Inverse ligand binding prediction provides insights into toxicity induced by Cyclosporine A

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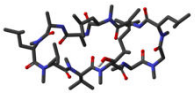
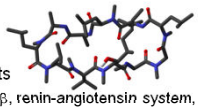
- Conclusions

Introduction



- Cyclosporin A (CSA)
  - cyclic nonribosomal peptide with 11 amino acids
  - immunosuppressant used in kidney, liver, bone marrow and pancreas transplantation; also used to treat heart failure, psoriasis, and rheumatoid arthritis
  - well understood mechanism of action
    - binds cyclophilin A & forms complex with calcineurin inhibiting its activity
    - this prevents dephosphorylation of nuclear factor of activated T-cells (NFAT), hampering their transit to the nucleus; thus immune response is shut down
  - also associated with severe side effects including nephrotoxicity, hepatotoxicity, and cardiotoxicity
    - mechanistic details behind these side effects remain unclear

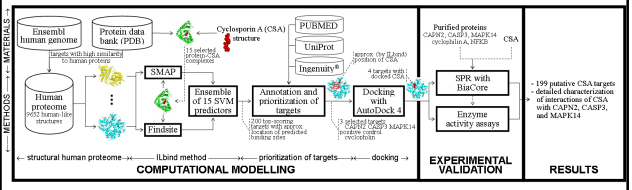
Introduction



- Cyclosporin A (CSA)
  - interacts with many protein targets
    - cyclophilin D, cyclophilin B, TGF-β, renin-angiotensin system, superoxide dismutase, etc.
  - these off-target interactions could be involved in the activation of signaling pathways that lead to the toxic responses
- we use inverse ligand binding predictions to perform structural human proteome-wide determination of putative binding partners of CSA
  - these putative interactions are linked to toxicities
  - selected three are investigated experimentaly in vitro

Pipeline

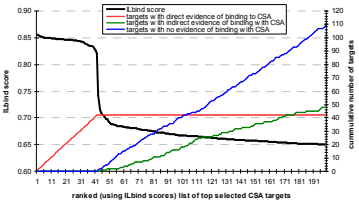
- Computational analysis

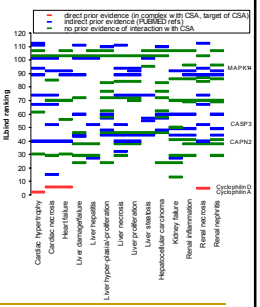


- Experimental validation
  - Surface Plasmon Resonance (SPR) with BiaCore to verify binding and assess binding kinetics
  - Enzyme activity assays

Results

- Analysis of putative targets predicted with ILbind





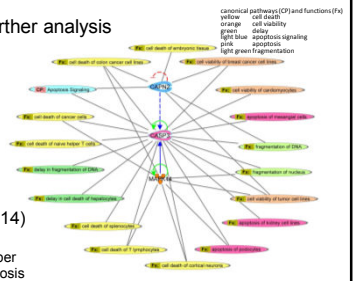
Results

- Analysis of putative targets predicted with ILbind

Top canonical pathways	*p*-values	# targets
IL-17 Signaling	8.51e-10	9
Acute Phase Response Signaling	9.31e-10	12
Dendritic Cell Maturation	1.48e-09	12
Top molecular and cellular functions	***p*-values**	**# targets**
Cell Death and Survival	3.91e-14 – 3.18e-04	64
Cellular Function and Maintenance	7.76e-14 – 2.52e-04	53
Cell Morphology	5.04e-11 – 2.76e-04	50
Cellular Movement	1.55e-10 – 2.95e-04	42
Lipid Metabolism	4.47e-10 – 2.46e-04	45
Top toxicity functions	***p*-values**	**# targets**
Hepatotoxicity		
Liver Necrosis/Cell Death	5.12e-10 – 1.93e-02	15
Liver Proliferation	7.24e-08 – 6.46e-03	12
Liver Inflammation	2.10e-06 – 2.10e-06	8
Liver Damage	6.43e-06 – 3.19e-02	9
Liver Hepatitis	2.36e-05 – 2.39e-01	8
Cardiotoxicity		
Cardiac Necrosis/Cell Death	3.02e-08 – 1.72e-01	13
Heart Failure	2.81e-05 – 1.88e-01	9
Cardiac Hypertrophy	8.03e-05 – 1.50e-01	13
Cardiac Proliferation	1.16e-04 – 1.16e-04	5
Cardiac Damage	6.90e-04 – 8.09e-02	4
Nephrotoxicity		
Kidney Failure	3.65e-08 – 6.46e-03	12
Renal Inflammation	3.66e-08 – 3.19e-02	13
Renal Nephritis	3.66e-08 – 3.19e-02	13
Renal Necrosis/Cell Death	3.06e-06 – 1.93e-02	14
Renal Fibrosis	2.38e-04 – 2.38e-04	4

Results

- Selection of targets for further analysis
  - calpain 2 (CAPN2)
    - calcium-dependent protease (breaks proteins)
  - caspase 3 (CASP3)
    - cysteine-aspartic acid protease
  - p38 mitogen-activated protein kinase 14 (MAPK14)
    - serine/threonine kinase that phosphorylates a number of proteins leading to apoptosis
  - involved in renal, cardiac and liver necrosis
  - linked to each other via apoptotic pathway

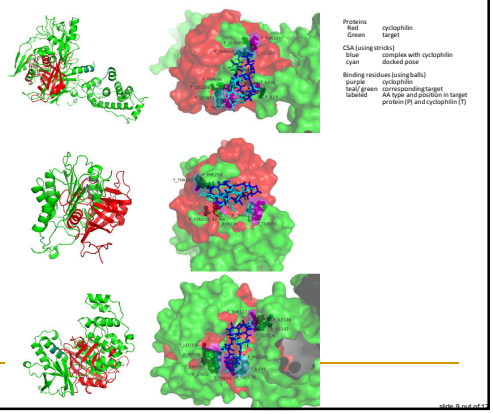


Results

CAPN2

CASP3

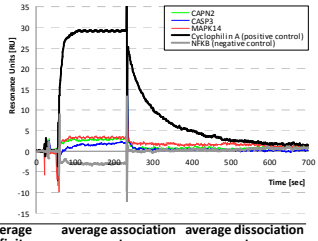
MAPK14



Results

- SPR validates interaction of CSA with CAPN2, CASP3, and MAPK14
- reproduces prior CSA results

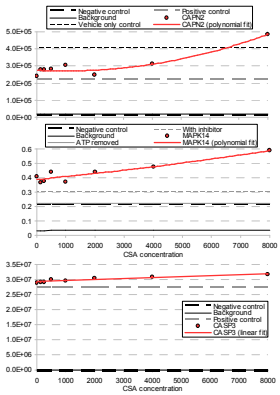
	prior	our
binding affinity	11	30
association rate	0.86	0.25
dissociation rate	10	6.6



Target type	Target	ILbind score	average affinity	average association rate	average dissociation rate
Positive control	Cyclophilin A	0.86	3.07E-08	2.53E+05 (fast)	6.60E-03 (medium)
Binding	CASP3	0.68	3.21E-04	2.36E+02 (slow)	2.73E-02 (fast)
Binding	CAPN2	0.67	3.22E-05	1.89E+04 (medium)	1.86E-03 (medium)
Binding	MAPK14	0.65	3.47E-05	4.01E+01 (slow)	1.04E-03 (medium)
Negative control	NFKB	0.58	N/A	N/A	N/A

Results

- CSA affects enzyme activity of CAPN2 and MAPK14
  - ~40% increase in CAPN2 activity with the addition of CSA
  - ~50% increase in MAPK14 activity with the addition of CSA



Conclusions

- Our unique and high-throughput approach successfully provides insights into side effects and mechanisms of action of small drugs that target proteins
- Putative targets of CSA were linked with an assortment of cellular functions, canonical pathways and toxicities that are typical for patients who take CSA
- Some of the toxic side effects of CSA may be mediated by direct binding of CSA to CAPN2, CASP3, and MAPK14
  - binding was shown experimentaly
  - binding with CAPN2 and MAPK14 changes enzymatic activity
  - treatment with CAPN2, MAPK14, and CASP3 inhibitors in combination with CSA treatment may eliminate some side effects