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Targeting protein–protein interactions as an anticancer strategy

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The emergence and convergence of cancer genomics, targeted therapies, and network oncology have significantly expanded the landscape of protein–protein interaction (PPI) networks in cancer for therapeutic discovery. Extensive biological and clinical investigations have led to the identification of protein interaction hubs and nodes that are critical for the acquisition and maintenance of characteristics of cancer essential for cell transformation. Such cancer-enabling PPIs have become promising therapeutic targets. With technological advances in PPI modulator discovery and validation of PPI-targeting agents in clinical settings, targeting of PPI interfaces as an anticancer strategy has become a reality. Future research directed at genomics-based PPI target discovery, PPI interface characterization, PPI-focused chemical library design, and patient-genomic subpopulation-driven clinical studies is expected to accelerate the development of the next generation of PPI-based anticancer agents for personalized precision medicine. Here we briefly review prominent PPIs that mediate cancer-acquired properties, highlight recognized challenges and promising clinical results in targeting PPIs, and outline emerging opportunities.

Rising interest in targeting PPIs

PPI interfaces represent a highly promising, although challenging, class of potential targets for therapeutic development [1]. In cancer, PPIs form signaling nodes and hubs that transmit pathophysiological cues along molecular networks to achieve an integrated biological output, thereby promoting tumorigenesis, tumor progression, invasion, and/or metastasis. Thus, pathway perturbation, through disruption of PPIs critical for cancer, offers a novel and effective strategy for curtailing the transmission of oncogenic signals. As our understanding of cancer biology has significantly increased in recent years, interest in targeting of PPIs as anticancer strategies has increased as well (Figure 1).

PPI interfaces constitute basic units in oncogenic signaling networks

A variety of environmental, genetic, and epigenetic factors induce the reprogramming of cancer-initiating cells and the acquisition of physical and molecular features that promote tumorigenesis and provide resistance to therapeutics. These characteristics, including sustained proliferative signaling and evasion of growth suppressors, permit the development and progression of cancer and have been recognized as distinctive hallmarks of cancer (Figure 2) [2]. These hallmarks provide a molecular framework for our understanding of cancer, linking molecular signaling events to pathological outcomes. The oncogenic potential of cells is determined by a combination of genetic and epigenetic alterations through the operation of well-orchestrated signaling networks. Importantly, PPIs represent the basic units within such vital networks.

On oncogenic stimulation, PPIs play essential roles in linking networks that relay oncogenic signals, allow the acquisition of hallmark features of cancer, and serve diverse roles in driving and maintaining the growth of cancer cells (Figure 2). From the engagement of receptors with dysregulated growth factors to dimerization of receptor tyrosine kinases triggered by gene amplification or mutations, PPIs initiate a cascade of reactions to promote uncontrolled cell proliferation [3]. Activated Ras, due to perturbations such as epidermal growth factor receptor (EGFR) activation, neurofibromin 1 (NF1) deletion, or intrinsic mutation, assumes a conformation that allows it to bind to multiple regulatory proteins and results in enforced proliferation and survival. Survival signaling, activated by proteins such as insulin-like growth factor 1 (IGF1) and phosphoinositide-3-kinase (PI3K) or disabled by the negative regulator PTEN, enables tumors to resist cell death through a number of different mechanisms. For example, the Akt–FOXO3a–14-3-3 complex mediates a transcription-dependent mechanism, whereas the Akt–Bad–14-3-3 interaction mediates a transcription-independent antiapoptotic mechanism [3]. In addition to providing resistance to cell death, Akt also regulates the mTOR complex to control cap-dependent translation, through the eIF4E–eIF4G PPI, of a large number of growth-promoting genes, including c-Myc. In turn, amplified c-Myc favors binding to Max over Mad and thereby drives transcription of growth-promoting genes such as cyclin D that modulate cell cycle progression [4].

For cancer progression, cells must acquire mechanisms to evade growth suppression. Several PPI complexes, including MDM2–p53 and CDK4–pRB, play key roles in neutralizing such tumor suppressive functions [2]. These tumor suppressor mechanisms are often hijacked by viral
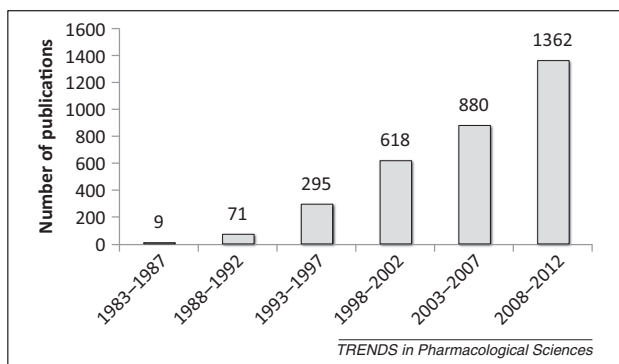
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Figure 1. Rising number of publications in the field of cancer-related protein–protein interactions. The PubMed database was searched using the following keywords: protein–protein interaction, tumor, cancer, and inflammation.

oncoproteins, such as human papillomavirus E7 protein, which binds to pRb, and E6 protein, which binds to p53, that allow the virus to induce tumors. Such PPIs offer tumor-specific targets. In addition to the examples given above, a large number of PPIs dictate signaling networks that allow the acquisition or maintenance of other hallmarks of cancer.

For instance, the VEGF–VEGFR and HIF1α–CBP PPIs mediate signals that induce angiogenesis, the catalytic activity of TERT dimers enables replicative immortality, and a variety of reprogrammed enzyme–substrate interactions, such as the onco-fusion gene-regulated PDHK1–PDHA1 PPI, play integral roles in dysregulated cellular metabolism by controlling a metabolic switch between glycolysis and oxidative phosphorylation [5]. In addition, mutated p53 and Myc also play key roles in the regulation of cancer metabolism. The IKK–NEMO–ASK1 complex integrates the proinflammatory function with stress response signaling initiated by reactive oxygen species [6]. It has recently been shown that epigenomic reprogramming is a critical part of cancer development [7], and PPIs involved in epigenomic dysregulation, such as SMARCA4 interactions, have been described [8].

As a result of oncogenic network reprogramming, some PPIs contribute to distinct features of cancer, whereas other PPIs are vital for multiple characteristics of cancer. For example, the MDM2–p53 and Myc–Max PPIs play key roles in evading growth suppression and cell death, as well as in promoting genomic instability and cancer metabolism. Thus, it is expected that interception of certain
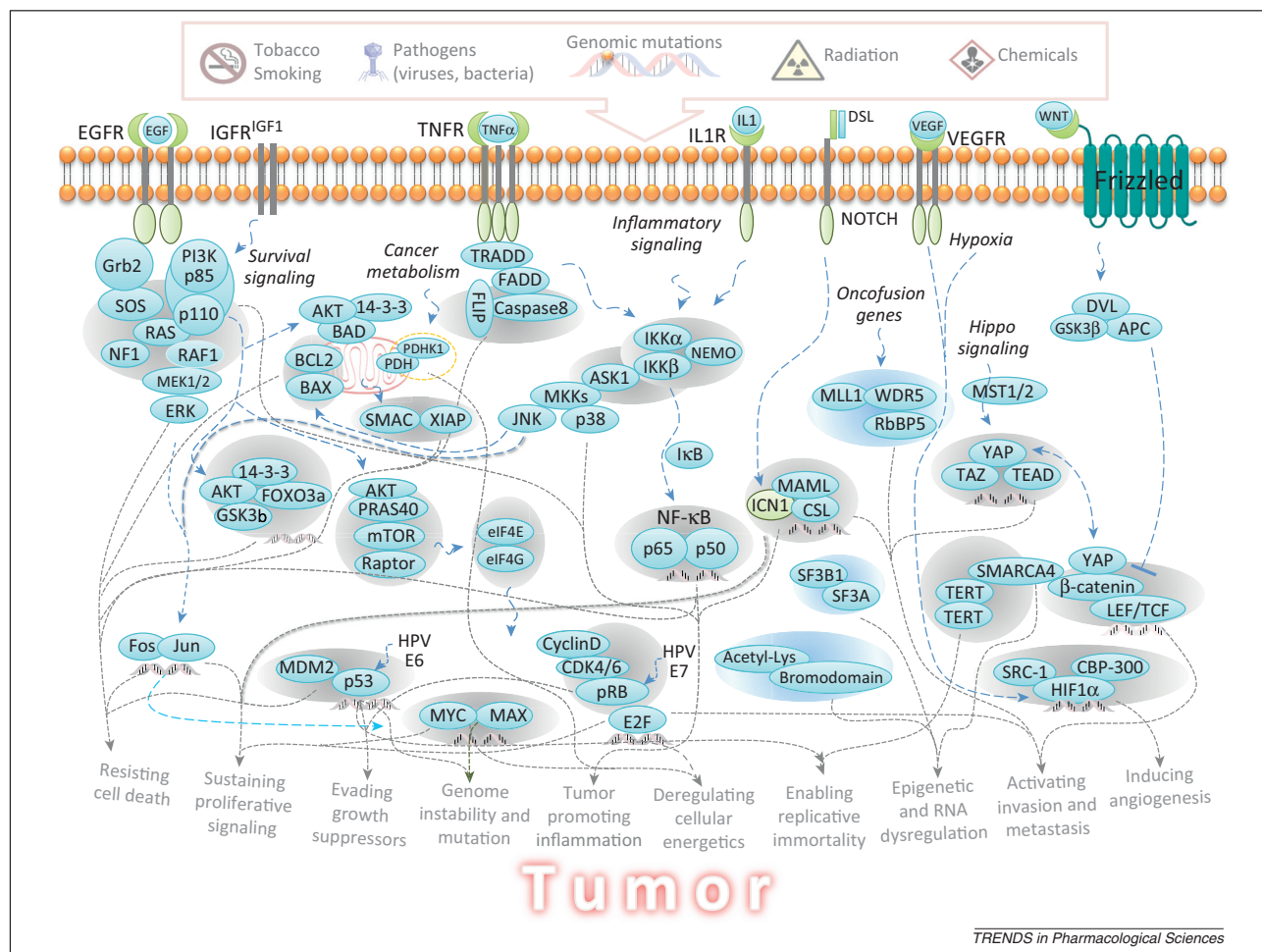

Figure 2. Representative PPIs in oncogenic signaling networks that drive the acquisition and development of hallmarks of cancer. Grey broken arrows connect PPIs to corresponding cancer hallmarks. Some PPIs contribute to multiple features of cancer. It should be noted that some PPIs may impact global processes of cell growth and their precise connections to cancer remain to be established.

critical PPIs may disable multiple mechanisms that cancer cells rely on for survival. A large number of PPIs are involved in driving tumorigenesis through the regulation of oncogenic networks, so these PPI interfaces represent fertile ground for anticancer therapeutic discovery and development.

Overcoming challenges and current strategies for targeting of PPIs

Challenges in discovering PPI modulators

A number of challenges and concerns exist regarding targeting of PPIs, some of which include: (i) large PPI interface areas, (ii) a lack of deep pockets, (iii) the presence of noncontiguous binding sites, and (iv) a general lack of natural ligands. In addition, PPI surfaces differ from small-molecule binding sites in their shape and amino acid residue composition. In contrast to the well-defined and normally hydrophilic ligand-binding cavities observed in the crystal structures of enzymes and G-protein-coupled receptors, the interface surfaces of many protein–protein complexes are typically hydrophobic and relatively flat and often lack deep grooves where a small molecule can dock. Recent studies have addressed some of these concerns, as detailed in several publications [1,9,10]. Although the PPI interface generally covers an average area of 1150–10 000 Å^2, which is larger than small-molecule binding sites of 100–600 Å^2, the presence of hot spots (small subsets of amino acid residues that contribute the most to the free binding energy) makes PPIs amenable to small-molecule perturbations [10,11]. In addition, PPIs are often mediated by post-translational modifications, such as the binding of 14-3-3 to phosphorylated Ser/Thr motifs and the interaction of bromodomain proteins to acetylated lysine, which simplifies definition of the targeting interfaces [12–14]. The typical lack of natural ligands for PPIs as starting points poses a significant challenge for structure-based drug design. However, promising examples of natural products that can act on PPIs do exist, such as rapamycin for mTOR and taxol for tubulin. There are also significant differences in the chemical space between PPI modulators (PPIMs) and conventional drug-like compounds [9,15,16]. In general, PPIMs have a higher molecular weight (> 400 Da) than that of typical drug-like compounds (200–500 Da) and they often violate the 'Rule of Five' [9]. Therefore, the application of commonly used high-throughput screening (HTS) methods for PPIMs has been limited because of the biased chemical composition towards classical target classes in current chemical libraries.

Current approaches for the discovery of PPI modulators

Structure-based design. Structural studies allow for the identification of peptide fragments and amino acid residues that are critical for PPI. This information, combined with that from functional assays, provides a basis for the rational design of PPIMs. Not surprisingly, mimicking the structure of binding peptides is one of the approaches widely used to design novel PPIMs [17]. Application of this approach led to the identification of potent inhibitors of BCL2, XIAP, NOTCH, and MDM2 [18–22]. General structure-based approaches include computational molecular modeling [23,24], peptide engineering with display technologies such as phage display [25], design of small molecules based on α-helix and β-sheet scaffolds [26,27], and synthesis of conformationally constrained 'stapled' peptides with a stabilized α-helical structure [28–31].

Small-molecule screening methods. In contrast to structure-based design, the screening approach allows the discovery of small-molecule PPIMs even if structural information is very limited or unavailable. Various screening approaches, including HTS, have been used to identify compounds that target 'hot spots' of PPI interfaces [1]. Furthermore, HTS can be used to reveal inducible pockets in PPI interfaces, as well as allosteric modulators. In many cases, the screening approach is combined with structure-based design to further enhance the physicochemical and pharmacological properties of identified PPI modulators.

The most widely used HTS techniques for PPIs include fluorescence polarization (FP) and Föster/fluorescence resonance energy transfer (FRET). FP measures the change in emitted polarization signals in solution on association of a small fluorescent molecule (such as a peptide) with a relatively large binding partner. FRET is a nonradioactive, photophysical effect in which energy absorbed by a donor fluorophore is transferred to an acceptor fluorophore. By coupling the donor and acceptor fluorophores with appropriate spectral properties to two interacting molecules, the fluorophores can be brought into close proximity (10–100 Å) and induce a FRET signal. Both FP and FRET methods are extensively used in HTS campaigns for the discovery of PPIMs [32]. Other HTS methods include ELISA, flow cytometry, surface plasmon resonance (SPR), and label-free platforms [33]. In addition to these biochemical HTS assays, intracellular PPIs can be coupled to readouts for cell-based reporter assays, which incorporate a more physiological cellular context and identify compounds that are cell-permeable. For example, the p53–MDM2 PPI has been linked to a reporter assay based on cytoplasm-nuclear redistribution [34]. Various biosensors, such as protein complementation assays, can also be used to monitor PPIs in a HTS format.

In addition to biochemical and cell-based HTS assays, fragment-based screening (FBS) is another approach commonly used for discovery of PPIMs. FBS aims to identify molecular fragments with binding activity for a target protein. Once the fragments have been identified, they, or the interactions they identify, are built into a drug-like compound [35]. The main advantage of the FBS approach is that a large chemical space can be targeted with approximately 10^3 fragments. The ligand efficiency (LE) of fragment hits is high. Moreover, FBS can be successfully utilized for many targets that were found to be challenging using traditional HTS [36]. Owing to their low molecular weight (∼200 Da) and thus limited contact area with a protein, the binding affinity of the fragments is relatively low (often in the millimolar range). Therefore, to detect a binding event, sensitive biophysical methods are required, including X-ray crystallography, nuclear magnetic resonance (NMR), and SPR [35,37,38]. Key advantages of NMR include automated sampling, high sensitivity, quantitative data on binding affinity, and the ability to obtain structural information about the binding site [39]. Examples of successful

application of NMR in FBS include discovery of XIAP [40], BCL2 [41], ZipA–FtsZ [42], and K-RAS–SOS [43] PPI inhibitors. A combination of FBS assays is often employed for a screening campaign. For instance, a combination of X-ray and NMR FB HTS led to identification of the Hsp90 inhibitor AT13387, which recently entered clinical trials [44,45]. Another assay commonly used for FBS is SPR, which detects changes in the refractive index near a sensor surface. SPR FBS has been utilized to identify novel inhibitors of Hsp90 interactions [46,47]. In contrast to methods for non-covalent binding, a 'tethering' approach is used to detect reversible covalent bonds formed between the cysteine of a target protein and fragment molecules containing a disulfide bond [48]. Tethering FBS has been employed to identify PPIMs such as IL2–IL2-αR inhibitors [1].

Two general approaches are used to perform computational screening of a 3D compound library: the ligand-based (also known as the pharmacophore-based) approach and structure-based virtual screening. A pharmacophore model represents the chemical features of a set of compounds critical for efficient protein binding [49]. These features or pharmacophore points (such as H-bond donors or acceptors, aromatic rings, and charges) have certain coordinates in a 3D space. The aim of this procedure is to identify compounds with a certain conformation and chemical composition that match the requirements of a pharmacophore model. The pharmacophore-based approach has been successfully utilized to identify novel inhibitors of the MDM2–p53 interaction [50,51], PPIs of BCL2 family proteins [52], and 14-3-3 inhibitors [53]. Conversely, the structure-based approach relies on structural information for the binding site on the target protein. For this type of screening, each compound in a chemical library has to be computationally docked to the binding site, and a binding affinity is estimated in terms of energy scoring functions. Application of structure-based virtual screening has led to the discovery of PPI inhibitors of Ubc13–Uev1 [54], MDM2–p53 [55], and TCF4–β-catenin [56].

Clinical validation of PPI targeting in cancer

Thousands of compounds have already been tested as potential inhibitors of various PPIs and the results are promising. Titrobifan, a glycoprotein IIb/IIIa inhibitor, and Maraviroc, an inhibitor of the CCR5–gp120 interaction, are currently available on the market as cardiovascular and anti-HIV drugs, respectively. These drugs demonstrate the feasibility of PPI targeting for the treatment of various diseases. In addition, several anticancer compounds have entered clinical trials, highlighting the potential of the PPI targeting approach in cancer.

Inhibitors of the MDM2–p53 interaction: a breakthrough in PPI targeting

p53 plays a critical role in cell cycle regulation, DNA repair, angiogenesis, and apoptosis [57,58]. Activation of p53 increases the expression of human protein double minute 2 (HDM2, MDM2 in mouse), which in turn directly binds to p53 and inhibits its tumor suppressive activity (Figure 2) [59,60]. Structurally, the N-terminal domain of MDM2 binds a short 15-residue α-helical peptide of p53 [61]. Three hydrophobic residues of p53 (Phe19, Trp23, and Leu26)

occupy a well-defined hydrophobic pocket of MDM2 (Figure 3). These structural features allow a strategy to target the MDM2–p53 PPI. Various approaches were used to identify drug-like inhibitors of MDM2–p53 interactions, including the design of peptidomimetics, HTS, and computational drug design. As a result, several MDM2–p53 PPI inhibitors have entered clinical trials [60,62]. For example, a series of *cis*-imidazoline analogs named Nutlins were identified by screening of compound libraries [59]. Nutlins employ the same binding mode as the p53 Phe19, Trp23, and Leu26 residues in the MDM2 binding pocket (Figure 3). Further chemical optimization of Nutlin-3 led to RG7112, the first MDM2 inhibitor to enter clinical trials in patients with advanced solid tumors in 2007 [63]. Another MDM2–p53 PPI inhibitor, RO5503781, is currently in a Phase I trial in patients with advanced malignancies (http://clinicaltrials.gov/ct2/show/NCT01462175%3Fterm%3DRO5503781%26rank%3D1). The exciting success of potent MDM2–p53 PPI inhibitors has significantly accelerated studies to target other PPIs with small chemical compounds as anticancer drugs.

Mimicking the structure of Smac peptides resulted in new XIAP antagonists in the clinic

One strategy widely used to identify lead PPIM compounds is to mimic the structure of binding peptides [26,27]. For example, the BIR3 domain of the XIAP binds to and inhibits proapoptotic caspase-9. In turn, the anti-apoptotic activity of XIAP can be neutralized by Smac, which is released from mitochondria during apoptosis (Figure 2). The XIAP–caspase-9 interaction can be disrupted by a Smac tetrapeptide (AVPI) [64,65], which provides a novel PPI target. A combination of structure-based design and targeted compound library generation led to the identification of GDC-0152, the first Smac mimetic to enter clinical trials in patients with locally advanced or metastatic malignancies [19]. GDC-0152 binds to BIR domains with low nanomolar affinity at the same binding site on IAPs as the SMAC AVPI peptide (Figure 3). A Phase I trial with another Smac mimetic, GDC-0917 (CUDC-427), has been completed in patients with advanced solid tumors and lymphomas. The structurally related LCL161 Smac mimetic has entered Phase II trials in patients with triple-negative breast cancer (Figure 3) [20,66]. Other non-peptide XIAP–Smac inhibitors include an orally available derivative, AT-406, and two bivalent Smac mimetics, TL32711 and HGS1029, all of which are currently in clinical trials for various cancers [67,68].

FBS-based discovery of mitochondrial apoptosis pathway modulators

BH3-containing proapoptotic proteins, such as Bax, bind to the hydrophobic pocket of antiapoptotic BCL2 proteins through a single α-helix (the BH3 domain). Mimicking of the BH3 domain with small-molecule compounds has shown significant therapeutic potential [18]. Several BH3 mimetics have been identified using NMR-based FBS combined with structure-based optimization. For instance, ABT-737 has high binding affinity (nanomolar range) for BCL2 and BCL-xL [69]. This compound occupies the same hydrophobic pocket on BCL-xL as a Bak-derived
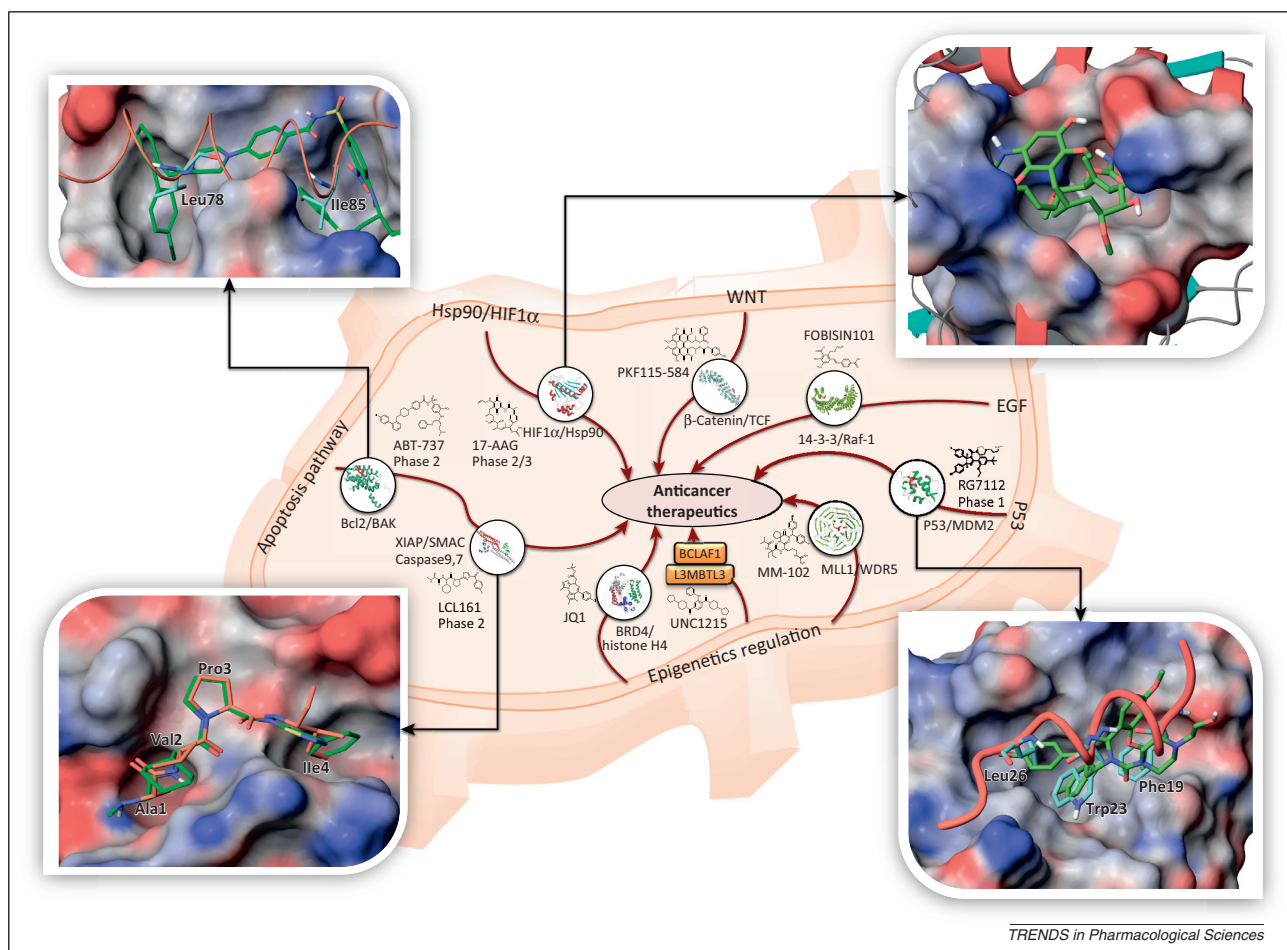
Figure 3. Examples of protein–protein interaction (PPI) inhibitors that have entered clinical trials and emerging agents. Inhibitors of MDM2–p53, BCL2, XIAP, and Hsp90 PPIs are in Phase I–III trials. Examples of promising PPI targets with recently identified novel inhibitors include MLL1–WDR5, β-catenin–TCF, BCLAF1–L3MBTL3, BRD4–histone H4, and 14-3-3 interactions. Chemical structures of representative inhibitors are shown, along with available crystal structures for protein–protein complexes. Top left: superimposition of Bak peptide (orange, carbon atoms colored cyan) and ABT-737 (carbon atoms colored green) bound at BCL-xL (PDB ID: 1BXL, 2YXJ). ABT-737 occupies the same hydrophobic pocket on the BCL-xL surface as the peptide, overlapping with Leu78 and Ile85 Bak residues critical for peptide binding. Top right: crystal structure of Hsp90 in a complex with geldanamycin (PDB ID: 1YET), the first Hsp90 inhibitor to enter clinical trials. Bottom right: superimposition of p53 peptide and Nutlin-2 (one of the first identified potent MDM2–p53 inhibitors) bound to the N-terminal domain of MDM2 (PDB ID: 1YCR, 1RV1). Phe19, Trp23, and Leu26 residues of p53 occupy the hydrophobic pocket of MDM2. The ethoxy and chlorophenyl groups of Nutlin-2 match the positions of Phe19, Trp23, and Leu26, respectively. Bottom left: superimposition of SMAC AVPI peptide (carbon atoms colored orange) and GDC-0152, the first SMAC mimetic to enter clinical trials (carbon atoms colored green) (PDB ID: 3UW5, 1G73). The molecular surfaces of Hsp90, BCL-xL, MDM2, and XIAP are colored according to their electrostatic potential (blue, positive; white, neutral; red, negative).

peptide, overlapping with the Leu78 and Ile85 of Bak, which are critical residues for peptide binding (Figure 3) [69–71]. ABT-737 was further improved to generate orally bioavailable ABT-263 (Navitoclax, Phase I) and ABT-199 (Phase I) with enhanced water solubility [72]. Another oral BH3-mimetic, Obatoclax (GS-01570), discovered by screening of natural product libraries, is currently in Phase II clinical trials in patients with small-cell lung cancer [18,73,74].

Allosteric regulation of PPIs: Hsp90 inhibitors
The Hsp90 chaperone protein regulates the activity and stability of numerous client proteins. Inhibition of Hsp90 can simultaneously shut down multiple oncogenic pathways, which has sparked interest in targeting of Hsp90 PPIs for cancer treatment [75–77]. The natural product geldanamycin (GM) inhibits Hsp90–Src complex formation by binding to a 15-Å deep ATP-binding site in the N-terminal domain of Hsp90 (Figure 3). A 17-allylamino,

17-demethoxy-substituted GM derivative, 17-AAG, was later developed as a clinical candidate, and is currently in Phase I and II trials in patients with multiple myeloma, lymphoma, stage IV pancreatic cancer, non-small-cell lung cancer, and solid tumors [78]. Other Hsp90 inhibitors in clinical testing include IPI-504 (Phase II), BIIB021 (Phase I and II), PU-H71 (Phase I), NVP-AUY922 (Phase II), AT13387 (Phase I/II), and KW-2478 (Phase I/II) [78].

Emerging opportunities for targeting of PPIs
Although validated PPIs remain active targets for therapeutic development, new concepts and promising PPIs have emerged for anticancer drug discovery (Figure 2). For example, increased knowledge of cancer genomics and PPI-mediated epigenetic mechanisms and identification of cancer-specific onco-fusion proteins have revealed a large number of new PPIs that are directly associated with pathology of cancer. Recent insight into the consequences of various cancer therapeutics and the induced therapeutic

resistance offers unanticipated PPIs as potential cancer targets to enhance therapeutic efficacy.

Cancer genomics

Large-scale genomics initiatives, such as The Cancer Genome Atlas (http://cancergenome.nih.gov) and The International Cancer Genome Consortium (http://icgc.org/icgc/cgp), have led to the discovery of a plethora of genomic alternations that drive tumorigenesis and/or progression [79,80]. It is not hard to imagine that such changes will lead to alteration of protein interaction networks that regulate cell growth. For example, Akt-activating mutations often rewire downstream phosphorelay systems via altered PPIs, such as enhanced 14-3-3 interactions with FOXO3a, Bad, and PRAS40 (Figure 2). To systematically examine PPI network changes in cancer, we have conducted large-scale experiments to establish cancer-associated PPI network maps based on genomic information from glioblastoma multiforme [81] and other tumor types. Such studies, along with predicted new PPIs [82], have revealed novel PPIs that act as major drivers of cancer and thus are potential targets for therapeutic exploration.

PPIs that regulate epigenetic mechanisms

Cancer genomics studies have not only validated the importance of classical hallmarks of cancer but have also revealed new characteristics that are intricately associated with cancer, such as epigenetic dysregulation and RNA splicing [8,79,80]. Recent advances outlining the contribution of dysregulated epigenetic mechanisms to cancer offer new opportunities for PPI targeting. For instance, it has been found that dysregulated histone methylation and acetylation are associated with tumorigenesis. These changes in turn dictate the specific recognition of modified histones by methyllysine-binding proteins and by acetyllysine-binding bromodomains (Figure 2) [7,13]. A potent and selective compound, UNC1215, was recently identified that effectively disrupts the interaction of methylated histone with the L3MBTL3 methyllysine binding protein [83]. UNC1215 demonstrated significant selectivity against more than 200 other analogous methyllysine recognition domains, making it a highly promising agent for probing L3MBTL3 function in cancer. For the interaction of acetylated histone with bromodomain-containing proteins, two small molecules, JQ1 and I-BET, that are pan-bromodomain and extraterminal domain (BET) family inhibitors have been developed [84]. Antitumor activity has been observed for JQ1 in a patient-derived xenograft animal model. It is particularly valuable for Myc-driven tumors [85]. I-BET-151 exhibited promising efficacy against onco-fusion-driven leukemia [86]. Cancer-associated mutations in the RNA-splicing machinery indicate the importance of PPIs in the regulation of RNA processing in cancer, such as the association of frequently mutated splicing factor 3b (SF3B1) with 3a (SF3A) in the U2 small nuclear ribonucleoprotein complex [80].

Onco-fusion PPIs offer cancer selectivity

PPIs are important for the catalytic activity of many enzymes, including epigenetic-modifying enzymes, which can be targeted. One example is the development of high-affinity peptidomimetics that antagonize the interaction of the histone methyltransferase mixed lineage leukemia (MLL1) and its activator WDR5. Dysregulated MLL1 is associated with various leukemias. Disruption of the MLL1–WDR5 PPI by peptidomimetics effectively decreased MLL1-fusion-mediated leukemogenesis [87]. Similarly, targeting of the MLL1–menin PPI led to the development of a series of lead compounds with therapeutic potential [88]. Importantly, fusion proteins such as MLL1 offer tumor-selective targets; thus, future efforts targeting onco-fusion-protein-specific PPIs are not only warranted but are much needed [89].

PPIs in protein complexes

As indicated for MLL1 and many other hub proteins that mediate oncogenic signaling, PPIs often involve multiprotein complexes. Selective inhibition of a particular PPI in the complex for a desired therapeutic effect is challenging. However, selective disruption of MLL1–WDR5 gave rise to promising antileukemogenesis activity [87]. Inhibition of MAML interaction with ICN1/CSL by a stapled peptide in NOTCH1 signaling is another example that offers a novel strategy for treating NOTCH1-dependent cancer [22]. Another challenge is experimental identification of selective agents via HTS. For example, 14-3-3 proteins interact with multiple partners, such as Raf-1, Bad, and FOXO [14]. Although these interactions engage a common binding groove, some partner-selective residues have been suggested. Technologies that can identify pan and specific modulators are expected to greatly accelerate the development of selective PPI inhibitors.

PPIs in combination therapies

Another emerging opportunity for PPI targeting in cancer is rewired PPIs in oncogenic signaling networks triggered by therapeutic insults. For example, inhibition of mTOR induces paradoxic activation of Akt [90]. Activated Akt triggers phosphorylation-dependent PPIs, such as 14-3-3-mediated PPIs [3,12]. Such induced PPIs may yield new cancer dependence and serve as new targets to overcome pharmacologically induced drug resistance. Interestingly, treatment of cancer cells with an MEK inhibitor renders them sensitive to the BCL2 PPI inhibitor ABT-263 [91]. PPI modulation is expected to have broad and important roles in future mechanism-based combination therapies.

Concluding remarks

Future efforts aimed at targeting of PPIs will be greatly accelerated by a number of recent advances. Understanding the nature of PPI interfaces and successful PPIMs may provide rationale design strategies for PPI-focused libraries. PPI assay technologies that closely reflect physiological conditions and address multiprotein complex issues are likely to shorten the process of lead discovery. PPI target discovery coupled with functional validation in genetically defined model systems is vital in moving PPIMs into the pipeline for clinical evaluation. These activities are fueled by a new US national initiative, the Cancer Target Discovery and Development (CTD²) network (http://ocg.cancer.gov/programs/ctdd.asp). CTD² aims to bridge the gap

between the vast amount of cancer genomics information and limited therapeutics by accelerating the discovery of new promising targets, including PPIs. Emphasizing collaborative interactions among members with complementary and unique expertise, the CTD^2 network focuses on rapid identification and characterization of potential targets for the development of cancer therapeutics. These scientific efforts will significantly accelerate the expansion of the PPI target landscape, which we hope will lead to a paradigm shift in targeting the once 'undruggable' for personalized cancer therapy and precision medicine.

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