Cancer Prognostics and Diagnostics

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Research Article

Cell Signaling & Cancer Therapy

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Abstract

During the course of tumor progression, cancer cells acquire a number of characteristic alterations. These include the capacities to proliferate independently of exogenous growth promoting or growth-inhibitory signals, to invade surrounding tissues and metastasize to distant sites, to elicit an angiogenic response, and to evade mechanisms that limit cell proliferation, such as apoptosis and replicative senescence. These properties reflect alterations in the cellular signaling pathways that in normal cells control cell proliferation, motility, and survival. Many of the proteins currently under investigation as possible targets for cancer therapy are signaling proteins that are components of these pathways. The nature of these signaling pathways and their roles in tumorigenesis were the subject of a recent Beatson International Cancer Conference.

Introduction

Cancer stem cells (CSCs) are a small subset of cancer cells with the capability of self-renewal and differentiation into heterogeneous into heterogeneous tumor cells, and they have been believed to be responsible for tumor initiation, growth, and recurrence. The first population of CSCs was identified in human acute myeloid leukemia (AML), where they displayed strong tumorigenic ability in an in vivo mouse model [1,2]. Subsequently, many laboratories across the globe have been able to capture and propagate CSCs from a variety of human tumors including brain cancer, melanoma and breast cancer, liver cancer, pancreatic cancer, colon cancer, and prostate cancer [3-9]. As CSCs can survive traditional cancer therapies and result in tumor recurrence and drug resistance [10-12], eradication of CSCs in tumors may represent an effective anticancer therapeutic strategy. Towards this goal, significant efforts have been made to explore the signaling mechanisms underlying CSCs' self-renewal and differentiation, as well as development of regimens targeting the CSCs. ln this review we focus on three key evolutionarily conserved CSC signaling pathways (Wnt, Hedgehog' and Notch pathways) and therapeutic strategies disrupting CSCs' stemness and functions by modulating these pathways.

Cellular signaling pathways are not isolated from each other but are interconnected to form complex signaling networks. Cells receive information from many different growth factor receptors and from cell-matrix and cell-cell contacts. They must then integrate this information to regulate diverse processes, such as protein synthesis and cell growth, motility, cell architecture and polarity, differentiation, and programmed cell death. The same signaling molecules are used to control different processes within different signaling complexes or at different intracellular locations. Moreover, signaling pathways are subject to developmental regulation and generate different outcomes in different cell types; the activation of a signaling molecule may have distinct consequences, depending on the cellular context. Understanding how these extraordinarily complex signaling networks function in vivo and how the5i 6ps altered in cancer cells represents a major intellectual challenge.

Material Methods

Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules ("molecular targets") that are involved in the growth, progression, and spread of cancer. Targeted cancer therapies are sometimes called "molecularly targeted drugs," "molecularly targeted therapies," "precision medicines," or similar names.

The Wnt, Hedgehog, and Notch pathways are inherent signaling pathways in normal embryogenesis, development, and hemostasis. However, Dysfunctions of these pathways are evident in multiple tumor types and malignancies. Specifically, aberrant activation of these pathways is Implicated in modulation of cancer stem cells (CSCs), a small subset of cancer cells capable of self-renewal and differentiation into heterogeneous tumor cells. The CSCs are accountable for tumor initiation, and recurrence. The this review, we focus on roles of Wnt, Hedgehog, and Notch pathways in CSCs' stemness and functions and summarize threrapeutic studies targeting these pathways to eliminate CSCs and improve overall cancer treatment outcomes.

Targeted therapies differ from standard chemotherapy in several ways

- Targeted therapies act on specific molecular targets that are associated with cancer, whereas most standard chemotherapies act on all rapidly dividing normal and cancerous cells.
- Targeted therapies are deliberately chosen or designed to interact with their target, whereas many standard chemotherapies were identified because they kill cells.
- Targeted therapies are often cytostatic (that is, they block tumor cell proliferation), whereas standard chemotherapy agents are cytotoxic (that is, they kill tumor cells).

Targeted therapies are currently the focus of much anticancer drug development, They are a cornerstone of precision medicine, a form of medicine that uses information about a person's genes and proteins to prevent, diagnose, and treat disease.

Many targeted cancer therapies have been approved by the Food

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and Drug Administration (FDA) to treat specific types of cancer. Others are being studied in clinical trials (research studies with people), and many more are in preclinical testing (research studies with animals).

The ability of intracellular signaling networks to integrate and distribute regulatory information requires that individual signaling proteins must act as nodes, responding to multiple inputs and regulating multiple effector outputs. One of the major advances in the last decade has been the recognition that many signaling proteins contain modular protein domains that mediate protein-protein interactions. These interaction modules serve to target signaling proteins to their substrates or to specific intracellular locations, to respond to posttranslational modifications, such as phosphorylation, acetylation and methylation, and to link polypeptides into multiprotein signaling complexes and pathways [13]. The same protein modules can also mediate intramolecular interactions that regulate signaling function, and a frequent theme is that upstream regulators may act by promoting or disrupting these intramolecular interactions. Thus, to understand the overall architecture of the signaling network, we will ultimately need to identify all of these inter- and intramolecular interantions.

Results

The intricacy of cellular signaling network$ has major implications for our understanding of tumor cell behavior and for our ability to use this knowledge for cancer therapy. Cell proliferation, motility. and survival are regulated by multiple pathways, and the changes that occur in cancer cells are the result of multiple alterations in cellular signaling machinery. Cancer cells are genetically unstable, undergo multiple genetic and epigenetic changes, and continuously evolve in response to selective pressures. Even if a mutationally activated pathway can be blocked by an inhibitor, tumor cells may evade the inhibitor by activating other pathways Thus, even though early stage malignancies may respond to a single 'inhibitor-the success of Gleevec (STl-571) being a prime example-effective therapies for more advanced malignancies may require combinations of signaling inhibitors, or signaling inhibitors in conjunction with traditional DNA-damaging chemotherapeutic reagents.

The complex architecture of signaling networks and the consequences of this complexity for possible cancer therapeutics were recurrent themes. (Figure 1)

This figure illustrates some of the signaling pathways involved in malignant transformation and tumor cell proliferation that were discussed at the meeting. Arrows (black) indicate activation or induction, T-bars (red) indicate inhibition.

The roles of Src and FAK in cell motility and invasion

The cellular *src* gene was the first protooncogene to be discovered in the vertebrate genome. ln the last few years, there has been increasing evidence that Src plays an important role in tumor cell invasion, in particular through its interaction with FAK (focal adhesion kinase). Src and FAK are nonreceptor tyrosine kinases that are localized to cellmatrix adhesions and mediate integrin signaling. Following integrin engagement, FAK undergoes autophosphorylation at Tyr 397 and Src becomes recruited to activated FAK via an interaction between the SH2 domain of Src and FAK pTyr397. Src then phosphorylates FAK at a number of tyrosine residues, creating docking sites for SH2 domain - containing signaling proteins, such as the adaptor Grb2. Genetic inactivation of Src family kinases or of FAK causes defects in cell motility, associated with an inability to turnover focal adhesions. ln addition, Src and FAK are found in membrane ruffles and in podosomes (also known as invadopodia), dynamic protrusions that are prominent in certain cancer cells and which are involved in the degradation of the extracellular matrix. Both Src and FAK exhibit elevated expression in a number of different epithelial tumors. e specially in invasive cancers. These observations have provided circumstantial evidence for a role of Src and FAK in tumor cell motility and invasion.

Margaret Frame (Beatson lnstitute, Glasgow) described the use of several different model systems to study the role of FAK in carcinoeenesis. ln the two-stage skin carcinogenesis model, mouse skin is initially painted with dimethylbenzanthracene (DMBA), which causes Ha-Ras mutations and the growth of papillomas. Subsequent treatment with the tumor promoter tetradecanoyl phorbol acetate (TPA) causes progression of the papillomas to malignant carcinomas. Experiments using *fak* knockout heterozygote s or a conditional *fak* allele indicate that the level of FAK protein appears to be a determinant of both initiation and progression. Reconstitution experiments with FAK-/- fibroblasts and mutant forms of FAK suggested that tyrosine phosphorylation of FAK is critical for cell motility. Moreover, when activated Src was introduced into noninvasive colon cancer cells, they were converted from an epithelial to a more invasive, mesenchymal

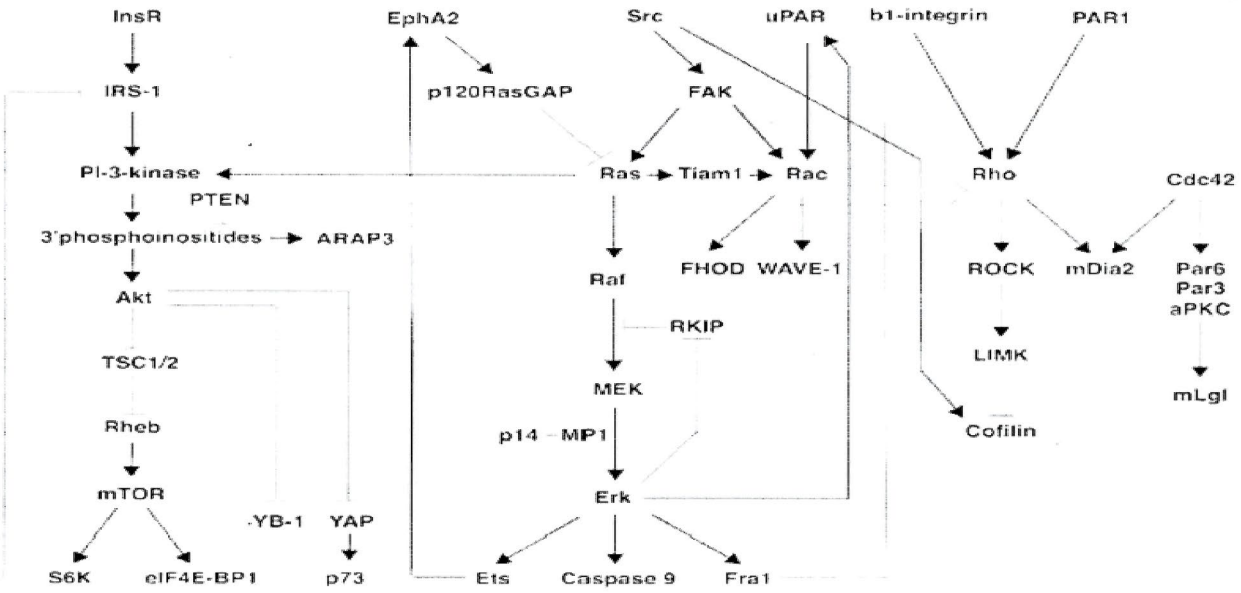

Figure 1: Cell signaling pathways.

phenotype, associated with a change from cadherin-dependent cell contacts to integrin-dependent matrix adhesions [14]. This alteration was also correlated with tyrosine phosphorylation of FAK. Thus, FAK activation and phosphorylation of FAK by Src appear to be important determinants of tumor cell motility and invasiveness.

Another mechanism by which the interaction between Src and FAK enhances tumor cell invasion was described by David Schlaepfer (Scripps lnstitute, La Jolla, California). Trans formation of FAK null cells by v-Src yields transformants that are noninvasive in Matrigel assays. The invasive properties of these transformants were restore d by reexpression of wild-type FAK but not by reexpression of FAK mutated at the Src docking site (Tyr397). Reconstitution experiments linked cell invasion to the formation of a signaling complex involving Src, FAK, the docking protein Cas, the adaptor Crk, and a guanine nucleotide exchange factor, Dock-180, which activates the small GTPase Rac. Activation of Rac was shown in turn to activate the Jun kinase (JNK) cascade, which leads to the activation and secretion of matrix metalloproteinases such as MMP-2 and MMP-9 [15]. Thus a Src-FAK-Rac pathway appears to promote the invasiveness of Src transformed cells. In addition, although expression of FAK does not promote the growth of Src-transformed cells in vitro, it does promote their growth as tumors in vivo, and this is correlated with increased expression of VIGF and increased tumor angiogenesis. Furthermore, the se effects of FAK are not restricted to cells transformed by Src in vitro, because a dominant-negative form of FAK (FAK-related non kinase or FRNK) blocks the growth of breast carcinoma cells as tumors in vivo. Finally, FAK plays still other roles in Src-induced focal adhesion turnover. Neil Carragher (Beatson lnstitute, Glasgow) reported that the protease calpain is activated in v-Src-transformed cells and that its recruitment lo focal adhesions promotes focal adhesion turnover and is dependent on FAK [16].

The Ras/MAP kinase pathway: Targets and scaffolds

Ras is mutationally activated in a significant fraction of human cancers. It interacts with multiple effectors, probably more than twenty. One of these, the serine/threonine kinase Raf, activates a MAP kinase pathway, the Raf-MEK-ERK pathway, which is required for transformation by oncogenic Ras in a number of systems. The ERK MAP kinases themselves have many substrates, and the identity of these substrates and their roles in tumorigenesis remain central questions. The pathway is regulated and organized by scaffolding proteins, in ways that are not yet fully understood. Finally, as discussed at the end of this report, components of this pathway are under intense investigation as possible targets for anti-cancer therapeutics.

The MAP kinase pathway activates Ets family transcription factors that in turn regulate the expression of multiple genes, including an ephrin receptor, the receptor-tyrosine kinase EphA2 (Frank McCormick, University of California, San Francisco). EphA2 activates pl20RasGAP [17], and so downregulates wild-type Ras but not mutationally activated Ras. Thus, EphA2 forms a component of a negative feedback loop, in which the Ras-MAP kinase pathway stimulates EphA2 expression, which in turn downregulales Ras. McCormick reported that breast cancer lines fall into two classes. More differentiated epithelial lines expressing wild-type Ras also express the EGF-related receptor tyrosine kinase ErbB3 and the EphA2 ligand ephrin, but do not express EphA2. The reason that EphA2 is not expressed in this class of breast cancer cell is partly because there is selection against FphA2 expression (since this downregulates wild-type Ras) and partly because ephrin itself downregulates EphA2. In contrast, the second class of breast cancer lines are more invasive and mesenchymal in phenotype and express both EphA2 and, in some cases, mutationally activated Ras. The invasive phenotype of this second class can be decreased by expression of ephrin.

Activation of MAP kinase can exert antiapoptotic effects that are important in tumor cell survival. Lindsey Allan (University of Dundee) described a novel target of MAP kinase signaling, caspase 9. This is a caspase that mediates mitochondrion-dependent apoptosis by activating caspase 3 in response to cytachrome c release . ERK MAP kinases phosphorylate caspase 9 at Thr125, and this phosphorylation inhibits proteolytic processing and activation of caspase 9. Okadaic acid, which inhibits protein phosphatases 1 and 2A, allows the accumulation of the phosphorylated form and also inhibits caspase 9 activation [18]. Thus, Map kinase phosphorylation of caspase 9 may represent one of the events by which oncogenic Ras promotes tumor cell survival.

The Raf-MEK-MAP kinase pathway is organized by a variety ol scaffold proteins, including Ksr, MP1, and 14-3-3 proteins. These scaffold proteins may regulate the activity of the pathway (positively or negatively), localize its output, increase the efiiciency of signal transmission, or isolate the pathway from crosstalk by other MAP kinase pathways. Two MAP kinase pathway scaffolds were discussed. One of these, identified by Walter Kolch (Beatson lnstitule, Glasgow), is termed the Raf kinase inhibitor protein or RKIP. This protein negatively regulates the pathway by blocking the interaction of Raf with MEK [19]. However, RKIP is itself phosphorylated by MAP kinase, reducing its affinity for Raf and causing its dissociation from Raf in vitro and in vivo. This appears to result in a positive feedback loop, in that MAP kinase activity inhibits an inhibitor of the pathway, and Kolch proposed that this loop may function to convert the pathway from a graded to a more switch-iike response. This represents another example of a phenomenon that is common to a number of signaling systems, namely the use of positive or double-negative feedback loops to create a switch-like “bistable” system [20]. As an inhibitor of the Raf-MAP kinase pathway, RKIP may function as a tumor suppressor, and indeed RKIP expression is downregulated in metastatic breast and prostate cancers. Thus, this scaffold appears to serve primarily a regulatory function. A different function appears to be exerted by the protein p14, which was described by David Teis (University of lnnsbruck). This is an endosomal protein that interacts with the MAP kinase scaffold MP1, which in turn interacts with both MFK and ERK kinases. p14 is required both for the endosomal localization of MAP kinases and for their activation on endosomes in response to EGF. It appears that the EGF receptor and Ras both traffic to endasomes and can activate the MAP kinase cascade at this intracellular site [21]. Thus, p14 is an endosomal adaptor for the MAP kinase scaffold MP1 and serves to localize MAP kinase activation to endosomes in response to EGF. It will be of interest to identify endosomal substrates of MAP kinase and to determine whether the endosomally localized enzyme has a distinct function.

P13-kinases and the regulation of cell growth, motility, and survival

The lipid kinase phosphatidylinositol 3-kinase (Pl3-kinase) generates 3’-phosphoinositides that recruit proteins containing lipid-recognition domains (PH domains. FYVE domains) to the membrane. Pl3-kinase signaling regulates cell growth, motility, and survival. PTEN, a phosphatase that dephosphorylates 3’-phosphoinositides, is a tumor suppressor, and the gene encoding p110o, the catalytic subunit of a type lA Pl3-kinase, is amplified in human cancers. Moreover, many downstream targets of Pl3-kinase signaling, such as the protein kinase Akt and the translation initiation factor elF4E, are transforming in cell culture. These and other observations indicate that pl3-kinase signaling is important in tumorigenesis.

In recent years, there has been increasing interest in the regulation of cell growth and cell size, stimulated in part by genetic analyses in Drosophila. The 3’-phosphoinositide dependent kinases PDK1 and Akt (also known as PKB) initiate a kinase cascade that plays a key role in growth regulation. PDK1 activates Akt by phosphorylation of a Thr residue in the activation loop, although full activation requires

phosphorylation of a Ser in a C-terminal hydrophobic motif. Akt in turn activates another kinase, the mammalian target of rapamycin, mTOR. mTOR activates translation in two ways. It activates the ribosomal S6 protein kinase, which has been implicated in the increased translation of 5'-terminal oligopyrimidine tract (TOP) mRNAs that encode ribosomal proteins and other components of the translational machinery. mTOR also inactivates 4EBP-1, an inhibitor of the initiation factor elF4E. In this way, Pl3-kinase signaling regulates the translational machinery and thus cell growth. Peter Vogt (Scripps lnstitute, La Jolla) described the role of mTOR in transformation by avian sarcoma viruses encoding activated and membrane targeted forms of Pl 3-kinase or Akt. Transformation by these viruses is inhibited by rapamycin, Indicating that mTOR activation is necessary for transformation [22]. Akt and Pl3- kinase downregulate an RNA and DNA binding protein, the Y box binding protein YB-1. Overexpression of YB-1 produces resistance to transformation by Akt, without affecting Akt-dependent phosphorylation of 4EBP-1. YB-1 inhibits both cap-and lRES-dependent translation. A mutant of YB-1 that is defective in RNA binding localizes lo the nucleus. Rather than the cytoplasm, and does not block transformation. Taken together, these observations suggest that the ability of YB-1 to block transformation is related to its ability to inhibit translation and that downregulation of YB-1 represents one of the pathways responsible for the transforming activity of Akt.

Recent observations indicate that mTOR is regulate d by the tuberous sclerosis proteins, hamartin (TSC-1) and tuberin (TSC-2). The tuberous sclerosis proteins are so called because they are growth inhibitors and tumor suppressors, and mutations in the TSC-1 and TSC-2 genes cause tubers in the brain or hardened (sclerotic) tumors in other organs. Ernst Hafen (University of Zurich) described a genetic and biochemical analysis of the relationship between TSC-1/2, TOR, and a small GTPase named Rheb (*Ras hornolog enriched in brain*) [23]. Mutations in the *Drosophila* Rheb gene inhibit growth, while overexpression of Rheb promotes cell growth. Epistasis tests indicate that Rheb functions downstream of TSC-1/2 and upstream of TOR and S6K. lndeed it appears that TSC-1/2 are GTPase-activating proteins for Rheb. So Pl3-kinase and Akt signaling appear to activate TOR by inhibiting TSC-1/2 and thereby activating Rheb. If this is the case, however, and inhibition of TSC1/2 mediates the effects of Pl3-kinase signaling on growth, why do mutations in TSC-1/2 produce only tumors and overgrowths, while mutations in PTEN also result in cancer susceptibility? A possible answer was provided by Richard Lamb (lnstitute of Cancer Research, London). Loss of TSC-1/2 not only activates TOR and S6K signaling, but also impairs Pl3-kinase signaling and Akt activation. This negative feedback loop is mediated in part by S6K phosphorylation of the insulin receptor substrate IRS-1, which impairs its interaction with receptor-tyrosine kinases, and in part by S6K- and TOR-mediated inhibition of lRS-1 transcription. Thus in tuberous sclerosis, loss of TSC1/2 leads to increased S6K and TOR signaling and increased growth, but the IRS-1-mediated negative feedback loop decreases Pl3-kinase signaling, blocking cancer development. These observations therefore provide an explanation for the different effects of loss of TSC-1/2 and inactivation of PTEN.

In addition to its effects on cell growth, the Pl3-kinase-Akt pathway can also promote malignant progression by enhancing survival signaling. The Pl3-kinase/Akt pathway promotes cell survival by Akt-dependent activation of mTOR, lx-kinase (IKK) and Mdm2, and by inactivation of pro-apoptotic Bcl-2 family members such as Bax and Bad and of proapoptotic transcription factors such as FKHR. .Julian Downward (Cancer Research UK London Laboratories) described the use of 14-3-3 proteins. which bind to a motif closely related to the phosphorylated Akt substrate motif, as affinity reagents to purify and identify novel Akt substrates. One of the Akt substrates so identified is YAP, an activator of the p53- related transcription factor p73. YAP enhances p73-induced transcription of the proapoptotic Bax protein and Akt reverses this effect, apparently by inducing cytoplasmic translocation of YAP. Another novel Aki substrate, Mad3, was identified by two-dimensional PAGF analysis of phosphoproteins recognized by an Akt substrate anti-phosphoepitope antibody. Mad3 is a component of the Myc/Max/Mad transcription network that inhibits Myc-dependent transcription. Akt appears to promote Myc function by phosphorylating Mad3 and sequestering it away from the Myc partner, Max. Paradoxicaily, Downward reported, Pl3-kinase and its downstream effector S6 kinase also mediate Ras-induced cellular senescence, a phenomenon observed in primary cells that is believed lo act as a tumor suppressor mechanism. Thus, Pl3-kinase signaling not only promotes tumor cell growth and survival but also elicits this protective checkpoint mechanism.

Pl3-kinase signaling also regulates cell motility and the actin cytoskeleton. A Ptdlns (3,4,5)P3 binding protein which may mediate some of these effects was described by Len Stephens (Babraham lnstitute, Cambridge). This protein, ARAP3, was identified by virtue of its ability to bind to matrices carrying 3'-phosphoinositides [24]. ARAP3 has five PH domains, of which one is required for translocation to membrane ruffles following PDGF treatment. Overexpression or RNAi knockdown of ARAP3 results in changes in cell spreading and in the ruffling response lo PDGF, suggesting that ARAP3 plays some role in the regulation of the actin cytoskeleton by 3'-phosphoincsitides. ARAP3 contains a 3'-phosphoinositicle stimulated GAP domain that is specific for Arf6, a small GTPase involved in de livery of an endosomal compartment to the lamellipodium, and the Arf6 GAP activity of ARAF3 blocks Arf6 transport to the cell surface. ARAP3 also contains a Rho GAP domain. which is stimulated by the Ras-related GTPase, Rap. Both Arf GAP and Rho GAP domains are required to mediate Pl3 kinase-dependent rearrangements in the actin cytoskeleton and cell shape. Thus, the GAP domains of ARAP3 appear to transduce signals from Pl 3-kinase to the actin cytoskeleton and cellular trafficking machinery.

Although it is convenient to refer to Pl3-kinase activity generically as though it we re a single enzyme, there are multiple isoforms of Pl 3-kinase, differing in mode s of regulation and pattern of expression. Class lA Pl 3-kinases are regulated by recruitment to tyrosinephosphorylated receptors or docking proteins and by direct interaction of the catalytic subunit with small GTPases such as Ras, while class lB Pl 3-kinases are regulated by the Gβγ subunits of heterotrimeric G proteins. The class lA Pl 3-kinases contain one of three catalytic subunits (p110α, p110β, and p110ð), plus one of five SH2 domain-containing regulatory subunits. Do these different isoforms have specific signaling mechanisms or physiological functions? Attempts to answer this question with knockout mice have been bedeviled by compensatory changed in the remaining isoforms. Bart Vanhaesebroeck (Ludwig lnslitute, London) described the generation of knockin mutations that render the molecule catalytically inactive and the use of these mutants to study isoform-specific function [25]. Whereas p110ð-deficient mice are viable but display defects in T and B cell signaling, p110α-deficient mice exhibit embryonic lethality at E10-11, and p110α- deficient fibroblasts do not proliferate. These results imply that Pl3-kinases have isoform specific functions: since they all carry out the same catalytic function, perhaps they interact with different regulators or function at different intracellular locations. It will be important to determine whether different isoforms play different roles in different cancers.

Activators and effectors of Rho family GTPases

In normal cells, Rho family GTPases regulate cell motility and proliferation. The levels of various Rho family members are elevated in different cancers, and Rho, Rac, and Cdc42 are required for transformation by Ras. However, whereas in human cancers Ras is

activated primarily by mutations that block GTP hydrolysis, Rho family members are activated by changes in upstream regulators. lndeed, Rho family GEFs are oncogenes in vitro. RhoGEFS have Dbl homology (DH) domains paired with adjacent PH domains, plus additional protein mudules that link the GEFs to upstream signals. For example, as Channing Der (University of Norlh Carolina at Chapel Hill) de scribed, the Rac GEF Tiam1 contains a Ras binding domain. Tiam1-deficient mice are resistant to Ras-induced Rac activation and to Ras-initiated tumorigenesis [26]. Moreover the anchorage-independent growth of pancreatic cancer cells carrying activated (Gl2V) K-Ras is de pendent on the continued expression of both activated Ras and Tiam1. Thus, Ras transformation appears to be dependent on Tiaml -mediated activation of Rac. Another example of Rho-dependent transformation is provided by the seven transmembrane G protein-coupled thrombin receptor, PAR1. PAR1 is an oncogene when overexpressed, and this transforming capacity is dependent on RhoA function. PAR1 internalization and degradation is impaired in invasive breast cancer cells, and antisense knockdown experiments indicate that the invasiveness of breast carcinoma lines is dependent on PAR1 function. PAR1 mediated invasion is dependent on Gai (which activates Ras) and Gs12l13 (which activate RhoA). Thus, the activation of Rac in pancreatic cancers and the activation of Rho in breast cancers are both due to changes in upstream signaling.

Both integrin signaling and growth factor signaling are required for the proliferation of anchorage-dependent cells. One of the mechanisms by which integrin and growth factor signaling are coordinated is through the adhesion-dependent targeting of Rac to the membrane [27]. Martin Schwar2 (Scripps lnstitute, La Jolla) described a mechanism by which integrin signaling modulates lipid rafts and caveolae at the plasma membrane and as a consequence, Rac activity.

The multiplicity of biological effects of Rho family GTPases is reflected in the multiplicity of effectors with which they interact. Actin stress fiber assembly driven by RhoA is mediated by two primary RhoA targets. One class, the Diaphanous-related formins, promote the polymerization of actin filaments and microtubules and are discussed below. The other class, the Rho kinases (ROCKs), promote actomyosin contraction by enhancing myosin light chain phosphorylation and, as noted earlier, promote actin filament stability by inactivating the actin filament severing protein cofilin. Chris Marshall (lnstitute of Cancer Research, London) reported that tumor cells exhibit two distinct modes of cell motility that differ in their requirement for Rho signaling through ROCK. The first mode of cell motility is the "classic" lamellipodial or mesenchymal motility driven by active Rac. This mode of motility requires downregulation of Rho-ROCK signaling. One way in which this can occur, as noted earlier for Src-transformed cells, is by dephosphorylation of cofilin. In cells transformed by activated Ras, downregulation of Rho-ROCK signaling is dependent on ERK MAP kinase activity [28]. ERK activates the transcription and translation of the AP-1 component Fra-1 , which inactivates integrin β1 signaling and thus downregulates RhoA. At the same time, ERK signaling upregulates the expression of uPAR (the receptor for urokinase plasminogen activator) which in turn activates Rac. In contrast to this lamellipodial, Rac-dependent type of movement, however. the invasiveness of some tumor cell types is actually dependent on Rho-ROCK signaling [29]. These cells have a rounded "blebby" morphology and a polarized distribution of ezrin, and their ability to invade is independent of matrix metalloproteinase secretion. Some tumor cell types exhibit both the mesenchymal, protease-dependent mode of invasion and the protease-independent, Rho-ROCK-dependent mode of cell movement. In these instances, inhibition of both proteases and ROCK is required to block cell motility. This suggests that an effective therapeutic strategy to inhibit tumor cell metastasis might be to simultaneously target both matrix metalloproteinases and ROCK.

The Diaphanous-related formins, which function as active scaffolds that direct actin filament and microtubule assembly, represent another major class of effectors for Rho family GTPases [29]. They are characterized by the presence of formin homology (FH) domains and regulatory domains thought lo confer sensitivity to active Rho GTPases: an amino-terminal GTPase binding domain (GBD) and a carboxy-terminal Diaautoregulatory domain (DAD). lnteraction of the GBD with the cognate GTPase disrupts the intramolecular GBD-DAD interaction and promotes the scaffolding function of the protein. Different formins appear to interact with distinct spectra of GTPases, suggesting that different GTPase-formin pairs are functionally distinct. To approach this question, Art Alberts (Van Andel lnstitute, Grand Rapids, Michigan) has used FRET (fluorescence resonance energy transfer) to analyze the sites of formin-GTPase interactions in intact cells. In one example. Cdc42 specifically interacts with mDia2 at the leading edge and MTOC of cells migrating into a wound. Demonstrating an effector role for mDia2, blocking mDia2 function inhibits the generation of filopodia induced by activated Cdc42. To explain these observations, Alberts proposed a model in which Cdc42 activates mDia2 at the base of the nascent filopodium and thus stimulates actin filament assembly and filament protrusion at the leading edge of migrating cells. In contrast to Cdc42, FRET analyses indicate that RhoB interacts with mDia2 on endosomes and RhoA interacts with mDia2 across the plasma membrane. Despite their ability to interact in vitro, mDia2 and RhoC did not interact in intact cells, suggesting that cells confer an additional specificity on GTPase-formin pairs. In another example, another formin protein (FHOD1) binds Rac in vitro and in vivo interacts with Rac on ruffles. Though recent studies have shown that formins nucleate nonbranched actin filaments in vitro, it appears that different formins participate in the formation of different cytoskeletal structures at discrete sites and in response to different GTPases. Surprisingly, however, it appears that the GTPase binding domain is not itself required for subcellular targeting. Thus, rather than the GTPase targeting the formin to the site at which it functions, it may be that formin localization precedes the activation of the GTPase. This is a different model than the general paradigm for small GTPase function, in which the GTPase is responsible for recruitinq its effectors to their site of function.

The WAVE/Scar proteins (WAVE-1, -2, and -3 in mammals) represent another class of scaffolding protein that mediates the effects of Rho family GTPases on the actin cytoskeleton. These proteins link Rho family GTPases to the Arp2l3 complex, which nucleates actin filament assembly and actin filament branching Rac promotes WAVE-1 activation, and signaling by Rac is terminated by a WAVE-1-associatecJ GTPase-activating protein named WRP. John Scott (Vollum lnstitute, Oregon Health and Sciences University, Portland) described the properties of WAVE-1 knockout mice [29]. These animals are viable but exhibit a variety of sensorimotor and learning deficits that are remarkably similar to those associated with haploinsufficiency of WRP in humans. Thus, alterations in Rac-regulated actin filament assembly may underlie this form of mental retardation.

Expression of activated Rho family GTPases has global effects on gene expression and function. Natalie Ahn (University of Colorado, Boulder) described a functional proteomics approach to characterize these changes. Cells expressing activated mutants of RhoA, Cdc42, or Rac1 were analyzed by2D-PAGE and spots identified by MALDI-qTOF peptide mass sequencing and peptide sequencing. The expression of some proteins was uniquely regulated by one specific GTPase, whereas others were regulated by more than one GTPase. The 2D-PAGE analysis also revealed covalent modifications induced by specific GTPases. For example, RhoA was found to inhibit PTP1B and thereby promote the phosphorylation of p130Cas Since Cas

phosphorylation promotes cell motility, it is conceivable that a Rho-PTP1B-Cas pathway might be involved in the Rho-dependent type of motility described earlier.

Novel and atypical protein kinase Cs in cell-matrix interactions and the control of cell polarity Conventional isoforms of protein kinase C (cPKCs: α, β, and y) are dependent on phospholipids, particularly phosphatidylserine, Ca^{2+}, and diacylglycerol. They have been the focus of intense interest since the discovery that they are targeted by tumor promoters such as TPA. The more recently identified novel PKCs (nPKCs: ð, Ɛ, ŋ, and Θ) are dependent only on phospholipids and diacylglycerol, while the atypical PKCs (aPKOs: l/ʎ and ζ)are dependent only on phospholipids. It now appears that the novel and atypical PKCs play critical roles in the response to cell-cell and cell-matrix interactions and in the development of cell polarity. Peter Parker (Cancer Research UK London Research lnstitute) described how the novel PKC PKCƐ integrates integrin and cytokine signaling. [29]. lntegrin engagement regulates the formation of a complex between PKCƐ, protein phosphatase 2A, and PDK1. such that PKCƐ is fully phosphorylated and active only in attached cells. PKCƐ activity in turn regulates interferon y-induced phosphorylation of STAT1 by Janus kinases (Jak1, Jak2) so that the interferon-y pathway is only active in attached cells. In this way, PKCƐ acts as a signal integrator mediating integrin regulation of cytokine responses.

The atypical PKCs appear to play an important role in the response to cell polarity signals. As first demonstrated by genetic studies on asymmetric division in C. *elegans,* one of the major determinants of cell polarity is a complex of the GTPase Cdc42, an atypical PKC and two scaffolding proteins, Par3 and Par6. Tony Pawson (Samuel Lunenfeld Research lnstitute, Toronto) described how a series of interactions assemble this complex at tight junctions between epithelial cells and at the leading edge of migrating astrocytes. In mammalian cells, there are direct interactions between aPKC, Par6, and mLgl, the mammalian homolog of the *Drosophila* tumor suppressor Lethal (2) giant larvae (Plant et al., 2003). mLgl contains aPKC phocsphrorylation sites that are important for fibroblasts to polarize in response to wounding. Thus, aPKC is directed to its substrate by specific protein-protein interactions. These interactions are part of a much larger network of interactions that connect the Par3-Par6 cell polarity complex to other complexes that regulate cell polarity, vesicle trafficking, microtubule stability, cell junction formation, and cell proliferation.

Signaling inhibitors as cancer therapeutics

Transformation of fibroblasts by activated oncogenes such as Ras is dependent on multiple pathways, and in some instances, inhibition of a single one of these pathways can inhibit transformation. If this is a valid model for carcinogenesis, single signaling inhibitors might be effective cancer therapeutics. However there are also precedents for believing that effective inhibition of some aspects of transformation may require multiple inhibitors. The synergy between metalloproteinase inhibitors and ROCK inhibitors in inhibiting tumor cell invasion, as described by Chris Marshall (see above), represents one such precedent. Moreover, as argued earlier, because tumor cells are genetically unstable and continually evolving, they may be able to evade blockade of a single pathway so that effective therapies may require the use of multiple inhibitors, or the use of signaling inhibitors as supplements to therapy with conventional DNA-damaging agents.

Raf proteins are under active investigation as therapeutic targets. There are three isoforms of Raf in mammals, Raf-1, B-Raf, and A-Raf. Mutations in the activation loop of B-Raf that activate catalytic activity have recently been found to occur in some 66% of melanomas and at a lower frequency in other cancers (Davies et al., 2002). The most common mutation is V599E, and this mutant form of Raf is transforming for NIH-3T3 cells. Maria Karasarides (lnstitute of Cancer Research, Landon) reported that transformation can be blocked by RNAi downregulation of B-Raf or by MFK inhibitors. Furthermore, downregulation of B-Raf in melanoma cells results in caspase 3 activation and apoptosis. Frank McCormick (University of California. San Francisco) discussed the therapeutic potential of a Raf inhibitor developed at the Bayer Corporation. Starting with a lead structure with an lC50 for Raf inhibition of 17 μM, the Bayer group, using combinatorial chemistry, developed an orally active compound, BAY43-9006, which has an lC50 for Raf inhibition of 12 nM. This inhibitor blocks MEK phosphorylalion by mutant B-Raf. Phase l trials suggest that the inhibitor may induce a partial response or stabilize disease progression when administered as single agent in the treatment of renal cell carcinoma or when used in combination with carboplatin and paclitaxel for the treatment of melanomas. These exciting but still preliminary findings suggest that Raf activity is a promising target for therapeutic intervention.

STI-571 (Gleevec) has proven extremely effective in the treatment of chronic myelogenous leukemia, in which Abl is activated by translocation, and is also effective in the treatment of gastrointestinal stromal tuntors, in which there are mutations in either c-Kit or the PDGF receptor o. Carl-Henrik Heldin (Ludwig lnstitute for Cancer Research, Uppsala, Sweden) described other uses of STI-571 as a PDGF receptor antagonist (Pietras et al., 2003). One such use is in the treatment of dermatofibrosarcoma protuberans, a disease of intermediate malignancy. It results from a fusion of the collagen 1A1 gene to the gene encoding the PDGF-B chain: the fusion gene product is processed to generate PDGF-B chain. STI-571 reduces the growth of subcutaneous dermatofibrosarcoma tumors in a xenograft model. Clinical trials suggest that STI-571 can induce tumor regression. Another use of STI-571 as. a cancer therapeutic stems from its effect on tumor stromal cells, which frequently express PDGF receptors. Activation of stromal PDGF-R causes an increase in tumor interstitial fluid pressure, which reduces the uptake of chemotherapeutic drugs. Heldin demonstrated that STI-571 reduces tumor interstitial fluid pressure and thereby increases the uptake and efficacy of drugs such as taxol and 5-FU (Pietras et al., 2002).

The response of a tumor cell to an inhibitor or drug depends on its particular genetic an epigenetic status. Tumor *cells* acquire resistance to apoptosis during the course of tumor progression, and enhanced survival signaling may be important in promoting resistance to chemotherapeutic agents (Johnstone et al. ,2002) for example, Scott Lowe (Cold Spring Harbor Laboratory. New York) reported that the introduction of various antiapoptotic lesions (e.9., p53 loss, or overexpression of Bcl-2 or Akt) in Eμ-Myc transgenic mice enhances lymphomagenesis and the chemoresistance of the lymphomas. Interestingly, the nature of the antiapoptotic iesion can have an impact on how the lymphoma responds to a combination of conventional and targeted agents. Thus, knowledge of apoptosis resistance mechanisms in cancer may allow the tailoring of therapies for individual patients. With this in mind, Lowe described the use of short-hairpin RNA libraries to identify genes that either sensitize or inhibit drug-induced apoptosis. In the same context, Margaret Frame and Caroline Dive (University of Manchester) reported that catalytically inactive mutants of Src sensitize metastatic colon cancer ceils to oxaliplatin- and Fas-induced apoptosis. These src mutants might act as either adaptor proteins or dominant-negatives and might either inhibit an antiapoptotic pathway or promote a proapoptotic pathway.

The findings described at this meeting indicate that our understanding of signaling pathways has advanced to the point where specific targets for therapeutic intervention can be identified. However, we need to understand how whole signaling networks function within the context of the intact cell if we are to develop rational strategies based on the genetic alterations of individual cancers. Based on the pace of the progress reported at this meeting, it is safe to predict that

the next few years should see exciting new developments in targeted cancer therapies.

Abnormal Notch Signaling Activation and CSC.s

Abnormal activation of Notch signaling plays a pivotal role in the CSCs of breast cancer, pancreatic cancer, and glioblastoma. For instance, Barnawi et al. reported that fascin (an actin-bundling protein) effectively regulates breast CSCs at least partially through Notch pathway [78]. Fascin knockdown significantly reduced breast stem cell-like phenotype (downregulation of stem cell pluripotent genes such as Oct4, Nanog, Sox2, and Klf4), and the cells became less competent in forming colonies and tumorspheres. Conversely, activation of Notch signaling induced the relevant downstream targets predominantly in the fascin-positive cells, and fascin-positive CSCs showed stronger tumorigenesis. In another study, immunohistochemical analysis of 115 breast tumor tissues from primary lesions was performed, and results showed that Notch positive tissues were significantly associated with a CSC marker aldehyde dehydrogenase I family member Al levels. Very recently, Choy et al. reported that Notch 3 signaled constitutively in a panel of basal breast cancer cell lines and in more than one-third of breast basal tumors.

Moreover, the important role of Notch signaling was also demonstrated in several other types of CSCs. In a study of patient-derived pancreatic CSCs, Notch ligands Notch 1, Notch 3, Jagl, Jag2, and Notch target gene Hes l were found to be highly expressed in the pancreatic CSCs, and an inhibitor of y-secretase (an important protease mediating Notch signaling by releasing the Notch I CD) significantly decreased the CSC's subpopulation and tumorsphere formation [81]. Moreover, activation of Notch signaling by delta/Serrate/Lag-2 peptide or inhibition of the signaling by knockdown of Hes l enhanced or decreased pancreatic CSC's tumorsphere formation, respectively [81]. In addition, Notch signaling dysregulation has also been recognized in glioblastoma CSCs [82]. It was found that Protein Kinase C Iota (PKCi) was highly expressed in glioblastoma patientderived CSCs, and silencing PKCi resulted in apoptosis and reduction of proliferation of the glioblastoma CSCs in vitro and tumor growth in vivo in a xenograft mouse model. Gene expression profiling of PKCi-silenced glioblastoma CSCs revealed a novel role of the Notch signaling pathway in PKCi mediated glioblastoma CSC's survival. In addition to its important roles in CSCs, Notch signaling is also involved in EMT to promote cancer cell acquisition of a stem-like phenotype and drug resistance. For instance, prostate cancer cells undergoing EMT displayed stem-like cell features characterized by increased expression of Notch 1 and other pluripotent genes such as Sox2, Nanog. Oct4, and Lin28.

Therapeutic Agents Targeting Notch Signaling

Therapeutics targeting the Notch pathway mostly consist of y-secretase inhibitors and anti-DLL4 antibqdies. Inhibition of the Notch pathway via y-secretase inhibitors prevents Notch receptor cleavage at the cell surface, thus blockrng activation of self-renewal target genes. ln a preclinical study, a y-secretase inhibitor RO4929091 significantly suppressed Notch target genes Hes1, Hey1, and HeyL [85]. Several phase I and phase II studies have been conducted in hopes of synergistically utilizing RO4929097 with other agents for cancer treatment. For instance, in a completed phase I trial, RO4929097 and Cediranib Maleate were used in tandem to determine the phase II dose and safety profile of RO4929097 in solid tumors (NCT Number: NCT01131234), and the clinical trial data shall be announced soon. Another y-secretase inhibitor is LY900009, developed by Eli Lilly, which is in phase I for patients with advanced cancer including leiomyosarcoma and ovarian cancer. A third y-secretase inhibitor (PF-003084014) was developed by Pfizer, and it is progressing in its phase I trials in patients with T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. In addition to y-secretase inhibitors, another category of Notch pathway molecules is monoclonal antibodies that target DLL4 (Delta-like ligand 4) to prevent ligand binding. Enoticumab (REGN42L) is an anti-DLL4 antibody that has been used to target advanced solid tumors with overexpression of DLL4 (such as ovarian cancer) [88]. In 2015, a recommended phase II dose of 4 mg /kg every 3 weeks or 3 mg/kg every 2 weeks administered intravenously was established based on PK profiles in patients diagnosed with ovarian, colon, or breast cancer. Another anti-DLL4 monoclonal antibody developed by OncoMed Pharmaceuticals and Celgene is Demcizumab, which has recently completed a phase I dose escalation clinical trial as well. In this study, Demcizumab was well tolerated at doses $\leq$5 mg with disease stabilization and tumor size decreases when administered weekly. The side effects of Demcizumab include hypertension and an increased risk of congestive heart failure in prolonged drug administration (NCT Number: NCT Number: NCT02722954).

Crosstalk among Pathways and Combination Treatments

Many pathways do not act as isolated units but rather often interact with other pathways as a biological network during development and homeostasis. Crosstalk among Wnt, HH, Notch, and other pathways have been reported in cancer and CSCs. For instance, in a colorectal cancer study, progastrin secreted by colorectal tumors was shown to activate Wnt signaling and result in expression of Wnt target genes including Jagged-1, one Notch ligand. Upregulation of Jagged-1 induces Notch signaling which in turn may further elevate B-catenin activity of progastrin-driven Wnt and Notch signaling in colorectal cancer cells. Similarly, in breast CSCs, Mel-18 was reported as a negative regulator of breast CSC's self-renewal. Knockdown of Mel-18 increased Wnt signaling, which subsequently upregulated Wnt target gene jagged-1's expression, leading to activation of the Notch pathway for CSC's self-renewal [93]. In addition, HH signaling can crosstalk with both Wnt and Notch pathways as well. In gastric cancer cells, HH signaling was shown to suppress Wnt signaling through the soluble frizzled-related protein 1 (sFrP1), a target gene of HH signaling capable of modulating Wnt pathway by directly binding to Wnt ligands. In another study of glioblastoma cells and patient specimens, Notch signaling inhibition was shown to downregulate its target gene Hes 1 which in turn upregulates GLI transcription in the HH pathway.

Complex signaling networks are known to contribute to the cellular diversity of stem cells during embryogenesis and tissue homeostasis and may play essential roles in the cancer and CSC's biology. In recent years, significant efforts have been made to develop combination therapies to target multiple signaling pathways for cancer treatments. For instance, a recent study demonstrated that combination inhibition of both Notch and HH signaling depleted the CSC subpopulation cells in a prostate cancer model. In addition, a clinical trial of combination of HH pathway inhibitor Vismodegib and Notch signaling inhibitor RO4929097 has bee conducted in patients with advanced breast and 'sarcoma. In another recent study, Sharma et al. showed that combination treatment with HH signaling inhibitor NVP-LDE225 and pI3/mTOR/Akt signaling inhibitor NVPBEZ235 inhibited self-renewal capacity of pancreatic CSCs by suppressing the expression of pluripotency maintaining factors Nanog, Oct-4, Sox-2, and c-Myc and transcription of GLI.

How are targets for targeted cancer therapies identified

The development of targeted therapies requires the identification of good targets-that is, targets that play a key role in cancer cell growth and survival. (lt is for this reason that targeted therapies are sometimes referred to as the product of "rational" drug design.)

One approach to identify potential targets is to compare the amounts of individual proteins in cancer cells with those in normal cells. Proteins that are present in cancer cells but not normal cells or that are more abundant in cancer cells would be potential targets, especially if they are known to be involved in cell growth or survival. An example of such a differentially expressed target is the human epidermal growth factor receptor 2 protein (HER-2). HER-2 is expressed at high levels on the surface of some cancer cells. Several targeted therapies are directed against HER-2, including trastuzumab (Herceptin®), which is approved to treat certain breast and stomach cancers that overexpress HER-2. Another approach to identify potential targets is to determine whether cancer cells produce mutant (altered) proteins that drive cancer progression. For example, the cell growth signaling protein BRAF is present in an altered form (known as BRAFV600E) in many melanomas. Vemurafenib (Zelboraf®) targets this mutant form of the BRAF protein and is approved to treat patients with inoperable or metastatic melanoma that contains this altered BRAF protein.

Researchers also look for abnormalities in chromosomes that are present in cancer cells but not in normal cells. Sometimes these chromosome abnormalities result in the creation of a fusion gene (a gene that incorporates parts of two different genes) whose product, called a fusion protein, may drive cancer development. Such fusion proteins are potential targets for targeted cancer therapies. For example, imatinib mesylate (Gleevec®) targets the BCR-ABL fusion protein, which is made from pieces of two genes that get joined together in some leukemia cells and promotes the growth of leukemic cells.

How are targeted therapies developed?

Once a candidate target has been identified, the next step is to develop a therapy that affects the target in a way that interferes with its ability to promote cancer cell growth or survival. For example, targeted therapy could reduce the activity of the target or prevent it from binding to a receptor that normally activates, among other possible mechanisms.

Most targeted therapies are either small molecules or monoclonal antibodies. Small-molecule compounds are typically developed for targets that are located inside the cell because such agents are able to enter cells relatively easily. Monoclonal antibodies are relatively large and generally cannot enter cells, so they are used only for targets that are outside cells or on the cell surface.

Candidate small molecules are usually identified in what are known as "high-throughput screens," in which the effects of thousands of test compounds on a specific target protein are examined. Compounds that affect the target (sometimes called "lead compounds") are then chemically modified to produce numerous closely related versions of the lead compound. These related compounds are then tested to determine which are most effective and have the fewest effects on nontarget molecules.

Monoclonal antibodies are developed by injecting animals (usually mice) with purified target proteins, causing the animals to make many different types of antibodies against the target. These antibodies are then tested to find the ones that bind best to the target without binding to nontarget proteins.

Before monoclonal antibodies are used in humans, they are "humanized" by replacing as much of the mouse antibody molecule as possible with corresponding portions of human antibodies. Humanizing is necessary to prevent the human immune system from recognizing the monoclonal antibody as "foreign" and destroying it before it has a chance to bind to its target protein. Humanization is not an issue for small-molecule compounds because they are not typically recognized by the body as foreign.

How is it determined whether a patient is a candidate for targeted therapy?

For some types of cancer, most patients with that cancer will have an appropriate target for a particular targeted therapy and, thus, will be candidates to be treated with that therapy. CML is an example: most patients have the BCR-IBL fusion gene. For other cancer types, however, a patient's tumor tissue must be tested to determine whether or not an appropriate target is present. The use of a targeted therapy may be restricted to patients whose tumor has a specific gene mutation that codes for the target; patients who do not have the mutation would not be candidates because the therapy would have nothing to target.

Sometimes, a patient is a candidate for a targeted therapy only if he or she meets specific criteria (for example, their cancer did not respond to other therapies, has spread, or is inoperable). These criteria are set by the FDA when it approves a specific targeted therapy.

What are the limitations of targeted cancer therapies?

Targeted therapies do have some limitations. One is that cancer cells can become resistant to them. Resistance can occur in two ways: the target itself changes through mutation so that the targeted therapy no longer interacts well with it, and/or the tumor finds a new pathway to achieve tumor growth that does not depend on the target.

For this reason, targeted therapies may work best in combination. For example, a recent study found that using two therapies that target different parts of the cell signaling pathway that is altered in melanoma by the BRAFV600E mutation slowed the development of resistance and disease progression to a greater extent than using just one targeted therapy.

Another approach is to use a targeted therapy in combination with one or more traditional chemotherapy drugs. For example, the targeted therapy trastuzumab (Herceptin ®) has been used in combination with docetaxel, a traditional chemotherapy drug, to treat women with metastatic breast cancer that overexpresses the protein HER2/neu.

Another limitation of targeted therapy at present is that drugs for some identified targets are difficult to develop because of the target's structure and/or the way its function is regulated in the cell. One example is Ras, a signaling protein that is mutated in as many as one-quarter of all cancers (and in the majority of certain cancer types, such as pancreatic cancer). To date, it has not been possible to develop inhibitors of Ras signaling with existing drug development technologies. However, promising new approaches are offering hope that this Iimitation can soon be overcome.

What are the side effects c:f targeted cancer therapies?

Scientists had expected that targeted cancer therapies would be less toxic than traditional chemotherapy drugs because cancer cells are more dependent on the targets than are normal cells. However, targeted cancer therapies can have substantial side effects.

The most common side effects seen with targeted therapies are diarrhea and liver problems, such as hepatitis and elevated liver enzymes. Other side effects seen with targeted therapies include:

. Skin problems (acneiform rash, dry skin, nail changes, hair depigmentation)

. Problems with blood clotting and wound healing

. High blood pressure

. Gastrointestinal perforation (a rare side effect of some targeted therapies)

Certain side effects of some targeted therapies have been linked to better patient outcomes. For example, patients who develop acneiform rash (skin eruptions that resemble acne) while being treated with the signal transduction inhibitors erlotinib (Tarceva®) or gefitinib (lressa®), both of which target the epidermal growth factor receptor, have tended to respond better to these drugs than patients who do not develop the rash. Similarly, patients who develop high blood pressure while being treated with the angiogenesis inhibitor bevacizumab generally have had better outcomes.

The few targeted therapies that are approved for use in children can have different side effects in children than in adults, including immunosuppression and impaired sperm production

What targeted therapies have been approved for specific types of cancer?

The FDA has approved targeted therapies for the treatment of some patients with the following types of cancer (some targeted therapies have been approved to treat more than one type of cancer):

Adenocarcinoma of the stomach or gastroesophageal junction: Trastuzumab (Herceptin®), ramucirumab (Cyramza®)

Bladder cancer: Arezolizumab (Tecentriq™), nivolumab (Opdivo ®), durvalumab (lmfinzi ™), avelumab (Bavencio ®), pembrolizumab (Keytruda ®)

Brain Cancer: Bevacizumab (Avastin ®), everolimus (Afinitor®)

Breast Cancer: Everolimus (Afinitor®), tamoxifen (Nolvadex), toremifene®), Trastuzumab (Herceptin®), fulvestrant (Faslodex®), anastrozole (Arimidex®), exemestane (Aromasin®), lapatinib (Tykerb®), letrozole (Femara®), pertuzumab (Perjeta®), ado-trastuzumab emtansine (kadcyla®). Palbociclib (Ibrance®), ribociclib (Kisqali®), neratinib maleate (Nerlynx™)

Cervical cancer : Bevacizumab (Avastin®)

Colorectal cancer: Cetuximab (Erbitux®) Panitumumab (Vectibix ®), bevacizumab (Avastin ®), zivaflibercept (Zaltrap®), regorafenib (Stivarga®), ramucirumab (Cyramza®), nivolumab (Opdivo®)

Dermatofibrosarcoma protuberans: Imatinib mesylate (GIeevec, ®)

Endocrine/neuroendocrine tumors: Lanreotide acetate (Somatuline ®Depot), avelumab Bavencio ®)

Head and neck cancer: Cetuximab (Erbituxi®), pembrolizumab (Keytruda®), nivolumab (Opdivo®)

Gastrointestinal stromal tumor: Imatinib mesylate (Gleevec®), sunitinib (Sutent®), regorafenib (Stivarga®]

Giant cell tumor of the bone: Denosumab (Xgeva®)

Kidney cancer: Bevacizumab (Avastin®), sorafenib (Nexavar®), sunitinib (Sutent®), pazopanib (Votrient®), temsirolimus (Torisel®), everolimus (Afinitor®), axirinib (lnlyta®), nivolumab (Opdivo®), cabozantinib (Cabometyx™), lenvatinib mesylate (Lenvima®)

Leukemia: Trerinoin (Vesarroid®), imalinib mesylate (Gleevec®), dasatinib (Sprycel®), nilotinib (Tasigna®), bosutinib (Bosulif®), rituximab (Rituxan®), alemtuzumab (Campath®), ofatumumab (Arzerra®), obinutuzumab (Gazyva®), ibrutinib (lmbruvica®), idelalisib (Zydelig®), blinatumomab (blincyto®), venetoclax (Venclexta™), ponatinib hydrochloride (lclusig®), midostaurin (Rydapt®), enasidenib mesylate (Idhifa®)

Liver cancer: Sorafenib (Nexavar®), rergorafenib (Stivarqa®)

Lung cancer: Bevacizumab (Avastin®), crizotinib (Xalkori®), erlotinib (Tarceva®), gefitinib (lressar®), afatinib dimaleate (Gilotrif®), ceritinib (LDK378/Zykadia™), ramucirunrab (Cyramza®), nivolumab (Opdivo®), pembrolizumab (Keytruda®), osimertinib (Tagrisso™), necitumumab (Portrazza™), alectinib (Alecensa®), atezolizumab (Tecentriq™), brigatinib (Alunbrig™), trametinib (Mekinist®), dabrafenib (Tafinlar®)

Lymphoma: Ibritumomah tiuxetan (Zevalin®), denileukin diftitox (Ontark®), brentuximab vedotin (Adcetris®), rituximab (Rituxan®), vorinostat (Zolinza®), romidepsin (lstodax®), bexarotene (Targretin®), bortezomib (Velcade®), pralatrexate (Folotyn®), ibrutinib (lmbruvica®), siltuximab (Sylvant®), idelalisib (Zydelig®), belinostat (Beleodaq®), obinutuzumab (Gazyva®), nivolumab (Opdivo®), pembrolizumab (Keytruda®)

Microsatellite instability-high or mismatch repair-deficient solid tumors: Pembrolizumab (Keytruda®)

Multiple myeloma: Bortezomib (Velcade®), carfilzomib (Kyprolis®), panobinostat (Farydak®), daratumumab (Darzalex™), ixazomib citrate (Ninlaro®), elotuzumub (Empliciti™)

Myelodysplastic/myeloproliferative disorders: Imatinib mesylate (Gleevec®), ruxolitinib phosphate (lakafi®)

Neuroblastoma: Dinuruximab (Unituxin ™)

Ovarian epithelial/fallopian tube/primary peritoneal cancers: Bevacizumab (Avastin®), olaparib (Lynparza™), rucaparib camsylate (Rubraca™), niraparib tosylate monohydrate(Zejula™)

Pancreatic cancer: Erlotinib (Tarceva®), everolimus (Afinitor®), sunitinib (Sutent®)

Prostate cancer: Cabazitaxel (Jevtana®), enzalutamide (Xofigo®), abiraterone acetate (Zytiga®), radium 223 dichloride (Xofiqo®)

Skin cancer: Vismodegib (Erivedge®), sonidegib (Odomzo®), ipilimumab (Yervoy®), vemurafenib (Zelboraf®), trametinib (Mekinist®), dabrafenib (Tafinlar®), pembrolizumab (Keytruda®), nivolumab (Opdivo®), cobimetinib (Cotellic™), alitretinoin (Panretin®), avelumab (Bavencio®)

Soft tissue sarcoma: Pazopanib (Votrient®), Olaratumab (Lartruvo™), alitretinoin (Panretin®)

Systemic mastocytosis: lmatinib mesylate (Gleevec®), midostaurin (Rydapt®)

Thyroid cancer: Cabozantinib (Cometriq®), vandetanib (Caprelsa®), sorafenib (Nexavar®), lenvatinib mesylate (Lenvima®)

Where can I find information about clinical trials of targeted therapies?

Both FDA-approved and experimental targeted therapies for specific types of cancer are being studied in clinical trials. Descriptions of ongoing clinical trials that are testing types of targeted therapies in cancer patients can be accessed by searching NCI's list of cancer clinical trials. NCI's list of cancer clinical trials includes all NCI-supported clinical trials that are taking place across the United States and Canada and around the world. For information about other ways to search the list, see Help Finding NCI-Supported Clinical Trials.

Conclusions

Since the first identification of CSCs in leukemia, the important roles of CSCs in cancer progression, metastasis, and relapse as well as drug resistance have been increasingly recognized. Eradication of CSCs by targeting the key signaling pathways underlying CSC's stemness and function represents a promising approach in cancer treatment. In this review, we mainly sumnrarized the three critical evolutionarily conserved pathways (Wnt, HH, and Notch signaling) in CSCs and potential therapies targeting these pathways for cancer treatment. To date, numerous agents have been developed to specifically target each

of these pathways for cancer treatments. Nevertheless, it has been recognized that the signaling pathways lnay interact with each other as a coordinated network to regulate CSC stemness and functions. Therefore, urderstanding the crosstalk among the signaling pathways in CSC regulation is critical for the development of therapies targeting CSCs.

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