Contribution from Dr. Amanda Harvey BSc PhD SFHEA

"Overview of Cell Signaling Pathways in Cancer".

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Abstract

There are many different signaling pathways that contribute to development and cellular homeostasis. In diseases, especially cancer, development components of these pathways often become mutated or overexpressed causing dysregulation of cellular signaling. This chapter provides an overview of the key pathways involved in tumor development and progression and studies some of the complications associated with therapeutic targeting, namely signaling cross talk and biomarker identification.

Keywords

EGFR, cancer, cell signaling, biomarkers, therapeutic targets, MAPK,

1. Introduction to Cancer Cell Signaling

Cell signaling is the 'catch-all' phrase that provides an overview of the communication system and is often linked to a single signalling pathway. In this one simple term, there is a sense of cells communicating with one another and changing their behavior as a result of such communication. This ability of cells to sense external signals and respond to them is a basic requirement for tissue development and repair, immunity, and homeostasis.

Signal transduction defines the precise series of molecular events that occur to convert an external stimulus into a cellular response. Most frequently these events involve phosphorylation of target molecules by enzymes with kinase activity. A signal transduction pathway is initiated when a ligand binds to its receptor resulting in a conformational change which then allows for activation of its kinase activity and receptor transphosphorylation (e.g. in the case of epidermal growth factor (EGF) mediated signaling, binding of downstream substrates and activation of the kinase activity. Often (but not always) the receptors cross the cell membrane allowing for ligand binding outside of the cell with the subsequent phosphorylation event occurring internally. This is a fundamental process by which cells can communicate with each other. One cell releases a ligand (e.g. growth factor or cytokine), which then binds to receptors on adjacent cells activating their internal signaling mechanisms.

Following receptor phosphorylation, and binding of an adaptor molecule, a signalling cascade becomes activated allowing for a series of phosphorylation events to occur transmitting the signal from the cell membrane to other parts of the cells, most often the nucleus where, upon phosphorylation, transcription factors become activated. Transcription factor activation results in changes in gene expression, subsequent translation and the production of a biological response by the cell.

Where nuclear receptors also act as transcriptional regulators, ligands diffuse into the cell and bind to the receptor in the cytoplasm resulting in a conformational change and subsequent nuclear translocation of the receptor. Once in the nucleus, these activated receptors are capable of binding to their respective consensus sequences within the promoter regions, altering gene transcription.
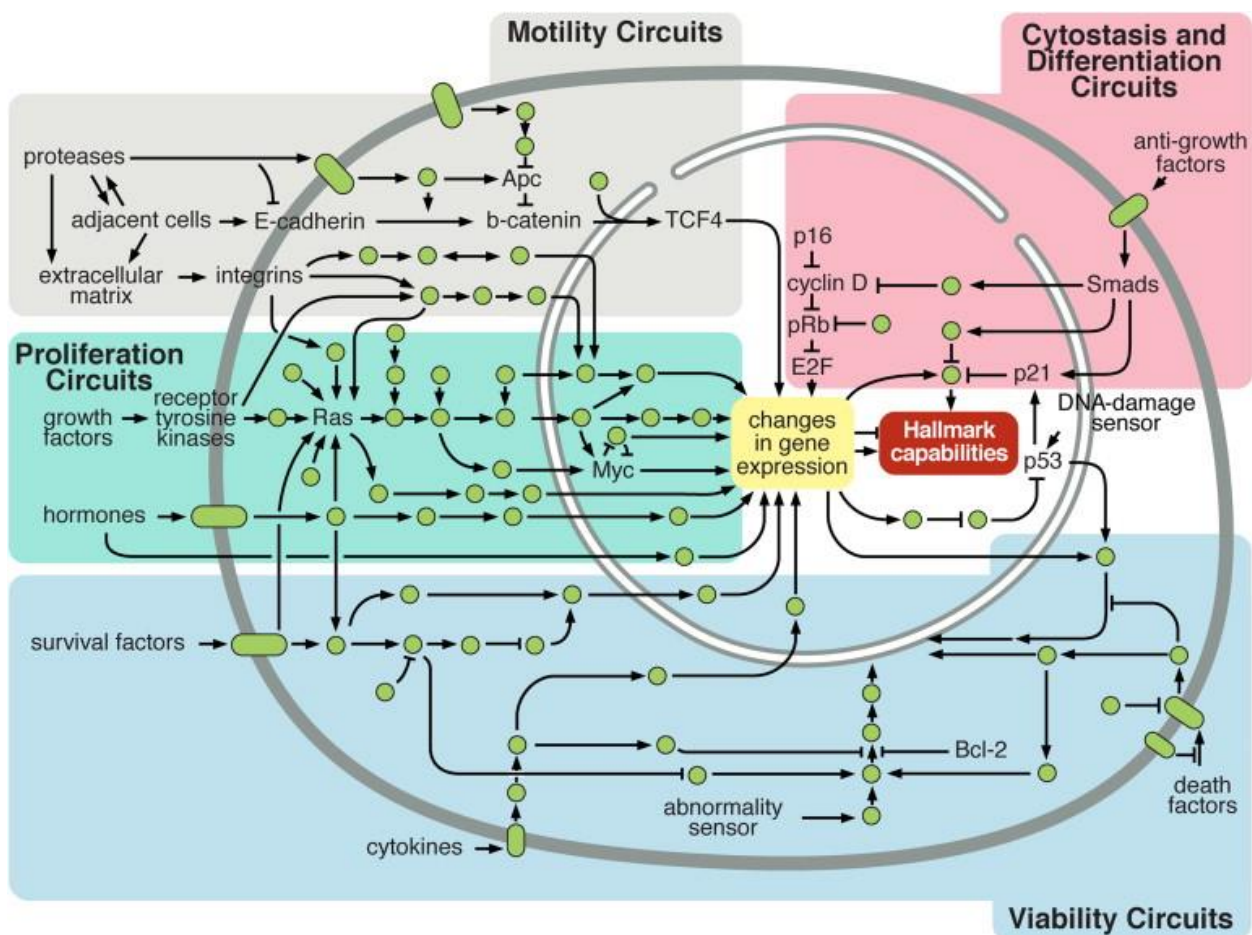

Figure1: Intracellular Signaling Networks Regulate the Operations of the Cancer Cell. An elaborate integrated circuit operates within normal cells and is reprogrammed to regulate hallmark capabilities within cancer cells. Separate subcircuits, depicted here in differently colored fields, are specialized to orchestrate the various capabilities. At one level, this depiction is simplistic, as there is considerable crosstalk between such subcircuits. In addition, because each cancer cell is exposed to a complex mixture of signals from its microenvironment, each of these subcircuits is connected with signals originating from other cells in the tumor microenvironment, as outlined in Figure 4. (Courtesy: Elsevier Inc. Reprinted from Fig. 2. Hanahan D and Weinberg RA. Cell 144, pg. 657, 2011)

Membrane Receptors

- *ErbB / HER Signaling Pathway*
  EGFR/HER family comprises four receptors and initiate signaling pathways (including PI3K/Akt, mTOR, and MAPK) involved in cell survival and proliferation. EGFR signaling is central to development.
  Roles in disease. These pathways have been implicated in several cancers (e.g. squamous-cell lung carcinomas, breast, colorectal and epithelial head and neck cancers).
  EGFR and HER2 are targets for kinase inhibitors (e.g. lapatinib, gefitinib) and monoclonal antibody (biological) therapies (e.g. trastazumab, pertuzumab). HER2 can also be targeted indirectly via inhibitors of Heat Shock protein 90 (Hsp90)
  (See chapter XYX in this book)

- *G Protein-Coupled Receptors (GPCRs) Signaling*
  GPCR signaling involves two principal signal transduction pathways: the cAMP signal pathway and the phosphatidylinositol signal pathway.
  GPCRs are the largest signaling receptor family; the receptors themselves are characterized by the seven transmembrane domains and they have broad physiological functions including cell proliferation and invasion as well as immune cell-mediated functions and nervous system transmission. Canonical signaling involves coupling with G-proteins resulting in phosphorylation of the receptor.
  Roles in disease. GPCRs are involved in numerous cancers, especially at secondary sites such as the lung, bone, lymph nodes and liver.
  GPCRs are potential targets for therapy but, currently, this has not been fully explored.

- *Fibroblast Growth Factor (FGF) Signalling Pathway*
  FGFs are considered to be either paracrine (locally acting) or endocrine (relating to hormones secreted into the blood) and signal through 4 receptors (FGFR1-2) to regulate several cell outcomes including survival, proliferation differentiation, and cell metabolism. They also regulate immunity, angiogenesis and epithelial to mesenchymal transition (EMT). Downstream signaling components include PI3K/Akt, mTOR, MAPK and phospholipase signaling.
  Roles in disease. FGF signaling is implicated in several cancers (e.g. gastric, lung and breast cancers).
  FGF23 is a target for biological (monoclonal antibody) therapy (e.g. KRN23) whilst the receptors are targets for numerous antibodies or small molecule inhibitors (e.g. NVP-BGJ398).

- *Insulin Receptor (IR) and Insulin-Like Growth Factor Receptor (IGFR) Signaling Pathways*
  Insulin is critical for regulation of glucose and energy metabolism, whilst IGF plays an important role in growth, through adapter proteins, the insulin receptor substrate (IRS)

family, both hormones mediate their effects via AMPK, PI3K/Akt, mTOR and MAPK signaling pathways.

Roles in disease. The IR and IGFR signaling pathways are widely implicated in many cancers (e.g. breast, prostate, ovarian, and colorectal cancers, Ewing's sarcoma, rhabdomyosarcoma, and non-small cell lung carcinomas).

IGFR1 can be targeted with both monoclonal antibodies (biological therapy) (e.g. cixutumumab) and small molecule tyrosine kinase inhibitors (linsitinib), and second generation anti-sense oligonucleotides are in development. As with FGF23, IGF1 and IGF2 are targets for anti-ligand antibodies (e.g. MEDI-573 or BI836845).

(See chapter XYX in this book)

- *Transforming Growth Factor-β (TGF-β) / Smad Signaling Pathway*
  TGF-β signaling has opposing roles in different cellular contexts. It plays key roles in embryonic stem cell renewal, differentiation, proliferation, immune system suppression, and homeostasis of mature cells. The canonical pathway is well characterized, and signaling is carried out via the Smad signaling cascade which links the transmembrane receptors with the cell nucleus.

  Roles in disease. TGF-β signaling is implicated in pathologies such as benign prostatic hyperplasia as well in various cancers (e.g. colorectal, gastric, endometrial, breast liver and pancreatic cancers). TGF-β is a target for ligand traps (by antibodies such as Lerdelimumab and Metlimumab) or anti-sense oligonucleotides (e.g. trabedersen), but translation into the clinical has been disappointing.
  (See chapter XYX in this book)

- *Vascular Endothelial Growth Factor (VEGF) Receptor Signalling*
  VEGF signaling is crucial during embryonic development as it is required for the formation of new blood vessels (angiogenesis). It is also required to restore oxygen levels in tissues when blood supply is compromised, and to create new blood vessels after injury. There are three receptors VEGFR1 (FLT-1), VEGFR2 (FLK-1) and VEGFR3 which homo- and hetero-dimerise.

  Roles in disease. VEGF signaling has been implicated in metastatic colorectal cancer (mCRC), metastatic renal cell carcinoma (mRCC, locally advanced, recurrent, or metastatic non-small cell lung cancer (NSCLC), progressive glioblastoma and breast cancer.
  VEGF receptors (VEGFRs) are targets for both kinase inhibitors (e.g. sorafenib) and biological (antibody-based) therapies (e.g. ramucirumab). VEGF is a target for ligand-blocking antibodies (e.g. bevacizumab). Small oligonucleotides (such as Veglin) are also being tested to prevent expression of VEGF genes.
  (See chapter XYX in this book)

- *Toll-like Receptors (TLRs) Pathway*
  The TLR family belongs to the larger group of pattern recognition receptors (PRRs). They are present on antigen presenting cells (APCs) and ligand binding results in maturation of

the cell, cytokine induction and the priming of naïve T-cells to drive acquired immunity because of downstream signaling causing nuclear translocation of NF-κB. TLR ligands have potential as vaccine adjuvants and could be co-administered with protein subunit vaccines to boost immune responses.

Roles in disease. TLR activation is linked to the pathology of immune diseases and cancer. Unlike other cancer targets where inhibition is key agonists of TLR2, such as SMP105 and Sumitomo, have potential as anti-cancer agents.

- *B Cell Receptor (BCR) Signaling Pathway*
  The BCR is central to regulating maturation and proliferation of, and antibody production by, B cells. Signalling from the receptor activates Src family members and PI3K with recruitment of Bruton tyrosine kinase (BTK), ultimately causing NF-κB to translocate to the nucleus inducing cytokine production.
  Roles in disease. B cell receptor cascade is implicated in the development of B-cell malignancies as upregulated signaling modulates cell migration and adhesion through remodeling of the microenvironment. BTK signaling plays a role in a number of auto-immune and inflammatory diseases such as rheumatoid arthritis and multiple sclerosis BTK is a B-cell-specific target for small molecule inhibitors and compounds such as PRN2246, which readily crosses the blood-brain barrier are in clinical trial.

- *T Cell Receptor (TCR) Signaling Pathway*
  TCRs recognize fragments of antigens and function as complex whose signaling is enhanced through a co-receptor (e.g. CD4 or CD8). As with BCR, signaling from the TCR activates Src family members resulting in phospholipase activation; MAPK and NF-κB pathways are also triggered
  Roles in disease. As well as being disease targets for drugs such as dasatanib that target the downstream elements of the pathway thereby inhibiting T-cell activation, T-cells themselves are being engineered for use in immunotherapy.

- *Hepatocyte Growth Factor (HGF)/Met Receptor Signaling*
  MET is a cell surface receptor tyrosine kinase found in both epithelial and endothelial cells. That Like other receptor tyrosine kinases MET signalling positively regulates a number of key cellular functions including proliferation, survival and cell migration; however, the MET receptor has a single ligand (HGF). There are several downstream pathways of MET signaling with the Ras-Raf-MAPK cascade and the PI3K-Akt axis being the most relevant to disease development.
  Roles in disease. In normal cells MET expression and activity is low with activation in tumor cells arising from gene amplification or increased HGF levels. In glioblastoma, MET activation is associated with the higher-grade tumors. Potential therapeutic strategies target different target different aspects of MET function. C-Met peptides bind to the receptor preventing HGF from binding, whereas antibodies such as Rilotummab bind to HGF directly, although clinical trials showed adverse effects with this agent. As with other receptor-tyrosine kinases small molecules (such as) can target the kinase activity of c-MET. [1]
  (See chapter XYX in this book)

- *Platelet-Derived Growth Factor (PDGF) Signaling*

  Platelet-derived growth factors are important during embryonic development where oligodendrcyte precursor cells are stimulated to proliferate din response to PDGF.

  There are two receptor monomers that dimerize, resulting in 3 possible receptor dimer combinations and, their kinase activity is activated by the binding of one of 4 ligand dimers. As with other receptors, downstream effectors include the MAPK cascades (via rash activation) and JAK/STAT signaling. During development negative feedback is limited, so signaling is controlled primarily through PDGF availability.

  Roles in disease. PDGF receptors are often mutated, or expression is amplified in glioblastoma, and increased activation of PDGFR α signalling may be a disease initiating event. PDGF

  Clinical trials with the signaling antagonist, imatinib, have not yielded the hoped-for results in glioblastoma although there has been more success with the same drug in some gastro-intestinal tumors. Quinine derivatives (e.g. NSC13316) may prove to be more successful. The inhibitor nintedanib is used to target PDGFR (as well as VEGFR and FGFR) in non-small cell carcinoma and pulmonary fibrosis [2].

  A nice animation of PDGFR activation can be accessed through the following link
  http://www.cellsignallingbiology.org/csb/001/csb001_movo16.htm

- *Death Receptor Signaling*

  The growth factor super-families that directly regulate cell death are large with 19 ligands and 29 receptors and are predominantly expressed by immune cells. Members such as tumor necrosis factor (TNF) and Fas Ligand (FASL/CD95) bind to their receptors, TNF receptor (TNFR) and Fas/CD95, initiating cell death through recruitment of adaptor proteins such as TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD), which both associate with their corresponding receptor death domain. This leads to the activation of caspases resulting in cell death. Receptors lacking in death domains, such as, recruit molecules such as TNF receptor-associated proteins (TRAF) to initiate cell death via signal transduction pathways and activation of transcription factors such as AP-1 and NF-κB.

  Ligands such as TNF-related apoptosis inducing ligand (Trail or Apo2L) and TNF-like weak inducer of apoptosis (Tweak or Apo3L) are also members of this superfamily with Trail binding to its own receptors and initiating cell death. Like TNF, Trail can also activate NF-κB, a pro-survival transcription factor, indicating the importance of signaling balance and activation of pro- and anti-apoptotic factors by this super-family.

  Roles in disease: Dysregultion of TNF occurs in rheumatoid arthritis and other inflammatory diseases such as ankylosing spondylitis, ulcerative colitis and Crohn's disease. The main target for therapy in this superfamily is TNF with infliximab (an anti-TNF antibody).

  Treating Chrons' disease patient with combinatorial therapy that includes TNF inhibition can result in an increased risk of non-Hodgkin's Lymphoma, skin and lung cancers,

potentially highlighting the requirement for functioning death pathways in normal tissue homeostasis [3]

Cytoplasmic Signaling molecules

- *Phosphatidylinositol-3-kinase (PI3K) / Akt Signaling Pathway*
  The PI3K/Akt pathway is downstream of several growth factor receptors, most notably the EGFR/HER family, and is upstream of mTOR. It plays an essential role in regulating growth, metabolism, and survival of normal cells and its activity is negatively regulated by the phosphatase and tensin homologue, PTEN.
  Roles in disease. Activating mutations in this pathway are some of the most common mutations in cancer and human pathologies. PI3K/Akt activation results in conditions of clinical overgrowth disorders (e.g. Proteus syndrome) and Cowden's disease (due to inactivation of PTEN) as well as, a range of solid tumors and hematological cancers (e.g. breast, colorectal, hepatocellular, and ovarian cancers and acute myeloid leukemia).
  PI3K is a target for inhibitors that either inhibit all PI3Ks (e.g. XL147) or are targeted to specific isoforms and several are in Phase II or Phase III trials (e.g. CAL-101/Idelalisib) . Akt inhibitors (e.g. GSK2141795) are less selective but are also in clinical trials [4].
  (See chapter XYX in this book)

- *mTOR Signaling Pathway*
  The mechanistic target of rapamycin (mTOR) pathway is a serine/threonine kinase belonging to the phosphoinositide-3-kinase-related kinase (PIKK) family. It forms two distinct complexes and is activated by PI3K/Akt signaling, so is therefore critical in cell growth, metabolism and survival, as well as protein synthesis. In addition, mTOR functions as a nutrient sensor so is central to the regulation of intracellular glucose and amino acids. In some animal models (e.g. *C. elegans* and *S. cerevisiae*) decreased mTOR activity is linked to an increase in life span.
  Roles in disease. mTOR signaling is implicated in central nervous system disorders and cancers. It is frequently up regulated in cancers including breast and renal cancers.
  mTOR is a target for inhibition in multiple cancers (by rapalogues such as everolimus, temsirolimus . Combined inhibitors that also target PI3K have also been designed (e.g. BEZ-235, XL765) [5].

  (See chapter XYX in this book)

- *Protein Kinase C (PKC) Signaling*
  The PKC sub group are a family of intracellular serine/threonine kinases, expressed in many different tissues types. They play a key role in many different signaling pathways

contributing to the formation and degradation of focal adhesions, as well as regulating cell proliferation and invasion.

Roles in disease. Because they act in a many different signaling pathways, PKCs have been implicated in a range of cancers including pancreatic cancers.

PKCs are potential targets for small molecule inhibitors (e.g. UCN-01) and compounds such as Bryostatin that induce membrane localization of PKC isoformsbut these have been unsuccessful in clinical trials.

(See chapter XYX in this book)

- *MAPK/Erk in Growth and Differentiation Signaling Pathway*
  The mitogen activated protein kinases (MAPK) and the extracellular signal-regulated kinases (Erk) are sub families of serine/threonine and tyrosine/threonine kinases which function in a canonical signaling cascade known as the MAPK cascade. MAPK/Erk signaling is downstream of several transmembrane receptors, including FGFR, IGFR, EGFR, VEGFR and GPCR, and controls vital functions such as proliferation, differentiation, apoptosis, development, inflammation and stress responses. MAPK also regulates the activities of transcription factors.

  Roles in disease. MAPK signaling is implicated in several pathologies including some neuropathologies and cancers (e.g. melanoma, renal cell carcinoma and Hodgkin disease), and elevated MAPK activity is common in all inflammatory diseases.

  RAF and MEK kinases are targets of FDA-approved small molecule inhibitors, and Erk is a current target for pre-clinical kinase inhibitors (e.g.AZD7624) [6].

- *Phospholipase Signalling*
  Phospholipases are widely occurring; they are a class of enzymes that cleave phospholipids and it is likely that that they signal through MAPKs and other kinase pathways to regulate differentiation, programmed cell death and immune cell activation.

  Roles in disease. Phospholipase signaling has a mixed role in tumor development. Some isoforms play key roles in cell migration and invasion so contribute to carcinogenesis, whereas others are linked to tumor suppression, especially in colorectal cancers.

  Phospholipases have potential as targets for inhibitors and molecules that target protein-protein interactions, but there are no compounds currently in clinical trial.

- *AMP-activated protein kinase (AMPK) Signaling Pathway*
  AMPK is an intracellular serine/threonine kinase that is widely expressed as a nutrient sensor. It is phosphorylated in response to stress and subsequently activates its downstream substrates. It is a critical regulator of metabolic homeostasis, as well as having a role in cell proliferation and cell cycle regulation.

  Roles in disease. AMPK is implicated in the pathology of Peutz-Jeghers syndrome and several cancers (e.g. lung, liver, and cervical cancers).

  It is a drug target in prostate cancer cell growth where metformin is believed to have both direct and indirect effects of AMPK activity.

- *Hedgehog Signaling Pathway*
  The hedgehog (Hh) pathway has a central role in segmental pattern formation and in development. Depending on the context it can induce both cell proliferation and differentiation, and its signaling is cross linked with the MAPK cascade and PI3K/Akt and mTOR signaling [7].
  Roles in disease. Hh is involved in developmental diseases such as abnormal tube development and cancers (e.g. medulloblastomas, neuroblastomas, gliomas and breast cancers).
  Smoothened (SMO) is a target for natural inhibitors and Vismodegib, the first Hh-targeting compound to get US FDA approval, entered clinical trial in 2017
  (See chapter XYX in this book)

- *Glycogen Synthase Kinase-3 (GSK-3) Signalling*
  GSK-3 is a serine/threonine kinase central to many cellular processes such as metabolism, apoptosis, cell cycle progression, migration, differentiation, and embryogenesis. It interacts with multiple signaling pathways including PI3K/Akt, MAPK, Wnt/β-Catenin, Notch and Hedgehog [7].
  Roles in disease. GSK-3 plays a role in several cancer types (e.g. breast, colorectal, pancreatic and ovarian cancers, and melanomas and glioblastomas) and is a target in Alzheimer's disease.
  GSK-3 can be therapeutically targeted by lithium and small molecule inhibitors (such as benzimidazoles and pyrimidines) and a potential target for miRNAs.

Signalling Molecules and Nuclear Receptors

- *Jak/STAT Signaling Pathway*
  The Janus kinase (JAK) family are non-receptor tyrosine kinases activated by cytokines. Cytokines phosphorylate the cell membrane cytokine receptors, causing binding and activation of the signal transducers and activators of transcription (STATs). STATs translocate to the nucleus where they regulate gene expression resulting in a wide range of biological effects that regulate T and B cell activities.
  Roles in disease. JAK/STATs play a role in numerous diseases including rheumatoid arthritis, colitis and Chrons disease, as well as in hematological malignancies such as leukemia and lymphoma and some solid tumors. JAKs are also targets for first and second generation small molecule inhibitors. A number of molecules targeting JAKS or STATS are in clinical trials such as sorafenib (STAT3 inhibitor in breast and thyroid cancer), WHI-P131 or WHI-P154 (JAK3 inhibitors in Glioblastoma).
  (See chapter XYX in this book)

- *Wnt / β-Catenin Signaling Pathway*

The Wnt/β-Catenin signaling pathway is important in normal cell growth and development. The presence of Wnt, β-Catenin forms a complex with transcription factors to regulate gene expression. In the absence of Wnt, β-Catenin is phosphorylated and subsequently degraded by the proteasome.

Roles in disease. Wnt / β-Catenin is involved in cancers such as medulloblastomas, ovarian and colorectal cancers. The most well-known genetic mutation in the pathway is in the *APC* gene resulting in familial adenomatous polyposis (FAP).

Wnt / β-Catenin is a target for traditional compounds such as iron chelators and non-steroidal anti-inflammatory drugs (NSAIDs). It is also a potential target for biological therapies (e.g. Vantictumab) and small molecules (e.g. LGK974) as well as for natural inhibitors that degrade β-Catenin (e.g. flavonoids) [7].

(See chapter XYX in this book)

- *Notch Signaling Pathway*
  Notch is critical in many cellular processes and is activated in response to cell-cell interactions. Activation occurs through cleavage of Notch to form Notch intracellular domain (NCID) which is capable of nuclear translocation where it regulates gene expression to control cell proliferation, survival, and differentiation [7].

  Roles in disease. Notch is involved in the development of gastrointestinal, gastric, colorectal and pancreatic cancersNotch is a target for gamma secretase inhibitors, a few which are in a clinical trial (e.g. RO4929097).

  (See chapter XYX in this book)

- *NF- κ B Signaling Pathway*
  Nuclear factor kappa B (NF- κ B) is a transcription factor that functions in a complex to regulate expression of genes involved in proliferation, apoptosis, inflammation, and immune responses. It is required at a low level for normal hematopoiesis [8].
  Roles in disease. NF- κ B is implicated in leukemia (e.g. acute myeloid leukemia)
  NF-κB is a target for inhibitors, and some of its regulators such as IRAK1, TAK1, Bruton tyrosine kinase (BTK) and IKK are also considered potential targets (e.g. by PCI-32765/ibrutinib).
  (See chapter XYX in this book)

- *Nuclear Receptor Signaling*
  The retinoic acid-related orphan receptors (ROR α-γ or NR1F1-3), the orphan receptor TAK1 (TR4 or NR2C2) and the estrogen receptor (ER) are members of the nuclear receptor superfamily of ligand-dependent transcription factors. These receptors exhibit critical functions in regulating embryonic development and many other physiological processes and have been implicated in a variety of pathologies

Roles in disease. The RORs, TAK1/TR4, and ER have been implicated in a number pathologies, including various cancers (e.g. breast cancer)

The ROR, TAK1/TR4, and ER nuclear receptors are targets for endocrine disruptors and drug therapy (e.g by Tamoxifen). ER activity can also be indirectly targeted through inhibition of the aromatase enzyme (e.g. by letrozole, anastrazole).

(See chapter XYX in this book)

- *Progesterone and Androgen Receptor Signaling*
  Like estrogen receptors, progesterone and androgen receptors are steroid hormone receptors. Progesterone and androgens (e.g. testosterone) bind to their respective receptors in the cytoplasm, initiating a conformational change and nuclear translocation. Once in the nucleus, the receptors predominantly function as DNA-binding transcriptional regulators.
  Roles in disease: The most notable examples of diseases involving these receptors are breast (progesterone) and prostate (androgen) cancers with the receptors being targets for drugs such as Tamoxifen (progesterone receptors) and bicalutamide (testosterone receptors).

- *Aurora Kinases*
  Aurora kinases became a focus of interest over the last 20 years after they were discovered during screens for proteins involved in mitotic spindle dysfuction; their role is to regulate mitosis. They are located at the kinetochores and their levels increase and decrease during the cell cycle, peaking between late S-phase and M-phase.

  Roles in disease. All three human aurora kinases play roles in the development of both hematological malignancies and solid tumors (e.g CML, AML, breast and colon cancer). They are targets for small molecule inhibitors such as danusertib and barasertib [9

2. Common Signaling Components in Cancer

   Most of the pathways discussed in Section 1 contribute to a more 'active' cellular phenotype; therefore, they are all implicated in cancer development in some way. What is also clear is that several of these pathways contribute to the development of multiple cancer types and that few cancer types arise from dysregulation of only a single pathway. For example, breast cancer can arise due to elevated ER, EGFR/HER or IGFR signaling and, on many occasions, dysregulation of more than one of these pathways is involved.

   Several of the cell membrane receptor families activate the same downstream intracellular pathways meaning there are common signaling components in the development of cancer. The MAPK cascade is activated by EGFR/HER, FGFR, IGFR, VEGFR, PDGFR and GPCR signaling (Figure 2).
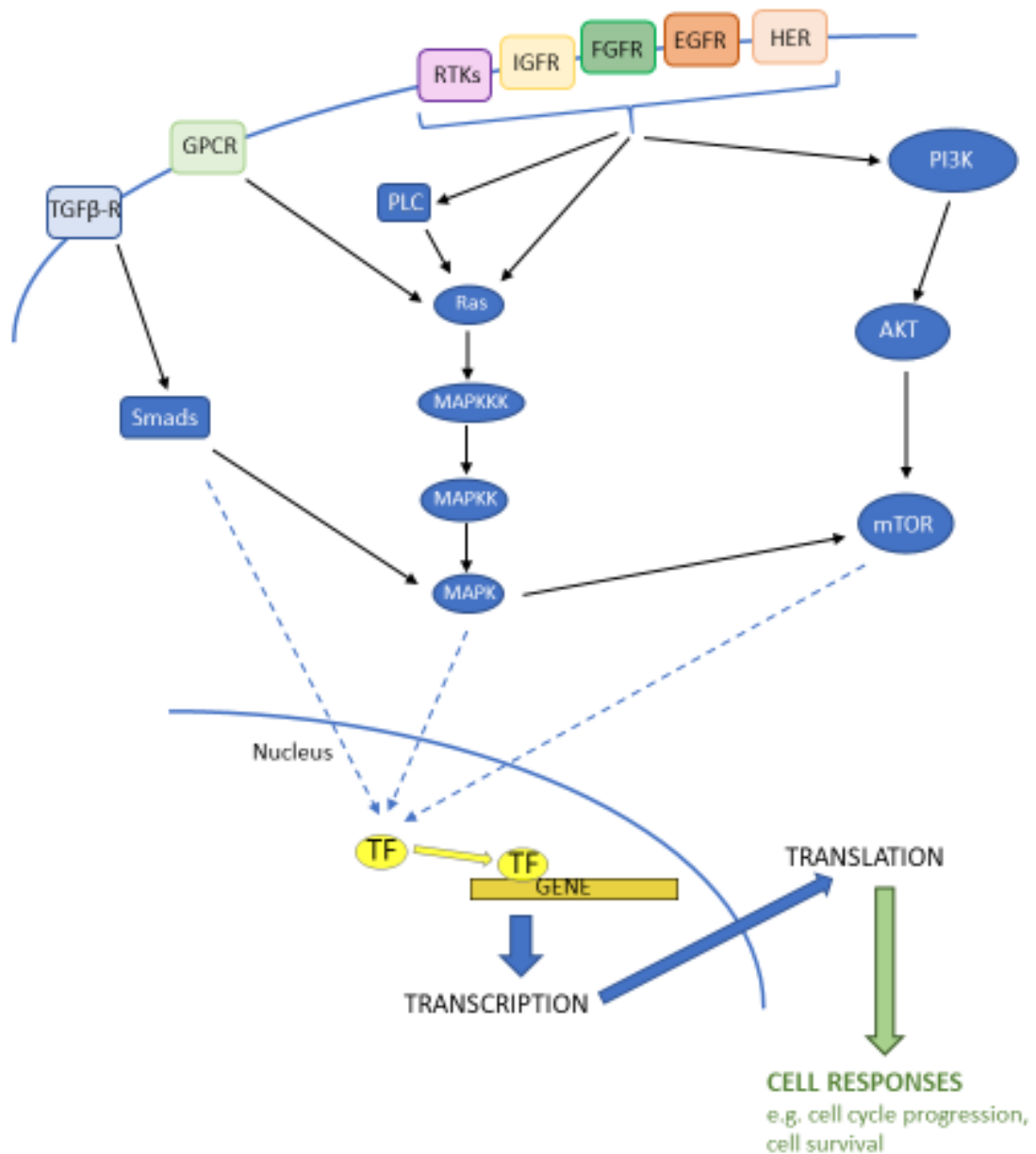
Figure 2: Common signaling components in cancer. In response to increased signaling from cell surface receptors, transcription of genes encoding of pro-survival proteins and positive regulators of cell cycle progression is increased, resulting in the cell adopting a more cancerous phenotype. TF= Transcription Factor

In the nucleus transcription of genes involved in cancer progression is increased; nuclear receptors are directly involved in mediating the transcriptional whereas activation of the other cell signalling pathways results in phosphorylation of transcriptional activators (for example STATs) which in turn increases transcription.

This often means that there can be increased activity of MAPK signaling in the absence of specific genetic or expression abnormalities, purely because an upstream receptor is more active. This point is nicely demonstrated in non-small cell lung carcinoma where, in 39 tumors with increased intracellular signaling due to activating mutations, 30% had mutations only in the EGFR/HER receptors and not in the Ras-Raf-MAPK cascade.

PI3K/Akt signaling has a long association with many types of cancer. Patients with Cowden's disease, characterized by PTEN mutations, have elevated PI3K/Akt signaling and are at a much-increased risk of developing cancers most notably, breast cancer. 70% of breast cancers have gene mutations resulting in increased PI3K/Akt activity. PI3K/Akt signaling is crucial in tumor development as it links receptor signaling with downstream effects such as MAPK and mTOR.

mTOR signaling is downstream of PI3K/Akt and therefore several upstream signaling pathways, including EGFR/HER, FGFR, IGFR, converge at this focal point. mTOR signaling is often over active as result of mutations in mTOR; however, in some cancers including breast cancer, activation of the EGFR/HER family of receptors and activating mutations in PI3K/Akt signaling also result in elevated mTOR activity [6].

JAK/STAT signaling tends to be more closely linked to the development of hematological malignancies, largely due to its involvement in cytokine signaling and the reliance of T and B cells on cytokines for their normal function. There is, however, a role for JAK/STAT signaling in the development of solid tumors as STAT5 can be activated by binding to EGFR and so could play a role in the signal cross talk (Section 3).

From a pharmacological perspective, activation of signaling pathways provides an opportunity for therapeutic intervention. The heterogeneity of signaling across cancers means drugs that are designed to inhibit specific signaling molecules have potential clinical benefit in more than one tumor type. The reality is, however, that some compounds are not as effective as predicted and this may well be due to the intricate balance of intracellular signaling required to maintain tumor growth is potentially different to that required to establish initial tumor formation, development, and metastasis. For example, VEGF signaling plays a niche role in the development of solid tumors. The barrier to a microscopic tumor progressing to a larger mass is the requirement for oxygen, delivered by a blood supply. VEGF signaling is therefore critical early on in tumor development for neo-angiogenesis (formation of new blood vessels). Once a solid tumor is established, the reliance on VEGF signaling is likely to diminish; however, as a tumor becomes metastatic and cells disseminate to distant locations VEGF signaling is once again required in the development of distant metastases. This potentially means that inhibition of VEGF signaling is maximal during the developmental stages or in treating tumor types where re-modelling, and therefore angiogenesis, is a common occurrence.

3. Signaling Cross-Talk

The commonality between the signaling pathways discussed in Section 1, and the fact that these provide many common signaling components in cancer development, also results in the biggest barrier to therapy, namely signaling cross talk and compensatory signaling [10].

Signalling cross-talk can occur via different mechanisms:
- A molecule in one pathway can affect the rate of activation of signaling molecules in a second pathway (signal flow cross-talk).
- Two pathways can compete for common components (substrate availability cross talk).
- Receptors can have altered ability to detect ligands, or if receptors are over expressed (as with HER2) signaling can happen in the absence of ligand (receptor function cross-talk).
- Individual pathways could have opposing effects on transcription factor activation (gene expression cross-talk).
- Ligand availability can be altered because of different mechanisms but often occurs in response to gene expression changes (intracellular communication cross-talk).

The cross-talk mechanisms are not mutually exclusive and will often influence each other. For example, because signaling pathways converge at focal points, inhibiting one route to the focal point still allows signaling to that point to be re-routed via a different path and potentially free components to be activated via the second pathway (examples of signal flow and substrate availability cross-talk).

Reducing PI3K/Akt or mTOR signaling, for example, through inhibition of membrane receptor activity, will initially achieve the desired outcome; however, overtime, tumor cells will adapt and find alternative mechanisms for increasing signaling. For example, if EGFR/HER is inhibited more PI3K/Akt becomes available for IGFR signaling.

In the development of drug resistance EGFR/HER inhibition could be mitigated through a compensatory increase in FGF, IGFR or GPCR signaling, all of which would sustain elevated PI3K/Akt or mTOR activity. Indeed, IGF-1R signaling reduces the sensitivity of breast cancer cells to anti-HER2 monoclonal antibody therapy; sensitivity to trastuzumab is increased through inhibition of IGF-1R [10].

What is also perhaps most surprising is the promiscuity of receptors in drug resistant cells. It is easy to presume that receptors only dimerize with their designated partners and that they only signal within their discreet pathways. This is not always the case. Both IGF-1R/HER2 dimers and IGF-1R/HER2/HER3 trimers have been detected in trastuzumab resistant cells suggesting firstly that compensation for EGFR/HER signaling inhibition could be mediated through insulin-like growth factor signaling and, secondly, that there is a

clinical rationale for combined EGFR/HER and IGF-1R targeting in tumors resistant to anti-HER2 or anti-EGFR therapy.

In addition to EGFR and IGFR, Wnt signaling also activates mTOR, where cross talk results in activation of both Notch and STAT signaling. Phosphorylation of EGFR/HER family receptors depends on the specific activating ligand. In some circumstances, phosphorylation of EGFR or HER4 will facilitate cross-talk through STAT5 binding and activation which, under normal conditions, is an infrequent event; however, in breast cancer, STAT5b could contribute to an increased proliferative phenotype through enhanced transcriptional activation.

Although canonical TGF-β signaling occurs via the Smad proteins, there is signal flow and gene expression cross-talk between TGF-β signaling and the MAPK pathways. MAPK signaling can activate expression of TGF-β target genes, and specific MAPK activity is central to breast cancer cell migration mediated by TGF-β [10].

4. Predictive Biomarkers and Therapeutic Targets

There is a very clear need for cancer biomarkers, both from a diagnostic and prognostic perspective. As our understanding of signaling has developed and the range of possible therapeutic options expands, it is vital to have reliable biomarkers that will predict which patients will benefit from specific treatment regimens. Many clinical trials now included evaluation of potential biomarkers as part of the study aims.

Unsurprisingly many prognostic biomarkers are also therapeutic targets, for example, the estrogen receptor (ER) predicts patient outcomes. Tumors lacking hormone receptors have worse outcomes, partly because triple negative breast cancers are more aggressive in nature and less responsive to chemotherapy, but also because the ER is itself a target for anti-hormone therapies such as Tamoxifen.

Given the broad nature of cell signaling and the variety of signaling pathways outlined in Section 1, there are many potential biomarkers in cancer. The discussion in this section will focus on EGFR/HER signaling (Figure 3), with other examples being illustrated in Table 1.
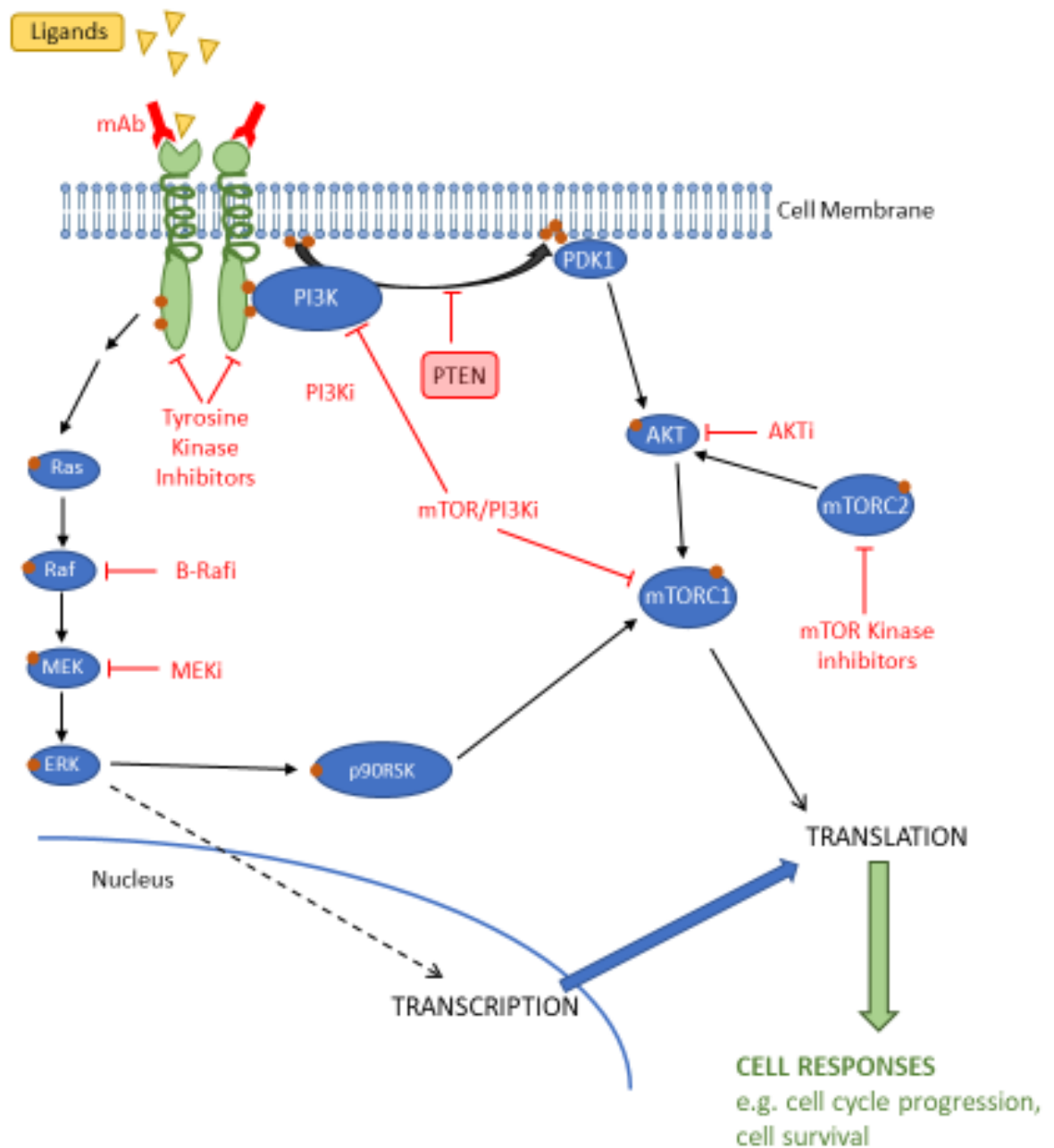
Figure 3: Examples of biomarkers and therapeutic targets in the EGFR/HER signaling pathways (adapted from [12]). In response to increased Her2 signaling, transcription of genes encoding of pro-survival proteins and positive regulators of cell cycle progression is increased, resulting in the cell adopting a more cancerous phenotype in response to transcription of pro-survival genes. When EGFR/HER signaling is inhibited by some of the compounds listed in the above figure, the increase in transcription is ablated with a down-regulation of the biological response.

As discussed above in signaling cross-talk, many patients do not respond to their targeted therapy, or they initially respond and then develop resistance. This is very evident in colorectal cancer, where patients with elevated EGFR signaling are offered anti-EGFR monoclonal antibody therapy. Elevated EGFR signaling in colorectal cancer can be categorized based on (i) increased upstream components, (ii) increased amount or aberrant EGFR, (iii) activation of downstream molecules or (iv) activation of alternative by-pass pathways. Only patients with tumors categorized in (i) or (ii) will respond to anti-EGFR monoclonal antibody therapy, so it is vital to have biomarkers that are predictive of response or resistance to treatment. So far, Ras has proved to be the most useful biomarker to predict resistance to anti EGFR monoclonal antibodies in colorectal cancers as Ras mutations are linked to resistance to anti-EGFR therapy in colorectal cancer [11].

Epiregulin (EREG) is an EGFR ligand that is initially released as a transmembrane precursor. It regulates angiogenesis, and cell proliferation and increased levels are associated with a more aggressive tumor phenotype. Colorectal carcinoma patients with wild-type Ras tumors and high EREG gene expression have better outcomes in response to anti-EGFR therapy (cetuximab), with and without chemotherapy than those with low EREG expression. When serum levels of EREG were considered the reverse was noted; both overall and progression-free survival times were shorter in patients with higher EREG levels than those with low. These inconsistencies are not surprising given the lack of correlation between protein levels and gene expression, but it does highlight the difficulties in identifying reliable prognostic biomarkers. More recent studies have indicated that BRAF mutations are more likely to serve as independent prognostic factors.

TGFα alpha activates the EGFR, stimulating the MAPK pathway resulting in increased proliferation invasion and metastasis in both colorectal carcinoma and breast cancer patients. High tumor levels of TGFα are linked with resistance to anti-EGFR antibodies in colorectal carcinoma patients. In breast cancer, high TGFα expression is linked with poorer outcomes and resistance to chemotherapy, whilst high serum levels correlate with a more aggressive tumor in non-small cell lung carcinoma (NSCLC). This illustrates that the same biomarker has potential in different tumor types, but it needs to be measured differently between the types; in some cases, tumor levels are required, in others it is serum levels that matter. In NSCLC, EGFR mutations are indicative of response to kinase inhibitors rather than absolute levels of EGFR.

In breast cancer, elevated HER2 is indicative of prognosis and relative HER2 and HER3 levels are predictive of patient responses to trastuzumab and pertuzumab respectively. This highlights that the complexities of signaling and receptor dimerization need to be considered alongside over expression and mutations when considering biomarkers, therapeutic targets and patient responses. A proportion of HER2 positive tumors also express a shorter form of HER2 (p95HER2). It lacks the extracellular domain, meaning it has no trastuzumab binding site, is hyperactive and very tumorigenic. In metastatic breast cancer p95HER2 expression correlates with intrinsic resistance to trastuzumab [12].

Other potential biomarkers in breast cancer are linked to IGF-1R; however, measuring levels of IGF-1R alone is not enough to select breast tumors that maybe sensitive to IGF signaling inhibition. It is the combined levels of IGF-1R and IRS-1 that maybe more informative especially as IRS-1 is associated with reduced disease free survival in breast cancers.

Table 1. Examples of cancer biomarkers and therapeutic targets and their relationship to the hallmarks of cancer identified by Hanahan and Weinberg [13].

Hallmarks of Cancer	Signaling Pathways	Example of Biomarkers	Example of a Major Therapeutic Target in Signaling
Sustaining proliferative signaling	EGFR/HER IGFR PKC MAPK	Breast cancer: ER PR HER2 p95HER2 IGF-1R/IRS-1 EREG (CRC) IRS1 (BC) IGF2 (CRC) PTEN (BC)	ER HER2
Activating invasion and metastasis	PKC MAPK EGFR/HER IGFR TGF-β	TGFα (CRC) TGFα / Amphiregulin (NSCLC)	EGFR
Evading Growth Suppressors	EGFR/HER MAPK	PTEN (BC)	EGFR
Resisting cell death	IGFR EGFR/HER	IGF2 (CRC) PTEN (BC)	 EGFR
Inducing Angiogenesis	VEGF EGFR/HER Ras	VEGF EREG (CRC)	VEGFR
Enabling Replicative Immortality	B-catenin	Telomerase length [14]	

BC: Breast Cancer, CRC Colorectal Carcinoma, NSCLC: Non-Small Cell Lung Carcinoma

5. New signaling pathways and Future Strategies

When signaling-molecule inhibitors were first developed, many lacked specificity and exhibited a variety of cross-reactivity. For this reason, they were not considered suitable for clinical use and researchers were skeptical about their value in *in vitro* pre-clinical studies as it was difficult to determine whether data generated was a result of a desired inhibitory effect, or as an artefact of an off-target. It is clear that single targeting has clinical benefit; however, it is also evident that cross-talk and compensatory signaling results in therapeutic resistance such that targeting of sole signaling molecules might not be a fruitful long-term treatment strategy. There are several clinical trials examining the combinatorial effects of multiple inhibitors and current thinking is that combined targeting strategies are likely to be the most successful for long-term patient survival.

In addition to multiple targeting, targeting adaptor molecules that link receptors to downstream effectors and signaling focal points are likely to have the most impact. To that end, multiple mTOR and dual mTOR/PI3K inhibitors are either undergoing clinical trials or are already in clinical use. Moreover, it is worth revisiting previous avenues that had previously been disregarded. The adaptor tyrosine kinases of the Src family were once perceived as potential drug targets. However, the amino-acid homology between family members meant designing specific inhibitors was difficult and, when Src was inhibited, lack of activity was compensated for by signaling via other family members. A broad-spectrum approach to kinase inhibitor design could ameliorate these issues.

There is also scope for novel drug targets to be identified and some, such as Brk/PTK6 may prove to be of therapeutic value as part of a combined therapeutic strategy especially in tumors for which there is currently no other viable signaling target (e.g. triple negative breast cancers) [15]. So far, this chapter has largely focused on intracellular signaling and cross-talk. To develop novel, more effective anti-cancer treatments the effects of the tumor microenvironment, and its interaction with tumor cells must be taken into consideration. The 'seed and soil hypothesis' is not new and it has long been known that certain tumor cell types 'prefer' to colonize specific extracellular environments to form metastases.

To colonize the microenvironment, cancer cells must be attached to the extracellular matrix (ECM) and signal to the cells within it, such as macrophages and fibroblasts which then become associated with the tumor and are referred to as tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs). Expression of factors that regulate the ECM can promote tumor formation; in addition, many factors within the ECM can enhance the ability of tumor cells to be invasive and re-model the microenvironment through a process termed epithelial to mesenchymal transition (EMT). Understanding the interplay between the microenvironment and tumor cells is critical in developing novel therapies. Although enhanced CAF activity by tumor secreted growth factors is well documented, it is still not clear what initiates CAF activation [16]. The link between tumor cells and the microenvironment could be mediated through NF-κB which, in addition to its role in proliferation and apoptotic control, can regulate the expression of pro-

inflammatory cytokines that will initiate signaling required for ECM remodeling, thereby promoting tumor progression. Targeting the production of such cytokines could have enhanced clinical benefit in comparison to focusing solely on the tumor cells.

As a result of their interaction with the microenvironment, tumor cells are also capable of evading detection by the immune system. Immunotherapeutics are being developed to re-activate the immune system to recognize and destroy tumor cells. Products such as Sipuleucel-T, a therapeutic immuno vaccine and Ipilimumab, a monoclonal antibody, both have FDA approval. At a cost of over $100,000 per individual treatment course, identifying patients who are most likely to benefit is crucial.
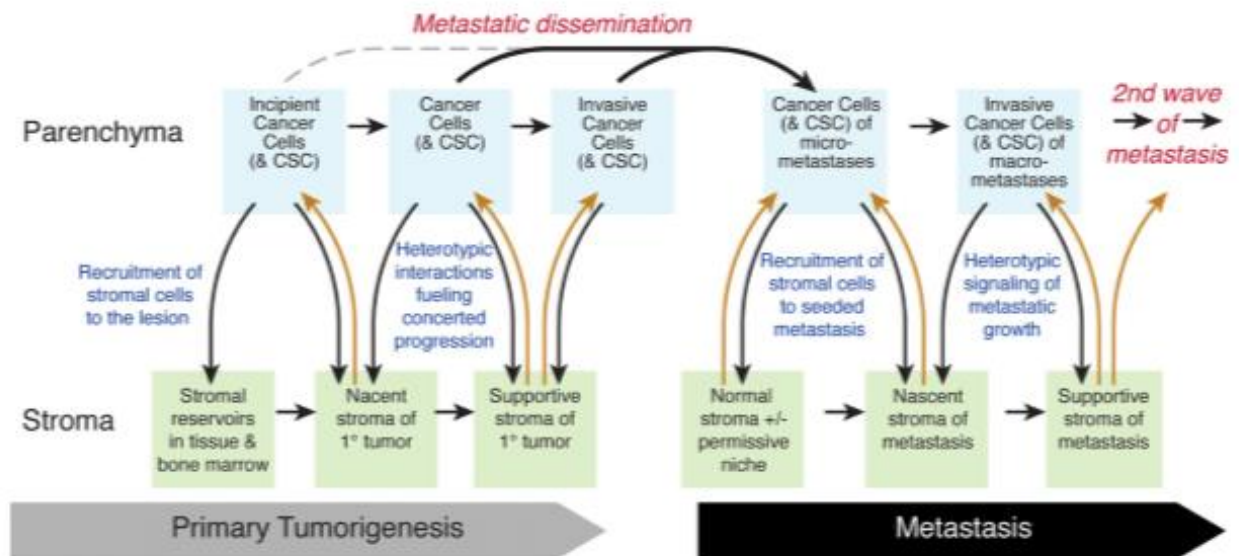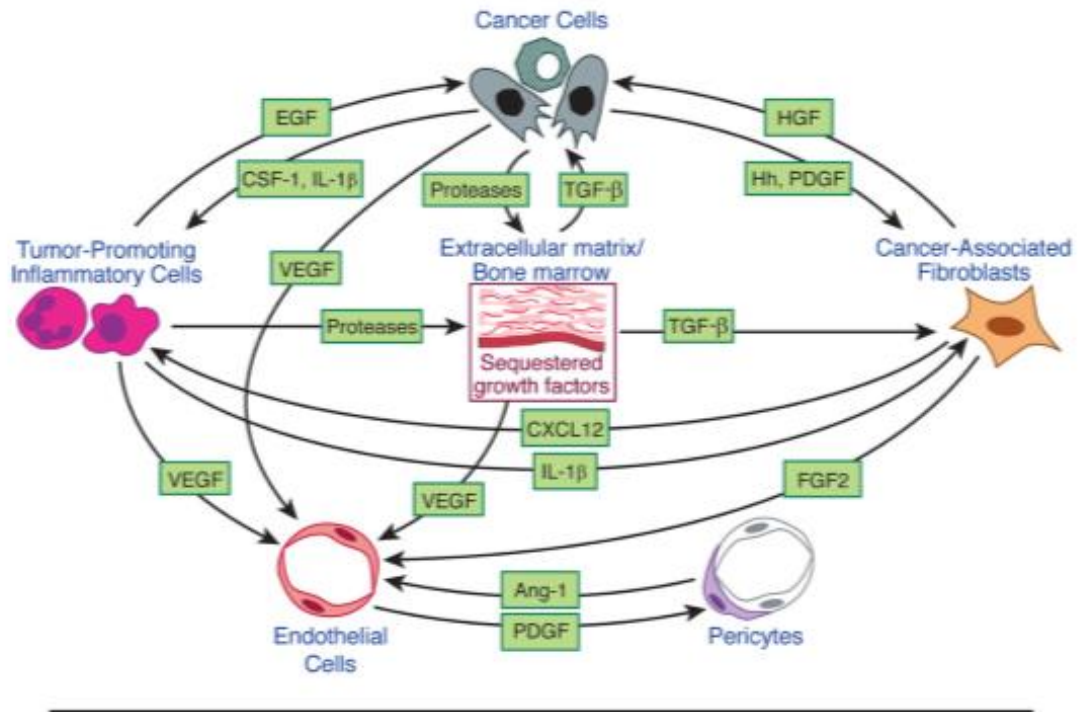
Figure 4. Signaling Interactions in the Tumor Microenvironment during Malignant Progression
(Upper) The assembly and collective contributions of the assorted cell types constituting the tumor microenvironment are orchestrated and maintained by reciprocal heterotypic signaling interactions, of which only a few are illustrated. (Lower) The intracellular signaling depicted in the upper panel within the tumor microenvironment is not static but instead changes during tumor progression as a result of reciprocal signaling interactions between cancer cells of the parenchyma and stromal cells that convey the increasingly aggressive phenotypes that underlie growth, invasion, and metastatic dissemination. Importantly, the predisposition to spawn metastatic lesions can begin early, being influenced by the differentiation program of

the normal cell-of-origin or by initiating oncogenic lesions. Certain organ sites (sometimes referred to as "fertile soil" or "metastatic niches") can be especially permissive for metastatic seeding and colonization by certain types of cancer cells,as a consequence of local properties that are either intrinsic to the normal tissue or induced at a distance by systemic actions of primary tumors. Cancer stem cells may be variably involved in some or all of the different stages of primary tumorigenesis and metastasis (Courtesy: Elsevier Inc. Reprinted from Fig. 5. Hanahan D and Weinberg RA. Cell 144, pg. 666, 2011)

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6. Conclusions and Perspectives

There is no doubt that the wealth of knowledge relating to cell signaling in cancer has vastly improved in last 20 years. More is known about cross-talk and how this could contribute to drug resistance or how it could influence treatment options and therapeutic combinations of the future. As a scientific community, there is still a tendency to consider signaling molecules in isolation and to teach students about individual pathways, largely for simplicity. There is a need to be much more aware of intracellular signaling networks and the cross-talk between pathways, as well as the extracellular cross-talk if the gains of the last 2 decades are to be continued in the next 2o years.

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