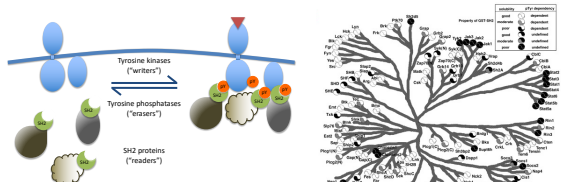


1048 SH2-Flow: A Multiplex Single Cell Phosphotyrosine Profiling Tool for B-cell Malignancies



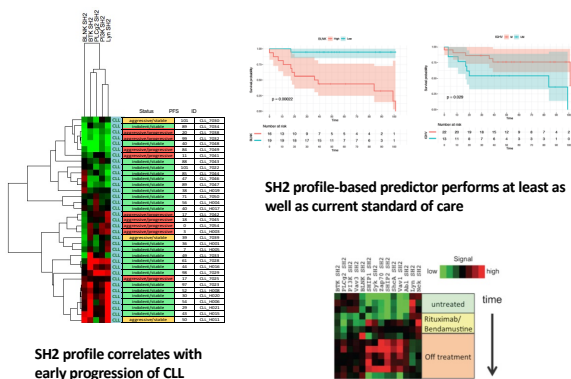
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SH2 Profiling



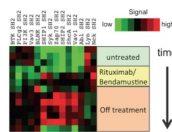
- Cells use SH2 domains to interpret changes in tyrosine phosphorylation
- Humans have 120 SH2 domains in 110 proteins
- Purified bacterially expressed SH2 domains can be used to quantify their binding sites in cell lysates or whole cells
- Quantitative pattern of binding sites for panel of SH2 domains provides a "snapshot" of tyrosine kinase mediated signaling in a cell

SH2 profiling in human CLL predicts clinical outcomes



SH2 profile correlates with early progression of CLL

SH2 profile-based predictor performs at least as well as current standard of care



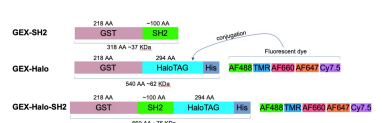
SH2 profile changes over treatment history

Barriers to clinical implementation of SH2 Profiling:

- Current platforms are labor-intensive and slow
- Requires relatively large amount of material
- No single-cell data (averages over population)

GOAL: Flow cytometry based SH2 profiling platform

Generating a panel of fluorescently labeled SH2 domain probes for flow cytometry

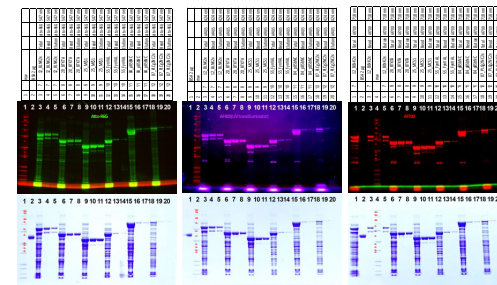


Probe Design:

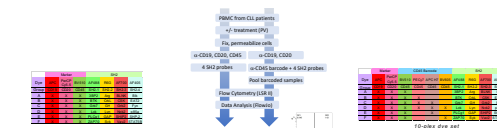
- SH2 domains expressed in *E. coli* as fusions with GST and Halo Tag
- GST for purification on GSH beads, and also for dimerization
- Halo Tag for labeling with Halo linker conjugated with fluorescent dyes → stoichiometric labeling

#	SH2	Gene symbol	Gene ID	Protein function
1	GST-Halo	na	na	Control
2	Arg	ABL2	27	Tyrosine kinase
3	BLK	BLK	640	Tyrosine kinase
4	Bcr	BLNK	25760	Adaptor
5	BRD01	STAP1	26229	Adaptor
6	Erk	ERK	655	Tyrosine kinase
7	CNA	CEL	887	Ubiquitin ligase
8	Ctk	CRK	1398	Adaptor
9	Csk	CSK	1399	Adaptor
10	Csk	CSK	1445	Tyrosine kinase
11	Src	SH2B3	112152	Adaptor
12	FynA	FYN	2534	Tyrosine kinase
13	Gib2	GRB2	2885	Adaptor
14	Gip7	GIP7	2896	Adaptor
15	Hck	HCK	3055	Tyrosine kinase
16	Lck	LCK	3052	Tyrosine kinase
17	Lyn	LYN	4367	Tyrosine kinase
18	Nck1	NCK1	4920	Adaptor
19	Nck2	NCK2	8440	Adaptor
20	p58a	PKC3R1	5295	Phosphoinositide kinase
21	PLC1	PLC1	5335	Phospholipase
22	PLC2	PLC2	5336	Phospholipase
23	RasGAP	RASA1	5921	GTPase activating protein
24	Shb102	SHB2	6452	Adaptor
25	ShcA	SHC1	6484	Adaptor
26	Shp1	INPP5D	3635	Inositol phosphatase
27	Shp2	INPP4	3636	Inositol phosphatase
28	Shp1	PTPNB	5777	Tyrosine phosphatase
29	Shp2	PTPNB1	5781	Tyrosine phosphatase
30	Stat1	STAT1	6772	Transcription factor
31	Stat3b	STAT3B	6777	Transcription factor
32	Syk	SYK	6959	Tyrosine kinase
33	Tec	TEC	7006	Tyrosine kinase
34	Vav1	VAV1	7459	Guanine nucleotide exchange factor
35	Vav2	VAV2	7410	Guanine nucleotide exchange factor
36	Vav3	VAV3	10451	Guanine nucleotide exchange factor
37	Zap70	ZAP70	7035	Tyrosine kinase

BCR SH2 Panel for SH2-Flow and other pTyr-SH2 Profiling

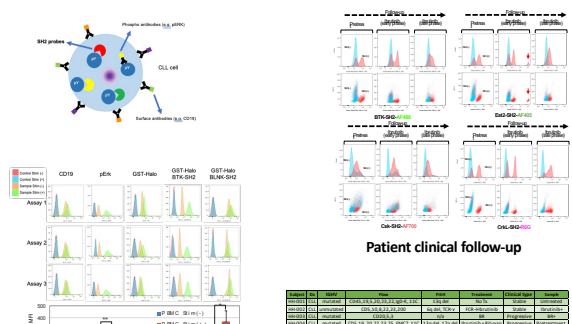


Crude bacterial lysates can be labeled and purified easily in multiplex format

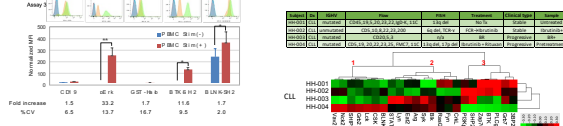


Bar-coding increases the number of SH2 domains per experiment

Multiplex Single Cell Phosphotyrosine-SH2 Profiling



Patient clinical follow-up



SH2-flow validation using single probes

Experimental variation: different aliquots of cells, labeling and flow on different days

Experimental variation: dye swap

Conclusions

- Single-cell SH2 profiling is feasible using recombinant fluorescently labeled SH2 domain probes
- Potential to analyze >50 SH2 domains in a single experiment
- Flow cytometry-based assays could easily be incorporated into standard diagnostic laboratory workflows for leukemia
- Signal strength in untreated cells needs to be optimized for some probes

Acknowledgements

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