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PERSPECTIVE

Neg-entropy is the true drug target for chronic diseases

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KEY WORDS

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Abstract Molecular mechanisms of chronic diseases are complicated, and it impedes drug target identification and subsequent drug discovery. We consider entropy increase in human body the root causes of chronic diseases. Accordingly, the inherent neg-entropic mechanisms, for instance the homeostatic mechanisms for metabolism, immunity, self-healing, etc., are true drug targets. Only very few molecules (such as proteins) are decisive for neg-entropy related functions, thus they are termed "head goose molecules" (HGMs) here. Identification of HGMs is key to activating neg-entropic mechanism(s), and drug intervention of the HGMs' functions might reprogram the disease process through a neg-entropy mediated drug cloud (dCloud) effect, resulting in a treatment of both symptoms and root causes of the diseases. Thus, we recommend, for the first time, the "HGMs−neg-entropy−dCloud" axis as an important strategy for discovering new drugs. Clinically proven effective drugs that target HGMs are given as examples to illustrate the concept. Different from most of the single-target drugs that interrupt disease signal pathway(s), neg-entropy drugs treat chronic diseases through converting disorderliness to orderliness in the body of patients. We hope it to be helpful in future drug discovery for chronic diseases.

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1. Introduction

In the current paradigm of drug discovery, identifying drug target is one of the major issues in finding novel drugs[1]. Therefore, a large number of proteins have been studied to learn their role in physiological function or pathological pathways, aiming to find drug targets[2,3]. Biophysical features of the 3D structure of speculative target proteins and related drug−target interaction are investigated for rational drug design (*i.e.*, chemicals or bioproducts), from the perspective of structure biology[4,5]. Indeed, this approach has led great successes for drugs against viral infections (Hepatitis C virus, HCV; Hepatitis B virus, HBV; Human immunodeficiency virus, HIV-1, etc.), as viral enzymes or surface proteins are effective drug targets to control viral replication[6,7]; in addition, the approach is also successful for those treating disease symptoms, for example, non-steroidal anti-inflammatory drugs (Celecoxib) that target COX-2[8] and antihypertensive drugs (Aliskiren) which inhibits renin[9]. However, the strategy has not produced desirable results yet when applied to chronic diseases, such as cancers, metabolic disorders, cardiovascular diseases and degenerative encephalopathy, although a large amount of research funds, time and manpower have been invested in these fields worldwide. We believe that the lack of satisfactory results might be attributed to the unknown disease mechanisms, multiple risk factors, as well as the complex and dynamic processes in pathogenesis, varying across different stages of diseases. The challenge to the single target theory is even bigger, if the effect of biological redundancy (for instance, genetic redundancy) is considered[10]. Indeed, unlike infectious diseases, many chronic diseases (as well as aging-related diseases) are resultant of risk factors inside the body, such as deviated energy metabolism, oxidative stress (ROS), inflammation, immune imbalances, endocrine disorders and psychiatric/psychological factors. The long-lasting alterations gradually disturb the orderliness at the molecular, cellular, organ-based and systemic levels before eventually effecting the entire body, leading to complex illness (or/and aging). Moreover, the abnormalities in various systems often overlapped, causing multimorbidity[11,12]. This pathogenic course could be viewed as an increase of entropy in the human body[9]. Indeed, the concept of entropy (see description below) has been recently used in disease study for cancers, brain diseases, infections, etc., and the increase of entropy appears to associate with aging, error accumulation of epigenetic and pathogen infection, etc.[13,14]. Human body factors like mitochondria function and ROS level are considered to be causes for the increase of entropy[15], and accordingly, drug investigation with the concept of neg-entropy has been reported[16-18].

2. Entropy

Entropy is a specialized terminology of the thermodynamic state in the second law of thermodynamics[19], and it has 3 key notions. The first is measurable physical property characterized with chaos, disorderliness, randomness, or uncertainty; the second is the “unavailable energy indicator” reflecting the irreversible dissipation of useable energy; and the third is ‘‘the principle of entropy increase” in isolated systems[20]. The concept of entropy has been used for living systems and diseases (termed bio-entropy), mainly implying the disorderliness of physical structures, for instance, disruption of macromolecules, cells, tissues or/and organs[21-24]. We assume that in terms of human health, entropy increases in the human body could be systemically accelerated by factors like oxidative stress, inflammation, infection, inappropriate energy metabolism, imbalance in immunity, or psychological pressures, etc. Entropy calculation is considered to be pivotal in analyzing health status with multifaceted risk factors (*e.g.*, heart rate, EEG, tissue/organ image, as well as biochemical indications in blood). There are several key entropy measures being used in experimental research, including the Shannon Entropy (foundation of information theory), Rényi Entropy (parameterized generalization), Approximate Entropy (ApEn, time-series irregularity assessment), as well as Sample Entropy (SampEn, improved noise robustness variant)[14]. These measures adapt to diverse clinical needs, after balancing computational efficiency and biological interpretability. Shannon Entropy is the most commonly used calculation for bio-entropy, defined as Eq. (1):

$$H(X) = -\sum_{i=1}^{n} p(xi)\log_2[p(xi)] \tag{1}$$

Where $p(xi)$ is the probability of discrete variable xi; for joint variables X and Y, the joint entropy extends to Eq. (2)[25]:

$$H(X,Y) = -\sum_{i=1}^{I}\sum_{j=1}^{J}\left\{p(xi,yj)\log_2[p(xi,yj)]\right\} \tag{2}$$

Bio-entropy measurement might give us a new angle and useful evaluation for drug efficacy, especially for drugs against multifaceted chronic diseases[17]. Still, the significance of entropy in living things or diseases needs further understanding and more appropriate computational algorithms for comprehensive bio-entropy calculation are under exploration. We have focused our drug discovery on candidates that could reduce entropy (termed "neg-entropy" drugs in this article), and been carefully interpretate calculation results. We believe that the strategy might enable us to discover effective drugs for chronic diseases[17].

The human body holds an inherent system that could efficiently sustain its biological complexity in good order and function for many years or decades, indicating the presence of systemic biological mechanisms of neg-entropy *in vivo*[19]. Indeed, the human body could spontaneously recover from illnesses or injury, and effective drug treatment should accelerate the recovery process *via* triggering the intrinsic neg-entropic mechanisms. In general, the neg-entropic mechanism could be classified into at least the following categories: metabolism & self-organization (*e.g.*, metabolism homeostasis, macromolecule synthesis/modification/organization, growth/differentiation, maintaining functions), defense system (*e.g.*, immunity, inflammation, barrier), self-healing ability (*e.g.*, stem cell, resolution of inflammation, injury repair, tissue growth), wear resistance (*e.g.*, errors correction, detoxification, DNA repair) and adaptability (*e.g.*, compensatory mechanism, rebuilding functions to cope with environment)[19]. We believe that true drug targets for chronic diseases are the systematic neg-entropic mechanisms, through which effective control or even cure of the diseases could be achieved. Fig. 1 shows the neg-entropy effect in the liver and myocardium of the animals treated with metabolism-regulating agent berberine[26,27]. Vaccine-elicited immune responses also exemplify a neg-entropic process. However, the question is how to trigger the innate neg-entropic mechanisms.

3. Head goose molecules

Mammalian cells are estimated to have more than tens of thousands of proteins per cell. Most of the proteins or their

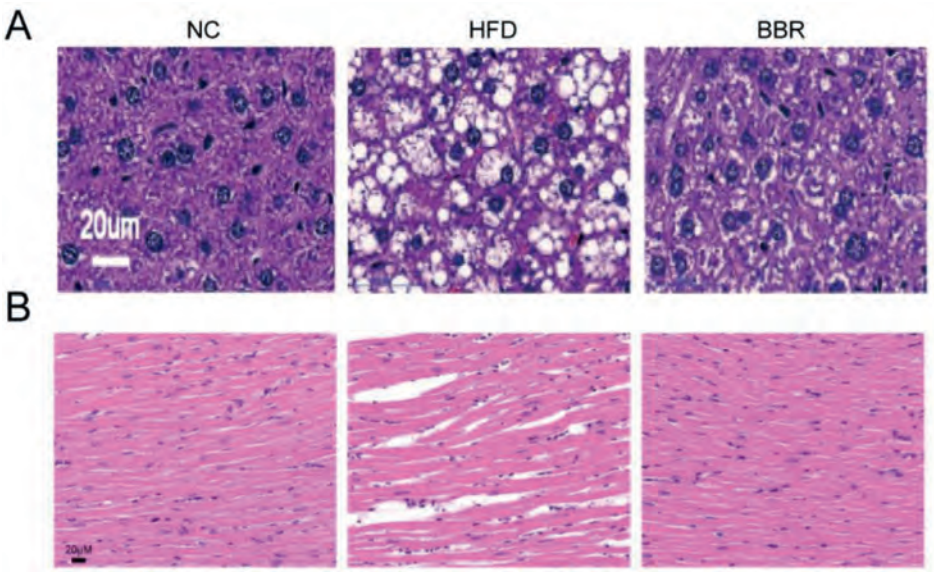

Figure 1 Neg-entropy effect in the liver and myocardium after berberine treatment. (A) H&E staining of mouse liver tissues of the normal control (NC), high-fat diet model (HFD), or berberine-treated HFD group (BBR). Reproduced with license of Creative Commons CC BY[26]. Copyright 2019 Springer Nature. (B) H&E staining of rat myocardium of the normal control (NC), high-fat diet model (HFD), or berberine-treated HFD group (BBR). Reproduced with license of Creative Commons CC BY[27]. Copyright 2025 MDPI. The results showed that BBR could reduce the disorderliness in the tissues of the animals fed with HFD.

modifications (such as those with glycosylation, phosphorylation, methylation, and acetylation) serve as single node within signal pathway networks of various cellular functions and are often interchangeable or replaceable. It suggests that regulating one of these nodes, with agonists or antagonists, might not be able to meaningfully change the disease progress. Thus, most of the proteins might not be effective drug targets. By principle, only a handful of intracellular proteins are able to function as pivotal front-runners in reprograming cellular networks and effectively suspending or reversing disease progress. Modulation of these proteins, through drug intervention, might be able to trigger a ripple effect throughout the network (*e.g.*, the adenosine 5′-monophosphate (AMP)-activated protein kinase, AMPK, for energy metabolism). The biological phenomena could be described as the "Head Goose Effect (HGE)" that is often seen in wild geese's long-distance migrations in nature. During the wild geese's seasonal migrations, heading either south or north, the flock is highly coordinated and typically flying in a "V" or "—" shape, with a strong, intelligent and experienced goose leading the way[28,29]. The aerodynamic benefits created by the lead goose's wing flaps assist the geese behind flying in an energy-saving model that allows them to traverse thousands of kilometers in an efficient fashion[30-32]. When the lead goose grows weary, another capable goose assumes the leadership position[29]. Similar phenomena are often seen in social organisms as well as in mammalian cells (Fig. 2), in which they act in accordance with the biological instruction from their organizers[33,34]. Therefore, we assume that only a very small number of cellular proteins are capable of reprograming their signal networks to halt the disease progress, and they are termed head goose molecules (HGMs) in the article. Serving as chief organizers of signal networks, HGMs should be able to reduce disease-related disorderliness (neg-entropic effect) after interacting with drugs. The following depicts HGMs examples in metabolic diseases, and drugs that target these HGMs have achieved positive clinical outcome globally.

AMPK is a key molecule in energy metabolism and composed of α (α1, α2), β (β1, β2) and γ (γ1, γ2, γ3) subunits, with the main function of sensing intracellular energy change[36,37]. AMPK's signaling pathways encompass a wide range of targets, including glycogen synthase 1/2 (GYS1/2, for glycogen), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2/3 (PFKFB 2/3, for glycolysis), phosphoenolpyruvate carboxykinase/

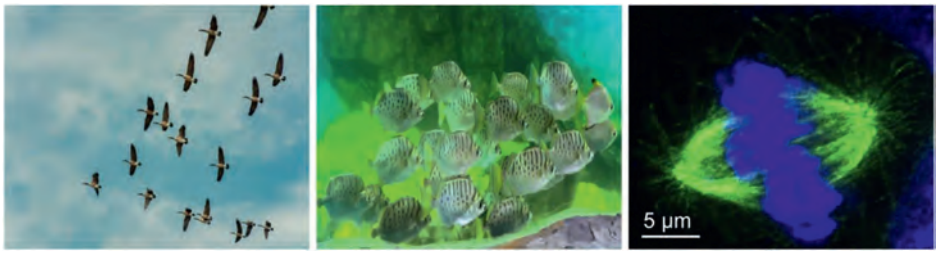

Figure 2 "Head goose effect" in nature. Social organisms are organized in good order for their life activities, and the leader organism plays a key role in the action. The examples in the figure are, in turn, goose, fish and cellular microtubules (Source: Goose, Photo by Mike Cox on Unsplash. Microtubules (mitotic spindles and chromosomes), Scale bar: 5 µm, © 2023 Qi et al.[35] originally published in *Journal of Cell Biology*. https://doi.org/10.1083/jcb.202210093).

glucose-6-phosphatase (PEPCK/G6pase, for gluconeogenesis), acetyl-CoA carboxylase 1/fatty acid synthase (ACC1/FAS, for fatty acid synthesis), carnitine palmitoyltransferase 1 (CPT1, for fatty acid oxidation), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR, for cholesterol synthesis), mammalian target of rapamycin (mTOR, for protein synthesis), UNC-51 like autophagy activating kinase 1 (ULK-1, for autophagy), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha/mitochondrial fission factor (PGC-1α/MFF, for mitochondrial biogenesis and fission)[37,38]. Apparently, these AMPK-coupled events are very closely associated with energy metabolism, an important neg-entropic mechanism. It has been well-documented that AMPK phosphorylation and activation regulate several downstream signal pathways, thus, it could be viewed as a HGM maintaining homeostasis of energy metabolism[39,40]. Indeed, drugs indirectly targeting AMPK, such as metformin and berberine (BBR), have demonstrated clinical efficacy in lowering blood glucose or lipids, anti-inflammation, as well as preventing cancer and cardiovascular events[41,42]. However, designed compounds that have strong affinity of direct interaction with AMPK failed in clinical trials[43], leaving us more room to think about AMPK-based drug design.

The glucagon-like peptide-1 receptor (GLP-1R) might be another example of HGM, as drugs that target GLP-1R have efficiently demonstrated benefit for sugar and lipid metabolism in human. Semaglutide, a well-known GLP-1R agonist, enhances insulin homeostasis in diabetes[44]. Further studies have shown that GLP-1R activation regulated several important pathways, such as cyclic adenosine monophosphate−protein kinase A−cAMP response element-binding protein (cAMP−PKA−CREB), phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), as well as nuclear factor-κB (NF-κB)[45,46]. The surprising clinical benefits of semaglutide, including weight loss, anti-inflammation and anti-tumor effect, might be attributed to the Head Goose Effect (HGE) of the GLP-1R molecule[45,47,48]. Additionally, the effect of semaglutide on different types of cells (for instance, pancreatic cell alpha, beta, or delta) varies, making its signal connection even more extensive[45].

Furthermore, the low-density lipoprotein receptor (LDLR) is primarily expressed on the membranes of hepatocytes, muscle cells et al. It facilitates the transport of low-density lipoprotein (LDL) from the blood to the liver. Since LDLR-associated pathways are linked to lipid metabolism (*e.g.*, 3-hydroxy-3-methylglutaryl-coenzyme A, HMG-CoA) and kinase activation (*e.g.*, epidermal growth factor receptor, EGFR)[49], up-regulation of LDLR expression has become a strategy to finding cholesterol-lowering agents, such as statins, BBR and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors that has been successfully used in clinic to treat patients with hyperlipidemia. In parallel, these drugs also exhibit anti-inflammatory, anti-oxidative and cardiovascular prevention properties in clinic[50-53], suggesting LDLR to be a potential HGM in lipid metabolism. However, among the mentioned LDLR up-regulators, statins increase LDLR expression through targeting HMG-CoA that causes side-effects in liver and muscle[54], suggesting that a good drug design for LDLR expression needs more understanding of molecular mechanisms.

To identify a HGM from the cellular protein pool is quite difficult if using conventional biotechnology. Thus, we approach HGMs from another perspective, and believe that multi-functional (or multi-target) drugs might be useful to probe HGMs. BBR is a drug with multiple pharmaceutical effects in metabolism and clinically effective in treating or preventing dyslipidemias, type 2 diabetes (T2D), fatty liver, as well as cardiovascular diseases[50,55-57]. The mechanism of BBR is associated with up-regulation of LDLR and insulin receptors (InsR), and activation of AMPK, inhibition of PCSK9[58-60], but the primary target(s) remains unknown. Recent studies have shown that BBR enters the hepatocyte mitochondria, and directly binds and activates Sirtuin 3 (SIRT3), thereby reducing acetylation of the catalytic subunit NDUFS1 in the N-module of mitochondria complex I (CI), ultimately leading to the dissociation of mitochondrial CI[61]. From literary research, SIRT3 was found to be connected with the AMPK and PCSK9 pathways[62-64]. We believe that SIRT3 (or CI of mitochondria) might be the primary target of BBR and could be an HGM to treat metabolic diseases. In fact, accumulating evidence has shown that SIRT3 is associated with several types of human diseases, such as age-related diseases, heart disease, cancer and metabolic diseases[65-68], supporting SIRT3 as a potential HGM. By principle, drugs treating disease through targeting HGMs might cause neg-entropic effects through reprograming biological functions that move toward homeostasis. We believe it agrees with the theory of dissipative structure in biology[69,70].

4. Drug cloud (dCloud)

Thus, we have proposed a drug cloud (dCloud) concept in our previous study on BBR[50]. In general, the dCloud concept is defined as a group of terminal biological events triggered by a drug (or/and its related metabolites), as well as the network connections among them, orchestrating coordinated therapeutic effect of one or more neg-entropic mechanisms. In this scenario, the clinical therapeutic efficacy of BBR in lowering blood-lipids/glucose and treating fatty liver[58,59] reflects a synchronized neg-entropic dCloud acting on both symptoms/signs (through its action on LDLR, InsR, AMPK, and PCSK9) and root causes of the diseases (*i.e.*, gut microbiota, inflammation). In fact, the dCloud effect is also applicable to other established multi-target drugs, such as aspirin and metformin[16,71]. The neg-entropy-based dCloud concept is different from the single-target strategy, and it might provide new ideas in drug discovery.

One of the examples is the anti-COVID-19 drug azvudine (FNC). Although COVID-19 is not a chronic disease, its mortality is closely related to chronic diseases and aging[72,73]. FNC is a nucleoside analogue and has been approved in China for treating AIDS patients, because of its inhibitory effect on viral RNA polymerase (RNAp)[74,75]. In the early outbreak of SARS-CoV-2, FNC was selected (from a number of agents effective against SARS-CoV-2) for clinical studies against COVID-19, mainly because it was found to be tri-phosphorylated (activated to inhibit RNAp) almost fully in the thymus and lymphocytes[76]. It protected thymic function and T cell immunity from SARS-CoV2 infection, in addition to its inhibitory effect on SARS-CoV replication[76,77]. After completion of the Phase III trials in patients with COVID-19, FNC was approved by the Chinese National Medical Products Administration (NMPA) for clinical use; and now, it has been repetitively validated in hospitals that FNC significantly reduced the all-cause mortality and improved T cell immunity in aged COVID-19 patients[78-81]. We believe that the clinical results of FNC in treating COVID-19 represents the combined effect of chemotherapy and immunotherapy[76,77], forming a synchronized neg-entropic dCloud against SARS-CoV2 infection. It appeared to be particularly helpful in aged COVID-19 patients who had reduced immunity[82-84].

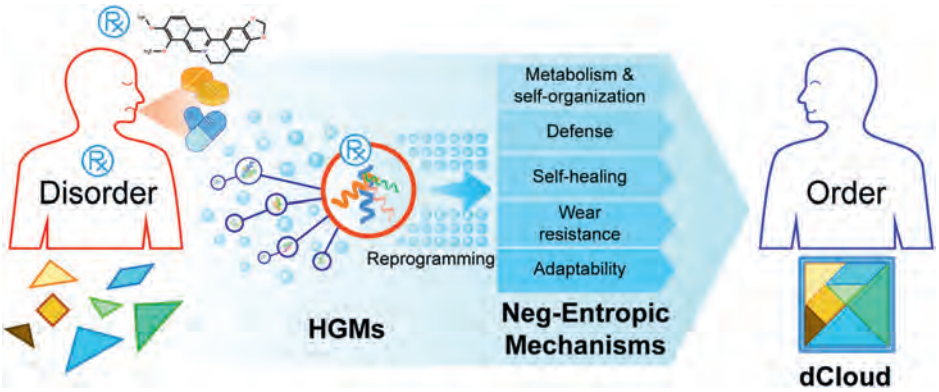

Figure 3 The "HGMs−Neg-entropy−dCloud" axis. Drugs (using BBR structure as an example) acting on the "HGMs−Neg-entropy−dCloud" axis might cure a patient from a disordered state to an ordered one. Rx, drug treatment; HGMs, head goose molecules; Neg-entropic mechanisms, negative-entropic mechanisms; dCloud, drug cloud.

Importantly, it has been observed that improvement in one neg-entropic mechanism might help other neg-entropic mechanisms, forming a joint neg-entropic dCloud. In the FNC case, enhancement of thymus-mediated T cell immunity appeared to be helpful in alleviating neutrophil-mediated inflammatory injury in the lung after SARS-CoV2 infection[85]. For BBR or metformin, improvement of lipid/glucose metabolism and gut microbiota composition promoted immunity and reduced inflammation[50,86,87], resulting in a reduced likelihood of developing cancer[56,88-94]. As the dCloud effect might yield therapy for both symptoms and root cause[95], we consider the "HGM−Neg-entropy−dCloud" axis (Fig. 3) an optimized strategy to discovering drugs for chronic diseases. Combination of neg-entropy drugs with single-target ones should yield better therapeutic efficacy [89,96-99].

5. Conclusions

Unlike single-target theory, the presented one views entropy-increase as the main cause of pathogenesis for chronic diseases; and accordingly, the neg-entropic mechanism(s) as the true and ideal drug target. In order to competently trigger the neg-entropic mechanisms, identifying HGMs is the key, because regulating HGMs might cause activation of the systemic neg-entropic mechanisms and reprogram the disease process (Fig. 3). The ultimate goal is to produce a therapeutic dCloud effect that might coordinate one or more neg-entropic mechanisms, and thus treating both clinical symptom and root cause of disease. Distinctive from the conventional single-target drugs, neg-entropy drugs target HGMs rather than ordinary molecules, and regulate one or more mechanisms, instead of one signal pathway. Here, for the first time, we demonstrate a "HGMs−neg-entropy−dCloud" axis for drug discovery, and hope it to be helpful for future drug research.

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Author contributions

Writing − original draft, writing − review and editing: Rui Li; Writing − original draft, writing − review and editing: Tian-Le Gao; Writing − original draft: Gang Ren; Conceptualization, supervision, validation: Lu-Lu Wang; Writing − original draft, writing − review and editing, conceptualization, supervision, validation: Jian-Dong Jiang.

Conflicts of interest

The author declares that there are no competing interests.

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