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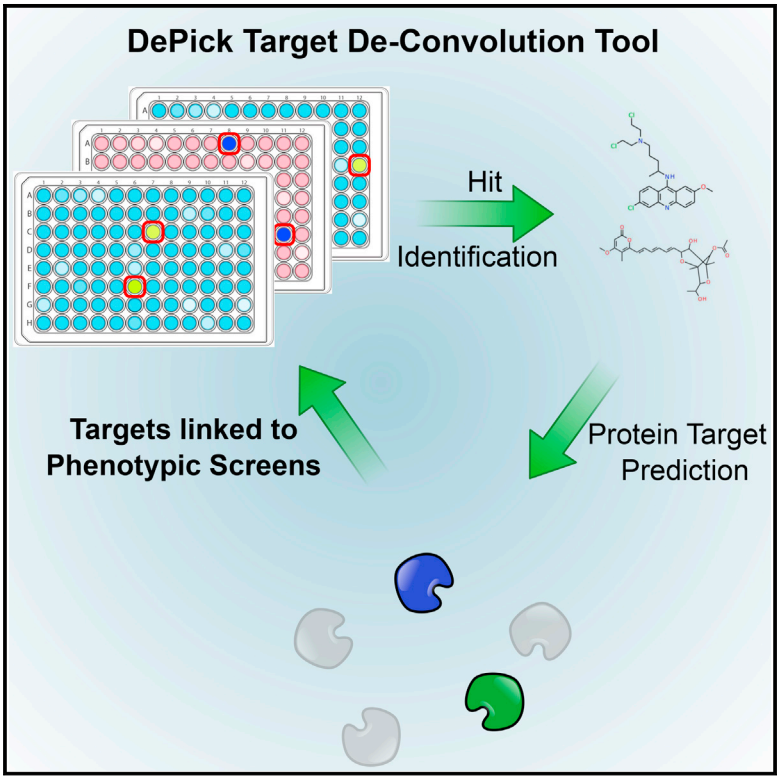
Cell Chemical Biology

Systematic Identification of Pharmacological Targets from Small-Molecule Phenotypic Screens

Graphical Abstract



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In Brief

Liu et al. present DePick, a novel target de-convolution approach to determine targets specifically linked to chemical phenotypic screens and illustrate that the application of DePick to public screens can expand the pharmacologically targetable molecular repertoire behind phenotypes with high efficiency.

Highlights

- DePick is a novel target de-convolution tool for chemical phenotypic screens
- DePick applied to 8 screens revealed 59 known and novel drug target-phenotype links
- DePick is publicly available at http://mips.helmholtz-muenchen.de/Depick/

Liu et al., 2016, Cell Chemical Biology *23*, 1302–1313
October 20, 2016
http://dx.doi.org/10.1016/j.chembiol.2016.08.011

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Please cite this article as: Liu et al., Systematic Identification of Pharmacological Targets from Small-Molecule Phenotypic Screens, Cell Chemical Biology (2016), http://dx.doi.org/10.1016/j.chembiol.2016.08.011

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Systematic Identification of Pharmacological Targets from Small-Molecule Phenotypic Screens

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http://dx.doi.org/10.1016/j.chembiol.2016.08.011

SUMMARY

Phenotypic drug discovery offers some advantages over target-based methods, mainly because it allows drug leads to be tested in systems that more closely model distinct disease states. However, a potential disadvantage is the difficulty of linking the observed phenotype to a specific cellular target. To address this problem, we developed DePick, a computational target de-convolution tool to determine targets specifically linked to small-molecule phenotypic screens. We applied DePick to eight publicly available screens and predicted 59 drug target-phenotype associations. In addition to literature-based evidence for our predictions, we provide experimental support for seven predicted associations. Interestingly, our analysis led to the discovery of a previously unrecognized connection between the Wnt signaling pathway and an aromatase, CYP19A1. These results demonstrate that the DePick approach can not only accelerate target de-convolution but also aid in discovery of new functionally relevant biological relationships.

INTRODUCTION

Target and phenotype-based high-throughput chemical screens are commonly used in the drug discovery field to detect chemical modulators of protein targets and disease phenotypes, respectively. Due to the limited success of target-based methods on the discovery of new medicines, the phenotypic high-throughput chemical strategy is re-emerging as a valuable drug discovery approach (Swinney and Anthony, 2011). The advantage of phenotypic methods over target-based approaches lies in the in vivo and more physiological conditions of the experiments, which allows distinct disease states to be modeled. This facilitates the discovery of chemical leads for disease treatment and presents chemical phenotypic screens as a promising technology for uncovering pharmacological targets of disease phenotypes. The development of publicly available tools to determine protein targets underlying readouts of chemical phenotypic screens, also called target de-convolution (Nijman, 2015) could thus rapidly expand the current druggable target-phenotype knowledge.

A target de-convolution approach requires two steps. The first consists of the identification of the interacting targets of active compounds in a screen (Gujral et al., 2014). Due to the polypharmacological property of compounds, that is, the well-known tendency of small molecules to bind multiple targets (Hopkins, 2008; Mestres et al., 2008; Paolini et al., 2006), a subsequent step is required. This involves the identification of the compound targets actually linked to the phenotype, since not all targets of a compound might be responsible for the phenotype.

Several strategies have been followed to determine targets of compounds, including direct biochemical, genetic interaction and computational inference methods (reviewed in Schenone et al., 2013). The identification of targets of compounds by experimental methods is a slow and arduous process, often restricted to a limited number of compounds with proven biological activity. In contrast, recently developed computational methods, including ligand and structured based prediction approaches (reviewed in Koutsoukas et al., 2011), allow prediction of targets for a large number of compounds. Ligand-based prediction methods such as the Similarity Ensemble Approach (SEA) (Keiser et al., 2007), SwissTargetPrediction (Gfeller et al., 2014), PASS (Poroikov et al., 2007), SOM-based prediction of drug equivalence relationships (SPiDER) (Reker et al., 2014), approaches using multiple-category Bayesian models (Nidhi et al., 2006), and support vector machines (Wale and Karypis, 2009) cluster compounds based on similarities in a multidimensional space of structure features. Structure-based methods including the docking approaches INVDOCK (Chen et al., 2003) and TarFisDock (Li et al., 2006) exploit protein structural information

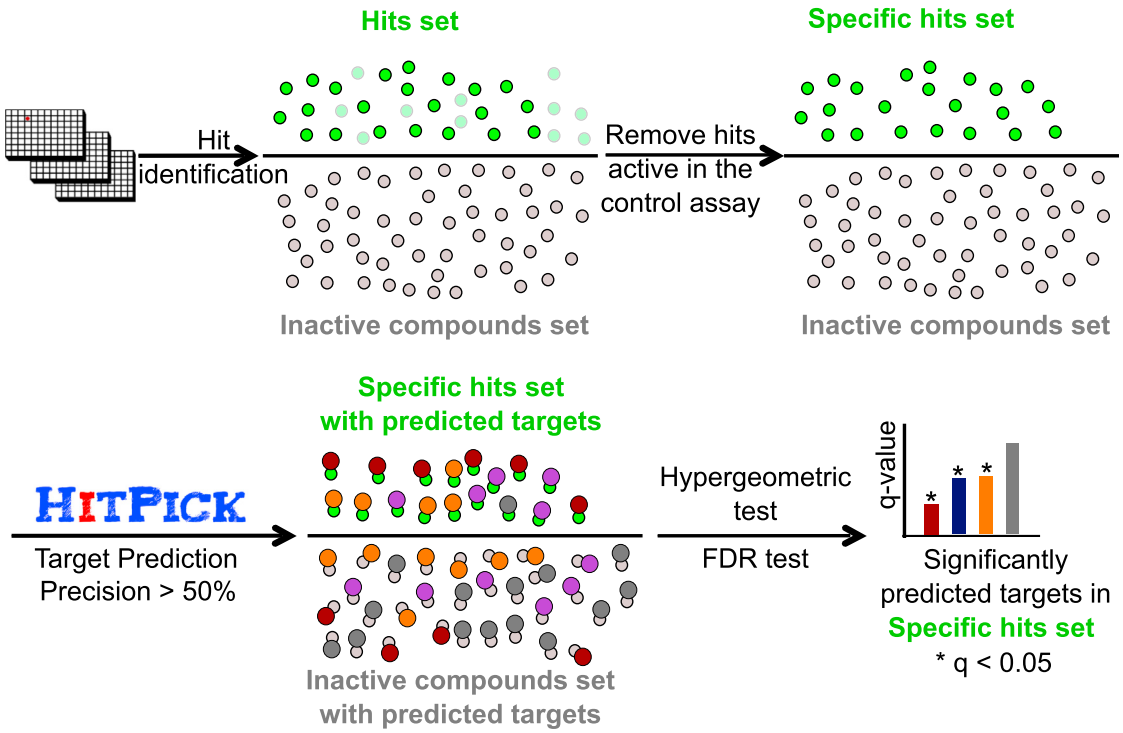

Figure 1. Steps of the DePick Method for the Identification of Drug Targets Associated with Phenotypic Screens

to predict ligand-target interactions. Other methods combine similarity methods such as TarFisDock (Li et al., 2006) and HitPick (Liu et al., 2013). An in silico target prediction approach followed by a statistical approach to determine targets linked to phenotypic screens offers huge potential for unraveling novel relationships between druggable targets and phenotypes in a fast and inexpensive manner, even for previously analyzed screens such as those stored in public repositories. Several of these target prediction methods have been applied to individual screens of anti-bacterial (Martínez-Jiménez et al., 2013), anti-malaria (Plouffe et al., 2008; Spitzmüller and Mestres, 2013), and anti-cancer activities (Flachner et al., 2012; Liggi et al., 2014; Lo et al., 2015) to propose links between pharmacological targets and phenotypes. However, a systematic analysis of the potential of de-convolution approaches to uncover relationship between targets and phenotypes has not yet been carried out.

To enable the systematic determination of protein targets linked to phenotypic screen readouts, we have developed a target de-convolution tool for phenotypic screens, DePick. This tool determines targets specifically linked to chemical phenotypic screens of mammalian organisms. DePick first identifies the compounds with specific activity on a screening assay and predicts their targets taking advantage of HitPick, an in silico target prediction method recently developed by our group (Liu et al., 2013). Then, it determines the predicted targets that are statistically enriched in the set of specific compounds.

In the last decade, an extensive number of chemical phenotypic assays have been deposited in public databases such as PubChem BioAssay (Wang et al., 2010) and ChemBank (Seiler et al., 2008), paving the way for a systematic analysis linking molecular and phenotypic information from phenotypic screens. Toward this aim, we have applied DePick to eight mammalian chemical phenotypic screens stored in the ChemBank repository where control screens were available, and therefore the specificity of the active compounds could be determined, and identified 59 target-phenotype relations. Manual curation of the scientific literature found direct support for 16 associations and strong indirect evidence for 20 additional connections. To reinforce the reliability of these predicted associations, we provide experimental support of the effect of pharmacological modulators of seven targets on the "Lipid Transfer" and "Wnt Inhibitors" screens. The results presented here confirm the feasibility of a target de-convolution approach applied to publicly available chemical phenotypic screens to confidently identify relationships between drug targets and phenotypes. Furthermore, the newly identified target-phenotype links open new therapeutic opportunities for diseases.

RESULTS

To facilitate the systematic determination of pharmacological targets linked to readouts of chemical phenotypic screens, we have devised a straightforward approach named DePick that detects the specific chemical hits of an assay (hereafter referred to as "selective hits"), confidently predicts targets for compounds using HitPick, and then identifies the predicted targets that are statistically associated with the specific hits of the screens (see Figure 1 and Experimental Procedures for details).

To test the capability of DePick to unravel drug target-phenotype links from chemical phenotypic screens, we applied our approach to chemical phenotypic assays from the ChemBank

Table 1. All the Predicted Targets of the Eight Assay Projects

Projects	Targets	Associations	Projects	Targets	Associations
Lipid Transfer	NCOA2	direct	E-Cadherin Synthetic Lethal	ATP1A1	direct
	RXRA	direct		ATP1B1	direct
	APP	indirect		CALM1	direct
	AGPAT2	indirect		ESRRG	direct
	FLT3	indirect		CALY	indirect
Wnt Inhibitors	MAOB	indirect		VDR	indirect
	ALDH2	indirect		TOP1	unexpected
	APP	direct		TOP2B	unexpected
	MCHR1	direct		Ribosome	unexpected
	ABL1	direct		CYP11A1	unexpected
	MTOR	direct		SLCO1A2	unexpected
	HMGCR	direct		SLCO1B3	unexpected
	CYP19A1	unexpected		SLCO1C1	unexpected
	CYP1B1	unexpected		SLCO4C1	unexpected
AR-NCoR Binding	APP	indirect	PGC1 Expression (Pre-adipocytes)	Ribosome	direct
	TUBB	indirect		TUBB	indirect
	TUBA4A	indirect		JUN	indirect
	ELANE	indirect		PRKCE	indirect
	HSD17B3	indirect		TUBA4A	indirect
	ALDH2	indirect		MT-ND4	unexpected
	MAOB	unexpected		ABCC6	unexpected
GSI Synthetic Lethal	PLA2G2A	unexpected		ABCB4	unexpected
	KCNH2	unexpected		ABCC1	unexpected
	HTR2A	unexpected		ABCB1	unexpected
	ADRA2B	unexpected		KCNJ1	unexpected
	CALM1	unexpected	PGC1 Expression (PSM)	NR3C1	direct
Glioblastoma Modulators	HDAC3	direct		Ribosome	direct
	HDAC2	direct		SERPINA6	indirect
	HDAC1	indirect		PLA2G4A	indirect
				CYP3A4	unexpected

repository. This resource stores raw data of an increasingly varied set of measurements derived from cells and other biological assay systems treated with small molecules (Seiler et al., 2008). In order to be able to detect drug targets modulating specifically phenotypes, we selected chemical phenotypic screens from ChemBank for which control assays accounting for non-specific hit effects were included. Then, we restricted the analysis to assays performed in mammalian organisms (see Supplemental Experimental Procedures) since HitPick, the target prediction method underlying DePick, can predict protein targets of human and evolutionarily close mammalian species for which a high conservation of human ligand-target binding associations has been reported (Gfeller and Zoete, 2015; Kruger and Overington, 2012). We could predict targets confidently for eight assays fulfilling these criteria. These include screens for disease areas for which pharmacological points of intervention are sought such as cancer (“Glioblastoma Modulators”, “Wnt Inhibitors”, “E-Cadherin Synthetic Lethal”, “Androgen Receptor - Nuclear Receptor Corepressor (AR-NCoR) Binding”, “Gamma Secretase Inhibitor (GSI) Synthetic Lethal”), cardiovascular (“Lipid Transfer”), and metabolic diseases (“PGC1 Expression”). We applied DePick to these assays and detected a total of 59 target-phenotypic screen associations (Table 1).

Analysis of the Hits and Predicted Targets

We found that an average of 30% of the selective hits of these screens interact or are predicted to bind to the significant targets detected by DePick, implying that for these compounds, we can propose the molecular mechanism underlying the phenotypes. This number contrasts with the marginal amount of information on the molecular mechanisms driving phenotypes that can be gained based only on statistical enrichment of only the known targets of the specific hits (Figure 2). This illustrates the potential of target prediction information to capture a large fraction of the molecular space covered by specific hits of a phenotypic screen. Interestingly, we observed that although the target prediction tool HitPick predicts at least one target confidently (50% precision) for an average of 55.6% of the selective hits in the eight screens (Figure 2), not all predicted targets appear to be responsible for the measured phenotype, highlighting the need to

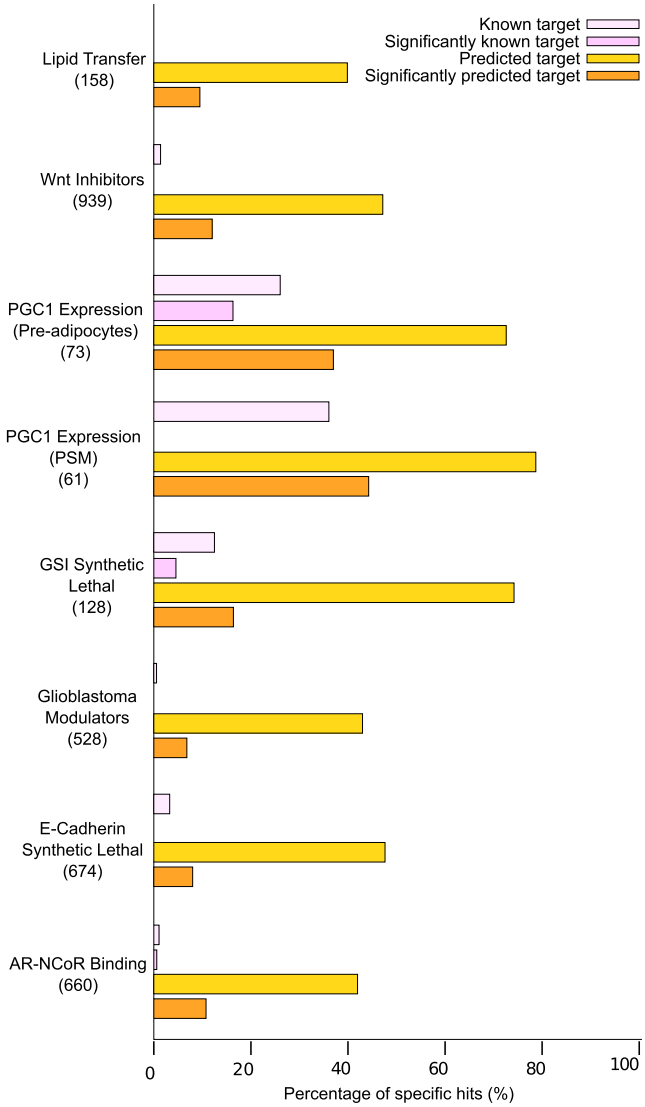

Figure 2. Known and Predicted Targets of the Specific Hits of the Assays

The number of specific hits in each assay project is displayed in brackets. The percentage of hits with at least one known target, the percentage of hits predicted to bind predicted targets with high confidence (>50%), and the percentage of hits binding the significantly known and predicted targets are displayed.

determine the causal relationships between targets and phenotypes in these screens.

The analysis of the targets involved in the 59 predicted target-phenotype associations revealed that 43 distinct protein targets are linked to only one phenotypic screen, implying that only a minority of the targets is associated with multiple screens. Among those, we found ribosomal proteins, amyloid-β precursor protein (APP), the tubulin, β class I (TUBB) and tubulin, α 4a (TUBA4A), monoamine oxidase B (MAOB), calmodulin 1 (CALM1), and aldehyde dehydrogenase 2 (ALDH2). (In this article, we use human gene names provided by EntrezGene to refer to genes as well as proteins.) Interestingly, the proteins APP, MAOB, and ALDH2 are linked to "AR-NCoR Binding", an assay that seeks enhancers of binding of androgen receptor to nuclear receptor corepressor, and also to the "'Wnt Inhibitors" screen. This points to the existence of common molecular links between the androgen receptor and Wnt pathways. Indeed, it is known that the crosstalk between androgen receptor signaling and β-catenin plays a notable role in prostate cancer (reviewed in Kypta and Waxman, 2012). APP was furthermore linked to an assay searching for modulators of the SR-BI lipid transporter process, denoting a more pleiotropic function of APP than its well-known role in Alzheimer's disease. Consistent with the key role of the ribosome in growth-related biological processes, ribosomal proteins were found to be associated with a screen searching for modulations with synergistic effects on cell growth upon E-cadherin inhibition ("E-Cadherin Synthetic Lethal") and two assays searching for modulations of PGC1-α gene expression, a key regulator of mitochondria biogenesis (Wu et al., 1999). The distinct and shared targets linked to these screens emphasize the potential of the approach to formulate hypotheses on specific as well as broad pharmacological modulators of disease phenotypes.

Literature Validation

In order to evaluate the reliability of our drug target-phenotypic screen pair's predictions, we first performed a manual curation of the scientific literature to retrieve the experimentally validated evidence on the relationship of drug targets and phenotypes. In the literature, we found experimental support that directly relates a chemical or genetic modulation with the phenotype ("direct" link) for 27% (16) of the connections. The links between the proteins calmodulin (CALM1) and estrogen-related receptor gamma (ESRRG) to the "E-Cadherin Synthetic Lethal" assay exemplify these relations. The former association is supported by the known effect of the calmodulin antagonist CGS9343B on E-cadherin adhesion (Li et al., 1999) and the latter by the induction of E-cadherin by the ectopic expression of ESRRG in mammary carcinoma cells, respectively (Tiraby et al., 2011). Interestingly, for 34% (20) additional associations, we found literature support linking an intermediate common protein or metabolite both to the drug target and the phenotype ("indirect" link). The connection between the drug target phospholipase A2, group IVA (PLA2G4A), and "PGC1 Expression (PSM)", a screen that searches for modulators of the PGC1-α gene expression in primary skeletal muscle cells, is one of these links. PLA2G4A and the phenotype are both related to the transcription factor CREB1 (cAMP responsive element binding protein 1). CREB1 is activated by the enzyme PLA2G4A (Hazan-Eitan et al., 2006) and is also known to modulate the promoter activity of PGC1-α, increasing the mRNA expression levels of the gene (Karamitri et al., 2009). Detailed information on "direct" and "indirect" relations are shown in Table S1 and Figure S1. Careful analysis of the literature reports supporting the "direct" and "indirect" links revealed that indirect mechanisms encompassing transcriptional processes, intermediate metabolites, or proteins are often involved in the modulation of the phenotype by the target. This illustrates that the de-convolution of chemical phenotypic screens can uncover a large variety of biological mechanisms that interfere with disease phenotypes that can be exploited pharmacologically.

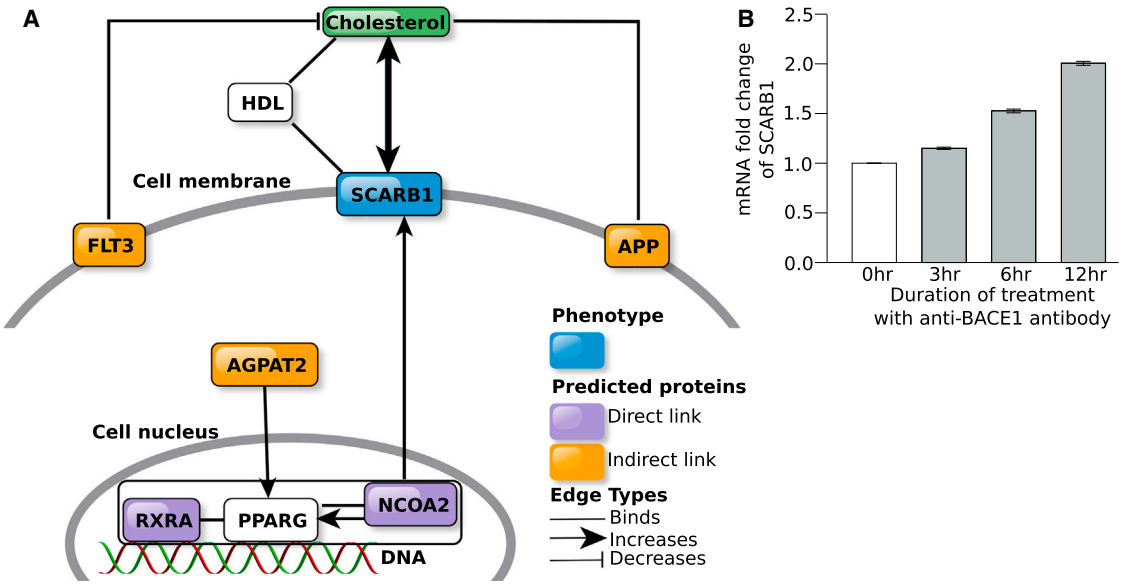

Figure 3. Analysis of the "Lipid Transfer" Screen

(A) Relationships between significantly predicted protein targets and the lipid transfer mediated by the SR-BI transporter phenotype assay. This interaction network has been created using the CIDeR database (Lechner et al., 2012) where all the human gene names are provided by EntrezGene. White and green rectangles indicate background proteins and chemicals related to the assay.

(B) Gene expression of SR-BI in SV-LECs was analyzed in the presence of anti-BACE1 neutralizing antibody or media alone (control). The experiment was repeated twice with the same observations (error bars represent SD).

For the remaining 39% (23) drug target-phenotype associations, we could not link the drug target to the phenotype by "direct" or "indirect" literature evidence ("unexpected" link). However, literature reports provide hints of possible connections between drug targets and the measured phenotype that merit further investigation. For example, DePick predicted that several members of the solute carrier organic anion transporter family (SLCO1A2, SLCO1B3, SLCO1C1, SLCO4C1) are related to the "E-Cadherin Synthetic Lethal" assay. These proteins increase the transport of the metabolite estrone 3-sulfate (Geyer et al., 2004; Maeda et al., 2010; Pizzagalli et al., 2002; Yamaguchi et al., 2010), a precursor of estradiol. Estradiol, in turn, has been shown to decrease the expression of E-cadherin (Oesterreich et al., 2003). Taken together, these pieces of evidence suggest that the synergistic effect of modulators of these transporters and inhibitors of E-cadherin activity on growth inhibition might be triggered by the modulation of intracellular estradiol levels by these transporters.

Case Studies: "Lipid Transfer" and "Wnt Inhibitors" Assays

The results from the literature validation of drug target-phenotype links provide strong support for the reliability of these predictions and indicate that the modulation of the phenotype by the alteration of the predicted targets is often due to indirect molecular effects. In order to provide experimental support for the predictions and prove this hypothesis experimentally, we inspected the "Lipid Transfer" and "Wnt Inhibitors" screens (see Table S2 for detailed information on these assays, including specific hits, inactive compounds, and predicted targets). These assays aim to detect modulators of the cholesterol transport mediated by the scavenger receptor, class B, type I, SR-BI (also known as SCARB1) and the canonical (β-catenin dependent) Wnt signaling pathway, respectively. We analyzed the five and nine targets significantly linked to these assays, respectively, and chose interesting drug target-phenotype links to be validated experimentally. We first tested if some of the ligand-target associations predicted by HitPick, the first step of the DePick target de-convolution method, could be confirmed experimentally. For that, we measured the activity of ten commercially available selective hits on five of the predicted targets for which commercially in vitro binding and enzymatic assays exist. These compounds were tested first at a single concentration of 10 μM in duplicate (Table S3). Four compounds showed an activity higher than 35%. Those were subsequently tested in dose-response curves to determine the half maximal inhibitory concentration (IC_{50}). The IC_{50} values obtained were in the micromolar and nanomolar range (35 μM, 6.7 μM, 2.9 μM, and 2 nM) (Figure S2), thus confirming the predicted compound-target associations. Afterwards, we sought literature evidence supporting the relationship of the targets to the screens reported by DePick. Lastly, we followed a pharmacological approach using potent ligands of predicted targets in experimental tests to support the predicted target-phenotype associations. The results of the literature and experimental analysis for the two assays are described below.

"Lipid Transfer" Assay

The "Lipid Transfer" phenotypic screen aimed to find selective modulators of the transfer of lipids mediated by the high-density lipoprotein (HDL) receptor SR-BI (SCARB1), which modulates both the selective uptake of cholesterol esters from HDL into cells and the efflux of cholesterol from cells to lipoproteins

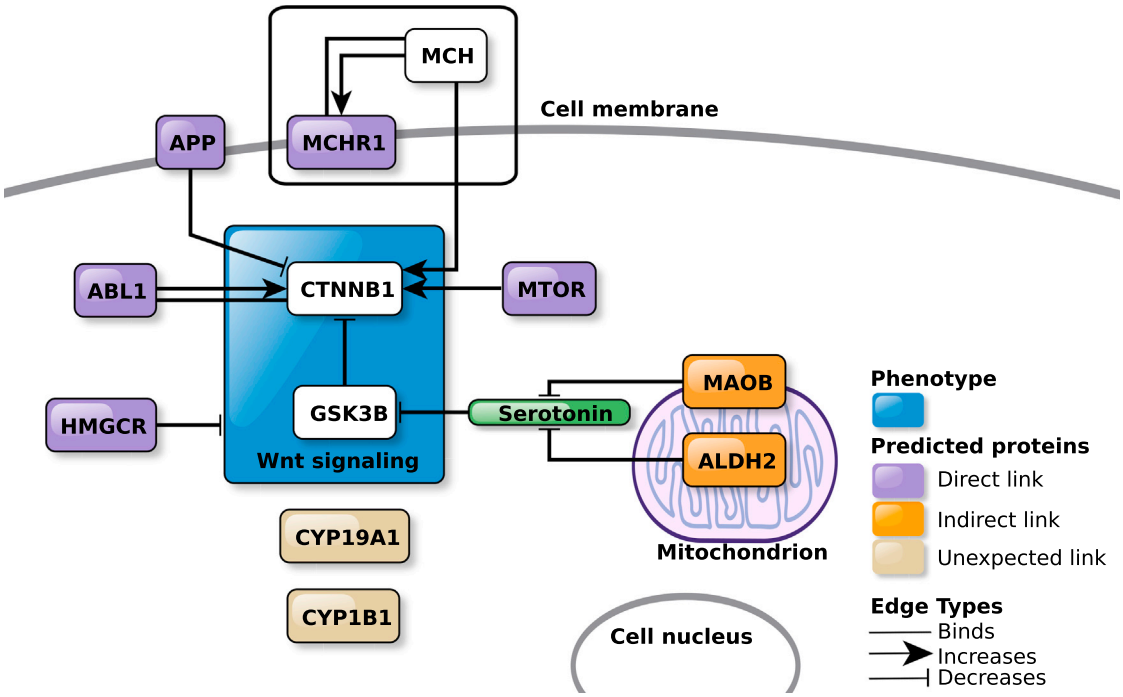

Figure 4. Analysis of "Wnt Inhibitors" Screen

Relationships between significantly predicted protein targets and the "Wnt Inhibitors" phenotype assay. This interaction network has been created using the CIDeR database (Lechner et al., 2012) where all the human gene names are provided by EntrezGene. White and green rectangles are proteins and chemicals related to the assay.

(Nieland et al., 2002). The five targets significantly enriched among the selective hits of this assay are nuclear receptor coactivator 2 (NCOA2), retinoid X receptor alpha (RXRA), 1-acylglycerol-3-phosphate *O*-acyltransferase 2 (AGPAT2), APP, and fms-related tyrosine kinase 3 (FLT3) (Figure 3A).

Two of the predicted proteins, namely NCOA2 and RXRA, are known to regulate SR-BI-mediated lipid transfer. NCOA2, a coactivator of steroid receptors, interacts with the complex formed by the peroxisome proliferator-activated receptor gamma (PPARG) and RXRA (Osz et al., 2012), thereby inducing the expression of SR-BI (Jeong et al., 2006). A third predicted protein, AGPAT2, which is mutated in patients with congenital generalized lipodystrophy, is indirectly influencing SR-BI activity via the modulation of PPARG (Subauste et al., 2012). These pieces of evidence provide strong support for the pharmacological modulation of SR-BI function by these predicted targets.

We found indirect literature evidence linking FLT3 and APP to SR-BI-mediated cholesterol transport via the common metabolite "cholesterol". Several studies have related FLT3, a gene frequently mutated in acute myeloid leukemia, to cholesterol transport (Soufi et al., 2012; Westerterp et al., 2012). APP, in turn, is known to bind cholesterol (Barrett et al., 2012) and inhibition of β-secretase (BACE1), the enzyme responsible for the processing of APP into amyloid β peptide, causes an increase in the membrane cholesterol level (Liu et al., 2009). We used a murine lymphatic endothelial cell line (SV-LEC), where SR-BI is responsible for the cholesterol transport (Lim et al., 2013) and also expressed APP (Figure S3) for experimental analysis (see Experimental Procedures). After blocking BACE1-mediated APP processing with a BACE1-neutralizing antibody, SR-BI expression increases up to 2-fold after 12 hr (Figure 3B). This result is in line with the involvement of APP on the regulation of SR-BI expression.

"Wnt Inhibitors" Assay

The Wnt signaling pathway is a highly conserved signaling pathway involved in organ growth and maintenance, which has also been implicated in several pathologies, including cancer and organ fibrosis (Baarsma et al., 2013). This screen seeks for chemicals modulating the canonical Wnt pathway using a reporter-based assay measuring the transcriptional response of the Wnt/β-catenin signal modulation in the colorectal adenocarcinoma cell line DLD1. We predicted nine targets specifically linked to this pathway, namely APP, ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1), mammalian target of rapamycin (MTOR), aldehyde dehydrogenase 2 (mitochondrial) (ALDH2), melanin-concentrating hormone receptor 1 (MCHR1), monoamine oxidase B (MAOB), two members of the cytochrome P450 family (i.e., cytochrome P450, family 1, subfamily B, polypeptide 1 [CYP1B1] and aromatase [CYP19A1]), and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (Figure 4).

Our literature review confirmed the pharmacological, genetic, or physical relationships of five of these proteins with the canonical Wnt pathway, namely ABL1, MCHR1, APP, HMGCR, and MTOR. ABL1 interacts with and stabilizes β-catenin (CTNNB1) (Coluccia et al., 2007). A positive effect on Wnt signaling has also been described for melanin-concentrating hormone

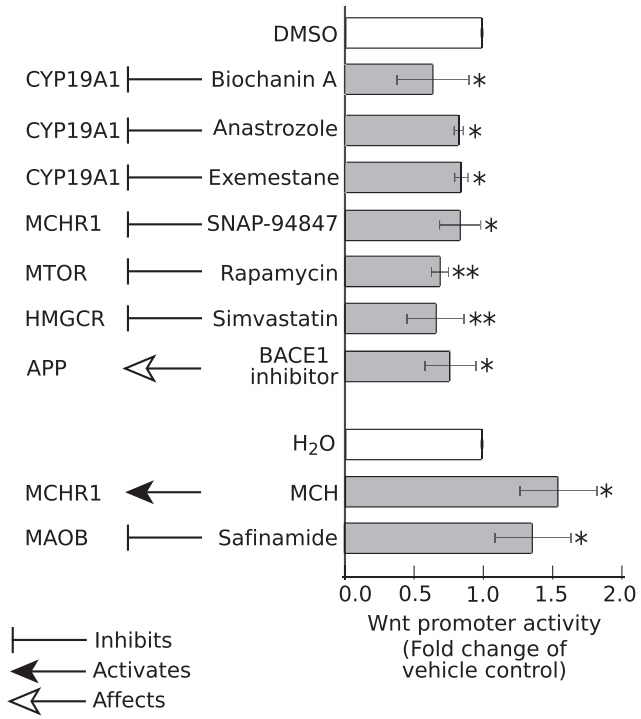

Figure 5. Fold Change of the Expression of the Wnt TCF/LEF Reporter Activity in A549 Cells after Treatment with Chemical Compounds Targeting the Predicted Targets

n = 4–6; *p < 0.05, **p < 0.01. p Values were calculated using a paired t test relative to the corresponding control. Error bars represent SD.

(MCH), the ligand of MCHR1 (Nagel et al., 2012). In addition, overexpression of APP as well as its processing products amyloid β peptide and amyloid precursor protein intracellular domain result in a significant reduction of β-catenin (Chen and Bodles, 2007; Magdesian et al., 2008). HMGCR is a central enzyme of the cholesterol biosynthesis pathway. A regulatory effect of statins, pharmacological inhibitors of HMGCR, on the Wnt signaling pathway is well described in the literature (Robin et al., 2014). Furthermore, there is experimental evidence for the activation as well as the inhibition of the pathway using the MTOR inhibitor rapamycin in H9 cells (Zhou et al., 2009) and hepatocellular carcinoma (Feng et al., 2011), respectively.

For the ALDH2 and MAOB proteins, indirect evidence from the literature suggests that the mechanism driving modulation of the canonical Wnt pathway by these proteins involves the intermediate metabolite serotonin (Rooke et al., 2000). This metabolite, in turn, is known to inhibit the central Wnt signaling inhibitor GSK3B (Sibilia et al., 2013). For the cytochrome P450 family members, CYP19A1 (aromatase) (Tao et al., 2007) and CYP1B1 (Li et al., 2000), an indirect connection to the phenotype involving only one intermediate molecule could not be established.

We opted to test the regulation of the canonical Wnt signaling experimentally by predicted targets involved in "direct" (MTOR, HMGCR, MCHR1, APP), "indirect" (MAOB), and "unexpected" (CYP19A1) associations in a Wnt TOPflash reporter assay using the human alveolar epithelial cell line A549 (see Experimental Procedures). We observed that the MTOR inhibitor rapamycin and HMGCR inhibitor simvastatin significantly attenuated canonical Wnt signaling (Figure 5). Furthermore, safinamide, a potent reversible inhibitor of MAOB, and MCH, the natural ligand of MCHR1, activated canonical Wnt signaling. The relationship between MCHR1 modulation and the canonical Wnt signaling pathway is further supported by the inhibition of the pathway after treatment with the MCHR1 antagonist SNAP94847. Biochanin A, anastrozole, and exemestane, which are inhibitors of CYP19A1 (aromatase) also significantly diminished the activity of canonical Wnt signaling. The same effect on the Wnt pathway was observed with a BACE1-inhibitor that blocks APP processing. These results substantiate the efficacy of the approach to detect targets associated with phenotypic screening readouts. The experimental results are in line with all tested predicted relationships of drug targets and phenotypes measured in the two assays, thereby illustrating the effectiveness of the target de-convolution approach in DePick to unveil novel targets of biological processes measured in bioassays. Lastly, we tested DePick in an assay from the PubChem BioAssay database (AID: 1672) (Table S4) and observed that DePick is also applicable to publicly available chemical screens from other repositories, reinforcing the potential of DePick to uncover drug target-phenotype links from phenotypic chemical screens.

In summary, we have demonstrated that application of the in silico target de-convolution approach DePick to chemical phenotypic screens reveals known and novel connections between molecular target and phenotypes. The application of this tool (http://mips.helmholtz-muenchen.de/Depick/) in a systematic way to more chemical phenotypic screens promises to expand the repertoire of target-phenotype links in an unprecedented manner.

DISCUSSION

The rapid increase of drug target information in the public domain in recent years is accelerating the creation of efficient computational methods for the prediction of targets for a large fraction of compounds (Keiser et al., 2007; Liu et al., 2013; Nidhi et al., 2006), facilitating the development of drug target de-convolution approaches for chemical phenotypic screens. In this study, we have shown that in silico de-convolution methods such as DePick are fast and efficient approaches to extract drug target-phenotype connections from chemical phenotypic screens stored in public repositories.

Despite their advantages, target de-convolution approaches for chemical high-throughput chemical screens face several challenges (Table 2). These are not restricted to methodological difficulties such as the development of efficient computational methods to detect the chemical hits and their protein targets. They also suffer limitations by the quality of information in chemical screens, including the presence of screening artifacts such as promiscuous compounds as well as by the availability of the screen information. For example, raw data of the experiments as well as control/counter screens are not always available, which hampers determination of compound selectivity as well as the specificity of the target-phenotype links. In the presented approach, to be able to detect targets specifically linked to phenotypic screens, we have analyzed screens with available

Table 2. Advantages and Weakness of DePick

Advantages	Weaknesses
Inexpensive, fast, and efficient method for the extraction of target-phenotype links from chemical screens	Dependence on the quality of the experimental data (presence of artifacts)
Applicability to any chemical phenotypic screens with available "control/counter" screens	Control/counter screens, essential to assess specificity, are not always available
Allows the determination of pharmacologically relevant protein targets	Protein targets limited to druggable targets (proteins with known ligands)

control assays in the ChemBank database. To determine selective compounds, we have removed compounds showing activity in the control assays. This procedure also removes promiscuous as well as non-specific compounds and is preferable over discarding promiscuous compounds based on their promiscuity index (Gamo et al., 2010), because it avoids disregarding non-artifactual compounds with multi-target activity, thereby enlarging the space of drug target information behind phenotypes.

We have tested DePick in eight phenotypic screens relevant to diseases such as cancer, diabetes, and cardiovascular diseases and have identified a total of 59 target-phenotype links. Around 30% of the specific hits are predicted to bind to the predicted targets of those links. This modest number is expected, since HitPick predicts at least one confident target only for 55.6% of the selective hits. We anticipate that the growing information on ligand-target pairs in the public domain will greatly contribute to increase the coverage of proteins assigned to hits by target-prediction methods, enabling the molecular space underlying phenotypic screens to expand.

A literature inspection of the 59 connections confirmed and provided strong indirect evidence relating the pharmacological targets to the measured phenotype for 64% of the target-phenotype links. For example, we found "indirect" literature evidence for the interesting connection between FLT3 and SR-BI. Overexpression of the SR-BI gene has been observed in 32Dcl3 cells stably transfected with FLT3-ITD (Mizuki et al., 2003). Furthermore, expression of the cholesterol transporter ABCA1 (ATP-binding cassette 1) is reduced in FLT3-ITD mice (Westerterp et al., 2012), which is consistent with a low cholesterol environment. Under low cholesterol conditions, the expression of SR-BI is induced to improve cholesterol uptake (Soufi et al., 2012). These findings suggest FLT3 as a novel potential target of lipid metabolism mediated by SR-BI, and encourage further research to elucidate the precise molecular mechanisms linking FLT3 and SR-BI.

The results of the experimental pharmacological approach using potent modulators of predicted targets linked to the "Lipid Transfer" and "Wnt Inhibitors" in cell lines are also aligned with predicted target-phenotype connections. We observed an increase in SR-BI mRNA expression after neutralizing with an antibody recognizing the APP processing enzyme BACE1 in SV-LEC cells. Similarly, chemical modulators of directly (MTOR, HMGCR, MCHR1, APP) and indirectly (MAOB) related targets to the regulation of either β-catenin or GSK3B activity in the literature are active on Wnt TOPflash reporter assays in A549 cells. Although these results are consistent with the predicted associations, they should be taken with caution since off-targets are known for some of the modulators, such as butyrylcholinesterase (Darvesh et al., 2004) for simvastatin, the sigma non-opioid intracellular receptor 1 (SIGMAR1) for safinamide (Fariello, 2007), as well as neural cell adhesion molecule L1-like protein (CHL-1) as a BACE1 substrate (Barão et al., 2015). Further research is needed to rule out unknown effects of these additional drug targets on the phenotypes studied.

The activity of potent modulators of predicted targets linked to the Wnt screen on human alveolar epithelia A549 cells provides novel understanding of the regulation of the Wnt pathway in lung cell lines. In this context, literature reports point to a cell-type-specific regulation (activation/inhibition) of some of the targets, such as HMGCR and MTOR (Feng et al., 2011; Hwang et al., 2014; Robin et al., 2014; Zhou et al., 2009). To our best knowledge, we are the first to show that rapamycin and simvastatin inhibit the Wnt signaling pathway in human alveolar epithelia A549 cells. As a result, these drugs might represent alternative pharmacological tools for the treatment of lung diseases. In addition, we observe for the first time that pharmacological modulation of MAOB and CYP19A1 (aromatase) activity regulates the canonical Wnt pathway. Although the exact molecular mechanism relating the predicted protein CYP19A1 (aromatase) and Wnt pathway merits further investigation, a possible mechanistic explanation might involve regulation of cellular levels of estradiol and consequently activation of estrogen receptor 1, a transcription factor that is co-activated by β-catenin (Kouzmenko et al., 2004).

The experimental evidence of the effect of three different aromatase inhibitors on Wnt activity supports the unexpected connection between CYP19A1 (aromatase) and the Wnt pathway, and reinforces the reliability of the 34% (20) "unexpected" drug target-phenotype associations lacking direct or strong indirect support from the literature. Although we could not explain some of the relationships based on the current knowledge in the literature, such as the link between five predicted targets and the "GSI Synthetic Lethal" assay, we have found distant yet plausible mechanistic explanations for other relationships. These include the link between several members of the solute carrier organic anion transporter family (SLCO1A2, SLCO1B3, SLCO1C1, SLCO4C1) and the "E-Cadherin Synthetic Lethal" assay and the association between four ATP-binding cassette proteins (ABCB1, ABCB4, ABCC1, and ABCC6) as well as MT-ND4, a component of the mitochondrial respiratory complex, to the "PGC1 Expression (Pre-adipocytes)" screen. We hypothesize that the changes in the intracellular ADP levels and the AMP/ATP ratio by the four ATP-binding cassette proteins and MT-ND4, respectively, are behind the predicted association linking these proteins to the "PGC1 Expression (Pre-adipocytes)" screen. Changes of ADP and AMP/ATP ratio levels are known to modulate the activity of AMP-activated protein kinase (AMPK) (Sanders et al., 2007; Xiao et al., 2011), an enzyme that regulates PGC1-α gene expression (Bortin et al., 1992). These pieces of evidence encourage further investigations to explore the pharmacological validity of these predictions.

An interesting advantage of this de-convolution approach (Table 2) is that the predicted targets are druggable proteins

and, and remarkably, therapeutic drugs are known for the majority of them. Consequently, the results presented here not only provide novel biological insights relating targets to phenotypes but also allow straightforward rationalized therapeutic strategies to interfere with diseases to be proposed. For example, the PGC1-α gene plays a central role in the regulation of cellular energy metabolism and has been proposed as a drug target candidate to treat metabolic disorders such as diabetes. Thus, the novel connections linking drug targets to PGC1-α gene expression found here can enlighten the molecular mechanism underlying the regulation of PGC1-α as well as propose therapeutic druggable hypotheses to modulate PGC1-α gene expression. Furthermore, knowledge of the pharmacological targets related to the same phenotype offers novel possibilities for drug combination treatment, which has been increasingly used to treat complex diseases such as cancer (Csermely et al., 2005).

In summary, we have shown that DePick, a target de-convolution method for chemical phenotypic screens, is able to uncover systematically hidden information on pharmacological targets modulating phenotypes. We have applied DePick to eight public phenotypic screens and discovered many potential molecular players of these phenotypes. The novel target-phenotype links increase current knowledge on the molecular mechanism connected to phenotypes and more importantly, expand the pharmacological possibilities to interfere with diseases related to these phenotypes, including cardiovascular disease and cancer. Our tool can help future research to focus on promising targets. The high quality of the results makes DePick a bona fide tool for investigation of cellular processes and the identification of novel therapeutic drug targets.

SIGNIFICANCE

In this work, we present DePick (http://mips.helmholtz-muenchen.de/Depick/), a drug target de-convolution tool to uncover pharmacological connections between drug targets and phenotypes measured in high-throughput chemical phenotypic screens. As a proof of concept, we have tested DePick in eight assays of the ChemBank repository for disease areas for which pharmacological points of intervention are sought such as cancer, cardiovascular, and metabolic diseases. We have detected a total of 59 target-phenotypic screen associations including novel targets modulating PGC1-α gene expression and proteins with synergistic effects on growth inhibition upon blockage of γ-secretase and e-cadherin function. An in-depth inspection of the literature found direct and strong indirect evidence for 27% and 34% of the links, respectively. These results provide support for the predicted relations and highlight the role of indirect molecular mechanisms connecting the protein targets to the phenotypes. The relevance of the predicted associations was further supported by experimental evidence. Our work illustrates that the systematic application of an in silico target de-convolution approach to chemical phenotypic assays, such as those stored in public repositories, offers huge potential for unraveling novel relationships between protein targets and phenotypes in a fast and inexpensive manner.

EXPERIMENTAL PROCEDURES

Target De-convolution Scheme DePick

We first detected chemical hits in the "experiment" and the "control" assays of a screen using the modified B-Score method (Liu and Campillos, 2014). The "experiment" and "control" assays measure the intended biological activity and the unspecific activity, respectively. Then, to assess the specificity of the chemical hits in a phenotypic screen, we defined the "Specific hits" and the "Inactive compounds" sets. The "Specific hits" set is composed of the compounds that specifically modulate the phenotypes of interest, that is, the compounds active in the "experiment" assay and inactive in the "control" assay. The compounds inactive in the experiment assay form the "Inactive compounds" set. Afterward, we applied HitPick (Liu et al., 2013) to predict the molecular targets of compounds in the "Specific hits" and "Inactive compounds" sets and selected those targets predicted with high confidence (precision >50%). HitPick is a ligand-based target prediction method that combines 1-nearest-neighbor (1NN) similarity searching and Laplacian-modified naive Bayesian machine learning to predict direct human binding targets using the STITCH database (Kuhn et al., 2008). In order to determine the targets of hits that are enriched in the specific hits of the assays, and thus, more likely to be relevant to the phenotypic response, we subsequently applied the hypergeometric test to detect predicted target(s) that are over-represented in the "Specific hits" set compared with "Inactive compounds" set. We associated to the phenotype those protein targets with a resulting p value lower than 0.05 after false discovery rate multiple testing correction (Benjamini et al., 2001) (Figure 1).

Experimental Testing

SR-BI mRNA Expression Assay

The murine lymphatic endothelial cell line, SV-LEC, was provided by Dr. J.S. Alexander (Shreveport, LA) and was cultured as previously described (Ando et al., 2005). SV-LECs were grown to confluence with DMEM culture medium containing high glucose (25 mM). In some experiments, cells were pre-incubated with 10 ng/mL of anti-BACE1 antibody (R&D Systems), diluted in media for 3, 6, or 12 hr prior to mRNA extraction for gene expression analysis. Total RNA from SV-LECs was extracted using an RNA extraction kit (NucleoSpin RNA II; Macherey-Nagel) and Maxtract tube (QIAGEN). cDNA was synthesized from 2 μg of RNA using a Taqman reverse transcriptase kit (Applied Biosystems). Real-time qPCR was performed in triplicate using SYBR Green PCR Master Mix (Applied Biosystems) and analyzed on an Abi Prism 7500 Detection System (Applied Biosystems). Data were normalized to GAPDH and the primers used are listed in Table S5. Consistent with our previous study (Lim et al., 2013), real-time qPCR analysis revealed the expression of SR-BI on SV-LECs, at similar levels as in the RAW macrophage cell line, which served as positive control (Lorenzi et al., 2008).

Canonical Wnt/β-catenin Activity Assay

Transcriptional activity of canonical Wnt signaling was determined with the M50 Super 8x TOPflash and M51 Super 8x FOPflash vectors, which contain a firefly luciferase gene under the control of seven TCF/LEF binding sites (TOPflash) or mutated TCF/LEF binding sites (FOPflash). A549 cells were plated in 48-well plates at a density of 1.5×10^5 cells per well. The next day, cells were transfected with either 500 ng/well of M50 Super 8x TOPflash plasmid or the negative control M51 Super 8x FOPflash using Lipofectamine LTX with PLUS reagent (Life Technologies) in serum-free Opti-MEM medium (Life Technologies). After 6 hr of transfection, cells were treated with the pharmacological inhibitors or vehicle control (H_2O or DMSO) diluted in DMEM/F12 medium supplemented with 0.1% (v/v) fetal calf serum. For each independent experiment, stimulations were performed in duplicate or more repetitions. After 24 hr of stimulation, the cells were lysed using Glo lysis buffer and luciferase activity was assayed using the Bright-Glo luciferase assay system (Promega). TOPflash activity was normalized to FOPflash activity and expressed relative to vehicle control conditions.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.chembiol.2016.08.011.

AUTHOR CONTRIBUTIONS

M.C. and X.L. conceived the project, designed the computational analysis, and wrote the manuscript. X.L. performed the computational analysis. J.-S.H. contributed to the computational analysis. H.A.B., C.H.T., M.K., and V.A. designed and performed the experiments. C.M., B.B., G.F., and A.R. performed the literature analysis and assisted in the writing of the manuscript. S.D. provided technical support. All authors revised the manuscript.

ACKNOWLEDGMENTS

The authors acknowledge members of the SBSM group at the Helmholtz Center Munich for helpful discussions, Jeremias Trapp for technical help, and Gianluca Adornetto for critical reading of the manuscript. This study was supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes research (DZD e.V) and a grant from NRF and NMRC to V.A.

Received: January 21, 2016
Revised: June 10, 2016
Accepted: August 5, 2016
Published: September 22, 2016

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