldosterone
  - salt & fluid (volume) homeostasis
- androgen receptor …… testosterone
  - sexual development, spermatogenesis, ↓ body fat, ↑ muscle mass, cognition & behavior
- progesterone receptor ….. progesterone
  - pregnancy, regulation of reproductive cycles

ligand binds to receptor in cytosol

- when ligand not bound, receptor complexes w/ Hsp90

causes ↑ incr or ↓ decr in gene expression

(+) agonists (dexamethasone)
(–) antagonist (tamoxifen)



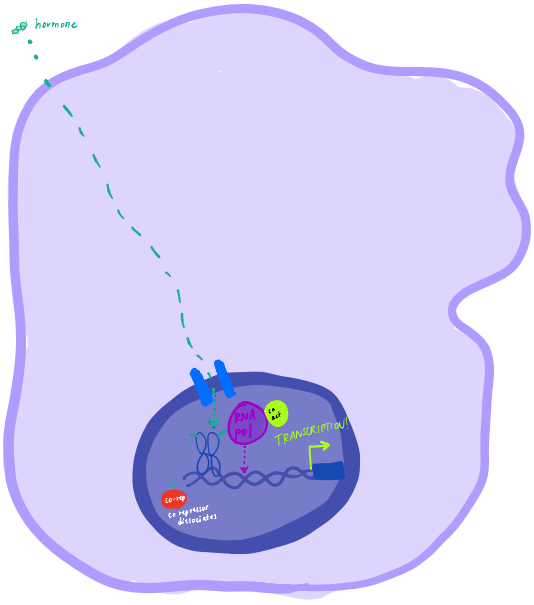
TYPE II

- thyroid hormone receptor …. T3 (active form) thyroid hormone
  - basal metabolic rate (metabolism), ↑ fat mobilization, ↑ fatty acid oxidation, ↑ glucose metabolism, ↑ insulin dependent glucose transport, ↑ glycogenolysis, ↑ gluconeogenesis
- (can be Type II) retinoid receptor …… retinoic acid (all-trans = ATRA)
  - Hox gene (development), growth (cancer), hematopoiesis
- vitamin D receptor …… 1,25 vitamin D
  - calcium homeostasis, phosphate homeostasis, bone remodeling, immune cell differentiation
- PPAR …… fatty acids, eicosanoids
  - lipid metabolism, glucose metabolism (insulin sensitivity)
- lipid sensors …… bile acids oxysterols
  - cholesterol homeostasis

ALWAYS and already bound to DNA, part of a complex that suppresses transcr. when ligand not bound
heterodimer

ligand binds to receptor in nucleus

causes ↑ incr in gene expression only



Background & Context

NUCLEAR RECEPTOR ligands

CORTISOL (produced in adrenal glands, the beanie of the kidneys) opposes actions of INSULIN

@ liver
- incr ↑ gluconeogenesis
- decr ↓ glucose uptake

@ skeletal muscle
- decr ↓ glucose uptake (↓ expression of GLUT4 transporter, proinsulin gene)
- decr ↓ protein intake
- incr ↑ proteolysis

@ adipose tissue
- incr ↑ lipolysis

insulin resistance

INSULIN
- ↓ decr gluconeogenesis
- ↑ incr glucose uptake
- ↑ incr glucose uptake
- ↑ incr protein intake
- decr ↓ proteolysis
- decr ↓ lipolysis

↑ PPARG — expression, activation

improved insulin signaling + sensitivity
- ↓ lipolysis
- ↓ free fatty acids
- ↓ TNF-α, IL-6, leptin
- ↑ adiponectin, GLUT4, fatty acid transport proteins

CUSHING'S vs ADDISON'S

CUSHING'S
cortisol (adrenal) excess

- moon facies
- buffalo hump
- weight gain
- slow wound healing
- thinning of hair on scalp
- thinskin, easy bruising
- muscle atrophy

ADDISON'S
cortisol (adrenal) insufficiency

- bronze pigmentation
- hypoglycemia
- postural hypotension
- weakness
- GI disturbances

→ **ADRENAL CRISIS**
- profound fatigue
- dehydration
- vascular collapse
- renal shutdown
- ↓ Na^+
- ↑ K^+ hyperkalemia

PI3 KINASE signaling pathway (PI3K)

leads to

mediating effects of insulin

- tumor cell survival
- cell proliferation
- invasiveness, cell migration
- tumor angiogenesis

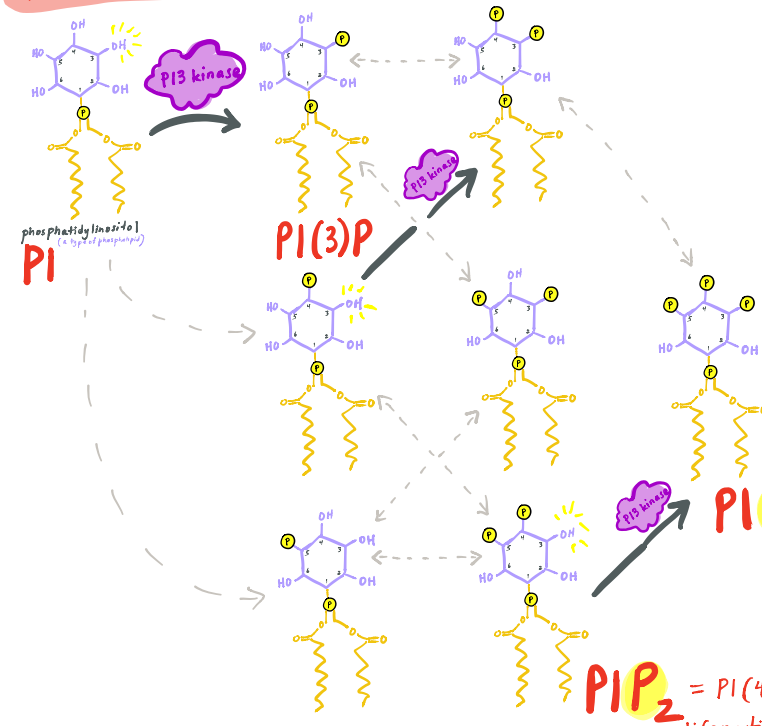
implicated in **CANCER** & **TYPE II DIABETES**

some activators of the PI3 signaling pathway: RTKs, cytokine receptors, GPCRs

main mediator = **Akt2**

other isoforms:
- Akt1: cell growth, angiogenesis
- Akt3: neuronal development

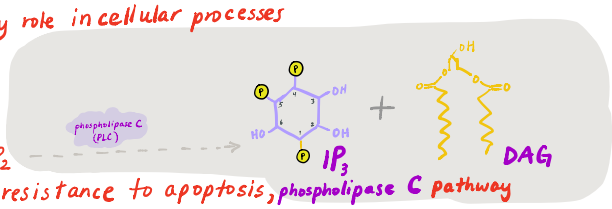
phosphoinositol structure

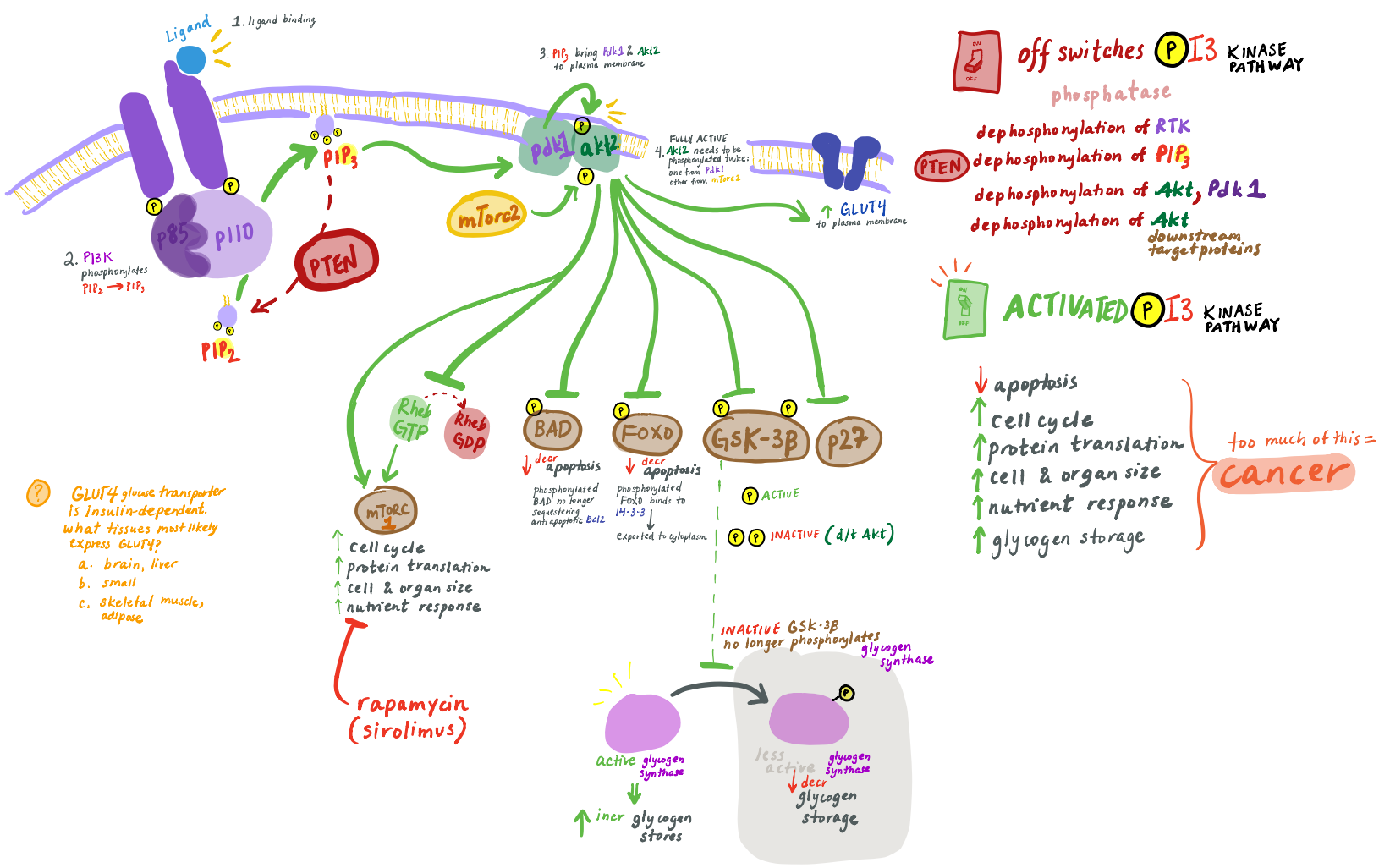


PI3 Kinase structure = p110 + p85

PIP_3 = PI(3,4,5)P_3
key role in cellular processes

PIP_2 = PI(4,5)P_2
cell proliferation, resistance to apoptosis, phospholipase C pathway




Ligand
1. ligand binding
3 PIP3 bring Pdk1 & Akt2 to plasma membrane
PIP3
pdk1
akt2
FULLY ACTIVE
4. Akt2 needs to be phosphorylated twice: one from Pdk1 other from mTorc2
mTorc2
GLUT4 to plasma membrane
P85
P110
2. PI3K phosphorylates PIP2 → PIP3
PTEN
PIP2
Rheb GTP
Rheb GDP
mTORC 1
↑ cell cycle
↑ protein translation
↑ cell & organ size
↑ nutrient response
rapamycin (sirolimus)
BAD
↓ decr apoptosis
phosphorylated BAD no longer sequestering anti apoptotic Bcl2
FOXO
↓ decr apoptosis
phosphorylated FOXO binds to 14-3-3
exported to cytoplasm
GSK-3β
P27
P ACTIVE
P P INACTIVE (d/t Akt)
INACTIVE GSK-3β no longer phosphorylates glycogen synthase
active glycogen Synthase
↑ incr glycogen stores
less active glycogen Synthase
↓ decr glycogen storage
GLUT4 glucose transporter is insulin-dependent. what tissues most likely express GLUT4?
a. brain, liver
b. small
c. skeletal muscle, adipose
off switches PI3 KINASE PATHWAY
phosphatase
dephosphorylation of RTK
PTEN dephosphorylation of PIP3
dephosphorylation of Akt, Pdk1
dephosphorylation of Akt
downstream target proteins
ACTIVATED PI3 KINASE PATHWAY
↓ apoptosis
↑ cell cycle
↑ protein translation
↑ cell & organ size
↑ nutrient response
↑ glycogen storage
too much of this = cancer

STEM CELLS

mesoderm endoderm ectoderm

1. can give rise to differentiated cells
2. can replicate itself (self-renewal)

differentiation

specificity / identity ↔ potency

∴ → ability to diff. into other cell types

As cells differentiate, they lose the ability to proliferate (without changing identity)

Physiologic Human Dev

Research Tools

engineered, do not occur naturally

self-renewal aka proliferation

differentiation

differentiation

e.g. zygote
totipotent stem cell
+ trophoblast

e.g. cell w/in inner cell mass
pluripotent stem cell
Should no longer exist in adult human body! By birth / after, these founder populations disappear.

e.g. hematopoetic stem cell (HSC)
adult multipotent stem cell
QUIESCENT (G_0) & RARE

e.g. common myeloid progenitor
oligopotent stem cell
RAPIDLY proliferate but only for a few rounds before committing to specialized fate

e.g. RBC

many types of adult stem cells in adult human body

RATE OF TURNOVER

HSC >10^6 blood cells every second
intestinal turnover every 3-4 days
skin turnover every 4 weeks

add cytokines & culture on dish (controversial)

embryonic stem cell

add 4 Yamanaka transcription factors (less controversial)

induced pluripotent stem cell

STEM CELLS & CANCER: what's the connection?

stem cell model of cancer = cancer is a neoplastic transformation of a stem cell and/or progenitor. If the source of cancer resides in quiescent stem cell, chemo agents that selectively target rapidly dividing cells WON'T WORK no 100% eradication

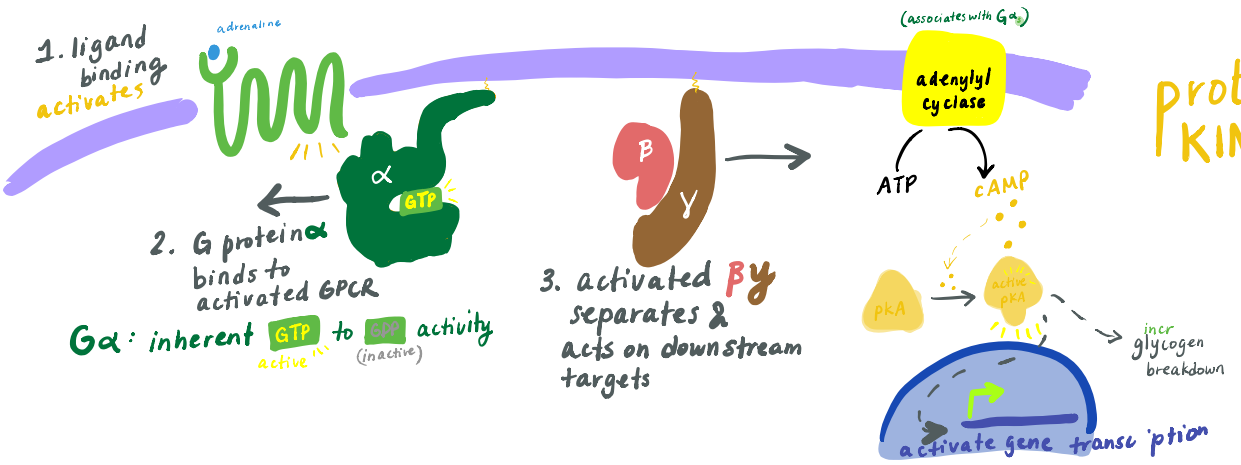
cancer is a clonal process = evidence of MONOCLONAL = identical genotype among pop'n
population of cells indicates one cell eenily out of control w/ cell prolif → likely neoplasm

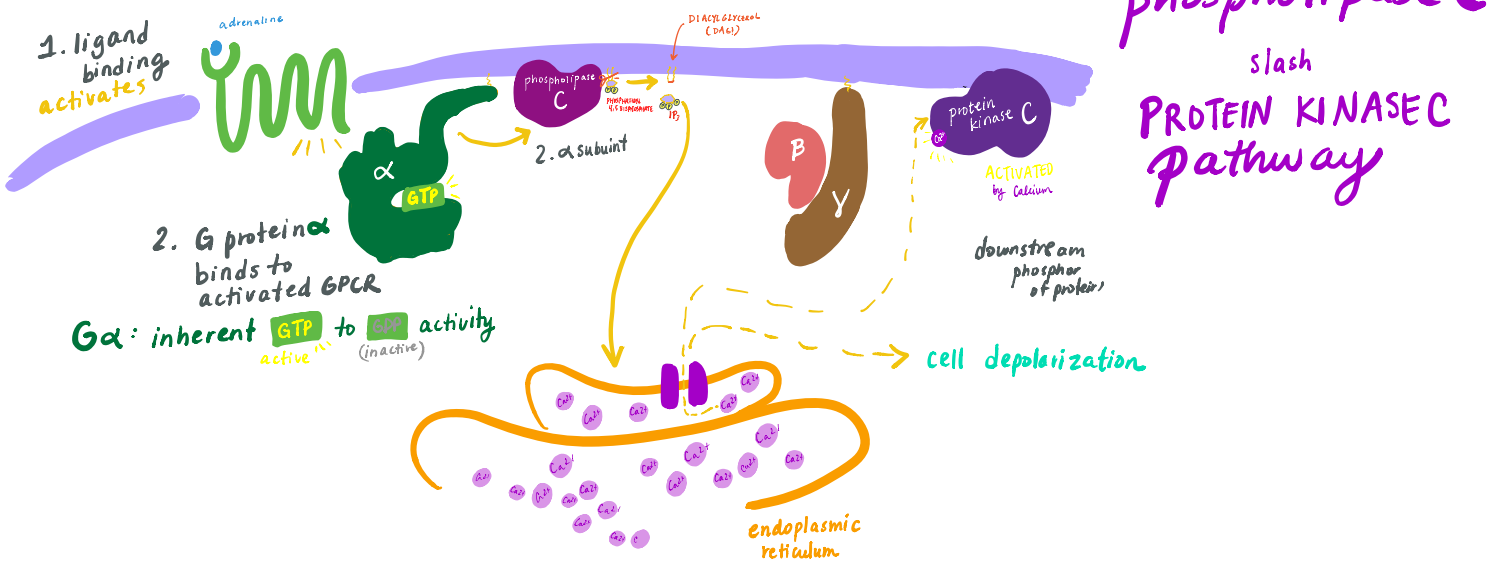
STEM CELL NICHE:

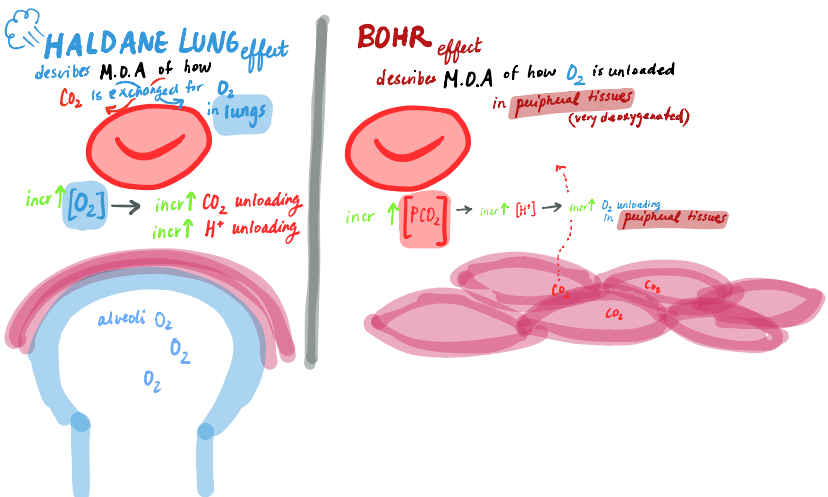
tissue-specific place where stem cells can reside & be themselves

factors there maintain potency

- quiescent (G_0) phase
- capacity for self renewal


1. ligand binding activates
adrenaline
α
GTP
2. G protein α binds to activated GPCR
Gα: inherent GTP to GDP activity
active
(inactive)
β
γ
3. activated βγ separates & acts on downstream targets
(associates with Gα)
adenylyl cyclase
ATP
cAMP
PKA
active PKA
incr glycogen breakdown
activate gene transcription
protein KINASE A


1. ligand binding activates
adrenaline
α
GTP
2. G protein α binds to activated GPCR
Gα: inherent GTP to GDP activity
active
(inactive)
phospholipase C
DIACYLGLYCEROL (DAG)
2. α subunit
β
γ
protein kinase C
ACTIVATED by Calcium
downstream phosphorylation
cell depolarization
endoplasmic reticulum
phospholipase C
slash
PROTEIN KINASE C
Pathway


HALDANE LUNG effect
describes M.D.A of how CO_2 is exchanged for O_2 in lungs
incr ↑ $[O_2]$
incr ↑ CO_2 unloading
incr ↑ H^+ unloading
alveoli O_2
BOHR effect
describes M.D.A of how O_2 is unloaded in peripheral tissues (very deoxygenated)
incr ↑ $[PCO_2]$
incr ↑ $[H^+]$
incr ↑ O_2 unloading in peripheral tissues
CO_2