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REVIEW

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Mapping oncogenic protein interactions for precision medicine

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Abstract

Normal protein-protein interactions (normPPIs) occur with high fidelity to regulate almost every physiological process. In cancer, this highly organised and precisely regulated network is disrupted, hijacked or reprogrammed resulting in oncogenic protein-protein interactions (oncoPPIs). OncoPPIs, which can result from genomic alterations, are a hallmark of many types of cancers. Recent technological advances in the field of mass spectrometry (MS)-based interactomics, structural biology and drug discovery have prompted scientists to identify and characterise oncoPPIs. Disruption of oncoPPI interfaces has become a major focus of drug discovery programs and has resulted in the use of PPI-specific drugs clinically. However, due to several technical hurdles, studies to build a reference oncoPPI map for various cancer types have not been undertaken. Therefore, there is an urgent need for experimental workflows to overcome the existing challenges in studying oncoPPIs in various cancers and to build comprehensive reference maps. Here, we discuss the important hurdles for characterising oncoPPIs and propose a three-phase multidisciplinary workflow to identify and characterise oncoPPIs. Systematic identification of cancer-type-specific oncogenic interactions will spur new opportunities for PPI-focused drug discovery projects and precision medicine.

KEYWORDS
cancer, drug discovery, genomic alteration, precision medicine, protein-protein interaction

1 | INTRODUCTION

Multimodal combinations of radiotherapy, surgery, biologicals and chemotherapy have become the mainstay of cancer therapeutics.[1,2] These modalities often lack selectivity which can lead to adverse effects such as chemotherapy-induced cardiotoxicity.[3,4] Quality of life in surviving patients is thereby compromised. In contrast, targeted molecular therapies offer a great potential to combat various cancer types with minimal harm to normal cells or organs.[5-7] The main purpose of molecular therapy in oncology is to target

Abbreviations: AP-MS, affinity purification mass spectrometry; APEX, ascorbate peroxidase; BioID, proximity-dependent biotin identification; cryo-EM, cryogenic electron microscopy; DLS, dynamic light scattering; FBDD, fragment-based drug discovery; FRET, Förster resonance energy transfer; HTS, high-throughput screening; NMR, nuclear magnetic resonance; normPPI, normal protein-protein interaction; oncoPPI, oncogenic protein-protein interaction; PBD, peptide binding domain; PL-MS, proximity labelling mass spectrometry; PPI, protein-protein interaction; SEC-MALLS, size exclusion chromatography—multiangle laser light scattering; TAD, topologically associating domain; XL-MS, cross-linking mass spectrometry; Y2H, yeast two-hybrid.

specific biological pathways activated by oncoproteins in tumour cells. Oncoproteins are involved in various signalling pathways and mediate a large number of oncogenic protein-protein interactions (oncoPPIs).[8-11] OncoPPI interfaces offer great potential for rational drug development and targeted cancer therapy.[8,9,12-19]

Recent technological advances in the fields of drug discovery and structural biology have accelerated the characterisation of oncogenic protein interfaces and rational drug design.[20,21] In recent years, several oncoPPI modulators have been tested in the clinical setting, with some now approved for clinical use.[17,22,23] However, most PPI-based drug discovery projects have focused on a relatively limited number of oncoPPIs detected in focused small-scale studies. Therefore, identification of more cancer-specific oncoPPIs in a proteome-wide manner will increase the potential for drug target discovery, improving options for targeted therapies.

Cancer is a highly complex disease featuring complex genotypes and gene dysregulation, which can result in the formation of complex oncogenic interaction networks.[19,24-26] Better understanding of the composition of protein interaction networks of each cancer will greatly enhance our molecular understanding of cancer biology and provide us with the ability to improve patient outcomes through precision and personalised medicine.[11,27] Efforts to map oncoPPI networks have already been initiated, for example, lung cancer-specific oncoPPI networks (~400 PPIs) have been generated on a small-scale for a few well-known cancer genes.[11]

Several publicly available databases such as BioGrid,[28] BioPlex,[29] IntAct,[30] MINT[31] and HIPPIES[32] regularly update human protein interaction networks by curating PPIs from both focused low-throughput experimental studies and large high-throughput mass spectrometry (MS) and yeast two-hybrid (Y2H) datasets. Databases such as PINA[33] integrate cancer-focused transcriptomics and proteomics information with PPI datasets to draw a cancer-specific protein interaction network. However, these maps do not distinguish normal PPIs (normPPIs) from oncoPPIs because nearly all affinity purification MS (AP-MS)-generated PPI datasets have been constructed using transformed cell lines, which contain abnormal karyotypes and thus an abnormal interactome profile. Hence, a focus on generating comprehensive maps of the oncoPPI networks for human cancers is crucial.

The protein interaction networks of oncoproteins which are referred to as "oncoPPIs" first appeared in the literature in 1997.[34] Since then, these terms have been used in many publications mostly for well-known oncoproteins (eg, KRAS) and tumour suppressors (eg, P53), which are categorised as cancer genes in many cancers.[35] Recent large-scale genome-wide analysis of 33 different cancer types involving 10 000 tumour samples reported that 55% (ie, 142 out of 258) of cancer genes and their mutations are associated with a single cancer.[35] This strongly suggests that oncoPPI networks could be unique for many cancer types as their cancer driver genes and genomic alterations vary. Here, we define oncoPPIs as any direct PPIs that arise from genomic alterations in tumour cells. Identification and characterisation of oncoPPIs for each cancer can guide targeted molecular therapies and precision medicine. In the following sections, we discuss the genomic alterations and approaches to build reference oncoPPI maps for various cancers.

2 | GENOMIC ALTERATIONS RESULT IN COMPLEX oncoPPI NETWORKS

The genomic landscapes of many tumours from diverse cancers have been described. Coding and noncoding mutations, as well as structural variants such as copy number variations, rearrangements, insertions, inversions and translocations have each been defined and characterised.[35-39] How pathogenic genomic alterations lead to complex oncogenic traits has remained one of the major challenges in cancer biology. Part of the answer lies in defining how these alterations are linked to oncogenic protein networks, thus enabling a network level understanding of the effects of genomic alterations on cellular functions.[40-43]

The majority of oncoPPIs arise from genomic alterations that directly affect protein interactions via different molecular mechanisms, including edgetic perturbation and/or node removal[42] (Figure 1A). In edgetic perturbation, mutations can change the folding free energy of interacting partners or perturb covalent interactions[43,44] and involves change of function. In node removal, genomic alterations lead to the aggregation or misfolding of the target protein, thereby causing the loss of its entire interaction network.[9] Nodal removal involves loss of function. Genomic alteration in regulatory regions can also indirectly affect protein interactions by dysregulating the expression or mislocalisation of target proteins.

2.1 | Genomic alterations can directly affect PPI networks

Analysis of pan-cancer genome studies show that noncoding mutations are less frequent than coding mutations. About 91% of tumours carry at least one driver mutation, with an average of 2.6 coding mutations per tumour.[36,37] The number of large-scale studies reporting the effects of coding mutations on disease-specific PPI networks has increased rapidly.[40,41] A recent comprehensive investigation of over 10 000 tumour exomes demonstrated that disease-associated germline variants as well as somatic missense mutations are significantly enriched in PPI interfaces compared to germline variants found in healthy individuals.[40] Further characterisation of some of the variants confirmed that disease-associated variants disrupt PPIs and regulate tumour growth.[40] In a large-scale study using the binary Y2H system, PPIs were assessed for ~2500 pathogenic missense mutations.[41] Approximately, 70% of the missense mutations had no effect on protein stability and folding, however half of these mutations resulted in edgetic perturbation. In a comprehensive assessment of somatic missense mutations within 304 cancer-specific PPI interfaces for which structural data was available, 16 interfaces (~5%) were disrupted.[45]

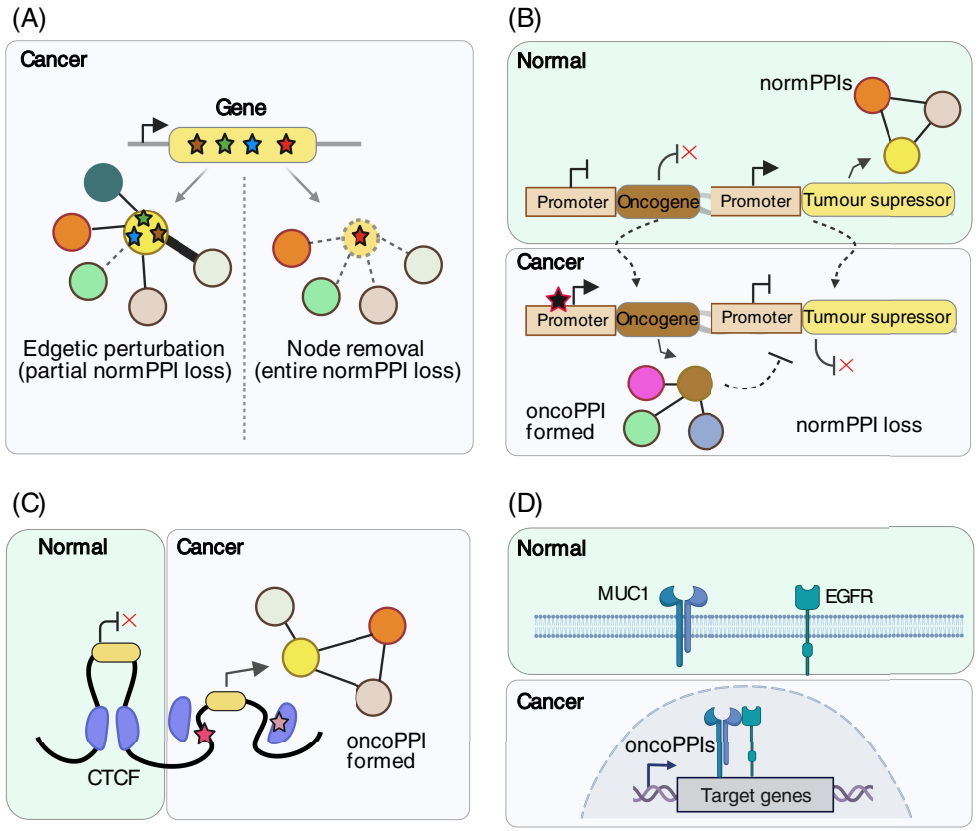

FIGURE 1 Genomic alterations, gene dysregulation and protein mislocalisation in cancer give rise to oncoPPIs. (A) Schematic illustration of edgetic perturbation and node removal in cancer and their impact on normal protein interaction networks. Missense mutations are depicted as coloured stars. Specific interactions can be interrupted (dotted line, blue star) or enhanced (thick line, brown star) or created (green star) by a missense mutation, whereas complete loss of the node protein (dotted circle) leads to the loss of the entire interaction network. (B) Gene dysregulation is another source of oncogenic network formation. In normal cells (upper panel), Gene 1 (ie, oncogene) is inactive whereas Gene 2 (ie, tumour suppressor) is active and normPPIs occur. In cancer (lower panel), Gene 1 is activated and initiates oncoPPIs whilst Gene 2 is silenced and normPPIs are lost. (C) Genome organising proteins such as CTCF regulate genome folding globally in a highly organised manner. In cancer, CTCF binding sites or CTCF itself can be mutated leading to loss of CTCF occupancy. This causes disorganisation of genome architecture and dysregulation of neighbouring genes. Dysregulated genes can potentially define new interaction networks. (D) In cancer, mislocalisation of proteins can also initiate new interaction networks. For example, MUC1 and EGFR are normally expressed separately at the cell surface. However, in cancer, MUC1 forms oncogenic PPIs with EGFR, regulating its nuclear localisation and hence transcriptional regulation of target genes

Missense or truncation mutations affecting protein interactions can rewire biological processes in cancer as well as developmental disorders.[41,46] This has been observed in melanoma where two missense mutations in cyclin-dependent kinase 4 (CDK4), R24H and R24C, have been shown to confer resistance to cyclin-dependent kinase inhibitor 2A (CDKN2A) inhibitors.[47] In a pairwise interaction study, it was shown that CDK4 R24C and R24H, but not other putative disease-causing mutations (N41S and S52N), disrupted CDK4 binding to CDKN2C.[48] Notably, perturbation of the CDK4-CDKN2C complex leads to drug resistance phenotypes in melanoma patients.

In a recent report in hereditary pancreatic cancer, it was shown that a truncated RAB-like protein 3 (RABL3) mutant markedly increased its binding affinity to RAP1 GTPase-GDP dissociation stimulator 1 (RAP1GDS1) protein, a chaperon involved in prenylation of RAS GTPases. Consequently, the RAS signalling pathway was disrupted leading to enhanced cell proliferation and tumour growth.[49]

In another study, it was shown that a missense mutation, R183W, in serine/threonine-protein phosphatase 2A regulatory subunit 1A (PPP2R1A) disrupted its binding to the canonical subunits of the PP2A complex B module, including B55 and B56 proteins, and abrogated growth suppressive effects.[50] However, mutant PPP2R1A also exhibited increased binding to striatin subunit family members, demonstrating that a missense mutation can confer both loss- and gain-of-function phenotypes.[50,51]

2.2 | Genomic alterations modulate PPI networks via gene dysregulation

Many cancers from diverse tissues exhibit dysregulated gene expression despite no significant genomic alterations in the protein coding region of the differentially expressed genes. Single nucleotide variants

within *cis*-regulatory regions of cancer genes have been shown to greatly modulate gene expression in a range of cancers.[52,53] These mutations can lead to transcriptional activation or repression of the target genes. For example, the promoter region of telomerase reverse transcriptase (*TERT*) is frequently mutated in a range of cancers[54-56] and linked to increased tumour growth and metastasis (Figure 1B). Genomic alterations can also occur in *trans*-regulatory elements, distant from *cis*-regulatory elements of the given cancer genes. For instance, mutations or structural variations in DNA motifs bound by genome-organising proteins, such as CCCTC-binding factor (CTCF) and cohesin, or transcription factors, indirectly lead to the dysregulation of many genes in various cancers[57-59] (Figure 1C). Mutations that occur within topologically associating domain (TAD) boundaries or in enhancer and insulator regions can result in coordinated dysregulation of a cluster of neighbouring genes located within the same TAD.[57-60] Mutations in CTCF itself, and other genome organising proteins such as the cohesin subunit STAG2, can have a similar impact.[61,62]

In gastrointestinal cancer, a CTCF-binding motif on chromosome 6 is disrupted by mutations at TAD boundaries flanking centromere protein Q (*CENPQ*) and methylmalonyl-CoA mutase (*MUT*) genes[57] (Figure 1C). In acute myeloid leukaemia, the oncogenic MDS1 and EVI1 Complex Locus (MECOM) protein is activated due to a genomic inversion event that occurs in neighbouring TADs which disrupts CTCF-mediated genome organisation.[63] Such aberrant activation of proteins can potentiate illicit oncoPPIs and redefine the oncogenic potential of cancer cells.[64] As another example, the aberrant expression of CTCF-like (CTCFL), a paralogue of CTCF,[65] in treatment-resistant neuroblastoma alters genome folding and leads to the formation of superenhancers that consequently activate proneural proteins and promote a resistance phenotype.[66] Another example is the bromodomain (BRD)-containing protein family members, which are readers of lysine acetylation, that are highly elevated in many cancers. BRD4 overexpression leads to the formation of an oncogenic complex with positive transcription elongation factor (p-TEFb) leading to activation of RNA Polymerase II (RNAPII) at late mitosis.[67]

Together, these examples highlight the impact that acquired mutations and structural variations can have on oncoPPI networks. These can directly rewire new oncoPPI networks or indirectly lead to the dysregulation of many genes which then form aberrant protein interaction networks to promote tumour growth and metastasis. Therefore, defining oncoPPIs for each cancer not only sheds light on molecular mechanisms of malignant phenotypes, but also paves the way to design more effective treatments.

2.3 | Mislocalisation of oncoproteins can initiate new classes of oncoPPI networks

Spatial compartmentalisation of proteins and organelle-specific interactions enable cells to conduct numerous biological processes in parallel. However, the subcellular relocalisation of the proteins as a rapid cellular response to internal or environmental stimuli can elicit new protein interaction networks. An exemplar is the receptor tyrosine-protein kinase HER2, an oncogene associated with progression of aggressive types of breast cancer. HER2 is expressed on the cell surface where it can function as a receptor or can localise to the nucleus upon stimulation to modulate transcription of target genes.[68,69] Another example is alpha-enolase (ENO1), a potential prognostic marker which is elevated in various types of cancers and is linked to tumourigenesis.[70] ENO1 can exhibit distinct functions in different cellular locations: (a) as a gene transcriptional repressor in the nucleus, where it exhibits enzymatic function in the cytosol regulating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signalling pathway; and (b) as a plasminogen receptor in the plasma membrane.[71,72] Similarly, mucin-1 (MUC1), a well-characterised oncoprotein extensively expressed on the surface of cancer cells, is also expressed in the nucleus, cytosol and mitochondria, and has been shown to interact with different protein partners in a location-dependent manner.[73-75] When MUC1 is localised to the nucleus in breast cancer cells it interacts with epidermal growth factor receptor (EGFR), facilitating chromatin binding and thereby rewiring oncogenic pathways[76] (Figure 1D). Therefore, specific biochemical and experimental strategies need to be designed to investigate multilocalising protein interactome networks as they can interact with a range of protein classes as they shuttle between different compartments.

3 | EXISTING CHALLENGES FOR ONCOGENIC PROTEIN INTERACTION STUDIES

Genomics,[36,37] transcriptomics[45] and proteomics[77,78] initiatives coupled with bioinformatics and biostatistics have extracted vast collections of biological data from comparatively small amount of patient material obtained at biopsy. In contrast, system-wide interactome studies using MS-based approaches (eg, cross-linking MS [XL-MS]) require a large amount of input materials (eg, microgram to milligramme protein sample in a native or seminative condition), which is nearly impossible to extract from limited patient samples.[79,80] In addition, AP-MS and proximity labelling MS (PL-MS) require gene transfer and genetic manipulation, which is not feasible in patient samples.[81-83] Therefore, a major bottleneck to systematic investigation of normPPIs vs oncoPPIs is having access to appropriate quantities of diverse input materials from patients. Besides the technical challenges, the lack of coordinated efforts between cancer biologists and other disciplines such as proteomics has been a hurdle. Together, these obstacles have slowed systematic investigations of normPPI and oncoPPI networks.

4 | ESTABLISHING WORKFLOWS FOR IDENTIFICATION OF ONCOGENIC NETWORKS

We propose a three-phase strategy to systematically characterise oncoPPIs in human cancers (Figure 2).

4.1 | Phase I: Establishment of patient-derived normal and cancer organoids

Over the past decades, numerous methods have been used to transform a range of patient cancer samples into immortalised cell lines for studies.[84] Tumour-derived cell lines are regularly used in pharmacogenomics and cancer biology to investigate biological pathways underlying tumour initiation and development.[85,86] Integrated analysis of proteogenomics data generated from hundreds of model cell lines have greatly enhanced our understanding of the molecular

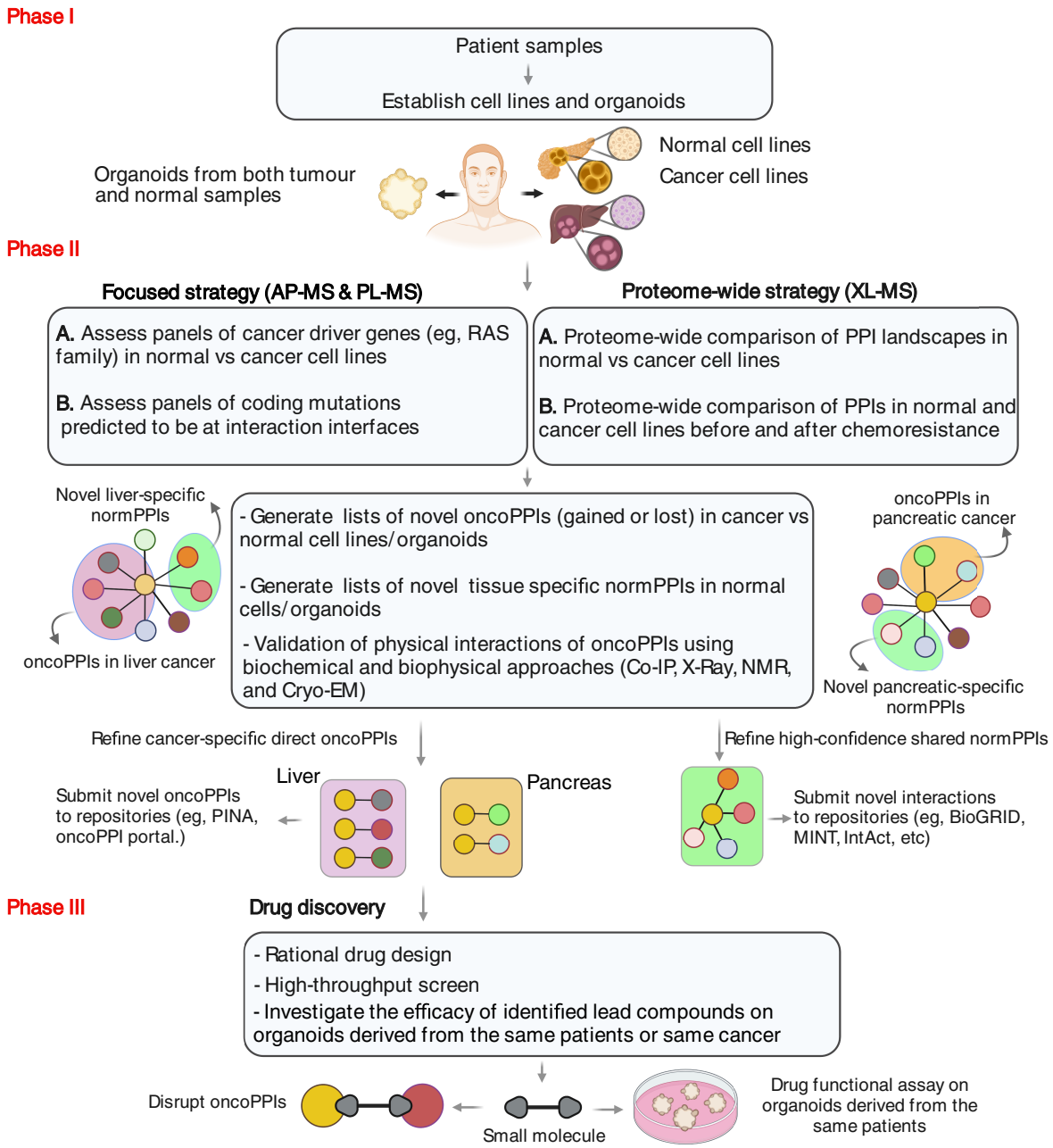

FIGURE 2 A cancer-focused human PPI workflow. A flowchart of the recommended procedures for systematic analysis of normPPIs and oncoPPIs. In Phase I, patient-derived tumour and normal tissue samples are used to generate cancer and normal cell lines as well as organoids; liver and pancreas have been depicted as examples. In Phase II, two different approaches can be chosen. First, a targeted approach where well-characterised oncoproteins are used to capture interactions in both patient-derived cancer and normal cells and organoids using AP-MS and PL-MS approaches. Second, an alternative approach can be undertaken on a proteome-wide scale using XL-MS, which can provide a picture of vicinal proteins within 30 Å. Phase II will define normPPIs and oncoPPIs in a stepwise manner. The novel normPPIs identified herein are reported to interactome repositories but oncoPPIs are further processed to characterise physical interactions. Physically interacting oncoproteins are subjected to structural analysis for rational drug design. In Phase III, a range of drug discovery approaches are employed to find small molecules that inhibit oncogenic interactions. Functional characterisation of drugs can be done in a personalised manner on the organoids from the same patient

mechanisms underlying cancer development.[85] Data generated from cell lines complement the large scale cancer genomics data generated from patient samples.[87] Cell lines offer several advantages including ease of maintenance and proliferation in cell culture with low cost and are amenable to high-throughput screening (HTS) of anticancer therapeutics. Therefore, establishing cell lines from patient samples would provide an important first step in mapping and identifying potential oncoPPI networks (Figure 2).

In addition, patient-derived organoids, which are three-dimensional structures with the capacity to self-organise into "miniorgans" resembling the tissue of origin, have been established in various cancers[88] (Table 1). Importantly, organoids maintain key cellular hierarchy and function,[111] and can genetically and phenotypically recapitulate the host cancer, with which therapeutic responses can potentially be predicted.[112] Their use as a cancer model for studying protein interactions as well as for screening oncoPPI inhibitors has so far been underutilised but show great potential for precision/personalised medicine. Small-scale studies using patient-derived colorectal cancer organoids has identified different proteomic signatures in all patients studied, highlighting the importance of PPI characterisation for personalised therapy.[113] In another study, autophagy impairment and disrupted pathways in Paneth cells was studied using mouse tumour organoids using computational PPI networks.[114] Mouse tumour organoids have also been used for functional validation of PPI inhibitors, Poloppin and Poloppin-II, which inhibit Polo-like kinase interactions.[115] Although patient-derived organoids are technically challenging and costly, the ability to gain a more clinically relevant understanding of a patient's tumour outweighs these challenges in the search for effective targeted therapies.

TABLE 1 Human organoid biobanks with the number of tumour and normal organoids

Tumour site	Tumour	Normal	Reference
Colorectal	22	19	89
	32	18	90
	52	41	91
Rectum	65	51	92
Pancreas	8	(2)	93
	114	(11) (19[a])	94
	39	(10[a])	95
	52	(5)	96
Stomach	37	7	97
	20	4	98
	46	17	99
	7	4	100
	10	8	101
	43	34	102
Prostate	7	2[a]	103
Breast	56	18	104
	95	5	105
Oesophagus	15	12	106
	10	1	107
Oral mucosa	25	9	108
Endometrium	14	66 2[a]	109
Kidney	54	47	110

[a]Normal-like, noncancer organoids that have a normal phenotype but grown from a cancer sample; brackets indicate that organoids were established but could not be propagated long-term.

4.2 | Phase II: Choosing an appropriate mass spectrometry approach for oncoPPI discovery

After establishment of patient-derived organoids, two different MS-based proteomics strategies can be utilised to study normPPI and oncoPPI networks: a focused or proteome-wide approach (Figure 2).

4.2.1 | Focused approach

A set of master oncogenes or tumour suppressors are selected for further characterisation in paired patient-derived normal and cancer cells or organoids (Figure 2). Depending on the protein of interest's function and localisation either AP-MS or proximity labelling (PL)-MS can be chosen.

4.2.1.1 | AP-MS is the method of choice to discover high affinity interactions

AP-MS is widely used to purify and characterise interactors of a protein of interest from a complex cell lysate mixture.[45,81,83] In AP-MS, either an immunoprecipitation-grade antibody is immobilised onto a solid-phase support and then is mixed with cell lysates to capture the target protein and its interactors, or the bait protein is either endogenously or ectopically tagged with epitopes (eg, FLAG, HA and myc) and used to capture interactions.[116,117] AP-MS using an epitope-tagged approach offers an excellent opportunity to undertake deep and region-specific interactome studies for most proteins. The most comprehensive human interactome, BioPlex 3.0, has been constructed using this approach.[29] In this project, ~15 000 genes were dual-tagged with FLAG and HA epitopes and stably overexpressed in HEK293 cells. Affinity-purified complexes were subjected to MS and network analysis. However, such interactome maps will not distinguish normPPIs from oncoPPIs. To do this, the protein of interest should be compared in both cancer and matched normal cell lines or organoids. For example, RAS gene family members *HRAS*, *KRAS* and *NRAS* carry missense mutations or display dysregulated expression patterns in various cancers and convey aberrant interaction networks.[118,119] Therefore, affinity purification and interactome studies of RAS family members or their mutants from both cancer and paired normal cells in pancreatic cancer or other cancers can extract more informative interaction networks and oncogenic pathways (Figure 2). Likewise, a different interaction profile might be observed for a panel of well-known

oncoproteins and tumour suppressors. Together, this approach will not only facilitate the comprehensive definition of the oncogenic map of cancer driver proteins in a tissue-specific manner but will also enable identification of novel tissue-specific normPPIs that have not been reported before. The novel PPIs can be reported in interaction repositories such as BioGRID.

MS-based high-throughput techniques cannot confidently identify direct interactions in a complex cellular milieu because there is always the possibility that interactions may be bridged by auxiliary proteins or by RNA and DNA molecules.[120] Multisubunit protein complexes are typified by the protein of interest directly interacting with one or two proteins within the same complex and indirectly with other subunits of the complex.[121] Therefore, highly enriched proteins need to be further validated for direct interaction using pairwise comparison experiments such as coimmunoprecipitation or biophysical methods. Such biophysical methods include nuclear magnetic resonance (NMR), X-Ray crystallography, size exclusion chromatography—multiangle laser light scattering (SEC-MALLS), dynamic light scattering (DLS) and Förster resonance energy transfer (FRET). Direct interaction interfaces which can be validated can then be used for rational drug design (Figure 2).

4.2.1.2 |PL-MS is the method of choice to discover transient and organelle-specific interactions

Engineered peroxidase and biotin ligase enzymes are now routinely applied to proximity labelling of protein interactions.[122,123] These include proximity-dependent biotin-identification (BioID)[124] and ascorbate peroxidase (APEX)-based methods[125] or derivatives of these. These approaches were initially developed to overcome the limitations of AP-MS approaches, in which mild lysis conditions may limit protein solubility and therefore accessibility.[123,124] Furthermore, weak and transient interactions, especially interactions involved in cell signalling, may remain undetected.[123,126] Therefore PL-MS is very useful to identify highly dynamic interactions that occur in various biological processes such as the cell cycle, transcription and translation initiation, elongation and termination. BioID, the most widely used approach over the past decade utilises engineered BirA enzymes that are covalently linked to a protein of interest to catalyse the conversion of biotin and ATP into highly reactive biotinoyl-5′-AMP molecules which bind lysine residues on neighbouring proteins. Proteins within a 10 nm radius are labelled depending on the type of fused BirA enzyme and duration.[127] Most recently, a BioID-based approach was used to define the interaction map for 4000 proteins within various intracellular compartments[82] highlighting BioID as a powerful tool for profiling PPIs in different organelles and compartments of live cells.

In mammalian cells, most signal transduction pathways are characterised by weak PPIs that typically occur between globular peptide binding domains (PBDs) of proteins with short, disordered peptide stretches. Over 1800 PBDs have been reported, which indicate their diversity and importance in mediating transient interactions.[128,129] Dissecting diversity, low binding affinities and the sensitivity of binding properties to minor sequence variation represent major challenges.[128] In cancer, somatic mutations can commonly occur in PBD-containing proteins and many of these proteins are drug targets.[128] BioID could be an ideal method to study the transient interactome of oncoproteins containing PBDs in different cancers.

A BioID-based screen for the "undruggable" SOX2 oncoprotein in patient-derived squamous cell carcinoma tissue identified SOX2 interaction with EP300 as a therapeutic vulnerability. Indeed, EP300 was shown to be a mediator of SOX2 activity and EP300 bromodomain inhibitors suppressed growth in several lung cancer cell lines.[130] Similarly, interrogation of the oncogenic $KRAS^{G12V}$ interactome using BioID coupled to a CRISPR-Cas9 loss-of-function screen revealed phosphatidylinositol phosphate kinase PIP5K1A as the most negatively enriched target. *PIP5K1A* depletion reduced KRAS-dependent proliferation and signalling in several pancreatic cancer cell lines, thus providing a potential drug target for the treatment of *KRAS*-mutant cancers.[118] Taken together, growing evidence supports a continual role for proximity labelling technology in the development of precision medicine.

4.2.2 | Proteome-wide approach

This alternative approach can be performed within cells using XL-MS,[131] whereby the lysine residues of physically-associating proteins or vicinal proteins (<30 Å) are cross-linked using homobifunctional chemicals.[132] Cross-linked proteomes are subjected to proteolytic digestion, size exclusion fractionation, and MS analysis to identify the cross-linked peptides. In contrast to AP-MS and BioID, XL-MS can provide a global snapshot of PPIs within cells in a native state.[133] In addition, it provides orthogonal information to structural maps generated by cryogenic electron microscopy (cryo-EM), revealing the conformations and dynamic topology of the protein complexes.[134] XL-MS is the only method that can capture structural and conformational changes of proteins on a global scale in response to stimuli.[134] This approach offers opportunities to study and distinguish the dynamic and protein interaction networks of cancer cells compared to normal counterparts on a proteome-wide manner.

Tumours acquire a chemoresistance phenotype by acquiring new protein interactions and conformations.[135,136] The ability to define interaction networks that drive chemoresistance will provide invaluable insights for developing new therapies and strategies for various drug-resistant cancers. Chemotherapy remains the standard of care treatment for many cancers. Despite significant advancements in cancer therapy, resistance to chemotherapy frequently occurs[137] and is responsible for poor patient outcomes and cancer relapse. XL-MS has successfully been used to investigate proteome dynamics and interactions in drug-sensitive and -resistant HeLa cells.[138] This supports using the XL-MS approach to study protein interaction networks in a proteome scale in normal and paired cancer cell lines (Figure 2).

5 | PHASE III: DRUG DISCOVERY

The development of new drug candidates is a very complex process and requires cross-disciplinary research efforts. Until recently, development of PPI-specific therapeutics was also a difficult assignment. Unlike typical receptor-ligand interactions involving a distinct binding

TABLE 2 List of PPI-specific drugs and their targets in clinical trials

Inhibitor name	Disease area	Developer	Clinical trial phase	ID
BCL-2/Bax				
Venetoclax (ABT199)	Chronic lymphocytic leukaemia	AbbVie	Approved in 2016	–
MDM2/p53				
Idasanutlin (RO5503781)	Acute myeloid leukaemia	Hoffmann-La Roche	Phase I/II (terminated)	NCT03850535
KRT 232 (formerly, AMG 232)	Chronic myeloid leukaemia	Kartos Therapeutics, Inc	Phase I/II (recruiting)	NCT04835584
NVP-CGM097	Solid tumour with p53 wild type status	Novartis Pharmaceuticals	Phase I (completed)	NCT01760525
Milademetan (DS-3032b)	Advanced solid tumour, lymphoma	Daiichi Sankyo, Inc	Phase I (completed)	NCT01877382
SAR405838	Neoplasm malignant	Sanofi	Phase I (completed)	NCT01636479
JNJ-26854165	Neoplasms	Johnson & Johnson Pharmaceutical Research & Development, L.L.C.	Phase I (completed)	NCT00676910
ALRN-6924	Advanced solid tumours or lymphomas	Aileron Therapeutics	Phase I/II (completed)	NCT02264613
LFA1/ICAM1				
Lifitegrast (SAR 1118)	Dry eye	Lifelong Vision Foundation	Phase IV (completed)	NCT03451396
XIAP/Caspase 9				
Debio 1143 (AT-406)	Squamous cell carcinoma of the head and neck	Debiopharm International SA	Phase I/II (active, not recruiting)	NCT02022098
LCL-161	Relapsed or refractory multiple myeloma	Novartis	Phase II (completed)	NCT01955434
Birinapant (TL32711)	Advanced or metastatic solid tumours	TetraLogic Pharmaceuticals	Phase I/II (completed)	NCT01188499
ASTX-660	Advanced solid tumours and lymphomas	Astex Pharmaceuticals, Inc	Phase I/II (recruiting)	NCT02503423
GDC-0917	Refractory solid tumours or lymphoma	Genentech, Inc	Phase I (completed)	NCT01226277
HGS1029 (AEG40826-2HCl)	Advanced solid tumours	Human Genome Sciences, Inc	Phase I (completed)	NCT00708006
Bromodomain/Histone				
Apabetalone (RVX-000222, RVX-208)	Coronary artery disease	Resverlogix Corp	Phase III (completed)	NCT02586155
Molibresib (GSK525762)	NUT midline carcinoma	GlaxoSmithKline	Phase I (completed)	NCT01587703
CPI-0610	Myelofibrosis	Constellation Pharmaceuticals	Phase I/II (recruiting)	NCT02158858
RO6870810 (formerly, TEN-010)	Acute myeloid leukaemia; myelodysplastic syndromes	Hoffmann-La Roche	Phase I (completed)	NCT02308761
OTX015 (MK-8628, Birabresib)	Haematologic malignancies	Oncoethix GmbH	Phase I (completed)	NCT01713582
B-catenin/CBP				
PRI-724	Liver cirrhosis	Komagome Hospital	Phase I/II (active, not recruiting)	NCT03620474
PD-1/PD-L1				
CA-170	Prostatic neoplasms	Astellas Pharma, Inc	Phase II (completed)	NCT01288911

Note: This table is not a comprehensive list of the PPI inhibitors on the market or undergoing clinical trials.

pocket, PPI interfaces are usually large, flat and featureless.[139] Recent technological advances in the field of cryo-EM,[20,21] high-resolution MS[140] and an urgent need for effective drugs to treat life-threatening diseases such as cancer have massively transformed PPI-focused drug discovery projects. Accordingly, numerous PPI-specific drugs have entered clinical trial with some already on the market[17,22] (Table 2).

Biological molecules (including antibodies, peptides, aptamers and proteins) and small molecules are the major drugs used in preclinical and clinical studies.[22] The specificity and potency of biological agents such as monoclonal antibodies are superior for targeting PPIs. However, antibody-based drugs can induce the host immune response and their usefulness is mostly limited to surface PPIs. In addition, antibody manufacturing is very complicated because opportunities for pharmacokinetic modifications are limited.[22] In contrast to antibodies, peptides and small molecules are cheaper and quicker to produce and generally do not induce a host immune response. Despite these features, peptides suffer from poor solubility, short half-life and poor oral bioavailability. However, peptide-based drugs are considered as a useful starting point for rational design of mimetics or nonpeptide inhibitors.[141,142] Traditionally, small molecules have successfully been used to target small interfaces and offer unique advantages such as oral administration and cell permeability, but they have more potential to exhibit side effects.[143]

5.1 | Strategies to identify lead compounds to target PPI interfaces

Strategies to identify and validate lead compounds to target PPI interfaces have been reviewed extensively elsewhere.[17,22,143] Hence, we briefly discuss three major platforms here.

5.1.1 | High-throughput screening

Both industry and academia have successfully used HTS technology for screening libraries of different chemical compounds for drug discovery purposes.[144] Lead compounds identified in HTS screens can be further modified to enhance the specificity and affinity in binding to their target. Compounds identified in HTS screens have been shown to best target PPIs mediated through helix-groove interactions, where a helical region of a protein binds to the groove of the second protein (eg, p53-MDM2 complex[145]). Recent advancements in technology and screening have also identified binders for nonhelix-groove interactions. For example, the XIAP-caspase-9 complex involved in early-stage regulation of the apoptosis cascade is an extended nonhelical-groove interaction and targeted by small molecules discovered from HTS studies (Table 2).[146,147]

5.1.2 | In silico screening

Computational methods are increasingly used in drug discovery programs. Here, machine learning and computer-aided tools are employed to screen chemical libraries for biological targets to find the best hits from millions of compounds.[148] One such approach is pharmacophore-based virtual scanning where steric and electrochemical factors are mapped in 3D space to provide a simple means to virtually screen compound libraries. Therefore, this approach is used as a foundation to find a hit based on pharmacophoric elements. For PPIs, the pharmacophore represents the molecular features of the protein domains directly involved in the interaction.

5.1.3 | Fragment-based drug discovery

The fragment-based drug discovery (FBDD) method has successfully been employed for intractable biological targets such as PPI interfaces.[149,150] FBDD is a powerful approach to develop effective small molecule compounds starting from weakly binding fragments. In this method, fragment hits (100-300 Da) with low affinity for the target protein are identified by screening libraries of synthetic or natural product low molecular weight (<300 Da) compounds.[18,151,152] Through chemical modifications, the identified fragment hits are further developed into drug-like molecules with higher affinity.[149,151,152] Recently, dozens of FBDD-based drugs have entered clinical trials and two have received Food and Drug Administration approval.[153] Using FBDD, PPI inhibitors for oncogenic interfaces such as XIAP-caspase 9,[154] and RAD51-BRCA2[155] complexes have been developed (Table 2).

6 | CONCLUDING REMARKS

Cancer patients exhibit widespread tumour heterogeneity, which drives an urgent need for precision and personalised treatments. Genomic characterisation of human tumours has led to the generation of vast amounts of high-resolution sequencing data. These data have enabled identification of many cancer-driver genes that are involved in the initiation and progression of tumour cells. However, it remains a challenging task to determine how these genomic signatures can be exploited to develop new therapies. Most cancer-driver genes or oncogenes, discovered to date, are essential for cell growth and maintenance pathways in both healthy and disease states. Therefore, exploiting them as new drug targets may result in adverse effects by perturbing normal physiological pathways. Given that proteins exert their function mostly through interaction with other proteins, it is highly plausible that targeting cancer-specific oncoPPIs could be a more advantageous approach which may result in a better clinical outcome with minimal side effects. Thus, a focus on generating a comprehensive map of the oncoPPI networks for various human cancers will stimulate targeted drug discovery efforts and will shed more insight into protein networks governing tumour growth.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

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