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Integrating network pharmacology: The next-generation approach in ocular drug discovery

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Abstract

With the spread of the "omics" sciences, the approaches of systems biology can be considered as new paradigms of pharmacological research for discovery of novel targets and/or treatments for complex multifactorial diseases. Data from omics sciences can be used for the design of biologic networks, that in turn can be quantitatively analyzed to identify new pharmacological targets. In this review, we will introduce the concept of network pharmacology, particularly the application of this innovative approach in the field of ocular pharmacology, with a focus on retinal diseases such as diabetic retinopathy (DR), age-related macular degeneration (AMD) and glaucoma.

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Network pharmacology

Canonical research and development (R&D) studies in pharmacology are longitudinal and are thus characterized by a first step of pharmacological target identification and its validation followed by several steps of screening mi(bi)llions of ligands for hit and lead identification, and finally efficacy and toxicity studies *in vitro*, *ex vivo* and *in vivo*. At the end of the non-clinical process, just one or a very small bunch of molecules would reach the clinical trial phases, even though a stop could happen any time during clinical drug development. Most R&D processes, focused on a selective mechanism of action, are mainly aimed at the design of drugs with high selectivity toward one pharmacological target, to minimize off-target effects and adverse events, and then maximize intrinsic and clinical efficacy of the drug. These consolidated R&D processes are strictly linked to the Paul Ehrlich's "Magic Bullet" concept, which states that the perfect drug is one that goes straight to a specific target. Pharmacological studies based on this concept have developed highly effective drugs, with undeniable clinical advantages such as precision therapies, and tumor-agnostic treatments [1].

On the contrary, looking at clinical experience in the field of neuropsychopharmacology, and specifically focusing on schizophrenia treatments, drugs with a polypharmacological profile have shown clinical advantages; e.g., drugs with high affinity towards two or more receptors, such as second-generation antipsychotics, have shown greater efficacy and safety compared to first generation antipsychotics. The discovery of drugs with polypharmacological profiles and specifically the discovery of best pharmacological targets to design effective drugs would take advantage of systems pharmacology approaches. Systems pharmacology, network pharmacology, and quantitative systems pharmacology can be considered as synonymous and are tightly linked to systems biology: a holistic approach (i.e., all-inclusive) which analyzes all elements of

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biological problems. Systems biology contrasts with reductionist biology, that analyzes a biological problem taking its elements apart and not altogether.

Specifically, systems biology analyzes a biological problem as a network of interactions at different scales, e.g., protein-protein in a cell type, cell-cell in a tissue, tissue-tissue, organ-organ, system-system. Systems biology directly exploits experimental data from omics sciences such as genomics, transcriptomics, proteomics, metabolomics, and lipidomics. Big data from omics sciences must be analyzed and screened at first through advanced statistical and computational approaches, for example through network computational approaches.

In this perspective, analysis of networks as models of biological systems evidenced that the Ehrlich principle of the "Magic Bullet" (e.g., perfect drug), targeting only a specific pharmacological target in a biological network, would be a limit, particularly for diseases with multifactorial and complex etiology, such as neuropsychiatric and neurodegenerative diseases. In fact, large-scale functional genomic studies on model organisms evidenced that silencing of one gene led to defective or pathogenic phenotypes only in 19% of cases. Additionally, studies on eukaryotic organisms (yeast) have shown that, over about 5900 tested genes, haploinsufficiency (i.e., pathologic mutation in heterozygosis) was evidenced only in the 3% of mutated genes in a single copy of chromosomes [2]. Besides, these data are coming from simple biological models, it has been evidenced by functional genomic studies that organisms are resistant to mutations or deletions due to redundancy of coding gene's functions, or due to compensatory mechanisms that are conserved. At the computational level, the biological robustness to single gene mutations has been proven through analysis of networks, representing biological systems. In fact, biological networks are scale-free networks, whose structure is maintained if a node (e.g., a gene, a protein, or a pathway) is randomly deleted; but network stability is compromised if a central element (e.g., a hub node or a cluster of nodes) is deleted [1].

Thereby biological networks, that represent complex multifactorial diseases, are resistant to single-node deletions. Thus, a complex disease would not be easily treated with a drug that selectively binds one receptor. Indeed, design of a highly selective drug toward one pharmacological target for complex diseases can be affected by attrition in the R&D processes that in turn would lead to failure in the clinical trial phases. In this perspective, the rational drug design could take advantage of a polypharmacological approach, specifically of the process of identification of more than one pharmacological target through analysis of the network, representing a given biological problem.

Biological networks can be built starting from experimental data (e.g., high throughput genomic, transcriptomic data) or through thirty-party re-analysis of data generated by others and deposited in free-access databases, such as Gene Expression Omnibus database (GEO) [3]. Third-party re-analysis is also defined as reverse engineering approach, since after advanced statistical analysis (e.g., through GEO2R analysis) of datasets (e.g., transcription analysis in pathological tissues in comparison to controls) and identification of top differential expressed genes (DEGs); these DEGs can be input of an enrichment of analysis (e.g., through GENEMANIA or STRING software). The enrichment analysis is necessary to rebuild or retrieve new interactions in the biological network that represent a model of the disease. Analysis of node's parameters, such as network centrality, can be carried out with open-access software, such as Cytoscape.

Network pharmacology and diabetic retinopathy

Diabetic retinopathy (DR), a severe complication of diabetes mellitus, is one of the major causes of irreversible vision loss [4]. Currently, there is an unmet medical need in terms of pharmacological tools to handle non-proliferative DR. In fact, pharmacological treatments are approved for proliferative DR and specifically for diabetic macular edema (DME), a complication of diabetes caused by fluid accumulation in the macula. Moreover, some patients are unresponsive to the therapies such as anti-vascular endothelial growth factor (VEGF), and steroids. Several factors are involved in the etiopathogenesis of DR, so studies are still needed for the identification of all genes and pathways dysregulated in this disease. Network pharmacology, combining systems biology with computational approaches, is able to identify gene-pathways networks involved in DR and to observe the influence of drugs on those pathways, revealing the molecular mechanism of drug effect and providing new hints for drug development. Different data have been presented regarding the mechanism of action of an investigational drug, the tert-butylhydroquinone (TBHQ), which has been found as a good candidate for the treatment of DR, acting as an antioxidant drug, since oxidative stress is a cause of microvascular endothelial injury. In this study network analysis was used to identify some targets, such as MAPK8, RELA, ESR1, APP, NOS3, Rap1 signaling pathway, arachidonic acid metabolism, VEGF signaling pathway, and renin-angiotensin system, which are strongly related to TBHQ treatment of DR [5]. In another study, bioinformatics is used to investigate the effect of *Eriocauli Flos* a Chinese herbal medicine, with already known anti-inflammatory and anti-bacterial effects. GO enrichment and KEGG enrichment demonstrated the anti-inflammatory effect of this extract by reducing TNF-α, VEGF-A protein, and regulating AGE-

RAGE and PI3K-Akt signaling pathways during diabetic complications [6]. Another compound has been identified as good candidate for DR, Huperzine A (Hup A) which is an alkaloid extracted from the *Huperzia serrata*. The protein−protein interaction (PPI) network for common targets of DR was analyzed to predict potential targets of Huperzine A. In this study, it has been demonstrated that HSPB1 (HSP27) and apoptosis-related proteins (Bax, Bcl-2, Caspase3) are influenced by HupA, representing potential targets for DR treatment [7]. Another study has identified a compound active against oxidative stress in DR, the andrographolide, a diterpenoid, and a component of traditional Chinese medicine. Through transcriptomics and network pharmacology based on an oxidative stress model on retinal endothelial cells, the authors identified 18 candidates and they confirmed that andrographolide, after *in vitro* validation, is able to regulate oxidative stress, inflammation, cell-adhesion, and other mechanisms unbalanced during DR [8]. The computational systems biology approach has been also applied in another study [3] where four different microarray datasets have been analyzed, and transcriptomic analysis was carried out comparing retinas of different animal models of DR. This study has also analyzed two datasets coming from clinical studies. The main pathways involved in the etiopathogenesis of DR have been identified from network approaches and were mainly linked to inflammation and fibrosis. Moreover, G protein-coupled receptors (GPCRs), such as adrenergic receptors, have been identified as intriguing pharmacological targets for treatment of DR. One of the conclusions of this study is related to identification of ADR1D and ADR2C receptors as potential innovative pharmacological targets for treatment of DR, but preclinical studies need to be carried out to confirm *in silico* data [3].

Network pharmacology and AMD

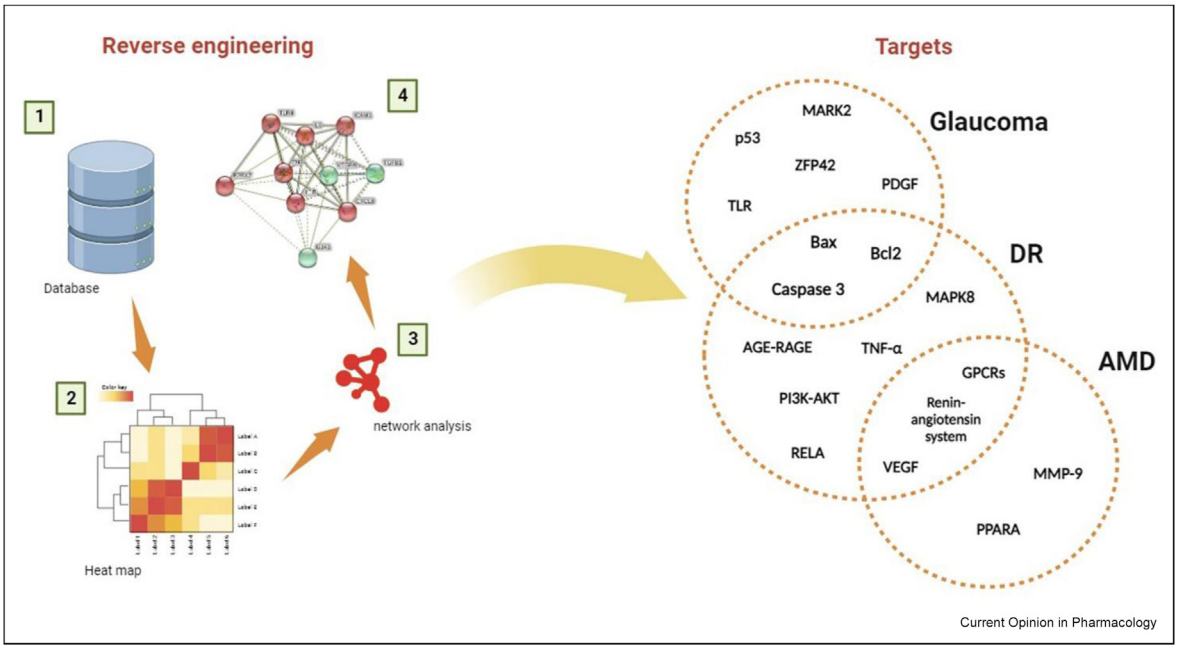
Age-related macular degeneration (AMD) is an ocular degenerative disease which affects the macula, the central area of the retina, leading to irreversible visual loss [9,10]. Currently, the neovascular form of AMD (wet-AMD) can be managed with drugs targeting VEGF. Whereas only two molecules inhibiting the complement C3 and C5 have been approved recently for the severe dry-AMD, pegcetacoplan, and avacincaptad pegol, respectively [11]. Due to the complex and multifactorial etiology of AMD, network pharmacology can be a powerful approach to discover new multitarget drugs, addressing interconnected signaling pathways potentially involved in AMD. Reverse engineering and network approaches helped also in the identification of miRNAs dysregulated in the retina of a rat model of AMD and in serum of AMD patients [12]. Moreover, network pharmacology could help to investigate the relationship between a drug and the associated mechanism of action, as reported in the study by Lazzara et al. [13], which has explained most of the pathways targeted by vitamin D_3 and mesozeaxanthin in three different *in vitro* models of AMD. Furthermore, Sha Liu et al. designed a novel retinoic acid drug, EYE-503, for the treatment of retinal neurodegeneration, which is involved in AMD [14]. In particular, the intravitreal injection of EYE-503 was protective in a mouse model of retinal degeneration, counteracting retinal ganglion cells (RGCs), and axonal degeneration, as well as reducing retinal reactive gliosis. Additionally, based on the chemical structure of EYE-503, the authors employed network pharmacology to identify the potential signaling pathways connecting EYE-503 and retinal neurodegeneration. Particularly, MAPK signaling pathway was predicted as EYE-503 target, which was confirmed by western blot analysis. Indeed, EYE-503 treatment significantly reduced JNK/p38 phosphorylation in RGCs, suggesting that EYE-503 neuroprotective effects are associated with JNK/p38 signaling [14]. An integrative approach to identify oxidative stress-related targets for AMD was proposed by Nishimura Y. et al. [15]. In particular, VEGF-A, matrix metallopeptidase 9 (MMP9), peroxisome proliferator-activated receptor α (PPARA), as well as several components of the renin-angiotensin system including angiotensin (ANG), angiotensin 1 converting enzyme (ACE1), ACE2 and angiotensin II receptor type 1 and type 2 (AGTR1 and AGTR2), were identified within the AMD network as potential therapeutic targets for AMD-related oxidative stress [15]. The combination of omics databases and biological knowledge is pivotal to generate disease-related networks and to identify potential therapeutic targets inside the network [15]. Indeed, based on network pharmacology approach and transcriptomic analysis, Chen Y et al. identified different GPCRs, associated with retinal degenerative disorders, which protect against cellular death and stress caused by connected signaling pathways [16]. Moreover, these authors evaluated the retinal protective effect of combined treatments that address different mechanisms. Specifically, drugs targeting Gq-coupled and Gi-coupled receptors were administered simultaneously in an *in vivo* model of retinal degeneration holding genetic modifications of retinoid cycle components, which are associated with an increased risk of AMD development [17]. In particular, the combination of guanabenz and doxazosin, which activate alpha-2A adrenergic receptor (ADRA2) and antagonize alpha-1A adrenergic receptor (ADRA1) respectively, protected more against retinal degeneration in comparison to drugs administered alone at the same dose [16,18].

Network pharmacology and glaucoma

Glaucoma is one of the most common causes of irreversible blindness in industrialized countries [19].

Cupping of the optic disc, along with apoptosis of retinal ganglion cells (RGCs), represents the hallmark of the disease. Current drug treatments are aimed at reducing the intraocular pressure (IOP), one of the most well-known glaucoma's risk factors, but cannot prevent optic nerve degeneration and consequently the loss of vision. To find out a neuroprotective therapy able to counteract the progression of the disease, identification of new drug targets is needed [20–23]. Specifically, a reverse engineering approach was used to confirm the mechanism of action of vitamin D_3, which preserved RGCs from neurodegeneration in a mouse model of glaucoma (i.e., DBA/2J mice) [24]. Network pharmacology approach has been proved as a powerful tool to improve the knowledge on pathological mechanisms in glaucoma, also bursting the drug discovery process. For instance, Yin et al., through this method, identified new regulatory axis putatively involved in glaucoma pathogenesis. Starting from the mining of microarray data on GEO database, 9 candidate genes have been identified as diagnostic marker for glaucoma. Finally, after the building of a glaucoma-specific transcriptional regulatory network, including the differential expressed transcription factors of the candidate genes, a correlation analysis showed that only ZFP42 and its target gene MARK2 were correlated with glaucoma. Indeed, transfection with a plasmid able to increase the expression of ZFP42 led to up-regulation of MARK2 and increased cell viability in transfected RGCs, exposed to H_2O_2 [20]. Moreover, besides the identification of new dysregulated genes, the network pharmacology approach could also burst the drug discovery of novel candidate drugs, as presented in the study of Zavarzadeh et al. [25]. After data mining from GEO dataset and the identification of the most significant DEGs, DAVID database was employed to carry out a functional enrichment and pathway analysis of the identified DEGs. This analysis showed that the most significant dysregulated pathways were related to extracellular matrix (ECM) organization, immune system, neutrophil degranulation, the platelet-derived growth factor (PDGF) signaling p53, and the toll-like receptor. Therefore, this enrichment information approach was effective in providing several biological pathways worthy of further investigation and validation. In addition, STRING database and ClusterVis were used to build the -PPI networks among the above-mentioned DEGs and to find protein hub modules, respectively. Then, Drug Gene Interaction Database (DGIdb) was employed to find drugs targeting the genes central in the network. This approach has revealed that metformin hydrochloride, bortezomib, ixazomib citrate, carfilzomib, carboplatin, and cisplatin could be used to treat glaucoma, but only after proper *in vitro* and *in vivo* validation studies [25]. Furthermore, network pharmacology approach could also be applied to examine the mechanism of action of investigational drugs or drug extracts, known to exert positive pharmacological effects. For instance, Yu et al. confirmed the pleiotropic mechanism of action of *Ginkgo biloba* extract, that promoted RGCs survival [26–28]. The network intersection between the known glaucoma therapeutic targets and validated targets of the *G. biloba* extract evidenced several overlapping nodes: p53, BAX,

Figure 1



Network pharmacology approaches in ocular pharmacology.

Table 1

Active compounds and pharmacological targets obtained from network pharmacology analyses.

Disease	Drug	Target
DR	TBHQ	MAPK8, RELA, ESR1, APP, NOS3, Rap1, arachidonic acid metabolism, VEGF, renin-angiotensin system
DR	*Eriocauli Flos*	TNF-α, VEGF-A, AGE-RAGE, PI3K-Akt
DR	Hup A	HSPB1 (HSP27), Bax, Bcl-2, caspase3
DR	–	ADR1D, ADR2C
AMD	–	miR-9, miR-23a, miR-27a, miR-34a, miR-146a, miR-155, TGFβ, mTOR, HIF-1α
AMD	Vitamin D3 and meso-zeaxanthin	APP, TLR4, IL6, TNF-α, PSEN1, CAT, IL-1β, VEGF-A
AMD	EYE-503	JNK/p38 signaling
AMD	–	VEGF-A, MMP9, PPARA, ANG, ACE1, ACE2, AGTR1, AGTR2.
AMD	Guanabenz and doxazosin	GPCRs
Glaucoma	–	ZFP42, MARK2
Glaucoma	Vitamin D3	VDR, BDNF, VEGF-A, PlGF (PGF), IL-6, IL-1β, CCL-3, IFN-γ, and p-65 NF-κB
Glaucoma	–	ECM organization, immune system, neutrophil degranulation, PDGF, p53, TLR
Glaucoma	Metformin hydrochloride, bortezomib, ixazomib citrate, carfilzomib, carboplatin, cisplatin	–
Glaucoma	*Ginkgo biloba* extract	p53, Bax, Bcl-2, caspase3, caspase9

BCL2, CASP3, and CASP9. In this study, the *in vitro* experiments confirmed the anti-apoptotic and antioxidant effects of *G. biloba*, targeting the p53, Bax, Bcl-2, Caspase-3, and Caspase-9 signaling pathways [26].

Summary

We hereby analyzed the studies regarding application of systems pharmacology for advances in R&D process in the field of ocular pharmacology (Figure 1). Network pharmacology approaches, hereby presented, were aimed at identification of innovative pharmacological targets of retinal diseases, characterization of novel pathogenic mechanisms, and profiling of multiple mechanisms of action of an investigational compound (Table 1). However, quantitative systems pharmacology approaches can also have other applications focused on decision-making approaches in clinics (Bayesian network models, network meta-analysis), although such applications have not been retrieved in the field of ocular pharmacology yet.

CRediT authorship contribution statement

CBMP: Conceptualization, Investigation, Writing – review & editing; FL, FC, EG: Investigation, Writing – review & editing; CME; FD: review & editing. CB Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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