Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Shedding light on complexity of protein–protein interactions in cancer

Tae-Young Yoon[1,3] and Hong-Won Lee[2,3]

Most cell signaling and surveillance circuits are physically maintained through a dense network of protein–protein interactions (PPIs). Genetic mutations, epigenetic changes as well as alterations in cellular microenvironment can markedly rewire the patterns of PPIs, which leads to neoplastic growth of cancer cells. There are accumulating evidences that drugs that target-specific PPI pairs may provide an opportunity to treat cancers with a higher specificity and efficacy than those inhibiting enzymatic activity of oncogenic proteins. Therefore, identification of driving PPIs in a given cancer not only improves our understanding for individual cancers, but it also provides therapeutic opportunities to cure the specific cancer. In this review, we introduce some examples of aberrant PPI complexes identified in several major types of cancers, and recent technical developments that permit assessment of PPI strength in clinical specimens. Finally, we discuss the potential use of such PPI profiling for the purpose of precision medicine.

Addresses
[1] School of Biological Sciences and Institute for Molecular Biology and Genetics, Seoul National University, Seoul 08826, South Korea
[2] Proteina Co., Ltd., Seoul 08826, South Korea

Corresponding authors: Yoon, Tae-Young (tyyoon@snu.ac.kr), Lee, Hong-Won (hwlee@proteina.co.kr)
[3] These authors contributed equally to this work.

Current Opinion in Chemical Biology 2019, **53**:75–81

This review comes from a themed issue on **Chemical biophysics**

Edited by **Yan Jie** and **Terence Strick**

https://doi.org/10.1016/j.cbpa.2019.07.001

Introduction

Cancer is an extremely heterogeneous disease, referring to a collection of vastly different cellular states with dysregulation in cell signaling and regulation circuits [1,2]. Recent interests in personalized medicine highlight the need to analyze these lesions at the molecular level, which can lead to a tailored, targeted therapy for given cancer tissues [3,4]. The current efforts for molecular cancer diagnostics primarily focus on large-scale genome sequencing, with the prominent examples being The Cancer Genome Atlas (TCGA) project and International Cancer Genome Consortium [5,6]. There are, however, accumulating evidences that each stage of the information flow in biological systems—the genome, transcriptome, proteome, and interactome—is all dynamically and independently regulated from one another. For example, it is increasingly clear that there exists a large mismatch between the transcriptome and the real proteome. A hypothetical assumption underlying the intensive study of transcriptomes is that after transcription, the mRNAs would be almost constitutively translated into corresponding proteins. Various molecular biology mechanisms are shown to widen the gap between the mRNA level and the quantity of corresponding protein, which includes regulation in the 5' and 3' untranslated regions and the stochasticity in gene expression [7–9].

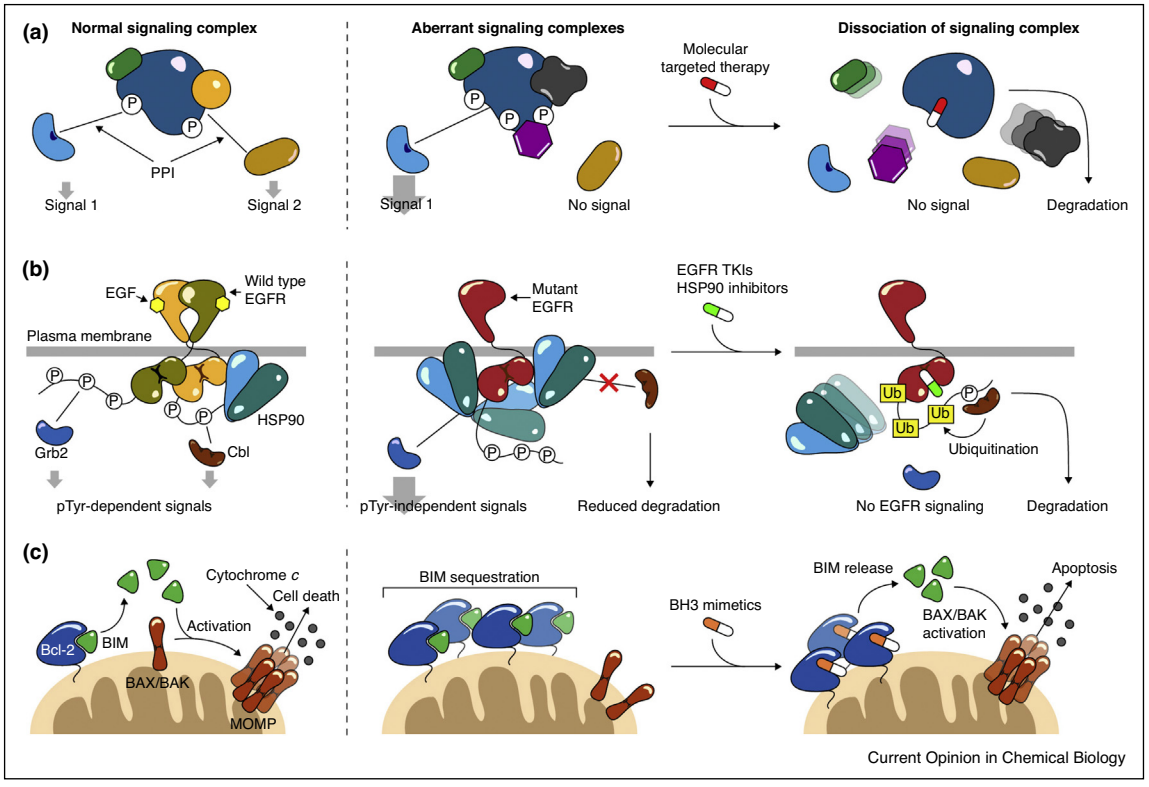
In addition, the amount of comprising proteins is not an absolute measure of the strengths of corresponding protein–protein interactions (PPIs), indicating a large discrepancy between the proteome and the resulting interactome. Cells employ various types of post-translational modifications to edit and modify the signaling proteins, which leads to dramatically different patterns of PPIs out of the same pool of signaling proteins [10]. In addition, the dysregulations in cell signaling and surveillance systems are, in many cases, physically reflected as changes in the patterns of PPIs (Figure 1a).

These all highlight the need to examine individual cancer tissues at the PPI level, which, if achieved, will reveal the latest rewiring status of the cellular circuits and help strategic decision on how to remedy a given cancer. Despite this emerging importance, there is a paucity of experimental tools that enable analysis of PPI patterns, in particular, for clinical specimens. In this review, we will introduce recent examples that demonstrate how PPIs are differently regulated in cancers for their neoplastic growth and how these different patterns of PPIs are therapeutically employed for selective treatment of cancers. We also discuss the recent development of experimental tools and outlook for the molecular cancer diagnostics at the PPI level.

Formation of aberrant PPI complexes in non-small cell lung cancers

It is recently shown that epidermal growth factor receptors (EGFRs) dramatically alter their PPI patterns in lung cancers (Figure 1b). Approximately, 20% of lung adenocarcinomas carries deletion in exon 19 (deletion from 746 to 750 amino acids) or genetic mutation in exon 21 (L858R) of the *EGFR* gene, which leads to

Figure 1



(a) Modulation of cell signaling depending on the formation of signaling complex. The formation of aberrant signaling complex enhances the level of post-translational modifications, rewires PPI networks and finally promotes cancer-specific signaling. Molecular targeted cancer drugs dissociate the aberrant signaling complex, which turns off cancer signals. **(b)** EGFR-mediated cell signaling. Mutant EGFR enhances the interaction with HSP90, which stabilizes mutant EGFR signaling complex. EGFR-TKIs or HSP90-specific inhibitors can dissociate EGFR–HSP90 interaction, promote Cbl-mediated degradation, and finally inhibit EGFR-signaling. **(c)** Regulation of apoptosis signal by Bcl-2 protein. Sequestered BIM proteins are released by BH-3 mimetic PPI drugs, which leads to the induction of apoptosis signal. *Abbreviations:* P, phosphorylation. Ub, ubiquitination. MOMP, mitochondrial outer membrane permeabilization.

strong oncogenic addiction of the corresponding cancer tissues to the EGFR signaling pathway [11,12]. When treated with EGFR-targeting tyrosine kinase inhibitors (TKIs), these lung cancers with the EGFR mutations are deprived of the proliferative signals and actively pushed to apoptosis [13].

EGFRs generate phosphorylated tyrosine (pTyr) residues in their C-terminal tails to recruit downstream interactors and initiate their signal transduction [14]. Because the exon 19/21 mutations are mapped to the tyrosine kinase domain of EGFR, it has been presumed that the EGFR mutations amplify the signaling activity of EGFRs and thus generate more pTyr residues. When tested in *in vitro* assays, however, the mutated EGFR kinase domains show catalytic rates increased by a factor of only three to four compared to their wild-type counterparts [15,16], raising a question that this marginal increase in the Tyr kinase activity can indeed lead to the observed strong oncogenic addiction.

It is increasingly clear that the strong proliferative signaling of the mutant EGFRs should be understood in the frame of their unique patterns of PPIs, rather than in a mere difference in the catalytic rate. The EGFR mutants build unique and large protein complexes around themselves [17,18•,19••]. These large protein complexes, at least, in part arise from cellular chaperoning efforts for the mutant EGFRs and thus include heat shock protein 90 (HSP 90) [20]. Association of HSP90 to the protein complex hinders recruitment of E3 ubiquitin ligases (Cbls) to EGFRs, thereby retarding degradation of the mutant EGFRs [20,21]. In addition, the large protein complex of the mutant EGFRs offers increased surfaces for PPIs and generates aberrant PPIs with downstream interactors in a largely pTyr-independent manner [19••]. This makes the signaling of the mutant EGFRs persistent even under prolonged absence of stimulatory ligands. Indeed, treatment of HSP90-specific inhibitors has been shown to destabilize the PPI complexes and downregulate the signaling activity of the mutant EGFRs in both preclinical studies clinical trials [22–25].

An immediate question is how the EGFR mutants show strong responses to EGFR-targeting TKIs when their PPIs become largely independent of the presence of pTyr residues. These two seemingly contradictory observations can be reconciled by recent experimental data that the large, aberrant signaling complex of the mutant EGFRs dissociates upon treatment of the EGFR-targeting TKIs for 24 hours [19••]. Thus, it is highly likely that initial construction of the protein complex requires the Tyr kinase activities of the mutant EGFRs. Once built, the protein complex makes largely pTyr-independent interactions with downstream signaling proteins, generating strong and persistent proliferative signaling that is minimally affected by the molecular actions of cellular phosphatases.

PPI inhibitors as anticancer drugs

As shown in the above example, inhibition of the cancer-specific PPIs (i.e. disintegration of the signaling complex of the mutant EGFRs) can be a therapeutic target because their inhibition selectively thwarts cancer growth while having a minimal side effect on normal cells. The most widely used approach to inhibit a target PPI is use of the monoclonal antibodies, with the immune checkpoint blockades being a recent notable example of PPI-inhibiting antibodies [26–28]. In tumors, immune cells are increasingly exhausted through chronic exposure to tumor-specific microenvironments [26,29]. In particular, checkpoint receptors, including cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), programed death 1 (PD1), lymphocyte-activation gene 3 (LAG3), T-cell immunoglobulin and mucin-domain containing 3 (TIM3) and T-cell immunoreceptor with Ig and ITIM domains (TIGIT), play key roles in suppressing immune cells, especially in CD8+ effector T-cells [26]. This suppression is essentially mediated by PPIs with corresponding ligands expressed on cancer cells or myeloid cells in the tumor microenvironment. The immune checkpoint blockades bind either to the checkpoint receptors on T-cells or the corresponding ligands to disrupt PPIs of the checkpoint receptors, which has a net effect of reinvigorating the suppressed effector T-cells that leads to therapeutic outcomes in many major cancer types [30–32].

Despite these remarkable successes, the use of therapeutic antibodies is largely limited to target proteins expressed on outer cell membrane. Thus, it has been actively pursued whether small molecule-based drugs also potently inhibit the target PPIs that occur deep inside cellular cytoplasm or nucleus. The interfaces for PPIs have large and featureless structures, thus thought of as largely 'undruggable' with small molecules [33•]. However, advances in high-throughput PPI screening, structural biology and bioinformatics lead to identification of novel small-molecules that inhibit target PPIs with sub-nM dissociation constants. Most notably, ABT-199, a selective inhibitor of the B-cell lymphoma 2 (Bcl-2) protein, showed a potent anti-tumor effect on chronic lymphocytic leukemia (CLL) cells [34,35]. Bcl-2, an anti-apoptotic protein, sequesters BH3 domain-only proteins such as BIM through specific PPIs, preventing the interaction of pro-apoptotic proteins, BAX (Bcl-2 associated X protein) and BAK (Bcl-2 homologous antagonist killer) [36] (Figure 1c). In the presence of an increased pro-apoptotic cue, Bcl-2 begins to release BIM, which in turn activates BAX and BAK. The activated BAX and BAK permeabilize the mitochondrial outer membrane, which constitutes the 'point-of-no-return' in the apoptosis process. In CLL, Bcl-2 is often overexpressed, which strengthens the anti-apoptotic PPIs among Bcl-2 and BH3 domain-only proteins. ABT-199 binds to the binding interface of Bcl-2 for the BH3 domain-only proteins with high affinity and selectivity [34]. This mimics an apoptotic stimulus, resulting in strong apoptosis for CLL, acute myeloid leukemia (AML) and multiple myeloma cancer types. On the basis of the positive clinical outcome, ABT-199 in combination with other drugs has been approved in the United States as an option for the first-line treatment of CLL patients [37–39].

Emerging technologies for analyzing PPIs in clinical specimens

As it is increasingly clear that PPI patterns can be markedly distorted in many cancer cells for survival under the nutritionally and metabolically competing environment within a tumor, profiling PPIs for an individual cancer will reveal its latest rewiring status of the cell signaling and regulation circuits, which in turn provides crucial information as to how to remedy the given specific cancer. Here we will review available technologies for PPI analysis with particular emphasis on those for clinical specimens (Table 1).

Affinity-purification mass spectrometry (AP–MS) is a unique platform for discovery of novel PPIs, which can be extended to a large-scale analysis [40,41] (Figure 2a). Using affinity tags, AP–MS isolates target proteins with interacting proteins physically bound to them, and induces fragmentation of these proteins into peptides by protease treatment. The resulting peptides are ionized and then separated with respect to their mass-to-charge ratios (m/z). Integration of liquid chromatography or tandem MS analysis further enhances the sensitivity of MS-based proteomics, which permits detection of post-translational modifications even at single residue resolution [42–44]. AP–MS is a library-independent analysis and offers an excellent throughput for identification of PPIs in a single sample [45•,46,47]. However, interpretation of the MS data is based on assigning the obtained m/z values to specific peptides in one-to-one manner, which makes the MS analysis largely dependent on available databases and thus researchers carrying out the analysis [48]. The MS-based approaches are also contamination-prone,

Table 1

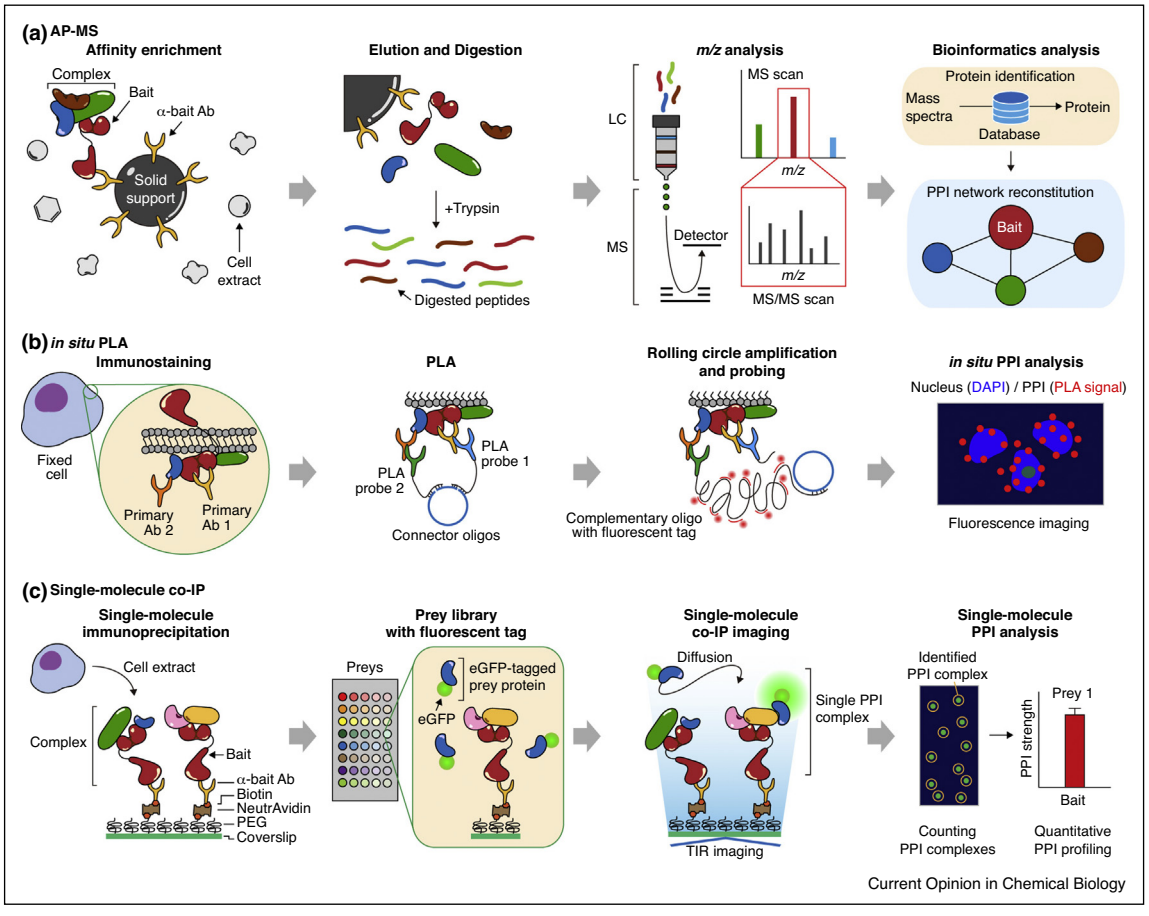
Comparison of techniques for PPI analysis

Technique	Detection	Advantages	Challenges
AP–MS	*m/z ratio*	- Proteomewide throughput - High sensitivity by mass analyzer	- Prone to contamination - High expertise with mass analyzer and bioinformatics toolkits
PLA	Ligated fluorescence oligos	- FFPE specimen compatible - *In situ* detection conserving spatial information	- Low throughput - Limited to the availability of antibodies for two proteins from different hosts
Single-molecule co-IP	Single fluorescence protein	- Quantification at single-molecule accuracy - Up to 40 multiplex assay on a single chip per 1 hour	- Requirement of fresh snap frozen specimens - Loss of spatial information for PPIs

known to generate false-positive results at a considerable rate. Thus, the throughput will be limited when the MS methods are used to compare multiple clinical samples that usually carry heterogeneous genetic and epigenetic changes.

Proximity ligation assay (PLA) is a promising method for the detection of PPIs in clinical specimens [49••] (Figure 2b). Typically, fixed samples are infused with two types of antibodies, with each antibody targeting one of the two proteins in the PPI pair, respectively. These

Figure 2



(a) Schematic representation of AP–MS workflow to identify PPIs. **(b)** Schematic workflow of *in situ* PLA for identification of PPIs. **(c)** Schematic workflow representing single-molecule co-IP analysis to profile PPIs. *Abbreviations:* Ab, antibody.

antibodies are either directly labeled with oligonucleotides (referred to as PLA probes) or probed with oligonucleotide-tagged secondary antibodies. Presence of the target PPIs bring the two PLA probes into a close proximity (generally, less than 40 nm). Hybridization of the two PLA probes produces a circular DNA template, which can be amplified by rolling circle amplification and then detected by complementary nucleotides with fluorescent tags.

The PLA method is recently used to visualize PPIs of the mutant EGFRs in non-small cell lung cancer patient specimens [49••]. One of the salient aspects of PLA is detection of PPIs at subcellular locations where the PPI complexes are assembled in cellular contexts. In addition, the PLA methods can be directly applied to deparaffinized formalin-fixed paraffin-embedded (FFPE) samples. Conversely, since PLA is essentially based on immunolabeling of cellular proteins in the dense cellular cytoplasm, the efficiency for the proper antibody binding to target proteins can largely vary depending on detailed sample conditions. Several factors—efficiency of the polymerase and ligase for rolling circle amplification, reaction time, background fluorescence, and density of resultant PLA spots—can further increase fluctuations in the PLA signals.

Single-molecule pull-down and co-IP is a novel toolkit developed for *in vitro* study of cellular protein complexes with various single-molecule fluorescence techniques [19••,50,51] (Figure 2c). Different from PLA that probes PPIs in fixed cells or tissues, the single-molecule co-IP includes cell or tissue lysis and immunoprecipitation of target protein complexes on an imaging surface of the single-molecule fluorescence microscope. Libraries of fluorescently labeled prey proteins—typically, tagged with enhanced green fluorescent protein (eGFP)—are subsequently added to induce PPIs with the immobilized target proteins. Formation of PPI complexes on surface appears as a point-spread function on a total internal reflection fluorescence microscopy. Because the single-molecule co-IP employs a dense polymer-coated surface to minimize non-specific bindings of cellular proteins, the eGFP-tagged prey proteins can also be directly added in an unpurified form (thus, in a cell/tissue lysate). This provides a facile way for assessing PPIs between the full-length proteins, while obviating the need to purify these large mammalian proteins.

With miniaturized reaction chambers and an automated imaging system, it is demonstrated that ~100 different kinds of PPIs can be assessed within 1 hour [19••]. Because the single-molecule co-IP requires a lysis step, it loses the original spatial distribution of the PPI complexes in a given tumor, thus not being suitable for analysis of the cell-to-cell heterogeneity in a single tumor mass. On the contrary, PPIs in single-molecule co-IP occur in a cell or tissue lysate, which is much more diluted than the original cellular cytoplasm. Thus, the PPI reactions in single-molecule co-IP are mainly governed by the simple law of mass action, and the resulting PPI counts show a much-reduced fluctuation when compared with those probed by immunohistochemistry and PLA that depend on immunolabeling in a dense cytoplasm. However, a major drawback of the single-molecule co-IP profiling is that it requires fresh snap-frozen samples, typically those preserved in liquid nitrogen.

Recent reports demonstrate the utility of PPI profiling in predicting the efficacy of targeted cancer drugs for clinical samples [19••,49••,52]. These studies convincingly show that the PPI counts of receptor tyrosine kinases (RTKs) are strongly correlated with the responses of individual cancer tissues to directed therapies targeting the corresponding RTKs. Remarkably, these PPI-based predictions are able to distinguish EGFR-TKI responders when there are no actionable mutations in the *EGFR* genes. Therefore, PPI profiling can monitor the strength of oncogenic signals involved in cancer progression, and may help guide therapeutic decision-making for the targeted cancer therapy along with genomic analysis.

Concluding remarks

Recent large-scale, genome-wide analysis revealed that almost half of human tumor lacks any potentially actionable mutations [53]. In addition, numerous regulations steps existing between the genome and the interactome make it extremely difficult to predict how a given genetic mutation is reflected at the PPI level, where the real signal transduction physically occurs. As discussed in this review, cancer actively distort patterns of PPIs for survival under the nutritionally and metabolically competing environments, and the PPI profiling provides a more direct avenue for identification of these dysregulated molecular lesions in given cancer specimens. In addition, recent examples of drug development demonstrate that disruption of these cancer-specific PPIs offers a novel therapeutic opportunity. Integrating with multi-omics data, PPI profiling will shed more comprehensive light to lesions of individual cancer tissues in the era of precision medicine.

Conflict of interest statement

T.-Y. Yoon and H.-W. Lee are inventors of patents related to the use of single-molecule co-IP technique. T.-Y. Yoon is a founder of Proteina Co., Ltd. H.-W. Lee is a full-time employee of Proteina Co., Ltd.

Acknowledgements

This work was supported by the National Creative Research Initiative Program (Center for Single-Molecule Systems Biology to T.-Y.Y.; grant number: NRF-2011-0018352) funded by the National Research Foundation of Korea, and Research Resettlement Fund for the new faculty of Seoul National University (T.-Y.Y.; grant number: 3344-20170013).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation**. *Cell* 2011, **144**:646-674.

2. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, Dimitriadoy S, Liu DL, Kantheti HS, Saghafinia S *et al.*: **Oncogenic signaling pathways in the cancer genome atlas**. *Cell* 2018, **173**:321-337.

3. de Bono JS, Ashworth A: **Translating cancer research into targeted therapeutics**. *Nature* 2010, **467**:543-549.

4. Collins FS, Varmus H: **A new initiative on precision medicine**. *N Engl J Med* 2015, **372**:793-795.

5. International Cancer Genome Consortium: **International network of cancer genome projects**. *Nature* 2010, **464**:993-998.

6. Cancer Genome Atlas Research Network, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM: **The cancer genome atlas pan-cancer analysis project**. *Nat Genet* 2013, **45**:1113-1120.

7. Blake WJ, Mads KA, Cantor CR, Collins JJ: **Noise in eukaryotic gene expression**. *Nature* 2003, **422**:633-637.

8. Yu J, Xiao J, Ren X, Lao K, Xie XS: **Probing gene expression in live cells, one protein molecule at a time**. *Science* 2006, **311**:1600-1603.

9. Bartel DP: **MicroRNAs: target recognition and regulatory functions**. *Cell* 2009, **136**:215-233.

10. Deribe YL, Pawson T, Dikic I: **Post-translational modifications in signal integration**. *Nat Struct Mol Biol* 2010, **17**:666-672.

11. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG *et al.*: **Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib**. *N Engl J Med* 2004, **350**:2129-2139.

12. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ *et al.*: **EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy**. *Science* 2004, **304**:1497-1500.

13. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, Tenen DG, Kobayashi S: **BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations**. *PLoS Med* 2007, **4**:1669-1679 discussion 1680.

14. Lemmon MA, Schlessinger J: **Cell signaling by receptor tyrosine kinases**. *Cell* 2010, **141**:1117-1134.

15. Zhang X, Gureasko J, Shen K, Cole PA, Kuriyan J: **An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor**. *Cell* 2006, **125**:1137-1149.

16. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, Eck MJ: **Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity**. *Cancer Cell* 2007, **11**: 217-227.

17. Li J, Bennett K, Stukalov A, Fang B, Zhang G, Yoshida T, Okamoto I, Kim JY, Song L, Bai Y *et al.*: **Perturbation of the mutated EGFR interactome identifies vulnerabilities and resistance mechanisms**. *Mol Syst Biol* 2013, **9**:705.

18. • Petschnigg J, Kotlyar M, Blair L, Jurisica I, Stagljar I, Ketteler R: **Systematic identification of oncogenic EGFR interaction partners**. *J Mol Biol* 2017, **429**:280-294.

Systematic identification of novel mutant EGFR interaction partners using high-throughput protein–protein interaction screening system.

19. •• Lee HW, Choi B, Kang HN, Kim H, Min A, Cha M, Ryu JY, Park S, Sohn J, Shin K *et al.*: **Profiling of protein-protein interactions via single-molecule techniques predicts the dependence of cancers on growth-factor receptors**. *Nat Biomed Eng* 2018, **2**:239-253.

This study demonstrated the capability of single-molecule co-IP analysis to profile protein–protein interactions from various systems. The authors revealed that the strength of protein–protein interaction is strongly correlated to the dependency of cancer on that signaling in individual cancers.

20. Yang S, Qu S, Perez-Tores M, Sawai A, Rosen N, Solit DB, Arteaga CL: **Association with HSP90 inhibits Cbl-mediated down-regulation of mutant epidermal growth factor receptors**. *Cancer Res* 2006, **66**:6990-6997.

21. Padron D, Sato M, Shay JW, Gazdar AF, Minna JD, Roth MG: **Epidermal growth factor receptors with tyrosine kinase domain mutations exhibit reduced Cbl association, poor ubiquitylation, and down-regulation but are efficiently internalized**. *Cancer Res* 2007, **67**:7695-7702.

22. Shimamura T, Li D, Ji H, Haringsma HJ, Liniker E, Borgman CL, Lowell AM, Minami Y, McNamara K, Perera SA *et al.*: **Hsp90 inhibition suppresses mutant EGFR-T790M signaling and overcomes kinase inhibitor resistance**. *Cancer Res* 2008, **68**:5827-5838.

23. Hendriks LEL, Dingemans AC: **Heat shock protein antagonists in early stage clinical trials for NSCLC**. *Expert Opin Investig Drugs* 2017, **26**:541-550.

24. Sawai A, Chandarlapaty S, Greulich H, Gonen M, Ye Q, Arteaga CL, Sellers W, Rosen N, Solit DB: **Inhibition of Hsp90 down-regulates mutant epidermal growth factor receptor (EGFR) expression and sensitizes EGFR mutant tumors to paclitaxel**. *Cancer Res* 2008, **68**:589-596.

25. Trepel J, Mollapour M, Giaccone G, Neckers L: **Targeting the dynamic HSP90 complex in cancer**. *Nat Rev Cancer* 2010, **10**:537-549.

26. Pardoll DM: **The blockade of immune checkpoints in cancer immunotherapy**. *Nat Rev Cancer* 2012, **12**:252-264.

27. Leach DR, Krummel MF, Allison JP: **Enhancement of antitumor immunity by CTLA-4 blockade**. *Science* 1996, **271**:1734-1736.

28. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL *et al.*: **Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates**. *J Clin Oncol* 2010, **28**:3167-3175.

29. Wherry EJ: **T cell exhaustion**. *Nat Immunol* 2011, **12**:492-499.

30. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN *et al.*: **Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients**. *Nature* 2014, **515**:563-567.

31. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E *et al.*: **Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer**. *N Engl J Med* 2015, **373**:123-135.

32. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS *et al.*: **Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer**. *Science* 2015, **348**:124-128.

33. • Nero TL, Morton CJ, Holien JK, Wielens J, Parker MW: **Oncogenic protein interfaces: small molecules, big challenges**. *Nat Rev Cancer* 2014, **14**:248-262.

Review summarizing techniques discovering PPI inhibitors and examples of small-molecule inhibitors of cancer PPIs in the clinic.

34. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, Dayton BD, Ding H, Enschede SH, Fairbrother WJ *et al.*: **ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets**. *Nat Med* 2013, **19**:202-208.

35. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, Kipps TJ, Anderson MA, Brown JR, Gressick L *et al.*: **Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia**. *N Engl J Med* 2016, **374**:311-322.

36. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ: **BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis**. *Mol Cell* 2001, **8**:705-711.

37. Seymour JF, Kipps TJ, Eichhorst B, Hillmen P, D'Rozario J, Assouline S, Owen C, Gerecitano J, Robak T, De la Serna J *et al.*: **Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia**. *N Engl J Med* 2018, **378**:1107-1120.

38. Fischer K, Al-Sawaf O, Bahlo J, Fink AM, Tandon M, Dixon M, Robrecht S, Warburton S, Humphrey K, Samoylova O *et al.*: **Venetoclax and obinutuzumab in patients with CLL and coexisting conditions**. *N Engl J Med* 2019, **380**:2225-2236.

39. Jain N, Keating M, Thompson P, Ferrajoli A, Burger J, Borthakur G, Takahashi K, Estrov Z, Fowler N, Kadia T *et al.*: **Ibrutinib and venetoclax for first-line treatment of CLL**. *N Engl J Med* 2019, **380**:2095-2103.

40. Aebersold R, Mann M: **Mass spectrometry-based proteomics**. *Nature* 2003, **422**:198-207.

41. Dunham WH, Mullin M, Gingras AC: **Affinity-purification coupled to mass spectrometry: basic principles and strategies**. *Proteomics* 2012, **12**:1576-1590.

42. Rush J, Moritz A, Lee KA, Guo A, Goss VL, Spek EJ, Zhang H, Zha XM, Polakiewicz RD, Comb MJ: **Immunoaffinity profiling of tyrosine phosphorylation in cancer cells**. *Nat Biotechnol* 2005, **23**:94-101.

43. Witze ES, Old WM, Resing KA, Ahn NG: **Mapping protein post-translational modifications with mass spectrometry**. *Nat Methods* 2007, **4**:798-806.

44. Zielinska DF, Gnad F, Wisniewski JR, Mann M: **Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints**. *Cell* 2010, **141**:897-907.

45. • Hein MY, Hubner NC, Poser I, Cox J, Nagaraj N, Toyoda Y, Gak IA, Weisswange I, Mansfeld J, Buchholz F *et al.*: **A human interactome in three quantitative dimensions organized by stoichiometries and abundances**. *Cell* 2015, **163**:712-723.
A comprehensive map of the human protein interactions using AP–MS in three quantitative dimensions.

46. Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, Colby G, Gebreab F, Gygi MP, Parzen H *et al.*: **Architecture of the human interactome defines protein communities and disease networks**. *Nature* 2017, **545**:505-509.

47. Barber KW, Muir P, Szeligowski RV, Rogulina S, Gerstein M, Sampson JR, Isaacs FJ, Rinehart J: **Encoding human serine phosphopeptides in bacteria for proteome-wide identification of phosphorylation-dependent interactions**. *Nat Biotechnol* 2018, **36**:638-644.

48. Carr S, Aebersold R, Baldwin M, Burlingame A, Clauser K, Nesvizhskii A, Working Group on Publication Guidelines for Peptide, Protein Identification Data: **The need for guidelines in publication of peptide and protein identification data: Working Group on Publication Guidelines for Peptide and Protein Identification Data**. *Mol Cell Proteomics* 2004, **3**:531-533.

49. •• Smith MA, Hall R, Fisher K, Haake SM, Khalil F, Schabath MB, Vuaroqueaux V, Fiebig HH, Altiok S, Chen YA *et al.*: **Annotation of human cancers with EGFR signaling-associated protein complexes using proximity ligation assays**. *Sci Signal* 2015, **8**:ra4.
Proximity ligation assay. They further showed that the amount of EGFR:Grb2 signals can be a predictive biomarker for the clinical outcome of EGFR-targeted therapy. This result is consistent with that of Ref. [19].

50. Jain A, Liu R, Ramani B, Arauz E, Ishitsuka Y, Ragunathan K, Park J, Chen J, Xiang YK, Ha T: **Probing cellular protein complexes using single-molecule pull-down**. *Nature* 2011, **473**:484-488.

51. Lee HW, Kyung T, Yoo J, Kim T, Chung C, Ryu JY, Lee H, Park K, Lee S, Jones WD *et al.*: **Real-time single-molecule co-immunoprecipitation analyses reveal cancer-specific Ras signalling dynamics**. *Nat Commun* 2013, **4**:1505.

52. Smith MA, Licata T, Lakhani A, Garcia MV, Schildhaus HU, Vuaroqueaux V, Halmos B, Borczuk AC, Chen YA, Creelan BC *et al.*: **MET-GRB2 signaling-associated complexes correlate with oncogenic MET signaling and sensitivity to MET kinase inhibitors**. *Clin Can Res* 2017, **23**:7084-7096.

53. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, Colaprico A, Wendl MC, Kim J, Reardon B *et al.*: **Comprehensive characterization of cancer driver genes and mutations**. *Cell* 2018, **173**:371-385.