Chapter 18

Network Pharmacology and Epilepsy

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Abstract

In contrast with the reductionist "one gene, one target, one drug" approach, network pharmacology proposes the use of multi-target therapies, a strategy that seems particularly suitable to treat disorders of complex etiology, among them epilepsy. As a matter of fact, most of the existing antiepileptic drugs are indeed multi-target unintended agents. Whereas a number of authors have recently advocated the use of network-based approximations in the antiepileptic drug discovery field, such strategy has so far not produced deliverables. Here, we review some practical considerations which could be used to assist in silico and wet screening for novel antiepileptic agents.

Key words Epilepsy, Network pharmacology, Systems biology, Multi-target drug, Tailored multi-target drug

1 Introduction

Some decades back, the pharmaceutical sector adopted a target-driven, reductionist approach to drug discovery. The idea was appealing: highly selective agents interacting with (and only with) a validated target would avoid off-target interactions, representing safer therapeutic solutions. Furthermore, the target-driven approach allowed the implementation of rational drug design campaigns and the bioethical (and cost-efficient) replacement of low-throughput animal models by high-throughput in vitro models as primary screening platform. Whereas such clean drugs seem well suited to find therapeutic agents to treat Mendelian disorders where a single gene is associated to the disease, they have generally shown limited efficacy for the treatment of complex disorders (e.g., cancer, psychiatric and neurological disorders). The former reductionist approach has once again been proposed as one of the possible explanations for the decline in productivity in the pharmaceutical sector [1–3], a reality that particularly hits the field of central nervous system (CNS) pharmacology [4, 5]. Curiously,

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the number of first-in-class small molecule drugs emerging from phenotypic screening seems to outnumber those from target-centered discovery [6].

From a systems biology viewpoint, living organisms are understood as robust entities, and disease can also be considered a robust state emerging from multiple and simultaneous perturbations of a resilient system [2]. Recently, a number of authors have discussed that epilepsy, being a multifactorial, polygenic, and dynamic disorder, could be particularly suited to be approached through network pharmacology [7–12]. Such belief is supported by the fact that, while it is currently thought that highly selective agents could be useful to treat specific syndromes [9], most of the existing antiepileptic drugs (AEDs) are actually fortuitous multi-target drugs which have emerged from phenotypic screening [8, 11]. On the other hand, a number of studies in animal models of seizure and epilepsy suggest that the combination of drugs associated with different mechanisms of action tends to enhance the efficacy of the treatment [9, 13–16]. At least, contrary to the general bioethical trend, in vivo models remain the primary screening assay to identify AEDs, which underlines the complex nature of the disorder. Whereas the majority of primary assays used within the pharmaceutical industry for the early drug discovery rely upon the creation of stable mammalian cell lines or upon the overexpression and purification of recombinant proteins to establish biochemical assays [17], the absence of immortalized cell lines mimicking the epileptic condition precludes this possibility. And while in vitro AED screening using animal or human ex vivo tissue (brain slice preparations) is also possible [18–21], such approach is limited by tissue availability, the rarity of spontaneous epileptiform activity in the tissue, and the viability of the tissue (a limitation that has lately been partially overcome thanks to the advances of organotypic culture techniques) [22].

Having said so, it is very surprising that the network pharmacology approach has yet not been fully embraced within the AED discovery field, which seems to have fallen a little behind in comparison with other complex disorders such as cancer or Alzheimer's disease. Stressing the previous statement, Fig. 1 displays the evolution over time (according to Scopus) of the number of scientific articles in periodicals that present the terms "multi-target" and "epilepsy" within their title, abstract, or keywords, in comparison with the coappearance of "multi-target" and "cancer" and "multi-target" and "Alzheimer." The previous observations are in agreement with the opinions of leading experts in the epilepsy field, who have stressed the urgent need of innovative approaches for AED discovery [9, 23]. While some recent articles have beautifully reviewed the experimental and theoretical basis that support adopting systemic, integrative approaches for AED discovery, here we will discuss some practical considerations for the implementation of this paradigm shift.

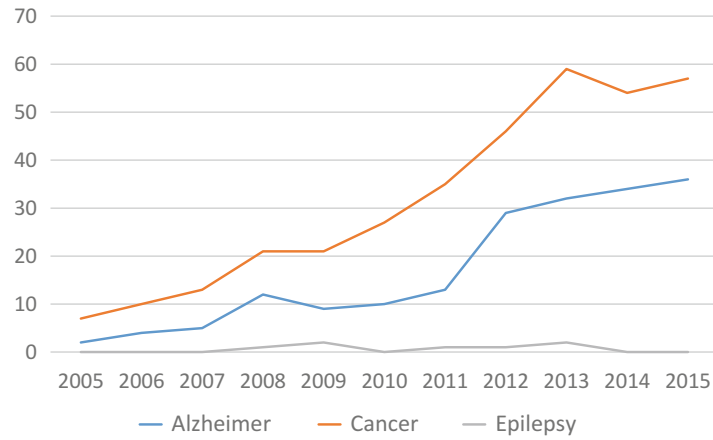

Fig. 1 Frequency of co-occurrence of the terms "multi-target" and "Alzheimer," "cancer," or "epilepsy" in scientific publications (2005–2015, Scopus). Whereas the relative frequencies could also reflect the comparative interest shown for each condition by the drug discovery sector, the epilepsy community seems to have embraced the network pharmacology paradigm more slowly

2 Tailored Multi-target Agents

Before target-focused drug discovery, new lead compounds emerged from serendipitous discovery, traditional medicine, and phenotypic/physiologic screening in cellular or animal models of disease. Though it was possible to find multi-target agents through such approaches, those targets were, hopefully, defined a posteriori, and the combination of targets attacked was unplanned and sometimes not fully known.

In the discussion on target- versus phenotypic-based strategies (or reductionist versus integrative approaches), tailored multi-target agents can be regarded as the dialectical synthesis that pick the best out of each paradigm. Multi-target therapies are an extension of the target-centered approach that incorporates the viewpoint of network pharmacology. Tailored (or designed) multifunctional agents are deliberately devised to selectively modulate a number of chosen targets, usually relying on computer-aided design and data analysis applications and simplifying the expensive target deconvolution. Theoretically, multi-target agents are equivalent to combined therapies with different single-target agents, but they are advantageous in terms of diminished chances of drug interactions, simpler pharmacokinetics, and improved patient compliance [24].

Multi-target agents could be a viable solution to deal with drug-resistant epilepsy linked to acquired or constitutive target modifications [25]. They could also be specifically designed to

address, in a simultaneous manner, the signs and symptoms and underlying causes of the disease. For example, it is suspected that seizures and inflammation take part in a complex interplay that results in a vicious circle [26, 27] where inflammation would be both cause and consequence of seizures. Clinical evidence shows that steroids and other anti-inflammatory treatments display anticonvulsant activity in some drug-resistant epilepsy syndromes, and some of the most widely used AEDs (e.g., valproate) have proven anti-inflammatory effects [28]. It could be guessed that a combination of anti-inflammatory and anticonvulsant properties in a single molecule could have a positive impact on epilepsy management. The preclinical evidence on the effects of anti-inflammatory agents in epilepsy is reviewed in a separate chapter of this same volume. Similarly, due to the high prevalence of comorbid mood disorders (mainly, anxiety and depression) in epileptic patients [29, 30], the simultaneous treatment of core and comorbid manifestations of epilepsy constitutes an additional potential application of multi-target agents in epilepsy management. Remarkably, many widely used antiepileptic drugs have shown efficacy against different psychiatric conditions, as clearly reviewed in Chap. 17 by Kubova.

2.1 Practical Considerations to Apply in the Design of Antiepileptic Multi-target Drugs

Etymologically, the word *pharmacophore* comes from the Greek and means "cure carrier" or "medicine carrier." Presently, it alludes to an abstract (geometrical) description of molecular features which are necessary for molecular recognition of a ligand by its molecular target. In other words, the pharmacophore is the molecular framework which is essential to elicit a biological response. A multi-target drug must either combine different pharmacophores in a single molecule (one for each specific recognition event intended) or present a common pharmacophore for different targets (which implies that the different targets display shared determinants of specificity). That is, different degrees of pharmacophore overlapping can be found in multi-target drugs [31] (Fig. 2).

Those multi-target drugs that use different sets of atoms (anchors) to interact with each target protein tend to violate drug-like criteria, presenting oral bioavailability issues [7, 32, 33]; understandably, the chance of violating drug-likeness rules and endangering bioavailability increases with the number of separate anchors. Thus, if designing multi-target drugs, watch carefully for violations of more than one of Lipinski's rules (no more than five H-bond donors, no more than ten H-bond acceptors, molecular mass below 500 g/Mol, and calculated Log*P* below 5) [34], and check if Veber's rules are accomplished (ten or fewer rotatable bonds and a polar surface area below 140 square Ångström) [35]. Note that, owing to the more challenging diffusion barrier posed by the blood–brain barrier, the physicochemical properties required to achieve brain bioavailability are even more stringent than those

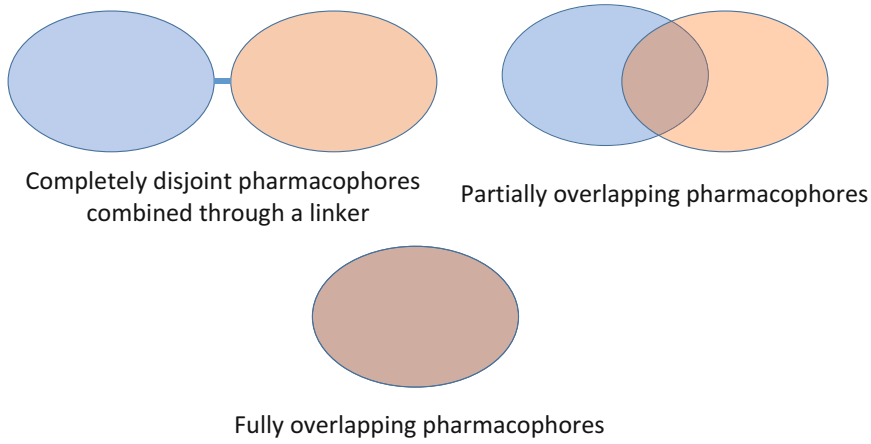

Fig. 2 Multi-target agents can display different degrees of merged pharmacophores

needed to attain oral bioavailability. Accordingly, the biopharmaceutical properties of multi-anchor ligands intended for the treatment of epilepsy or other central nervous system conditions must be specially watched. As discussed elsewhere in this volume, the very interesting work from Wager and coworkers presents a central nervous system multiparameter optimization approach that delivers a desirability score that can be especially useful to assist the design of multi-anchor multi-target AEDs on the basis of readily computable physicochemical properties [36]. The score is easily computed by calculating six theoretical properties, most of which are available in almost every modern chemoinformatics software package and also frequently provided by public chemical databases as PubChem or ZINC: calculated partition coefficient (clog*P*), calculated distribution coefficient at pH = 7.4 (clog*D*), molecular mass, number of H-bond donors, topological polar surface area, and the pKa of the most basic center. A direct relationship between Wager's desirability score and key in vitro attributes (absence of permeability issues, P-glycoprotein efflux, safety, metabolic stability) has been observed in marketed central nervous system drugs as well as Pfizer's candidate set. Remarkably, almost all the approved AEDs present high desirability scores (around 5).

Ligand efficiency metrics are another aspect that should not be disregarded during the design of multi-target agents [7, 33]. The empirical analysis by Juntz et al. revealed that, across a wide variety of ligand-macromolecule complexes, maximal contributions to binding free energy per ligand non-hydrogen atom are similar to −1.5 kcal/mol; the authors also observed a trend to a smaller free energy contribution per atom as the molecular mass of the ligand increases [37]. Ligand efficiency metrics have gained increasing acceptance within the drug discovery community, with retrospective analysis of recently marketed oral drugs showing that they usually have highly optimized ligand efficiency values [38]. In the case

of the bianchor agents, it might be speculated that efficiency metrics will tend to be low since only a fraction of the molecule participates in each independent binding event between ligand and each molecular target. Consequently, the density of efficient contacts between the drug and the targets is expected to be low. Merged pharmacophores may serve to solve the binding efficiency and bioavailability issues that characterize fragment-based approximations to multi-target drugs.

A number of physicochemical molecular properties could be tuned to promote promiscuity. Several reports suggest that ligand promiscuity is inversely related to molecular weight [39–41], but others have failed to find a correlation or have even shown an opposite trend [42, 43] suggesting that the relationship between molecular mass and promiscuity might be context dependent [44]. Sturn et al. observed a class of multi-target compounds that they called "superpromiscuous," which could bind to nonhomologous targets and shared some of the atoms involved in direct interactions with each target [39]. Curiously, these superpromiscuous ligands tend to present either low or high complexity. Direct correlations have also been found between promiscuity and calculated log*P* [43, 44]. Bases and quaternary bases are markedly more promiscuous than acids, neutral compounds, or zwitterions [43]. The molecular topology can also influence promiscuity: the number of rings and the fraction of molecular framework (f_{MF}) have also shown to be directly correlated with promiscuity at least for large (above 0.65) molecular framework values [43, 44]. The f_{MF} is defined as the atom of heavy atoms in the molecular framework (MF) divided by the total number of heavy atoms in the molecule. In other words, a smaller molecular framework and more side-chain atoms will improve selectivity. A clear (and graphical) definition of the molecular framework can be found in the original work by Bernis and Murcko [45].

In the particular case of AEDs, our group, while looking for quantitative structure-activity relationships (QSAR) to identify anticonvulsants with activity in the maximal electroshock seizure (MES) model, observed that compounds with anti-MES effects tend to be small and display low complexity [46]. Figure 3 shows the distribution of molecular mass for AEDs approved between 1912 and 2012. Observe that the average molecular mass is 243.5, with a standard deviation of 70. If we compare these data with the average values of molecular mass for the compounds patented by 18 big pharmaceutical companies between 2000 and 2010, across the major drug-target classes [47], we will observe that the average molecular mass for approved AEDs is considerably lower (note that a definite trend toward higher molecular mass and clog*P* values, within Lipinski's rule limits, has been observed between 2000 and 2010, when the target-centered approaches still prevailed). Interestingly, Yang et al. [44] have studied the

interplay between promiscuity, log*P*, and molecular topology. They defined four different topological classes according to the number of terminal ring systems and the presence of a molecular bridge. The "one terminal ring system" (1TR) class includes molecules with only one ring system. The "two terminal ring systems" (2TR) contain molecules with two ring systems directly connected to each other. The 2TR + B class comprehends molecules with two terminal ring systems and a molecular bridge. Finally, the 3TR + B class contains molecules with three terminal ring systems and a molecular bridge. While, in general, promiscuity showed an uptrend from simpler (1TR) to more complex (2TR + B) motifs, the topology class did not influence promiscuity for compounds with clog*P* values ranging from 1 to 3. In the case of approved AEDs, Fig. 3 also shows that, for most AEDs, the f_{MF} tends to be high (with some exceptions, e.g., aliphatic compounds such as valproic acid or pregabalin). About 80% of the approved AEDs belong to the simpler topological classes (TR or 2TR), which a priori suggests a reduced tendency to promiscuity, but they also show, in most cases, clog*P* values in the range of 1–3 (Fig. 3), which is optimal for passive diffusion through the blood–brain barrier and where the degree of promiscuity seems to be indifferent to the topological class (with some remarkable exceptions like vigabatrin, tiagabine, or perampanel, which emerged in the context of the target-centered paradigm).

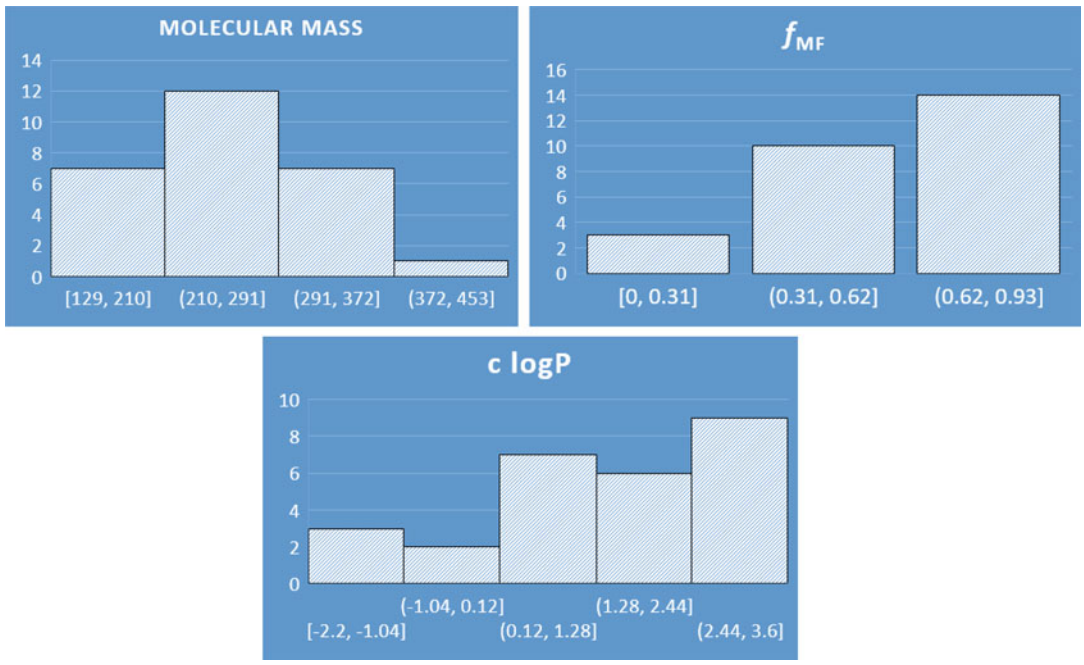

Fig. 3 Molecular mass, fraction of molecular framework and clogP distribution for AEDs approved between 1912 and 2012. The analyzed drugs have been extracted from ref [9]

The previous analysis confirms our observations and provides some keys to the proven promiscuity of most AEDs: low molecular mass and low complexity, high f_{MF}, and an adequate interplay between clog*P* and molecular topology. The analysis also suggests that novel scaffolds should simultaneously explore the 3TR+B topology and higher lipophilicity, while keeping high f_{MF} values (i.e., few side chains), which represents an unexplored and promising region of the chemical space for AEDs.

Regarding virtual screening campaigns focused on multi-target agents, one should remember that application of independent models to identify multi-target agents is expected to yield lower *positive predictive values* than virtual screening campaigns focused on single-target drugs [4, 32, 33, 48]. If it is assumed that being a ligand for one of the intended targets does not enhance or reduce the probability of being a ligand for another one (a situation that corresponds to nonoverlapping pharmacophores), each model applied in the virtual screening process works as a structural restriction that filters out all the molecules that do not accomplish the model requirements; subsequently, finding chemical compounds accomplishing all the model structural constraints becomes increasingly difficult as the number of target increases. Having this in mind, when optimizing the score thresholds, it is advised to gain sensitivity at the expense of specificity (be thus prepared to observe higher false-positive rates: such strategy results in an increment of experiment-related costs due to reduced active enrichment).

Also keep in mind that the pharmacophores correspondent to two targets could be mutually exclusive. Some pharmacophoric features are irreconcilable (they cannot coexist in the same point of the molecule, e.g., a charged moiety and a lipophilic one), while others are not (e.g., negative charge and H-bond acceptor). Choosing the pursued targets on the basis of empirical or theoretical evidence on common determinants of specificity (resulting in overlapping ligand specificity due to common pharmacophores) could be a good advice to expand the likelihood of success (remember that multi-target ligands with merged pharmacophores are preferred from the ligand efficiency and bioavailability perspectives). Bioinformatics tools capable of detecting protein coevolution [49] can be useful for detecting molecular targets with similar binding sites.

2.2 The Most Potent, the Better?

From a network pharmacology standpoint, attacking hubs (highly connected nodes in a biochemical network) may not be the best strategy, particularly if we are targeting sensitive organs like the brain. Designing low-affinity multi-target drugs to modulate multiple nonessential nodes nearby key nodes seems a more rational approach to restore the network to its normal functioning without serious toxic effects that could otherwise be expected when

blocking a key node [50]. As openly voiced by Bianchi and coworkers, "the complexity of neural processes underlying seizure activity may be more amenable to multiple small perturbations than a single dominant mechanism" [11]. At this point it may be worth highlighting the difference between potency and efficacy. Potency is related to the amount of drug that is needed to produce a given effect and is related to the affinity of the drug for its molecular target(s) and the number of units of the molecular target(s) available. In contrast, efficacy is linked to the maximum effect that a drug can produce, regardless of dose. From these definitions, it follows that *a drug could be more efficacious than others without being more potent*.

Memantine represents an outstanding example of the potential benefits of low-affinity multi-target ligands on CNS disorders [51, 52]. This drug is presently prescribed for the treatment of moderate to severe Alzheimer's disease and other dementias when acetylcholinesterase inhibitors are not well tolerated. In contrast, high-affinity uncompetitive inhibitor of the *N*-methyl-d-aspartate receptors (NMDARs) dizocilpine has not reached the market due to serious adverse reactions including Olney's lesions, cognitive disruption, and psychotic reactions. Memantine possesses low-affinity binding to NMDARs (in the high nM to low μM range), fast on/off kinetics, and almost no selectivity among subtypes [51], being consequently much better tolerated. Memantine also shows uncompetitive antagonism on other receptors, e.g., serotonin 5-HT3 [53] and dopamine D2 receptors [54], with similar affinity than for the NMDA receptors.

The old paradigm (the more potent, the better) still prevails in the primary screening for novel AEDs. The NIH's Anticonvulsant Screening Program considers the potency of drug candidates in acute seizure models as one of the criteria to select which drug candidates will advance to further testing [55]; this decision-making scheme may underestimate the efficacy of the drugs in on a long-term basis [56]. An equivalent principle is often applied in the context of some computer-aided screening campaigns (e.g., those based on docking and regression models), where the hits with higher predicted affinities are more likely to be selected for experimental validation. In the light of this paradigm shift, it could be appropriate to discard mere potency as selection criteria of AED candidates, preferring drugs with multiple (small) actions on different targets. Protein network analysis could prove useful to reveal weakly and moderately connected nodes as potential new targets for epilepsy therapies. In this line, it has been stressed that levetiracetam (a new generation AED that enjoys increasing attention within the epilepsy community and perhaps the third generation AED with the most innovative pharmacologic profile) exerts various mild modulatory actions on neurons [8].

2.3 What Response Should Be Modeled When Building Computational Models to Detect Novel AEDs

In the background of classical QSAR theory and more reasonably in the case of 3D QSAR, the compound dataset used to build and validate the model should present a common mode of action and even the same binding mode [57–59]. Alignment-dependent 3D QSAR methods have been conceived to describe one specific interaction step in the lifetime of ligands [57]. Obviously, the same principle applies for molecular docking and other structure-based approaches that explicitly predict the ligand-target interaction. Consequently, in vitro affinity data could be considered the gold standard for traditional QSAR modeling. However, considering the possible benefits of network pharmacology in the field of epilepsy and as an alternative to tailored multi-target agents, it is possible to detect novel antiepileptic drugs by modeling "dirty" responses obtained in phenotypic/physiologic models, instead of "clean" affinity data. Molecular descriptors reflecting more general structural patterns than those required for the specific ligand-recognition event by a certain target could be probably more suitable for this job. Many of the successful QSAR models and virtual screening applications for the discovery of AEDs have relied on in vivo biological data for modeling purposes [46, 60–70], and they include work by leading experts in the QSAR field [60].

Since most of the previously cited articles report models to predict the effect of a drug in seizure models (prominently, MES test), the current challenge is probably to face modeling campaigns based on biological data obtained from actual models of epilepsy (e.g., pilocarpine and kindling models), refractory epilepsy, and acute models so far understudied through the QSAR theory (e.g., 6 Hz test).

3 Comparing Gene-Wide Profiles

It is now known that drugs, particularly those administered in a repeated manner (e.g., AEDs), do not only provoke a given biological response in a direct way; they also indirectly elicit regulatory effects on gene expression profiles which could be even more relevant than the direct drug-target interaction.

Gene expression profiles offer a snapshot of globally measured transcript levels in a given cell, tissue, or organism at a specific point of time [71]; gene signatures are representative of specific conditions, i.e., exposure to a given xenobiotic or a disease state. Gene signatures are particularly relevant to characterize the phenotypic response to long-term exposure, shed light about the modes of action of a drug, and identify potential treatments for a certain disorder. The Broad Institute has pioneered such applications through its Connectivity Map, a publicly available resource designed to link disease and drugs through gene profiles [72]. This resource collects gene signatures derived from the exposure of human cells to a huge number of xenobiotics, including 1300

FDA-approved drugs. Query signatures can be compared to the stored ones through similarity matching algorithms: those at the top and bottom of the resulting ranking are thought to be related to the query state by shared or opposite expression changes. Compounds eliciting similar expression changes would exacerbate such condition; compounds displaying inverse signatures would function as therapeutic agents.

A diversity of microarray-based gene expression profiling studies have been conducted to elucidate the molecular changes underlying epilepsy and epileptogenesis [73–77]. They and other similar works could be a fair starting point to find novel drugs with disease-modifying properties by application of the inverse similarity idea proposed by the Connectivity Map.

4 Final Remarks

In spite of recent authoritative opinions on the potential contribution of network pharmacology to the development of more efficacious AEDs, such considerations have so far not been translated into new drug candidates, contrasting advances in other complex diseases such as neurodegenerative conditions or cancer.

We have presented four strategies to incorporate a network pharmacology perspective in the field of AED discovery: tailored multi-target agents, reexamining the validity of "the more potent, the better" paradigm, building QSAR models based on biological responses emerging from phenotypic models, and gene signature comparison.

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