

Novel anticancer drug discovery

John K Buolamwini

There is at present, much optimism about the possibility of finding selective anticancer drugs that will eliminate the cytotoxic side effects associated with conventional cancer chemotherapy. This hope is based on uncovering many novel molecular targets that are 'cancer-specific', which will allow the targeting of cancer cells while normal cells are spared. Thus far, encouraging results have been obtained with several of these novel agents at the preclinical level, and clinical trials have begun. These targets are involved at one level or more in tumor biology, including tumor cell proliferation, angiogenesis and metastasis. Novel targets for which advances are being made include the following: growth factor receptor tyrosine kinases such as the epidermal growth factor receptor and HER-2/neu (proliferation); the vascular endothelial growth factor receptor and the basic fibroblast growth factor receptor (angiogenesis); the oncogenic GTP-binding protein Ras (especially agents targeting Ras farnesylation, farnesyltransferase inhibitors) (proliferation); protein kinase C (proliferation and drug resistance); cyclin-dependent kinases (proliferation); and matrix metalloproteinases and angiogenin (angiogenesis and metastasis). Less explored, but potentially useful targets include the receptor tyrosine kinase platelet-derived growth factor receptor, mitogen-activated protein kinase cascade oncogenes such as Raf-1 and mitogen-activated protein kinase kinase, cell adhesion molecules such as integrins, anti-apoptosis proteins such as Bcl-2, MDM2 and survivin, and the cell life-span target telomerase.

Addresses

Department of Medicinal Chemistry and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS 38677, USA; e-mail: mcjkb@olemiss.edu

Current Opinion in Chemical Biology 1999, 3:500–509

<http://biomednet.com/elecref/1367593100300500>

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Abbreviations

bFGFR	basic FGFR
CDK	cyclin-dependent kinase
CDKI	CDK inhibitor
EGFR	epidermal growth factor receptor
ERK	extracellular-signal-regulated kinase
FGFR	fibroblast growth factor receptor
FTase	farnesyltransferase
Grb2	growth-factor-receptor-binding protein 2
MAPK	mitogen-activated protein kinase
MEK	MAPK kinase
MMP	matrix metalloproteinase
PDGF	platelet-derived growth factor
PDGFR	PDGF receptor
PKC	protein kinase C
RTK	receptor tyrosine kinase
SH2/3	Src homology 2/3
Sos	son of sevenless
uPA	urokinase
VEGFR	vascular endothelial growth factor receptor

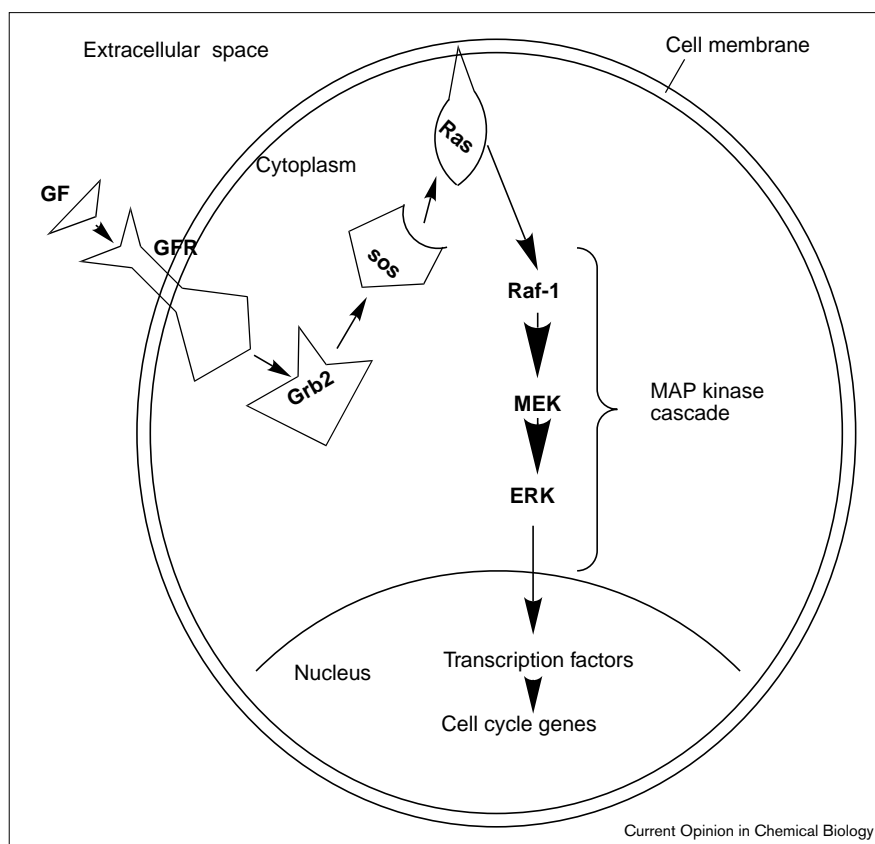
Introduction

Conventional cancer chemotherapy is highly inadequate as a result of the lack of selectivity between cancer cells and normal cells. This calls for novel cancer therapies for selectively targeting cancers without toxicity to normal tissues. The discovery of novel anticancer agents that will hopefully provide the desired degree of selectivity for cancer cells versus normal tissues has been fueled by the unveiling of a host of novel potential molecular targets through the application of molecular biology methods to cancer biology. These novel targets include genes involved in malignant transformation, cancer progression and metastasis [1*]. In addition to the identification of many novel anticancer targets, molecular biology methods have facilitated the investigation of the potential of these targets for drug discovery, by allowing functional expression or production of the targets for use in high-throughput screening assays of natural and synthetic molecule libraries. This has also allowed the production of sufficient quantities of target proteins for X-ray crystallographic studies that provide pertinent three-dimensional structural information on the targets and their interaction with ligands/inhibitors for structure-based rational drug design. Interesting and creative approaches to specifically killing cancer cells are also emerging, such as the use of engineered adenoviruses like ONYX-015 (in clinical trials, ONYX Pharmaceuticals, Richmond, CA, USA) which selectively replicate in, and kill cells that have lost p53 function but are unable to replicate in, and therefore do not affect, cells with normal p53 function.

This is a selective review highlighting developments in anticancer drug discovery based on novel molecular targets that are envisaged to hold promise for providing the long sought-after selectivity in anticancer therapy. Contemporary anticancer drug discovery follows the main paradigm of current drug discovery in general, which is largely molecular-target-based [2*]. Global genomic and proteomic approaches that are being employed in conjunction with bioinformatic tools to identify novel drug discovery targets, and to probe mechanisms of action and toxicity of potential drug molecules have been reviewed recently [2*]. These include molecular target discovery by expressed sequence tag (EST) database searching, proteomic molecular profiling through high-resolution quantitative two-dimensional protein gel electrophoresis, and functional genomics through cDNA microarray expression analysis. Antisense, ribozyme and antibody methods comprise the main means of molecular target validation. The National Cancer Institute of the United States, with its 60 human cancer cell line screen, has been prominent in this information-intensive approach to cancer pharmacology, as well as the Fred Hutchinson Cancer Research Center (Seattle, WA, USA), which is exploiting

Figure 1

General growth factor mitogenic signalling through the Ras–MAPK pathway. GF, growth factor, GFR, GF receptor.



yeast genetics for cancer drug discovery [2*,3]. Combinatorial chemistry and high-throughput screening against pure molecular targets and cancer cells are established methods for primary anticancer drug discovery. Computational structure-based drug design, utilizing X-ray crystallographic information, is also becoming rapidly established in cancer drug discovery. A prime illustration of how these methods are being integrated in anticancer drug discovery is provided by the recent report of Gray *et al.* [4**]. Most of the potential novel molecular targets for anticancer drug discovery can be grouped into the following categories: growth factor receptor tyrosine kinases (RTKs) and serine/threonine kinase signal transduction pathway targets; cell cycle targets; apoptosis-related targets; extracellular matrix targets, tumor angiogenesis and metastasis targets; and cell life-span targets.

Growth factor receptor tyrosine kinases and serine/threonine kinase signal transduction pathway targets and inhibitors

The potential for inhibiting RTK function to achieve an anticancer effect stems from their important role in proliferative signal transduction, their overexpression in cancers, and their oncogenic potential, as revealed within the past decade. Following, is a summary of the major mitogenic signaling pathway involving growth factor RTKs (see

Figure 1). The binding of endogenous ligands (growth factors) to their RTKs results in receptor dimerization, which triggers tyrosine phosphorylation in the cytoplasmic domains of the RTKs. This receptor phosphorylation allows the binding of the growth-factor-receptor-binding protein 2 (Grb2) adapter protein via its Src homology 2 (SH2) domain to the intracellular domain of RTKs. The bound Grb2 is then activated to bind to the proline-rich region of guanine nucleotide exchange factor Sos (son of sevenless) protein via its SH3 domain, and cause Sos to translocate to the cell membrane and bind to the Ras-GTP-binding protein. The binding of Sos to Ras leads to Ras activation by allowing it to undergo a molecular switch releasing GDP and binding GTP in its place. Activated Ras, in turn, triggers the mitogen-activated protein kinase (MAPK) cascade by binding to and activating the MAPK kinase (MEK) kinase (MAPKKK) Raf-1 kinase. Activated Raf-1 kinase then phosphorylates MEK kinase, which in turn phosphorylates the ultimate MAPK in this cascade, extracellular-signal-regulated kinase (ERK) kinase. Activated ERK translocates into the cell nucleus where it propagates the mitogenic signal by way of phosphorylating and activating the appropriate transcription factors to induce the expression of genes necessary for initiating the cell division cycle. Several other upstream regulators and downstream effectors of Ras have been identified [5**,6**].

The various other downstream effectors of Ras and the involvement of Rho family of proteins (Ras-related GTP-binding proteins) have been reviewed recently [7•]. Several of these interactions are currently being targeted for anticancer drug discovery as discussed below.

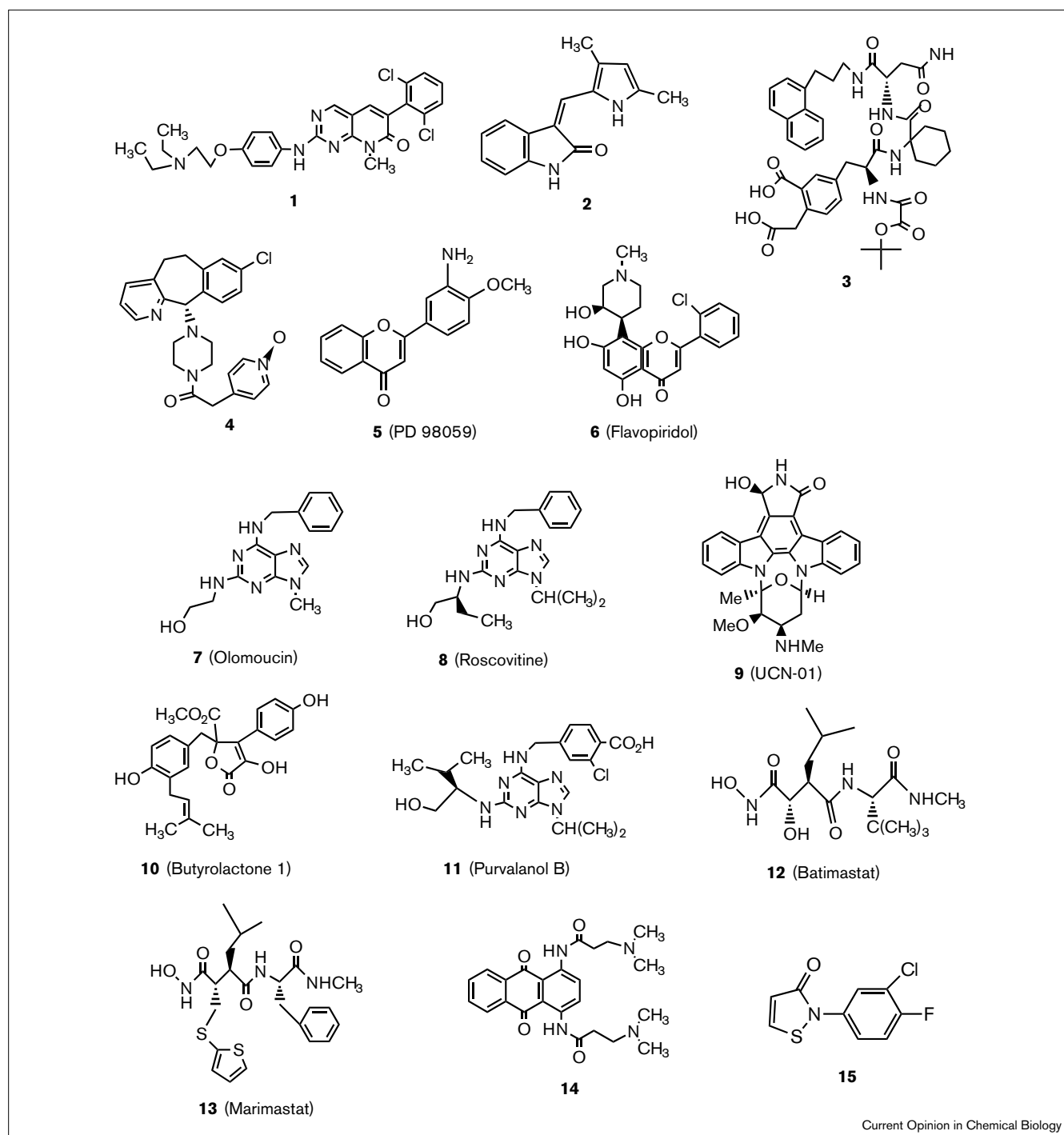
The epidermal growth factor receptor (EGFR), *c-erbB-2*/HER-2/neu receptor, platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR) are the most widely explored RTKs for novel anticancer drug discovery. Neutralizing antibodies against these receptors have been investigated in the clinic for various solid tumors. Recently, Herceptin (Genentech, Inc., San Francisco, USA), a humanized antibody against HER-2/neu [8•], was approved in the United States for treatment of metastatic breast cancer. Trials are also underway for therapies combining antibody therapy with other cancer therapy modalities [9••]. Antibody combination therapy for increased antitumor effect has been demonstrated with the combination of EGFR and HER-2/neu antibodies [10••]. Furthermore, immunotoxins involving conjugates of antibodies to toxins such as *pseudomonas* exotoxin are also being developed, as reviewed recently [9••]. The small-molecule inhibitors targeting RTKs that have been identified are largely inhibitors of receptor kinase activity. They may be mimics of the tyrosine, or ATP substrate, or a hybrid structure. None of these are on the market yet, but intense efforts are being made by several pharmaceutical companies to develop RTK inhibitors for cancer therapy [1•].

In addition to several chemical classes such as tyrostopins and quinazolines that have been identified as potent RTK inhibitors, a new series of 2-substituted aminopyrido[2,3-*d*]pyrimidinones tyrosine kinase inhibitors represented by **1** (Figure 2) have been reported recently [11••] that showed *in vivo* anticancer activity against ovarian and colon cancers, and selective inhibitory activity against several tyrosine kinases, including EGFR, PDGFR and Src. The structure/activity relationships (SARs) data, as well as molecular modeling, have been used to develop a tyrosine kinase binding model for this series [12•]. RTK inhibitors against endothelial cell growth factor receptors, particularly VEGFR-2 (Flk-1/KDR) and basic FGFR (bFGFR) are being pursued primarily as novel antiangiogenic anticancer agents. For example, an inhibitor identified recently as a potential antiangiogenic agent, SU5416 (3-[(2,4-dimethylpyrrol-5-yl)methylidene]-indolin-2-one, **2** [Figure 2]), has been shown to inhibit tumor vascularization and the growth of multiple types of tumor xenografts in mice [13••]. The use of a new homogeneous time-resolved fluorescence assay for tyrosine kinase inhibitor discovery has been reviewed [14], and is said to eliminate many of the problems associated with conventional screening assays, such as false positive and false negative results. It is expected to improve high-throughput tyrosine kinase inhibitor discovery in terms of higher efficiency and fewer false positives or negatives.

The interaction of the Grb2 adapter protein with RTKs is also being targeted for cancer drug discovery. Peptidyl phosphotyrosine analogs are being designed to bind at the Grb2 SH2 site and inhibit Grb2 binding to activated RTKs. Recently, nonphosphate-containing phosphotyrosine mimetics have been reported that effectively inhibit Grb2–HER-2/neu interaction without requiring prodrug derivatization for effective delivery (e.g. **3** [Figure 2], with an IC₅₀ of 1.3 μM) [15••]. The interaction of Grb2 with Sos via the Sos SH3 domain has not yet been the focus of anticancer drug discovery, and neither has the interaction of Sos and Ras. In this latter context, the availability of an X-ray structure of the Ras–Sos complex interface [16••] should facilitate the design of inhibitors of this interaction. It has been suggested that this X-ray structural information may be used to design inhibitors by making or identifying nucleotide analogs that bind to the altered nucleotide-binding site in the Ras–Sos complex in order to stabilize it, thereby mimicking the action of dominant negative alleles of Ras, or by designing hydrophobic compounds that will bind to the core hydrophobic region of the Ras–Sos binding interface [16••]. In terms of SH2 domains other than that of Grb2, a focused parallel combinatorial library of phosphotyrosine peptides was used to identify for the first time ligands with selectivity enough to discriminate among the Src kinase family [17•].

Intense efforts have been concentrated on developing Ras-targeted agents as novel anticancer drugs. This derives from the discovery of the oncogenic properties of mutant Ras, and that Ras mutations occur in about 30% of human tumors; Ras mutations are particularly prevalent in pancreatic, colon and lung cancers, as well as leukemias. Oncogenic mutation causes Ras to be permanently activated and continuously stimulate its downstream effectors, leading to mitogenic activity without the need for upstream mitogenic signals. Anticancer drug discovery based on the inhibition of post-translational modification of Ras has been pursued vigorously; particular effort has been made to identify inhibitors that target the Ras farnesyltransferase (FTase) enzyme, as reviewed recently [18••]. FTase is required to transfer a farnesyl moiety from cytosolic farnesylpyrophosphate to a cysteine residue at the carboxyl terminus in the CAAX motif (where C is cysteine, A is any aliphatic amino acid and X is any amino acid) of newly translated Ras protein. This farnesylation is necessary to anchor Ras to the cell membrane and allow it to perform its signal relay functions. FTase inhibitors have been effective in blocking Ras function, and have demonstrated potent antitumor activity both *in vitro* (in cell culture) and *in vivo* (in animal tumor models). Recently, further structural modifications on tricyclic CAAX competitive FTase inhibitors (such as **4**, Figure 2) that were discovered previously [19••] provided orally bioavailable analogs with improved *in vivo* anticancer activity and pharmacokinetic profiles [20••]. The recent report of the first X-ray crystal structure of a FTase (rat) in complex with a farnesylpyrophosphate substrate [21••] should provide three-dimensional information on the

Figure 2



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The structures of some of the inhibitors discussed in this review.

binding site and facilitate rational structure-based design of novel FTase inhibitors.

Surprisingly, FTase inhibitors have so far not been associated with toxicity problems; however, their actual mechanism of action appears more complicated than was originally envisaged. Growth inhibitory responses to FTase

inhibitors have been obtained in cancers with either mutant or wild type Ras. This notwithstanding, H-Ras mutant cancers are the most responsive to the current FTase inhibitors. They appear to cause antiproliferative effects through Ras-dependent and Ras-independent mechanisms [22•]. Effective FTase inhibitors should discriminate between FTase and geranylgeranyl transferase,

another protein prenylation enzyme with which it shares structural similarity. Lack of discrimination may lead to toxicity due to interference with the normal function of geranylgeranylated proteins. So far, FTase inhibitors have proven effective in many animal tumor models, and have reached the stage of human clinical evaluation [18**].

The possibility of combining FTase inhibitors with other cancer treatment modalities has also been demonstrated. In humans, the ras GTP-binding protein family is made up of three members, K-ras, H-ras and N-ras which play a critical role in mitogenic signal transduction pathways that lead to cell proliferation and differentiation. Inhibition of Ras prenylation was shown to increase the radiosensitivity of human tumor cell lines with oncogenic Ras mutations [23**], H-Ras mutants being more sensitive than K-Ras mutants. Ras-related GTP-binding proteins such as Rho, have also been proposed as potential anticancer drug discovery targets. p21^{WAF1} is one of several genes encoding proteins that function as cyclin-dependent kinase inhibitors (CDKIs) to regulate their activity and thereby cause cell cycle arrest. The p21^{WAF1} gene product is induced primarily by functional p53 gene product to cause cell cycle arrest in G1. Among the rest of the members of the Ras–MAPK signal transduction cascade, Raf-1 and MEK are the noted oncogenic members [24]. However, drug discovery targeting these MAPKs downstream of Ras has lagged far behind that of Ras. While antisense oligonucleotide strategies are being pursued for Raf-1 modulation [25], a selective small-molecule inhibitor of MEK, PD 98059 (5, Figure 2), which is also able to inhibit cell growth and reverse Ras transformation, has been reported [26].

Among other cytoplasmic signal transduction protein kinases, the protein kinase C (PKC) family has received considerable attention in terms of anticancer drug discovery, as reviewed recently [27]. PKC overexpression has been observed in estrogen-receptor-negative breast cancer, thyroid cancer, gliomas and melanoma, and is also implicated in tumor angiogenesis and multidrug resistance [27]. It has also been shown that PKC is an upstream regulator of Ras, and an activator of the ERK MAPK cascade [5**]. PKC inhibitors induce apoptosis, making them potentially useful for enhancing the efficacy of current cancer chemotherapy, as reviewed by Schwartz [28]. The availability of the X-ray crystal structure of PKC δ complexed with phorbol 13-acetate (which activates it) has been valuable in structure-based design of PKC ligands, leading to the recent design of novel γ -lactam PKC activators ([29**] and references therein).

Cell cycle targets and inhibitors

After being activated as part of the RTK- or non-RTK-initiated mitogenic signaling cascades, MAPKs translocate to the nucleus where they activate transcription factors that cause the expression of genes to initiate the cell division cycle. Many oncogenic transcription factors involved in

cell proliferation and differentiation have been identified such as Myc, Ets, Fos, Jun, Rel/NF- κ B and Myb, [24,30], but have not yet been a major focus for anticancer drug discovery. Cell cycle research has shown that CDKs (serine/threonine kinases) are key regulatory molecules that work as binary complexes with various activating cyclins (regulatory units), to drive the progression of the cell cycle through the different phases (i.e. G1, S and G2/M phases). Different CDKs, individually or as groups, bind to different cyclins or subsets of cyclins as follows: CDK1 (Cdc2) binds cyclins A and B1–B3; CDK2 binds cyclins A, D1–D3 and E; CDK4, CDK5 and CDK6 all bind cyclins D1–D3, and CDK7 binds cyclin H [31].

CDKs are regulated by endogenous proteins known as CDK inhibitors (CDKIs). Insights into the interactions of CDKIs with CDKs, provided by recent X-ray crystallographic studies, have been reviewed recently [32]. The inappropriate expression and/or mutations of cyclins and CDKs, and the common cancers in which these occur, as well as drug discovery efforts targeting them for cancer therapy, have been reviewed recently [33**]. Oncogenic amplification and overexpression of CDKs have been reported in cancers such as gliomas and soft tissue sarcomas. CDKI discovery has been one of the intense areas of novel anticancer drug discovery [31]. The most prominent small-molecule CDKIs are flavopiridol (6), olumoucine (7), and its analog roscovitine (8), the staurosporine derivative UCN-01 (9) and butyrolactone 1 (10) [31]. Flavopiridol appears to be the most widely evaluated among these. It is a flavone derivative that inhibits CDK2 and CDK4; it causes cell cycle arrest in G1 independent of functional p53 or Rb, and cell cycle arrest in G2, which is attributed to the alteration of the phosphorylation state of CDK1, and/or inhibition of the kinase activity of cyclin B–CDK1 [34]. p53 is a tumor suppressor gene involved in cell cycle arrest at G1 and apoptosis. The p53 gene product is a nuclear phosphoprotein transcription factor which causes cells to arrest at the G1 checkpoint, or to die by apoptosis in response to DNA damage. The retinoblastoma gene Rb is a tumor suppressor gene that contributes in controlling the entry of cells into the S phase by binding, in the hypophosphorylated state to the E2F transcription factor family. Phosphorylation of Rb gene product by cyclin-cyclin-dependent kinase complexes releases E2F from the Rb complex, allowing the transcription of genes required for entry into the S-phase of the cell division cycle.

New powerful technologies in drug discovery and design are being applied to develop more specific and potent purine inhibitors (related to the CDK inhibitor olumoucine, 7 [Figure 2]) of CDKs, as reported recently [4**]. This report describes the use of combinatorial chemistry to explore the effects of a diverse array of substituents at the 2-, 6- and 9-positions of the purine ring, and high-throughput screening in 24 purified protein kinase systems. This led to the discovery of highly specific purine inhibitors of human Cdc2 (i.e. CDK1–cyclin B, CDK2–cyclin A,

CDK2–cyclin E and CDK5–p35 complexes, as well as yeast [*Saccharomyces cerevisiae*] Cdc28p). The most potent of these inhibitors was purvalanol B (**11**) with an IC₅₀ value (i.e. concentration effecting 50% inhibition) of 6 nM, a 1000-fold more potent than olomoucine against CDK2–cyclin A complex. The binding interactions of these inhibitors at the ATP-binding site of the CDK complexes were also characterized by X-ray crystallography, and the cellular effects of the compounds in mammalian cells were characterized using high density oligonucleotide probe arrays.

Apoptosis-related targets and inhibitors

The p53 tumor suppressor gene's involvement in cell cycle arrest and apoptosis have been investigated extensively. The p53 gene product is a nuclear transcription factor that functions primarily to cause cell cycle arrest or apoptosis in response to DNA damage [35]. One mechanism by which p53 induces apoptosis is the transcriptional induction of the *Bax* gene product, which competes with the anti-apoptotic *Bcl-2* gene product [36••], thereby disrupting its anti-apoptotic function. Mutations in p53, which occur in more than 50% of human cancers, cause it to lose its tumor suppressor function. Alternatively the function of wild type p53 can be abrogated by the *Mdm2* gene product, which is transcriptionally induced by p53 in a negative feedback control loop [37••]. *Mdm2* has been shown to be an oncogene in its own right independent of its inhibition of p53 [37••].

The Bcl-2 oncoprotein and several of its family members, such as Bcl-X_L, Bcl-W, and Mcl-1, act as anti-apoptotic factors through extensive interaction with multiple apoptosis-related proteins, of which some are also Bcl-2 family proteins such as Bax, Bak, Bcl-X_S, Bad and Bid [36••]. Bcl-2 also cooperates with Myc to cause oncogenic transformation, and is also implicated in anticancer drug resistance by inhibiting apoptosis [38••]. In addition to more than 80% of B-cell lymphomas, Bcl-2 overexpression has been observed in 90% of colorectal adenocarcinomas, 30–60% of prostate cancers, 70% of breast carcinomas, 80% of undifferentiated nasopharyngeal cancers, 70% of chronic lymphocytic leukemias, as well as other cancers including small-cell lung and non-small-cell lung cancers, neuroblastomas, renal cancers and melanomas [38••,39••]. There are several ways to target Bcl-2 for enhancement of apoptosis and cancer therapy, including direct methods such as downregulation of its expression, and the use of competitive ligands to block its negative interactions with pro-apoptotic proteins, or positive interactions with cell proliferation-promoting proteins such as Raf-1 [36••], or by indirect methods using compounds such as somatostatin, bromocriptine, melatonin, vitamins A or B, or retinoic acid [38••]. To date, however, no potent direct inhibitors of Bcl-2 interactions have been reported. One concern to keep in mind when attempting to use Bcl-2 inhibitors in cancer therapy may be their adverse effects in patients with ischemic cardiac disease, where the anti-apoptotic effects of Bcl-2 are beneficial [39••].

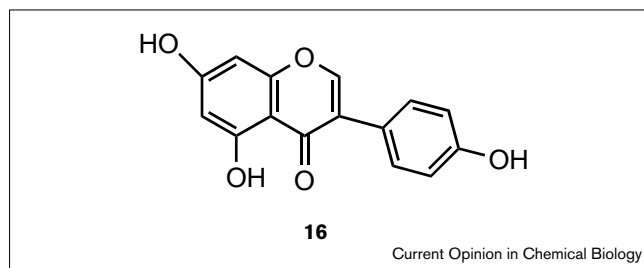
Another important oncogenic molecular target in the p53 pathway is the product of the *Mdm2* gene. MDM2 is a zinc finger protein that is transcriptionally induced by p53 in a negative feedback control loop to regulate p53 [37••]. The regulation of p53 by MDM2 is thought to be by interference with transcriptional activity and by nuclear to cytoplasmic shuttling of p53 by MDM2, leading to p53 degradation (see [37••]). Not only does MDM2 repress p53, but it also inactivates the tumor suppressor *Rb* gene product, and stimulates the E2F1/DP-1 transcription factors to promote G1 to S phase transition. The *Ink4a* tumor suppressor gene product, p19^{Arf}, has been shown recently to interact with MDM2 and neutralize its inhibition of p53 [40••]. *MDM2* overexpression has been observed in many human tumors, including sarcomas, glioblastomas, astrocytomas, leukemias, non-Hodgkin's lymphomas, squamous cell and breast carcinomas and malignant melanomas [37••]. These observations demonstrate the potential of MDM2 as a novel molecular target for cancer therapy. The proof of principle for this has already been demonstrated by antisense oligonucleotide inhibition of MDM2 translation [41••]. Peptides have been identified that competitively inhibit p53–MDM2 binding [37••]. The availability of an X-ray crystal structure of MDM2 bound to the transactivation domain of p53 [37••] may be useful for structure-based design of inhibitors of the MDM2–p53 interaction.

A new and interesting potential anti-apoptosis molecular target reported recently is *survivin* [42••]. Down-regulation of *survivin* has been shown to increase apoptosis and to inhibit the growth of transformed cells [42••]. This protein is reported to be expressed in the most common human cancers but not in normal adult tissues [42••]. Work on this target is still in the early stages, and it will be interesting to see how research on *survivin* pans out. Although no small-molecule direct inhibitors of these anti-apoptosis proteins have been discovered, this is certainly a worthwhile research area for novel anticancer drug discovery.

Angiogenesis and metastasis targets and inhibitors

Angiogenesis is critical for cancer progression and metastasis. Recent reports of the highly effective elimination of tumors in mice by the anti-angiogenic molecules angiostatin and endostatin, peptidyl compounds that antagonize the angiogenic actions of angiogenin, have resulted in an increased attention on angiogenic targets for novel cancer chemotherapy [43••,44••]. In addition to pursuing the anti-angiogenic polypeptides angiostatin and endostatin [43••], considerable anti-angiogenesis cancer drug discovery has been directed at growth factors and growth factor receptors involved in endothelial cell proliferation. The most prominent of these are VEGF and its receptor VEGFR2 (flk-1), and bFGF and its receptor. One other important angiogenic factor is angiogenin, a polypeptide that can both induce or suppress angiogenesis, but does not appear to be mitogenic towards endothelial cells. Many small-molecule angiogenesis

Figure 3



The structure of genistein.

inhibitors have been discovered [45]. They include suramin and its analogs, which are nonspecific agents that block growth factor binding to their cognate receptors, selective inhibitors affecting receptor kinase activity of VEGFR-2 (flk-1), bFGFR, or PDGF β receptor and other small molecules of diverse structural classes with yet unclear mechanisms of action such as thalidomide and fumagilins, as well as monoclonal antibodies [46••]. The X-ray crystal structure of the bFGFR tyrosine kinase domain in complex with inhibitors was solved recently [47] and may pave the way for structure-based design of novel bFGF RTK inhibitors. Some of these anti-angiogenic agents are now undergoing clinical evaluation, such as SU5416 (SUGEN, San Diego, CA, USA).

Extracellular matrix proteinases particularly matrix metalloproteinases (MMPs), urokinase (uPA) and cell adhesion molecules are also the targets of much anticancer drug discovery activity because of their involvement in tumor invasion and angiogenesis (which culminate in cancer progression and metastasis) [48••,49•]. MMPs are a large family of zinc-binding proteins that can be divided into five classes, on the basis of substrate preference as follows: type I collagenases, comprising MMP-1 and MMP-8, MMP-13; type IV collagenases, MMP-2 and MMP-9; stromelysins, MMP-3, MMP-7, MMP-10 and MMP-11; elastases, MMP-12; and membrane-type MMPs, MT-MMPs, which are regulated by endogenous inhibitors known as TIMPs (tissue inhibitors of metalloproteinases) [48••]. uPA is a serine protease formed initially as high molecular weight uPA (HMWuPA) that is cleaved into an amino terminal fragment (ATF) and low molecular weight uPA (LMWuPA). uPA and the uPA receptor have been shown to cooperate with MMPs, especially MMP-9, to cause tumor cell intravasation [50,51••]. Many small-molecule potent MMP inhibitors have been discovered with nanomolar to picomolar IC₅₀ values, as reviewed recently [52•]. Notable among these are the hydroxamate-based inhibitors batimastat (**12**, Figure 2) and its more water-soluble analog marimastat (**13**, Figure 2), which are now under advanced clinical evaluation against many human cancers [53••]. The clinical results appear promising, except for the troubling side effects of musculoskeletal pain and stiffness.

A recent review of uPA receptor antagonists in metastatic disease shows the potential utility of such antagonists in

patients with malignant melanoma, colon, nonsmall-cell lung, stomach, breast and ovarian cancers [49•]. Genistein (**16**, Figure 3), an isoflavone known to inhibit tyrosine kinases, was recently shown to inhibit both constitutive and EGF-stimulated invasion in estrogen-receptor-negative human breast cancer cells by mechanisms involving down-regulation of MMP-9 and upregulation of TIMPs 1 [54••].

Another important class of extracellular matrix targets in connection with cancer progression are the cell adhesion molecules, integrins. These are transmembrane heterodimeric proteins comprising α and β subunits that function as receptors for matrix proteins such as fibronectin, vitronectin, laminin and collagen. The potential of adhesion molecules for cancer chemotherapy has also been reviewed recently [55]. Synthetic peptides designed to antagonize adhesion interactions, especially those incorporating a RGD (Arg-Gly-Asp) motif, are being investigated with some success in preventing metastasis [55]. Interestingly, it has been shown recently that RGD peptides can induce apoptosis independently of integrin binding, by activation of caspase-3 [56••]. Caspase-3 is a key member of the cysteine aspartyl protease family that has been shown to be involved in the end stages of the programmed-cell death (apoptosis) process. Potent nonpeptidyl small-molecule integrin inhibitors have been described and shown to act as angiogenesis inhibitors as well [57••]. The evidence indicates a real potential for exploiting cell adhesion interactions in treating metastatic disease, but more research into the specific functions and interactions of cell adhesion molecules is needed for their rational targeting in cancer therapy.

Cell life-span targets and inhibitors

There is much current interest in the enzyme telomerase in connection with the prolongation of the proliferation life-span in cells. Telomerase is a ribonucleoprotein DNA polymerase that lengthens telomeres (specialized nucleotide sequences at the ends of chromosomes comprising long tandem repeats of the sequence TTAGGG). It is believed that telomere length progressively shortens with each cell division until a critical length is achieved beyond which the cells cannot divide anymore. This places a cap on how many cell division cycles can be attained for any cell capable of division, even immortalized cell lines. Telomerase activity is said to be elevated in about 85% of all cancers studied, prompting the investigation of telomerase as a potential cancer therapeutic target [58•]. In addition to porphyrins and nucleotide analogs, a series of anthraquinone telomerase inhibitors represented by **14** (Figure 2) have been reported recently, showing antiproliferative activities against human cancer cell lines with IC₅₀ values as low as 16 nM [59••]. A series of potent isothiazolone and benzisothiazolone telomerase inhibitors such as **15** (Figure 2) were also discovered recently, using a new time-resolved fluorescence-based assay [60••]. The occurrence of telomerase in renewable tissues such as the liver and lymphocytes, as well as germ-line cells, appears to pose a potential toxicity problem [58•,61]. This notwithstanding, encouraging results have been obtained at

the preclinical level to warrant the entry of telomerase inhibitors into clinical trials, although more preclinical investigations are warranted.

Conclusions

Drug discovery efforts to harness novel anticancer targets with potential to provide more selective and safe anticancer drugs have advanced significantly. There is much excitement in the cancer drug discovery field at the moment, and as the first generation of these new agents enter clinical trials it remains to be seen whether the present optimism will be confirmed. The occurrence of different novel targets in different cancers means that therapy will have to be tailored according to individual cancer target profiles. Cocktails of these agents may also be required in some cases for optimal therapy. Furthermore, these new anticancer agents are not cytotoxic but rather cytostatic. This will mean that they have to be administered over long periods of time to be effective, and therefore the agents should have minimal toxicity. In cases of aggressive cancers, these agents may have to be combined with conventional cytotoxic agents initially to reduce tumor burden. It is also being contended that the present models and the endpoints that are used to evaluate the preclinical and clinical efficacy of newer compounds may not be appropriate because these models were developed for the old cytotoxic paradigm of cancer chemotherapy. That issue has to be addressed to allow for the proper evaluation of the efficacy of the new agents. These issues notwithstanding, much progress is being made in developing novel therapeutics based on the novel targets, as exemplified by many clinical trials and the approval in the United States of Herceptin, a humanized antibody to HER-2, for the treatment of metastatic breast cancers expressing the HER-2 oncogene.

Acknowledgements

The author acknowledges Tomoko Mineno for interpreting journal articles written in Japanese.

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- of special interest
- of outstanding interest

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