Lecture 4: Drug targets (receptors, enzymes, antigens, DNA, tubulin)

Key objectives:
1. Be able to describe several receptor type drug targets.
2. Be able to describe several enzyme type drug targets.
3. Understand antigen type drug targets.
4. Understand the nature of DNA type drug targets.
5. Understand replication targets such as tubulin

In the discipline of oncology, anything can be viewed as a target to kill or inhibit cancer cells. Supposed joke at FDA: "In God We Trust, but everyone else bring data!"

Receptors: Most commonly, receptors are proteins on the surface of cells that cause a signal following the binding of a specific ligand. Binding of the ligand can increase in the transport of specific ions (e.g. sodium, chloride, calcium, potassium) or the amplification of a signaling cascade (e.g. kinase cascade).

Enzymes: Enzymes are proteins on the surface or inside of cells (more often inside) that perform specific metabolic transformations to convert substrates into products. The products formed possess different chemical properties than those of the substrates. Note: Receptors and enzymes can work together to create signals.

DNA: Deoxyribonucleic acid, which (as the component bases) make up the chromosomes within the nuclei of mammalian cells. All the information required for normal cellular homeostasis is encoded the DNA.

Antigens: Antigens are proteins on the surface of cells; the protein might have a known function (or not) but the important thing is that it is expressed only (or mainly) on a specific cell type to allow targeting of a drug (usually an antibody). Almost all the effects of drugs turn off or decrease a cellular signal. This is because it is more difficult to increase a signal than inhibit it. Gene therapy (e.g. gene insertion into DNA) can increase a signal, but this technology is much more complicated to implement. It remains an active research area.

Ia. Extracellular receptors as targets

Acetylcholine (Ach; Figure 1) binds to cholinergic receptors and is important as a neurotransmitter in the brain of animals. It is also important in the stimulation of skeletal muscles for physical movement and in autonomic stimulation. There are two major types of acetylcholine receptors which are classified as muscarinic (mACHR) or nicotinic (nACHR) due to the ability of these two compounds to act as selective agonists for the receptor in question (Figure 2).

These two major classes are further divided into subclasses so the actual situation is complicated. We note right away that muscarine is unusual as it has a quaternary
nitrogen (always positively charged just like acetylcholine itself) so we wouldn’t expect it to cross membranes particularly well. Nicotine has two basic ionizable groups (pyridine pKa around 5) and an alkylamine (pKa around 9). Both compounds can exist as stereoisomers but only single enantiomers are found in nature.

![Figure 1. The structure of acetylcholine](image)

![Figure 2. Structures of muscarine and nicotine](image)

![Figure 3. Muscarinic and nicotinic receptors embedded in a membrane.](image)

There are 5 types of muscarinic receptors (Figure 3) and all are G-coupled receptors. M1, M3 and M5 control intracellular free calcium concentrations via the phosphoinositol pathway. Binding to M2 and M4 inhibits the formation of the second messenger of the adrenergic system cAMP. The quantity and distribution of these receptor subtypes depends on the exact nerve under discussion.
Acetylcholinesterase (AChE) hydrolyses ACh (Figure 4). So inhibition of AChE causes increased levels of ACh and this causes agonist effects. Receptor selectivity can be enhanced by selective distribution of the inhibitors (e.g. the blood brain barrier). Also, drug companies have developed drugs that are selective agonists and antagonists of the major classes of acetylcholine receptors.

![Figure 4. Hydrolysis of ACh to acetic acid and choline](image)

Extensive structure activity relationship (SAR) studies have been carried out for cholinergic agonists and antagonists. The main goal of these kinds of studies is to develop ligands that differentiate between the various types of receptors. Below we see the results of a study that sought to differentiate between the five mAChR receptor subtypes by developing selective antagonists for each subtype. Results are shown for the most selective inhibitor (9i; left values, right displacement curves) in the displacement of a radioactive ligand from each individual receptor subtype (Figure 5). Overall selectivity for M1 is significantly improved over the other receptor subtypes and over atropine.
Table 1. IC50 values for various analogs and muscarinic receptors M1-M5

Many analogs that were tested in this experiment against the receptor subtypes to arrive at the structure of 9i. In these cases, size and lipophilicity of a single R group were varied often with dramatic and surprising effect on binding affinity.

Table 2. $K_i$ determinations and binding fold selectivity for 9i

<table>
<thead>
<tr>
<th>mACHR</th>
<th>$K_i$ (nM)</th>
<th>Fold selectivity (vs M1)</th>
<th>Atropine $K_i$ (nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>12.7 ± 1.7</td>
<td>27</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>M2</td>
<td>338.0 ± 13.5</td>
<td>6</td>
<td>2.69 ± 0.20</td>
</tr>
<tr>
<td>M3</td>
<td>74.8 ± 4.3</td>
<td>6</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>M4</td>
<td>445.1 ± 23.8</td>
<td>35</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>M5</td>
<td>85.7 ± 15.9</td>
<td>7</td>
<td>1.80 ± 0.11</td>
</tr>
</tbody>
</table>

$^a$ $K_i$ am an average of three independent experiments using rat mACHR (CHO) cell lines.

Figure 5. Determination of selectivity of compound 9i for different muscarinic receptors
<table>
<thead>
<tr>
<th>Type of Action</th>
<th>Receptor Type</th>
<th>Compounds Optimized for Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist</td>
<td>Nicotinic (nAChR) Na/Ca channel</td>
<td>Chantix (α4β2 subtype) Smoking cessation Issues with bizarre behavior</td>
</tr>
<tr>
<td></td>
<td>Muscarinic (mAChR) G-coupled receptor</td>
<td>Methanocholine Diagnostic for bronchial hypersensitivity in asthma (S) enantiomer more potent</td>
</tr>
<tr>
<td>Antagonist</td>
<td>Succinyl Choline Depolarizing neuromuscular blocking agent</td>
<td>Atropine Classic antagonist (S)-enantiomer 120 times more potent</td>
</tr>
</tbody>
</table>

**Figure 6.** Receptor subtypes and compounds optimized for activity

Similar experiments have allowed the identification of specific compound for each receptor subtype (Figure 6).

**lb. Intracellular receptors as drug targets**
Steroids readily enter cells and bind to receptors inside the cells (in the cell cytosol). They exert their effects by subsequent translocation of the receptor complexes into the nucleus (Figure 7) and binding to regulatory regions of DNA called response elements (GRE, MRE, ERE, ARE). Most often, this binding activates transcription of specific mRNA products and these are exported to the cytosol and translated into proteins. However, the binding to a response element can also inhibit transcription. For instance RU-486 is an antagonist of the progesterone response system and has no effect on the mineralocorticoid response system.

Typically steroid antagonist-receptor complexes can also enter the nucleus, but they do not bind the response elements productively and are not active. So steroid type drugs may be either receptor agonists or antagonists.

All steroids are biosynthesized starting from cholesterol (Figure 8). Note the differences between the progestins, glucocorticoids, mineralocorticoids, androgens, and estrogens. Also note the large number of chiral centers in the steroids and the different functional groups. These differences give the different steroids the ability to bind selectively to their specific receptors and cause their specific effects. Perhaps the best illustration of
the differences between steroids is between the androgens (male hormones) and estrogens (female hormones), which give rise to male and female sex characteristics.

Later in this course, we will discuss the importance of steroids and steroid antagonists in lectures that focus on anti-hormonal oncology agents.

**Figure 8.** Biosynthetic scheme for the production of the steroid hormones