Drug targets – on overview

Lecture 5. 1st Year Graduate students.
2016
Drug target classes
(classification may vary)

Proteins
- G protein-coupled receptors (target of 50% of drugs)
- enzymes
  (especially protein kinases, proteases, esterases, and phosphatases)
- ion channels
- nuclear hormone receptors
- structural proteins such as tubulin
- epigenetic targets (enzymes)

Nucleic acids
- DNA coiling (topoisomerasers)
- intercalating drugs and alkylators
Picture of the day
Interesting (10-year old) statistics

<table>
<thead>
<tr>
<th>Molecular targets of FDA-approved drugs</th>
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</thead>
<tbody>
<tr>
<td>Class of drug target</td>
</tr>
<tr>
<td>Targets of approved drugs</td>
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<tr>
<td>Human genome targets of approved drugs</td>
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<tr>
<td>Targets of approved small-molecule drugs</td>
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<tr>
<td>Targets of approved therapeutic antibodies</td>
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<tr>
<td>Targets of approved biologicals</td>
</tr>
</tbody>
</table>

From Overington et al. How many drug targets are there? Nat Rev Drug Discov 2006, 5, 993-996 (posted in A MUST)
In the ‘Overington report’…

>21,000 drug products considered

↓

1,375 unique drugs

(after removal of duplicate AIs, salt forms, supplements, vitamins, imaging agents)

↓

1,375 = 1,204 small-molecule drugs* + 166 ‘biological drugs’

↓

1,204 ‘small molecules’ = 803 administered p.o.,** 421 administered i.p., 275 topical

* 192 drugs are pro-drugs (16%)

* 885 small molecules are Ro5-compliant (only 619 are dosed p.o.)

** 159 of orally dosed drugs fail at least one Ro5
Two other useful cartoons

Gene-family distribution of current drugs per drug substance.
GPCRs

Aka:
• seven-transmembrane domain receptors
• 7TM receptors
• heptahelical receptors
• serpentine receptors

The binding ligands:
• light-sensitive compounds
• odors
• pheromones
• hormones
• neurotransmitters

(from small molecules to peptides to large proteins)
The 2012 Nobel Prize in Chemistry was awarded to Brian Kobilka and Robert Lefkowitz for their work that was "crucial for understanding how G protein–coupled receptors function".

There are two principal signal transduction pathways involving the G protein–coupled receptors:

- the cAMP signal pathway and
- the phosphatidylinositol (PI) signal pathway
cAMP pathway

- too complex to go into
- a universal pathway to activate intracellular processes by binding of an extracellular ligand
- many BIOASSAYS to measure small-molecule (de)activation of GPCRs are based on measuring ↑ levels of cAMP
Phosphatidylinositol (PI) pathway

Phosphorylation-dephosphorylation of the inositol unit is the major signal transduction event

PIP₂
phosphatidylinositol-4,5-bisphosphate

Cleavage by
Phospholipase C

PIP₂
phosphatidylinositol-4,5-bisphosphate

IP₃
inositol-1,4,5-trisphosphate

“second messengers”
Ca++ flux

many BIOASSAYS to measure small-molecule (de)activation of GPCRs are based on measuring ↑ levels of intracellular Ca++ ("Ca flux assays")
FLIPR screening platform

FLIPR® (fluorometric imaging plate reader)

- uses Ca++ sensitive dye (change in fluorescence)
- extremely high-throughput
What does a chemist see?

- Activation by saturating endogenous ligand
- Constitutive activity of receptor in absence of ligand

![Graph showing different types of agonists: Full agonist, Partial agonist, Neutral antagonist, Inverse agonist]
Varenicline (Chantix®)

Cytisine
(from Cytisus plant = ракитник)

Nicotine

Chantix

Bupropion

Graph:
- Full agonist
- Partial agonist
- Neutral antagonist

Response vs [Drug] graph with concentrations from $10^{-10}$ to $10^{-6}$.
Chantix horrors

Alarm I -- In November 2007, the US FDA announced it had received post-marketing reports of thoughts of suicide and occasional suicidal behavior, erratic behavior, and drowsiness among people using varenicline for smoking cessation. → BLACKBOX WARNING
(A 2014 systematic review did not find evidence of an increased suicide risk.)

Alarm II – May 22, 2008 – the use of Chantix was prohibited to pilots and traffic controllers (New York Times)

Alarm III – Feb 4 2009 – Canadian Ministry of Health reported 800 complaints from Chantix users about mood swings, aggression, suicidal thoughts.
Protein Kinases – examples of enzymes

Ser, Thr – only those
+ Tyr – dual-specificity kinases
+ a relatively rare class of His kinases.

Chemical structures:
- Proteins
- ATP
- ADP
- Various amino acids and chemical groups.
As with any other enzymes...

- orthosteric site – where the native substrate binds
- ATP-binding site (where most inhibitors work)
- allosteric site – any other binding pocket discovered on the protein, binding at which inhibits the enzymatic activity

\[ E + S \rightleftharpoons ES \rightleftharpoons ES^* \rightleftharpoons EP \rightleftharpoons E + P \]

\[ v_0 = \frac{V_{\text{max}} [S]}{K_M + [S]} \]

*(Michaelis–Menten equation)*

*(Chapter on Enzyme Kinetics potested in A MUST)*
ION CHANNELS

Main points to remember:

- what is an ion channel
- IC can be made of subunits
- IC gating
- types of gating control
- case Study: capsaicin and TRPV1 ion channel
What is an ion channel?

- Ions CANNOT diffuse through the cell membrane lipid bilayer

- Ion channels provide a polar environment (a pore) that facilitates the flux of ions across the lipid membrane
What ions?

<table>
<thead>
<tr>
<th>Cation Permeable</th>
<th>Anion Permeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>Cl⁻</td>
</tr>
<tr>
<td>K⁺</td>
<td></td>
</tr>
<tr>
<td>Ca⁺⁺</td>
<td></td>
</tr>
<tr>
<td>Na⁺, Ca⁺⁺, K⁺</td>
<td></td>
</tr>
</tbody>
</table>
Numerous transport functions:

- Transmit electrical signals in CNS
- Control the release of neurotransmitters and hormones
- Initiate muscle contraction
- Transfer small molecules between the cells
- Fluid transport in secretory cells
- Control cell motility (new/growing cells)

Ion channels ARE an important class of targets!
Ion Channels are made of subunits

Several individual proteins that are expressed within the cell and migrate toward and combine within the membrane to form functional ION CHANNEL.
IC can be closed or open

Extracellular side

Cytoplasmic side
Ion channel gating – what is it?

- regulation “OPEN/CLOSED” by external factors is called GATING
- there are FIVE types of GATING
1. Ligand-gated ion channels
2. Phosphorylation-gated IC

Phosphorylate

Dephosphorylate

G protein–coupled receptor

Transmitter

G protein

Second-messenger cascade
3. Voltage-gated Ion Channel
4. Mechanical force gated ion channel
5. Temperature-gated ion channels

Current

Temperature (° C.)

Cold-Sensitive

Heat-Sensitive
Case study: Capsaicin – the TRPV1 ion channel activator

TRPV1 = transient receptor potential cation channel subfamily V member 1 also known as capsaicin receptor
Capsaicin and its analogs

<table>
<thead>
<tr>
<th>Capsaicinoid name</th>
<th>Abbrev.</th>
<th>Typical relative amount</th>
<th>Scoville heat units</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>C</td>
<td>69%</td>
<td>16,000,000</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Dihydrocapsaicin</td>
<td>DHC</td>
<td>22%</td>
<td>15,000,000</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Nordihydrocapsaicin</td>
<td>NDHC</td>
<td>7%</td>
<td>9,100,000</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Homodihydrocapsaicin</td>
<td>HDHC</td>
<td>1%</td>
<td>8,600,000</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Homocapsaicin</td>
<td>HC</td>
<td>1%</td>
<td>8,600,000</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Nonivamide</td>
<td>PAVA</td>
<td></td>
<td>9,200,000</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
</tbody>
</table>
Scoville scale

- A scale to measure of ‘heat’ of chilly peppers
- Done by Scoville Organoleptic Test
- e.g. Carolina Reaper – the Guinness World Record hottest pepper

Carolina Reaper 1,569,300 SHU (2013)
Bhut jolokia 1,041,427 SHU

= +

Red Habanero 100,000–350,000 SHU

- Upon prolonged exposure to capsaicin, TRPV1 activity decreases, a phenomenon called desensitization.
- Desensitization of TRPV1 is thought to underlie the paradoxical analgesic effect of capsaicin.
TRPV1 as a drug target

- primary area – nociception (i.e. development of new analgesics)

**Antagonists**

- Block TRPV1 → suppress nociception
- Effective against neuropathic pain (amputation, multiple sclerosis, cancer)
- Main obstacle → hyperthermia (TRPV1 regulates body temperature)

![AMG-517](image)

AMG-517 → clinical trials stopped due to hyperthermia in patients

**Agonists**

- work mostly through desensitization mechanism
- numerous ointments (0.025 - 0.075% capsaicin) are available over the counter (OTC)
- up to 10% capsaicin patches are in clinical trials

![Resiniferatoxin](image)

Resiniferatoxin 16,000,000,000 SHU

- TRPV1 in CNS is explored as target for treatment of anxiety and long-term depression
Nuclear hormone receptors

- located in the nucleus
- responsible for sensing steroid and thyroid hormones
- regulate gene expression
- sometimes considered transcription factors

![Diagram of gene transcription process](image.png)
Most common NHR ligands

- estrogens
- progesterone
- androgens
- corticosteroids
- thyroid hormones
- vitamin D
- retinoids

Estradiol

Testosterone

Cortisol (glucocorticoid)

Aldosterone (mineralocorticoid)

Vitamin D3

Progesterone

(S)-triiodothyronine (T3, also called liothyronine in Pharma)

Retinol
What is this?
Tubulin

- Proteins (α and β) that polymerize into microtubules that form cytoskeleton.
- Microtubules are essential for cell division.
- Therefore, drugs that bind to tubulin and block cell division can be applied in proliferative disorders such as cancer.
- Most of the tubulin binding drugs stabilize microtubule and block mitosis.
Examples of microtubule binders

Natural

- Taxol
- Discodermolide
- Epothilone A
- Dictyostatin
- Colchicine

Domontet et al. Nat Rev Drug Discov 2010, 9, 790 (posted in A MUST)
Acetylation of histones – epigenetic regulator of gene expression

- Any deviation from the ‘NORMAL’ state of histone acetylation can lead to pathology
- HDACs (~20 isoforms) are primary drug targets
- Areas: psychiatry and neurology, cancer, parasitic and inflammatory disease
Classes of HDAC inhibitors

**Hydroxamic acids**

- **Vorinostat (SAHA)**
  - Zolinza® for cutaneous T-cell lymphoma

**Benzamides**

- **Entinostat (SNDX-275)**
  - FDA was working on PIII design in cancer in Sept 2013

**Aliphatic carboxylic acids**

- **Na phenylbutyrate**
  - in research for cancer and cystic fibrosis

**Valproic acid**

- **Depakote® for treatment of bipolar disorder, epilepsy, migraine**

  + eletrophilic ketones
  + cyclic tetrapeptides
  + depsipeptides
Other epigenetic events targeted by drugs

- Cytosine methylation and hydroxymethylation (post-translational DNA modification)
- Histone Lys or Arg methylation (histone methyl transferases)
- Threonine phosphorylation (post-translational DNA modification)
- Lys ubiquitylation*
- Lys sumoylation**

* ~8.5 kDa regulatory protein found in all eukaryotes
** SUMO protein (small ubiquitin-like modifier protein)
DNA topoisomerase as drug target

Inhibition of topoisomerases halts the replication and can be used to treat cancer

https://www.youtube.com/watch?v=EYGreIVyHnU

Double helix supercoiling following the replication fork

Topoisomerases release the supercoiling

(A) Type I

(B) Type II
Known topoisomerase I inhibitors

Camptothecin

Lamellarin D

Topotecan (Hycamtin®)

Cyprofloxacin
(bacterial topo inhibitor, antibiotic)
Known topoisomerase II inhibitors

- Etoposide (Etopophos®) Approved in 1983
- Teniposide (Vumon®)
- Podophyllotoxin

Mark Cushman (Purdue U) & Yvves Pommier (NCI):

\[
\text{MeO} \quad R \quad \text{N} \quad \text{MeO} \quad \text{SOCl}_2
\]

\[
\begin{align*}
\text{MeO} &+ \tedone[1,2-c]\text{isoquinolines} \quad \text{ready for phase II (!!!)} \\
\end{align*}
\]

The Castagnoli-Cushman reaction
DNA intercalating and alkylating drugs

- both classes of drugs stop DNA replication by binding to actively replicating DNA in cancer cells

**Intercalation:**

**Alkylation:**

\[
\begin{align*}
\text{Intercalation:} & \\
\text{Alkylation:} & \\
\end{align*}
\]
Examples of DNA intercalators

(in complexes with transition metals)

Ethidium bromide
Proflavine
Daunorubicin or daunomycin (Cerubidine®)
DNA alkylators in clinical use

Busulfan
(in clinic since 1959)

Melphalan

Chlorambucil
(Leukeran® by GlaxoSmithKline)
QUESTIONS?