

Computational Systems Biology: Biology X

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Cancer and Signals

Outline

1 Biological Signaling

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Micro-Environment Story

- How does the rest of our body influences the cancer cell?
What is the role of the **microenvironment**: the normal cells and molecules that surround a tumor cell.
- When a normal cell transforms into a benign tumor, it loses some of its growth regulation. For it to invade and metastasize, it has to have the ability to move through the bloodstream and end up at distant sites.

- *For example*, on the outside of the skin are epithelial cells. Underneath the epithelial cells there is supporting tissue, called **connective tissue**, which is there to support the epithelial cells.
- At the beginning of skin cancer, epithelial cells start to grow uncontrollably, start to accumulate mutations and develop their own growth signals. As they do this they take along the supporting structure, called **stroma**.
- As a result, *a tumor contains both stroma and the malignant tumor cells themselves*. Within the stroma there are things like blood vessels, which bring the nutrients that the tumor cell needs to grow, and immune or inflammatory cells that come from the bloodstream and get into the tumor. This increases a tumor's complexity.

- **So what is in the tumor and microenvironment?** There are normal epithelial cells — those that are not cancer yet. There are fibroblasts and the cells that make up the blood vessels. There are the infiltrating immune cells that come from the bloodstream. There are the structural components comprised of proteins — little strands of fibers that hold our cells together — which are called the extracellular matrix (ECM) because it's outside of the cell. Then there are lots of molecules. Some of the molecules are special kinds of growth factors called chemokines and cytokines, which are chemical activators and cellular activators. There are also chemicals, like oxygen, and chemicals that can change the acidity or the alkalinity of the tissue. All of these different things make up the tumor microenvironment.

- The communication between the tumor cells and the surrounding cells — the microenvironment — helps drive the process of tumor progression. So going from normal to benign, benign to malignant, malignant to metastatic is driven not just by what's happening inside the tumor cell itself but by what's happening around it.
- Two of the key hallmarks of cancer are dependent on the surrounding microenvironment. One of these is **angiogenesis** and the other is **invasion and metastasis**. Angiogenesis creates the blood vessels that give the tumor its oxygen supply. Invasion and metastasis give the tumor the ability to invade into other areas and to travel to different parts of the body. If a cancer cell didn't have these special characteristics, it would not be able to continue to grow.

Signaling

Key to these is a complex **signaling** equilibrium — which was achieved through an **evolutionary signaling game**.

- Intracrine (within a cell)
- Autocrine (originating from the same cell)
- Paracrine (originating from nearby cells)
- Endocrine (system-wide)

Signal

- Growth Factors (Kinases)
- Motility (Integrin)
- Apoptosis (Caspases)
- Metabolism (Hypoxia, Anoxia, etc.)
- Autophagy
- Metaplasia (Transdifferentiation, Dedifferentiation)
- Meta-signals (Mutators?)

Multicellularity

- In a multicellular organism (or even a tumor), a group of cells must work together to accomplish a particular “function.”
- No single cell can perform the entire function, but only its “component” of the function: **action**.
- The appropriate **action** depends upon the global state: microenvironment, stress, oxygen, pH, etc.
- No single cell may know the global state: but only some “component” of the state: **type**.

Sender-Receiver Game

- A sender cell or ECM (extra-cellular matrix) knows the type, and based on it sends a subset of few available **signals**.
- A receiver cell receives the **signals** and activates kinases, transcriptional factors to turn on certain genes to perform certain **actions**.
- Sender wants the signals to carry as much information as possible, and specific actions to be carried out as a result of the signals.
- Receiver wishes the signals to encode the global state as best as possible, and the actions to confirm to the state as informatively as possible.

Correlation of Interaction

- Malignant tumor cells grow in its microenvironment in an anchorage-independent manner. (Normal cells – without proper attachment – commit **Anoikis**, a form of programmed cell death which is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix (ECM).)
- Usually cells stay close to the tissue to which they belong since the communication between proximal cells as well as between cells and ECM provide essential signals for growth or survival. However, metastatic tumor cells must escape from anoikis to invade other organs.
- ECM ... consists of various *glycoproteins* — Collagen, Laminin, Fibronectin, Proteoglycan etc. These are the ligands, sensed by **integrin**. They also provide the *geometry*, necessary for *correlation of interaction*.

Integrin

- Integrins are *heterodimeric* (use α and β subunits)... In mammals, eighteen α and eight β subunits have been characterized (whereas the *Drosophila* genome encodes only five α and two β subunits, and *Caenorhabditis* nematodes possess genes for two α subunits and one β . The α and β subunits each contain two separate tails, both of which penetrate the plasma membrane and possess small cytoplasmic domains. Through different combinations of these ? and ? subunits, some 24 unique integrins are generated, although the number varies according to different studies.
- Integrins cluster to create **focal adhesion** and activate *Actin*, *vinculin*, *Talin* & *Paxilin*...
- In this way, integrins activate different signaling pathways for **Migration**, **Proliferation** and **Survival**.

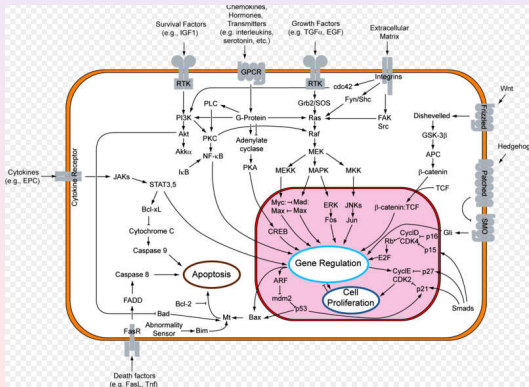
Cell Motility

- Cell motility is thus controlled by FAK (**Focal Adhesion Kinases**), as they remodel focal contacts.
- Through these signaling, population density is controlled in a multicellular structures: Growth factors (EGF, PDGF etc.) sense soluble growth factors; Integrins sense insoluble scaffolding of ECM.
- Preconditions for growth and division: *mitogenic growth factors* and *adequate anchoring*... We need to think about how we model them in an *Abstract Signaling Machine* (ASM).
- A key component is the proto-oncogene: RAS. We need to ask: Why is RAS so important?

RAS

- It has been known that *ras-transformed cells* can grow in an environment with low concentration of serum; they can also proliferate in an *anchorage-independent manner*. Why?
- Important: Understanding structure and function of normal and oncogenic **Ras protein**.
- Ras is a G-protein, and is activated by a tyrosin kinase (e.g., cytoplasmic tail of an EGF receptor). How does it activate the down-stream signaling cascade? Who are the participants there?
- There are 3 ras genes and 4 Ras proteins; K-Ras is the result of alternate splicing...

Ras Pathway



Ras

- The Ras subfamily (an abbreviation of RAAt Sarcoma) is a protein subfamily of small GTPases that are involved in cellular signal transduction, and is also used to designate gene subfamily of the genes encoding those proteins. Activation of Ras signalling causes cell growth, differentiation and survival. Ras is the prototypical member of the Ras superfamily of proteins which are all related in structure and regulate diverse cell behaviours.
- 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

- Because these signals result in cell growth and division, dysregulated Ras signaling can ultimately lead to oncogenesis and cancer. 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 hydrolyzes 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 GTPase allows it to turn itself off.
- Ras-oncoprotein becomes constitutively active when it loses all GTPase activities... **Constitutive Signaling by RAS**: Receiver carries out an action without any sender sending any type.

Cytoplasmic Signaling Circuitry Programs

- *In summary*, Difference between normal and neoplastic cells — minor modifications — yet with significant changes in phenotypes... Note, constitutive activation of Ras oncoprotein (through GTPase inactivation) gives the cell a proliferative phenotype.
- Key elements:
 - Loss of Control in Signal Processing circuitry: E.g., loss of synchronization among various cell-cycle clocks..
 - Loss of Control in Specificity or Rate of Access: E.g., in a linear signaling cascade the partner proteins must communicate
- **Cancer is a disease of aberrant signal processing.**

Ras-Mediated Transmission

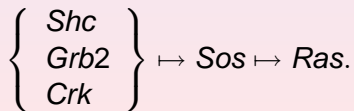
- How does Ras transmit signal from the cell surface to nucleus?
- Experiment: “Serum Starvation” of fibroblasts... Leads to the cells entering into G_0 . Upon exposure to fresh serum (& mitogenic growth factors), cells start to replicate *synchronously*.
- The signaling cascade triggers expression of IEGs (“Immediate Early Genes”).
- Shortly after that there is expression of DEGs (“Delayed Early Genes”).

Growth Factors

- Growth factors induce other actions too:
 - (1) Increased levels of cellular protein synthesis
 - (2) Motility
 - (3) Reorganization of actin fibers (cell shape control)
 - (4) Survival signals (Resistance to apoptosis)

Sos

- Recall: Ras operates like a binary switch; it has two states (1) Active (GTP-bound) and (2) Inactive (GDP-bound).
- *Sevenless* is a gene that plays an important role in ommatidia development in fly eyes (light sensing components in its compound eye). It also encodes “EGF Receptor”
- Sos (Son-of-sevenless) acts via nucleotide exchange by G-proteins... Mediated by GEF (Guanine nucleotide Exchange Factors). Sos then turns on Ras.



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)
- The first mechanism (change of conformation) gets the signaling molecule in an actively signaling state, while the second (change of location) brings the signaling molecule to a favorable site for signaling.
- The way Ras acts is determined by its SH domains (Src Homology domains) 1, 2, and 3: SH1, SH2, & SH3. Of these SH2 is the most important as it acts as an “Intracellular Receptor.”

SH2 Domain

- SH2 domain enables partnering with specific phosphotyrosine (plus flanking amino acid sequences)...
Note: some of these proteins have other catalytic sites:
Phosphotase Catalytic Domain.
- The sequence of events: (i) GF Receptors start ligand induced trans-phosphorylation, (ii) In the cytoplasmic tail, they display a characteristic phosphotyrosine residues, (iii) These tails become attractive homing sites for various SH2 containing cytoplasmic proteins (e.g., Ras), (iv) They initiate appropriate action related to signaling type. (They can also shut down receptor signaling... An interesting action by itself.)

Membrane Clustering

- Through this process, SH2 containing proteins travel closer to membrane surface (where the receptor has its 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.

SH Family

- SH3 and SH2 domains are important in signaling.
- SH3 domain interacts with proline-rich sequence domains in partner proteins, while SH2 is triggered by tyrosine phosphorylation. There are 253 genes in human genome coding proteins containing SH3, while there are only 117 for SH2. SH3 is old (1.5 Bya) while SH2 is more recent (660 mya), arising with multicellularity in metazoan.

Example: Ras-GAP

- Ras-GAP (Ras GTPase Activating Proteins) attaches to phosphorylated receptors... In close proximity to Ras, which are anchored to plasma membrane (through c-terminal lipid tails). They hydrolyze Ras's bound GTP

Ras(Active) \mapsto *Ras(Inactive)*.

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 Effectors**
 - **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).

[End of Lecture #10]