Sustaining proliferative signaling

Resisting cell death

Evading growth suppressors

Inducing angiogenesis

Activating invasion and metastasis

Enabling replicative immortality

Hanahan and Weinberg, 2011
The Development and Metastasis of Human Colorectal Cancer and its Genetic Basis

Lodish et al., Molecular Cell Biology
Signal transduction is the process of converting extracellular signals into cellular responses
Signal Transduction

- Signal transduction was initially viewed as a collection of linear information transporting pipelines.

- Now we know that pathway crosstalk enables signals to propagate through a tangled network of interconnecting networks and cascades.

- Recent findings from genome research reveal a new problem: there are fewer genes than biological processes.

- Spatiotemporal control is an important means by which Signal transduction is regulated. Our understanding in this area is lagging behind.
Integrated Circuit of the Cell

Hanahan and Weinberg, 2000
Figure 5.1  *The Biology of Cancer* (© Garland Science 2007)
General Schemes of Intercellular Signaling

(a) Endocrine signaling

(b) Paracrine signaling

(c) Autocrine signaling

(d) Signaling by plasma membrane-attached proteins
Structure of the Epidermal Growth Factor (EGF) Receptor

- EGF receptor
- ligand binding
- ectodomain
- transmembrane domain
- cytoplasmic domain
- C-terminal tail
- region of homology
- Src

621 aa  23 aa  542 aa
The EGF Receptor and v-ErbB (Oncoprotein Specified by erbB Oncogene of Avian Erythroblastosis Virus)
**erb-B Receptor family**

- Derives its name from its sequence homology with the avian erythroblastosis retroviral oncogene v-erb-B
- The receptors of the family are also frequently referred to as HERs: **Human EGF Receptor**
- Members of this family include EGFR/HER1, HER2, HER3, and HER4

**EGFR: Epidermal Growth Factor Receptor**
Structure of Membrane Tyrosine Kinase Receptors

Figure 5.10 The Biology of Cancer (© Garland Science 2007)
PDGF was found to be closely related in sequence to the v-sis oncogene of simian sarcoma virus.

Simian sarcoma virus transform fibroblasts, but not epithelial cells.
-because PDGF receptor is expressed on the surfaces of mesenchymal cells, and not on epithelial cell surface.
Effect of Platelet-Derived Growth Factor (PDGF) on Cells

Wt. Fibroblasts
PDGFR+

Mut. Fibroblasts
PDGFR-
(PDGF receptor lost)
### Table 5.2 Tyrosine kinase GF receptors altered in human tumors

<table>
<thead>
<tr>
<th>Name of receptor</th>
<th>Main ligand</th>
<th>Type of alteration</th>
<th>Types of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF-R/ErbB1</td>
<td>EGF, TGF-α</td>
<td>overexpression</td>
<td>non-small cell lung cancer; breast, head and neck, stomach, colorectal, esophageal, prostate, bladder, renal, pancreatic, and ovarian carcinomas; glioblastoma</td>
</tr>
<tr>
<td>EGF-R/ErbB1</td>
<td>NRG, EGF</td>
<td>truncation of ectodomain overexpression</td>
<td>glioblastoma, lung and breast carcinomas</td>
</tr>
<tr>
<td>ErbB2/HER2/Neu</td>
<td>various</td>
<td>overexpression</td>
<td>30% of breast adenocarcinomas</td>
</tr>
<tr>
<td>ErbB3, 4</td>
<td>FL</td>
<td>tandem duplication amino acid substitutions</td>
<td>oral squamous cell carcinoma</td>
</tr>
<tr>
<td>Kit</td>
<td>SCF</td>
<td>fusion with other proteins, point mutations</td>
<td>acute myelogenous leukemia</td>
</tr>
<tr>
<td>Ret</td>
<td></td>
<td></td>
<td>gastrointestinal stromal tumor</td>
</tr>
<tr>
<td>FGF-R3</td>
<td>FGF</td>
<td>overexpression; amino acid substitutions</td>
<td>papillary thyroid carcinomas, multiple endocrine neoplasias 2A and 2B</td>
</tr>
</tbody>
</table>

See also Figure 5.17.
How do Receptor Tyrosine Kinases (RTKs) Transduce Signals from the Extracellular Space into the Cytoplasm of Cells?
Receptor Dimerization Following Ligand Binding
Deregulation of Receptor Activation

- Normal cell:
  - GF binding to receptor
  - Ligand binding
  - Tyrosine kinase domain
  - Ligand-dependent firing

- Cancer cell:
  - Mutations affecting structure
  - Overexpression
  - Ligand-independent firing

Figure S.11a The Biology of Cancer © Garland Science 2014
Deregulation of Receptor Activation

normal cell

autocrine signaling in cancer cell

activation of GF gene
Autocrine Signaling in Cancer

Certain lung cancers produce:
Growth factors such as tumor growth factor α (TGFα),
stem cell factor (SCF),
Insulin-like growth factor (IGF).

These cancers also express the receptors for these ligands:
EGF receptor, Kit, and IGF-1 receptor.
Autocrine Signaling in Kaposi’s Sarcoma

• In Kaposi’s sarcoma (discovered by Moritz Kaposi, 1872; caused by human herpes virus 8 (HHV-8); a tumor of cells closely related to the endothelial cells that form lymph ducts), the tumor cells produce PDGF, TGF-β, IGF-1, Ang2, CC18/14, CXCL11, and endothelin (ligands of cellular origin) as well as receptors for these ligands.

• Also, the HHV-8 genome produces two additional ligands-vIL6 (viral IL6) and vMIP (viral macrophage inflammatory protein), and their receptors
### Table 5.3 Examples of human tumors making autocrine growth factors

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Receptor</th>
<th>Tumor type(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF</td>
<td>Met</td>
<td>miscellaneous endocrinial tumors, invasive breast and lung cancers, osteosarcoma</td>
</tr>
<tr>
<td>IGF-2</td>
<td>IGF-1R</td>
<td>colorectal</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL-6R</td>
<td>myeloma, HNSCC</td>
</tr>
<tr>
<td>IL-8</td>
<td>IL-8R A</td>
<td>bladder cancer</td>
</tr>
<tr>
<td>NRG</td>
<td>ErbB2/ErbB3</td>
<td>ovarian carcinoma</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>PDGF-Rα/β</td>
<td>osteosarcoma, glioma</td>
</tr>
<tr>
<td>PDGF-C</td>
<td>PDGF-α/β</td>
<td>Ewing's sarcoma</td>
</tr>
<tr>
<td>PRL</td>
<td>PRL-R</td>
<td>breast carcinoma</td>
</tr>
<tr>
<td>SCF</td>
<td>Kit</td>
<td>Ewing's sarcoma, SCLC</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>VEGF-R (Flt-1)</td>
<td>neuroblastoma, prostate cancer, Kaposi’s sarcoma</td>
</tr>
<tr>
<td>TGF-α</td>
<td>EGF-R</td>
<td>squamous cell lung, breast and prostate adenocarcinoma, pancreatic, mesothelioma</td>
</tr>
<tr>
<td>GRP</td>
<td>GRP-R</td>
<td>small-cell lung cancer</td>
</tr>
</tbody>
</table>

*Also known as HER2 or Neu receptor.*
Deregulation of Receptor Firing: Autocrine Signaling in Human Breast Carcinoma

Over-expression of EFG receptor (EGFR)

Over-expression of TGF-α (ligand of EGFR)

Superimposed image
Important Features of Membrane Receptor Tyrosine Kinases (RTKs)

- Lateral mobility in the membrane
- Dimerization
- Autophosphorylation
**erb-B Receptor family**

- Derives its name from its sequence homology with the avian erythroblastosis retroviral oncogene *v-erb-B*
- The receptors of the family are also frequently referred to as HERs, based on their homology to the Human EGF Receptor
- Members of this family include EGFR/HER1, HER2, HER3, and HER4
- **HER2 is the preferred dimerization partner for all erb-B receptors**

EGFR: Epidermal Growth Factor Receptor
Erb-B Receptor Family, Ligands and Dimers

AR, amphiregulin; BTC, betacellulin; EPR, epieregulin; HB-EGF, heparin-binding EGF; NRGs, neuregulins; TGF, transforming growth factor-α.
Factors Contributing to Abnormal HER2 Signaling

1. Receptor gene amplification

2. Increased receptor gene transcription

3. Mutations in the receptor

4. Increased production of growth factor (ligand)

5. Increased life-time of receptor at the cell surface
Overexpression of HER2 can Result from Increased DNA, RNA, or Protein
HER2 Amplification is Associated with Poor Patient Prognosis (Kaplan-Meir Plot)

D. J. Slamon et al., Science 235:177-182
Figure 4.4 B  The Biology of Cancer (© Garland Science 2014)
Chimeric Receptors Leading to Aberrant Signaling

Figure 5.16 The Biology of Cancer (© Garland Science 2014)
How Cells Communicate with their Surroundings

Figure 5.1  The Biology of Cancer (© Garland Science 2007)
Complexity of Signaling Circuitry

• Of the Approximately 22,000 genes in the human genome, 518 genes encode various types of protein kinases; 40% of these genes generate alternatively spliced mRNAs encoding slightly different variant structures of kinase proteins, leading to more than 1,000 distinct kinase proteins.

• Of the 518 kinase genes, 90 encode tyrosine kinases, the remainder being serine/threonine kinases.

• Among the 90 tyrosine kinases, 58 encode proteins with general structures of the EGF and PDGF receptors (ligand-binding ectodomain, a transmembrane domain, and a cytoplasmic tyrosine kinase domain).
Each protein component of a signaling circuit must solve two problems:

(1) **Specificity**: how can it exchange signals only with the small subset of cellular proteins that are its intended signaling partners in the circuit?

(2) How can this protein acquire rapid, almost instantaneous access to these signaling partners, doing so while operating in the viscous soup in the cytoplasm and nucleus?
Domain Structure of the Src Protein
The human genome encodes at least 117 distinct SH2 groups each constituting a domain of a larger protein and each apparently having an affinity for binding a particular phosphotyrosine with a flanking peptide sequence.
Structure and Function of SH3 Groups

Figure 6.8c: The Biology of Cancer (© Garland Science 2014)
Protein Interaction Domains as Modular Units of Protein Structure

Figure 6.10a  The Biology of Cancer (© Garland Science 2014)
Table 6.2 Binding domains that are carried by various proteins

<table>
<thead>
<tr>
<th>Name of domain</th>
<th>Ligand</th>
<th>Example of proteins carrying this domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH2</td>
<td>phosphotyrosine</td>
<td>Src (tyrosine kinase), Grb2 (adaptor protein), Shc (scaffolding protein), SHP2 (phosphatase), Cbl (ubiquitylation)</td>
</tr>
<tr>
<td>PTB</td>
<td>phosphotyrosine</td>
<td>Shc (adaptor protein), IRS-1 (adaptor for insulin RTK signaling), X11 (neuronal protein)</td>
</tr>
<tr>
<td>SH3 14-3-3</td>
<td>proline-rich phosphoserine</td>
<td>Src (tyrosine kinase), Crk (adaptor protein), Grb2 (adaptor protein), Cdc25 (CDK phosphatase), Bad (apoptosis regulator), Raf (ser/thr kinase), PKC (protein kinase C ser/thr kinase)</td>
</tr>
<tr>
<td>Bromo PHb</td>
<td>acetylated lysine</td>
<td>P/CAF (transcription co-factor), chromatin proteins</td>
</tr>
<tr>
<td></td>
<td>phosphorylated inositides</td>
<td>PLC-8 (phospholipase C), Akt/PKB (ser/thr kinase), BTK</td>
</tr>
</tbody>
</table>

*AAt least 32 distinct types of binding domains have been identified. This table presents six of these that are often associated with transduction of mitogenic signals.

bThe phosphoinositide-binding groups include, in addition to the PH domain, the Fab1, YOTB, Vac1, EEA1 (FYVE), PX, ENTH, and FERM domains.*
Receptor Dimerization and Autophosphorylation Following Ligand Binding

Figure 5.15  *The Biology of Cancer* (© Garland Science 2007)
Formation of Phosphotyrosine on the EFG-R Following Ligand Addition

Inhibitor (AG1478) of EGFR Kinase

Red: Below basal level
Blue: Above basal level

Figure 5.13  The Biology of Cancer (© Garland Science 2007)
Attraction of Signal Transducing Proteins by Phosphorylated Receptors
Which proteins are affected thereafter by the receptor phosphorylation events?

How does this phosphorylation lead further to a mitotic response by the cell – its entrance into a phase of active growth and division?
The Ras Signaling Cycle

GTP hydrolysis and Ras inactivation induced by GAP

blockage caused by oncogenic mutation

upstream stimulatory signal and Ras activation triggered by GEF (Guanine nucleotide Exchange Factor)

GAP (GTPase Activating Protein)

ACTIVE downstream signaling

Figure 5.30  The Biology of Cancer (© Garland Science 2007)
Ras Activation by GEF

Receptor $\rightarrow$ Grb2 $\rightarrow$ Sos $\rightarrow$ Ras or Receptor $\rightarrow$ Shc $\rightarrow$ Grb2 $\rightarrow$ Sos $\rightarrow$ Ras
Ras Activation

1. Binding of hormone causes dimerization and autophosphorylation of tyrosine residues.
2. Binding of GRB2 and Sos couples receptor to inactive Ras.
3. Sos promotes dissociation of GDP from Ras; GTP binds and Sos dissociates from active Ras.

Lodish et al., Molecular Cell Biology
The Structure of the Ras Protein
The Ras Effector Loop

Figure 6.13  *The Biology of Cancer* (© Garland Science 2007)
The Ras—Raf—Map kinase Pathway

Figure 6.14 The Biology of Cancer (© Garland Science 2014)
Activation of Ras Pathway Following Binding of a Hormone/Ligand (e.g., EGF) to An RTK
Activation of Ras Pathway Following Binding of A Hormone (e.g., EGF) to An RTK
(From Cell Membrane to the Nucleus)
Expression of Immediate Early Genes - Analyzed by Gene Expression Array

Figure 6.2  *The Biology of Cancer* (© Garland Science 2007)
Activity of Two Proto-Oncogene Products Following Serum Stimulation of Quiescent Cells

Lodish et al., Molecular Cell Biology
The PI3 Kinase Pathway

- PI3K
  - PI3K
    - PI(3,4,5)P3
      - Akt/PKB
      - Rho-GEFs
        - Bad inhibition of apoptosis
        - mTOR stimulation of protein synthesis (cell growth)
        - GSK-3β stimulation of cell proliferation
        - FOXO cell-cycle progression; inhibition of apoptosis
  - Raf (MAPKKK)
    - MEK (MAPKK)
      - Erk1 or 2 (MAPK)
      - Msk1/RSK
      - Mnk1
      - Ets/Elk-1/SAP-1
      - elf4E (protein synthesis)
      - chromatin remodeling

- Ras
  - Ras-GTP
  - Raf (MAPKKK)
    - MEK (MAPKK)
      - Erk1 or 2 (MAPK)
      - Msk1/RSK
      - Mnk1
      - Ets/Elk-1/SAP-1
      - elf4E (protein synthesis)
      - chromatin remodeling

- RaI-GEFs
  - RaIA
  - RaIB
  - Sec5
  - Exo84
  - RaIBP1
    - Cdc42
    - Rac
    - filopodia
    - lamellipodia

Figure 6.15 The Biology of Cancer (© Garland Science 2014)
Figure 6.16 The Biology of Cancer (© Garland Science 2014)
Activation of PI3K by Ras

PI3K

catalytic domain
N-terminal lobe

PI3K

RBD

Ras

catalytic domain
C-terminal lobe

Figure 6.17  The Biology of Cancer (© Garland Science 2007)
Docking of PH (Peckstrin Homology) Domains of Akt/PKB to PIP3

Figure 6.19 The Biology of Cancer (© Garland Science 2014)
New Somatic Mutation in AKT Identified

A somatic mutation in human breast, colorectal, and ovarian cancers that results in glutamic acid to lysine substitution at aminoacid 17 (E17K) in the lipid-binding pocket (that includes the PH domain) of AKT1 has been identified. This results in activation of AKT by pathological localization to the plasma membrane stimulating downstream signaling, transforms cells and induces leukemia in mice.

This mutation decreases the sensitivity to an allosteric kinase inhibitor.
Phosphorylation by Serine/Thrreonine Kinase Akt/PKB

Figure 5.7b  *The Biology of Cancer* (© Garland Science 2007)
<table>
<thead>
<tr>
<th>Biological effect</th>
<th>Substrate of Akt/PKB</th>
<th>Description</th>
<th>Functional consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-apoptotic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad (pro-apoptotic)</td>
<td>Bcl-X antagonist; like Bad, belongs to Bcl-2 protein family controlling mitochondrial membrane pores (Section 9.13).</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td>caspase-9 (pro-apoptotic)</td>
<td>Component of the protease cascade that affects the apoptotic program (Section 9.13).</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td>IκB kinase, abbreviated IKK (anti-apoptotic)</td>
<td>Activated by Akt/PKB phosphorylation (Section 6.12.).</td>
<td>activation</td>
<td></td>
</tr>
<tr>
<td>FOXO1 TF, formerly called FKHR TF (pro-apoptotic)</td>
<td>Phosphorylation prevents its nuclear translocation and activation of pro-apoptotic genes.</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td>Mdm2 (anti-apoptotic)</td>
<td>Activated via phosphorylation by Akt/PKB; it triggers destruction of p53 (Section 9.7).</td>
<td>activation</td>
<td></td>
</tr>
<tr>
<td><strong>Proliferative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSK-3β (anti-proliferative)</td>
<td>Phosphorylates β-catenin, cyclin D1, and Myc (Sections 7.11, 8.3, 8.9), causing their degradation; inactivated via phosphorylation by Akt/PKB.</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td>FOXO4, formerly called AFX (anti-proliferative)</td>
<td>Induces expression of the CDK inhibitor p27kip1 (Section 8.4) gene and some pro-apoptotic genes; exported from the nucleus when phosphorylated by Akt/PKB.</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td>p21cip1 (anti-proliferative)</td>
<td>CDK inhibitor, like p27kip1 (Section 8.4). Exits the nucleus upon phosphorylation by Akt/PKB; in the cytoplasm, phosphorylated p21cip1 inhibits caspases, thereby acquiring anti-apoptotic functions (Section 9.13).</td>
<td>inhibition</td>
<td></td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsc2 (anti-growth)</td>
<td>Phosphorylation by Akt/PKB causes Tsc1/Tsc2 complex to dissociate, allowing activation of mTOR, which then up-regulates protein synthesis (Section 16.15).</td>
<td>inhibition</td>
<td></td>
</tr>
</tbody>
</table>
Akt/PKB and the Control of Cell Growth

A. Normal Pancreatic Islet

B. Pancreatic Islet with Constitutively Active Akt/PKB kinase

Figure 6.20 A&B The Biology of Cancer (© Garland Science 2007)
### Table 6.4 Alteration of the PI3K pathway in human tumors

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Akt/PKB hyperactive</th>
<th>PIK3CA hyperactive</th>
<th>p85α</th>
<th>PTEN-mutant or repressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma</td>
<td>6–27%</td>
<td></td>
<td>8%</td>
<td>20%</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>-2%</td>
<td>4–12%</td>
<td>4%</td>
<td>8%</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>22%</td>
<td></td>
<td></td>
<td>42–54%</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>6–36%</td>
<td></td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>-80%</td>
<td>-9%</td>
<td>40–50%</td>
<td></td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>3–4%</td>
<td></td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>3%</td>
<td></td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>5%</td>
<td></td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>3%</td>
<td></td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>2%</td>
<td></td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>-6%</td>
<td>14–32%</td>
<td>2–8%</td>
<td>13–54%</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>-8%</td>
<td>18–40%</td>
<td>2%</td>
<td>20–33%</td>
</tr>
<tr>
<td>Bladder</td>
<td>23%</td>
<td></td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Pancreatic</td>
<td>25%</td>
<td></td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Gastric</td>
<td></td>
<td></td>
<td>8%</td>
<td></td>
</tr>
</tbody>
</table>

*aThe percentages in this table are approximate, since the proportion of tumors bearing the indicated alteration increases progressively as tumor progression proceeds, often dramatically, and because many reports do not distinguish between inactivation by mutation and inactivation by promoter methylation.

*bPIK3CA appears to be the only gene of the 16 members of the PI3K-encoding gene family to undergo somatic mutation during tumor development. These mutations affect the p110 catalytic subunit of PI3 kinase; frequently occurring amplifications of this gene are not registered in this table.

*cPI3KRI mutations affect the regulatory subunit of PI3K kinase and are most commonly observed in human cancers; alterations of the four other members of this family of PI3K regulatory subunits are not registered here. Alterations of the encoded p85α subunit cited here were few in number and the indicated percentages are likely to change dramatically as more data are collected.

*dPTEN nonsense mutations and deletions are registered here and, in many cases, the even more frequent shutdown of expression through promoter methylation or the actions of microRNAs. (Promoter methylation often results in shutdown of transcription of a gene; see Section 7.8.)


---

Table 6.4 The Biology of Cancer (© Garland Science 2014)
Viral Strategy to Subvert Cellular Signaling
Autocrine Signaling in Kaposi’s Sarcoma

- In Kaposi’s sarcoma (discovered by Moritz Kaposi, 1872; caused by human herpes virus 8 (HHV-8); a tumor of cells closely related to the endothelial cells that form lymph ducts), the tumor cells produce PDGF, TGF-β, IGF-1, Ang2, CC18/14, CXCL11, and endothelin (ligands of cellular origin) as well as receptors for these ligands.

- Also, the HHV-8 genome produces two additional ligands-vIL6 (viral IL6) and vMIP (viral macrophage inflammatory protein), and their receptors
E6 protein inhibits p53
E7 protein inhibits Rb
E5 protein causes sustained activation of PDGF receptor

Estrogen increases E5’s ability to cause cervical cancer
Signal Transduction and Cancer Therapy
Inhibition of Tumor Growth by Targeting Signaling Proteins

- JAK inhibitors
- Src inhibitors
- PDK1 inhibitors
- mTOR inhibitors
- Akt inhibitors
- PDK1
- mTOR
- Akt
- PI3K
- MEK1/2
- MAPK
- anti-ErbB2 (HER2) receptor MoAbs
- anti-HER1, HER2, HER4 tyrosine kinase inhibitors
- RAS farnesyltransferase inhibitors
- PI3K inhibitors
- RAF inhibitors
- BTK inhibitors

Cell cycle progression
Survival
Proliferation
<table>
<thead>
<tr>
<th>Agent</th>
<th>Molecular target</th>
<th>Mechanism of action</th>
<th>Highest development status (protocol number or NCT ID number)</th>
<th>Licensed indications</th>
</tr>
</thead>
</table>
| Trastuzumab (Herceptin; Genentech) | ERBB2 | Suppression of ERBB2 signalling, ERBB2 stabilization, marks cells for immunological attack | * Launched for breast cancer  
* ERBB2-positive gastric cancer — Phase III (BO10255) | ERBB2-positive metastatic breast cancer, ERBB2-positive early breast cancer |
| Pertuzumab (Genentech/ Hoffmann-La Roche) | ERBB2 | Dimerization inhibitor, marks cells for immunological attack | * Breast cancer — Phase III (NCT00567190)  
* Ovarian cancer — Phase II (NCT00096993, NCT00058552) | None |
| Trastuzumab—DM1 (Genentech) | ERBB2 | Targeted delivery of a potent anti-microtubule cytotoxic agent | Breast cancer — Phase III (NCT00829166) | None |
| Ertuxamomab (Fresenius Biotech GmbH) | ERBB2 | Bispecific affinity allows recruitment of T cells | Breast cancer — Phase II (NCT00351858, NCT00522457, NCT00452140) | None |
| AMG 888 or U3-1287 (Amgen) | ERBB3 | Not yet defined | Phase I (NCT00730470) | None |
| **TKIs** | | | | |
| Lapatinib (Tykerb; GlaxoSmithKline) | ERBB2 | TKI | * Launched for breast cancer  
* ERBB2-positive gastric cancer — Phase III (NCT00486954, NCT00660901)  
* NSCLC — Phase II (NCT00528581)  
* Head and neck cancer — Phase II (NCT00490061, NCT00367127, NCT00424255)  
* Colorectal adenocarcinoma — Phase II (NCT00574171) | In combination with capecitabine for advanced ERBB2-positive breast cancer previously treated with an anthraclycine, a taxane or trastuzumab |
| HKI-272 (Wyeth) | EGFR, ERBB2 | Irreversible TKI | Breast cancer — Phase III (NCT00777101) | None |
| ARRY-334543 (Array BioPharma) | EGFR, ERBB2, ERBB4 | Reversible TKI | Breast cancer — Phase II (NCT00710736) | None |
| BIBW-2992 (Boehringer Ingelheim) | EGFR, ERBB2 | Irreversible TKI | * Breast cancer — Phase II (NCT00425854, NCT00326267, NCT00705214)  
* NSCLC — Phase III (NCT00425854, NCT00326267, NCT00705214)  
* Head and neck cancer — Phase II (NCT00514943) | None |
| **Heat-shock protein inhibitors** | | | | |
| 17-AAG (Bristol-Myers Squibb) | HSP90 | Inhibitory activity reduces the stability of ERBB2, causes abrogation of ERBB2 signalling | * Multiple myeloma — Phase III (NCT00514371)  
* Breast cancer — Phase II (NCT00817362) | None |
| IPI-504 (Infinity Pharmaceuticals) | HSP90 | Inhibitory activity reduces the stability of ERBB2, causes abrogation of ERBB2 signalling | * Multiple myeloma — Phase II and III (NCT00514371)  
* Breast cancer — Phase II (NCT00817362)  
* NSCLC — Phase II (NCT00431015)  
* Melanoma — Phase II (NCT00087386)  
* Ovarian cancer — Phase II (NCT00093496) | None |

EGFR, epidermal growth factor receptor; HSP90, heat shock protein 90; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor.
### Cancer drugs that act against tyrosine kinases

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cancer</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small-molecule drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imatinib (Gleevec)</td>
<td>Leukemia (CML)</td>
<td>BCR-ABL tyrosine kinase</td>
</tr>
<tr>
<td>Gefitinib (Iressa)</td>
<td>Lung cancer</td>
<td>EGFR tyrosine kinase</td>
</tr>
<tr>
<td>Erlotinib (Tarceva)</td>
<td>Lung cancer</td>
<td>EGFR tyrosine kinase</td>
</tr>
<tr>
<td>Sunitinib (Sutent)</td>
<td>GIST/renal carcinoma</td>
<td>KIT receptor tyrosine kinase</td>
</tr>
<tr>
<td>Dasatinib (Sprycel)</td>
<td>Leukemia (CML)</td>
<td>BCR-ABL tyrosine kinase</td>
</tr>
<tr>
<td>Lapatinib (Tykerb)</td>
<td>Breast cancer</td>
<td>ERBB2 receptor tyrosine kinase</td>
</tr>
<tr>
<td>Many in clinical trials</td>
<td>Several types/AML</td>
<td>Angiogenesis/FLT3 receptor tyrosine</td>
</tr>
<tr>
<td><strong>Monoclonal antibody drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trastuzumab (Herceptin)</td>
<td>Breast cancer</td>
<td>ERBB2 receptor tyrosine kinase</td>
</tr>
<tr>
<td>Cetuximab (Erbitux)</td>
<td>Breast/renal cancer</td>
<td>EGFR tyrosine kinase</td>
</tr>
<tr>
<td>Cevacizumab (Avastin)</td>
<td>Colon cancer</td>
<td>VEGF</td>
</tr>
</tbody>
</table>

More than 70 protein kinase inhibitors are in cancer clinical trials, including several directed against serine/threonine kinases implicated in cancer. The RAF inhibitor sorafenib (Nexavar) has recently been approved for treatment of renal cell carcinoma. Rapamycin, an mTOR kinase inhibitor, and rapamycin analogues are also in clinical trials for several cancers.
Figure 6.19 The Biology of Cancer (© Garland Science 2014)
Resistance to Trastuzumab Due to PTEN Deficiency.

In Panel A, the overexpression of ErbB2 leads to its activation through autophosphorylation (P). As a result, Src kinase and phosphatidylinositol 3’ kinase (PI3K), with its regulatory subunits p85 and p110, are recruited to the receptor and kept in their active state. The activation of PI3K leads in turn to the activation of the proto-oncogenic signaling pathway consisting of Akt and the mammalian target of rapamycin (mTOR). Nagata and colleagues\(^2\) show that when active, Src can inactivate PTEN through the phosphorylation of its C-terminal end. This triggers the production of elevated levels of phosphatidylinositol 3,4,5-triphosphate (PIP3), further potentiating the activation of PI3K. On binding to the ErbB2 receptor, trastuzumab causes the dissociation of the receptor from Src and its inactivation through unknown mechanisms (Panel B). PTEN thus becomes free to antagonize the activation of the PI3K–AKT–mTOR signaling pathway through the dephosphorylation of PIP3. Trastuzumab could be combined with drugs such as sirolimus (rapamycin) and its analogues, everolimus (RAD001) and CCI-779, which inhibit mTOR, to block this critical signaling pathway at two different points. Nagata et al. show that a partial or total deficiency of PTEN (Panel C) may account for resistance to trastuzumab.\(^2\)

Underlying Major Themes

Modulation of:

• **Intrinsic activity** of signaling molecules
• **Concentration** of signaling molecules
• **Intracellular localization** of signaling molecules
• **Post translational modifications** (PMT)
p53 is activated by an array of cellular stresses and responds by activating various signalling pathways that are involved in diverse cellular mechanisms, from apoptosis to DNA repair. The protein level, localization, post-translational modifications and the cofactors of p53 are crucial to the function and regulation of p53. We propose that each individual aspect of p53 regulation represents a bar from a barcode that directs p53 activity. Different combinations of bars form different barcodes, and the barcode dictates which response p53 induces, be it apoptosis, cell-cycle arrest or senescence. Importantly, this allows p53 to be activated in a manner that is stress and cell-type dependent. The diagram shows a range of p53 regulations that control p53 activity and, ultimately, determine the cellular response. This response may be transcription dependent or independent. Each regulator is illustrated with its own bars. The number and width of the bars was assigned arbitrarily and has no relevance to the importance of each aspect in the regulation of p53 activity.

Murray-Zmijewski et al., 2008, 9: 702
Complexity of Cancer

• In each type of cell, the signaling pathways work in a combinatorial fashion to ensure that proliferation occurs in the right place at the right time during development and repair. Moreover, different cell types use different combinations of pathways to regulate their growth and division. This explains why the biochemistry of cancer is so complex.
There can be differences in signaling pathways among various cancer cells. In other words, defects that caused conversion of a normal cell to a cancer cells need not be the same in all cancers. Moreover, signaling pathways can change during progression of cancer.

Further, variation in drug delivery, drug metabolism, drug resistance and drug specificity on cancer cells with minimal effect on normal cells are also major concerns to be confronted in developing effective drugs.
Prevention is better than Cure

The challenge is how to develop an effective prevention strategy with minimal side effects.

Safety
Timing

Better understanding of signaling abnormalities in cancer cells should help develop effective prevention strategies.
The general organization and behaviour of cell signalling circuits.

Cells generally sense environmental stimuli through sensors, such as receptors. This information is then processed by intracellular signalling networks, which engage various cellular outputs, including gene expression, secretion, cytoskeletal changes and cell growth. Some of the major challenges in the evolution or engineering of novel signalling circuits are achieving the correct linkage between specific inputs (A, B and C) and outputs (X, Y and Z), tuning the quantitative behaviours (dose response and dynamics) of the signalling response so that they are optimal for their physiological function, and generating robust spatially self-organizing processes, such as those associated with cell polarization, directed motility, division and compartmentalization.
References

for

Growth Factors/Signal Transduction Lecture

(1) The Biology of Cancer, Chapters 5 & 6
Robert Weinberg
Garland Science, 2014

(2) Molecular Cell Biology, Chapters 13, 14, and 23
H. Lodish, A. Berk, S. Zipursky, P. Matsudaria,
C. Kraiser, M. Krieger, M. Scott, J. Darnell.
or
Molecular Cell Biology, Chapters 20 & 24
H. Lodish, A. Berk, S. Zipursky, P. Matsudaria,
D. Baltimore, J. Darnell.

Other Recommended reading:
2. S. Mukherjee, “The Emperor of All Maladies: A Biography of Cancer”

- Winner of the 2011 Pulitzer prize for general nonfiction.
- “The Emperor of All Maladies is a magnificent, profoundly humane “biography” of cancer—from its first documented appearances thousands of years ago through the epic battles in the twentieth century to cure, control, and conquer it to a radical new understanding of its essence. Physician, researcher, and award-winning science writer, Siddhartha Mukherjee examines cancer with a cellular biologist’s precision, a historian’s perspective, and a biographer’s passion. The result is an astonishingly lucid and eloquent chronicle of a disease humans have lived with—and perished from—for more than five thousand years”. —Extracted from the book description.