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Leveraging Years of Data on GPCRs for Transformative Drug Discovery

Boston - GPCRs, which play a role in most biological processes, remain one of the most vibrant fields for drug discovery and perhaps the single largest source of untapped therapeutic potential. Years of data are increasingly being leveraged to overcome the complexity of GPCR biology and pharmacology and accelerate GPCR drug discovery to the next level. It is now possible to hope to rationally design drugs with optimal effects, thanks to a better understanding of the concept of functional selectivity or biased signalling. A rapidly growing volume of structural data also allows researchers to perform virtual screens with ever-increasing numbers of compounds and suggest new small molecule candidates in mere hours instead of months, allowing the exploration of a chemical space that seems infinite, with potentially 10^{60} drug-like molecules.

KEY WORDS: GPCR, drug discovery, biosensors, bioSensAll™, Functional selectivity, Virtual screening

In the past two decades, compounds targeting GPCRs have led to FDA approval of 116 new drugs for 42 different indications. However, the full potential of GPCRs remains underexploited due to incomplete understanding of their complex pharmacology.

In his opening remarks, at a lunchtime panel event at this year's Discovery on Target conference in Boston, Chair Michel Bouvier PhD, CEO of the Institute for Research in Immunology and Cancer (IRIC) and Professor, Biochemistry and Molecular Medicine, Université de Montréal, touched on GPCR pluridimensionality or biased signalling. Each GPCR can respond to multiple ligands and each ligand may activate distinct combinations of downstream signaling pathways. "Unlike what we previously thought, GPCRs are not wired linearly," Pr. Bouvier said. Knowledge of the plethora of signals from a single GPCR could yield ligands that selectively activate or block the pathway(s) of therapeutic focus while avoiding undesirable signalling pathways also controlled by the receptor, he said. This concept known as bias signaling, or functional selectivity, creates an opportunity to change the way drugs are designed. Pr. Bouvier presented an update on one approach to tease out these signals: a panel of 70 BRET-based biosensors that serves to monitor the signalling pathways engaged by GPCRs in living cells in real time. Now out-licensed and used by Domain Therapeutics under the name bioSensAll™, the platform grew out of a Quebec, Canada-based academic-industry collaboration funded by Consortium Québécois sur la Découverte du Médicament (CQDM) and spearheaded by Pr. Bouvier with a team of researchers composed of Drs. Laporte and Hebert (McGill University), Pineyro (Ste-Justine hospital research center), Leduc (Sherbrooke University) and coordinated by Dr. Le Gouill in the Bouvier Laboratory.Pr. Bouvier presented results for 13 different angiotensin ligands over several different pathways including Gαq, Gαi2 and Gαi3 that were obtained in collaboration with the laboratory of Dr. Stéphane Laporte at McGill University. "There are textures in the ability of these compounds to engage these different pathways," he said. The pattern was respected even for the cascade of downstream effectors: "So we can have the confidence that each of these biosensors really is informing us of what we think they are informing us about," Pr. Bouvier said. Subsequently, a large-scale mutagenesis analysis, in which clustered mutations were overlaid on the structure of the receptor, allowed the team to visualize the allosteric connectors likely to be responsible for the functionally selective actions of these receptors. Pr. Bouvier concluded, "All this opens the path for the rational design of drugs."

GPCR mutations: Exploring the Lab of Evolution:

"Rather than relying on experimental data from the wet lab we have tried to rely on experiments done by evolution," said panelist Olivier Lichtarge MD PhD, of the Computational and Integrative Biomedical Research Center, Baylor College of Medicine in Houston, Texas. In his talk, Pr. Lichtarge focused on the structural determinants of GPCR signalling complexity. "The crosstalk, overlap and feedback between the different pathways downstream of the GPCR makes signalling very complex. The fact that different ligands can bias signalling in different ways complicates the picture even further," he said. To understand which amino acid positions control which part of signalling – thereby identifying which variants drive which traits and diseases – Pr. Lichtarge's team has developed the Evolutionary Action (EA) approach defined by a formal equation of the genotype-phenotype relationship. "To evaluate the mutational size," said Pr. Lichtarge, "the team used simple transition matrices between amino acids and for the sensitivity of the mutated site, they used phylogenetic analysis of the importance of each amino acid in a given protein in light of evolutionary divergences."

Over time, his group has used the guidance of EA to engineer GPCRs with decreased ligand binding and with constitutive activity, for example in the case of rhodopsin. EA has also been employed to reprogram allosteric specificity; for example, swapping amino acids between

a dopamine and serotonin receptor to create a dopamine mutant that responded much more to serotonin than to dopamine. "Essentially," said Pr. Lichtarge, "we switched functions like you might do with two keys by changing the notches."

Importantly, EA is helpful in decoding the switches responsible for GPCR biased signalling. Pr. Lichtarge cited work headed by collaborator Anne-Marie Schönegge in the laboratory of Michel Bouvier of the Université de Montréal, Canada, that measured experimentally the impact of mutations across five different pathways, following identification of mutation position by EA. All mutations were close to conserved class A GPCR motifs such as E/DRY, PIF and NPxxY. The work was published in *Nature Communications* in 2017. "The group titrated the mutations by cranking up the degree to which the mutation was less and less conservative," said Pr. Lichtarge. Clustering revealed the responses fell into one of three distinct profiles. The extent to which the signalling was perturbed "correlated reasonably well with the prediction from the evolutionary equation that we had," said Pr. Lichtarge. In the active conformation of the perturbed sites, each of the three mutational clusters had different amino-acid neighbours. Each cluster also affected the conserved motifs in the GPCR in distinctly different ways. "So mutations in the region near functional motifs can rewire GPCR signalling," said Pr. Lichtarge.

Pr. Lichtarge elaborated on the technique of supervised clustering. He and his collaborators have examined whether clustering can help clarify, classify and even predict drug side-effect profiles by deconvoluting the complexity of ligand responses. Typically, a ligand will activate a mix of desired and deleterious pathways, either within the target receptor itself and/or in homologous receptors. Pr. Lichtarge presented work by lab colleague Dr. Jonathan Gallion, Pr. Michel Bouvier and Pr. Graciela Pineyro of the Université de Montréal, published earlier this year in *Nature Communications*. The investigators clustered the extensive signalling diversity of mu-opioid receptor ligands using machine learning (or artificial intelligence, AI), then compared the resulting categories to drug side effects reported to the FDA's Clinical Adverse Event Reporting System (AERS; FDA.gov). They found that the in-vitro signalling profiles, clustered by AI, were significantly associated with well-known opioid side effects such as muscle wasting, GI motility and deadly respiratory depression. "So altogether these data show a clustering approach is general, scalable, flexible… This is a good example of robust deconvolution of large, complex data from high-throughput screens in a way that can be predictive of drug biological actions," Pr. Lichtarge concluded.

Work such as this, by disentangling GPCR biased signalling, brings novel drugs with custom-designed signalling properties one step closer. Pr. Lichtarge concluded, "We have shown that we can efficiently engineer and guide the design of G-protein coupled receptors by interpreting properly the enormous body of mutations that have already been done for us – for free – by evolution."

Virtual screening: A Post-structural Era

"Docking libraries are getting bigger and bigger and not stopping – it's an explosion," said panelist John Irwin PhD, of the University of California San Francisco. Structure-based docking programs can screen large, diverse virtual libraries, allowing researchers to select only the best-fitting molecules for synthesis and testing. Pr. Irwin's team's make-on-demand docking library, ZINC (http://zinc15.docking.org), is expected to reach 1 billion molecules in 2020.

Pr. Irwin said that the team has been focusing on GPCRs since 2007. "We also look at kinases and nuclear receptors, but GPCRs are really where it's at," he said.

An analysis of 138-million library molecules in the docking program UCSF DOCK3.7 recently yielded one of the most potent, selective full agonists ever characterized for the D4 receptor. To get to this point, researchers Drs. JianKun Lyu, Trent Balius and Anat Levit docked 138 million virtual molecules against the structure of the receptor, with approximately 70 trillion complexes sampled at the orthosteric site and ranked them according to binding score. This required 43,563 core hours – the equivalent of 1.2 calendar days on their cluster at UCSF. All ranked molecules were clustered by topology and scaffold to focus on novel chemotypes. Molecules that resembled known adrenergic, dopaminergic or serotonergic ligands were excluded. The team led by Dr. Yurii Moroz at Enamine, Kyiv, Ukraine (enamine.net) went on to synthesize 549 of the 589 top-ranking virtual molecules. The compounds were tested by Drs. Sheng Wang and Tao Che in the lab of Pr. Bryan Roth at UNC Chapel Hill. Visual inspection showed that 124 molecules had favourable and diverse interactions with the D4 site with no internal strain. Compound ZINC621433144 proved to be one of the most potent, selective full agonists ever characterized for the D4 receptor. The breakthrough work was published in *Nature* on February 14 this year.

Virtual molecules are generated using building blocks provided by companies such as Enamine. Pr. Irwin's library, which started with approximately 70,000 building blocks from the company, focuses on compounds that have a reliable chemical pedigree: "The secret is you take reliable reactions – reactions all chemists know – and then you spend time making weird building blocks with synthetic handles that behave well, then you multiply these together, and that's how we go from 70,000 building blocks to 700 million or a billion molecules," Pr. Irwin said. His current computing power can build 3D models of about 3 million molecules a day. Docking campaigns vary in their success, but a 20% hit rate is typical based on recent work. "The good news is this is only the beginning," Pr. Irwin said. Clearly, virtual libraries and virtual screening are now a crucial part of GPCR-related drug discovery.

Summary

At the 17th Annual Discovery on Target Conference, a group of KOLs discussed different approaches to unlocking the potential of GPCR drug discovery. The development of a panel of BRET-based biosensors teasing out the diversity of GPCR signaling pathways represents a significant step towards better understanding the complex biology of these receptors. Combining such technology with evolutionary-action analyses helps elucidate the structure-function relationship in GPCR signaling, which brings the development of drugs having customized signaling properties one step closer. In parallel to these new insights in the GPCR signaling, GPCR drug discovery is also being revolutionized by the advent and refinement of virtual screening methods. Libraries of millions of molecules can now be tested within a few hours. This technological breakthrough allows to continuously explore the vast chemical space to discover more efficacious and safer drugs. All in all, the capacity to combine a better molecular understanding of functional selectivity, with the ever-increasing number of GPCR ligands discovered through virtual screening approaches, indicates that we are on the eve of a new GPCR drug discovery era that could have an important impact on human health. □

Based on presentations at the luncheon panel "GPCRs: Leveraging Years of Data for Transformative Drug Discovery" at Discovery on Target 2019. Key references included: [Pr. Bouvier's presentation] Namkung Y, LeGouill C, Kumar S, *et al*. Functional selectivity profiling of the angiotensive II type 1 receptor using pathway-wide BRET signaling sensors. *Science Signaling* 2018; 11. DOI: 10.1126/scisignal.aat1631. [Pr. Lichtarge's presentation] Katsonis P and Lichtarge, O. CAGI5: Objective performance assessments of predictions based on the Evolutionary Action Equation. *Human Mutation* 2019 Jul; PMID: 31317604 DOI: 10.1002/humu.23873; [Pr. Irwin's presentation] Lyu J, Wang S, Balius TE, *et al*. Ultra-large library docking for discovering new chemotypes. *Nature* 2019; 566: 14 February, 2019, https://doi.org/10.1038/s41586-019-0917-9.

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