Network pharmacology

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Review

Network pharmacology: curing causal mechanisms instead of treating symptoms

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For complex diseases, most drugs are highly ineffective, and the success rate of drug discovery is in constant decline. While low quality, reproducibility issues, and translational irrelevance of most basic and preclinical research have contributed to this, the current organ-centricity of medicine and the 'one disease–one target–one drug' dogma obstruct innovation in the most profound manner. Systems and network medicine and their therapeutic arm, network pharmacology, revolutionize how we define, diagnose, treat, and, ideally, cure diseases. Descriptive disease phenotypes are replaced by endotypes defined by causal, multitarget signaling modules that also explain respective comorbidities. Precise and effective therapeutic intervention is achieved by synergistic multicompound network pharmacology and drug repurposing, obviating the need for drug discovery and speeding up clinical translation.

Highlights

Current diseases are defined by a phenotype rather than by a disease mechanism. Thus, we hardly understand any disease mechanistically and treat symptoms chronically with low precision.

When a mechanism is described, it often involves single targets (e.g., rare, typically monogenic diseases).

In the case of complex diseases, the current 'one disease–one target–one drug' dogma will hardly yield any result when in fact, their causes are small signaling networks.

Signaling pathways are currently defined by highly curated mind maps capturing our current understanding of (patho)biology. However, many pathophysiologically relevant signaling mechanisms are likely unknown and can be revealed by unbiased *de novo* interactome modules.

These knowledge gaps will be overcome by systems and network medicine, redefining what we call disease, how we diagnose it, and how we cure, not treat, it.

The one symptom–one target–one drug problem

For several drugs already on the market, population-based studies fail to show patient-relevant benefits [1]. In fact, the ten highest-grossing drugs in the USA fail to improve the conditions for most patients, leading to high numbers needed to treat (NNT) [2]. In high-risk patients, the NNTs are smaller, but the problem persists [3]. Thus, a move from chronically treating symptoms towards a more precise and ideally curative therapy, effective for almost every patient, is of utmost importance.

Since the 1950s, we have observed a constant decline in our efficacy to translate biomedical research into successful drug discovery, coined as Eroom's law [4–6]. Overcoming this requires entirely new approaches to medicine and the acknowledgment of at least two key factors contributing to this innovation roadblock. One factor is the irreproducibility of preclinical and basic research [7], where poor study quality, such as lack of statistical power and positive publication bias by scientific journals, are the main contributors [8,9]. The second factor is our conceptual knowledge gap concerning most current disease definitions. Except for infectious and rare diseases, chronic disease definitions are based on phenotypes (i.e., symptoms manifesting in an organ). In fact, medicine is currently structured primarily in an organ-by-organ manner. Moreover, our preclinical animal models of disease can often only mimic these symptoms, without any evidence that the mechanism causing the symptoms in the animal model matches the human disease [10–15]. Therefore, we lack a mechanistic understanding of the causes of disease and hence we chronically treat symptoms but do not cure the disease.

For example, high blood pressure is, in 95% of the cases, diagnosed as primary hypertension, meaning that the blood pressure is elevated, but we do not know why. These patients are treated with blood vessel-dilating drugs, such as thiazide-type diuretics or calcium channel blockers, targeting mechanistically unrelated proteins until the symptom, elevated blood pressure,

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disappears. However, the molecular cause of hypertension remains unknown; thus, we do not treat high blood pressure but aim to prevent myocardial infarctions and strokes. Nevertheless, although most patients at risk are successfully treated with antihypertensives, they will still experience these adverse outcomes. Thus, our current treatment options for complex diseases are neither curative nor precise and require chronic treatment [16]. Noteworthy exceptions to these limitations and shortcomings are, again, rare diseases, where a precise, typically monogenetic, mechanism is known.

From phenotype and symptom to endotype and cause

The fundamental and conceptual breakthrough to redefine diseases is to move from symptom and organ to mechanism and cause, as conceptually shown in the network of all human diseases, the **diseasome** (see Glossary) (Figure 1) [17,18]. In the first version, diseases were linked by joint risk genes in a scale-free network and clustered by several shared risk genes. These clusters of diseases thus hinted towards a common causal mechanism [17]. Later, other multiscale disease networks were formed based on shared symptoms, drugs, or comorbidities [19]. Interestingly, most **disease clusters** contain disease phenotypes of different organs, which substantiates the notion that organ- and symptom-based disease classifications are obsolete and rather obstruct innovation.

Thus, these phenotypes are no longer considered the disease definitions but rather the symptoms of their underlying common causal molecular mechanisms. Once elucidated, these mechanisms will become the new disease definitions, the **endotypes**. These endotypes are constructed from associated risk, driver genes, proteins, and drug targets to form a *de novo* disease signaling network or **disease module** [19]. One disease phenotype or symptom may be caused by different mechanisms that may be acting together (Figure 2).

The validity of these disease modules is essential for precision medicine because they represent new targets for both: (i) diagnostic strategies for patients-at-risk identification and subsequent mechanistic stratification, and (ii) therapeutic strategies to modulate the disease module by **network pharmacology**. Once all current disease phenotypes are fully endotyped and mechanistically understood, they will segregate into several distinct molecular disease mechanisms and endotypes [20]. Consequently, many common or complex disease phenotypes will split up into several rarer and less complex endotypes.

Unlike in monogenetic rare diseases, endotypes are caused by a signaling network's dysregulation rather than a single protein [19]. Given the redundancy and resilience of signaling networks [21], the current practice of modulating a single target per disease explains why the 'one disease–one target–one drug' approach has been insufficient. Even **combination therapy** with drugs targeting single, mechanistically unrelated, and noncausal proteins is no exception to this. Instead, concerted network modulation with multiple mechanistically related drugs will be much more effective [22].

Defining these signaling modules is not trivial, despite the availability of extensive literature and highly curated signaling pathway databases such as Kyoto Encyclopedia of Genes and Genomes (**KEGG**)[i] [23] or **WikiPathways**[ii] [24] (see Outstanding questions). These databases are primarily collections of manually curated pathway maps that represent our current knowledge of molecular interactions. Importantly, they fail to reflect that biological pathways are not isolated but are connected in different functional contexts. Moreover, curated pathways imply that all its components are in direct contact, which is not the case. Instead, signaling elements such as cAMP and calcium are typically distributed in different parts over several subcellular compartments. Indeed,

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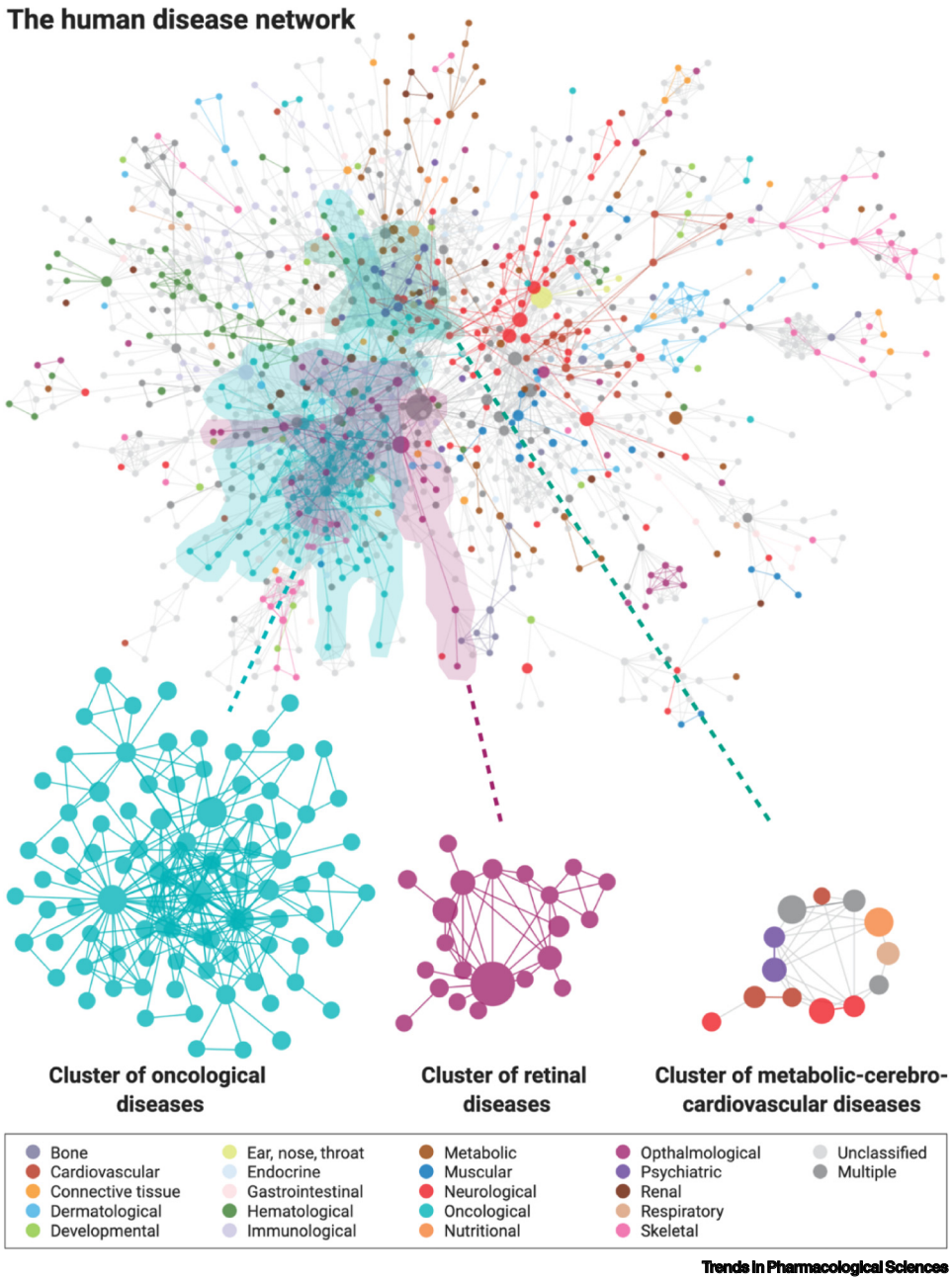

Figure 1. Illustration of mechanistic disease clusters in the human disease network. The upper half of the figure shows the human disease network, adapted from [17]. In the human disease network (i.e., the diseasome), diseases are represented by nodes and connected by edges when they share disease-associated risk genes [17,18]. The size of the disease nodes is proportional to the number of disease-associated genes. Nodes are colored according to their primary disease phenotype shaping mechanism-based disease clusters, defined by shared risk genes such as a cluster of oncological diseases (light blue) or a cluster of retinal diseases (purple). Moreover, disease-heterogenous clusters can also be appreciated, such as the reactive oxygen species (ROS)/cGMP (ROCG) cluster of metabolic-cerebro-cardiovascular disease phenotypes [76].

recent developments in cAMP signaling have highlighted the existence of nanodomains, although still from a canonical signaling pathway point of view [25,26]. Moreover, these signaling elements also interact with different pathways (e.g., the cAMP–cGMP crosstalk) and form hybrid domains

Glossary

Basket trials: clinical trials that focus on patients with a single genomic alteration or type of alterations in multiple, organ-independent, or so-called 'histology-independent' tumor types, allowing the inclusion of rare cancer types for precision intervention [112]. Umbrella and basket trials are novel examples of mechanism-based, genomically driven therapeutic interventions, mainly used for cancer. Umbrella trials still adhere to an organ-based focus but stratify patients and their therapy according to predicted responses using genomic markers [113].

Combination therapy: pharmacological treatment where drugs acting symptomatically on unrelated targets are combined, independently of the causal disease mechanism. Combination therapies are at best additive and will not show pharmacological synergy.

CUSP9v3 Treatment Protocol: clinical trial assessing the safety of nine repurposed drugs combined with temozolomide to increase its efficacy in glioblastoma patients.

Disease cluster: in the diseasome, diseases cluster based on shared risk genes. Disease clusters are mechanistically defined or endotyped by genes and proteins, creating a disease signaling network.

Disease module: localized perturbations in a protein–protein interaction network that characterize diseases. In the disease module, proteins mechanistically define pathological signaling by their neighborhood in the interactome.

Diseasome: the human disease network where nodes represent diseases and are linked to each other if they share a common genetic component. The size of the nodes is proportional to the number of disease-associated genes.

Driver mutation: a mutation that provides a growth advantage on the carrying cells and has been positively selected during the evolution of the cancer tumor.

DrugBank: database resource containing information about drugs and drug targets.

Endotype: new disease definition, defined by a causal disease mechanism or disease module rather than simply by symptoms.

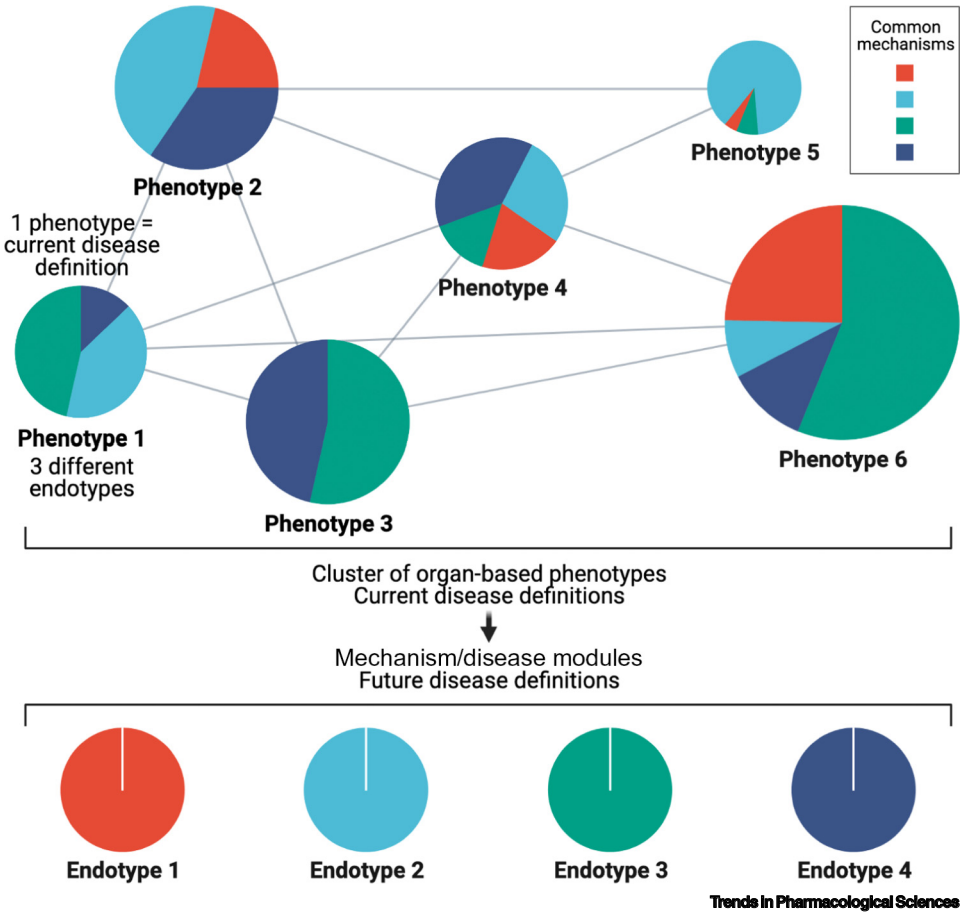

Figure 2. Endotyping disease clusters for common mechanism identification guides precision medicine. Schematic representation of the principle by which multiscale disease networks and the clusters therein lead to the identification of common mechanisms. In the illustrated example, a cluster of disease phenotypes (pie charts), that is, the current organ-based disease definition, is formed by shared risk genes, shared drugs, shared symptoms or comorbidity, or several of these (multiscale). These shared features form connections ('edges') between the diseases ('nodes'). Each phenotype is then endotyped through genes and drug targets, leading to different endotypes. Eventually, these endotypes will replace the previous phenotypic disease definition, allowing for precision diagnosis and intervention.

Interactome: complete set of molecular interactions within a cell (i.e., gene–gene, gene–protein, protein–protein, etc.).
I-PREDICT study: personalized cancer therapy study that uses genomic testing on primary tumor tissue to determine the best therapy for the patients.
KEGG: database resource for biological interactions. The data available range from molecular and cell-level interactions to ecosystems.
Network pharmacology: pharmacological treatment where two or more drugs mechanistically targeting the same causal disease module or signaling network are combined, synergistically acting on key network proteins.
PISCES: server tool for culling protein sequences from the Protein Data Bank that provides the longest list of the highest resolution structures according to sequence identity and structural quality criteria.
Retinal dystrophies (RD): a class of retinal diseases that cause degeneration of the outer retina.
Seed nodes: clinically validated, that is, disease-relevant genes or proteins used as a guided search for modules in gene–gene and protein–protein interaction networks.
STRING: database of known and predicted protein–protein interaction.
WikiPathways: open scientific database of biological pathways and interactions.

composed of elements from distinct signaling principles. Nevertheless, subcellular compartmentalization and even their transition over time matter in defining disease modules [19].

Thus, for pharmaco-therapeutic purposes, not only the present concept of disease but also of cellular signaling must be revised. Classical, canonical, or curated pathways are close to meaningless if we want to define disease modules. Leveraging the power of networks in the context of complex diseases requires conceptually novel experimental and, above all, computational approaches that have been uncommon to pharmacology.

How to construct disease modules

To construct *de novo* disease modules, we need to discern between methods using existing molecular interaction networks, such as, for instance, protein–protein interaction (PPI) or gene-regulatory networks, and methods that infer context-specific networks directly from disease-specific data. Such networks can be dissected using community detection or network module

identification methods. Recently, the DREAM challenge has demonstrated that such methods are generally suited to discover disease modules [27]. Alternatively, *de novo* network enrichment is a popular strategy in which omics data such as gene expression or single-nucleotide variants are projected onto a network for extracting disease modules enriched with genes or proteins for some physiologically relevant measure, such as differential gene expression or high somatic mutation load [28]. Although these methods hold great promise for disease module detection, context-specific networks are urgently needed to improve their performance [29]. Network inference methods use bulk or single-cell transcriptomics together with other omics data to determine associations between genes, typically using a (partial) correlation, (conditional) mutual information, or machine learning approaches [30]. The inferred networks offer insights into disturbed gene regulation within signaling pathways in diseases and lead to the identification of putative drug targets and experimentally testable hypotheses. In the context of complex age-related diseases, experimental approaches need to focus on the study of homeostasis processes and the identification of key ubiquitous signaling proteins that are sensitive to protein activity or abundance changes. Manipulation of protein abundance at those 'tipping points' may steer networks to a more physiologically effective state and slow disease emergence [31]. For instance, computational models assisted in determining large-scale network behavior in complex retinal degeneration [32]. Notably, disease modules require clinical proof-of-concept and pharmacological validation.

Opportunities for rational drug repurposing

Networks provide a broader selection of pharmacologically relevant targets. If a preferred target is not druggable, a neighboring target protein may compensate for this. Moreover, with 4196 approved drugs (of which 2700 are small molecule drugs; **DrugBank**[iii] [33]), it is quite likely that at least one drug is already available for any given causal disease module, obviating the need for time-consuming drug discovery and development. Based on the **PISCES**[iv] dataset, registered drugs bind with high affinity to conserved binding pockets of, on average, 39 proteins [34,35]. Thus, small-molecule drugs are highly promiscuous and can even be repurposed from one to many other target proteins with similar binding sites. Repurposing registered drugs with a known safety profile may be so powerful that it may rapidly address therapeutic needs in many different causal disease modules and outcompete classical drug discovery. Thus, we may already have almost all the drugs we need [36,37]. Rather than relying on serendipitous drug repurposing or high-throughput screening of small compounds to identify candidates, computational approaches leverage molecular networks and known drug–target interactions. Such methods first need to identify suitable drug targets that lie in one or several disease modules. Here, prior knowledge of a disease can be incorporated to guide the search (i.e., in the form of **seed nodes**) [38]. Subsequently, drugs targeting the disease module can be extracted. For example, the web application CoVex[v] integrates drug–target interaction and PPI data to facilitate drug target discovery as well as the search for repurposable drug candidates for severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and SARS-CoV-2 using known virus–host PPIs, as well as transcriptomics data [39]. An advantage of identifying disease modules is that multiple actionable drug targets can often be identified and leveraged for the development of network pharmacology therapy [40].

Curative network pharmacology

Network pharmacology approaches use two or more drugs acting mechanistically on the same causal signaling disease module, thus targeting key network proteins in a synergistic manner (Figure 3). This allows network pharmacology-based treatments to substantially lower the dose of each drug as compared with monotherapy and still achieve the same or even a more significant therapeutic effect while reducing: (i) side effects of each individual drug, and (ii) possible unwanted

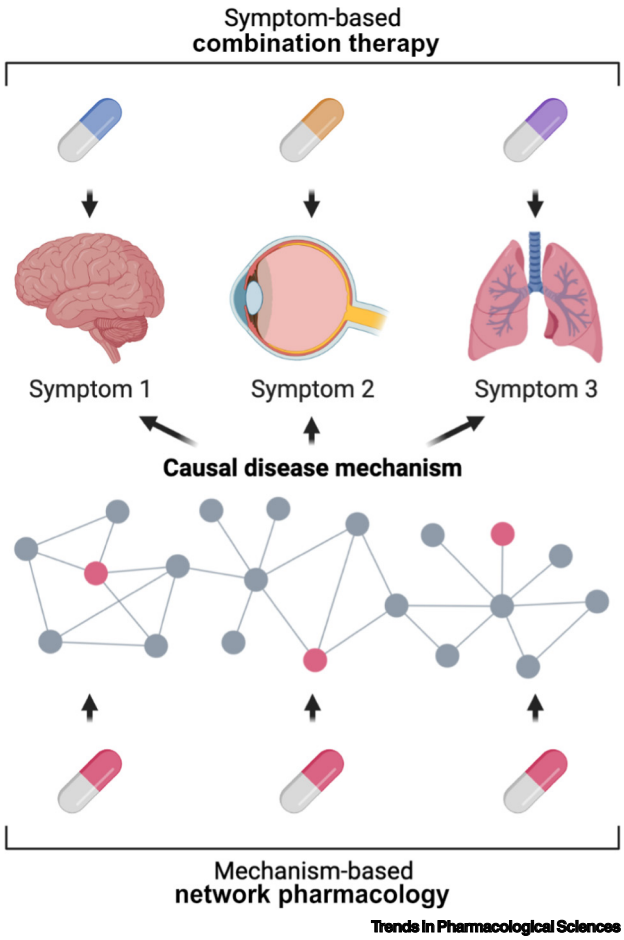

Figure 3. Differences between a mechanistic network pharmacology and a symptomatic combination therapy drug intervention. In symptom-based combination therapy (upper panel), different drugs are combined targeting symptom phenotypes, independently of the causal disease mechanism. On the contrary, in curative mechanism-based network pharmacology approaches (lower panel), low-dose drug combinations target the causal disease mechanism and reach a synergy effect. The network pharmacology example shows how in a protein–protein (gray nodes) interaction network, drugs are combined targeting proteins (pink) that are causal of the disease mechanism. The triple drug therapy shown thus interacts with key components within the same disease module or signaling network.

drug–drug interactions [41–43]. Notably, the concept of network pharmacology must not be confused with combination therapy (Figure 3), where drugs acting symptomatically on unrelated targets are combined, but none of them acts on a causal disease mechanism. Such combination therapies are, at best, additive and will not show any pharmacological synergy. Moreover, drug combinations can easily get out of control when polypharmacy results in four or more drugs being prescribed with unwanted drug–drug interactions and side effects [44]. In complex diseases that harbor robust biological networks, such as cancer, single target intervention has been proved ineffective and insufficient [45,46]. In these cases, network pharmacology approaches are instrumental, since they can simultaneously target two or more proteins within disease signaling module(s) [47]. In the clinical pharmacological workflow of network pharmacology, the reliable detection of a disease-relevant dysfunction in the signaling module is essential.

Biomarkers for molecular pathology and patient stratification

The challenge for successful therapy is not to select the right treatment but rather the right patients (i.e., a subset of patients that present both the phenotype and the endotype). Only those will most likely benefit from a specific network pharmacological intervention. Biomarkers thus become a critical diagnostic tool in disease identification, indicating a biological state and resulting in precision medicine [48–50]. Currently, biomarkers are mainly used as correlative surrogates or omics-based indicators. Less frequently do current biomarkers represent validated risk factors.

Moreover, a complete functional analysis of a patient's activity state of a causal disease module has so far not been reported [48]. Thus, predictive biomarkers that guide precision medicine, and accurately predict the response to a given treatment, represent a crucial knowledge gap between mechanism-based disease definition and clinical intervention [51,52]. Currently, this gap causes inefficient or imprecise drug therapy and clinical trials with a high failure rate.

Early adopters and proof-of-concept

The precision medicine approach reviewed earlier incorporates, to different degrees, signaling modules as disease definition and a low-dose multidrug therapeutic approach to synergistically target several proteins within these modules [41,53–56]. Ideally, this strategy will be implemented in patients stratified not only based on symptoms but also based on the diagnosis of a dysfunction of the defining disease module (i.e., the patient's disease endotype).

Cancer

Genomic profiling rather than anatomic or histologic staging of cancers has led to unprecedented opportunities for precision oncology and targeted cancer therapies [57]. It matches patients to mechanism-based cancer therapies, independent of the primary tumor location [58,59]. One example is larotrectinib, approved for fusions in the neurotrophic receptor tyrosine kinase (NTRK) gene but no longer for a specific tumor. In **basket trials**, larotrectinib demonstrated significant efficacy indeed in both children and adults [60]. In general, precision oncology trials first analyze patients' genetic makeup for later designing personalized treatments based on druggable variants. Typically, they still focus on single genetic variants [61,62], such as neratinib in tumors with variants in the human epidermal growth factor receptors (HER) [63] and capivasertib in *AKT1*-mutant breast cancers [64].

Recently, the molecular profile-related evidence to determine individualized therapy for advanced or poor prognosis cancers **(I-PREDICT) study**[vi] (NCT02534675) administered individualized drug combinations targeting several molecular alterations, resulting in improved disease control rates, more prolonged tumor-free survival, and overall survival rates in approximately half of the patients [65]. Moreover, in the Phase I/II trial in patients with recurrent glioblastoma, the **CUSP9v3 Treatment Protocol**[vii] (coordinated undermining of survival paths by nine repurposed drugs combined with metronomic temozolomide; NCT02770378), temozolomide, typically prescribed as maintenance therapy for glioblastoma patients [66], was safely combined with low doses of nine repurposed drugs, which block survival paths known in glioblastoma to render temozolomide more effective [67,68].

Tumors vary in complexity and exposure [69]. Breast and brain tumor tissues are probably protected by blood–breast and blood–brain barriers, respectively, harboring only a few alterations [70,71]. Others, such as colon and lung cancer, are presumably exposed to more carcinogens and present more variable biological networks, making them more complex and resistant to pharmacotherapy [72]. A recent whole-exome analysis of somatic alterations of at least ten canonical mitogenic pathways on more than 9000 patient samples profiled by The Cancer Genome Atlas[viii] across 33 cancer types, observed significant over-representation of individual and co-occurring actionable alterations in ten canonical pathways. In 89% of tumors, at least one **driver mutation** within these pathways was reported, suggesting that all cancers could be defined by one or more of these affected pathways. Of therapeutic relevance, 57% of samples had at least one alteration potentially targetable by currently available drugs [55]. While the principle is highly innovative, there were two limitations concerning the clinical translation of this approach. First, the analysis *a priori* excluded several cancer-relevant pathways. Second, the highly curated canonical pathways represent particular signaling principles and not experimentally validated PPI networks or functional

disease modules. Validated disease modules, however, comprise only fragments and often mixtures of two or more canonical pathways. Thus, classical pathways are misleading when defining a disease module. When reanalyzing the ten selected mitogenic pathways based on the **interactome** (Figure 4A), they are actually highly interconnected and do not represent distinct entities (Figure 4B). Thus, complex tumors most likely contain more than ten disease modules [55]. Assuming that each module is ideally treated with at least two synergistic drugs through precision network pharmacotherapy, complex tumors will probably require at least combinations of up to ten or more different drugs, depending on the cancer modules affected in each patient. The use of the aforementioned combinations will not only control the perturbed modules, but

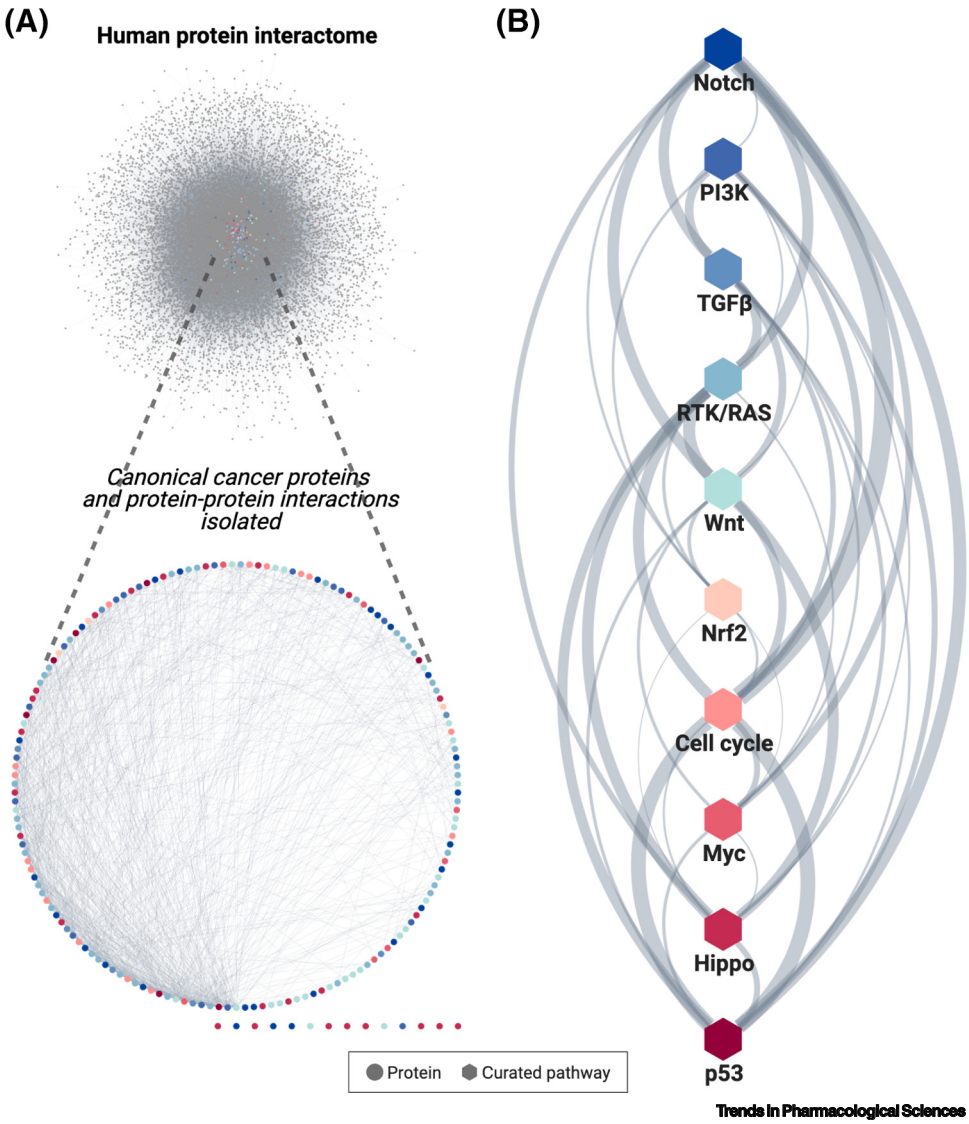

Figure 4. Canonical cancer pathways are heavily interconnected. (A) Canonical cancer proteins, adapted from [55], are mapped in the human protein–protein interactome and isolated [114]. Proteins are colored according to the classical cancer pathway that they have traditionally been associated with (i.e., Notch, PI3K, TGFβ, RTK/RAS, Wnt, Nrf2, Cell cyclase, Myc, Hippo, or p53). Although classically separated in ten different canonical cancer pathways [55], these cancer proteins appear to be heavily connected by protein–protein interactions. (B) Such interconnection between canonical pathways is even more apparent when these proteins are mapped and grouped to their respective classical pathways.

also prevent the activation of alternative survival pathways, hence, prevent emergence of resistance [73]. Thus, interventions such as the CUSP9v3 trial will rather be the rule than the exemption to cover all modules affected in a specific patient.

Immune diseases

Genome-wide association studies across autoimmune and immune-mediated diseases discovered possible underlying causal mechanisms but also endotypes within classical disease phenotypes, which also explains differential drug responses [74]. Rheumatoid arthritis is a typical case of within-disease variation with respect to clinical manifestations and severity, age of onset, number of affected joints, and their distribution or extra-articular complications. Some of this heterogeneity correlates with rheumatoid factor, anticitrullinated antibody, or human leukocyte antigen associations. A typical example of convergence between different autoimmune diseases is the rate-limiting tumor necrosis factor (TNF) expression and the success of anti-TNF therapy in rheumatoid arthritis, Crohn's disease, psoriasis, ulcerative colitis, and ankylosing spondylitis, but not in multiple sclerosis, where it may worsen symptoms.

An analysis of associations across phenotypes revealed that comorbidities between different immune diseases are best explained by biological pleiotropy [56,75]. When linking the gene- and SNP set-derived PPI network of five chronic inflammatory diseases to drugs from DrugBank and ranked these according to (pre)clinical evidence, nine drug target genes overlapped, forming a loose network with possible subnetworks (modules) with opportunities for drug repurposing and presumably synergistic, multitarget network pharmacology. Indeed, these nine druggable genes are linked to several registered drugs allowing for direct clinical translation [56]. This highlights a new evidence- or *in silico*-based approach to drug repurposing beyond the initial scope of immune diseases. It further reaffirms the argument that disease endotyping, and not drug discovery, is the limiting factor and knowledge gap in identifying new therapeutics for precision medicine.

ROCG cluster of metabolic-cerebro-cardiovascular disease phenotypes

The diseasome surprisingly revealed a heterogeneous cluster of cerebro-cardiovascular and metabolic disease phenotypes sharing a common underlying pathomechanism [17] (Figure 5A). Indeed, several genes related to reactive oxygen species (ROS) dysfunction [41] and cGMP signaling [76] (ROCG), currently separated fields, linked these phenotypes in one joint signaling network ideally suited as a test case for the mechanistic redefinition of diseases [77,78]. Four disease phenotypes (i.e., ischemic stroke [8,41,76,79–81], hypertension [38,82], diabetes [83,84], and atherosclerosis [85,86]) have already been validated within the ROCG endotype. Moreover, heart failure [87,88], myocardial infarction [89], and asthma [90–92] appear to be causally linked to ROCG dysfunction.

A recent approach built the ROCG network *de novo*, starting with seed proteins for which high-quality clinical evidence was available and their first-neighbor PPI [38]. Surprisingly, the resulting network was highly distinct from all currently curated ones and segregated into different, disconnected subnetworks, some of which are related to diseases other than those in the ROCG cluster (Figure 5B,C). These results were further validated with the two top-performing algorithms in the Module Identification DREAM Challenge [27]. With all three *in silico* methods yielding the same result, the NADPH oxidase (NOX) 5, and not the other NOX enzymes, is a direct neighbor of endothelial nitric oxide-cyclic GMP signaling. A similar *de novo* first-neighbor PPI approach has been used to identify disease modules for ROS-associated disease states (i.e., ROSopathies) [93]. Twelve distinct human interactome-based signaling modules were isolated, including novel non-ROS-related proteins, forming functional hybrids. This has significant consequences concerning which targets should be selected for network pharmacology and related diagnostic

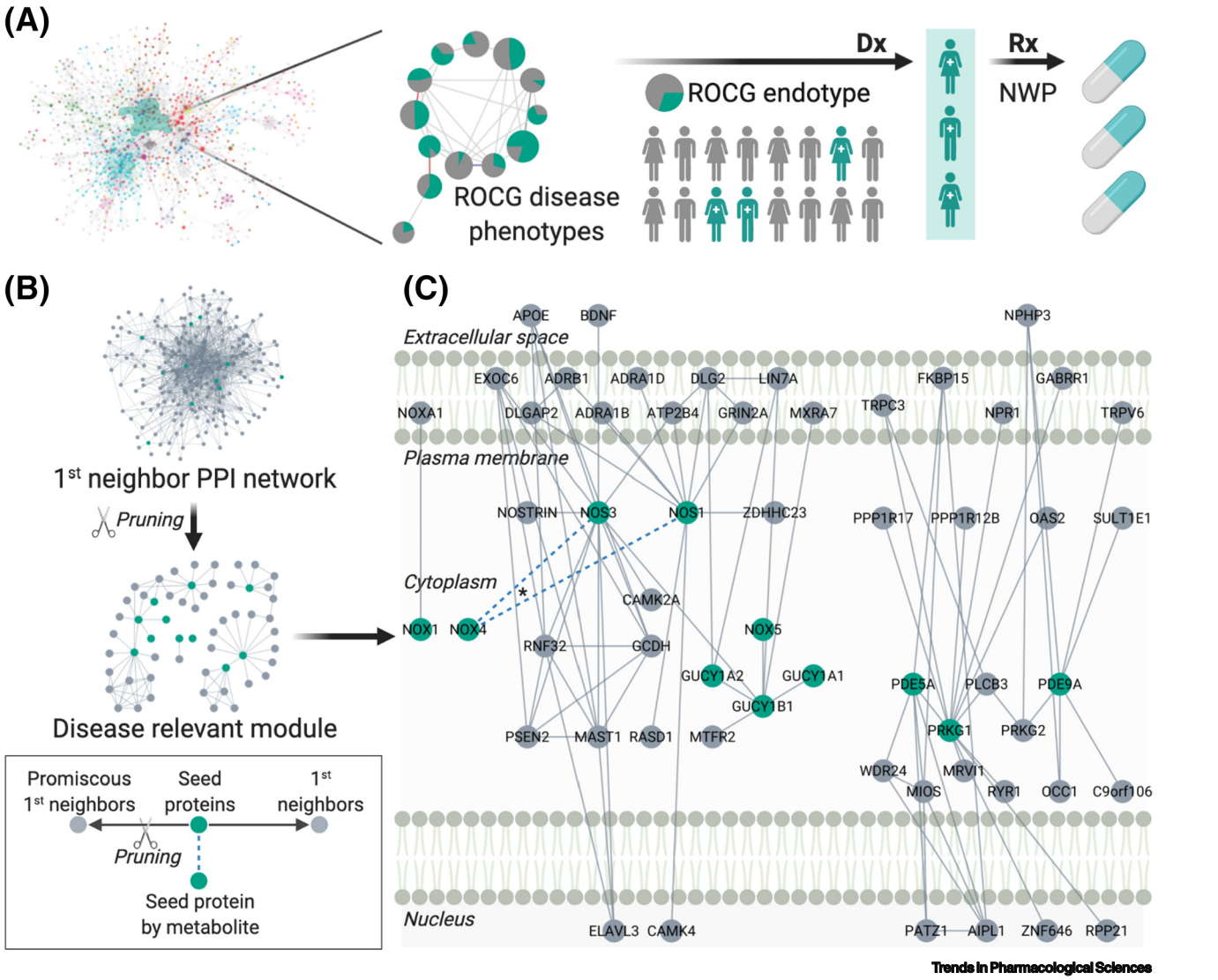

Figure 5. The reactive oxygen species/cGMP (ROCG) cluster of metabolic-cerebrovascular-pulmonary disease phenotypes. (A) The ROCG cluster of metabolic-cerebrovascular-pulmonary disease phenotypes in the diseasome (i.e., atherosclerosis, hypertension, myocardial infarction, ischemic stroke, asthma, obesity, Alzheimer's disease, dementia, migraine, Parkinson's disease, diabetes, heart failure, major affective disorder, and unipolar depression). Patients with one (or more) of the 13 phenotypes are depicted, of whom some (in green) carry the ROCG endotype, other patients have a different causal disease mechanism (gray). ROCG positive patients are endotyped with predictive biomarkers (Dx), stratified, and treated with curative mechanism-based network pharmacology (Rx). (B) In a previous study [38], clinically validated seed proteins were used as seeds to build a first-neighbor protein–protein interaction (PPI) network that characterized the ROCG causal mechanism. The network was pruned to remove highly connected proteins that are not relevant for the ROCG module and, thus, a disease-relevant module was isolated. (C) This network has been rearranged in a classical pathway manner. Previous guilt-by-association analysis data [41] has shown that NADPH oxidase (NOX) 4 is linked to NOS3 and NOS1.

biomarkers. Moreover, these results also most likely pertain to all disease mechanisms, requiring a paradigm shift and efforts to be focused on reconstructing unbiased *de novo* networks that are disease-specific (i.e., an entirely new approach to signaling and clinical pharmacology).

In the oxidative stress arm of the ROCG network, the field has drifted from antioxidant therapy, now proven to be clinically without benefit, to identifying and inhibiting disease-relevant enzymatic sources of ROS [94–96]. Pharmacological inhibition of NADPH oxidases in ischemic stroke has been shown to be neuroprotective, leading to a significant reduction of infarct volume

[79–81,97]. Similar observations have been made in diabetic nephropathy [83,84], atherosclerosis [85], and ischemic retinopathy [98]. Additionally, NOS1 knockout or inhibition appears to be protective against ischemic damage of the brain [8]. In combination, a recent network pharmacology approach in ischemic stroke targeted both enzymes, moving away from the current 'one disease–one target–one drug' paradigm of drug discovery that is becoming increasingly inefficient [41]. Indeed, NOX4 and NOS inhibitors at subthreshold concentrations showed pharmacological synergy, resulting in supra-additive improvement of all neuroprotection markers [76]. Such therapies have also been explored in preclinical models of other disease phenotypes of the cluster [99].

Cystic fibrosis (CF)

Prior to newer cystic fibrosis transmembrane receptor (CFTR) modulating small molecules, CF therapies have exclusively focused on managing the disease symptoms and controlling the infections. Ivacaftor is the first small molecule approved targeting the pathomechanism of CF. However, ivacaftor monotherapy has not shown any improvement in patients with the most common *F508del-CFTR* mutation [100], present in roughly 85% of CF patients [101] and resulting in the transcription of a truncated protein. Newly developed corrector drugs facilitate the folding of the truncated channel and its transport to the cell membrane [102,103]. In fact, a triple combination CFTR modulating therapy has been recently approved composed of two corrector drugs, elexacaftor and tezacaftor, and the potentiator ivacaftor [104,105]. Altogether, these drugs rescued the chloride ion transport function [54] acting on different sites of the mutated CFTR protein and stabilizing the folding and presentation to the cell surface, where the potentiator increases chloride ion flow [54]. Even though network pharmacology conventionally employs multiple drugs to modulate different proteins within the same network synergistically, the triple-drug combination and the recent evolution of CF therapies greatly illustrate the necessary shift in medicine towards targeting causal disease modules. The three-drug network pharmacotherapy increased eligibility for CFTR modulating drugs to up to 90% of all CF patients. In cases where the disease mechanism is due to a single protein variant, gene therapy will be a much better and curative therapeutic option [106] than chronically treating patients with CFTR drugs.

Retinopathies

Retinopathies, also termed **retinal dystrophies (RD)**, are a class of retinal diseases that cause degeneration of the outer retina. Simple (monogenic) RD develop due to rare genetic mutations and tend to manifest early in life. Complex RD, such as age-related macular degeneration (AMD), become apparent later in life and emanate from a combination of genetic, aging, environmental, and lifestyle risk factors. For both simple and complex retinopathies, disease–gene networks have been identified [53]. Surprisingly, despite their similar manifestations in the same organ/tissue and the presence of overlapping retinal phenotypes, the diseasome partitions RD into three distinct clusters. Genes associated with the different forms of RD, in general, do not overlap to any great extent [53]. Not all disease genes have an expression profile restricted to cell types in the outer retina [53] and a large proportion of genes are expressed in all nonretinal tissues (see HPA[ix] [107]). Thus, the RD diseasome overlaps with genes associated with other nonretinal phenotypes/diseases. AMD endotypes were characterized by generating a knowledge repository of PPI networks implicated in AMD pathogenesis [108]. Interestingly, the network analysis revealed two clusters, one linked to para-inflammation and the other to extracellular matrix homeostasis, which may each represent different underlying molecular pathology mechanisms, possibly resulting in patient-specific phenotypic manifestations. The network also demonstrated that bioenergetics (energy metabolism) is critical for homeostasis and repair mechanisms in general.

Therapeutically, the application of single drugs may be appropriate for targets that tend to be explicitly expressed in the retina linked to specific functions. By contrast, proteins expressed in

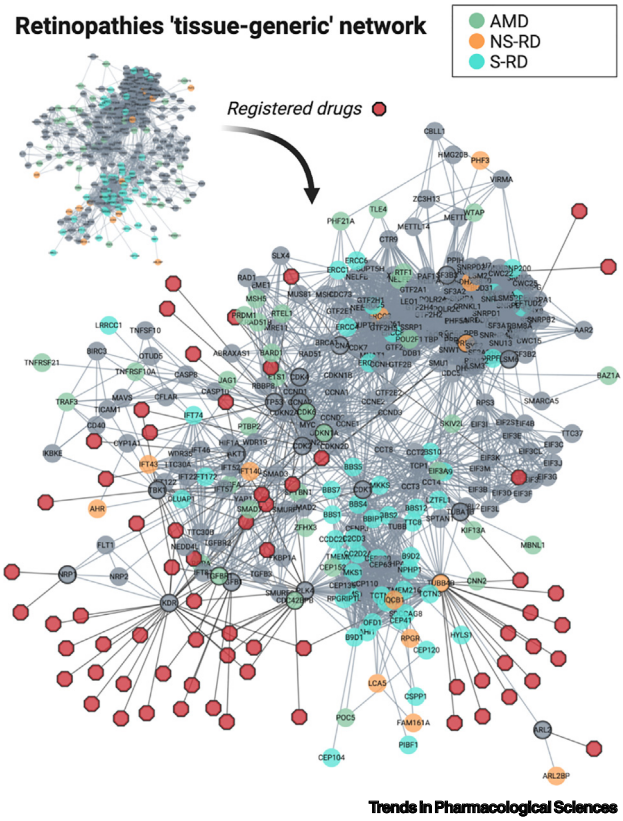

Figure 6. Suggested pharmacological interventions in retinal dystrophies. Protein–protein interaction network of gene products belonging to the 'tissue-generic' network. Interactions are obtained from the **STRING**[xi] database. The data shown has been adapted from [53,111]. Nodes are colored according to their association to retinopathies and gray colors correspond to first-neighbor interactors. Registered drugs are added from DrugBank and DrugCentral[x]. Abbreviations: AMD, age-related macular degeneration; NS-RD, non-syndromic retinal dystrophy; S-RD, syndromic retinal dystrophy.

multiple cells (tissue-generic) are part of PPI networks involved in tissue homeostasis processes, the function of which is an emerging property of the PPI network and might be better suited for network pharmacology therapy (Figure 6). Taking advantage of a recent single-cell gene expression dataset of 17 cell types in the outer retina [109,110], a systems approach narrowed down PPI networks in AMD to more specific protein drug targets [111].

Concluding remarks and future perspectives

We believe that a new era of pharmacology has commenced. While many molecular, structure–activity, and chemical aspects of drug–target interactions are understood, the target choice and definition prevents pharmacology and drug therapy from becoming an exact science. Thus, a realistic and urgent task is to revisit and redefine our current concept of diseases. Similar to rare diseases, which are mostly named after a causal gene or protein, the new disease definitions will be molecular and cross current organ borders and scientific silos. Reflecting the complexity of diseases, these mechanisms are, in most cases, not single proteins but small networks or disease modules. Moreover, we observe that disease-related signaling networks often do not overlap with our current concept of signaling pathways, possibly because these signaling networks comprise elements of more than one canonical curated pathway. Because of their network structure and the fact that they are better modulated by targeting it at several sites, we will increasingly see synergistic network pharmacology, which is not to be confused with current combination therapy, characterized by mechanistically unrelated drugs that do not target causal genes. Finally, such effective targeting of causal disease modules will develop pharmacology and drug therapy from chronically treating

Outstanding questions

How can we better select pharmacological targets and design precise pharmacological interventions?

How do we define and limit the number of disease-associated seed and driver genes?

What is the best bioinformatics/network science method to identify and define a disease module from these seed and driver genes?

How will we name those modules of polygenetic disease if no longer by a leading symptom or group of symptoms or comorbidities?

How many disease-relevant genes are there? According to the Pareto principle, one can modulate every network with about 20% of its components. Will thus 20% of all 22 000 proteins (i.e., 4400) suffice to regulate and deregulate our interactome?

What is then the average size of a module? If it is, for example, between ten and 30 proteins, will we then have 4400/20 (i.e., only 220) polygenetic diseases in total?

If classical homogenous pathways are nothing but mind maps, what will replace or best represent them; heterogenous nanodomains?

Do current disease definitions block innovation and precision medicine? How do we redefine a disease from symptom to mechanism?

How do we then identify these disease mechanisms and modules, ideally in a point-of-care setting, by genetics, blood tests, biopsies, or all of these? Are molecular disease diagnostics the next big research gap?? Would signaling modules better reflect the complexity of diseases? And, if so, what is the average size of a module, from ten to 30 proteins?

Will we need mainly new drugs to cover those disease modules, or can we, given also the promiscuity of small molecules, repurpose a sufficient number of registered drugs obviating the need for drug discovery?

disease symptoms to curing diseases, thereby complementing gene therapy of monogenic diseases. Collectively, this will gradually lower the NNT towards the ultimate goal of reaching precision medicine.

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Declaration of interests

No interests are declared.

Resources

[i]www.genome.jp/kegg/

[ii]www.wikipathways.org

[iii]www.drugbank.com

[iv]http://dunbrack.fccc.edu/pisces/PISCES_OptionPage.php

[v]https://exbio.wzw.tum.de/covex/

[vi]https://clinicaltrials.gov/ct2/show/NCT02534675

[vii]https://clinicaltrials.gov/ct2/show/NCT02770378

[viii]www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga

[ix]www.proteinatlas.org

[x]https://drugcentral.org

[xi]https://string-db.org

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Will we be then able to cure or prevent diseases with high precision (i.e., NNTs of close to 1) by treating causal mechanisms rather than symptoms as we do now? With only 4400 disease genes and the average size of a module being from ten to 50 proteins, will we have only 150–440 diseases in the end?

If we know the causal human disease mechanism, but animal models mimic only a symptom of the disease but not the causal mechanism, will we then see a profound shift in pharmacological research from basic and preclinical animal-based work to human big data and rather Phase I and II clinical trials?

How do we then identify a therapeutic strategy? Would a mechanism-based network pharmacology approach restore the healthy signaling of the module?

The ultimate proof of this fairly radical new concept has to come from clinical evidence. Will we thus see a profound shift in pharmacological research from basic and preclinical animal-based work to human big data and clinical studies?

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