Tyrosine Kinase Network Signaling in Normal Physiology and Cancer
Tyrosine Kinase Network Signaling in Normal Physiology and Cancer

• Introduction of PTKs
• Src Family Kinases
• FAK/RAFTK(=PYK2) Family Kinases
• ErbB Family Kinases and Their Role in Breast Cancer: ErbB2 and EGFR
Protein Tyrosine Kinases (PTKs)

1. PTKs are enzymes that transfer gamma-phosphate groups from ATP to the hydroxyl group of tyrosine residues on signal transduction molecules.

2. PTKs play an important role in the control of most fundamental cellular processes including the cell cycle, cell migration, cell metabolism and survival, as well as cell-cell proliferation and differentiation.

PTKs = Protein Tyrosine Kinases
RTKs = Receptor Tyrosine Kinases
The human genome contains 518 protein kinase genes (~1.7% of total)

Manning et al. (2002)  
*Science* 298, 1912-1934

Tyrosine kinases account for 17% of the total kinases
Two major divisions of tyrosine kinases are “receptors” and cytoplasmic “nonreceptors.”
Protein Tyrosine Kinases (=PTKs)

- PTKs are important regulators of intracellular signal-transduction pathways mediating development and multicellular communication.
- PTK activity is normally tightly controlled and regulated.
- Perturbation of PTK signaling by mutations and other genetic alterations results in deregulated kinase activity and malignant transformation.
Cell Signaling by PTKs

• Membrane receptors can be classified into distinct families based upon the ligands they recognize, the biological response they induce and their primary structures.
Receptor Tyrosine Kinases (20 families)
Nonreceptor tyrosine kinases
(32 total in 10 families)

Src family
- SRC, YES, FYN, LYN, LCK, BLK, HCK, FGR,
- FRK (M KK3), BRK, SRM
- BTK, ITK, TEC
- M KK2 (BM X), TXK

CSK family
- CSK, CTK
- ABL, ARG
- ZAP70, SYK
- FES/FPS, FER

FAK family
- FAK, PYK2
- JAK1, JAK2, TYK2, JAK3
- ACK, ACK2

JAK family
- MYR, SH3, SH2, Kinase
- PH
- DBD, BD
- f-Actin
- FAT
- Kinase-like
- CDC42, Proline-rich

DBD: DNA binding domain
PH: Pleckstrin homology
FAT: Focal adhesion targeting
BD: Binding domain
Signal transduction by membrane-bound receptor tyrosine kinases (TKs)

Growth factor or other ligand binding to the receptor leads to receptor dimerization and autophosphorylation. The phosphorylated tyrosine residues on the receptors bind to adaptors, which then recruit downstream signal transduction molecules. This process leads to cell proliferation and other effects.
Receptor TKs as rational targets

<table>
<thead>
<tr>
<th>Receptor TK</th>
<th>Example ligands</th>
<th>Example receptor TK-expressing cells</th>
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<tbody>
<tr>
<td>c-KIT</td>
<td>SCF</td>
<td>Wide range of tissues</td>
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<tr>
<td>EGFR</td>
<td>EGF, TGFα</td>
<td>Epithelial cells</td>
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<tr>
<td>FGFR</td>
<td>FGF</td>
<td>Fibroblasts, endothelial cells</td>
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<tr>
<td>PDGFR</td>
<td>PDGF</td>
<td>Fibroblasts, endothelial cells</td>
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<tr>
<td>VEGFR</td>
<td>VEGF</td>
<td>Vascular endothelial cells</td>
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<tr>
<td>HER2/ErbB-2</td>
<td>–</td>
<td>Wide range of tissues</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>–</td>
<td>Myeloid cells containing Philadelphia chromosome (e.g. chronic myeloid leukemia)</td>
</tr>
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</table>
RTKs are considered not only as receptors with tyrosine kinase activity but also act as a platform for the recognition and recruitment of a specific complementary set of signaling proteins.
Two Functions for the Tyrosine Kinase Domain

1) Autophosphorylation (Transphosphorylation)- Generate docking sites for signaling molecules

2) Transphosphorylation- Activate an enzyme
Protein Domains Used For Protein/Protein Interactions
Adaptors Link TKR to Ras

**Function of SH2 Domains:**
1) Found in many signaling molecules
2) Recognize p-Tyr

![Diagram of signaling molecules and proteins]
SH3 Domains

- Binding site is poly-proline
- SH3/PPP interaction can be constitutive (GRB2/SOS) or the SH3 domain only becomes exposed for binding to the poly-proline region after some stimulation
Several types of proteins involved in signaling have SH2 and SH3 domains.

- c-Src
- c-Abl
- PTP1C
- PLCγ
- p85 (PI3K)
- GAP
- GRB2/sem-5

SH2 SH3 Catalytic activity

Autophosphorylation of the cytosolic domain of the PDGF receptor creates SH2-binding sites for several proteins. Some sites can bind more than one type of SH2 domain. Some SH2-containing proteins can bind to more than one site. The kinase domain consists of two separated regions (shown in blue), and is activated by the phosphorylation site in it.
Protein Modules Implicated in the Control of Intercellular Signaling Pathways
Paradigms for Activation of Signaling Proteins in Response to RTK Activation

- Activation by membrane translocation (PDGF R – PIP$_2$/PIP$_3$)
- Activation by a conformational change (PDGF R-Src-PI-3 kinase)
- Activation by tyrosine phosphorylation (EGF R-binding of PLC)
Activation of PKB by Membrane Translocation
Activation by a Conformational Change

[Diagram showing the activation process involving PTK, PIP2, PIP3, Targets, PI-3 kinase, and the transition from active to inactive states.]
Activation by Tyrosine Phosphorylation
Activation of Different Signaling Pathways by PTKs

Mechanisms for Attenuation and Termination of RTK Activation

• Short-term phosphorylation
• Long-term phosphorylation
• Attenuation
• Termination
Factors that determine the Specificity of Signaling Pathways

Question: How are the myriad of extracellular cues transmitted to induce specific biological responses?

Several mechanisms have been proposed to control specificity in cell signaling:

1. Combinational Control: recruitment and activation of unique sets of signaling based in tyrosine autophosphorylation sites and by means of tyrosine phosphorylation sites on closely associated proteins (such as Gab1)

2. Scaffold Proteins: scaffolding proteins that bind simultaneously to several proteins are able to insulate key components of signaling pathways (MAP kinases)

3. Cellular Compartmentalization: membrane translocation, membrane rafts

4. Signal Duration and Amplitude: (EGFR->transient MAPK stimulation ->PC12 cell proliferation; PC12 cell differentiation. NGF R-> late and sustained MAPK activation

5. Cellular Context: cell lineages, tissue specific, stage of differentiation, ligands.
**Src family of non receptor protein tyrosine kinases**

<table>
<thead>
<tr>
<th>Gene</th>
<th>kDa</th>
<th>Expression</th>
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<tr>
<td>c-Src</td>
<td>60-61</td>
<td>NK cells, mast cells, monocytes, high in platelets</td>
</tr>
<tr>
<td>c-Yes</td>
<td>62</td>
<td>T cells, NK cells, mast cells, platelets</td>
</tr>
<tr>
<td>Fyn</td>
<td>59-60</td>
<td>T cells, B cells, NK cells, monocytes, platelets</td>
</tr>
<tr>
<td>Lyn</td>
<td>55-58</td>
<td>B cells, NK cells, mast cells, monocytes, platelets</td>
</tr>
<tr>
<td>c-Fgr</td>
<td>58</td>
<td>monocytes, granulocytes</td>
</tr>
<tr>
<td>Lck</td>
<td>56</td>
<td>T cells, NK cells, low in B cells</td>
</tr>
<tr>
<td>Hck</td>
<td>59-64</td>
<td>B cells, mast cells, monocytes, granulocytes, platelets</td>
</tr>
<tr>
<td>Blk</td>
<td>55-56</td>
<td>B cells</td>
</tr>
<tr>
<td>Yrk</td>
<td>60</td>
<td>monocytes, neurons</td>
</tr>
</tbody>
</table>

*: increased activities in human primary breast carcinoma
What Is the Cellular Function of c-Src?

1. Encoded by a proto-oncogene.
   - deregulated activity promotes aberrant proliferation

2. Expressed ubiquitously.
   - highest in blood platelets, neurons, and osteoclasts
   - suggests widespread function(s) not necessarily related to proliferation

3. src −/− mice are healthy, born and survive up to one year.
   - no obvious defects in platelet and neuronal function
   - compensation by other Src-family kinases?
**Expression of Src Family Kinases**

<table>
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<th>Kinase</th>
<th>Expression</th>
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<tr>
<td>Src</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>Fyn</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>Yes</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>Lyn</td>
<td>Brain, B-cells, myeloid cells</td>
</tr>
<tr>
<td>Hck</td>
<td>Myeloid cells</td>
</tr>
<tr>
<td>Fgr</td>
<td>Myeloid cells, B-cells</td>
</tr>
<tr>
<td>Blk</td>
<td>B-cells</td>
</tr>
<tr>
<td>Lck</td>
<td>T-cells, NK cells, brain</td>
</tr>
</tbody>
</table>

4. *src/fyn/yes* triple knockout is embryonically lethal at mid-gestation.
   - cells have defects in motility but not proliferation
   - motility defects are rescued by reexpression of c-Src

5. c-Src and v-Src localize predominantly to cellular *focal adhesions*.
   - consistent with their role in cell motility
Key Events

Timeline | Key events in hunting the Src

- Discovery of Rous sarcoma virus (RSV)
- Development of the focus assay for RSV
- Bryan strain of RSV found to be replication defective
- Discovery of c-src proto-oncogene
- Src shown to be a protein-tyrosine kinase
- Identification of SH2 domain
- SH-2 domains shown to bind phosphotyrosine
- Crystal structures of Src and Hck

- Transformation by RSV in cell culture
- Isolation of fusiform mutants of RSV
- Genetic and physical identification of the v-src gene
- Identification of the v-Src protein
- Ras shown to mediate Src signalling
- Identification of SH3 domain
- src knockout
- src mutations detected in colon cancer

Comparison between v-Src and c-Src

<table>
<thead>
<tr>
<th>M</th>
<th>U</th>
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<th>SH2</th>
<th>L</th>
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<td>R</td>
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<td>L</td>
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<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Tyr</td>
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<td>COOH</td>
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<table>
<thead>
<tr>
<th>v-Src</th>
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<tbody>
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<td>H2N</td>
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<tr>
<td>Tyr</td>
<td></td>
</tr>
<tr>
<td>COOH</td>
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</tr>
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</table>

Regulation of Src family PTKs

- SH3 domain
- SH2 domain
- PTK domain

Active Src

**PTPase**

Cell activation

Inactive Src

**Csk**

PTPase
Activation of c-Src

Regulation of c-Src by Surface Receptors

Identification of Src Substrates

1990; Tom Parsons raised antibodies against tyrosine-phosphorylated proteins in v-Src transformed cells

- mice immunized with a mix of cellular pTyr-containing proteins purified using affinity-chromatography to anti-pTyr antibodies

- monoclonal antibodies recognizing several putative Src substrates were isolated

Figure: - immunoprecipitate with different mAbs,
- immunoblot with anti-pTyr antibody

What are p210, p130, p125, etc?
Focal Adhesions:
Specialized regions of the plasma membrane formed at sites where cultured cells adhere tightly to the underlying ECM substratum.
- sites of clustering of integrin receptors for ECM adhesive glycoproteins
- site of anchorage of actin filaments >> cell spreading and locomotion
Tyrosine-Phosphorylated Proteins Are Enriched in Focal Adhesions

Green = actin
Red = pTyr
Yellow = costain (focal adhesions)
Review of Key Concepts

Tyrosine kinases make up a large family of receptor and cytoplasmic protein kinases that function to regulate cellular responses to extracellular stimuli associated with metazoan complexity (ECM, cytokines and growth factors, antigens, etc).

Cellular phosphotyrosine is rare, compared to phosphoserine and phosphothreonine, indicating that tyrosine phosphorylation of proteins is transient and highly regulated.

Tyrosine phosphorylation can be assessed by phospho-amino acid analysis or, more commonly, using phosphotyrosine-specific antibodies.

Mutations that result in deregulated tyrosine kinase signaling often lead to uncontrolled cell proliferation associated with cancer.

Tyrosine kinase signaling is frequently involved in the recruitment of SH2-containing effector proteins to phosphorylated tyrosine sites.

Different SH2 domains exhibit different binding preferences based on amino acid residues close to the C-terminal of the phosphotyrosine.
FAK/RAFTK (=Pyk2)
Schematic Presentation of FAK and RAFTK/Pyk2 and their Respective Binding Partners

FAK
- Y (397)
- Kinase
- Paxillin
- Talin
- Pi3K
- GRB2
- Y Y
- Lyn
- Y881 Y906 ppp
- p44/42 MAPK, JNK, p38 MAPK
- Gene Activation
- Cytoskeletal changes

RAFTK/Pyk2
- Y402
- Kinase
- Paxillin
- Talin
- FRNK
Focal Adhesion Protein Tyrosine Kinase Family

1. This family consists of FAK and RAFTK/Pyk2 kinase (also designated FAK2, CAK-β or CADTK).

2. Both kinases have a molecular mass between 110-125 KDa and are closely related in their overall structures.

3. Neither kinase contains SH2 and SH3 domains.

4. Analyses of FAK and RAFTK/Pyk2 activation events have shown that phosphorylation occurs at several sites in vivo (next table).

5. The presence of two proline-rich motifs within the C-terminal domains of RAFTK/Pyk2 and FAK is also conserved. The proline-rich motifs are sites for SH3-mediated protein-protein interactions.

6. FAK is expressed in almost all tissues while RAFTK/Pyk2 is expressed mainly in the central nervous system and in hematopoietic cells.

7. FAK is localized to focal adhesion sites in adherent cells, while RAFTK/Pyk2 is mainly diffused throughout the cytoplasm and is concentrated in the perinuclear region.
FAK and RAFTK/Pyk2

<table>
<thead>
<tr>
<th>Site in FAK</th>
<th>FAK motif</th>
<th>Site in RAFTK/Pyk2</th>
<th>RAFTK/Pyk2 motif</th>
<th>Function</th>
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<tbody>
<tr>
<td>Tyr&lt;sup&gt;397&lt;/sup&gt;</td>
<td>YAE1</td>
<td>Tyr&lt;sup&gt;402&lt;/sup&gt;</td>
<td>YAE1</td>
<td>Src-family SH2 binding</td>
</tr>
<tr>
<td>Tyr&lt;sup&gt;407&lt;/sup&gt;</td>
<td>YTMP</td>
<td>—</td>
<td>—</td>
<td>Unknown</td>
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<td>Tyr&lt;sup&gt;576/577&lt;/sup&gt;</td>
<td>STYYKAS</td>
<td>Tyr&lt;sup&gt;579/580&lt;/sup&gt;</td>
<td>EDYYKAS</td>
<td>Regulatory-kinase activation loop</td>
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<tr>
<td>Tyr&lt;sup&gt;861&lt;/sup&gt;</td>
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<td>YENV</td>
<td>Tyr&lt;sup&gt;881&lt;/sup&gt;</td>
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<td>Grb2-SH2 binding site</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;712/713&lt;/sup&gt;</td>
<td>APPKPSPG</td>
<td>Pro&lt;sup&gt;714/715&lt;/sup&gt;</td>
<td>PPPKPSPK</td>
<td>p&lt;sub&gt;130&lt;/sub&gt;&lt;sup&gt;Cas&lt;/sup&gt; SH3-binding site</td>
</tr>
<tr>
<td>Pro&lt;sup&gt;876/877&lt;/sup&gt;</td>
<td>PPKKPPRPG</td>
<td>Pro&lt;sup&gt;859/860&lt;/sup&gt;</td>
<td>PPQKPPRLG</td>
<td>p&lt;sub&gt;130&lt;/sub&gt;&lt;sup&gt;Cas&lt;/sup&gt;/Graf SH3-binding site</td>
</tr>
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</table>
Schematic of Signaling Cascades Downstream of FAK and RAFTK/Pyk2

ECM

α

β

GRB2

P-

GRAF

FAK

P-

Src

RAFTK

P-

Pax

GRB2

P-

Ras

MAPK

Rho

CRK

C3G

NCK

PAK1

p38

PI3 Kinase

R-Ras

JNK

p38

 RAFTK

FAK

CAS

-P

α

β
Integrin Signaling Through the FAK/Src/CAS Complex

FAK autophosphorylates Tyr-397, then acts as a scaffold to recruit and activate Src to phosphorylate the CAS “substrate domain”
Potential RAFTK/Pyk2/CAK-β/CADTK Mediated Signaling Pathways
VEGF Regulates Focal Adhesion Assembly Through FAK & RAFTK/PYK2
Focal Adhesion Assembly

* is a complex comprised of scaffolding and signaling proteins organized by adhesion to the ECM
* links the actin cytoskeleton to ECM via the integrin receptor complex
* includes actin-binding proteins (such as talin, vinculin and tensin) that co-localize with integrins

VEGF & Focal Adhesion Assembly

VEGF-induced migration of ECs: is a key step in angiogenic response & is mediated by an accelerated rate of focal adhesion complex assembly & disassembly
VEGF

* a basic, heparin-binding, homodimeric glycoprotein of 45kD

* VEGF<sub>121</sub>, VEGF<sub>165</sub>, VEGF<sub>189</sub>, VEGF<sub>206</sub> (alternative exon splicing of a single VEGF gene)

Functions

* Potent vascular permeable activity
* Induction of plasma protein leakage in BBB
* Induction of fenestrations in endothelial cells
VEGF Signaling Pathways in ECs

BEC: blood EC  LEC: lymphatic EC

Focal Adhesion Assembly in HBMECs

A. FAK/Ctl-0h
   FAK/VEGF-1h
   FAK/VEGF-4h

B. FAK/Ctl-0h/FN
   FAK/Ctl-1h/FN
   FAK/VEGF-1h/FN
   FAK/Ctl-4h/FN
   FAK/VEGF-4h/FN

C. RAFTK/Ctl-0h/FN
   RAFTK/VEGF-1h/FN
   RAFTK/VEGF-4h/FN
Tyrosine Phosphorylation of Flk-1/KDR, Flt-4, FAK and RAFTK/Pyk2 upon VEGF Treatment in HBMECs

A. 

<table>
<thead>
<tr>
<th>Flk-1</th>
<th>VEGF</th>
<th>IP: Flk-1</th>
<th>WB: 4G10</th>
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B. 

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C. 

<table>
<thead>
<tr>
<th>RAFTK</th>
<th>VEGF (min):</th>
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D. 

<table>
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<tr>
<th>FAK</th>
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E. 

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<th>NCK</th>
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<td>KDR</td>
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Effects of VEGF and Integrins on Focal Adhesion Assembly in HBMECs

A.

B.

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Effects of VEGF and Integrins on Focal Adhesion Assembly in HBMECs
RAFTK/Pyk2 Kinase Activity is Critical for HBMEC Spreading and Migration

A. Spreading assay

B. Migration

- Vector
- WT
- Y402F

Control
VEGF
Effects of FRNK Overexpression on VEGF-Induced Focal Adhesion Assembly and Migration

A. Cells positive for focal adhesions (%)

B. Migration

- **Control**
- **VEGF**
* Conclusions *

These studies define a mechanism for the regulatory role of VEGF in focal adhesion complex assembly in HBMECs, via activation of FAK & RAFTK/Pyk2

1. VEGF treatment of HBMECs plated on laminin or fibronectin stimulated cytoskeletal organization and increased focal adhesion sites.

2. VEGF induced formation of focal adhesions in HBMECs when cells were seeded on fibronectin.

3. FAK and RAFTK/Pyk2 kinases were tyrosine-phosphorylated by VEGF.

4. Overexpression of wild-type RAFTK/Pyk2 increased cell spreading and the migration of HBMECs.

5. Overexpression of catalytically-inactive mutant RAFTK/Pyk2 markedly suppressed HBMEC spreading (~70%), adhesion (~82%) & migration (~65%).

6. Inhibition of FAK by dominant-interfering mutant FRNK (FAK-related non-kinase) significantly inhibited HBMEC spreading and migration as well as disrupted focal adhesions.
Receptors and Growth Factors

- Activation by an autocrine loop
- Constitutive ligand production leading to tyrosine kinase activation
The EGF Receptor (ErbB) Family and Its Ligands

- **EGF**: TGFα, Amphiregulin, β-cellulin, HB-EGF, Epiregulin
- **Herregulins**:erbB3, HER3
- **EGF**: No specific ligands - often acts as dimer partner
- **Heregulins**:erbB1, HER1, EGFR, erbB2, HER2, neu, erbB3, HER3, erbB4, HER4

**Cysteine-rich domains**

**Tyrosine kinase domain**

**C-terminus**

**Extracellular**

**Intracellular**

**membrane**
The ErbB Signaling Network

1) There are four members of the ErbB family:
   - Epidermal growth factor-EGF receptor (=EGFR); also termed ErbB1/HER1
   - ErbB2/Neu/HER2
   - ErbB3/HER3
   - ErbB4/HER4

2) A family of ligands, the EGF-related peptide growth factors bind the extracellular domain of ErbB receptors leading to the formation of both homo and heterodimers.

3) Dimerization consequently stimulates the intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation of specific tyrosine residues with the cytoplasmic domain.

4) These phosphorylated residues serve as docking sites for signaling molecules involved in the regulation of intracellular signaling cascades.

5) The downstream effects on gene expression determine the biological response to receptor activation.
Expression of ErbB receptors

1) ErbB receptors are expressed in a variety of tissues of epithelial, mesenchymal and neuronal origin.

2) ErbB receptors play fundamental roles in development, proliferation, and differentiation.

3) Deregulated expression of ErbB receptors, in particular ErbB1 and ErbB2, has been implicated in the development and malignancy of several types of cancer.

4) The primary function of ErbB2 is as a coreceptor. ErbB2 is the preferred heterodimerization partner for all other ErbB family members and plays a role in the potentiation of ErbB receptor signaling.

5) ErbB heterodimers provide signal diversification.
ErbB-2 Is the Preferred Dimerization Partner for the Other ErbB Receptors

Olayioye, M.A. et al. The EMBO Journal, 2000;19:3159-3167
Specific Phosphotyrosine Residues and Binding of Signaling Molecules to the ErbB Receptors

Olayioye, M.A. et al. The EMBO Journal, 2000;19:3159-3167
The Power of ErbB Receptor Heterodimerization

1. ErbB heterodimerization provides for signal amplification
2. ErbB heterodimerization provides for signal diversification
3. The interplay of the ErbB family and their ligands in the context of the entire signaling network as present in live cells:
   - Cross-talk between different pathways
   - ErbB receptors as signal integrators
Ductal carcinoma *in situ* (DCIS)

- 90% express EGFR
- 70% express c-erbB2
- Comedo DCIS is estrogen independent
- Role of adjuvant treatment unclear

Holland et al, JNCI, 1997
Human Breast Cancer

- Leading cause of death in women 30 to 70 years of age

- Acquisition of several successive distinct genetic changes
  - inherited: BRCA1, BRCA2 mutations
  - acquired: overexpression of oncogenes
    - HER-2/neu
    - Src family
    - p53
    - PTEN
  
  mutations of tumor suppressor genes

...
HER (ErbB) family in cancer

**HER-1**  
?

**HER-2** overexpression in  
- breast carcinomas (25-30%)  
  - ovarian carcinomas  
  - lung carcinomas  
  - gastric carcinomas  
  - oral carcinomas

**HER-3** overexpression in non small cell lung carcinomas (NSCLC)

**HER-4** overexpression in papillary thyroid carcinomas
HER (ErbB) family of protein tyrosine kinase receptor

**HER-1**  
ligands =  - epithelial growth Factor (EGF)  
  - betacellulin (BTC)  
  - epieregulin (EPR)  
  - amphiregulin (AR)  
  - transforming growth factor alpha (TGF-α)

**HER-2**  
no ligand

**HER-3**  
ligands = heregulin (HRG) = neu differentiation factor (NDF) = neuregulin (NRG)  
kinase-dead

**HER-4**  
ligands =  - HRG (NDF)(NRG)  
  - EGF  
  - BTC  
  - EPR
HER-2/neu (c-erbB2) protein tyrosine kinase receptor

- HER-2/neu activation
  
  normal cells = heterodimers:  
  HER-2 / EGF-R ← EGF  
  HER-2 / HER-3 ← HRG (NDF) (NRG)  
  
  cancer cells = homodimers:  
  HER-2 / HER-2 ← constitutively active  

- HER-2/neu signaling
  
  transmembrane protein tyrosine kinase receptor  
  activation → auto(P)  
  → binding to SH2 containing proteins  
  = Shc, PLC-γ, Ras-GAP, PI-3K, c-Src, ...
Regulation of ErbB2 by CHK and CSK Kinase
Csk Homologous Kinase (CHK)

บทบาทในการคัดค้าน

- Cloning

  - Matk: human megakaryocytes (1994)
  - Ctk: mouse brain (1994)
  - Hyl: human megakaryocytes (1994)
  - Ntk: mouse fetal thymus (1994)
  - Lsk: PHA-stimulated human peripheral T cells (1994)
  - Batk: rat hippocampus (1994)
    - >> CHK (1997)

- 2 isoforms

  alternative splicing

- p57 Chk (human)
- p52 Chk (mouse) lacks 40 N-terminal residues
**CHK / Csk similarities**

- **Homology**
  - 53% amino acid identity overall
  - 59% amino acid identity within the catalytic domain

- **Structure**
  - SH3 domain - SH2 domain - Catalytic domain
  - no amino-terminal myristoylation site >> cytoplasmic
  - no autophosphorylation site, no negative regulatory site >> constitutively active

- **Function**
  - phosphorylation and downregulation of Src family PTKs
  - CHK -> in vitro phosphorylation of Lck, Fyn, c-Src, Lyn
CHK / Csk differences

✦ Expression

Csk = ubiquitous

constitutive

CHK = restricted to neuronal and hematopoietic cells

induced - by IL-2 in NK cells

- by PHA in T lymphocytes

- by SCF in megakaryocytes

- by IL-4 and IL-13 in monocytes

✦ Activity

Csk = constitutive

CHK = ?
**CHK in Breast Cancer**

- **CHK expression**
  - no expression in human normal breast tissues (0/19)
  - expression in human primary breast cancer tissues (70/80)
  - co-localization CHK / HER-2 in human primary breast cancer tissues (6/6)

- **Role in HER-2/neu signaling**

  upon HRG stimulation (not upon EGF stimulation)

  >> binding of CHK to auto(P)-Tyr1253 of HER-2/neu via CHK SH2 domain

  >> downregulation of HER-2/neu-activated Src kinases
Model for the regulation of HER-2/neu-activated Src kinases by CHK

Growth Factors

Express mutant or un-regulatable forms of receptors which are constantly active in breast, lung, bladder and brain tumours.

e.g. c-erbB encoding EGF tyrosine kinase

Oncogenes may code for secreted proteins, transmembrane proteins, cytoplasmic proteins, or nuclear proteins.

- Growth factors
  - sis
  - K5/HST
  - wnt1
  - int2
  - PDGF B chain
  - related to FGF
  - related to wingless
  - related to FGF

- Growth factor receptors
  - c-erbB
  - EGF receptor kinase
  - erbB2.3
  - EGF-like receptor kinases
  - c-fms
  - CSF-I receptor kinase
  - c-kit
  - steel receptor kinase
  - mas
  - angiotensin receptor

- G protein/signal transduction
  - c-ras
  - GTP-binding protein
  - G\textsubscript{o} and G\textsubscript{i}

- Intracellular tyrosine kinases
  - c-src
  - membrane-associated
  - c-abl
  - cytosolic
  - c-fps
  - cytosolic

- Serine/threonine kinases
  - c-raf
  - cytosolic
  - c-mos
  - cytosolic

- Signaling
  - crk
  - SH2/SH3 regulator
  - vav
  - SH2 regulator

- Transcription factors
  - c-myc
  - HLH protein
  - c-myb
  - transcription factor
  - c-fos
  - leucine zipper protein
  - c-jun
  - leucine zipper protein
  - NF-kB family
  - c-rel
  - thyroid hormone receptor
  - c-erbA
Activating mutations in RTKs takes several forms but all lead to ligand-independent dimerization and thus activation.
Gene Amplification is also a Common Mechanism of Inappropriate Gene Activation in Human Tumors

Double minute chromosomes

Tandem duplications
Mutant Receptor May Lack the Ligand Binding Domain (e.g. c-ErbB and Breast Cancer)

Activation of a growth factor receptor involves ligand binding, dimerization, and autophosphorylation. A truncated oncogenic receptor that lacks the ligand-binding region is constitutively active because it is not repressed by the N-terminal domain.
ErbB-Directed Therapies

1) ErbB2 is a target for cancer therapy
2) A mAb that targets the extracellular domain of ErbB2 specifically inhibits in-vitro growth of ErbB2 overexpressing tumor cells.
3) The humanized version of this antibody (Herceptin) has been validated in the clinic as an ErbB2-directed therapeutic approach.
4) However, although all patients treated with Herceptin do have tumors exhibiting ErbB2 overexpression, not all respond to treatment.
How Herceptin Works

What is a monoclonal antibody?
An *antibody* is a protein made by the body’s own natural immune system. They are directed against foreign and infectious agents, called antigens. *Monoclonal antibodies* engineered through biotechnology are produced to provide specific anti-tumor action within the human body.

Monoclonal antibodies targeting a HER2 protein overexpressing cell
In first-line combination use with paclitaxel in this landmark Phase III clinical trial, Herceptin achieved a 38% response rate vs 15% for paclitaxel alone ($P<0.001$).\cite{1} In addition, overall response rates (ORR) (complete response + partial response) increased by 55%. Forty-five percent of patients with Herceptin plus chemotherapy responded, vs 29% of patients receiving chemotherapy alone ($P<0.001$).\cite{1}
Intracellular Transducer

Member of the expanding family of closely related tyrosine kinases
Functions in relaying information to the nucleus
Non-receptor tyrosine kinase with at least 9 members expressed in
a variety of cells.

Loss of regulation of c-src is closely associated with colon carcinomas
Intracellular Transducer

e.g. src tyrosine kinase

Multi-domain protein

- Myristate anchors protein to plasma membrane
- Unique domain
- SH-2 domain for tyrosine phosphate binding
- SH-3 domain for protein:protein interaction
- Catalytic domain
- Regulatory domain basally phosphorylated at Y527
Model for the activation of c-src in response to growth factors

For activation of src, the protein requires:

Localisation to the plasma membrane

De-phosphorylation of tyrosine 527

Autophosphorylation of tyrosine 416
Model for the activation of c-src in response to growth factors

When a receptor tyrosine kinase is activated, autophosphorylation generates a binding site for the Src SH2 domain, Tyr-527 is released and dephosphorylated, Tyr-416 becomes phosphorylated, and Src kinase is activated.

Y527 interacts with src SH-2 domain in a low-affinity interaction
Growth factor receptor activation creates a high affinity binding site
Src associates with the activated receptor and changes conformation
Y527 is de-phosphorylated by phosphatases recruited to the receptor
Y416 is phosphorylated and src is activated
Tyrosine phosphorylation is important for the activation of c-src

Two tyrosine residues are targets for phosphorylation in Src proteins. Phosphorylation at Tyr-527 of c-Src suppresses autophosphorylation at Tyr-416, which is associated with transforming activity. Only Tyr-416 is present in v-Src. Transforming potential of c-Src may be activated by removing Tyr-527 or repressed by removing Tyr-416.

Y527P suppresses phosphorylation at Y416
v-src has lost the last 19 amino acids so that regulation via Y527 is lost

v-Src    c-Src
## Tumors Showing High EGFR Expression

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>40-80%</td>
</tr>
<tr>
<td>Prostate</td>
<td>40-80%</td>
</tr>
<tr>
<td>Gastric</td>
<td>33-74%</td>
</tr>
<tr>
<td>Breast</td>
<td>14-91%</td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>90-100%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>25-77%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>30-50%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>35-70%</td>
</tr>
</tbody>
</table>
Signal Transduction Through EGFR-TK

Ligand binding induces receptor dimerization and autophosphorylation, creating docking sites for adaptor molecules leading to the activation of downstream effector molecules. A variety of signaling pathways results in pleiotropic effects, including cell proliferation, control of the cell cycle, regulation of apoptosis and survival, and alterations in cell migration and invasiveness.

Vlahovic, G. et Al. The Oncologist, 2003;8:531-538.
EGFR Expression in Human Tumors

High expression is generally associated with

- Invasion
- Metastasis
- Late-stage disease
- Chemotherapy resistance
- Hormonal therapy resistance
- Poor outcome
EGFR and Cancer

1) EGFR is overexpressed or aberrantly activated in the most common solid tumors including non-small cell lung cancer (NSCLC) and cancers of the breast, prostate and colon.

2) There are several small molecule EGFR-TK inhibitors approved in clinical development (Table 2).

3) Randomized clinical trials of the EGFR-TK inhibitor gefitinib have demonstrated clinical benefits in patients with advanced non-small cell lung cancer (NSCLC).

4) As TK inhibitors become available for clinical use, new challenges include predicting which patients are most likely to respond to these targeted TK inhibitors.
EGFR Expression or Overexpression in Human Tumors

% of tumors with high EGFR expression

- NSCLC
- Prostate
- Pancreatic
- Breast
- Colorectal
- Gastric
- Ovarian

2020
Measurement of EGFR Expression

- Immunohistochemistry
  - EGFR autophosphorylation
- RT-PCR, ELISA, labelled ligand
- Downstream markers of EGF signaling
  - proliferation markers
  - maturation markers
Mechanisms of Increased EGFR Activation

1. Overexpression of EGFR protein
2. Ligand/autocrine loop
3. Heterodimerization and cross-talk
4. Phosphatase
5. Mutant EGFR

Mitogenic signals
Strategies for EGF Signaling Inhibition

- Immune effector cell
- Anti-receptor mAbs (e.g., C225)
- Bispecific Abs
- Anti-ligand mAbs (e.g., ZD1839)
- Ligand/toxin conjugates
- scFv/toxin conjugates
- Ligand-genistein conjugates
- EGFR-TKIs (e.g., ZD1839)
- Intracellular scFvs
- Antisense
- Nucleus
Protein Kinase Inhibitors

1) Protein kinases are targets for treatment of a number of diseases.

2) Structures of some PTKs have provided insights into drug design aimed at the inactive or active form of the kinase targeted and mechanisms of inhibition.

3) The specific PTKs include: EGFR, VEGFR, and FGFR.

4) Nonreceptor tyrosine kinase Bcr-Abl.

5) Cancer therapeutic agents include herceptin and Gleevec.

6) Among the serine-threonine kinases, p38, Rho-kinase, and cyclin-dependent kinase have been targeted for inflammation and cancer
## EGFR-TK Inhibitors Approved or in Development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Molecular target</th>
<th>Tumor types</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>gefitinib</td>
<td>EGFR-TK selective</td>
<td>NSCLC, breast, head &amp; neck</td>
<td>Approved (NSCLC)</td>
</tr>
<tr>
<td>OSI-774</td>
<td>EGFR-TK selective</td>
<td>NSCLC, head &amp; neck, ovarian</td>
<td>Phase II, III</td>
</tr>
<tr>
<td>CI-1033</td>
<td>Pan-ErbB-TK</td>
<td>SCLC, solid tumors</td>
<td>Phase I</td>
</tr>
<tr>
<td>PKI-166</td>
<td>EGFR-TK selective</td>
<td>Solid tumors</td>
<td>Phase I</td>
</tr>
<tr>
<td>GW-572016</td>
<td>Pan-ErbB-TK</td>
<td>Healthy volunteers</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

Vlahovic, G. et Al. The Oncologist, 2003;8:531-538.
NSCLC
Breast
Prostate
CRC
H & N
Ovarian

ZD1839
Structure of ZD1839 (‘Iressa’), an EGFR-Tyrosine Kinase Inhibitor

MW = 447
ZD1839 Mode of Action

- Cell proliferation
- Decreased apoptosis
- Angiogenesis
- Metastasis

Signalling molecules

Ligand

Cancer cell membrane

Extracellular

Intracellular

ZD1839
ZD1839: a Selective and Potent Inhibitor of EGFR Tyrosine Kinase

- *In vitro* cell growth inhibition IC$_{50}$ = 0.08 µM
- Selective *in vitro* tyrosine kinase enzyme inhibition

<table>
<thead>
<tr>
<th>Kinase</th>
<th>ZD1839 IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine kinases</td>
<td></td>
</tr>
<tr>
<td>erbB1 (EGFR)</td>
<td>0.033</td>
</tr>
<tr>
<td>erbB2</td>
<td>1.2-3.7</td>
</tr>
<tr>
<td>KDR</td>
<td>3.7-33</td>
</tr>
<tr>
<td>c-flt</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Serine/threonine kinases</td>
<td></td>
</tr>
<tr>
<td>PKC</td>
<td>&gt;100</td>
</tr>
<tr>
<td>raf</td>
<td>~100</td>
</tr>
<tr>
<td>MEK-1</td>
<td>23-30</td>
</tr>
<tr>
<td>ERK 2</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

AstraZeneca: data on file; Woodburn JR et al. Proc AACR 2000; 41: abs 2552
Potential Treatment Options for Solid Tumors

- Localized tumors:
  - Surgery
  - Radiotherapy
  - Hormonal therapy

- Regional spread:
  - Chemotherapy
  - Radiotherapy
  - Hormonal therapy

- Distant metastases:
  - Chemotherapy
  - Hormonal therapy

Biological agents (e.g. ZD1839)
Available data from preclinical, pharmacokinetic, and pharmacodynamic studies provide strong support for clinical trials of ZD1839, and other therapies targeting EGFR-TK, in a range of cancers.
Type III EGFR Mutation

Wild type EGFR

- Domain I
- Domain II
- III - ligand binding
- IV
- V - transmembrane

EGFRvIII

- Part of ligand binding domain deleted
- TK domain constitutively phosphorylated

- TK sites constitutively active
- May provide a tumor-cell-specific target for selective small-molecule tyrosine kinase inhibitors

EGFR: Mutation and Modes of Resistance to Therapy

- Type III EGFR mutation (EGFRvIII) is common in solid tumors
  - breast (77%), ovarian (75%), NSCLC (16%)
- EGFRvIII is often amplified in genome, resulting in overexpression
- EGFRvIII can also arise by differential mRNA splicing
- EGFRvIII heterodimer formation may have significant effects on EGFR signaling
- Mutation may lead to reduced activity of EGFR inhibitors

Summary

• Protein kinases are targets for treatment of a number of diseases.

• PTKs are important for cell development, differentiation and proliferation.

• PTKs are important components of signaling cascades, via protein networks that are subjected to multiple positive and negative feedback mechanisms.

• New development will be in the measurement of the kinetics of cellular reactions in the context of living cells and live animals, to gain insights into signaling networks and in-vivo cell signaling.