Review

Why should we still care about oncogenes?

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Abstract

Although oncogenes and their transformation mechanisms have been known for 30 years, we are just now using our understanding of protein function to abrogate the activity of these genes to block cancer growth. The advent of specific small-molecule inhibitors has been a tremendous step in the fight against cancer and their main targets are the cellular counterparts of viral oncogenes. The best-known example of a molecular therapeutic is Gleevec (imatinib). In the early 1990s, IFN-α treatment produced a sustained cytologic response in $\sim$33% of chronic myelogenous leukemia patients. Today, with Gleevec targeting the kinase activity of the proto-oncogene abl, the hematologic response rate in chronic myelogenous leukemia patients is 95% with 89% progression-free survival at 18 months. There are still drawbacks to the new therapies, such as drug resistance after a period of treatment, but the drawbacks are being studied experimentally. New drugs and combination therapies are being designed that will bypass the resistance mechanisms. [Mol Cancer Ther 2007;6(2):418–27]

Introduction

In 1911, when Peyton Rous injected healthy chickens with a filtrate of chicken tumors and observed the formation of new tumors (1), the field of viral oncology was born. Rous observed that the more times the tumor filtrate was injected into the chickens, the shorter the latency period between injection and tumor generation. This prompted Rous to propose that a chemical was present in the filtrate that was causing the tumors; however, the causative agent was later discovered to be a virulent form of a known avian retrovirus and was renamed Rous sarcoma virus. Duesberg and Vogt (2) showed that, compared with its nontransforming counterpart, Rous sarcoma virus contained an extra sequence of DNA, which they termed SARC (for sarcoma; later shortened to SRC). The extra sequence was called an oncogene or cancer-causing gene. During this time, it was believed that viruses deposited their oncogene into the host cells; in other words, no cellular oncogenes were believed to exist. In 1976, Stehelin et al. (3) showed that the SARC sequence of Rous sarcoma virus was found in bird species that could not be infected with the virus, indicating that the viral oncogenes were normal cellular genes whose protein products controlled cellular proliferation, which were incorporated into viruses and moved to other cells.

In 1981, Weinberg et al. showed that the human homologue of the viral oncogene v-*ras* was a cause of bladder cancer. Shih et al. (4) isolated DNA from a human bladder cancer and transfected it into mouse cells. DNA isolated from the resulting transformed mouse cells was transfected into new mouse cells and the procedure was repeated, each time taking some of the DNA and doing Southern blot analysis for the human *alu* sequence to indicate how much human DNA was present. This procedure was repeated until only one *alu* band was seen in the transformed cells. Because this band was isolated from a human cancer and was linked to transformation of the mouse cells via their experiments, they reasoned that the *alu*-associated human DNA played a role in the transformation process. At the time, the only DNA sequences known to cause cancer were the viral oncogenes from animal viruses, so they probed this band with a variety of known oncogenes, identifying a homologue of v-*ras*[3] as the gene that played a role in the transformation. This discovery led molecular biologists to study other viral oncogenes and the role of their human counterparts in cancer.

Categories of Oncogenes

Oncogenes can be categorized into many different protein classes. These classes are based on function and include receptor tyrosine kinases (RTK), adapter proteins, small G-proteins, downstream cytoplasmic signaling molecules, and transcription factors. Figure 1 shows the most common subcellular localizations of each of the oncogene classes.

Received 9/29/06; revised 11/13/06; accepted 12/6/06.

Grant support: NIH grant R01 CA098513.

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doi:10.1158/1535-7163.MCT-06-0603

[3] Traditional nomenclature is used in this review. Viral oncogenes will be designated as v-*onc*. Proto-oncogenes, or cellular homologues to viral oncogenes, will be designated as c-*onc*. Proteins will be designated as the gene name starting with a capital letter. The term ''oncogene'' is sometimes used to describe a cellular gene that has been shown to play a role in tumorigenesis.

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Examples of each class include the RTK v-*erb*B [the cellular homologues being epidermal growth factor (EGF) receptor (EGFR), erbB-2 (HER-2), erbB-3, and erbB-4; ref. 5], the small G-protein v-*ras* (the cellular homologues being H-*ras*, K-*ras*, and N-*ras*; ref. 6), the cytoplasmic tyrosine kinase v-*src* (the cellular homologue being c-*src*; ref. 7), the adapter protein v-*crk* (the cellular homologue being c-*crk*; ref. 8), and the transcription factor v-*myc* (the cellular homologues being c-*myc* and N-*myc*; ref. 9).

The first category of oncogenes, the RTKs, contains an extracellular domain that binds ligand, a transmembrane domain, and a cytoplasmic domain that contains the kinase domain. The kinase is activated by phosphorylation of a regulatory tyrosine via transfer of a phosphate group from ATP to the tyrosine (10). This phosphorylation event occurs when the protein dimerizes with a similar molecule or a member of its family. Unstable dimers of RTKs occur in the fluid plasma membrane; ligand binding to the extracellular domain stabilizes the dimer and the subsequent signals. Overexpression of a RTK leads to the formation of many unstable dimers causing inappropriately amplified growth signals.

*erb*B Family

In 1984, the cellular homologue of viral oncogene v-*erb*B, from an avian erythroblastosis virus, was shown to be a receptor that binds EGF (11–13). EGFR is a RTK found on cells of epithelial origin. Because v-*erb*B was found in the erythroblastosis virus, EGFR and its family of RTKs are known as erbB proteins. EGFR has six ligands: transforming growth factor-α, EGF, β cellulin, heparin-binding EGF-like factor, epiregulin, and amphiregulin (14). The reason EGFR binds so many ligands is not known, but there is some evidence that the binding of different ligands to EGFR results in different intracellular signals being activated.

EGFR is overexpressed and constitutively active in a variety of tumors, including breast, brain, and lung, but the *EGFR* gene is amplified in only a small percentage of tumors. Overexpression of EGFR correlates with a poor prognosis for the cancer patient (15). The *EGFR* gene is not usually found to be mutated in cancer (16), although it has been shown recently that lung cancers which have a mutated EGFR are more susceptible to the small-molecule EGFR inhibitor Iressa (17). There is also a variant of EGFR, vIII, expressed in some tumors. The vIII variant lacks the majority of the ligand binding domain but is constitutively active, producing ligand-independent growth signals. However, the existence of this variant and its role in tumorigenesis remains controversial. Activation of EGFR is a known survival signal and is considered to be seminal to the survival of tumors that overexpress it (18–23).[4]

HER-2 (erbB-2) is a member of the *erb*B family of protein tyrosine kinases and is an orphan receptor that requires heterodimerization with another *erb*B family member for activation (24). The *HER-2* gene is amplified and overexpressed in ~30% of human breast cancers (25), and its overexpression correlates with a poor prognosis. HER-2 is a homologue of the rat *Neu* gene, which has been shown to cause mammary tumors in rats when mutated (26, 27). Unlike the rat *Neu* gene, activating mutations in HER-2 have not been associated with cancer (28–30). By using human mammary epithelial cells that overexpress HER-2, we have shown that HER-2 overexpression and activation up-regulates a variety of pathways (31–33), including growth factor–independent and anchorage-independent growth.[4] HER-2–overexpressing cells require additional changes to circumvent EGF-dependent survival signals; these changes include EGF-independent activation of EGFR and activation of alternative survival signaling pathways (23, 34).[4] These data suggest that HER-2 overexpression in combination with EGFR overexpression causes cells to be extremely aggressive, supporting clinical data indicating that breast cancers that overexpress both HER-2 and EGFR have a very poor prognosis.

In the past, therapies for patients with overexpressed *erb*B receptors have been scarce. Recently, trastuzamab, Herceptin (35), and IMC-225 (36), monoclonal antibodies to HER-2 and EGFR, respectively, have shown promise clinically. However, not all patients with *erb*B overexpression benefit from these therapies for several reasons (37). It is known that the extracellular domain of HER-2, where trastuzamab is targeted, is shed thereby removing the target from the tumor (38). In addition, monoclonal antibodies are large and may not arrive at the tumor site or may not be able to penetrate past the outer layers of tumor. However, because antibodies exhibit some efficacy, there are several in trials and approved for therapy (Table 1).

Because many tumors have specific genes overexpressed or constitutively activated that are required for tumor growth, the newest method of treatment for cancers is molecular therapeutics. Molecular therapeutics are small molecules that target the active site of the signature proteins of tumors, thus blocking their activity (for examples, see Table 2). Because EGFR and HER-2 are overexpressed in a variety of tumors, small-molecule inhibitors to these proteins are in clinical trials. Most of the inhibitors targeted to the *erb*B family are quinazolones that block the ATP binding site in the kinase domain of the receptor. Blocking ATP binding stops the transfer of phosphate groups to molecules that are downstream of the receptors in signaling cascades, thus blocking the growth and survival signals from the receptors. Examples of small-molecule *Erb*B inhibitors (reviewed in refs. 39–41) are ZD1839 (Iressa), which blocks mainly EGFR but can also block HER-2, OSI-774 (Tarceva), PKI-166, and EKB-569, all of which block EGFR specifically, GW-2016 which blocks both EGFR and HER-2, and CI-1033, which blocks all *erb*B receptors. All of these drugs have been in at least phase I clinical trials with favorable toxicity profiles.[5] However, these drugs, along

[4] K.M. Diehl, N.K. Grewal, S.P. Ethier, K.M.W. Ignatoski. p38MAPK-activated AKT in HER-2-over expressing human breast cancer cells acts as an EGF-independent survival signal. J Surg Res, in press.

[5] http://www.clinicaltrials.gov/

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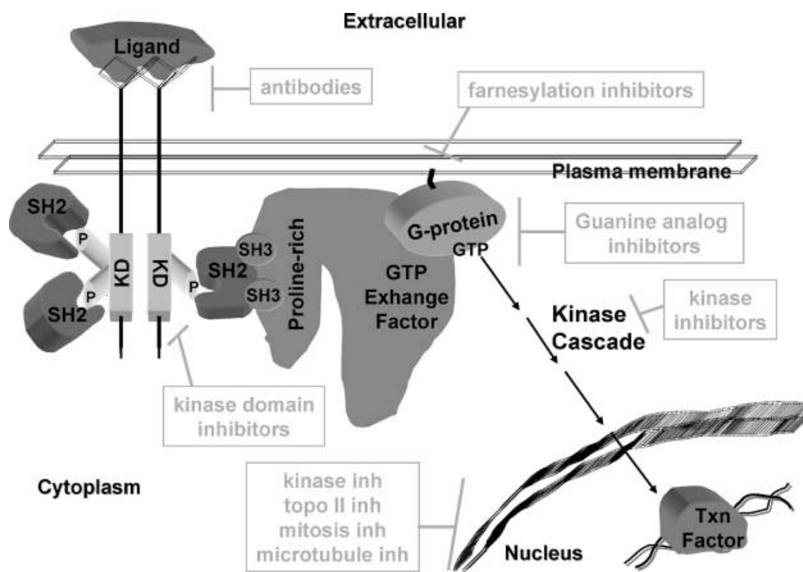

Figure 1. Cell signaling pathways and their inhibitors. Generic illustration of the subcellular localizations of oncogene protein products and classes of small-molecule inhibitors used in cancer treatments.

with the monoclonal antibodies, seem only to work in a subset of patients (37), indicating that the survival of tumors is more complex so that simply blocking one seemingly important pathway will not completely stop the cancer. More work is needed to identify the subset of patients that would benefit most from the use of these drugs.

Adapter Proteins

Adapter proteins consist of small proteins that are mainly composed of protein-protein interaction domains, such as SH2 and SH3 domains. SH2 domains bind phosphorylated tyrosines in a sequence-specific manner (42), and SH3 domains bind a site consisting of a hydrophobic patch that contains a cluster of conserved aromatic residues and is surrounded by two charged and variable loops (43). Adapter proteins bind to the activated receptor with one binding domain and then bind downstream effectors with another domain, linking the start of the pathway to downstream effectors to transmit the signal to the nucleus.

Mitogen-Activated Protein Kinase

Small G-proteins are signaling molecules found downstream of RTKs. The classic example of small G-proteins is *Ras*. V-*ras* was identified as the transforming agent when murine sarcoma viruses were used to transform rat cells. Two different viruses were used; therefore, two different v-*ras* genes were found: *Harvey-ras* (*H-ras*) and *Kirtsen-ras* (*K-ras*). The *Ras-Raf*-mitogen-activated protein kinase (MAPK) pathway is, arguably, the most studied pathway downstream of activated tyrosine kinase receptors. When a RTK becomes activated, tyrosines in its cytoplasmic tail are phosphorylated, often creating a binding site for the SH2 domain of the adaptor molecule GRB-2 (44). GRB-2 in turn binds the guanine nucleotide exchange factor son-of-sevenless via the interaction of the SH3 domain of GRB-2 and the proline-rich sequences of son-of-sevenless (45). These interactions bring son-of-sevenless into close proximity to the small G-protein *Ras* at the plasma membrane where *Ras* is located due to the addition of a fatty acid at its NH_2 terminus (46). Son-of-sevenless exchanges GDP on *Ras* for GTP, thereby activating *Ras. Ras* then activates the MAPK kinase (MEK) kinase *Raf*. V-*raf* was identified as the transforming agent of murine sarcoma virus 3611. *Raf* is a serine/threonine kinase that phosphorylates the MEKs MEK-1 and MEK-2, which are also serine/threonine kinases; these kinases then phosphorylate the MAPKs extracellular signal-regulated kinase-1 and extracellular signal-regulated kinase-2 (47–50). The MAPKs then activate several downstream molecules, including transcription factors, to regulate cell growth and differentiation (the MAPK pathway is reviewed in ref. 51).

The canonical MAPK pathway (the pathway described above) is not as simple as it seems. Several other pathways interact with each member of the MAPK pathway; however, the classic pathway remains the most studied and presents several targets for chemotherapeutic intervention. In fact, chemical inhibitors of this pathway have been used in the laboratory for years. For example, PD98059 is a commonly used inhibitor of MEK, but it only inhibits MEK-1; UO126 inhibits both MEK-1 and MEK-2. These drugs are not useful in the clinic due to either harmful side effects or nonspecificity or both (52, 53).

New inhibitors of the canonical MAPK pathway have been investigated recently as potential chemotherapeutic agents. The drug CI-1040 (Pfizer, Ann Arbor, MI) blocks the activation of MAPK and has been proven effective in blocking tumorigenesis in phase I and II trials (54). The kinase activity of *Raf* can be blocked with Nexvar (BAY 43-9006, Onyx Pharmaceuticals, Richmond, CA) The *Raf* inhibitor has shown promising results against *BRaf* (55, 56), a commonly mutated gene in melanoma (57). In addition, the *Ras* molecule is farnesylated because of a CAAX box at

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Table 1. Antibodies targeted to oncogene products

Agent	Mechanism of action	Sponsor	FDA approval	Trial stage	Fast track
Erbitux (cetuximab)	EGFR inhibitor	ImClone/BMS/Merck KGaA (New York, NY)	Yes	N/A	N/A
ABX-EGF (panitumumab)	EGFR inhibitor	Abgenix/Amgen (Thousand Oaks, CA)	No	III	Yes
h-R3	EGFR inhibitor	YM Biosciences (Canada)	?	?	?
Herceptin* (trastuzumab)	HER-2/*neu* inhibitor	Genentech (South San Francisco, CA)	Yes	N/A	N/A
HuMax-EGFr	EGFR inhibitor	Genmab (Denmark)	No	I/II	Yes
Matuzumab	EGFR inhibitor	Merck KGaA (Whitenhouse Station, NJ)	No	I/II	No
Omnitarg* (pertuzumab)	HER-2 heterodimerization inhibitor	Oncotest GmbH/Genentech (South San Francisco, CA)	No	II	No

NOTE: Agents with mechanism of action block oncongene activity are in italics.
Abbreviation: N/A, not available.
*Blocks a molecule closely related to an oncogene.

the NH_2 terminus of the protein that identifies the molecule for addition of a fatty acid by farnesyl transferase. Fatty acid addition targets the molecule to the cell membrane where it is in close proximity to *Raf*. Therefore, blockage of farnesylation would prevent the activation of the MAPK pathway. There are several drugs in clinical trials, including R115777, which prevent NH_2-terminal fatty acid additions (58–60).

Many cytoplasmic signaling molecules can be activated directly by RTK activation or by small G-protein activation. Cytoplasmic signaling molecules are frequently tyrosine kinases, such as the *Src* family of cytoplasmic tyrosine kinases, or serine/threonine kinases, such as *Raf, MAPK*, and *AKT*. Protein kinases are not the only cytoplasmic signaling molecules, there are also phosphatidylinositol kinases, phosphatases (which dephosphorylate sites), cell cycle control elements, structural proteins, and translation factors.

Phosphatidylinositol 3-Kinase

The phosphatidylinositol 3-kinase, originally found as the oncogene in avian sarcoma virus 16, phosphorylates phosphatidylinositide 2′ phosphate to make phosphatidylinositide 3′ phosphate in the plasma membrane (61). Formation of phosphatidylinositide 3′ phosphate creates a binding site for the serine/threonine kinase AKT. AKT was originally identified as the transforming agent of the virus AKT 8 in a mouse thymoma. When AKT is present at the cell surface, it can be phosphorylated, and subsequently activated, by two membrane-bound kinases, PDK1 and PDK2 (62, 63). Activation of AKT results in the activation of several downstream molecules, including mammalian target of rapamycin (mTOR), p70 S6 kinase, forkhead, glycogen synthase kinase-3, and nonclassic protein kinase Cs (64–68). AKT activation also results in the phosphorylation and inactivation of the antiapoptosis gene *BAD* (69). Activation of AKT, therefore, mediates cell survival. The phosphatidylinositol 3-kinase pathway is controlled by phosphatase with homology to tensin, a phosphatase that dephosphorylates phosphatidylinositide 3′ phosphate back to phosphatidylinositide 2′ phosphate (70).

Alterations in the phosphatidylinositol 3-kinase pathway have been seen in many cancers. Mutations in the *PTEN* gene have been implicated in the development of several types of cancer, including endometrial, prostate, thyroid, and brain (reviewed in ref. 71). AKT activation has been shown to occur in many types of cancer as well (72). However, drugs, such as wortmannin and LY294002, which act against phosphatidylinositol 3-kinase, have only been used in the laboratory due to specificity issues (73).

The AKT/mTOR pathway is an important cell survival pathway that has potential targets for adjuvant therapy. In this pathway, environmental signals from insulin, hormones, growth factors, and nutrients are integrated into signals regulating cellular proliferation and survival. Phosphorylation and stimulation of AKT phosphorylates the tuberous sclerosis complex, which in turn then releases its inhibition of the small G-protein Ras homologue enriched in brain. When Ras homologue enriched in brain is activated and GTP is bound, it activates the mTOR complex TORC1 or mTOR/raptor. This sways the balance between the rapamycin-sensitive mTOR complex TORC1, which includes mTOR, raptor, mLs8 (GβL), and the rapamycin-insensitive complex TORC2, which includes mTOR, rictor, and GβL toward increasing TORC1. This in turn stimulates phosphorylation ribosomal p70 S6 kinase leading to cap-dependent translation. These proteins are required for G_1 cell-cycle progression and S-phase initiation (74–79).

Rapamycin is a natural antibiotic originally developed as an antifungal agent. Clinically, it is best known for its use as an immunosuppressive agent in solid organ transplantation. Rapamycin *in vivo* combines with FK506 binding protein-12, and this complex inhibits the mTOR complex TORC1 or mTOR/raptor (76). Based on promising preclinical studies, testing on rapamycin as an adjuvant cancer treatment has begun in the clinical setting. In addition, rapamycin derivatives have been developed, including

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Table 2. New targeted agents with various mechanisms of action

Agent	Mechanism of action	Sponsor	FDA approval	Trial stage	Fast track
PX-12	Inhibition of thioredoxin reductase	ProLx (Tucson, AZ)	No	II	No
SAHA	Inhibition of histone deacetylase	Aton (Tarrytown, NY)	No	III	No
2C4/pertuzum*	Humanized Ab to HER-2/*neu*	Genentech (South San Francisco, CA)	No	II	No
AG2037	GAR transformylase inhibitor	Pfizer (New York, NY)	No	I	No
HMN214	Polo kinase inhibitor	Nippon Shinyaku (Japan)	No	I	No
ILX651	Tubulin-interactive agent	Genzyme (Cambridge, MA)	No	II	No
Brostallicin	Activated by GST	Pfizer	No	II	No
ABT 510	Thrombospondin mimetic	Abbott (Abbott Park, IL)	No	II	No
NM-3	Oral VEGF inhibitor	Genzyme	No	II	No
NB1011	Agent activated by thymidylate synthase	NewBiotics (San Diego, CA)	No	II	No
Troxacitabine	L-Nucleoside targets mitochondria DNA	Shire (England)	No	II	No
SGN-15	cBR96 doxorubicin immunoconjugate	Seattle Genetics (Bothall, WA)	No	II	No
TLK286	Activated by GST (DNA-dependent protein kinase)	Telik (Palo Alto, CA)	No	III	No
Clofarabine	Nucleoside	Genzyme	Yes	N/A	N/A
Tesmilifene	Mechanism of action?	YM Biosciences (Canada)	No	III	No
G17DT	Gastrin analogue vaccine	Aphton (Philadelphia, PA)	No	III	Yes
GW 572016/lapatinib	Topo I receptor kinase inhibitor	GSK (Philadelphia, PA)	No	II	Yes
CRX-26	Pattern agent	CombinatoRx (Cambridge, MA)	?	?	?
MGI114/irofulven	DNA interactive (ERCC3 deficient)	MGI Pharma (Bloomington, MN)	No	II	Yes
CC5013	Imide mechanism of action?	Celgene (Summit, NJ)	No	III	No
SCH 66334	Farnesyl transferase inhibitor	Schering (Kenilworth, NJ)	?	?	?
R115777/Zarnestra	Farnesyl transferase inhibitor	Ortho (Puerto Rico)	No	II	No
Psorospermine	Topo II inhibitor	Cyternex (San Diego, CA)	?	?	?
IGN 241	Adeno-MDA-7 induces apoptotic death	Introgen (Austin, TX)	No	II/III	No
Ab EGFR	Antibody to EGFR	Abgenix (Freemont, CA)	?	?	?
Alanosine	Active in MTAP-deficient tumors	Salmedix (San Diego, CA)	No	II	No
Aplidine	Inhibits palmityl thioesterase	PharmaMar (Spain)	No	I	No
Kahalalide F	Lysosomal destabilization	PharmaMar	No	II	No
R-etodolac (SDX 101)	Mechanism?	Salmedix	No	II	No
Hh-1	Antibody to hedgehog protein	Curis (Cambrige, MA)	No	I	No
Remicade	Anti-TNF Ab	Centocor (Horsham, PA)	Yes	N/A	N/A
Diflomotecan	Topo I inhibitor	Biomeasure (Milford, MA)	No	I	No
CEP 751	NGF/TRK A antagonist	Cephalon (Frazer, PA)	No	I/II	No
Amonafide derivative	Topo II inhibitor	Xanthus (Cambridge, MA)	No	II	No
AGN 195183	RAR antagonist	Allergan (Irvine, CA)	No	I	No
HGS-ETR1/mapatumumab	Anti-TRAIL effect	Human Genome Sciences (Rockville, MD)	No	II	No
NX211	Liposomal Topo I inhibitor	OSI (Mellville, NY)	No	I	No
CT2106	Polyglutamated camptothecin	Cell Therapeutics (Seattle, WA)	No	II	No
RSR-13	Oxygen unloader (2-3 DPG)	Allos (Westminster, CO)	No	III	No
Aroplatin	Liposomal DACH platinum	Antigenics	No	I/II	No
C1033	erbB antagonist	Pfizer	?	?	?
GW506U78	Guanine analogue	GSK	No	II	No
HIF inhibitor	Inhibits HIF	ProLx	No	IB	No
G3139	Antisense to bcl-2	Genta (Berkeley Heights, NJ)	No	I	No
Iressa (Gefitinib)	EGFR-specific inhibitor	AstaZeneca (England)	Yes	N/A	N/A
Gleevec	Abl kinase inhibitor	Novartis	Yes	N/A	N/A
Oncophage	HSP vaccine	Antigenics	No	III	No
Sprycel (dasatinib)	Multi-RTK inhibitor for Gleevec-resistant CML	Bristol-Myers Squibb	Yes	N/A	N/A
Sutent	Multi-RTK inhibitor	Pfizer	Yes	N/A	N/A
PKC412	c-kit, pkc, flt3, tyrosine kinase receptor inhibitor	Novartis	No	II	No
Nexavar (BAY 439006/Sofafenib)	Raf kinase inhibitor	Bayer (Germany)	No	III	No
CanFite 1	Adenosine A3 receptor	CanFite (Israel)	No	II	No

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Table 2. New targeted agents with various mechanisms of action (Cont'd)

Agent	Mechanism of action	Sponsor	FDA approval	Trial stage	Fast track
Apomine	Farnesoid X receptor interactive	Genzyme	No	II	No
SKB40875	Maytansine Mo Ab conjugate	GSK	?	?	?
MB-8	Methylase	MGI	?	?	?
LY293111 (MEPM)	Leukotriene antagonist	Eli Lilly (Indianapolis, IN)	No	II	No
LDP341	Proteosome inhibitor	Millenium (Cambridge, MA)	No	II	Yes
DJ-927	Taxane not affected by MDR	Daiichi (Japan)	No	II	No
ABT-751	β-tubulin inhibitor	Abbott	No	I	No
AEE788	Receptor tyrosine kinase inhibitor	Novartis	No	I/II	No
Alimta	Antifolate	Eli Lilly	Yes	N/A	N/A
BMS-214662	Farnesyl transferase inhibitor	Bristol-Myers Squibb	No	I	No
BMS-354825/Dasatinib	Abl kinase inhibitor	Bristol-Myers Squibb	No	I	No
Bortezomib/Velcade	Proteosome inhibitor	Millenium Pharm	Yes	N/A	N/A
EKB-569	EGFR inhibitor	Wyeth-Ayerst	No	I/II	No
Erlotinib/Tarceva	EGFR inhibitor	OSI/Hoffman-LaRoche/ Genentech	Yes	N/A	N/A
Femara (Letrozole)	Aromatase inhibitor	Novartis	Yes	N/A	N/A
GW-786034	Tumor angiogenesis inhibitor	GlaxoSmithKline	No	II	No
Fludarabine		Berlex (Wayne, NJ)	Yes	N/A	N/A
Ixabepilone	Microtubule stabilizer	Bristol-Myers Squibb	No	II	No
Lapatinib	EGFR/HER-2 inhibitor	GlaxoSmithKline	No	II	Yes
Lenalidomide		Celgene Corp.	Yes	N/A	N/A
Letrozole/femara	Aromatase inhibitor	Novartis	Yes	N/A	N/A
ONYX-015	Oncolytic virus	Onyx (Emeryville, CA)	No	II	No
OSI-211		OSI	No	II	No
OSI-7904L	Thymidilate inhibitor	OSI	No	I	No

NOTE: Agents with mechanism of action block oncongene activity are in italics.
Abbreviations: Ab, antibody; TNF, tumor necrosis factor; GAR, glycinamide ribonucleotide; GST, glutathione *S*-transferase; MTAP, methylthioadenosine phosphorylase; NGF, nerve growth factor; RAR, retinoic acid receptor; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand; HIF, hypoxia-inducible factor; HSP, heat shock protein.
*Blocks a molecule closely related to an oncogene.

CCI-779 by Wyeth Ayerst (Madison, WI), RAD001 by Novartis (Switzerland), and AP23573 by Ariad (Cambridge, MA). Thus far, the dose-limiting toxicity in clinical trials has been dermatologic reactions, mucositis, neutropenia, and thrombocytopenia. Tumor response has been seen in patients with sarcoma, as well as lung, renal cell, breast, and endometrial carcinoma (80–83). Early trials indicate a correlation between activation of the AKT/mTOR pathway or expression of the insulin-like growth factor receptor and response to rapamycin. *In vitro* and *in vivo* studies show that treatment with rapamycin is associated with G_1 arrest and down-regulation of CCND1 (cyclin D1) and cyclin-dependent kinase 4 (80, 84–86).

Breakpoint Cluster Region-Abl

Nowell and Hungerford (87) observed an abnormally small chromosome in cells from chronic myelogenous leukemia (CML) patients. The small CML chromosome was labeled the Philadelphia chromosome because these researchers were from Philadelphia. It was subsequently shown that the Philadelphia chromosome resulted from a translocation between the long arms of chromosomes 9 and 22 [t(9;22)(q34;q11); ref. 88]. The chimeric protein formed by the chromosomal joining contains a region termed the breakpoint cluster region (Bcr) fused to the c-*abl* tyrosine kinase, originally identified as the oncogene of the Abelson murine leukemia virus, to create Bcr-Abl (89). The Bcr-Abl protein has deregulated kinase activity compared with wild-type *Abl* and has been shown to play a causative role in CML (90–92).

Because the causative agent for CML, Bcr-Abl, is an overactive kinase, it was plausible to make a small-molecule inhibitor to the *Abl* kinase to treat the disease. A high-throughput screen of chemical libraries identified CGP-57148 (STI1571, now imatinib, Gleevec, or Glivac) as having inhibitory activity against the *Abl* kinase (93). Imatinib binds in the kinase domain of the *Abl* portion of the protein and blocks its activity with IC_{50} values in the 0.025 μmol/L aliquot in *in vitro* assays (93, 94). The inhibitory activity of imatinib toward platelet-derived growth factor receptor and c-*Kit* was found to be similar; however, activity against other tyrosine and serine/threonine kinases was at least 100-fold lower (95). Dose-dependent inhibition of mouse tumor growth expressing Bcr-Abl occurred at 10 to 50 μg/kg daily with no effect on v-*src*–induced tumors or on normal cells (93). In phase I trials, 98% of CML patients treated with imatinib had a

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complete hematologic response (96). In phase II trials, 30% of patients with myeloid blast crisis had 1-year survival (97), 74% of accelerated phase patients also had a 1-year survival (97–99), and 95% of patients in chronic phase had a complete hematologic response (95).

As stated above, imatinib is also a potent inhibitor of platelet-derived growth factor receptor and c-*Kit* (94). Most gastrointestinal stromal tumors contain activating c-*Kit* mutations. Kit is the oncogene of the Hardy-Zuckerman 4 strain of the feline sarcoma virus. Gastrointestinal stromal tumors, which are typically unresponsive to standard chemotherapy, respond dramatically to imatinib (100). Platelet-derived growth factor receptor, the receptor for the oncogene v-*sis*, a platelet-derived growth factor homologue from simian sarcoma virus, is activated in a variety of tumors, including glioblastoma, dermatofibrosarcoma, and CML (reviewed in ref. 101). Imatinib has also been shown to be effective in the laboratory against tumors that express an activated platelet-derived growth factor receptor (102).

Imatinib has been a breakthrough treatment for both CML and gastrointestinal stromal tumors; however, drug resistance has been a problem (94, 103). Disease relapse has been seen in patients whose tumors were initially responsive to imatinib (104). It seems that the most common method of resistance is a point mutation in the *Abl* kinase domain, which blocks the binding of the inhibitor but does not block kinase activity (95, 104–106). In addition, in a small subset, genomic amplification of *bcr-abl* is seen. This implies that the amount of protein increases to a point where its combined kinase activity overwhelms the inhibitory effects of the drug. Improved versions of imatinib and other inhibitors should circumvent the problem of mutation-directed resistance.

The signals emanating from the cytoplasmic signaling molecules ultimately activate the transcription factors. Transcription factors bind DNA and initiate transcription of downstream genes. Transcription factor oncogenes usually are not found to be mutated but are overexpressed in human cancers. For example, the proto-oncogene c-*myc*, the oncogene is from chicken myelocytomatosis 29 virus, is translocated to the transcription control sequences of IgG in B cells in Burkitt's lymphoma (107, 108). Because IgG is highly expressed in B cells, this translocation results in tremendous overexpression of c-*myc*, which drives tumorigenesis.

Finding New Targets

In the past, genes involved in human tumorigenesis were identified because of their homology to known oncogenes (4). Then, as advancements in molecular biology grew, tumorigenesis-related genes were identified because they were amplified in several cancers. These genes were isolated and expressed in nontransformed cells. *In vitro* assays on the cells expressing the genes were done to determine motility, invasion, and anchorage-independent growth; all of these phenotypes are hallmarks of transformed cells. If the cells became transformed, they were placed *in vivo* in mice to see if they grew into tumors. These processes were tedious as only one gene could be analyzed at once.

Today, multiple genes can be examined simultaneously for their transforming potential. For example, all of the expressed genes from a cancer cell or genes from a region of amplification that is common among cancers are cloned into viruses. These viruses are used to infect nontransformed cells. Transformed cells are then selected by various criteria, including the ability to grow without anchorage to a substrate or to grow without growth factors. The genes responsible for the transformation are then isolated. This approach is called expression cloning (109).

Another multigene approach is microarrays. With this technique, expressed RNA is isolated from a normal cell and a cancer cell, labeled different colors, and used to probe a glass slide (a chip) that contains fragments of as many as 64,000 genes. The intensity of the different colors or of a combined color indicates whether a gene is overexpressed or underexpressed in the cancer cells. Recently, protein microarrays have been developed where antibodies are spotted on a glass slide and proteins from normal and tumor cells are labeled. Again, color intensity indicates what proteins are overexpressed or underexpressed in the cancer cells. All of these assays have the same drawbacks as the original assays but are less time consuming because multiple genes can be identified at once.

The microarrays can also yield tumor signatures: groups of genes that are overexpressed in certain subsets of tumors. In the future, these tumor signatures will be used to identify cancers that will likely respond to one drug or another, providing individualized, specific treatments for the cancer patient.

New Therapeutics

An increase in potential molecular targets in cancer has occurred with the development of high-throughput screens for genes involved in cancer. Genes identified by high-throughput screens are able to be analyzed quickly for their potential to be transforming because of the background knowledge gained from the study of oncogenes. For example, a gene whose product is a ribosomal structural protein does not have the same apparent transforming potential as a gene whose product is a tyrosine kinase and, therefore, would be discarded as a candidate. Due to these advancements, the percentage of new agents that proceeded to phase I trials and were later Food and Drug Administration (FDA) approved has increased from just 8% between 1978 and 1983 to 61% between 1996 and 2001 (110).

Many of the newer molecular targeted drugs are antibodies that block the activation of transforming proteins (Table 1). However, the extracellular domain of the targeted protein may be shed, thereby removing the target from the tumor (38). In addition, monoclonal antibodies are large and may not arrive at the tumor site or may not be able to penetrate past the outer layers of tumor.

The small-molecule inhibitors are the newest class of drug to enter trials. These drugs inhibit a molecule thought

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to be a causative agent in tumorigenesis by inhibiting kinase activities, fatty acid additions, growth factor actions, DNA unwinding, proteosome degradation, mitosis, or other enzymatic activities. There are several drugs that have been approved recently by the FDA, including the EGFR kinase inhibitors Tarceva and Eribitux (Table 2). There are also many new drugs entering trials that have new or improved mechanisms of action (Table 2). Among these are SCH 66334 and R115777, which inhibit farnesyl transferase activity, thereby inhibiting *Ras* localization and its signaling, and CI-1033, which is a pan-*Erb*B kinase inhibitor. Several of the new drugs have been shown to induce dramatic responses in phase I trials. If the response rates and magnitudes hold up in later trials, these drugs will have a significant effect on cancer treatment. For an illustration of the actions of targeted therapies and cellular localization of their targets, see Fig. 1.

Summary

Although oncogenes and their transformation mechanisms have been known for almost 30 years (3), we are just now using our understanding of protein function to abrogate the activity of these genes to block cancer growth. The advent of specific small-molecule inhibitors has been a tremendous step in the fight against cancer. The best-known example is Gleevec (imatinib), which acts against the oncoprotein *Abl* in CML. In the early 1990s, IFN-α treatment produced a sustained cytologic response in ~33% of CML patients (111). Today, with Gleevec, the hematologic response rate in CML patients is 95% with 89% progression-free survival at 18 months (95). There are still drawbacks to the new therapies, such as drug resistance after a period of treatment, but the drawbacks are being studied experimentally. New drugs and combination therapies are being designed that will bypass the resistance mechanisms.

The phenomenon that tumors may be depended on one protein for survival has been termed recently as ''oncogene addiction'' (112) as was recognized 25 years ago in the classic experiments by Shih et al. (4). This addiction is essential to the success of small-molecule inhibitors because single oncogenes are the targets of many of the new drugs on the market. Twenty-five percent of the 11 FDA approved small-molecule inhibitors listed are against oncogenes and 20% of fast-tracked drugs also inhibit oncogenic proteins (Table 2). Taking the two tables together, 31% of the 83 inhibitors listed in Tables 1 and 2 inhibit oncogene proteins. Finally, 30 years after the identification of *Src* as the transforming agent in Rous sarcoma virus, oncogenes are being recognized as prime targets for anticancer therapies.

Acknowledgments

We thank Jonathan Lubin for his research on FDA approvals for therapeutics and Elizabeth Talbot for her editing assistance.

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