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Pragmatic Approach To Drug Discovery Through Biophysical Perspective

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Abstract
Drug discovery process is a long drawn time and resource consuming process. The conventional drug discovery process involves potential lead from either traditional system of medicine or from the in vitro experiments leading to a lot of animal experimentations wahich culminates in human trials. These whole processes usually take years of time and resources. Many a times, when a drug accreditation is accorded, the pharmacoeconomic values outnumbers the drug addition value to the drug library. With the advent of the usage of computers in biology, the discovery process shortened with the help of uniform and defined in silico parameters, thus providing an alternative to the laborious and variable in vitro experiments. While, this was a welcome move in the field of drug discovery but simultaneously it also opened the flood of potential molecules which often matched the defined in silico parameters but provided variable results in in vivo experiments. This article provides an overview of the drug discovery processes and its efficacy with a check on pharmacoeconomic values. The system of selection of potential lead molecule is based on several parameters out of which the most important aspect is that it should have no toxicity in vivo.

Key Words: Drug discovery, Crystallization, Clinical trial

Introduction

New era of drug discovery beckons. Tools and technology derived from biotechnology, genetics and computer science has forwarded the impetus of drug discovery process. The biophysical methods and techniques like NMR, X-Ray Crystallography, genome mapping etc have accelerated the drug discovery process. The diligent and discerning integration of the modern scientific knowledge has cut down the necessary time required to bring new drugs to the market. Around 1980's, a lot of investment has been done by private sectors like pharmaceutical industries in research for validated drug targets (1). More than one lakh sequences of potential drug targets have been submitted after the successful completion of human genome project. The genome project completion has led to a lot of raw information which could not be translated to the therapeutic drug discovery (2). Thus, a gap was created between genomic discovery and drug discovery which still exist today (3). The probable reason behind this mismatch could be because gene sequence does not provide full information about disease as these are the areas on which extensive research are going on. Moreover, researchers are not completely aware of the complete functioning of all the encoded proteins which is mainly the cause of delay in converting the genomic information to product in the form of therapeutic drug. The new concept of proteomics emerged after genomics as it refers to any protein based approach that provides new insight on protein structure or functioning on a genome wide scale. The proteomic discovery is more tedious than genomic discovery as majority of predicted proteins have no known cellular function and predicting their function *in silico* is like predicting multiple proteins for the same function *in vivo*. However, as we know that all enzymes are protein, therefore discovering new proteins may lead to discovery of new enzyme, new signaling molecules which might lead to the discovery of new pathways for therapeutic targets (4). Proteomics technology (5) is used not only to validate the drug discovery but it also increases the drug discovery process. The discovery of all the proteins encoded by genes leads to a high throughput library where one can screen the validation of drugs but the proteins have another twist which genes do not have i.e. their structure. The structural biology of proteins through the technique like crystallography and

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NMR provides information about the structure of protein which defines the functioning of protein at site (6). Therefore, proteomics is a means by which the drug discovery time could be shortened as it bridges the gap between genomics and proteomics (7). As discussed earlier, the crystal structure determination at atomic resolution provides the most conclusive basis for drug discovery (8). The crystal structures of the binding of a ligand with its receptor provide the interaction distance as well as binding affinity at atomic level resolution (9). There have been a lot of instances of translation of the lead from crystal to the level of therapeutic drug (10). This article is an overview of the technologies most relevant to the drug discovery process and provides some ideas about developing proteomics technology in the quest for the discovery of novel drugs.

Proteins as important drug targets

Proteomics is thought of more as a basic science having some applied value. *In silico* docking studies could not be relied upon for drug development as it has not been able to predict properly the protein function. Proteomics mainly requires samples in high quantity if one wants a high throughput proteomics screening. Several structural protein banks are coming up so that every time everyone does not have to go through the process of purification (11). In the experimental set up, a large scale of proteins have been purified, cloned and expressed in bacteria and yeast and have been predicted to be cytoplasmic with no structural homologs (12). Hence, these are proteins with no known function. The main problem faced in *in vitro* studies is the heterogeneous folding of proteins which differ in our *in vivo* system. The folding of proteins mainly defines its function and if it differs then function is bound to be different. Majority of proteins have been observed to have a unstable structure (13). The databank containing such improperly folded proteins should not be used for high throughput screening as it often provides incorrect results and scientists who use them do waste their time and money on fruitless targets.

Target Discovery

Out of more than a lakh of proteins, only a fraction of these are expressed in any given cell type. Therefore, the expression of protein is very important which should be documented both quantitatively as well as qualitatively in cases of expression in disease related process. As we have discussed how difficult and time consuming it is to correctly predict the protein quantity as well as quality, therefore researchers thought of finding another means which could help to relate with protein expression. The DNA microarray technology came as a tool to correlate the mRNA and protein expression. This microarray technique is very sensitive and detects even a poorly expressed gene and provides information of the type of gene expressed in the type of tissue. The mRNA formed after transcription *in vivo* undergoes post translational modifications. To discover and monitor the relevance of a protein to a disease-related process, it is important to catalog where, when, and to what extent a protein is expressed. DNA microarray technology (14), which monitors the relative abundance of mRNA in a cell, is a powerful way to accomplish this because mRNA and protein concentrations are often correlated. DNA microarray technology can measure even poorly expressed genes, ensuring a comprehensive assessment of which genes are expressed in which tissue. However, since mRNA and protein levels do not always correlate in the cell and many regulatory processes occur after transcription, a direct measure of relative protein abundance is more desirable. There are different types of proteomics technology available to quantify the different levels of proteins expressed in different tissues. The most commonly used technique for resolving and sequencing a mixture of proteins is Mass Spectroscopy (MS). MS resolves a mixture of proteins and then individual protein bands could be sequenced through peptide fingerprinting (15). The main problem with this wonderful technique is that the MS spectra of abundant proteins generally dominate and overlaps the lower abundant proteins. Therefore, it is not possible to identify low abundant proteins without interference. Although, a lot of research is undergoing to make MS more and more efficient but it is proving difficult to fractionate cell extracts based on MS (16). While identifying the composition of proteins in the cell, it must be taken into account that protein isoforms are often formed after mRNA splicing and its further covalent modifications which contribute to the regulatory processes within the cells. Thus, a therapeutic intervention by protein first requires cataloguing all such isoforms expressed in disease causing pathways so that a strategy could be designed for the same. The other approaches include the modification specific antibodies. Technique involving recording all the changes in gene expression, protein levels, or any modification caused by cellular perturbations are powerful methods to identify protein targets for drug discovery (17). However, these methods do not tell if a particular gene product i.e. protein is related to disease or it is to be used for targeting drug development. To address these issues, the approach is needed which addresses protein function as well which is known as structural proteomics.

Drug development through structural proteomics

Structural proteomics is mainly used for target validation. Since, the protein function is determined by its three dimensional structure, therefore, proteins of similar function shares structural homology. There are a lot of expressed proteins which shows some sort of structural homology to the known proteins. Hence, the protein structure database is increasing exponentially with newer submission which

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provides information on the functional aspects of newly discovered proteins. Structural proteomics work under the principle that structure underlies function. Its main objective is to find a three dimensional structure of all the discovered proteins (18). Many pharmaceutical companies have this private structural database which can often be used to identify potential drug targets or to invalidate targets with structural properties that do not let themselves to the binding of a drug. The database of a public domain is open but the pharmaceutical company's database is restricted and that is where the companies invest on research. The more enriched database of the companies are discovering better and newer drugs. The scientists predict that the addition of a lot of compounds will help in zeroing down to the perfect structure prediction algorithms, which will eventually allow scientists to predict structure and function from sequence.

Target validation through Interaction proteomics

The most cellular process including cell-cell interaction, signaling pathways, metabolism and cellular architecture involves protein – protein interaction. The full characterization of the complex network of protein-protein interaction is required to fully understand the cellular functions. Many human diseases like cancer, autoimmune disorders and viral infections do occur because of aberration in protein-protein associations. Therefore, finding a complete set of protein-protein interactions within the cells as well as interaction of proteins with their targets has potential to reveal newer biological function and new targets for therapeutic intervention (19). The commonly used technology for the discovery of protein interaction is yeast two hybrid system. Although, this technique has a great potential but it also has several limitations among which the most prominent one is false positive results as the technique can only characterize interaction between two molecules and is not useful for multi molecular techniques.

Interaction Proteomics

Most of the cellular processes involve protein-protein interaction. Therefore, full characterization of the complex network of cellular protein interaction is required for the complete understanding of the cellular processes. Many human diseases like different kinds of autoimmune disorders and cancers occur because of aberrations in protein-protein interaction. Therefore, protein-protein interaction in diseased state provides new targets for therapeutic intervention. The most commonly used technology for determining protein-protein interaction is yeast two hybrid system. The yeast two hybrid systems have been used to discover many new protein-protein interactions but it also has several shortcomings. There are several false positive interactions for every legitimate and valid interaction. Only a fraction of known interactions have been identified in whole genome wide two hybrid screens. The yeast two hybrid system can identify only interaction between two proteins while it is not applicable in multi protein interaction set up. To overcome the limitation of yeast two hybrid systems, protein affinity chromatography is often used (20). In this chromatography technique, the pure protein samples are used and it is better in the sense that it generates very few false positive interactions. It could be used for high throughput screening as it immobilizes the target of purified protein on solid support which could be eluted by gel electrophoresis or Mass Spectrometry (MS). This protein interaction method is suitable to explore the protein-protein interaction whose levels in cells are very low as 1/100,000.

Bioinformatics

The main aim of genomics and proteomics is to characterize and to dig out all the pharmacology related information using computational methods (21, 22). However, as majority of proteins have no known function, therefore, this goal cannot be reached for all the proteins. Therefore, to increase the efficiency of bioinformatics predictive ability, the requirement is to first characterize and complete the proteomics of all the genome. The challenge is that it cannot be done in one go, therefore, a database needs to be created which pools all the unbiased genome wide proteomic results. This database capability could be enhanced by pattern recognition algorithms coupled with addition of new knowledge which could decipher the protein's cellular function. This unbiased approach provides more reliable platform of hypothesis generation as compared to the traditional approach. But such unbiased database cannot be comprehensive and there are chances of repeated value of data generation which provides confidence to the unbiased approach. In the proteomic sector a database has been created which links protein function with its biophysical properties. The protein solubility and its crystallization properties are also linked to it. Therefore, as it is evident that the linkage of the function of individual protein to protein pathways and the protein pathways to the cellular pathways is happening and happening fast, therefore, we might be knocking the doors of an era which will see a dramatic increase in the efficiency of the drug discovery process. This strategy is bound to make an impact in the coming decade.

Lipophilic Compounds in drug discovery

The era is witnessing a high throughput screening computationally and for that we need to augment the database with valid and unbiased experimental results. The lipophilic compounds are unique as most of the drugs are lipophilic and acts via lipophilic carriers (23). Therefore, formulation strategies for compounds inadequately soluble in water are coming up. Many drug delivery vehicles for lipid soluble compounds have been suggested. The targeted drug delivery and drug delivery for nano compounds often require lipophilic medium. Emulsion is liquid in liquid system in which one liquid is dispersed in a second, immiscible liquid in the form of droplets. Intralipid

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emulsion consists of a water phase with droplets composed of triglyceride core that is stabilized with a phospholipid monolayer. The two forces that plays a pivotal role in stabilization are long range repulsive electrostatic forces and a short-range repulsive hydration forces. The excess phospholipid exists as dispersed liposomes in a closed aggregate with water core. NMR and electron microscopy helps to elucidate detailed structure (24). Droplet emulsions of micromolar range are characterized by DLS (Dynamic light scattering methods) (25). The formulated and processed nutritional emulsions are stabilized by phospholipids for longer periods. The stability of emulsion is an important factor in clinical nutritional practice as emulsions are often used under circumstances that put a severe strain on the stability of the molecule. The patient needs supplementary nutrition as per the diet requirement and the nutrients often added to the emulsion are glucose, electrolytes, amino acids, the trace elements and vitamins. Despite emulsion being loaded extensively, it remains stable for prolonged period.

The production of formulated intralipids became the starting point for using lipid emulsions as a delivery matrix for a lipid -soluble drugs (26). The 1970's witnessed the development of drugs based on this technology that includes formulation of and manufacturing capabilities. The Diazepam emulsions which are used as sedatives were developed as a intravenous formulation using barbiturates. In the field of anesthesia, a propofol containing drug emulsion Diprivan is used successfully for inducing and maintaining anaesthesia which is one of the most prominent examples of lipid emulsions applied to drug delivery (27-29). The solubility of propofol is an important aspect in its success. Propofol readily dissolves in soyabean oil and has low concentration in water phase. Since, the pain while injecting the drug is mainly dependent on its concentration, therefore, the drug in water as emulsion is less painful in comparison to its soyabean only counterpart. There are a lot of other examples of drugs such as Cyclosporin (30, 31), Amphothericin B (32, 33) and Taxol (34). Taxol has been formulated using mixtures of nutritional emulsions is another example of several lipophilic compounds which has been synthesized based on lipophilic character (34).

Discussion

The drug discovery process is a time and resource consuming which needs to be updated and shortened in view of the upcoming tools and technologies. The screening and validation of new drug molecules for new therapeutics must be hastened. Fig 1 depicts a flow of how a new compound can be tested first by virtual screening and then by deciphering its structure so that the binding interaction can be validated. This is followed by clinical trials and a new drug could be deciphered after regulatory clearance.

Future aspects

The future of the drug discovery lies in the economics of drug discovery considering financial as well as timing aspects. Therefore, there is a need to expedite the discovery by investing in the *in silico* designs to find the best suitable molecule against the target. The target could be synthesized and validated by binding studies as well as crystallography or NMR methods. The *in vitro* testing followed by *in vivo* studies covering toxicity, efficacy and safety aspects makes the molecule suitable to be applied for the regulatory clearance. The other approach is the repurposing of approved drugs. As of now, serendipity has played a major role in drug repurposing which was mainly based on clinical observations but now days a rationalized approach is being taken for drug repurposing.

Conclusion

A lot of novel approaches are being tried so that a drug discovery process could be shortened both in terms of time and money. The computational approaches provide a theoretical model based on which newer drugs could be theoretically tested *in silico*. The synthetic chemists then make the peptides and check the design in-vivo. Once the peptide is successfully made and shows the required binding then hey! We have discovered a new molecule which could be a potent drug after successful clinical trials

References

1. Gibson SG & Lemmens T (2014) Niche markets and evidence assessment in transition: a critical review of proposed drug reforms. *Medical law review* 22(2):200-220.
2. La Cognata V, *et al.* (2014) Increasing the Coding Potential of Genomes Through Alternative Splicing: The Case of PARK2 Gene. *Current genomics* 15(3):203-216.
3. Janero DR (2014) The future of drug discovery: enabling technologies for enhancing lead characterization and profiling therapeutic potential. *Expert opinion on drug discovery* 9(8):847-858.
4. Zhou X, Liao W, Liao JM, Liao P, & Lu H (2015) Ribosomal proteins: functions beyond the ribosome. *Journal of molecular cell biology*. doi: 10.1093/jmcb/mjv014.
5. Ryall KA & Tan AC (2015) Systems biology approaches for advancing the discovery of effective drug combinations. *Journal of cheminformatics* 7:7.
6. Tulpule A (2005) Multidrug resistance in AIDS-related lymphoma. *Current opinion in oncology* 17(5):466-468.

DOI: 10.18535/ijmsci/v3i4.3

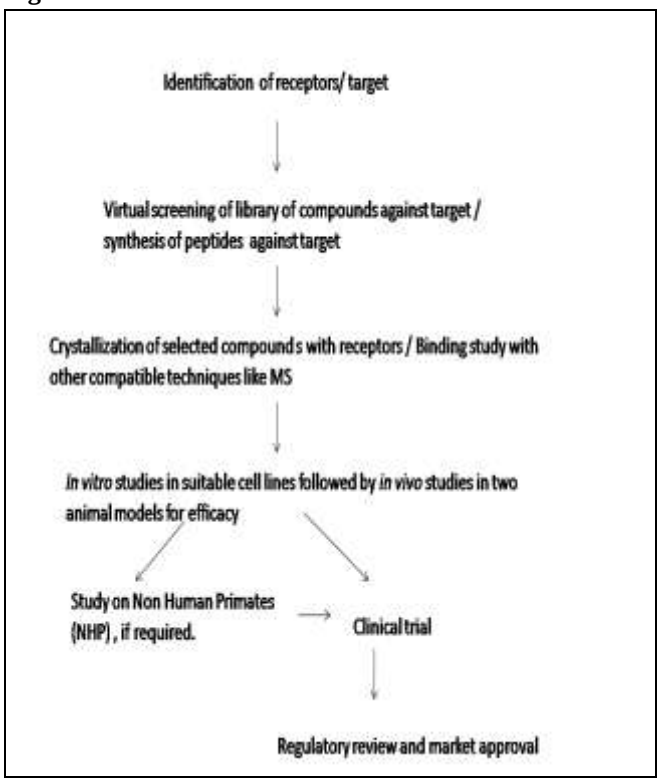
7. Zhou Y & Huang N (2015) Binding site druggability assessment in fragment-based drug design. *Methods in molecular biology* 1289:13-21.
8. Rogers WB & Manoharan VN (2015) DNA nanotechnology. Programming colloidal phase transitions with DNA strand displacement. *Science* 347(6222):639-642.
9. Tanabe M, Nimigean CM, & Iverson TM (2010) Structural basis for solute transport, nucleotide regulation, and immunological recognition of Neisseria meningitidis PorB. *Proceedings of the National Academy of Sciences of the United States of America* 107(15):6811-6816.
10. Li F, Liu J, Zheng Y, Garavito RM, & Ferguson-Miller S (2015) Protein structure. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. *Science* 347(6221):555-558.
11. Gokulrangan G, Zaidi A, Michaelis ML, & Schoneich C (2007) Proteomic analysis of protein nitration in rat cerebellum: effect of biological aging. *Journal of neurochemistry* 100(6):1494-1504.
12. Anonymous (2007) Proteomics' framework for success? *Nature methods* 4(1):1-2.
13. Meuwis MA, *et al.* (2007) Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling. *Biochemical pharmacology* 73(9):1422-1433.
14. Zimenkov DV, *et al.* (2015) Evaluation of a low-density hydrogel microarray technique for mycobacterial species identification. *Journal of clinical microbiology*. doi: 10.1128/JCM.02579-14.
15. Andrilenas KK, Penvose A, & Siggers T (2015) Using protein-binding microarrays to study transcription factor specificity: homologs, isoforms and complexes. *Briefings in functional genomics* 14(1):17-29.
16. Li G, Pei J, Yin Y, & Huang G (2015) Direct sequencing of a disulfide-linked peptide with electrospray ionization tandem mass spectrometry. *The Analyst*.
17. Lee JJ, Park YS, & Lee KJ (2015) Hydrogen-deuterium exchange mass spectrometry for determining protein structural changes in drug discovery. *Archives of pharmacal research*.
18. Burg JS, *et al.* (2015) Structural biology. Structural basis for chemokine recognition and activation of a viral G protein-coupled receptor. *Science* 347(6226):1113-1117.
19. Anania VG & Lill JR (2015) Proteomic tools for the characterization of cell death mechanisms in drug discovery. *Proteomics. Clinical applications* doi: 10.1002/prca.201400151..
20. Miles J & Formosa T (1992) Protein affinity chromatography with purified yeast DNA polymerase alpha detects proteins that bind to DNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America* 89(4):1276-1280.
21. Vyas N, *et al.* (2008) Nanoscale organization of hedgehog is essential for long-range signaling. *Cell* 133(7):1214-1227.
22. Czekaj T, Wu W, & Walczak B (2008) Classification of genomic data: some aspects of feature selection. *Talanta* 76(3):564-574.
23. Beig A, Agbaria R, & Dahan A (2013) Oral delivery of lipophilic drugs: the tradeoff between solubility increase and permeability decrease when using cyclodextrin-based formulations. *PloS one* 8(7):e68237.
24. Krishna G, Chen K, Lin C, & Nomeir AA (2001) Permeability of lipophilic compounds in drug discovery using in-vitro human absorption model, Caco-2. *International journal of pharmaceutics* 222(1):77-89.
25. Wadhwa J, Nair A, & Kumria R (2012) Emulsion forming drug delivery system for lipophilic drugs. *Acta poloniae pharmaceutica* 69(2):179-191.
26. Jeppsson R & Ljungberg S (1975) Anticonvulsant activity in mice of diazepam in an emulsion formulation of intravenous administration. *Acta pharmacologica et toxicologica* 36(4):312-320.
27. Rivera B (2015) The Current Status of Nurse-Administered Propofol Sedation in Endoscopy: An Evidence-Based Practice Nurse Fellowship Project. *Gastroenterology nursing : the official journal of the Society of Gastroenterology Nurses and Associates*.
28. Bibian S, Dumont GA, & Black I (2015) Closed-loop target-controlled infusion systems: stability and performance aspects. *Military medicine* 180(3 Suppl):96-103.
29. Papp Z (1975) [Amniocentesis in prenatal genetic diagnosis]. *Kinderarztliche Praxis* 43(10):471-476.
30. Kim K, *et al.* (2015) The effect of cyclosporine A in hemorrhagic shock model of rats. *The journal of trauma and acute care surgery* 78(2):370-377.
31. Goy JJ & Deruaz JP (1992) Cyclosporin and muscle. *Lancet* 339(8787):254.
32. Badali H, Mohammadi R, Mashedi O, de Hoog GS, & Meis JF (2015) In vitro susceptibility patterns of clinically important Trichophyton and Epidermophyton species against nine antifungal drugs. *Mycoses*.
33. Morales BP, *et al.* (2015) In vitro susceptibility testing of amphotericin B for Cryptococcus neoformans variety grubii AFLP1/VNI and

DOI: 10.18535/ijmsci/v3i4.3

Cryptococcus gattii AFLP6/VGII by CLSI and flow cytometry. *Mycoses*.

34. Yang FH, *et al.* (2015) Bioavailability Enhancement of Paclitaxel via a Novel Oral Drug Delivery System: Paclitaxel-Loaded Glycyrrhizic Acid Micelles. *Molecules* 20(3):4337-4356.

Fig 1



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