1 # ANAT3231 - Cell Biology Lecture 12

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3 ## A Sample Signaling

4 ## Signaling Text References

- Essential Cell Biology
  - Chapter 15
- Molecular Biology of the Cell
  - Chapter 15
- Molecular Cell Biology
  - Chapter 20
- Nature Signaling Gateway
  - http://www.signaling-gateway.org/molecule/

5 ## Lecture Summary

- Continuation of Lecture 11 Signaling
- Specific examples of signaling
  - Pathways
  - Mechanisms
    - Note steroid and lipid signaling covered last week

6 ## Common Signals

7 ## Signals and Receptors

8 ## Signaling between Tissues

- Regulation of cells and tissues
- Hormones
  - secreted by one tissue to regulate function of other cells or tissues
- Chemical Signal Types
  - water soluble
  - lipid soluble

9 ## Extracellular Signal Steps

- Signaling Molecule
  - Synthesis
  - Release by signaling cell
  - Transport to target cell
  - Detection by a specific receptor protein
- Change by receptor-signal complex (trigger)
  - Metabolism
  - Function
  - development
- Removal of the signal

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- often terminates cellular response

10 Messenger /Receptor Interaction

- Binding of messenger (ligand) has to lead to a change in the receptor
  - like enzyme and substrate
  - Specific recognition
  - Receptor affinity
- activation
- signal transduction
  - Signal cascade
  - Secondary messengers

11 Chemical Signals

- Water Soluble
  - bind to surface receptors
- Lipid Soluble
  - bind to cytoplasmic or nuclear receptors
  - steroid hormones
    - Covered in Lecture 11

12 Receptor Pathways

13 Second Messengers

14 Second Messengers

- Cyclic nucleotides
  - cAMP, cGMP
- Calcium Ions
- Protein Kinase A
  - PKA, B, C
- diacylglycerol (DAG)
  - modified lipid activates PKC
- Kinase cascades
- small GTP binding proteins
  - related to RAS which is G protein family

15 Membrane Receptors

- embedded in plasma membrane
- ligand binding
  - leads to conformational change in receptor
  - activation of intracellular pathway
- G Protein Linked receptors

16 Cell Surface Receptor Types

17 G Protein Receptors

18 G Protein-Coupled Signal Pathways

- Transmembrane proteins transduce extracellular signals
  - common structural motif of 7 membrane spanning regions
- Receptor binding promotes interaction
  - between receptor
  - G protein on interior surface of membrane

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19 G Protein-Coupled Signal Pathways

- induces an exchange of GDP for GTP on G protein α subunit and dissociation of the α subunit from the $\beta\gamma$ heterodimer
- Depending on isoform, GTP-α subunit complex mediates intracellular signaling either
  - **indirectly** by acting on effector molecules
    - adenylyl cyclase (AC)
    - phospholipase C (PLC)
  - **directly** by regulating ion channel or kinase function

20 G Protein Linked

21 Growth Factors

22 Receptor associated with Kinase

- Many growth factors use this pathway
  - Vascular Endothelial Growth Factor
  - Epidermal Growth Factor
  - Nerve Growth Factor
  - Bone Morphogenic Protein
  - Transforming Growth Factor-beta

23 Receptor Kinase Pathway

- Ligand binding
- Receptor association
- Phosphorylation
- Kinase cascade
  - Multiple intracellular tagets

24 Proto-oncogenes

- proto-oncogenes
  - Normal cell proteins that have potential to cause uncontrolled growth when mutated
- loss of receptor regulation
- cells grow out of control
- mutation in TK Receptor
  - receptor always activated
- mutation of activating protein
  - always active
- Oncogenes
  - Ras
  - mutants detected in 30% cervical cancers

25 Fibroblast Growth Factors

- 19 FGF family of growth factors
- 2 originally characterized FGFs were identified by biological assay
  - FGF1 (acidic-FGF, aFGF)
  - FGF2 (basic-FGF, bFGF)

26 FGF Receptors

- family of related tyrosine kinase receptors
  - FGFR1, FGFR2, FGFR3, FGFR4
- Protein structure

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- 3 extracellular immunoglobulin-like domains
- 1 membrane spanning segment
- cytoplasmic tyrosine kinase domain

27 FGF Receptors

- transmembrane receptors with tyrosine kinase activity
  - Like EGF and PDGF receptors
- immediate response to FGF binding is receptor autophosphorylation
- Following activation numerous signal-transducing proteins associate with receptor and become tyrosine-phosphorylated
- Example: expressed in developing bone
  - autosomal dominant disorders of bone growth result from mutations in FGFR genes
  - FGFR3 (mutant in achondroplasia)

28 Nerve Growth Factor

- polypeptide involved in regulation of growth and differentiation of sympathetic and certain sensory neurons
  - Levi-Montalcini, R. The nerve growth factor thirty-five years later. *Science* 237: 1154-1162, 1987

29 Nerve Growth Factor Receptor

- p75 (NTR)
  - molecular mass and ability to bind at low affinity not only NGF but also other neurotrophins
    - brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NTF3), and neurotrophin-4/5 (NTF5)
- tumor necrosis factor receptors share overall structure of NGFR
  - 4 extracellular ligand-binding, cysteine-rich repeats (CRs), and signaling through association with, or disassociation from, cytoplasmic interactors
- Associated with nuclear factor kappa-B (NFKB) and apoptosis pathways

30 Nerve Growth Factor Receptor

- Low affinity
  - monomer NGFR binds NGF
- High affinity binding
  - by association with low-affinity neurotrophin receptors (tropomyosin receptor kinases- TRK)
    - TRKA (NTRK1), TRKB (NTRK2), and TRKC (NTRK3)
  - Specificity
    - TRKA - NGF
    - TRKB - NTF5 and BDNF
    - TRKC - NTF3
      - NTF3 also binds to TRKA and TRKB with significantly lower affinity

31 TrkA Receptor

- Trk proto-oncogenes
  - TrkA, TrkB, TrkC, TrkE
- variably expressed in CNS and PNS
- TrkA binds to nerve growth factor (NGF) and autophosphorylates
  - leading to activation of multiple downstream effector proteins

32 VEGF Receptor and Ligands

33 EGF Receptor Transduction Pathway

34 Transforming Growth Factors

- are a family of biologically active polypeptides that reversibly confer transformed phenotype on cultured cells
  - TGFA (alpha)

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    - shows homology (40%) with epidermal growth factor (EGF) and competes with EGF for binding to the EGF receptor stimulating its phosphorylation and producing a mitogenic response
  - TGFB (beta)
    - acts synergistically with TGFA in inducing transformation

35 Signaling Pathway of TGF-β

36 Transforming Growth Factor- beta

- Transforming growth factors (TGFs) are biologically active polypeptides that reversibly confer the transformed phenotype on cultured cells
  - Many cells synthesize TGFB and also have specific receptors for this peptide
- Function
  - controls proliferation, differentiation, and other functions in many cell types
  - TGFB acts synergistically with TGFA in inducing transformation
  - acts as a negative autocrine growth factor
  - Dysregulation of TGFB activation and signaling may result in apoptosis
  - TGFB1, TGFB2 and TGFB3 all function through same receptor signaling systems

37 Insulin-like Growth Factors

- Insulin-like growth factors I and II
  - also known as somatomedin C and A
  - polypeptides which share an amino acid sequence homology with insulin (47%) and relaxin (31%) insulin family of polypeptide growth factors.
- Functions
  - mediation of growth hormone action, stimulation of growth of cultured cells, stimulation of insulin action, and involvement in development and growth
  - autocrine regulators of cell proliferation

38 Erythropoietin

- Main regulator of red cell production
  - synthesized and released by the kidney
  - circulates to the bone marrow where it stimulates resident erythroid progenitors via a specific receptor
- acidic glycoprotein hormone (Mr 34 kD)
- Receptor
  - Related to cytokine receptors
  - protein phosphorylation an early component of transmembrane signaling

39 Cytokines

- Secreted by leukocytes
  - stimulate humoral and cellular immune responses
  - activation of phagocytic cells
    - lymphokines secreted by lymphocytes
    - monokines secreted by monocytes or macrophages
- produced by other cells of the body
- lymphokines also known as interleukins (ILs)
  - not only secreted by leukocytes also able to affect cellular responses of leukocytes

40 Interleukins

- growth factors targeted to cells of hematopoietic origin
- now 22 identified interleukins
  - Number still growing with individual activities

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42 Online References

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- Sigma Apoptosis Brochure
  - http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cell_Signaling.html
- Growth factors
  - http://web.indstate.edu/thcme/mwking/growth-factors.html

43 Signal Transduction Research Labs

1

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- Bruce Zetter (Children's Hospital, Harvard Medical School) * # H, M, MV

44 Reference: Molecular Biology of Cell

- III. Internal Organization of the Cell
  - 15. Cell Signaling
    - Introduction
    - General Principles of Cell Signaling
    - Signaling via G-Protein-linked Cell-Surface Receptors
    - Signaling via Enzyme-linked Cell-Surface Receptors
    - Target-Cell Adaptation
    - The Logic of Intracellular Signaling: Lessons from Computer-based "Neural Networks"
    - References

45 Reference: Molecular Cell Biology

- 20. Cell-to-Cell Signaling: Hormones and Receptors
  - 20.1 Overview of Extracellular Signaling
  - 20.2 Identification and Purification of Cell-Surface Receptors
  - 20.3 G Protein –Coupled Receptors and Their Effectors
  - 20.4 Receptor Tyrosine Kinases and Ras
  - 20.5 MAP Kinase Pathways
  - 20.6 Second Messengers

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- 20.7 Interaction and Regulation of Signaling Pathways
- 20.8 From Plasma Membrane to Nucleus
- PERSPECTIVES
  - Future
  - Literature

46 Reference: The Cell

- IV. Cell Regulation
  - 13. Cell Signaling
    - Signaling Molecules and Their Receptors
    - Functions of Cell Surface Receptors
    - Pathways of Intracellular Signal Transduction
    - Signal Transduction and the Cytoskeleton
    - Signaling in Development and Differentiation
    - Regulation of Programmed Cell Death
    - Summary
    - Questions
    - References and Further Reading

47 Reference: Developmental Biology

- Part 1. Principles of development in biology
  - 6. Cell-cell communication in development
    - Induction and Competence
    - Paracrine Factors
    - Cell Surface Receptors and Their Signal Transduction Pathways
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    - References