Lecture 5: Drug targets (continued)

IIa. Enzymes as drug targets (HMG-CoA reductase example)

Many drugs are inhibitors of enzymes that catalyze biologically important reactions.
The conversion of HMG-CoA to mevalonic acid (Figure 9) is a good example because the production of mevalonic acid is the rate limiting step for production of cholesterol in mammals. This biochemistry knowledge was exploited in the development of the statin drugs for the treatment of high cholesterol.

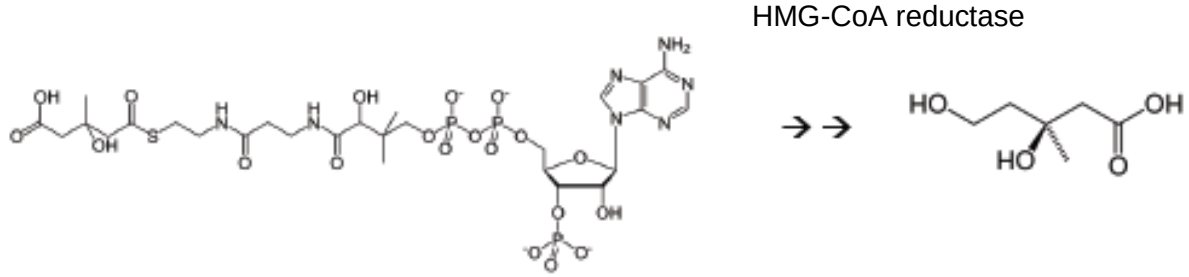

Figure 9. The conversion of HMG-CoA to mevalonic acid by HMG-CoA reductase

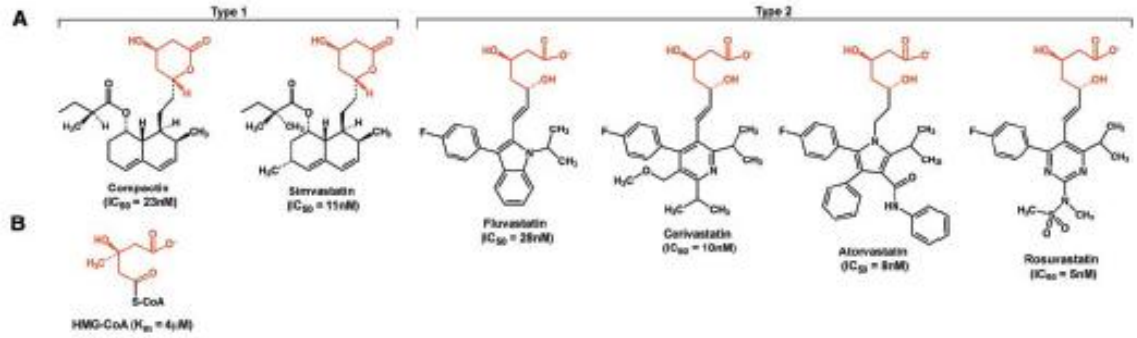

Figure 10. The structures of several statin drugs

The statin drugs (Figure 10) are reversible, tight binding (IC50's are low nM), competitive inhibitors of HMG-CoA reductase. This enzyme is present in the cytosol of many cells (especially high in liver cells) and the drugs must enter the cells to do their job. The drugs bind the enzyme active site and prevent the binding of HMG-CoA. The statins bind only a portion of the active site and the CoA portion of HMG-CoA lies outside of the binding area for the statins (Figure 11).

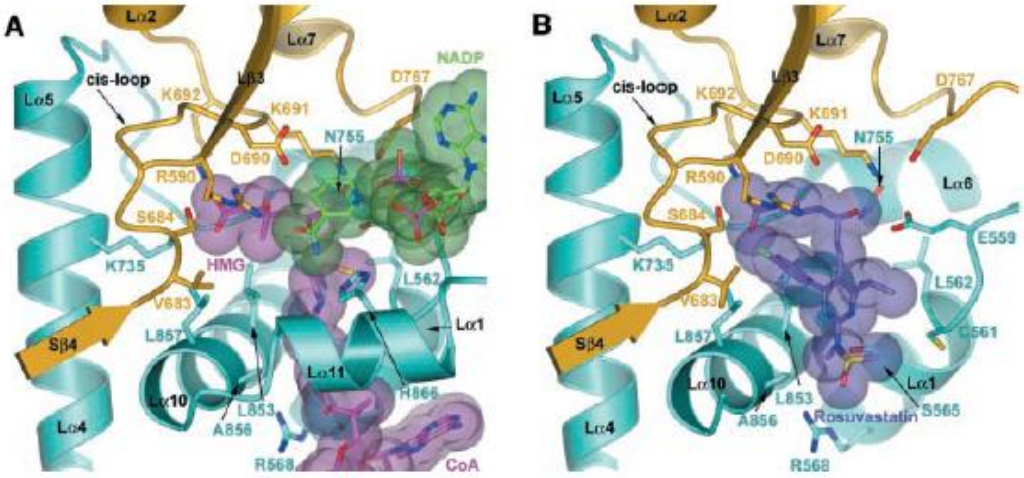

Figure 11. Example of HMG-CoA reductase and the statin binding in the active site

Note in the structure on the left (Figure 11) that the cofactor NADPH is also shown (in green) at top right. The statins have a rigid core and bind to the enzyme in a similar configuration that expands the active site. Successful drugs of the Type 2 class contain the para-fluoro phenyl group which associates with an arginine side chain (R-590). This ring must bind orthogonal to the rigid core.

The ring-opened form that mimics the natural substrate (Type II statins) is required for binding to the enzyme. Simvastatin (a prodrug) must undergo hydrolysis to be active. Note that all of the inhibitors do not have a methyl group in the 3 position. The systemic bioavailability of the statins is generally poor (5-50%). The affinity of the statins for the enzyme active site are actually much better than for HMG-CoA (the endogenous substrate) itself.

Generally, enzyme inhibitors are structurally similar to the endogenous substrates. However, in some cases only a portion of the substrate structure is found in the inhibitor.

Most inhibitors of enzymes are competitive inhibitors that bind <u>reversibly</u> to the enzyme and compete with the natural substrate for the enzyme active site. Major goals of drug design are to design compounds that have increased affinity (lower IC50) of the drug for the enzyme and that have minimal off-target effects.

Typically, we will see many drugs that are designed to do the same thing and we can understand a class of compounds based on structure activity relationships (SAR). Other inhibitors react covalently with a critical amino acid residue and inhibit the enzyme <u>irreversibly</u>. Here the reversible affinity is less important as the substrate cannot be processed by the inhibited enzyme because it is permanently inhibited.

IIb. Enzymes as drug targets (kinase example)

The importance of kinase enzymes to intracellular signaling cannot be overstated. This is especially true in the case of several tyrosine kinases, to which specific inhibitors have been developed as anti-cancer agents. In brief, kinase enzymes work by catalyzing the attachment of a phosphate group (phosphorylation) at a tyrosine residue according to the equation below:

Kinase: Enzyme-Tyr-OH + ATP → Enzyme-Tyr-O-PO_3^{-2} + ADP

We will discuss several kinase enzymes and their inhibitors later in this course. For now, it is important to appreciate that kinase enzymes are commonly part of a kinase cascade signaling process that leads to signal amplification (Figure 12). Each kinase enzyme can be activated by phosphorylation and go on to phosphorylate (activate) several other kinase enzymes. In this way, the signal generated by a single ligand-receptor complex can generate an amplified signal very quickly. As you might expect, there is a rapid mechanism for turning off this signal as well. This turning off process is accomplished by phosphatase enzymes, which remove the phosphate groups. More discussion of kinases and phosphates will come later.

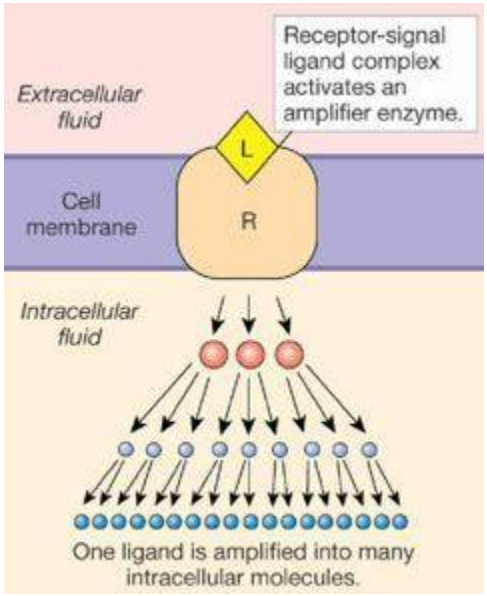

Figure 12. Depiction of signal amplification that starts at the cell membrane but is amplified by different kinase enzymes

III. Antigens as drug targets

All cells have proteins expressed on their surfaces. In some cases, the presence (abundance) of these proteins is over-expressed in certain cell types. In the case of cancer therapy, it can be very advantageous if certain cancer cells have a protein over-expressed on their surface. This allows for the protein (and therefore the cell itself) to be targeted more selectively by an antibody type of drug. When a protein is identified as a potential target for developing a drug, we call the protein an antigen.

In some cases, we understand the roles for these proteins (antigens), but for others will still do not. One situation we understand quite clearly is the human endothelial receptor 2 (HER2), which is also called the endothelial growth factor receptor 2 (EGFR2). Actually, it is an important receptor for stimulating growth in cells, both in normal but also in certain cancer cells. However, it is actually the <u>over-expression</u> of HER2 that is important in approximately 30% of breast

cancer cases (Figure 13). This over-expression provides a useful target for treating this type of breast cancer. But the fact that normal cells also possess some of the protein (antigen), this can lead to side effects.
Anyway, the antibody drug that has been developed to HER2 is called Herceptin (trastuzumab). It is worth noting that normal heart tissue also possesses a fair number of HER2 antigens. As a consequence, cardiotoxicity can occur with use of Herceptin. This toxicity is usually manageable and reversible if identified early. Oncologists simply have cardiac function tests done on patients undergoing therapy with Herceptin and if a drop in cardiac function is observed, treatment is simply stopped for a month or two and then restarted.

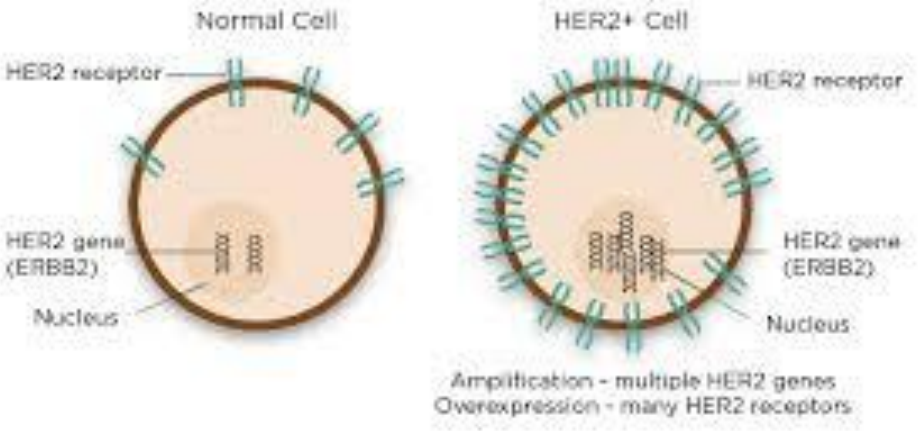

Figure 13. Expression of HER2 on a normal cell and an over-expressing cancer cell

Useful antigens in several types of leukemias and lymphomas have also been identified and antibody drugs have been developed to treat them. Much more discussion on these antibody drugs will occur in later lectures.

IV. DNA as drug targets

Another critical target for treating many types of cancer is cellular DNA which resides inside the nucleus of cells (Figure 14). Because all replicating cells must generate more DNA to keep the process of cell replication rolling, DNA is a very good target for killing or at least slowing the growth of cancer cells. As usual, the challenge is managing the attending toxicities because normal cells require replicating DNA as well. And DNA is very electron rich, so this has allowed for the development of agents that attack DNA. These agents attack DNA because they are electron poor. Again, there will be more discussion of this later when we take up specific agents.

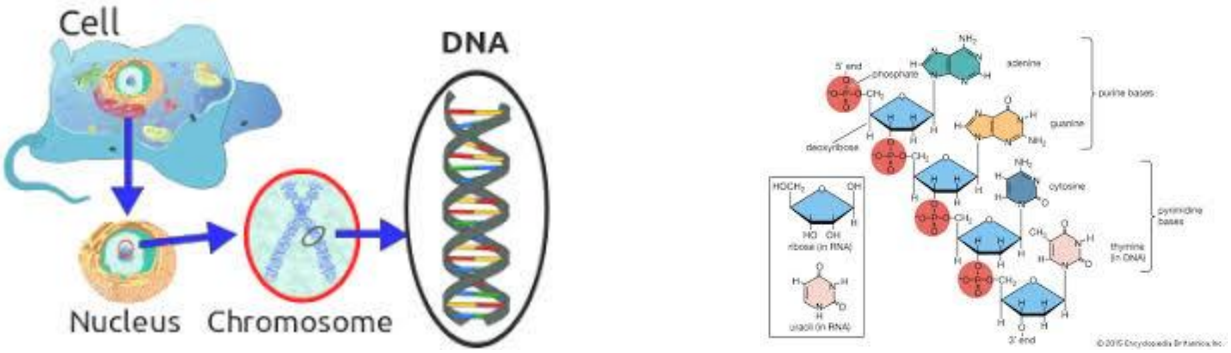

Figure 14. Location of DNA inside of a cell and the individual base pairs of DNA

The oldest type of cancer chemotherapy involves the attack and damage of DNA by chemical agents. We will discuss this soon. As you might expect, achieving specific damage to DNA in cancer cells is challenging (impossible actually) so side effects are a major problem with these older agents. But they work and so they are still used, especially in combination with other agents.

Targeting regions of DNA by classic gene therapy involves the direct insertion of a segment of DNA into a chromosome (Figure 15). Of course, such a technique involves targeting the correct chromosome and targeting the new gene segment into the correct location into a given chromosome. The target DNA inside the nucleus must first be clipped to allow the insertion, and then the new DNA segment is re-annealed at both ends. If time allows, a bit more of this will be discussed later in the course. This is an active area of research, but so far clinical success has been limited.

Figure 15. Simplistic depiction of gene therapy to insert a segment of DNA directly into a specific chromosome; this is easy to draw but hard to do.

V. Replication machinery as drug targets

As stated above, all replicating cells must generate more DNA to maintain the process of cell replication. In addition to DNA, any structure associated with the cell replication process is potentially a good target in the oncology setting.

Microtubules (tubulin proteins) are critically important in the mechanism of cell division (mitosis). These proteins are also called “tubulin” and they are responsible for separating strands of DNA that were duplicated in the mitotic process (Figure 16). Tubulins are very dynamic proteins that need to have the capacity to elongate and then contract to pull the strands of DNA apart. Any molecule that can interfere with the dynamics of these proteins is a potential anti-cancer drug.

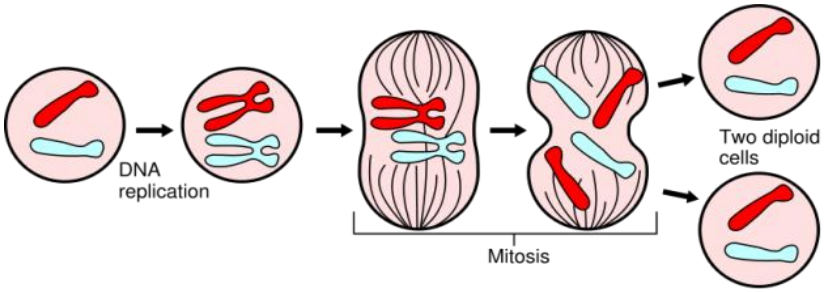

Figure 16. A simplistic depiction of DNA strand replication followed by separation by the action of the microtubules (tubulin)

Also, the process of reading a certain region of DNA (reading a gene) is called transcription. It involves a complicated process whereby the DNA helix must first be unfolded. This unfolding is catalyzed by enzymes called topoisomerases (Figure 17). More on these enzymes later.

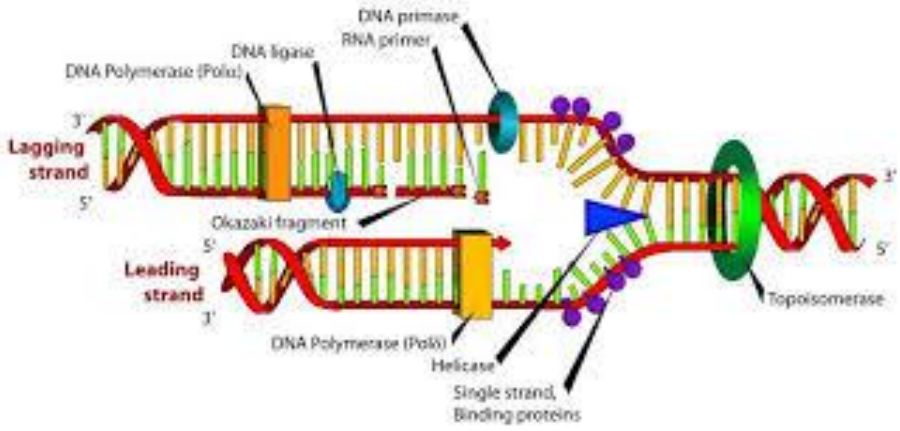

Figure 17. Depiction of a topoisomerase (green ring) knicking and allowing the unfolding of DNA so the DNA can be read properly

Finally, remember that many more potential targets exist but they remain the subject of research and clinical studies. Due to time limitations, these targets will not be discussed in this course.