Computational Systems Biology: Biology X

Bud Mishra

Room 1002, 715 Broadway, Courant Institute, NYU, New York, USA

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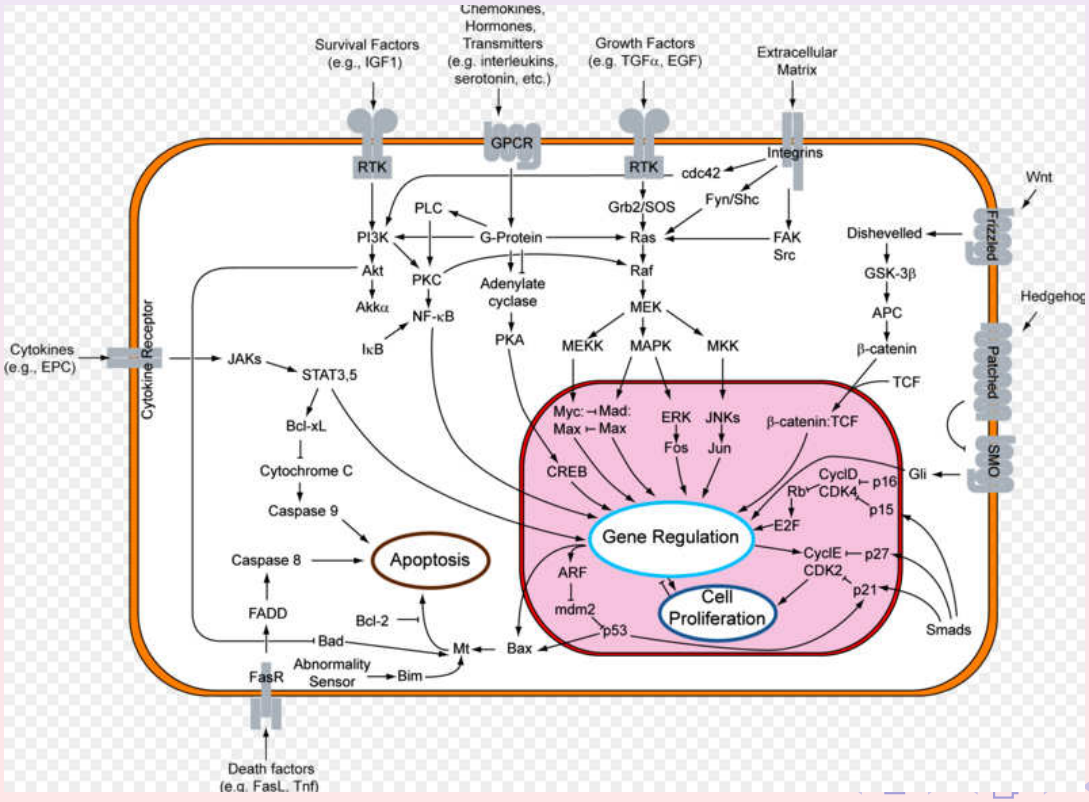
Cancer and Signals

Outline

Ras Pathway


Survival Factors (e.g., IGF1)
Chemokines, Hormones, Transmitters (e.g. interleukins, serotonin, etc.)
Growth Factors (e.g. TGFα, EGF)
Extracellular Matrix
RTK
GPCR
RTK
Integrins
cdc42
Fyn/Shc
PLC
Grb2/SOS
PI3K
G-Protein
Ras
FAK
Src
Akt
PKC
Adenylate cyclase
Raf
Akkα
NF-κB
MEK
IκB
PKA
MEKK
MAPK
MKK
Cytokines (e.g., EPC)
Cytokine Receptor
JAKs
STAT3,5
Bcl-xL
Cytochrome C
Caspase 9
Caspase 8
Apoptosis
FADD
Bad
Bcl-2
Mt
Bax
FasR
Abnormality Sensor
Bim
Death factors (e.g. FasL, Tnf)
Myc: Mad: Max Max
ERK
JNKs
Fos
Jun
β-catenin:TCF
CREB
CyclD
CDK4
p16
p15
Rb
E2F
Gene Regulation
CyclE
CDK2
p27
p21
ARF
mdm2
p53
Cell Proliferation
Smads
Wnt
Frizzled
Dishevelled
GSK-3β
APC
β-catenin
TCF
Hedgehog
Patched
SMO
Gli

Ras

- The Ras subfamily (an abbreviation of RAt Sarcoma) is a protein subfamily of small GTPases that are involved in cellular signal transduction.
- Since Ras communicates signals from outside the cell to the nucleus, mutations in ras genes can permanently activate it and cause inappropriate transmission inside the cell even in the absence of extracellular signals.

RAS

- Activating mutations in Ras are found in 20-25% of all human tumors and up to 90% in specific tumor types.
- The clinically most notable members of the Ras subfamily are HRAS, KRAS and NRAS, mainly for being implicated in many types of cancer.... However, there are many other members of this subfamily as well: DIRAS1; DIRAS2; DIRAS3; ERAS; GEM; MRAS; NKIRAS1; NKIRAS2; NRAS; RALA; RALB; RAP1A; RAP1B; RAP2A; RAP2B; RAP2C; RASD1; RASD2; RASL10A; RASL10B; RASL11A; RASL11B; RASL12; REM1; REM2; RERG; RERGL; RRAD; RRAS; RRAS2.

RAS

- Ras proteins attach to the cytoplasmic face of the plasma membrane; their c-termini have lipid tails.
- Ras binds and hydorlizes Guanosine nucleoside (GTP). It acts as a binary switch... Bound to GDP it's inactive.. signaling cascade binds a GTP to make it active. After a fixed delay, intrinsic FTPase allows it to turn itself off.
- Ras-oncoprotein becomes constitutively active when it loses all GTPase activities... **Constitutuvel Signaling by RAS**: Receiver carries out an action without any sender sending any type.

Tyrosine Phosphorylation

- Tyrosine kinase by phosphorylation transmits the signal: It could do this in two different ways: (a) Change conformation or (b) change physical location. Ras acts by the second mechanism (change of location).
- SH2 domain in Ras enables partnering with specific phosphotyrosine (plus flanking amino acid sequences)...
- Through this process, SH2 containing proteins travel closer to membrane surface (where the receptor has it cytoplasmic tail with tyrosine kinase). They interact with other membrane associated proteins and phospholipids.
- The signal is then transmitted to various down-stream signaling transducing cascades.

RAS

- **Pathways controlled by Ras.**
- In this pathway, growth factor binds to a cognate receptor; this triggers a specific combination of downstream signaling molecules.
- These may get turned on in a mutant cancer cell in several ways:
  - Amplification of oncoprotein
  - Autocrine signaling loop
  - Constitutively activated receptor

Major pathways

- There are 3 major downstream signaling cascades radiating from the Ras protein:
  + Cell Cycle Progression/Transcription/Translation
  + Membrane Trafficking Vescicles
  + Cytoskeleton Cell Motility
  − Apoptosis

Effector Loops

- After Ras binds to GTP, it begins to interact with several downstream signaling partners: **Ras Efectors**
  - **Raf Kinase**
  - **PI3K**
  - **Ral-GEF**

Raf Kinase

- RAF (proto-oncogene) serine/threonine-protein kinase (also known as proto-oncogene c-RAF or simply c-Raf) is an enzyme, encoded by the RAF1 gene. The c-Raf protein functions in the MAPK/ERK signal transduction pathway as part of a protein kinase cascade.
- c-Raf is a MAP kinase kinase kinase (MAP3K) which functions downstream of the Ras subfamily of membrane associated GTPases to which it binds directly. Once activated Raf-1 can phosphorylate to activate the dual specificity protein kinases MEK1 and MEK2 which in turn phosphorylate to activate the serine/threonine specific protein kinases ERK1 and ERK2.
- ERKs are pleiotropic effectors of cell physiology and control gene expression involved in the cell division cycle, apoptosis, cell differentiation and cell migration.

PI3K

- Phosphatidylinositol 3-kinases (PI 3-kinases or PI3Ks) are a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which in turn are involved in cancer.
- PI3Ks are a family of related intracellular signal transducer enzymes capable of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns) — inositol lipid embedded in the membrane.
- The pathway, with oncogene PIK3CA and tumor suppressor PTEN (gene) is implicated in insensitivity of cancer tumors to insulin and IGF1, in calorie restriction.
- **Warburg Effect**

Ral-GEF

- It is coordinated by two Ras-like proteins Ral-A and Ral-B.
- Ral pathways activates two Rho proteins: Rac and CDC42.
- They influence: mitogenic signals, production of Reactive Oxygen Species (ROS), and motility (necessary for invasion and metastasis).

Raf Kinase

- Raf phosphorylates substrate proteins on their serine/threonine residues
- The pathway critically depends on **Raf localization** within cytoplasm. Raf becomes tethered via Ras to the plasma membrane.

$$Raf \mapsto MEK\,.$$

- Raf phosphorylates a second kinase MEK... which is a "*dual specifixity kinase*" ... [It can phosphorylate both tyrosine residues as well as serine/threonine residues.

ERK 1/2

$$Ras \mapsto Raf \mapsto MEK \mapsto ERK1/2.$$

- **Linear cascade**: MEK activates ERK 1/2, which then phosphorylates substrates and thus, regulates various cellular processes.
- **MAPK pathway**: Mitogen Activated Protein Kinase Pathway... Kinase cascading signals. Very often, a constitutively activated MAPK pathway (in mutant oncogenic) is present in transformed cancer cell.
- Note: $Ras \mapsto Raf$ is governed by tyrosine-phosphorylation (**Relocalization**) ... whereas $Raf \mapsto MEK$ is governed by serine/threonine phosphorylation (**Functional Activation**).

ERK

- Finally, ERK (by phosphorylation of various Transcriptional Factors: Ets-1, SAP-1, Jun, c-fos, etc.) turns on Immediate and Delayed Early Genes (IEGs and DEGs)...
- These genes are then responsible for *protein synthesis* and cell-growth.
- Fos dimerizes with Jun to form the AP-1 *transcription factor*, ... which upregulates transcription of a diverse range of genes involved in: **proliferation**, **differentiation**, & **defense against invasion and cell damage**.
- Damage to this pathway can result in
  – Anchorage Independence & Loss of Contact Inhibition
  – Change in Cell Shape
  giving the affected cell motility.

Inositol Lipid Control

- *Plasma Membrane*: It is a lipid bilayer... Forms the barrier between interior of the cell and exterior aqueous environments... It has to be monitored for cell's proper performance and disfunction results in initiation of cellular apoptosis program.
- Plasma membranes are constructed out of phospholipids, glycolipids, and steroids... Phospholipids are amphipathic... Amphipathic lipids are molecules that are mostly lipid-like (hydrophobic) in structure, but at one end have a region that is polar or ionic (hydrophilic). The hydrophilic region is usually referred to as the head group, and the lipid portion is known as the tail(s).

Phospho-Inositol

- Some of these hydrophilic heads contains an **inositol** group... the inositol group is made up of water soluble carbohydrates... Its moiety is controlled by the additions of phosphate groups... IP3 (phosphoinositol) can be cleaved and acts as **intracellular hormone**.
- With IP3, the cell sends signals from the plasma membrane to distant part of cells

Second Messengers

- Second Messanger: Diacylglycerol (DAG)... it is a glyceride consisting of two fatty acid chains covalently bonded to a glycerol molecule through ester linkages.
- It functions as a second messenger signaling lipid, and is a product of the hydrolysis of the phospholipid PIP2 (phosphatidyl inositol-bisphosphate) by the enzyme phospholipase C (PLC) (a membrane-bound enzyme) that, through the same reaction, produces inositol trisphosphate (PIP3).
- Inositol trisphosphate (IP3) diffuses into the cytosol... diacylglycerol (DAG) remains within the plasma membrane, due to its hydrophobic properties.

$$Ras \mapsto PI3K \mapsto PIP3.$$

- Ras activates PI3K, which attaches phosphate groups to 3' hydroxyl of the inositol moiety (converts $PIP2 \mapsto PIP3$)
- The production of DAG in the membrane facilitates translocation of protein kinase C (PKC) from the cytosol to the plasma membrane. PKC is a serine/threonine kinase.

PIP3

- PIP3 carries phosphates at its three 3', 4' and 5' positions...
- **PH (Pleckstrin Homology) Domains** in cytosolic proteins attract them to PIP3. Pleckstrin homology domain is a protein domain of approximately 120 amino acids ... involved in intracellular signaling or as constituents of the cytoskeleton...
- AKT (also known as PKB, protein kinase B) is one such PH domain carrying protein and acts as a serine/threonine kinase. ...AKT gets phosphorylated by PIP3... results in functional activation of Akt/PKB as a kinase.
- Properties
  - a) Anti-apoptotic
  - b) Pro-proliferation
  - c) Pro-cell-growth (promote size increases)

PTEN - Phosphatase

- Phosphatase and tensin homolog (PTEN) is a protein that keeps the level of PIP3 under control by removing phosphates... Transformed cells are often characterized by
  – Hyperactivity of PIP3
  or
  – Inactivity of PTEN
- PTEN acts as a tumor suppressor gene through the action of its phosphatase protein product... and thus, by negatively regulating Akt/PKB signaling pathway.
- This phosphatase is involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly.[

$$Akt/PKB \dashv \text{(inactivates)} TSC2 \dashv \text{ mTOR kinase}$$
$$\begin{cases} \dashv 4E - BP \text{ (inhibitor of translation)} \\ \mapsto p7056 \text{ (activator of translation)} \end{cases}$$

- PTEN thus controls the rate of protein synthesis...

Rho GTPase Family

- The Rho family of GTPases is a family of small (21 kDa) signaling G protein (more specific, a GTPase)
- It is a subfamily of the Ras superfamily...
- Three members of the Rho GTPase family have been studied a extensively: Cdc42, Rac1, and RhoA.
- Rho proteins have been described as “molecular (binary) switches” ... switch between GTP-bound active state and GDP-bound inactive state...
- They play a role in **cell proliferation**, **apoptosis**, **gene expression**, and multiple other common cellular functions.

Rho-GEF

- It is activated by binding to PIP3... Acts on Rho to jettison GDPs. Thus Rho becomes GTP-bound and active. (Similar to Ras-SOS)
- Rho ... Restructures the cytoskeleton... Thus controls the cell shape, motility and invasiveness
- Structural changes
  – **Filopedia** (CDC42)
  – **Lamellipedia** (Rac)

Ras regulated Ral Pathway

- Key components: two Ras-like proteins... Ral-A and Ral-B (share 80% homology with Ras)
- Functional activation of Ral proteins... achieved by replacing bound GDP with GTP.
- Ral-GEF: Functional property as a GEF (Guanine-nucleotide Exchange Factor) + Localization property through a pocket to bind activated Ras...
- Binding to activated Ras: causes localization of Ral-GEFs near the membrane surface + Conformal shift (by binding to Ras) turns on GEF activity...

Ral

- Ral pathway activates two Rho proteins: Rac and CDC42...
- Pleiotropy:
  - Mitogenic Signals
  - Stimulates production of reactive oxygen species (ROS)
  - Enables mobility (thus, invasiveness and metastasis)

Jak-STAT pathway

- Necessary for transmission from plasma membrane directly to the nucleus...
- Jak (Janus-Kinase class)... it is a non-covalent tyrosine kinases... It is a receptor for
  – TFN (Interferon)... immune system
  – EPO (Erythropoietin) .. Erythropoiesis... Red blood cells
  – TPO (Thrombopoietin).. Thrombopoiesis .. Platlet formation
- **Jak Pathway activation**: Ligand binding ... results in phosphorylation of tyrosine on the cytoplasmic tail... the activated tail attracts SH2 containing transcriptional factors (TFs) STAT...

STAT

- STAT (Signal Transducers and Activation of Transcription). It contains both (i) SH2 domain and (ii) phosphotyrosine.
- Thus, it can dimerize (through SH2-STAT phosphotyrosine binding) to make STAT-STAT dimers.
- It then migrates to nucleus where it acts as a transcription factor, which activates proliferation and survival genes: myc, cyclin D2 & D3 (cell cycle genes), genes for BCL-X (anti-apoptotic gene)
- Jak can also phosphorylate other substrates: RAs-MAPK pathway & other mitogen pathway.

Mutant STATs in Cancer Cells

- Reengineering of ATAT3 proteins in cancer cells through cysteine residues.
- Mutant STAT3 dimerizes spontaneously by stable covalent disulfide cross-linking bonds and become a constitutively active nuclear Transcriptional Factor... **Oncoprotein**.
- In melanoma, Src constitutively activates STAT... as it can be activated by tyrosine kinases other than receptors associated with Jak...
- In breast cancer, STAT is activated by Src and Jaks acting collaboratively (Chimeric Signaling)
- In head and neck cancer, STAT constitutively activated via EGFR

Wnt pathway

- The canonical Wnt pathway is activated when Wnt proteins bind to cell-surface receptors of the Frizzled family, causing the receptors to activate Dishevelled family proteins and ultimately resulting in a change in the amount of β-catenin that reaches the nucleus.
- Dishevelled (DSH) is a key component of a membrane-associated Wnt receptor complex.. it inhibits a second complex of proteins that includes axin, GSK-3, and the protein APC.

Wnt pathway

- The axin/GSK-3/APC complex normally promotes the proteolytic degradation of the β-catenin intracellular signaling molecule. After this "β-catenin destruction complex" is inhibited, a pool of cytoplasmic β-catenin stabilizes, and some β-catenin is able to enter the nucleus and interact with TCF/LEF family transcription factors to promote specific gene expression...
- Wnt-β-catenin pathway is a mitogenic pathway... It allows cells to remain in a relatively undifferentiated state

G protein-coupled receptors (GPCRs)

- G protein-coupled receptors (GPCRs)...also known as seven-transmembrane domain receptors, heptahelical receptors, and serpentine receptor...
- There are two principal signal transduction pathways involving the G protein-coupled receptors: the cAMP signal pathway and the Phosphatidylinositol signal pathway.
- When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR can then activate an associated G-protein by exchanging its bound GDP for a GTP.

G protein-coupled receptors (GPCRs)

- The G-proteins are hetero-timeric: $G\alpha$, $G\beta$ and $G\gamma$ subunits... Stimulated by GPCR, α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the ? subunit type...
- It activates various cytoplasmic enzymes.

Outline

Cancer Etiology

- **Etiology**: Causative mechanisms of a disease... We still lack one for cancer.
- Treatments have been based on evidence (and triage)...
  – Surgery (radical)
  – Radiation Therapy
  – Chemotherapy
- The preceding techniques were all developed before 1975... Before the genetic and biochemical mechanisms of cancer was understood.

Cancer Etiology

- **Can we do better?**
- **Can understanding signaling and its role in multi-cellularity help?**
- **Can ideas from game theory be used?**

Strategies for Therapy

- Small molecular-weight drugs
- Proteins
- Monoclonal anti-bodies
- Gene Therapy (e.g., viral vectors)
- Synthetic Biology
- Information-Based/Model-Based Therapy

[End of Lecture #11]