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COMMENTARY

Molecular switches in signaling networks as a mechanism of action for oncogenic mutations in proximity of tyrosine residues

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ABSTRACT

We developed a mass spectrometry-based proteomics strategy to study oncogenic phosphotyrosine signaling networks in tissues. We outlined epidermal growth factor-dependent phosphotyrosine signaling in lung tissue and discovered that cancer mutations in vicinity of phosphotyrosine sites can induce molecular switches in recruited protein complexes, which ultimately alter the signaling outcome of the network activation.

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Auto-commentary

Tyrosine phosphorylation is a central post-translational modification that regulates multi-layered signaling networks. Dysregulation of these networks is intricately involved in cancer. Oncogenic mutations in the epidermal growth factor receptor (Egfr) are, e.g., prevalent in lung adenocarcinomas,[1] and tyrosine kinases have accordingly become prominent anti-cancer drug targets.[2,3] Functional investigations of the molecular consequences of aberrant wiring of phosphotyrosine signaling in pathophysiological states remain experimentally challenging, mainly due to the complexity of measuring multiple signaling layers. To facilitate investigations of oncogenic phosphotyrosine signaling networks, we recently developed a mass spectrometry-based proteomics strategy to investigate such networks directly in tissue samples. We applied the approach to study epidermal growth factor (Egf)-dependent phosphotyrosine signaling in rat lung tissue and outlined which phosphorylation sites are regulated as well as which proteins are recruited to phosphorylated sites. In this, we discovered that cancer mutations in vicinity of phosphotyrosine sites can induce molecular switches in recruited protein complexes that ultimately alter the signaling outcome of the network activation.[4] As a result, we presented molecular switches in protein complex assembly at phosphotyrosine sites as a mechanism of action for surrounding oncogenic mutations (Figure 1(a)).

The immediate signaling response elicited by tyrosine kinases induces changes in protein phosphorylation site stoichiometry. Next, the succeeding signaling layer engages the dynamical formation of protein-protein complexes at the regulated phosphotyrosine sites. This recruitment of adaptor proteins to phosphotyrosine sites is a critical part of the signaling network as it wires an assembly of protein complexes that each expands the signaling response. We developed a mass spectrometry-based proteomics strategy to resolve these two layers of phosphotyrosine signaling in tissues: quantitative analysis of phosphotyrosine sites and systematic examination of the dynamically regulated protein complexes they recruit. Phosphorylation site stoichiometry changes can be analyzed by quantitative mass spectrometry-based phosphoproteomics, which has previously been achieved in tissue samples for the much more abundant serine- and threonine-phosphorylation cascades.[5,6,7] Analysis of the less abundant, but functionally important, tyrosine phosphorylation sites requires a separate enrichment strategy based on phosphotyrosine-specific antibodies. With the experimental strategy we developed, we additionally determine the identity of recruited protein complexes to phosphorylated tyrosine residues. Synthesizing peptides corresponding to regulated phosphotyrosine sites and their six flanking amino acids along with a biotin tag and a hydrophilic linker, we use these peptides as baits in pull-down experiments in tissue lysates (Figure 1(b)).[4] Pulldowns are performed in a 96-well format and interacting proteins are enzymatically digested on-beads and subsequently analyzed by high-resolution liquid chromatography–tandem mass spectrometry (LC-MS/MS).

With our approach, we depicted the Egf-dependent phosphotyrosine signaling network in lung tissue and determined the protein complexes assembled at Egf-dependent phosphotyrosine sites.[4] It is well described, that adapter proteins are recruited to phosphotyrosine sites through specificity granted by the amino acid sequences surrounding the site.[8,9] This led us to ask, if the molecular consequence of cancer mutations near phosphorylated tyrosine residues may be linked to changes in protein complexes recruited to the phosphotyrosine site. We indeed confirmed this phenomenon. We found that the lung cancer mutation Egfr P1019L induces a switch in adaptor protein interactions at position pY1016, which leads

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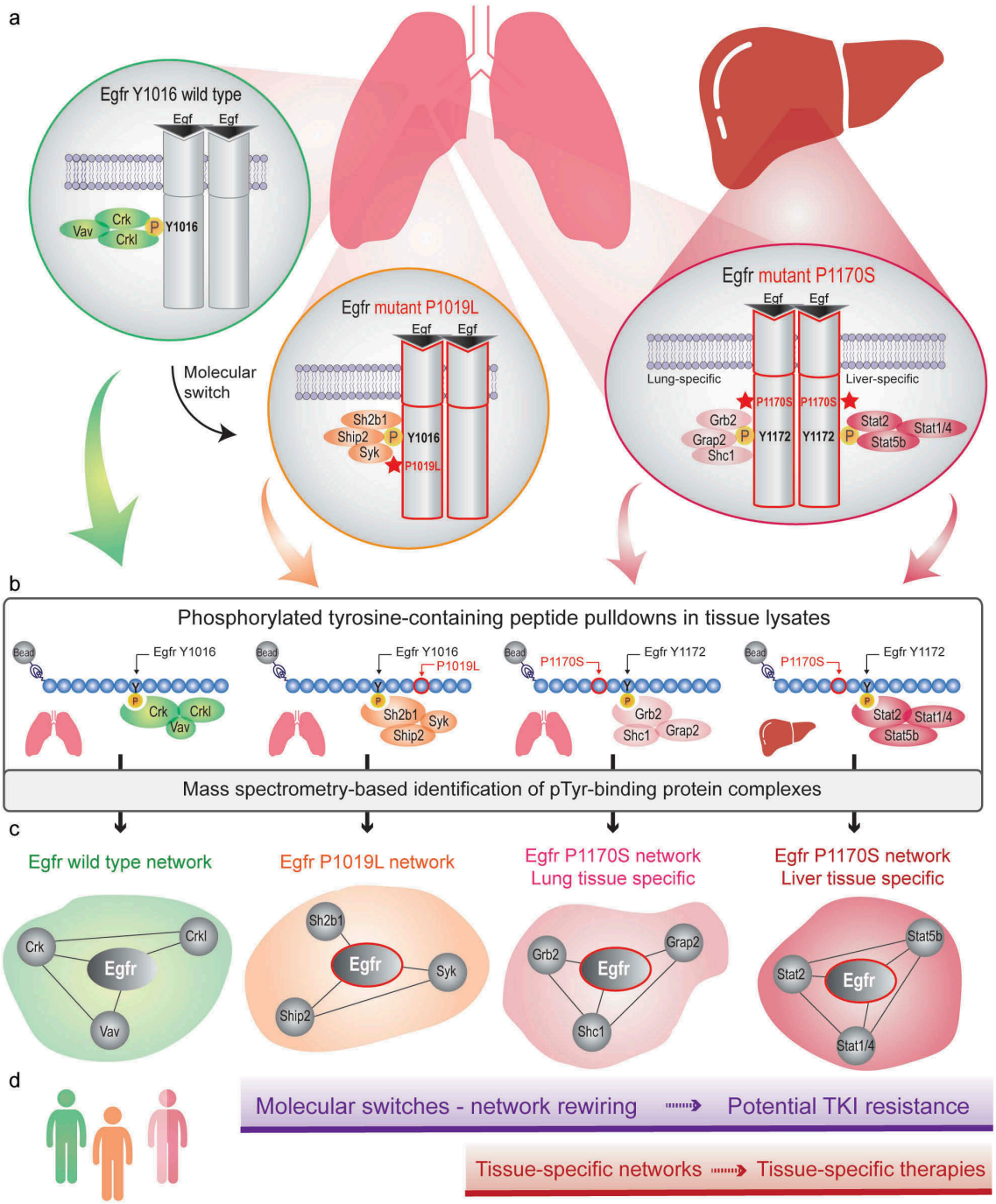

Figure 1. Mechanisms whereby oncogenic mutations near phosphorylated tyrosine residues introduce molecular switches in signaling networks. (a) Oncogenic mutations, such as P1019L (P; proline, L; leucine) and P1170S (S; serine) in epidermal growth factor receptor (Egfr), alter the protein complexes recruited to nearby tyrosine (Y) residues (Y1016 and Y1172, respectively) compared to wild type Egfr, when these are phosphorylated. The wild type complex includes proteins Vav, Crk and Crkl whereas mutant P1019L recruits proteins Sh2b1, Ship2 and Syk. Also, the protein complexes binding the phosphotyrosine site are tissue-specific as exemplified for P1170S. The lung-specific complex includes Grb2, Grap2 and Shc1 whereas the liver-specific complex includes Stat1/4, Stat2 and Stat5b. Data from a recently published paper by Lundby and colleagues.[4] (b) Experimental investigation of recruited protein complexes is achieved by peptide-based pulldowns exemplified for phosphorylated tyrosine-containing peptides (wild type and mutants as shown in (A)) and subsequent analysis by high-resolution mass spectrometry. (c) From the mass spectrometry-based proteomics strategy the protein signaling networks are determined for wild type and oncogenic mutations near phosphorylated tyrosine residues. The network components depend on the identity of the cancer mutations as well as tissue expression. (d) The molecular switches, e.g., signaling networks alter the ultimate signaling outcome and hence may affect response to tyrosine kinase inhibitor (TKI) treatment. Knowledge of such altered signaling activity may be used to generate personalized treatment strategies.

to sustained activation of downstream kinase signaling pathways ultimately resulting in enhanced cell migration and invasiveness.[4] We identified another eight cancer mutations in vicinity of phosphotyrosine sites and demonstrated that their detrimental effect can alike be explained by the introduction of molecular switches that alter protein signaling networks. By detailed investigation of single amino acid cancer mutations located near regulated phosphotyrosine sites we showed that these mutations induce a switch in the signaling response by altering the recruitment of protein binding

partners to a completely different protein complex upon receptor activation (Figure 1(c)). That is, we discovered a concept of how oncogenic mutations in vicinity of phosphotyrosine sites introduce a molecular switch that rewires intracellular signaling cascades. This finding presents a new mechanism linking dysregulated phosphotyrosine signaling to cancer.

The methodology we developed illustrates how quantitative phosphotyrosine interaction proteomics can be deployed to assign novel functions to regulated phosphorylation sites in a large-scale manner exemplified by Egf-signaling in rat lung tissue. We performed more than 1,000 peptide-based pulldowns in tissue lysates and analyzed them using a 'data-independent acquisition' (DIA) approach and cutting-edge mass spectrometry instrumentation holding the potential to be applied to functionally evaluate patient cancer mutations. To stress the potential of the technology in a clinical context with regards to identifying altered signaling of patient cancer mutants both at large scale and in a rapid fashion, we streamlined the methodology to utilize the latest advancements in mass spectrometry, which enables analysis of 60 peptide pulldown experiments in just one day. Our finding of altered protein complex signaling by cancer mutations suggests mutation-specific possibilities for kinase inhibitor treatment strategies, e.g. to bypass resistance to tyrosine kinase inhibitor (TKI) treatment (Figure 1(d)). Investigation of these molecular switches allows for identification of tissue-specific signaling pathway re-wiring induced by cancer mutations, and has as such the potential to contribute to tissue- and patient-specific therapies. The outlook of the developed strategy is an application in a clinical context where results can be used to determine a strategy for personalized medicine for cancer patients.

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