CHAPTER FIFTEEN

Targeting Protein–Protein Interactions to Treat Cancer—Recent Progress and Future Directions

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Contents

1. INTRODUCTION

Protein–protein interactions (PPIs) are a vast, complex network of reactions important to the regulation and execution of most biological processes. Recently, this network was labeled the "interactome." [1] The number of binary relations between proteins may be 200,000 PPIs[2] or greater with only about 8% identified.[3] The interactome is a target-rich but relatively

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unexplored source for new drugs for untreatable diseases or for drugs superior to currently used agents. The activity of many marketed drugs with unknown or ill-defined mechanisms of action (MOA) is also likely the result of affecting PPIs.

This chapter does not address any analytical issues associated with discovering PPIs or detecting inhibition or stabilization of PPIs. The chapter does highlight PPI-specific issues relevant to drug design and recent progress addressing PPIs with a focus on oncologic applications. The evaluation of PPIs associated with the initiation, growth, and spread of cancer is very active[4,5] because of the limitations of current targets, for example, success inhibiting growth promoting protein kinases, enzymes, and G protein-coupled receptors (GPCRs), are limited because of frequent mutations of the target and the heterogeneous nature of tumors. Increased interest in PPIs among medicinal chemists has resulted from recent success addressing PPIs previously considered intractable, and the intense competition with traditional targets, for example, protein kinases and GPCRs. In addition, the therapeutic effect of inhibitors or stabilizers of PPIs that utilize multiple, relatively low-energy interactions with a protein surface to achieve activity is potentially less sensitive to escape of the target protein through mutation. However, to succeed the chemist and team addressing a specific PPI must fully understand the associated biology and protein biochemistry as well as expanding their chemical creativity.

2. PPIs IMPORTANT TO THE GROWTH AND SPREAD OF CANCER

Cancer is a multistep process of cellular transformations leading to unregulated cell growth. The acquired capabilities of cancer cells[6] are listed in Table 15.1 along with selected examples of PPIs associated with each capability.

These selected but broadly pertinent examples illustrate how significant PPI targets are to cancer.

3. CHALLENGES IN TARGETING PPIs

Most PPI inhibitors fit structurally in chemical space not well served by traditional lead generation approaches and compound libraries.[21] In addition, the need to expand the chemical space explored for PPI modulators is amplified because of the greatly expanded population of targets. The surfaces associated with PPIs are featureless and large (contact surface generally

Table 15.1 Hallmarks of cancer and related protein–protein targets

Acquired capability	Example	Example PPI target
Increased proliferation	Overexpression growth-factor receptors, for example, Her2/neu	c-Myc/Max,[7] ESX/Sur-2[8]
Decreased/inactivation of tumor suppressors	Inactivation retinoblastoma protein, inactivation p53	β-catenin/T-cell factor (TCF),[9] RB/Raf-1,[10] MDM2/p53[11]
Evading immune surveillance	Desensitizing T-cells	PD-L1/PD-1[12]
Increased angiogenesis	Induction vascular endothelial growth factor	E-protein/inhibitor of differentiation (Id)[13]
Activating tissue invasion and metastasis	Alteration cell adhesion and motility	Rac/Tiam1,[14] E-protein/Id[15]
Enabling replicative immortality	Activation of telomerase	TPP1/telomerase[16]
Resisting programmed cell death (apoptosis)	Overexpression of anti-apoptotic proteins	XIAP/caspase,[17] Bcl2[18]
Reprogramming of energy metabolism	Increased uptake and conversion of glucose to lactate by cancer cells	PPARγ and PKM2 and/or PGK1[19]
Recruitment of supportive healthy cells, for example, innate immune cells	Infiltration of pro-growth secreting neutrophil/macrophages into tumors	CXCR2/IQGAP1[20]

750–1500 A^2). The energetics of binding are predominantly hydrophobic best suited for orthosteric interaction with large lipophilic molecules which is not the situation with traditional drug targets. Less than 1% of the roughly 30,000 unique protein sequences that comprise the human proteome have been successfully manipulated to date for therapeutic use[22]; that is, all currently approved small-molecule drugs interact with just over 200 protein targets and ~50% of these fall into just four protein classes: GPCRs, nuclear receptors, voltage-gated, and ligand-gated ion channels.[23] The sites for these targets comprise deep clefts with clearly defined binding sites containing the critical amino acids required for ligand interaction. The molecules that interact with these targets emerge from a relatively small number of molecular scaffolds that, unsurprisingly, are also the basis for most current chemical libraries. Furthermore, active-site clefts tend to shield binding sites from

water that can otherwise hinder ligand interactions. This shielding is less possible on the typical surface involved in a PPI.

The targeting approach for classical binding sites will likely not be effective with most PPI, but it can be used to target "hot spots" present on some proteins. These shallow binding cavities provide small subset of residues that contribute most of free energy of binding in the protein partner/small-molecule interaction. The classical approach and associated chemical libraries are expected to find success addressing allosteric binding sites which if occupied will disrupt PPIs if the site resembles a classic site. However, libraries containing very different chemicals than those used to address "classical" targets will probably be required to be routinely successful with PPIs. In particular, libraries containing larger molecular weight (MW) molecules are more suitable as PPI inhibitors because of the possibility of multiple interaction sites over a relatively large surface area. Fortunately, the identification of tractable PPIs and which type of molecule to use to address the PPI will be greatly aided by the new tools to explore protein surfaces emerging from structural biology,[24] computational methodologies[25] and molecular dynamics simulations efforts.[26]

4. METHODS FOR DISCOVERING SMALL-MOLECULE INHIBITORS OF PPIs

What makes PPIs such a challenge for small-molecule intervention is the diverse array of observed molecular moieties at the protein–protein interfaces. Fortunately, a PPI often involves only a few key residues that contribute the majority of the binding affinity to the interaction although the interaction itself takes place over a large surface area. These small subsets of protein surface residues that contribute most of the free energy of binding associated with PPI serve as the starting point for the rational design of orthostatic PPI inhibitors. The approaches typically employed to identify PPI modulators are:

- *Traditional HTS.* Conventional screening has been generally unsuccessful at routinely identifying PPI inhibitors, presumably because conventional libraries are optimized for molecules that bind to enzyme-like or GPCR-like cavities. Nonetheless, combinatorial chemistry and HTS have produced useful inhibitors of PPIs and additional advances with this approach are likely.[27] Potency expectations for "hits" also must be recalibrated: low μM instead of nM or pM to reflect lower binding energetics. Traditional HTS remains an attractive approach for discovering allosteric inhibitors that affect a remote binding site that disrupts the

principal PPI interface by promoting a conformational change that modifies the manner in which one of the proteins subsequently behaves.

- *Virtual screening*. *In silico* screening has been employed to identify inhibitors of PPIs.[28,29] For instance, this application to the (H/M)DM2/p53 cancer target led to a large and diverse set of inhibitors whose validity was confirmed by obtaining co-crystal structure of the inhibitors and the protein.[30]
- *Fragment screening*. ABT-737, an inhibitor of Bcl-2 PPIs that induces regression of solid tumors and was at one time in Phase II clinical trials, was identified by screening a collection of traditional organic molecules for binding to the protein using NMR followed by chemically linking together the best binders or binding regions of the best binders.[31] Recently, inhibitors of the chemokine CXCL12 PPI with the CXCR4 GPCR, a high-priority target because of its involvement in metastatic cancers, were discovered using fragment-based optimization.[32]
- *Hot spot-based design*. Computational docking,[33] a form of virtual screening, and visual inspection are often used to discover and explore sites for PPI inhibition. Systematic mutations of amino acids in the protein pairs are the most compelling and thorough method for identifying hot spots.[34]

Various chemical scaffolds have been suggested as a more systematic approach to affect PPI inhibition than using *ad hoc* organic chemicals.[35]

- *Constrained secondary structures*. Stable peptides with defined secondary structure are theoretically ideal inhibitors of PPIs. However, those with less than around 15 amino acid residues rarely adopt a defined conformation in isolation and so recent research has focused upon generating peptides with constrained configurations to disrupt PPIs. A recent advance has been synthesis of peptides that are covalently constrained using hydrocarbon "stapling." [36]
- *β-Turns*. β-Turns play an important role in inhibiting or stabilizing PPIs. Thus, there is significant interest in identifying stable β-turns scaffolds useful for inhibiting PPIs.
- *Constrained α-helices and β-sheets*. To satisfy hydrogen binding requirements, α-helices and β-sheets form recognition elements in many naturally occurring PPIs. Thus, small-molecule mimetics or constrained peptides that satisfy this hydrogen binding requirement for either protein of the interaction protein pair can disrupt the PPI. Stabilization of α-helical structure in a peptide chain has been achieved using covalent linkages and noncovalent interactions residues, for example,

metal–ligand interactions, salt bridges, π–π stacking and cation–π interactions.

- *Foldamers*. Foldamers, discrete artificial oligomers that adopt a secondary structure stabilized by noncovalent interactions, have been proposed as scaffolds suitable for near universal use in designing PPI inhibitors.[37] Foldamers mimic the ability of proteins to fold into well-defined conformations, such as helices and β-sheets.
- *Surface mimetics*. Surface mimetics rely upon multisite presentation of recognition domains capable of binding "hot spots" on a protein surface over a sufficiently large surface area to be able to bind and block interaction with the partner protein.[38]

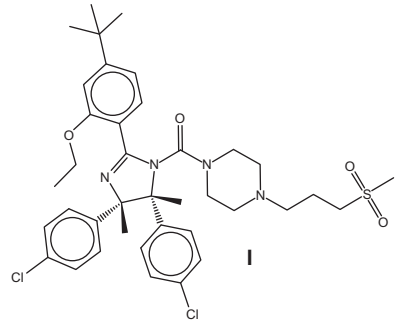
5. EXAMPLES OF TARGETING PPIs IMPORTANT TO ONCOLOGY

5.1. Small-molecule inhibitors

Several examples of oncology-relevant, clinical stage applications of small molecules to inhibit PPIs are provided below. The examples are representative and not an exhaustive list.

5.1.1 RG-7112

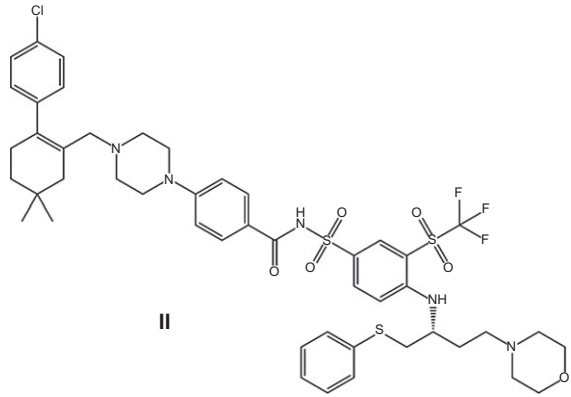
p53—a potent tumor suppressor and "guardian of the genome" with central roles in the regulation of the cell cycle, apoptosis, DNA repair and senescence[39]—is one of the most frequently mutated proteins in human tumors.[40] Inhibition of p53 by the oncoprotein (H/M)DM2 regulates the basal level and activity of p53 in cells.[41] More specifically, p53 binds to a promoter of the (H/M)DM2 gene and transcriptionally induces expression of (H/M)DM2 which inhibits the transactivation activity of p53 resulting in export of p53 from the nucleus with subsequent proteasomal degradation. Several inhibitors of the p53/(H/M)DM2 PPI have advanced into clinical trials, for example, the "nutlins." [42] The current lead clinical candidate is RG-7112/RO5045337 (**I**),[43] an orally active agent being evaluated in both solid and hematologic malignancies.[44] These inhibitors all require active p53, a situation not always present in tumors.



I

5.1.2 Navitoclax/ABT-263

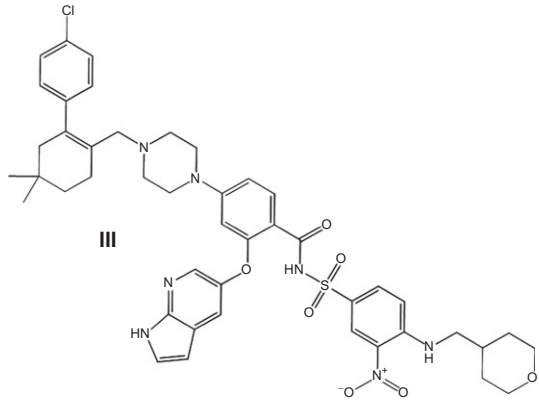
Cancer cells overexpress antiapoptotic proteins like Bcl-2[45] to promote tumor maintenance, progression, and chemoresistance. Agents designed to target the binding grooves of antiapoptotic Bcl-2 proteins are predicted to induce apoptosis in cancer cells by antagonizing their protective effect that cooperate through PPIs to mediate the intrinsic apoptotic pathway.[46–49] Navitoclax (**II**) binds to the BH3-binding groove of one member of the Bcl-2 family, Bcl-2-L1. Navitoclax is a potent inhibitor of various Bcl molecules by disrupting the PPI between proapoptotic Bcl-2 proteins that block apoptosis-enabling cytochrome *c* release. Navitoclax demonstrated clinical activity as a single agent or used in combination with radiation or other chemotherapeutics. This agent is currently in Phase II clinical trial for the treatments of chronic lymphocytic leukemia (CLL).[50] The clinical development of ABT-737, the precursor to Navitoclax, was dropped because of poor oral bioavailability.[51]



II

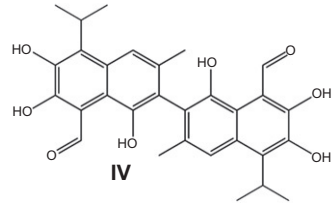
5.1.3 GDC-0199/ABT-199[52]

Bcl-2 is highly expressed in various lymphomas. The first-generation Bcl-2 inhibitor Navitoclax showed activity in lymphoma, but co-inhibition of Bcl-xL by Navitoclax resulted in dose-limiting thrombocytopenia that hindered the use of drug in lymphomas. ABT-199 (**III**) is an orally bioavailable, second-generation BH3 mimetic that inhibits Bcl-2, but has 500-fold less activity against Bcl-xL. ABT-199 demonstrated antitumor activity against a variety of human cell lines and xenograft models that include B-cell non-Hodgkin lymphoma and myeloid cell leukemia. Enrollment of CLL patients in a clinical trial with ABT-199 was recently suspended after the death of a patient due to tumor lysis syndrome but dosing of already enrolled patients continues.[53]



5.1.4 R-(−)-Gossypol/AT-101

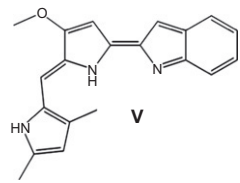
The R-(−)-enantiomer of gossypol (**IV**) mimics the BH3 domains of various BcL-2 molecules including Mcl-1 (myeloid cell leukemia 1 protein), a dominant member of the BcL-2 antiapoptotic protein family.[54,55] AT-101 disrupts heterodimerization of Bcl-2 with proapoptotic family members. The molecule also induces apoptosis *in vitro* through the activation of caspase-9 and is cytotoxic to multiple myeloma and drug-resistant cell lines. AT-101 delays the onset of androgen-independent growth of VCaP prostate cancer xenografts *in vivo*. Akt and inhibitor of apoptosis (XIAP) are downregulated in the presence of AT-101. Treatment with bicalutamide and AT-101 increased apoptosis by reducing the expression of prosurvival proteins. AT-101 is being evaluated in Phase 2 studies in various cancers.[56]



IV

5.1.5 Obatoclax/GX15-070

Obatoclax (**V**), another BH3 mimetic, is an inhibitor of Bcl-2 with high nM potency *in vitro*.[57] For Mcl-1, Obatoclax displaces BH3 domains by activation of a pocket of the BcL-2 family member.[58] Obatoclax also binds to a broad spectrum of Bcl-2 family members, including Bcl-2 and Bcl-xL. Inhibition of Obatoclax leads to release of apoptosis-inducing cytochrome *c*. Obatoclax enhances the antimyeloma activity of melphalan, dexamethasone or bortezomib. Obatoclax also potentiates TRAIL-mediated apoptosis in cancer cells as well as exhibiting antitumor activity in severe combined immunodeficiency (SCID) mice bearing various human cancer cell lines. The current, major clinical effort with Obatoclax is an evaluation in combination with bortezomib in patients with relapsed or refractory non-Hodgkin lymphoma.[59]

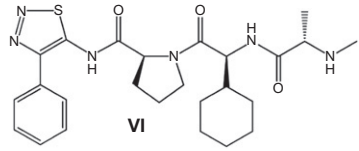


V

5.1.6 GDC-0152

Inhibitors of apoptosis proteins (IAPs) are endogenous caspase inhibitors that bind and inhibit caspases 3, 7, and 9. Eight members of the IAP family are known to date of which the X-linked inhibitor of apoptosis protein (XIAP) is the best characterized. Endogenous inhibition of the IAP family can occur via DIABLO (also known as Smac) protein released from the mitochondria in response to apoptotic stimuli, which directly interacts with XIAP.[47] The finding that the levels of IAP family members were elevated in several tumors suggested the possibility to treat cancer by inactivating the IAP family to induce apoptosis. A series of compounds mimicking the N-terminus of DIABLO (Smac) were designed and synthesized as antagonists of XIAP and related family members. GDC-0152 (**VI**) had the best profile of these

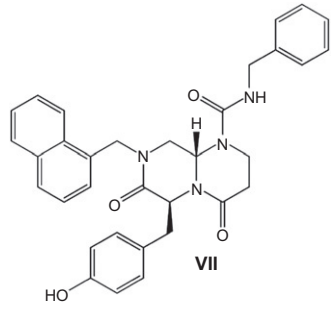
compounds.[60] This compound promoted degradation of relevant IAP molecules, induced activation of caspase 3 and 7, decreased viability of breast cancer cells without affecting normal mammary epithelial cells and inhibited tumor growth in a human breast cancer xenograft model. GDC-0152, dosed orally, recently completed a Phase 1 safety/pharmacokinetic evaluation in cancer patients.[61]



VI

5.1.7 PRI-724/ICG-001[62]

The Wnt/β-catenin signaling pathway regulates cell morphology, motility, and proliferation. Aberrant regulation of this pathway leads to neoplastic proliferation and prevention of apoptosis in a number of human cancers. β-catenin/CBP binds to the Wnt-responsive element and activates transcription of a wide range of target genes associated with Wnt/β-catenin signaling. PRI-724 (**VII**) is a potent, antiproliferative small molecule that specifically inhibits the canonical Wnt signaling pathway in cancer stem cells while also providing antineoplastic activity. By blocking the interaction of β-catenin and CBP, its coactivator, PRI-724 prevents gene expression of many proteins necessary for growth. PRI-724 substantially inhibits tumor growth in animal studies. PRI-724 is currently in early clinical testing for treating solid tumors and myeloid malignancies.[63]

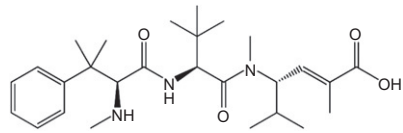


VII

5.1.8 Talbtobulin

During cell division, α/β-tubulin polymerizes into dynamic structures called microtubules. Inhibitors of tubulin either target polymerization (vinca

alkaloids and colchicine) or depolymerization (taxanes and epothilones). These two functional classes bind to different regions of the α/β-tubulin heterodimer and allosterically regulate tubulin oligomerization. Although small-molecule inhibitors of tubulin have been in clinical use since 1965, the identification of new inhibitors is still an active area of research, especially because current therapeutics are susceptible to a common mechanism of induced drug resistance (efflux by P-glycoprotein). One novel tubulin polymerization inhibitor undergoing clinical evaluation is talbtobulin (**VIII**).[64] Many classes of tubulin inhibitors are known; for example, colchicine buried and at the heterodimer interface and taxanes binding to a shallow groove found on the β-subunit. The presence of these multiple allosteric binding sites makes tubulin particularly amenable to inhibition by small molecules.



VIII

5.2. Small-molecule stabilizers

Although the vast majority of PPI modulators are inhibitors, an increased lifespan for a PPI associated with an anticancer effect is desirable. Some PPI-stabilizing natural products have already found application as important drugs. One of the most intensely studied modulators of microtubules is paclitaxel. Paclitaxel stabilizes the polymerized microtubule structures by allosteric binding with high affinity to a hydrophobic pocket of polymerized tubulin located on the β-subunit thereby strengthening the lateral contacts of neighboring β-subunits. One common mechanism for stabilization observed for direct PPI stabilizers[65] is binding of a small molecule to one of the proteins, thereby creating or modifying the interaction surface for the second protein. This stabilizing effect can be so strong that two proteins can be induced to dimerize that do not bind to each other in the absence of these molecules. This extreme case is observed for the FKBP binding molecules, FK506 and rapamycin. For cancer, a small molecule that stabilizes the interaction between 14-3-3, a family of conserved regulatory molecules and p53 would be therapeutically desirable because the 14-3-3 proteins bind to the regulatory C-terminal domain of p53 and prevent MDM2-dependent degradation, thereby enhancing p53 stability.[66] The feasibility of achieving

this result was recently demonstrated with the discovery by systematic screening of a small molecule that inhibits the PPI of p53 with PMA2, a plant H^+-ATPase.[67]

5.3. Other

Phosphorylation-dependent[68] signaling events govern large multiprotein complexes to regulate cell growth, progression of the cell cycle, differentiation, motility, gene expression, and apoptosis. Both phosphorylation and dephosphorylation of protein substrates have critical functions. In many cases, phosphorylated residues on the substrates create binding sites for phospho-protein binding domains that link upstream kinases and downstream effectors to form protein complexes that regulate the activity, binding partners, and localization of the specific protein.

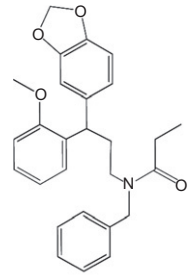
EGFR–HER2 and HER2–HER3 PPIs are important in cancer for regulation of the human epidermal growth factor receptor. Recently, a small molecule that modulates HER2-mediated signaling was discovered that inhibits the phosphorylation of HER2 kinase domain resulting in inhibition of activation-dependent heterodimerization.[69] Molecular modeling was used to design the inhibitor.

6. TWO RECENT SUCCESSES

6.1. Inhibitor of differentiation 1

The Id genes/proteins are critically important during embryonic development but are only active in adults when cancer is present. The Ids function as negative regulators of transcription (see Fig. 15.1), and are expressed in virtually all cancers. Overexpression of Id genes in tumors is associated with an aggressive phenotype and poor clinical outcome.[70–74] This is not surprising since the effects of Id proteins are linked to almost all signaling elements critical to the initiation and progression of cancer.[75–79] Recently, AGX51 (**IX**), a small-molecule anti-Id agent, was disclosed.[80,81] AGX51 was discovered using X-ray crystallography, gel shift assays, Matrigel evaluations, and xenograft studies (Fig. 15.2). As expected, AGX51 completely blocked tumor-associated angiogenesis in nude mice implanted with human breast cancer cells and also treated briefly with a taxane, blocked new blood vessel formation into implanted vascular endothelial growth factor (VEGF)/fibroblast growth factor (FGF)-treated Matrigel plugs, decreased metastases of injected Lewis

Lung cancer cells, etc. AGX51 also restored levels in cancer cells of mediators of cell cycle regulators, such as p16, p21 and p27.



IX

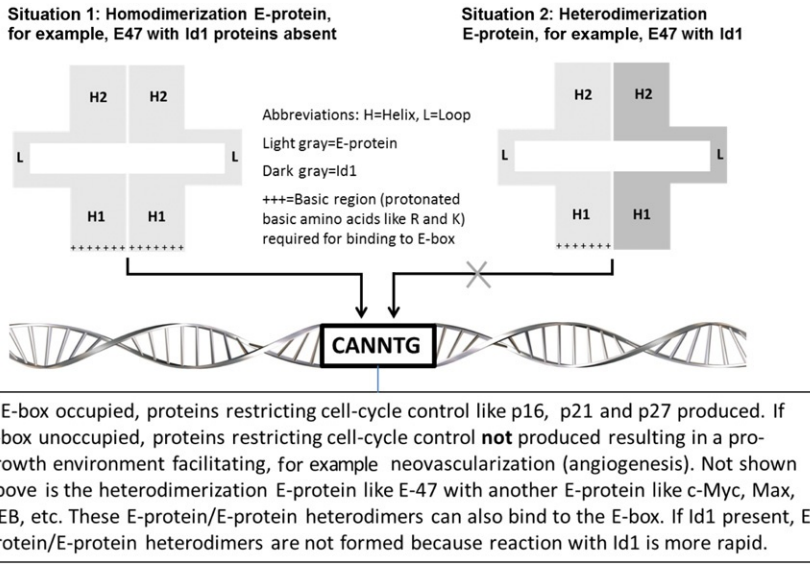


Figure 15.1 Scheme for interaction of E-proteins with E-box on DNA.

6.2. BRD4–histone

In late 2010, Bradner and colleagues reported that JQ1 (**X**) could block the interaction between BRD4 and histones, providing proof-of-principle evidence that PPI inhibition could provide epigenetic control.[82,83] JQ1 was discovered by HTS. A BRD4–NUT translocation has been linked to an aggressive form of human squamous carcinoma, and the BRD4–histone

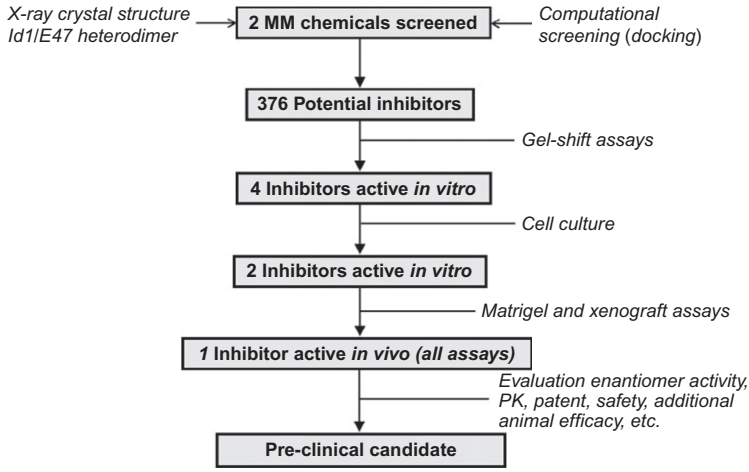
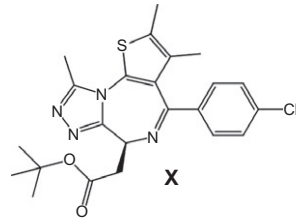


Figure 15.2 Process used to discover small-molecule inhibitors of Id1–E47 interaction.

team was able to generate *in vivo* data demonstrating the potential therapeutic applications of their compound in this setting. Further studies unveiled its potential in other indications, including MYC-associated cancers.[84]



X

7. FUTURE DEVELOPMENTS

Industry and government scientists have produced improved technologies in several areas important to drug discovery, such as automation, stereo-selective organic syntheses, sophisticated screening assays, analysis of genetic targets, computational strategies and structural biology. However, these advances have not appreciably improved the success of drug discovery in targeting oncogenic PPIs. Most disappointing has been progress with promising oncology targets like Myc.[85] Most of the tractable targets to date, for example, the inhibitors of the p53-(H/M)DM2 PPI or BCL-2 and related molecules have protein interfaces in which a small, linear region

of one protein binds into a hydrophobic cleft of the other, not too different than traditional disruption of receptor-substrate fit.

Areas for improvement relevant to discovering new PPI modulators include:

- *PPI-friendly libraries*. The development of chemical libraries is costly and time consuming. However, current chemical libraries are optimized for interaction with classic targets and as a result are consistent with the Lipinski guidelines.[86] However, the interface for PPI is much broader with relatively weak and scattered binding sites (few, or no, highly energetic binding sites). Consistent with this understanding, successful PPI inhibitors possess chemical properties shifted toward higher molecular weight, increased hydrophobicity, and a higher unsaturation index and ring complexity than common drugs.[87] The design of inhibitors that obey the Lipinski guidelines and possess the optimum features for inhibition of PPIs therefore appears to require opposing criteria. Poor oral bioavailability and cell penetration are risks with higher MW agents.
- *PPI-friendly scaffolds*. Because of the need for providing multiple, often widely spaced interactions, scaffolds that can easily be manipulated to move interacting chemical moieties on the scaffold to provide favorable binding are very desirable. The design and production of these flexible scaffolds will likely require new synthetic chemistry approaches and techniques. A similar approach, designated "credit card" libraries, has been proposed.[88] The "credit cards" are defined as flat and rigid small molecules further functionalized to install elements of chemical diversity needed to disrupt a specific PPI.
- *PPI-friendly computational/cheminformatic tools*. Many tools are now available to facilitate the analysis of protein–protein interfaces for small-molecule drug design. Surprisingly, many successful PPI inhibitors have been designed from just visual inspection of the surfaces of the interacting protein without associated computational docking studies. Protein structure determinations have become routine and widespread as have automated docking routines using commercial chemical libraries. Many programs for viewing protein surfaces are now available. However, user-friendly software for seamless integration of all these tools for PPI evaluations is still lacking. In addition, the commercial libraries used for computational docking evaluations have the same previously discussed structural limitations. Besides help in suggesting inhibitors of PPIs, the integrated computational/cheminformatic package could also suggest PPIs whose chemical characteristics are too daunting to attempt

manipulation. Such a powerful tool could also be used to rationally design PPI-friendly focused libraries.

- *Covalently bound modulators*. Because of the weak energetics associated with binding to a protein surface associated with a PPI, covalent binding of the inhibitor with the protein should be considered.[89]
- *Mutated proteins and PPIs*. A critical gap in understanding PPIs associated with cancer is the role of protein mutation, which is so important with protein kinases.[90] This possibility is relatively unexplored. However, if new findings suggest mutations play a role in oncologic PPIs, the overall challenge of manipulation PPIs for anticancer activity will become even more complex.

8. CONCLUSIONS

Protein complexes in the interactome provide practical drug targets for oncology drug discovery. Research on a handful of PPIs important to the growth and spread of cancer has produced agents with sufficient potency and cellular activity to become clinical candidates. The list of "tractable protein–protein targets" is growing although still small compared to the list of "considered intractable protein–protein targets." With numerous possible cancer drug targets in the interactome, thoughtful selection of PPI targets that are amenable to inhibition or stabilization by small molecules will become a critical task for researchers. However, the true test of the evolving emphasis on PPI in cancer will be the availability of PPI-based drugs to treat cancer patients.

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