Quantitative proteomics for elucidating protein-protein interactions and pathways

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Outline

- SILAC for quantitative proteomics
- Characterizing protein complex association with an adapter protein, Odin, in EGFR signaling
- Signaling pathways activated by TSLPR, a receptor involved in the pathogenesis of asthma and acute lymphoblastic leukemias

SILAC for in vivo labeling

- Simple
- Does not require any extra processing steps
- All proteins are uniformly labeled
- Complete and predictable incorporation
- Choice of labeled amino acids
- De novo sequencing can be performed
- www.silac.org

5-state SILAC experiment to study proteome dynamics



A Mass Spectrum From a 5-Plex Experiment



Protein Dynamics of >100 Proteins Measured by SILAC



Function of Odin in Growth Factor Signaling



Odin is a negative regulator of growth factor signaling



NIH3T3 fibroblasts





Primary mouse embryo fibroblasts treated with or without 20ng/ml PDGF-BB for 8h

Pandey et al., Oncogene, 2002 Kristiansen et al., DNA Research 2004

Odin undergoes tyrosine phosphorylation in receptor tyrosine kinase signaling





Reprobe: anti-FLAG

Identifying Odin Interactors Using SILAC



Odin is identified from Transfected Cells



Specific and non-specific Odin Interactors



Specific Odin Interactors

	Gene Symbol	Protein
1	YWHAG	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide (14-3-3 gamma)
2	YWHAZ	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta polypeptide (14-3-3 theta)
3	YWHAB	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide (14-3-3 beta)
4	YWHAH	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, eta polypeptide (14-3-3 eta)
5	YWHAE	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, epsilon polypeptide (14-3-3 epsilon)
6	SFN	Stratifin (14-3-3 sigma)
7	SH3KBP1	SH3-domain kinase binding protein 1
8	CD2AP	CD2-associated protein (CMS)
9	RASAL2	RAS protein activator like 2
10	YWHAQ	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, theta polypeptide (14-3-3 theta)
11	CAPZB	F-actin capping protein beta subunit
12	DAB2IP	DAB2 interacting protein
13	TLN2	Talin 2
14	GART	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase,
		phosphoribosylaminoimidazole synthetase
15	VAPA	Vesicle-associated membrane protein (VAMP)-associated protein A
16	ARHGAP10	Rho GTPase activating protein 10
17	HSPA9	Heat shock 70 kDa protein 9 (mortalin)
18	UACA	Uveal autoantigen

Validation of protein interactions



Known Interaction Network between Odin and its Interactors



Expanded Odin Interaction Network



Summary

- It is possible to derive 'clues' about the function of a protein from protein complex studies
- Odin is possibly involved in endocytosis pathway (guilt by association) but additional directed experiments need to be carried out

Not all protein complexes are stable...

Thymic Stromal Lymphopoietin (TSLP)

- A novel IL-7-like cytokine
- Originally identified from a murine thymic stromal cell line
- Important in the pathogenesis of asthma
- Mutations recently identified in certain types of leukemias

Biological Functions of TSLP



TSLP receptor complex

- Consists of two receptor subunits
 - Interleukin 7 receptor alpha chain (shared with another cytokine, IL-7)
 - A unique receptor designated TSLPR



TSLP Signaling

- JAK family kinases shown not to be activated based on Western blotting data (lack of increased tyrosine phosphorylation in phosphotyrosine Western blots)
- Scientists in the field trying to identify the 'elusive' kinase(s)

TSLP signaling – a Western blot perspective



TSLP Signaling



IL-2 Signaling: Several Decades of Studies



How do we start to map the detailed circuitry in signaling pathways?

- Experimentally identify components of signaling pathways
- Determine the ordering of the components by biochemical experiments
- Determine the interactions among the components
- Mapping of enzymes and their substrates in signaling is a key step

Profiling of activated kinases: Identifying direct kinase substrates is difficult

- Kinase-substrate interactions are transient
- Stoichiometry of phosphorylation is low
- Analytical methods to identify phosphorylation sites are difficult and not comprehensive
- Establishing a protein as a direct substrate of a kinase is not trivial

TSLP requires IL-7R and TSLPR to transmit signals



TSLP requires at least one cytoplasmic tyrosine residue to transmit proliferative signals



SILAC-based pTyr and pSer/pThr proteome profiling



TSLP induces tyrosine phosphorylation of signaling molecules

Protein	Phosphopeptides with sites	Fold upregulation
STAT5A	AKAVDG <mark>pY</mark> VKPQIK	3.2
STAT5B	AKAADG <mark>pY</mark> VKPQIK	10.0
TEC	YVLDDQ pY TSSSGA	1.4
ВТК	HYVVCSTPQSQ <mark>pY</mark> YLAEK	1.4
SHIP1	L <mark>pY</mark> DFVKTERDESSGMK	2.0
	EVGD pY GQLHKTEVLLK	1.4
JAN2	REVGD <mark>pY</mark> GQLHK	1.8
FcRγ	SQET <mark>pY</mark> ETLK	1.8
FYB	TTAVEIDpYDSLK	2.0
SHP2	IQNTGD <mark>pY</mark> YDLYGGEK	1.8
	EKAEERPTFDYLQSVLDDFYTATEGQpYQQQP	0.5
	SLDNGG <mark>pY</mark> YISPR	1.5
ERK1	IADPEHDHTGFLTE pY VATR	1.3
CDK3	VEKIGEGT <mark>pY</mark> GVV pY K	1.0
GSK3A	GEPNVSpYICSR	1.0

Phosphorylation changes in Lyn reflect activation



Src family kinase inhibitor, SU6656, inhibits TSLP-induced cell proliferation



Bruton's tyrosine kinase



BTK kinase inhibitor, LFM-A13, inhibits TSLP-mediated proliferation



JAK2 inhibitor inhibits TSLP-mediated proliferation



JAK2 inhibitor inhibits TSLP-induced Stat5 phosphorylation



A partial list of serine/threonine phosphorylated peptides identified from SCX fractionation experiments

Protein Name	Phosphopeptides	Fold upregulation with TSLP treatment
Pumilio homolog 2	pTPGSRQA <mark>pS</mark> PTEVVER	4.3
Programmed cell death protein 5	KVMD <mark>pS</mark> DEDDADY	10.0
Protein transport protein Sec61 subunit beta	PGPTPSGTNVG <mark>pS</mark> SGR <mark>pS</mark> PSK	6.6
60S ribosomal protein L8	GAG <mark>pS</mark> VFR	4.5
Plectin1	SS pS VGSSSSYPISSAGPR	5.5
Heterogeneous nuclear ribonucleoprotein A3	SSGSPYGGGYG pS GGGSGGYGSR	5.2
Programmed cell death protein 4	FV <mark>pS</mark> EGDGGR	3.8
Rho guanine nucleotide exchange factor 1	pSESLRVpSDR	6.7
FcRγ	SQE <mark>pT</mark> YETLK	2.5
Nucleolar RNA helicase 2	SNSSDAPGEE <mark>pS</mark> SpSETEKEIPVEQK	1.0
Eukaryotic translation initiation factor 4E transporter	SS <mark>pS</mark> PVGLAK	1.0

TSLP Signaling: BEFORE



TSLP Signaling: AFTER



Lessons Learnt

- Exercise caution in interpreting negative data 'not detectable' is not the same as 'not occurring in cells'
- It is possible to take unbiased discovery approaches to dissect signaling pathways in a global fashion using quantitative proteomics
- Activated kinases identified using this proteomic approach are candidate therapeutic targets in asthma and leukemias

NetPath

Net	Path	PRKCA UNNO SPT GRIM19	EGFR GRB2 RGS16 MIG6 MIG6 PRKAR1A		
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			TGF beta Receptor	T Cell Receptor	
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			 Alpha6 Beta4 Integrin 	• IL2	
			• ID	• IL3	
			 Hedgehog 	• IL4	
			• Notch	• IL5	
			• Wnt	• IL6	
			• AR	• IL7	
			Kit Receptor	• IL9	
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Vimentin – tissue expression from *Human Proteinpedia*

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