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How do oncoprotein mutations rewire protein-protein interaction networks? Perspectives and techniques

Emily Bowler¹, Zhenghe Wang², and Rob M. Ewing^{1,*}

¹Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK

²Department of Genetics and Genome Science, Case Western Reserve University, Cleveland, Ohio 44106, USA

Abstract

The acquisition of mutations that activate oncogenes or inactivate tumour suppressors is a primary feature of most cancers. Mutations that directly alter protein sequence and structure drive the development of tumours through aberrant expression and modification of proteins, in many cases directly impacting components of signal transduction pathways and cellular architecture. Cancer-associated mutations may have direct or indirect effects on proteins and their interactions and whilst the effects of mutations on signalling pathways have been widely studied, how mutations alter underlying protein-protein interaction (PPI) networks is much less well understood. Systematic mapping of oncoprotein protein interactions using proteomics techniques as well as computational network analyses is revealing how oncoprotein mutations perturb PPI networks and drive the cancer phenotype.

Keywords

protein-protein interaction; mutation; cancer; network

Overview

There is increasing interest in understanding molecular networks in biological systems both from a fundamental perspective and for improved targeting and efficacy of therapeutics (Csermely et al., 2013). Protein-protein interaction (PPI) networks are of particular interest because of their intrinsically functional nature; interactions between proteins assemble proteins into functional protein complexes or link them into pathways and larger networks, and these higher-order assemblages of proteins are at the heart of most cellular processes. Significant efforts have been made to map PPI networks, using both computational and experimental approaches. Experimental approaches such as yeast two-hybrid and mass-spectrometry proteomics have been applied on a large-scale to map thousands of protein-protein interactions (Gavin et al., 2002; Rolland et al., 2014; Rual JF Venkatesan K et al., 2005). In most cases, however, these studies have been conducted under standard laboratory conditions, in which the dynamic nature of PPI networks is not considered (Ideker and

*For correspondence: rob.ewing@soton.ac.uk, Phone: +44(0)2380594401.

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Krogan, 2012). Environmental signals, tissue-type and genotype (e.g. mutation status) are some of the important features that determine cellular “context”, and the state and structure of PPI networks. In cancer, somatic mutations are important determinants of tumour phenotype. Although tumour genome sequencing is rapidly cataloguing the heterogeneity of somatic mutations across different cancers, we know very little about the molecular effects of the vast majority of these mutations. Somatic mutations that affect protein function may result in stabilization or destabilization, altered interaction interfaces and/or alter sub-cellular localization of the mutant protein. Gain or loss of interaction partners is an important consequence of protein mutations, and in turn may result in ‘rewiring’ of PPI networks, with associated alteration in cellular behaviour. For example, although p53 is best known as a tumour suppressor, certain mutations promote p53 oncogenic properties (Muller and Vousden, 2013). These gain-of-function effects may be mediated via altered specificity or affinity for interaction partners, as illustrated by a recent study showing how an interaction between mutant p53 and Pontin (RUVBL1) promotes invasion and migration of tumour cells (Zhao et al., 2015). Similarly, a proteomic comparison of the interaction partners of mutant p53 with wild type identified several new mutant p53 partners, one of which, nardilysin, was shown to promote invasiveness (Coffill et al., 2012). To facilitate the identification of oncoprotein mutant-specific interaction partners, we developed a proteomic approach using cancer cell lines with endogenously epitope-tagged oncoproteins (Song et al., 2012). We used this technique to show how oncogenic variants of the p100 α subunit of PI3K interact with insulin receptor substrate I (IRS1), and how mutant β -catenin interacts with DNA methyltransferase I (Dnmt1), in both cases the mutant interactions activate downstream signalling pathways (Hao et al., 2013; Song et al., 2015). These examples illustrate how analysis of protein-protein interaction analysis is revealing the altered interaction profiles of oncoproteins, and how in turn this may promote tumorigenesis. In this review, we focus on interaction proteomics, rather than other types of proteomic experiment that can be used to delineate PPI networks (e.g. phosphoproteomics), and discuss how oncoprotein and other cancer-associated mutations alter proteins, their interactions and PPI networks.

Interaction proteomics techniques: large-scale approaches

Binary interactions between pairs of proteins of interest have long been studied using the yeast two-hybrid (Y2H) technique (Vidal and Fields, 2014), and recent large-scale applications of Y2H have enabled the construction of reference human interaction networks (Rual JF Venkatesan K et al., 2005). Applications of Y2H have contributed enormously to our understanding of oncoprotein PPI networks by identifying interaction partners for known oncoproteins (Stelzl et al., 2005) or by focusing on systematically mapping PPI networks that define specific cancer-relevant pathways such as the MAP kinase pathway (Bandyopadhyay et al., 2010). Although Y2H provides a powerful means of analysis of protein interaction networks, interactions are by necessity identified in yeast cells rather than in a cancer-relevant context, and it cannot therefore provide a view of the complexity of the proteome in an endogenous cellular environment. This drawback has been somewhat mitigated by the development of similar methods in mammalian cells (Lievens et al., 2009). For example, a recent application of a mammalian two-hybrid assay showed how changes in

the interaction partners of wild type and oncogenic variants of Epidermal Growth Factor Receptor could be detected (Petschnigg et al., 2014).

Mass-spectrometry proteomics methods are a highly complementary technique to Y2H for large-scale PPI analysis. Affinity-Purification Mass-Spectrometry (AP-MS) is the method-of-choice for focused analysis of oncoproteins and their interaction partners. In AP-MS, 'bait' proteins of interest are isolated from biological samples using antibodies and then associated 'prey' proteins identified and quantified by mass-spectrometry (Dunham et al., 2012). In principal, antibodies with high affinity and specificity for the bait proteins can be used to affinity-purify associated protein complexes under physiological conditions. However, such antibodies are not available for most proteins and generation of high quality antibodies is time-consuming, expensive, and often not successful. More pragmatically, bait proteins are typically epitope-tagged, expressed in cultured human cells, and then associated protein complexes recovered using an antibody against the epitope-tag (Dunham et al., 2012). This approach will be especially important for the analysis of oncoprotein PPIs since antibodies specific to different mutant or variant alleles of a target oncoprotein are typically unavailable. AP-MS has been widely used to analyse PPI networks associated with human disease. For example, we previously used AP-MS to survey protein-protein interactions of 338 epitope-tagged bait proteins in human cells (Ewing et al., 2007). Most of the selected bait proteins have known disease associations, and 15% of them correspond to known tumour suppressors or oncoproteins in the Tumor Associated Gene database (Chen et al., 2013). AP-MS has also been widely used to map PPI networks for specific diseases (for example, identification of interaction partners for proteins linked to vascular conditions in the brain (Goudreault et al., 2009).

Whilst large-scale approaches such as Y2H and AP-MS provide "reference" PPI networks, they are often limited by the biological context in which the experiments are performed. Cancer is context-sensitive; the state of the cell in terms of its genotype (mutation landscape) as well as its immediate environment and most importantly, the interaction between these features, are critical determinants of its phenotype. Proteomic and transcriptomic analyses of clinical samples (e.g. tumours) has contributed enormously to understanding the complexities of the cancer cell state, however, pinpointing the specific effects of individual oncoprotein mutations in the resulting data is challenging, in part due to the genotypic complexity of the samples in which a multitude of mutations may contribute to the phenotype. Engineered cell-lines provide a more controlled system for analysing oncoprotein PPI networks when used in conjunction with quantitative, phospho-, or interaction proteomics analyses. For example, proteomic analyses of the network-level effects of the APC tumour suppressor have been performed using an isogenic pair of APC-null and APC-expressing cell-lines (Halvey et al., 2012). Similarly, an integrated protein-expression and phospho-proteomic approach was used to analyse isogenic cells edited with different KRAS mutations to reconstruct allele-specific PPI networks (Hammond et al., 2015).

An important experimental consideration for AP-MS experiments is the levels and methods of expression of the bait protein. Experimental systems that ensure expression of bait proteins at endogenous levels are to be preferred and several methods for achieving this

have been demonstrated. For example, BAC clone recombineering has been used to engineer epitope-tags into the target ORF so that flanking regulatory regions are preserved. These clones are then transfected into the desired cells and AP-MS experiments performed (Poser et al., 2008). Alternatively, the genome of target cells (e.g. cancer-cell lines or primary cells) may be directly engineered to knock-in epitope-tags at loci of interest. This method has been applied to identify protein phosphatase (PPP1CC2) interaction partners in embryonic stem cells (MacLeod and Varmuza, 2012), and previously by us to identify oncoprotein interaction partners in colorectal cancer cells (Song et al., 2012; Zhang et al., 2008). The singular advantage of “knock-in AP-MS” is that specific alleles may be epitope-tagged thus providing a technique for analysing allelic-specific (e.g. mutant) PPIs. We previously used this technique to identify mutation-specific partners for the p110 α subunit of PIK3CA, and showed that two mutations with different mechanisms of action and oncogenic properties also interacted differentially with their downstream partner, IRS1 (Hao et al., 2013). These types of techniques will likely become increasingly important as the field of proteomics moves towards understanding how biological context and cellular state impact PPI networks (Ideker and Krogan, 2012).

Constructing PPI networks

Assembly of protein-protein interactions into PPI networks is a pre-requisite for understanding the effects of mutations at the network-level, and significant effort has gone into developing tools and methodologies as the number of identified protein-protein interactions has increased. PPI networks are typically constructed as weighted or unweighted graphs with nodes representing individual proteins, and edges representing the interactions between proteins (Pržulj, 2011). Using this formulation, PPI networks can then be analysed using graph-theoretic concepts such as node degree which measures the number of edges connected to a node, clustering, which measures the interconnectivity of that node, and betweenness, which measures the centrality, or the number of shortest paths that transect a node (Koyutürk, 2010). An important question has been to understand what the biological correlates are of these network features. For example, nodes with high degree represent hub proteins in the network, and sets of highly connected nodes may represent a functional module or protein complex (Gulati et al., 2013). The effects of mutations on PPI networks, have been classified as “node removal” events, whereby a mutation would cause the loss of a protein and its associated edges in the network (e.g. a null mutation) or alternatively “edgetic” mutations, whereby edges are selectively removed from the network, corresponding to mutations that impact specific interaction interfaces but not others (Zhong et al., 2009).

A relatively new development in the field of PPI network analysis is to integrate the increasingly large volumes of protein structural information to create ‘3D’ PPI networks (Das et al., 2013; Lu et al., 2013). This is particularly relevant for understanding how mutations impact PPI networks, since it places detailed structural descriptions of mutant proteins in their network context. By integrating gene-disease associations and known mutations with 3D PPI networks, it was found that certain classes of disease-associated mutations are enriched on interaction interfaces (Wang et al., 2012). Furthermore, mutations on corresponding interaction interfaces of interacting proteins were significantly more likely

to be linked to the same disease, and mutations of different interfaces of the same protein were less likely to cause the same disease than mutations on the same interface, thus providing molecular explanations for the phenomena of gene pleiotropy and locus heterogeneity (David et al., 2012; Wang et al., 2012). A long-standing challenge in constructing PPI networks has been to discriminate low confidence (or false positive) protein-protein interactions from bona fide ones. Homologous, co-crystal, and predicted protein structures, can all add to the confidence of mapped interactions by assessing their actual or predicted solvent accessible areas and therefore the likelihood of interactions between proteins (Aloy and Russell, 2002; Lu et al., 2013). Using computational approaches to combine functional and structural data can be used to classify interactions according to their confidence, and these classifiers have prediction accuracy as high as experimental data in both yeast and human (Zhang et al., 2012). Finally, 3D PPI networks also enhance the understanding of a protein's position and interactions within its environment, facilitating identification of central multi-domain proteins, commonly occurring interaction pairs and possible unspecific target affects (Blundell et al., 2006; Engin et al., 2012).

The effects of mutations on proteins and PPI networks

Mutations that alter protein structure may have consequences at different levels, from the function and structure of the individual protein, knock-on effects on interacting partner proteins, broader global effects through the PPI network, and ultimately phenotypic effects at the cellular level and beyond. Here we consider some of the important consequences of cancer-associated mutations on proteins and PPI networks. Primary protein sequence may be altered by mutations in diverse ways including substitution, insertion, deletion or truncation. In turn, this can have far-reaching effects on proteins, such as alterations of structural conformation, stability, and interactions. Although there is a huge body of knowledge detailing how mutations and variants alter protein structure, much less is known of the PPI network-level consequences of mutations.

Changes in protein stability are an important consequence of mutations by altering the abundance and sub-cellular localization of proteins and their associated interaction partners. β -catenin, the primary effector of Wnt signalling, is a well studied example of this phenomenon; mutations that alter or delete key serine residues in β -catenin allow β -catenin to escape phosphorylation and degradation by the destruction complex resulting in stabilization and aberrant accumulation of β -catenin (MacDonald et al., 2009). This promotes β -catenin accumulation in the nucleus, with concomitant activation of Wnt gene-expression programs (Liu et al., 2002; Morin et al., 1997; Valenta et al., 2012). Stabilization also alters β -catenin interaction partners. For example, we found that stabilized mutant β -catenin interacts with DNA methyltransferase I (Dnmt1) in the nucleus of colorectal cancer cells. This interaction promotes the stability of both proteins, is associated with new β -catenin interaction partners including lysine-specific demethylase I (LSD1), and impacts Wnt signalling activity and DNA methylation activity (Song et al., 2015). Importantly, mutations that constitutively activate or stabilize oncoproteins are likely to have more complex downstream activities that can be explained by simple activation of the associated signalling pathway (Kreeger and Lauffenburger, 2010). For example, analyses of the

network-level effects of oncogenic RAS mutations has revealed complex positive and negative feedback mechanisms (Kreeger et al., 2009).

Specific changes in physicochemical properties of substituted amino acids are an important determinant of the resulting effects. For example, large changes in amino acid charge typically resulting in protein destabilization, with the opposite true of small changes (Nishi et al., 2013). Mutational phenotypes may also be dependent on their destabilizing energy changes, as is the case with RASopathies. Mutations in the same 15 genes can result in phenotypes ranging from cancer to developmental disorders. (Kiel and Serrano, 2014) found that cancer-associated mutations had a higher energy change and were randomly distributed within the protein sequence, whereas mutations causing RASopathies were clustered in specific structural regions responsible for protein signalling activation. There may not be a simple, linear relationship between the immediate molecular effects of a mutation and the phenotypic or network-level consequences. Through analysis of mutations in yeast, it has been found that mutations that subtly alter binding properties of interacting partners actually may have more serious phenotypic consequences than knock-out mutations (PCNA) (Fridman et al., 2010).

Mutations that result in protein destabilization may not necessarily result in inactive or degraded protein, but rather in a dependence on other proteins for their stability. For example, wild-type B-Raf proteins do not require the stabilizing and folding activity of the chaperone Hsp90, whereas their cancer-associated mutant counterparts do (Grbovic et al., 2006). Indeed, mutant but not wild-type B-Raf, can be completely silenced by targeted inhibition of Hsp90 leading to cell cycle arrest and apoptosis (Grbovic et al., 2006). Finally, protein dimers, such as IDH1, can be stabilized by point mutations resulting in a change of residue accessibility that impairs ligand interaction, locking the dimer in an inactive conformation lacking catalytic activity (Pace et al., 2009; Zhao et al., 2009).

Mutations may occur in both functional and accessory regions of the protein, potentially directly affecting protein interactions through alteration of the favourability of electrostatic interactions (Nishi et al., 2013). Interaction site mutations can cause changes, such as hydrophobic destabilization, loss of electrostatic salt bridges, changes in the main-chain protein conformation, and formation of steric clashes (David et al., 2012).

An in depth understanding of the effects of mutations on protein structure and function requires significant investment of time and effort, and is available for relatively few proteins. High-throughput techniques such as next-generation sequencing of whole genomes are able to identify large numbers of mutations very rapidly. This is nowhere more apparent than in the field of cancer genomics, where sequencing of whole tumour genomes is rapidly identifying thousands of somatic mutations across diverse cancers (Forbes et al., 2011; Hammerman et al., 2012). For the vast majority of these mutations, their effects on protein structure, function and interactions are unknown. Cancer mutations may be classified as “drivers” or “passengers” where drivers are those mutations that provide selective growth or other advantage to cancer cells and passengers are neutral mutations that accumulate in cancer cells (Stratton et al., 2009), and several computational methods have been developed to classify mutations according to their functional effects. For example, the CHASM method

uses a machine learning method trained on multiple predictive features to specifically identify deleterious missense cancer mutations (Carter et al., 2009). CanPredict combines metrics that predict whether non-synonymous amino-acid substitutions are tolerant or intolerant, based upon evolutionary conservation with domain-based conservation and gene annotations to create a classifier for identification of deleterious mutations (Kaminker et al., 2007). Whilst these methods aim to identify mutations that alter protein function, they do not explicitly consider the effects of mutations on PPI networks. A recent study focusing on cancer-associated SH2 domain – phosphotyrosine interactions sought to predict the effects of mutations on these interactions as well as their more global effects at the network-level (AlQuraishi et al., 2014). The authors used a statistical mechanics framework to predict the effects of mutations on SH2 domain – phosphotyrosine residues by integrating experimental peptide-domain interaction as well as known PPI networks. Intriguingly, the effect of mutations on SH2-phosphotyrosine interactions was not correlated to the mutation frequency, suggesting that many low frequency somatic mutations in cancer may be functionally important.

Finally, there is considerable interest in understanding how the topology of PPI networks relates to biological function. For example, network hubs (highly connected proteins) have specific features in PPI networks, having been shown to be more essential, and with a higher likelihood of driver mutations in cancer (Jonsson and Bates, 2006; Rambaldi et al., 2008). Hub proteins may be further defined as ‘party’ hubs or as ‘date’ hubs. These categories were originally defined according to whether their interacting partners were co-expressed (party hubs) or incoherently expressed (date) (Han et al., 2004), although there has been considerable discussion about whether these categories represent real distinction in biological networks (Chang et al., 2013). They are at the very least a useful framework for considering the function of proteins in PPI networks, since hubs may also be viewed as either single-interface nodes or multi-interface nodes. Single-interface nodes typically bind transiently to a wide range of partners whilst multi-interface nodes are more likely to have high-affinity interaction partners and often are the central members of protein complexes (Kim et al., 2006). Clearly understanding how these topological network features relate to biological function is highly relevant to understanding how mutations affect PPI networks, since mutations of single-interface hubs might have significantly different impacts at the network level than multi-interface proteins.

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