Signal Transduction 3

Ron Bose, MD PhD
BBSB and MCB Programs
Lab: 4515 McKinley Research Building, 3rd floor
Washington University School of Medicine
Introduction – Part 1

Pathways

1. G-Protein signaling
2. Receptor Tyrosine Kinase
3. Cyclic AMP and other Second Messenger Pathways
4. Nuclear Hormone Receptors
5. Cytokine receptors and JAK-STAT pathway

Human Physiology and Diseases

Pain and Pain relief
Heart Function
Asthma
Cancer
"Signal Transduction is a Pain"

1. Pain is a complex process.
2. Signal transduction pathways play a key role in it.
3. The 2 most commonly used classes of pain medications are:
   - Anti-inflammatory
   - Opiates

Nakahata, Pharmacology & Therapeutics 2008
Anti-Inflammatory Medicines inhibit Thromboxane Synthesis

Cyclooxygenase (COX) Inhibitors

Aspirin

Ibuprofen

Nakahata, Pharmacology & Therapeutics 2008
Opiate Receptors are GPCR’s

- Morphine and related drugs are opiates and are commonly used pain medications.
- The major opiate receptors (δ, μ, and κ) are G-protein coupled receptors.
- The endogenous ligands for opiate receptors are peptide hormones like enkephalin, endorphins, and dynorphin.

Brunton et al., Goodman & Gilman, The Pharmacological Basis of Therapeutics, 12th Ed., 2011
Opiate Receptors are GPCR’s

- Opiate receptors can homo- and heterodimerize. Additionally, cross-talk between different GPCR’s occurs.
- A common side effect of morphine is itching.
- Itching is mediated by cross-talk between an alternately spliced µ opiate receptor (MOR1D) and the GRPR protein.

Miyamoto et al., Cell 2011, Liu et al., Cell 2011
Cyclic AMP and GPCR signaling

STUDIES ON THE MECHANISM OF HORMONE ACTION
Nobel Lecture, December 11, 1971

by

Earl W. Sutherland

Cyclic AMP

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Adrenergic Receptors bind Adrenaline/Epinephrine

There are also $\beta_2$ and $\beta_3$ receptors.
β-Adrenergic Receptors stimulate cAMP production

\[ \beta_1 \] 

Adenylate Cyclase

ATP

cAMP

Protein Kinase A (PKA)

\( G_{\alpha_s} \)
Physiologic Effects of $\beta$-Adrenergic Signaling

1. Heart – increased heart rate and contractility
2. Vascular - Dilation of the coronary arteries and arteries to skeletal muscles.
3. Dilation of the airways in the lung

Commonly used medications:

$\beta$-Blockers: control heart rate and blood pressure.

Albuterol – The most common medicine for asthma. It is a $\beta2$ adrenergic agonist.
Chronic Myeloid Leukemia (CML) and the Philadelphia Chromosome

- CML is diagnosed in 5,000 new patients each year in the US.
- A classic chromosomal rearrangement between chromosomes 9 and 22, named the Philadelphia chromosome, defines CML.
- This 9;22 translocation produces a fusion protein between the ABL tyrosine kinase and the BCR gene.
Chronic Myeloid Leukemia (CML) and BCR-ABL

- Abl can be inhibited with tyrosine kinase inhibitors.
  - Imatinib (Gleevec)
  - Dasatinib
  - Nilotinib

- Tyrosine kinase inhibitors have revolutionized the treatment of CML, and greatly prolonged patient lifespans.

Crystal structure of ABL tyrosine kinase with Imatinib (orange) bound.

The EGFR family of Receptor Tyrosine Kinases

EGFR  Her2/neu  Her3  Her4

Extracellular
Transmembrane
Cytoplasmic

Drugs to Target Receptor Tyrosine Kinases

Monoclonal Antibodies

Extracellular domain

Tyrosine-kinase domains

HER2

HER2

HER2

EGFR

ATP-mimetic Tyrosine Kinase Inhibitors

Homodimer

Heterodimer
Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib

Figure 1. Example of the Response to Gefitinib in a Patient with Lung Cancer. A computed tomographic scan of the chest in Patient 6 shows marked shrinkage of cancer and improved lung aeration with Gefitinib.
Her2/neu and Breast Cancer

• Her2 first identified as an oncogene from a carcinogen-induced rat brain tumor model.

• Her2 is gene amplified in about 25% of human breast cancers.

• Overexpression of Her2 in the mammary gland of transgenic mice causes breast cancer.

• Herceptin, a monoclonal antibody to Her2/neu, effectively treats Her2 gene amplified human breast cancer.
Therapeutic Antibodies Target Her2

ErbB2

Herceptin

ErbB2

Pertuzumab

Cho et al. (2003) Nature

Franklin et al. (2004) Cancer Cell
Successful treating Her2 amplified Breast Cancer

• The combination of chemotherapy (AC→T) plus Herceptin markedly improves patient survival as compared to chemotherapy alone.

• Treatment of women with Herceptin has saved THOUSANDS of lives.

![Graph showing disease free survival rates for AC→TH and AC→T treatments]

- AC→TH: 87% at 2 years, 85% at 5 years
- AC→T: 75% at 2 years, 67% at 5 years

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Events</th>
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<tr>
<td>AC→T</td>
<td>1679</td>
<td>261</td>
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<tr>
<td>AC→TH</td>
<td>1672</td>
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p < 0.0001
Outline – Part 2

1. Nuclear Hormone Receptors

2. Cytokine Receptors – JAK/STAT Pathway

3. PI3-kinase – Akt – mTOR

4. Regulation of Protein Kinases
Resources: Nuclear Hormone Receptors

https://www.nursa.org/nursa/index.jsf

Online Course:

# Nuclear Hormone Receptor Superfamily

1. 48 Human genes
2. Major Categories:

<table>
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<tr>
<th>Thyroid Hormone Receptor (TR)- like</th>
<th>TR, RAR, PPAR, Vitamin D receptor, LiverX Receptor</th>
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</thead>
<tbody>
<tr>
<td>Estrogen Receptor (ER)- like</td>
<td>ER, PR, AR, Estrogen Receptor Related, Glucocorticoid receptor, Mineralocorticoid receptor</td>
</tr>
<tr>
<td>Retinoid X Receptor (RXR) like</td>
<td>RXR, Hepatocyte nuclear factor-4, etc.</td>
</tr>
</tbody>
</table>

Knock-out in mice causes reproductive, developmental, or metabolic abnormalities.
| DNA Binding Domain | Ligand Binding Domain |
AF: Activation Function.
Mediate transcriptional activation

Zinc Finger DNA Binding Domain

Ligand binding domain (LBD)
Hormone response elements are inverted repeats.

Hormone response elements are direct repeats.
Ligand Present

Ligand Absent

Activation

mRNA

Protein

Repression
Movie: https://nursa.org/nursa/about/tutorial.jsf
Tab 12. Nuclear Hormone Action Model

Nuclear Receptor Signaling: Concepts and Models

12. Nuclear Receptor Action Model

INTRODUCTION

NR SIGNALING

3. Introduction
4. Identification and Cloning of Nuclear Receptors
5. The Nuclear Receptor Superfamily
6. Functional Domains of Nuclear Receptors
7. Coregulators
8. Cloning and Characterization of Coactivators
9. Corepressors
10. Coregulators in vivo
11. Regulating Coregulators
12. Nuclear Receptor Action Model
13. Rational Drug Design
14. Self Test Quiz

RESOURCES
Outline

1. Nuclear Hormone Receptors
2. Cytokine Receptors – JAK/STAT Pathway
3. PI3-kinase – Akt – mTOR
4. Regulation of Protein Kinases
Cytokine Receptors

Baker et al., Oncogene (2007) 26, 6724–6737
JAK = Janus kinases

4 genes in humans and mice
- TYK2 (first gene in this family to be identified)
- JAK1, JAK2, JAK3

STAT= Signal Transducers and Activators of Transcription

Cytokine Receptors – JAK/STAT Pathway

Receptor Tyrosine Kinases
Examples – EGFR, Her2, etc
Outline

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PI3-kinase – Akt – mTOR

PI3-kinase – Akt

PtdIns(4,5)P$_2$ (PIP2) $\leftrightarrow$ PtdIns(3,4,5)P$_3$ (PIP3) $\rightarrow$ Akt

PI3K $\rightarrow$ PtdIns(3,4,5)P$_3$ (PIP3) $\rightarrow$ PDK1

PTEN $\leftarrow$ PtdIns(4,5)P$_2$ (PIP2)

**mTOR complexes**

**mTORC1**
- Rapamycin sensitive
- Responds to nutrient level, growth factors, energy, and stress.

**mTORC2**
- NOT rapamycin sensitive
- Inputs into mTORC2 less well known.

mTORC1 substrates

- S6 kinase 1 (S6K1)
- eIF-4E binding protein (4E-BP)

mTORC2 substrates

Akt

mTORC2

PDK1

PH domain

Kinase Domain

Downstream substrates: TSC complex, PRAS40, etc.

Bringing it all together

mTOR is a signal integrator, like the chips and circuits in your smart phone

1. Nuclear Hormone Receptors
2. Cytokine Receptors – JAK/STAT Pathway
3. PI3-kinase – Akt – mTOR
4. Regulation of Protein Kinases
More information available at:
http://kinase.com/web/current/

Manning et al., Science 2002
Regulation of Protein Kinases

1. Post-translation modifications.
   - Phosphorylation-dependent
   - Activation Loop

2. Protein-protein interactions
   - Regulatory Subunits
   - Dimers
Structure of PKA catalytic domain

α Helices

N-lobe

C Helix

β Sheets

C-lobe

Caplan, Science STKE 2005
Structural features of the PKA Activation Loop
Phosphorylation of the MAP Kinase activation loop

- Phosphorylation on threonine and tyrosine
- Phospho-Thr 183 contacts $\alpha$-C and promotes active conformation
- Phospho-Thr 183 promotes ERK2 dimerization via conformational changes in C-terminal extension


AKT phosphorylation at T308 is also Activation Loop Phosphorylation
MAP Kinase Structure

Unphosphorylated

Phosphorylated

Canagarajah et al Cell 90, 859-869 (1997)
MAP Kinase Structure

Unphosphorylated

Phosphorylated

Canagarajah et al Cell 90, 859-869 (1997)
Cyclin - Cyclin-dependent kinase (CDK) Complex

Cyclin A

Cyclin-dependent kinase (Cdk2)


Cdk2

Cdk2.CyclinA

C-helix

Activation Loop

Activation Loop
Asymmetric Dimer Formed by the EGFR Kinase Domain

1. Nuclear hormone receptors consist of a DNA-binding domain and ligand-binding domain.

2. Cytokine receptors signal through the JAK kinases, which have 2 kinase domains, and the STAT transcription factors.

3. mTOR is a signal integrator for metabolic and growth factor signaling.

4. Protein kinases are regulated by PTM’s and protein-protein interactions.