Signal Transduction Pathways in Human Diseases

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# Introduction

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The EGFR family of Receptor Tyrosine Kinases

EGFR  Her2/neu  Her3  Her4

- Extracellular
- Transmembrane
- Cytoplasmic

Kinase Domain

C-terminal tail

ErbB Receptor Homo- or Heterodimerization

from Marmor, Skaria, and Yarden 2004
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.
Her2/neu and Breast Cancer

- 1987 – Southern blots of genomic DNA from breast cancer patients shows Her2 gene amplification.
  - Sample 3 & 4: normal level
  - Sample 1 & 2: 2-5 x normal
  - Sample 6 & 26: >5 x normal
  - Sample 18: > 20 x normal

- Correlation between Her2 gene copy number and patient survival

Slamon, et al., Science 1987
Her2/neu and Breast Cancer

- Transgenic mice bearing the MMTV-Her2/neu construct develop breast cancer in all 5 pairs of mouse mammary glands.

- Tumor formation with Her2 in this tg model is more rapid than with the Myc oncogene.

Muller et al., Cell 1988
Drugs to Target Receptor Tyrosine Kinases

Homodimer

Heterodimer

Monoclonal Antibodies

Extracellular domain

Tyrosine-kinase domains

HER2

HER2

HER2

HER2

EGFR

ATP-mimetic Tyrosine Kinase Inhibitors
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.
Alterations of ErbB receptors in Cancer

Overexpression/Gene Amplification

• Her2/neu - 25% of breast cancers, rarer in gastric and ovarian cancers.
• EGFR – many cancers including lung, breast, GI, ovarian, brain

Mutations

• EGFR del E746-A750
• EGFR L858R
  Both found in Lung cancer cases from Non-smokers. These cancers respond very well to Gefitinib (an anti-EGFR tyrosine kinase inhibitor).
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.
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

- The BCR-ABL fusion protein activates several signaling pathways.
- Introduction of BCR-ABL gene in mice causes myeloid leukemias.

Druker, Blood 2008
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, reducing the death rate to only 270 deaths in the U.S. in 2011 (about 5% of incidence rate).

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

Targeting MAPK pathway in Cancer

(A) 
plasma membrane \\ TK 
Grb2 
P SH2 SH3 
Sos 

(B) 
plasma membrane \\ TK 
Grb2 
P Shc P SH2 SH3 
Sos 

(C) 
Ras-GTP 
\downarrow 
Raf (MAPKKK) 
\downarrow 
MEK (MAPKK) 
\downarrow 
Erk 1 & 2 (MAPK) 

Raf inhibitors 

MEK inhibitors 

Figure 6.12 The Biology of Cancer (© Garland Science 2007)
Targeting the PI 3-kinase/Akt pathway

From Engelman, Luo, and Cantley, Nature Reviews Genetics 2006
Nuclear Hormone Receptors

- Nuclear hormone receptors bind ligands like steroids, thyroid hormone, retinoids, etc.
- They have DNA binding and hormone binding domains.
- Ligand binding causes transcriptional de-repression or activation.

In the absence of T3, thyroid hormone receptor (TR) heterodimerizes with retinoid X receptor (RXR), binds to a thyroid hormone response element (TRE) and recruits co-repressor (CoR), resulting in gene silencing.

The RARα Nuclear Hormone Receptor in Acute Promyelocytic Leukemia (APL)

- APL has a characteristic translocation 15;17 that forms the PML-RARα fusion protein.
- Retinoic Acid (RA) binding converts PML-RARα from a transcriptional repressor to a transcriptional activator.
- All-trans retinoic acid (ATRA) has made APL the most treatable and best prognosis form of adult acute leukemia.
G-Protein Coupled Receptors in Melanomas

- Metabotropic glutamate receptors (mGluR) are GPCR’s.
- mGluR1 or mGluR5 transgenic mice develop melanomas.
- Other GPCR’s implicated in cancer:
  - Chemokine receptors: CXCR2 and CXCR4
  - Endothelin receptors

Marin and Chen, J. Mol Med 2004
Signaling in Heart and Kidney Diseases

1. β-Adrenergic signaling via GPCR’s

2. Renin-Angiotensin signaling via the Angiotensin II receptor

3. Erythropoietin signaling in anemia of chronic renal disease.
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 β2 and β3 receptors.
β-Adrenergic Receptors stimulate cAMP production

Gαs

Adenylate Cyclase

ATP → cAMP

Protein Kinase A (PKA)
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 $\beta_2$ adrenergic agonist.
Downregulation/Desensitization of β-Adrenergic Receptor

McDonald and Lefkowitz, Cell Signaling 2001
GPCR signaling Controls Blood Pressure via the Renin-Angiotensin System

Angiotensinogen

\[ \text{Angiotensinogen} \rightarrow \text{Renin} \ (\text{kidney}) \]

Angiotensin I

\[ \text{Angiotensin I} \rightarrow \text{ACE} \ (\text{lung}) \ (\text{Angiotensin Converting Enzyme}) \]

Angiotensin II

\[ \text{Angiotensin II} \rightarrow \text{Angiotensin II Receptor (GPCR)} \]

Common Blood Pressure Medicines

- **ACE inhibitors**

- **Angiotensin Receptor Blockers**
GPCR signaling Controls Blood Pressure via the Renin-Angiotensin System

Timmermanns, Pharm Review 1993
Other Second Messengers

- Cyclic GMP
- IP₃
- Small Lipids like:
  - Diacylglycerol
  - Ceramide
Cytokine Receptors activate the JAK-STAT pathway

Cytokine receptor

JAK tyrosine kinase (a cytoplasmic tyr kinase)

STAT transcription factors shuttle from cytoplasm to nucleus

Nucleus

DNA
Erythropoietin (EPO) binds to a Cytokine Receptor

Munugalavadla and Kapur, Reviews in Onc-Hem, 2005
Chronic kidney disease causes a fall in EPO secretion and this results in decreased red blood cell production (i.e.- anemia). Therefore patients with chronic kidney disease are given recombinant EPO to prevent anemia.
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

TXA₂

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 (ð, 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
Summary

1. Signaling pathways play central roles in cell functioning and in human physiology.

2. Altered signaling pathways cause or contribute to human diseases.

3. Many drugs that are given to people directly affect signal transduction pathways.