Chemical genetics to chemical genomics: Utilizing small molecules

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Chemical Genetics

Study of biological systems using small molecule (chemical) intervention, instead of only genetic intervention. Such techniques useful for:

• Cell permeability and higher selectivity

• Perturbing protein function rapidly, reversibly, and conditionally

• Temporal and quantitative control
Forward/Reverse Genetics/Chemical Genetics

**Forward Genetics**
- phenotype $\rightarrow$ gene
- normal bacterial cell
- (i) random mutations
- (ii) select phenotype
- different cell morphology
- discover gene responsible for desired phenotype
- DNA

**Forward Chemical Genetics**
- phenotype $\rightarrow$ protein
- normal bacterial cell
- (i) screen small molecules
- (ii) select phenotype
- different cell morphology
- discover protein responsible for desired phenotype
- protein $\rightarrow$ Small molecule

**Reverse Genetics**
- gene $\rightarrow$ phenotype
- normal DNA
- delete specific gene
- normal DNA
- observe phenotype
- different cell morphology

**Reverse Chemical Genetics**
- protein $\rightarrow$ phenotype
- normal protein
- discover small molecule partner
- protein $\rightarrow$ Small molecule
- observe phenotype
- different cell morphology

Scheme 1 Comparing genetics with chemical genetics.
Challenges of Chemical Genomics

• Estimated 10x as many proteins than genes in humans, since genes can code for a multiple number of proteins.

• Most proteins have more than one function, hence a protein may require a different small molecule target for each function.
Advantages of small molecules

- Most act reversibly. Difficult to construct conditional alleles (i.e., temperature sensitive mutations) or diminish pleiotropic effects.

- Conditional, quantitative, and temporal control, whereas gene knockout often produce steady-state effects or none of the expected effects due to redundancy.

- Cell permeability and higher selectivity, unlike antisense oligonucleotides and siRNA’s, which also neglect post-translational modification and individual protein domain/sub-domain functions.
Disadvantages of small molecules

No general applications to discover cellular targets or mechanisms of any one small molecule!

- Selective small molecule ligands have highly specific protein binding partners
- Only a fraction of proteins have an identified ligand partner.

Must increase throughput of protein binding screens with a collection of small molecules to find viable protein-ligand pairs and correlate with phenotypic results to eventually make chemical genetics as generally applicable as genetics.
Searching for protein-ligand compounds

- Enzymes and receptors typically have small molecule partners that are easier to identify.

- Natural products (cyclosporine, rapamycin) are typically highly selective and complex. However, most useful natural products come as mixtures that must be purified. They are also so complex that it is difficult to chemically synthesize (DOS: Diversity-Oriented Synthesis is an initiative to provide detailed information on chemically diverse and structurally complex molecules).
Pre-selecting a target protein in protein binding screens

Scheme 2  Small molecule microarrays. Different small molecules can be covalently attached to glass slides and probed with fluorescently labelled proteins requiring a small molecule partner. After the slides are washed, to remove non-specific interactions, they can be scanned for spots of fluorescence, indicating a protein–small molecule interaction.
Small molecule target for Ure2p protein

Fig. 1  Uretupamine was discovered from a small molecule microarray as a ligand to the protein Ure2p; the primary alcohol was attached covalently to the glass surface.
Scheme 3  Biochemical target protein identification. The target protein becomes covalently labelled (tagged) by using labelled small molecules that attach themselves covalently to their protein partner. Covalent crosslinking is involved in the normal mechanism of some small molecules such as penicillin. Other small molecules can be derivatised with chemical crosslinking reagents that have the ability to unmask reactive function groups such as a nitrene, which are capable of sigma bond metathesis with nearby bonds.
Eliminating known targets or unwanted actions

• Screen small molecules in organisms to induce fatality then prioritize those “hits” with a variety of mode of action assays to eliminate unwanted modes of action.

• For example, most inhibitors of DHFR are not selective enough and are probably toxic to most mammalian cells.
Applying Genetics

• Random mutations in a gene product to resist effects of small molecules. Mutants are screened with different small molecules and the mutant that offers resistance is identified.

• A combination of non-lethal gene deletions can also be identified that when present together can produce an inviable phenotype, whereas individual gene deletions lead to viable mutants (synthetic lethal screen).
Matching synthetic lethal profiles

Scheme 4 Synthetic lethal screens. Genetic interactions can be similar to chemical genetic interactions. In the synthetic lethal interaction (left), individual deletions of genes (represented by the black X) lead to viable mutants (alive), but double mutants are not viable (dead). In the chemical genetic interaction (right) the deletion mutant is hypersensitive to a normally sub-lethal treatment of the small molecule. A gene deletion that is lethal when cells are treated with the small molecule should also be lethal with a mutation in the compound’s target gene. Therefore, comparing the matrix of synthetic lethal interactions of all non-essential genes with the profile from the small molecule treated cells should identify the pathways and targets that the small molecule is modulating.
Signaling proteins

• Protein kinases typically have well conserved active sites. Mutating active sites creates greater selectivity for small molecules.

• Temperature sensitive genetic studies Cdc28 kinase suggested that primary function was to control G1 to S phase transition in the cell cycle. Chemical genetics revealed that inhibitor-sensitive Cdc28 mutant arrested at G2/M transition.
To induce mitotic arrest, monastrol was synthesized, which led to the reorganization of the mitotic spindle. Monastrol inhibits an Eg5 kinesin protein which diminishes microtubule motility. The interaction is highly reversible and specific, making this small molecule particularly useful for studying Eg5.
Studying zebrafish embryos:

The 31N3 protein inhibits otolith development, which move in response to gravity and allow the fish to maintain balance. 31N3 allowed identification of critical time point of otolith development via temporal and quantitative control.
Sonic hedgehog pathway

Sonic hedgehog pathway implicated in many developmental processes including midline facial structures and limbs. Antagonists of the SHH pathway include:

Cyclopamime

Cyclopaia
Stem Cells

Small molecule can reverse the differentiated state of muscle cells back into stem cells which can then be re-differentiated into different types of cells.
Protein-Protein Interactions

• Enzyme/receptor proteins are generally the easiest to modulate with small molecules. Challenges arise from the fact that:

  – Proteins generally have more than one protein-binding surface.
  – Proteins may lack small molecule binding sites since they are often flat and too large (800 Å²).

However, there are often “hot spots” on the binding surfaces that account for most of the binding energy, which makes chemical genomics more practical for such applications.
More examples

• Serves as a molecular antagonist of the oncogenic T cell factor (Tcf/β-caretin protein complex (possible cancer therapy)

• Inhibition of LFA-1 and ICAM-1 binding interaction has potential to inhibit both the immune and inflammatory response.
Gene transcription

Small molecules can also disrupt transcription and translation factors. For example, small molecules can be used for activating ribosome activity or changing RNA secondary structure. These domains are known as “riboswitches”
Conclusions

Chemical genetics is useful for many properties, including cell permeability, high selectivity, rapid perturbation of protein function, reversibility, and condition temporal and quantitative control. These techniques, when combined with genetic tools, will yield a more complete understanding of biological processes, diseases, and future drug treatments. More studies and higher throughput is required to before chemical genetics is as applicable and generalizable as modern day genetics.