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Ligandomics: a paradigm shift in biological drug discovery

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Abstract

As productivity of pharmaceutical research and development (R&D) for small-molecule drugs declines, the trend in drug discovery strategies is shifting towards biologics, which predominantly target secreted or cell surface proteins. Receptors and ligands are the most-valuable drug targets. In contrast to conventional approaches of discovering one ligand at a time, the emerging technology of ligandomics can systematically map disease-selective cellular ligands in the absence of molecular probes. Biologics targeting these ligands with disease selectivity have the advantages of high efficacy, minimal adverse effects, wide therapeutic indices, and low safety-related attrition rates. Therefore, ligandomics represents a paradigm shift to address the bottleneck of target discovery for biologics development.

Graphical abstract

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Conflict of interest

W.L and H.T are founders and equity stockholders of LigandomicsRx, LLC and Everglades Biopharma, LLC. W. Li is the inventor on patent applications related to this work and will be entitled to royalties if licensing and/or commercialization occurs.

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Keywords

ligandomics; ligandome; quantitative ligandomics; comparative ligandomics; disease-selective angiogenesis blocker; OPD-NGS

Introduction

The pharmaceutical industry faces a plethora of challenges, including declining productivity to develop small-molecule drugs, increasing attrition rates, rapidly rising R&D costs, and losses of revenues because of patent expirations and healthcare cost control [1–3]. The key to addressing these challenges is to develop more innovative and cost-effective drugs. Over the past two decades, it has become increasingly clear that biologics are one of the important answers to these challenges, with proven medical and commercial successes [4]. The trend in pharmaceutical R&D strategies is shifting from small molecules to biologics. In 2000, the US Food and Drug Administration (FDA) approved only six biologics versus 27 new molecular entities (NMEs). This ratio had increased to 14:13 by 2016 [5,6]. The sales of biologics topped US\$140 billion in 2014, representing approximately 19% of total drug sales [4,5].

Biologics, such as antibodies (Abs) and recombinant proteins, are mainly distributed in the extracellular space, thereby mostly targeting secreted or cell surface proteins [7,8]. Of these extracellular proteins, cell surface receptors and ligands are particularly important as drug targets. This is highlighted by the fact that approximately one-third of all approved drugs target G-protein-coupled receptors (GPCRs) [7]. Other ligands and receptors, such as insulin, erythropoietin, vascular endothelial growth factor (VEGF), and programmed cell death protein 1 (PD-1), are well-known targets for biologics [7,9,10]. Ligands with protective or detrimental roles could be overexpressed or blocked, respectively, for disease therapy.

With well-developed technologies to engineer and manufacture Abs and recombinant proteins [11], the bottleneck to develop biologics is how to efficiently identify therapeutic targets. It was widely anticipated that the advent of proteomics technologies at the turn of the new millennium would expand the target space for pharmaceutical industry [12]. However, more than a decade later, the whole industry still struggles with the paucity of new drug targets, as highlighted by a precipitous drop in the number of approved drugs by the FDA in 2016 [6]. Part of the reason is that proteomics technologies are mainly developed to map intracellular protein interaction networks, rather than ligand–receptor interactions [13]. Consequently, cell surface ligands are traditionally identified and characterized on a case-by-case basis, with associated technical challenges. It is even more daunting to delineate pathogenic or therapeutic ligands and receptors. Therapeutics targeting molecules with minimal disease selectivity often have narrow therapeutic indices with high attrition rates because of adverse effects.

To address these challenges, ligandomics was recently developed as the only high-throughput technology to globally profile cell-wide ligands [14]. Comparative ligandomics can systematically map disease-associated ligands as therapeutic targets for robust and cost-

effective biologics drug discovery. In this review, we describe the unique capacities of comparative ligandomics compared with other technologies, propose new criteria for target selection, and outline the advantages of biologics with high disease selectivity for targeted therapy.

Quantitative ligandomics

Ligandomics technology was developed from open reading frame phage display (OPD) (Figure 1) [14,15]. Whereas conventional phage display identifies a high percentage of out-of-frame unnatural short peptides from cDNA libraries of cellular proteins, OPD can efficiently delineate endogenous binding proteins, including cellular ligands [16–18]. When combined with next-generation DNA sequencing (NGS) and cell-based screens, OPD-NGS emerges as the first paradigm of ligandomics for the global mapping of cell-wide ligands with simultaneous binding or functional activity quantification [15,19,20].

The quantification capacity of ligandomics is critical to delineate disease-associated cellular ligands. The copy numbers of the cDNA inserts identified by NGS are equivalent to the binding or functional activity of their cognate ligands [15,19]. The validity of this quantification by ligandomics was established by quantifying the differential endothelial binding activities of VEGF and GFP (Figure 1d) [14]. This binding activity quantification can be globally applied to all enriched ligands in the absence of receptor information. When coupled with phagocytes, ligandomics can simultaneously quantify the phagocytosis activity of all enriched ligands [19,20].

The binding activity determined by NGS reflects not only the binding affinity of individual ligands, but also the expression level of their cognate receptors. Some ligands may bind to multiple receptors. Thus, the copy number of each bound ligand is the summation of its binding to all interacting receptors with different affinities and expression levels. Furthermore, this relative binding activity can be influenced by various experimental conditions, including the total number of sequences identified by NGS and washing conditions. Owing to these variations, it is inappropriate to compare the relative binding activities among different ligands, even within the same ligandome data sets. Despite the limitations, this quantification capacity is critical for comparative ligandomics to identify disease-associated targets.

From comparative ligandomics to disease-selective targets

Unlike functional proteomics [21], an exceptional capacity of comparative ligandomics for target discovery is that it does not require receptor information or molecular probes, and only paired disease and normal cells or animal models are needed. To illustrate this unique feature, we recapitulate below the discovery and preclinical development of a novel disease-selective angiogenesis blocker in a recent study [14].

Comparative ligandomics was applied to diabetic and control mice to identify diabetic retinopathy (DR)-associated retinal endothelial ligands. After three rounds of *in vivo* binding selection, NGS identified a total of 489 126 and 473 965 valid sequence reads that aligned to 1548 (diabetic) and 844 (control) proteins, respectively. Comparative ligandomics globally

compared entire ligandome profiles for diseased versus healthy cells and systematically mapped 458 disease-associated ligands. Binding activity plots further categorized disease-associated ligands into 353 'DR-high' ligands with increased binding to diabetic retinal vessels and 105 'DR-low' ligands with decreased binding (Figure 1e). Scg3 was identified as a DR-high ligand (1731:0 copy for diabetic:control) (Figure 1d). HRP-3 was a disease-low ligand (48:11 140). VEGF was found as a 'disease-unchanged' (or minimally changed) ligand (408:2420) in 4-month-old diabetic mice. GFP as a background control had the same low binding to diabetic and control retinal vessels (10:10). Scg3 was independently characterized as a novel angiogenic and vascular leakage factor using various *in vitro* assays [14].

The validity of comparative ligandomics was supported by the distinctively different angiogenic activity patterns of Scg3, HRP-3, and VEGF in diabetic and control mice. Corneal pocket assays showed that diabetes-high Scg3 selectively induced angiogenesis in diabetic but not in normal mice. Diabetes-low HRP-3 preferentially stimulated corneal angiogenesis in control but not diabetic mice, whereas diabetes-unchanged VEGF promoted angiogenesis in both diabetic and control mice. These three distinct angiogenic activity patterns were closely correlated to their binding activity patterns (Figure 1d), strongly supporting the validity of comparative ligandomics.

Of all the identified endothelial ligands, Scg3 had the highest binding activity ratio to diabetic versus control retina and the lowest background binding to control vessels [14]. Without the technology of comparative ligandomics, Scg3 with minimal binding to normal vessels would be missed by conventional approaches. Perhaps this is why Scg3 had not been reported as a cellular ligand for so long before the ligandomics analysis [22,23].

Another important question is whether comparative ligandomics identified Scg3 as a disease-related angiogenic factor by serendipity. Comparative ligandomics also discovered pleiotrophin as a DR-high angiogenic factors, albeit with relatively low disease selectivity (38:0) [14]. The therapeutic potential of pleiotrophin for DR and retinopathy of prematurity (ROP) was demonstrated in a recent study using pleiotrophin-neutralizing Abs [24]. Comparative ligandomics also uncovered amyloid precursor protein (App) as a DR-high ligand (206:1) [14]. Amyloid β derived from App is one of the ligands for the receptor for advanced glycation end products (RAGE), which is markedly upregulated in diabetes [25]. These findings suggest that comparative ligandomics is capable of identifying different diabetes-associated endothelial ligands.

Criteria of target selection for biologics development

To date, nearly all conventional angiogenic factors have been discovered and verified based on their functional activity on normal vessels and, therefore, their activators and inhibitors target both normal and diseased vessels. This could result in untoward effects on normal vessels with narrow therapeutic indices and high safety-related attrition rates. Narrow therapeutic indices could also become a major barrier to achieving maximal efficacy at high doses. Based on a recent study of comparative ligandomics [14], we propose the following two principles to uncover disease-associated angiogenic factors as promising targets for

biologics development. These principles are broadly applicable to other ligands beyond vascular biology.

First, 'disease-high' ligands with increased binding to disease vessels are more-promising drug targets than are 'disease-low' ligands with decreased binding or 'disease-unchanged' ligands with minimal binding activity changes. The receptors of disease-high ligands are upregulated in pathological conditions, whereas disease-low ligands indicate downregulation of their cognate receptors. When receptors of disease-low ligands are reduced to undetectable levels, no agonists or antagonists, regardless of their potency, are capable of evoking any pharmacological response. By contrast, activators and inhibitors of disease-high ligands can elicit augmented cellular response owing to the induction of the cognate receptors in disease conditions. Therefore, drug targets should be selected from disease-high ligands for optimal efficacy.

Second, cellular ligands with the highest disease selectivity are likely to have the least-nonspecific adverse effects, largest therapeutic windows, and lowest safety-related attrition rates. Disease selectivity is defined by the ligand-binding activity ratio to diseased versus healthy cells as well as background binding activity to normal cells (Box 1). The binding activity ratio is expected to be proportional to the therapeutic index. The higher the ratio, the larger the therapeutic index. Additionally, the background binding activity should be directly related to potential adverse effects. Ligands with undetectable background binding activity to normal cells or vessels are likely to have minimal adverse effects, even at excessively high doses for maximal efficacy (Figure 2). Therefore, therapeutics targeting highly disease-selective cellular ligands might have reduced efficacy and/or safety-related attrition rates.

Box 1

Disease selectivity versus disease relevance

Disease selectivity and disease relevance of cellular ligands are two distinct concepts. Disease relevance refers to whether cellular ligands have a role in disease pathogenesis. Disease selectivity is attributed to the preferential expression of surface receptors on diseased but not normal cells, such that their cognate ligands preferentially stimulate cells in disease conditions.

There are two mechanisms for detrimental ligands to contribute to disease pathogenesis (Figure I): (i) ligand upregulation (type 1); or (ii) receptor induction (type 2) on diseased cells. For example, VEGF is markedly induced 36–110-fold in the vitreous fluid of patients with PDR, whereas soluble VEGF receptor 1 (VEGFR1) is upregulated moderately by ~1.5-fold [59,60]. By contrast, Scg3 binding to retinal vessels of diabetic mice increases by >1700-fold, whereas Scg3 expression is induced only by 1.38-fold [14]. These two examples also indicated that these two types of mechanism could coexist for individual ligand–receptor pairs and jointly contribute to pathogenesis. Importantly, VEGF and Scg3 exacerbate pathogenesis predominantly through type 1 and 2 mechanisms, respectively.

Ligands and their antagonists can travel extracellularly and regulate both diseased and normal cells. Therefore, disease selectivity is defined by receptor expression or ligand

binding, but not by ligand upregulation in disease cells or tissues. Disease selectivity of cellular ligands is defined by the following two criteria: (i) the binding activity ratio of diseased versus healthy cells or organs; and (ii) the background binding activity to healthy cells or organs. In this regard, VEGF is highly disease relevant but has relatively low disease selectivity. By contrast, Scg3 is not only disease relevant, but also highly disease selective.

Figure I.

Two models of pathogenic ligands. In the type 1 model, cellular ligands, such as vascular endothelial growth factor (VEGF), are markedly upregulated to trigger pathogenesis. In the type 2 model, receptors of cellular ligands (e.g., secretogranin III; Scg3), but not the ligands themselves, are upregulated to exacerbate pathogenesis.

Conventional approaches for ligand discovery lack systematic criteria to predict the disease selectivity of identified targets. Coupled with comparative ligandomics, the above two principles set the first guidelines to delineate disease-selective cellular ligands for biologics discovery and targeted therapy.

Disease-associated ligands as targets for biologics development

Cellular ligands with high disease selectivity are promising targets for both small-molecule drugs and biologics. Compared with small molecules with possible promiscuous targets, biologics generally have higher specificity and lower off-target toxicity [26]. This could be part of the reason why biologics had an overall success rate of 18%, twice the 9% success

rate of small-molecule drugs [27]. Although small molecules are able to access intracellular targets, biologics have the ability to exploit the therapeutic potential of secreted and cell surface proteins [26]. Therefore, disease-high ligands identified by comparative ligandomics are ideal targets for the rapid development of biologics, thereby overcoming the bottleneck in drug discovery. Coupled with intrinsic target specificity, biologics targeting disease-high ligands are likely to have advantages of high efficacy, minimal adverse effects, wide therapeutic windows, and low safety-related attrition rates.

According to the aforementioned criteria of target selection for disease therapy, Scg3 as a diabetes-high angiogenic factor was selected for biologics development. A Scg3-neutralizing monoclonal Ab (mAb) was developed as a selective angiogenesis blocker, showing some similarity to selective β adrenergic blockers [14]. Intravitreal injection of the Scg3-neutralizing mAb alleviated retinal vascular leakage in streptozotocin-induced diabetic mice. The therapeutic activity of anti-Scg3 mAb to inhibit DR leakage was independently verified in Ins2^{Akita} spontaneous diabetic mice. These results suggest that Scg3 has an important role in diabetic retinal vascular leakage and that anti-Scg3 mAb could be further developed for clinical therapy of diabetic macular edema.

Diabetic rodents do not develop proliferative diabetic retinopathy (PDR), probably because of their relatively short lifespan. Mice with oxygen-induced retinopathy (OIR), a disease model of ROP, are often used as a surrogate model of PDR. Both PDR and ROP are characterized by pathological retinal neovascularization (RNV), but with different underlying pathogenic mechanisms (i.e., with or without hyperglycemia). Intravitreal injection of a Scg3-neutralizing mAb prevented RNV in OIR mice [14]. These results suggest that Scg3 is involved in OIR pathogenesis and that Scg3 mAb has therapeutic potential to treat both ROP and PDR.

Angiogenic factors contribute to vascular pathogenesis by upregulating the expression of the ligands themselves or their receptors (Box 1). Given that Scg3 expression is not induced in OIR [14], the therapeutic activity of anti-Scg3 mAb implies that Scg3 binding to OIR vessels also increases in the absence of hyperglycemia. Future ligandomics analyses are needed to support this notion. Nonetheless, this suggests that disease-high Scg3 identified by comparative ligandomics is 'disease-associated or selective', but might not be 'diabetes specific'. Scg3 is predominantly expressed in endocrine, neuroendocrine, and neuronal cells [23,28,29]. Upregulated expression of Scg3 was reported in multiple sclerosis and tumors, including neuroendocrine tumors, prostate cancer, small cell lung cancer, and hepatocellular carcinoma [30–35]. Whether upregulated Scg3 stimulates angiogenesis in these pathological conditions is yet to be investigated.

A major advantage for therapies targeting disease-selective angiogenic factors is the expected safety. Safety is the leading cause of drug attrition (51%, including both preclinical and clinical), whereas insufficient efficacy accounts only for 9% of failure [36]. Two important underlying causes of adverse effects are the lack of target specificity and disease selectivity. An example of the former is small-molecule drugs, which can nonspecifically target other proteins to trigger adverse effects [26]. An example of the latter is the EGF inhibitor bevacizumab. Despite its high target specificity, systemic administration of

bevacizumab can trigger severe or fatal adverse effects because VEGF is a growth factor in not only tumor vessels, but also normal endothelium and other cells [37–39]. Highly disease-selective ligands identified by comparative ligandomics, such as Scg3, are ideal targets to develop biologics that will circumvent adverse effects related to the lack of target specificity and disease selectivity. In this regard, comparative ligandomics will improve the transition success rates of drug development from mice to humans.

The safety of anti-Scg3 therapy is supported by Scg3-knockout mice. Mice with homozygous deletion of the Scg3 gene (i.e., equivalent to 100% depletion of Scg3) have a normal phenotype [40]. By contrast, mice with deletion of a single VEGF allele are embryonic lethal with severe defects in blood vessel formation and developmental abnormalities [41], highlighting the critical role of VEGF in vasculogenesis and embryogenesis. The safety concerns have so far precluded VEGF inhibitors from receiving regulatory approval for ROP therapy in preterm infants. These differential phenotypes of Scg3^{-/-} and VEGF^{-/+} mice support the safety of anti-Scg3 mAb for ROP therapy [40,41]. This safety advantage is yet to be confirmed with an anti-Scg3 mAb in preclinical and clinical studies.

Comparison of ligandomics and antibody phage display

Another approach to directly discover therapeutic Abs targeting cell surface proteins is ‘function-first’ Ab phage display [42–45]. In general, this platform comprises two high-throughput screens: (i) a differential cell-based phage display screen for Abs preferentially recognizing tumor cells; and (ii) a subsequent high-throughput functional screen to identify Abs capable of inducing tumor cell apoptosis. The major advantage of this approach is to directly isolate therapeutic Abs from Ab phage display libraries in the absence of cell-surface antigen information [42,43,46]. Furthermore, therapeutic Abs can be used as molecule probes to identify tumor-specific antigens, such as intercellular adhesion molecule 1 (ICAM-1) and CD73 [43,45].

However, this strategy is complicated because of several reasons. First, emergence of dominant phage clones led to identification of Abs recognizing only a limited number of antigens. Most of these antigens, such as EGFR, HER2, CD44 and transferrin receptor [42,43,47–52], were already known as cancer markers at that time. Second, the versatility of this approach to identify therapeutic Abs targeting receptors with diverse functions is restricted by high-throughput functional assays. Nearly all reported studies screened candidate Abs using cancer cell apoptosis assays. However, Abs targeting other surface receptors, such as angiogenic receptors, cannot be identified by these screens. Third, nearly all high-throughput apoptosis assays require Ab cross-linking or Ab-dependent cell-mediated cytotoxicity (ADCC) to induce tumor cell apoptosis [42,43,45]. In these cases, Abs might not directly activate surface receptors and are not suitable to treat diseases other than cancer, such as DR and ROP. Finally, Ab crosslinking might favor targeting receptors requiring dimerization, such as receptor tyrosine kinases, but not other receptors (e.g., G-protein-coupled receptors). These limitations of high-throughput functional assays could markedly reduce the versatility of this ‘function-first’ Ab-screening approach.

Another approach is peptide ligands identified from phage display random peptide libraries. Arap *et al.* discovered a peptide binding to the interleukin (IL)-11 receptor on blood vessels of a terminally ill patient with cancer [53]. This strategy is useful to understand the progression of a disease by its differential expression patterns and to develop therapeutic targeting strategies [54]. However, identified unnatural peptide ligands cannot reveal extrinsic regulations in pathological conditions and systematically map therapeutic targets.

Similarly, comparative ligandomics also has several drawbacks. First, OPD-based ligandomics might miss some cellular ligands that require proper post-translational modifications for receptor binding. This limitation affects only a small percentage of cellular ligands, as previously discussed [14]. Second, ligandomics can only delineate disease-associated ligands but not their functional roles. Consequently, ligandomics cannot determine whether identified ligands are detrimental or protective. Third, ligandomics cannot elucidate receptors of identified ligands. As a result, Scg3 and HRP-3 receptors remain elusive. Nonetheless, identified disease-high ligands can be used as molecular probes to elucidate cognate receptors as additional disease-selective targets (Figure 1f). Fourth, unlike Abs that can be readily expressed and purified, it is difficult to prepare a large number of identified ligands for functional characterizations. Fifth, not all ligands identified by ligandomics are extracellular proteins and might not be biologically relevant. Thus, ligandomics data should be appropriately filtered by bioinformatics analyses to identify only cellular ligands with extracellular trafficking. Finally, neutralizing Abs against detrimental ligands might not be immediately available and need to be generated with extra research effort.

Despite these limitations, OPD-based ligandomics is the only high-throughput technology to globally profile extracellular ligands [15,19,20]. Furthermore, ligandomics can identify a large number of 'disease-high' ligands, whose receptors are upregulated on diseased cells [14]. In general, ligand–receptor interactions have lower affinity than does Ab–antigen binding. This could explain why ligandomics screens discover more-diverse ligands than do Ab phage displays [14,41]. In contrast to Abs recognizing only linear epitopes with seven to ten amino acids, ligand–receptor interactions usually require conformational epitopes. As a result, ligands identified by ligandomics are more likely able to directly activate surface receptors without Ab crosslinking or ADCC. Perhaps the most important advantage of ligandomics is to reveal the identities of disease-selective endogenous ligands. These identities enable searches through available databases and literatures to understand their physiological role, assess their pathological contribution, predict their therapeutic potential, and estimate safety of new therapies. For example, Scg3 identified by comparative ligandomics has never been reported as a cellular ligand. However, its family members, including chromogranin A (CgA), CgA-derived peptides, and Scg2-derived secretoneurin, are involved in angiogenesis regulation [55]. Scg3 was also reported to have increased expression in several tumors [30–33], implicating its potential role in the regulation of tumor angiogenesis. The differential phenotypes between VEGF^{-/+} and Scg3^{-/-} mice also imply the better safety profile of anti-Scg3 therapy [40,41]. Based on these analyses, Scg3 was chosen as a target to develop the disease-selective angiogenesis blocker for DR therapy [14].

Similar to phage display [53], ligandomics can be performed in terminally ill patients with appropriate ethical approval. However, performing such studies in two different patients for comparative ligandomics analysis is technically difficult. An alternative approach for ligandomics analysis in human organs is organ-on-chip models that can reconstitute complex organ-level physiological functions and clinically relevant disease phenotypes [56].

Ligandomics versus proteomics

Functional proteomics technologies are mainly developed to map intracellular protein–protein interaction networks (Table 1) [57]. For example, Krogan *et al.* successfully tagged, expressed, and purified 2357 bait proteins in yeast to reveal 4087 associated proteins by mass spectrometry [58]. Using a similar approach, Ewing *et al.* performed the first large-scale analysis of protein interactomes in human cells and identified 24 540 potential protein interactions for 338 bait proteins [21]. However, such an approach of functional proteomics cannot be applied to ligand–receptor interactions on the cell surface.

By contrast, expressional proteomics can detect cell surface proteins but with limited sensitivity. For example, a previous study detected only 37 proteins located at the plasma membrane of MDA-MB-231 cells by mass spectrometry [43]. Cell-surface proteins with low abundance cannot be detected by proteomics. To our knowledge, no proteomics technologies can globally profile cellular ligands.

Ligandomics as an equivalent of functional proteomics is an efficient approach to mapping cell-binding ligands and has the sensitivity to detect a single copy of individual bound ligands (Table 1) [16]. This is particularly advantageous to identified ligands for low-abundance receptors. Owing to the binding quantification capacity, comparative ligandomics analysis of diseased versus healthy cells or organs can systematically identify disease-associated ligands. Biologics targeting ligands with high disease selectivity have the advantages of minimal adverse effects on normal cells with large therapeutic indices, thereby providing opportunities to increase doses within therapeutic windows for maximal efficacy. In this regard, disease-selective biologics can have relatively low efficacy and/or safety-related attrition rates. Importantly, comparative ligandomics can systematically identify disease-selective targets without the need for any molecular probes or receptor information. As a result, ligandomics is broadly applicable to any type of cell or disease in both *in vitro* and *in vivo* settings.

Versatility of comparative ligandomics

Besides vascular diseases, comparative ligandomics could be applied to cancer, neurodegenerative, and immunological diseases. Quantitative comparison of entire ligandomes for healthy versus diseased, young versus aged, or receptor-expressing versus receptor-deficient and/or silenced cells will systematically delineate disease-, age- or receptor-related ligands. Comparative ligandomics should have versatile applications with different ligand selection strategies [15,19], cell lineages, tissues, organs, and disease conditions. For example, comparative ligandomics could be applied to tumors in live animals and systematically identify tumor-associated angiogenic factors. If applied to tumor

models at different disease stages, comparative ligandomics can dynamically map tumor-high endothelial ligands throughout the disease course and identify targets for the stage-specific antiangiogenic therapy of cancer. Alternatively, comparative ligandomics can be applied to stem cells versus nonstem cells to globally profile stem cell-binding ligands as extrinsic regulators to understand the complexity of niche environments. Taken together, comparative ligandomics is a powerful technology with versatile applications to discover pathogenic and therapeutic ligands for different diseases.

Concluding remarks

Ligandomics is the first high-throughput technology to globally profile ligand–receptor interactions and systematically identify disease-high ligands. Biologics targeting ligands with high disease selectivity have the advantages of high efficacy, minimal adverse effects, wide therapeutic windows, and reduced safety-related attrition rates. Therefore, ligandomics represents a potential paradigm shift for drug target discovery. Although biologics has the advantage of high target specificity, ligandomics is capable of identifying highly disease-selective ligands as druggable targets. The combination of these two technologies could become a winning strategy to improve markedly the productivity of pharmaceutical R&D. However, this notion should be ultimately tested further using different disease-selective ligands to determine both preclinical and clinical success rates.

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References

1. Pammolli F, et al. The productivity crisis in pharmaceutical R&D. *Nat Rev Drug Discov.* 2011; 10:428–438. [PubMed: 21629293]
2. Paul SM, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat Rev Drug Discov.* 2010; 9:203–214. [PubMed: 20168317]
3. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov.* 2004; 3:711–715. [PubMed: 15286737]
4. Walsh G. Biopharmaceutical benchmarks 2014. *Nat Biotechnol.* 2014; 32:992–1000. [PubMed: 25299917]
5. Evaluate Pharma. Evaluate Pharma World Preview 2015, Outlook to 2020. Evaluate Pharma; 2015.
6. Evaluate Pharma. Evaluate Pharma World Preview 2017, Outlook to 2022. Evaluate Pharma; 2017.
7. Santos R, et al. A comprehensive map of molecular drug targets. *Nat Rev Drug Discov.* 2017; 16:19–34. [PubMed: 27910877]
8. Shi S. Biologics: an update and challenge of their pharmacokinetics. *Curr Drug Metab.* 2014; 15:271–290. [PubMed: 24745789]
9. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. *Nat Rev Drug Discov.* 2016; 15:385–403. [PubMed: 26775688]
10. Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. *J Clin Invest.* 2015; 125:3384–3391. [PubMed: 26325035]
11. Shukla AA, Thommes J. Recent advances in large-scale production of monoclonal antibodies and related proteins. *Trends Biotechnol.* 2010; 28:253–261. [PubMed: 20304511]

12. Shen Z, et al. Use of high-throughput LC-MS/MS proteomics technologies in drug discovery. *Drug Discov Today Technol.* 2006; 3:301–306. [PubMed: 24980532]
13. Ngounou Wetie AG, et al. Protein-protein interactions: switch from classical methods to proteomics and bioinformatics-based approaches. *Cell Mol Life Sci.* 2014; 71:205–228. [PubMed: 23579629]
14. LeBlanc ME, et al. Secretogranin III as a disease-associated ligand for antiangiogenic therapy of diabetic retinopathy. *J Exp Med.* 2017; 214:1029–1047. [PubMed: 28330905]
15. LeBlanc ME, et al. Hepatoma-derived growth factor-related protein-3 is a novel angiogenic factor. *PLoS ONE.* 2015; 10:e0127904. [PubMed: 25996149]
16. Li W. ORF phage display to identify cellular proteins with different functions. *Methods.* 2012; 58:2–9. [PubMed: 22836128]
17. Caberoy NB, et al. Efficient identification of tubby-binding proteins by an improved system of T7 phage display. *J Mol Recognit.* 2010; 23:74–83. [PubMed: 19718693]
18. Li W. Eat-me signals: keys to molecular phagocyte biology and ‘Appetite’ control. *J Cell Physiol.* 2012; 227:1291–1297. [PubMed: 21520079]
19. Guo F, et al. ABCF1 extrinsically regulates retinal pigment epithelial cell phagocytosis. *Mol Biol Cell.* 2015; 26:2311–2320. [PubMed: 25904329]
20. Ding Y, et al. Reticulocalbin-1 facilitates microglial phagocytosis. *PLoS ONE.* 2015; 10:e0126993. [PubMed: 25992960]
21. Ewing RM, et al. Large-scale mapping of human protein–protein interactions by mass spectrometry. *Mol Syst Biol.* 2007; 3:89. [PubMed: 17353931]
22. Hosaka M, Watanabe T. Secretogranin III: a bridge between core hormone aggregates and the secretory granule membrane. *Endocr J.* 2010; 57:275–286. [PubMed: 20203425]
23. Ottiger HP, et al. 1B1075: a brain- and pituitary-specific mRNA that encodes a novel chromogranin/secretogranin-like component of intracellular vesicles. *J Neurosci.* 1990; 10:3135–3147. [PubMed: 2204688]
24. Wang W, et al. Pathogenic role and therapeutic potential of pleiotrophin in mouse models of ocular vascular disease. *Angiogenesis.* 2017; 20:479–492. [PubMed: 28447229]
25. Manigrasso MB, et al. Unlocking the biology of RAGE in diabetic microvascular complications. *Trends Endocrinol Metab.* 2014; 25:15–22. [PubMed: 24011512]
26. Oo C, Kalbag SS. Leveraging the attributes of biologics and small molecules, and releasing the bottlenecks: a new wave of revolution in drug development. *Expert Rev Clin Pharmacol.* 2016; 9:747–749. [PubMed: 26933755]
27. Smietana K, et al. Trends in clinical success rates. *Nat Rev Drug Discov.* 2016; 15:379–380. [PubMed: 27199245]
28. Sakai Y, et al. Immunocytochemical localization of secretogranin III in the endocrine pancreas of male rats. *Arch Histol Cytol.* 2004; 67:57–64. [PubMed: 15125023]
29. Hosaka M, et al. Identification of a chromogranin A domain that mediates binding to secretogranin III and targeting to secretory granules in pituitary cells and pancreatic beta-cells. *Mol Biol Cell.* 2002; 13:3388–3399. [PubMed: 12388744]
30. Jongsma J, et al. Different profiles of neuroendocrine cell differentiation evolve in the PC-310 human prostate cancer model during long-term androgen deprivation. *Prostate.* 2002; 50:203–215. [PubMed: 11870798]
31. Moss AC, et al. SCG3 transcript in peripheral blood is a prognostic biomarker for REST-deficient small cell lung cancer. *Clin Cancer Res.* 2009; 15:274–283. [PubMed: 19118055]
32. Portela-Gomes GM, et al. Secretogranin III in human neuroendocrine tumours: a comparative immunohistochemical study with chromogranins A and B and secretogranin II. *Regul Pept.* 2010; 165:30–35. [PubMed: 20550951]
33. Wang Y, et al. The oncoprotein HBXIP up-regulates SCG3 through modulating E2F1 and miR-509-3p in hepatoma cells. *Cancer Lett.* 2014; 352:169–178. [PubMed: 24882622]
34. Lloyd RV, et al. Analysis of chromogranin/secretogranin messenger RNAs in human pituitary adenomas. *Diagn Mol Pathol.* 1994; 3:38–45. [PubMed: 8162254]

35. Teunissen CE, et al. Identification of biomarkers for diagnosis and progression of MS by MALDI-TOF mass spectrometry. *Mult Scler*. 2011; 17:838–850. [PubMed: 21505015]
36. Waring MJ, et al. An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nat Rev Drug Discov*. 2015; 14:475–486. [PubMed: 26091267]
37. Byeon SH, et al. Vascular endothelial growth factor as an autocrine survival factor for retinal pigment epithelial cells under oxidative stress via the VEGF-R2/PI3K/Akt. *Invest Ophthalmol Vis Sci*. 2010; 51:1190–1197. [PubMed: 19834034]
38. Rosenstein JM, et al. Neurotrophic effects of vascular endothelial growth factor on organotypic cortical explants and primary cortical neurons. *J Neurosci*. 2003; 23:11036–11044. [PubMed: 14657160]
39. Falk AT, et al. Bevacizumab: a dose review. *Crit Rev Oncol Hematol*. 2015; 94:311–322. [PubMed: 25703583]
40. Kingsley DM, et al. Genetic ablation of a mouse gene expressed specifically in brain. *EMBO J*. 1990; 9:395–399. [PubMed: 2303033]
41. Ferrara N, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature*. 1996; 380:439–442. [PubMed: 8602242]
42. Kurosawa G, et al. Comprehensive screening for antigens overexpressed on carcinomas via isolation of human mAbs that may be therapeutic. *Proc Natl Acad Sci U S A*. 2008; 105:7287–7292. [PubMed: 18474866]
43. Rust S, et al. Combining phenotypic and proteomic approaches to identify membrane targets in a ‘triple negative’ breast cancer cell type. *Mol Cancer*. 2013; 12:11. [PubMed: 23406016]
44. Frendeus B. Function-first antibody discovery: embracing the unpredictable biology of antibodies. *Oncoimmunology*. 2013; 2:e25047. [PubMed: 24083074]
45. Fransson J, et al. Rapid induction of apoptosis in B-cell lymphoma by functionally isolated human antibodies. *Int J Cancer*. 2006; 119:349–358. [PubMed: 16477633]
46. Veitonmaki N, et al. A human ICAM-1 antibody isolated by a function-first approach has potent macrophage-dependent antimyeloma activity *in vivo*. *Cancer Cell*. 2013; 23:502–515. [PubMed: 23597564]
47. Heitner T, et al. Selection of cell binding and internalizing epidermal growth factor receptor antibodies from a phage display library. *J Immunol Methods*. 2001; 248:17–30. [PubMed: 11223066]
48. Ayat H, et al. Isolation of scFv antibody fragments against HER2 and CEA tumor antigens from combinatorial antibody libraries derived from cancer patients. *Biologicals*. 2013; 41:345–354. [PubMed: 23845693]
49. Mazuet C, et al. Breast carcinoma specific antibody selection combining phage display and immunomagnetic cell sorting. *Biochem Biophys Res Commun*. 2006; 348:550–559. [PubMed: 16890194]
50. Shukla GS, Krag DN. Phage display selection for cell-specific ligands: development of a screening procedure suitable for small tumor specimens. *J Drug Target*. 2005; 13:7–18. [PubMed: 15848950]
51. Goenaga AL, et al. Identification and characterization of tumor antigens by using antibody phage display and intrabody strategies. *Mol Immunol*. 2007; 44:3777–3788. [PubMed: 17498801]
52. Sui J, et al. Identification of CD4 and transferrin receptor antibodies by CXCR4 antibody-guided Pathfinder selection. *Eur J Biochem*. 2003; 270:4497–4506. [PubMed: 14622278]
53. Arap W, et al. Steps toward mapping the human vasculature by phage display. *Nat Med*. 2002; 8:121–127. [PubMed: 11821895]
54. Christianson DR, et al. Techniques to decipher molecular diversity by phage display. *Methods Mol Biol*. 2007; 357:385–406. [PubMed: 17172704]
55. Helle KB, Corti A. Chromogranin A: a paradoxical player in angiogenesis and vascular biology. *Cell Mol Life Sci*. 2015; 72:339–348. [PubMed: 25297920]
56. Esch EW, et al. Organs-on-chips at the frontiers of drug discovery. *Nat Rev Drug Discov*. 2015; 14:248–260. [PubMed: 25792263]
57. Collins MO, Choudhary JS. Mapping multiprotein complexes by affinity purification and mass spectrometry. *Curr Opin Biotechnol*. 2008; 19:324–330. [PubMed: 18598764]

58. Krogan NJ, et al. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature*. 2006; 440:637–643. [PubMed: 16554755]
59. Aiello LP, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med*. 1994; 331:1480–1487. [PubMed: 7526212]
60. Matsunaga N, et al. Role of soluble vascular endothelial growth factor receptor-1 in the vitreous in proliferative diabetic retinopathy. *Ophthalmology*. 2008; 115:1916–1922. [PubMed: 18718666]

Research Highlights

- A major bottleneck of biologics discovery is how to identify drug targets.
- Cellular ligands and receptors are the most valuable targets for biologics.
- Disease-selective ligands are promising drug targets.
- Ligandomics can systematically identify disease-selective ligands.
- Biologics targeting such ligands likely have low attrition rates.

Figure 1.

Comparative ligandomics to identify secretogranin III (Scg3) as the first disease-selective angiogenic factor. **(a)** *In vivo* selection to enrich retinal endothelial ligands in live mice by open reading frame phage display (OPD)-based ligandomics [14]. **(b)** Quantitative ligandomics globally maps cell-wide endothelial ligands with simultaneous binding activity quantification by next-generation sequencing (NGS). **(c)** Comparative ligandomics. Quantitative comparison of the entire ligandome profiles for diabetic versus healthy retina systematically identifies disease-associated endothelial ligands. **(d)** Comparative ligandomics identified Scg3 as a diabetes-high ligand, hepatoma-derived growth factor-related protein-3 (HRP-3) as a diabetes-low ligand, and vascular endothelial growth factor (VEGF) as a diabetes-unchanged ligand. GFP was used as a background control. **(e)** Binding activity plot for comparative ligandomics analysis further categorizes all identified endothelial ligands into four groups: (i) DR-high ligands with increased binding to diabetic retinal vessels; (ii) DR-low ligands with decreased binding; (iii) DR-unchanged ligands with minimal binding activity change; and (iv) background binding with low binding activity. The positions of Scg3, HRP-3, VEGF, and GFP are indicated in the plot. **(f)** Characterization of disease-selective angiogenic factors could lead to disease-selective angiogenesis blockers. Identified ligands can also be used as molecules probes to delineate cognate receptors as additional disease-selective targets for biologics discovery. Reproduced, with permission, from [14] (A–E)..

Figure 2.

Nonselective versus selective angiogenesis blockers. The receptors for disease-selective angiogenic ligands (e.g., secretogranin III; Scg3) are predominantly expressed on diseased vessels, whereas the receptors for nonselective angiogenic factors (e.g., vascular endothelial growth factor; VEGF) are expressed on both diseased and normal endothelium. Selective angiogenesis blockers preferentially exert therapeutic activity on diseased vessels with minimal adverse effects on normal vessels, similar in some ways to selective β -adrenergic blockers. By contrast, nonselective angiogenesis blockers have therapeutic and detrimental effects on diseased and normal vasculature simultaneously. Therefore, nonselective blockers have limited therapeutic indices, whereas selective blockers have wide safety windows.

Table 1

Comparison of ligandomics and functional proteomics

Characteristic	Functional proteomics	Ligandomics
Technical platform	Mass spectrometry	OPD-NGS
Detection sensitivity	Attomolar range (10^{-18})	Single bound ligand (10^{-23})
'Protein-PCR' for multirounds of target amplification	No	Yes
Number of ligands identified	~100/cell	>1000/cell
Target interaction mode	Interactomes with direct or indirect binding	Direct receptor binding
Protein subcellular location	Intracellular	Cell surface
Drug type for identified target	Small molecules	Biologics and small molecules
Global binding activity quantification	No	Yes
Quantitative binding activity comparison	No	Yes
High-throughput identification of disease-associated targets	No	Yes
Main technical barrier	High cost of mass spectrometry instruments	High-quality OPD libraries