DNA Damaging Agents

The preservation of DNA is crucial to the body. A simple change or mutation in one of the nucleobases in DNA can result in the development of cancer. Cancer occurs when the genetic code for replication of DNA is damaged somewhere, and thus, cells begin to divide uncontrollably, ultimately leading to the formation of a tumor. DNA damaging agents are usually small, organic molecules that react with the DNA strands and cause them to split. There are some different requirementss a molecule must fulfill in order to be a DNA damager, and these will be discussed later in the section. When DNA damaging molecules cause cancer, they are known as carcinogens. However, properly designed DNA damaging molecules are used to target cancer cells, and thus are known as chemotherapeutics during treatment. It is kind of cool that we can use something bad against itself. However, cancer chemotherapeutics have not quite advanced to the level where they can target specific cancer cells very well. Chemotherapeutic drugs attempt to target cells that divide rapidly (cancer cells), but because of their lack of perfect specificity other rapidly dividing cells all over the body can get the wrath of these DNA damaging agents, especially hair, gastrointestinal, and immune system cells.

Below is the typical pathway a carcinogen must take to create a cancerous cell.

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So, a potential carcinogen enters the system. Obviously it must interact with DNA if it is even going to have a chance of reacting with it. If it reacts and damages DNA it may result in an altered protein expression, which leads to broken replication control, which leads to cancer and then most likely tumor formation.

However, everytime a carcinogen damages your DNA it does not mean you are going to develop cancer. In fact, our DNA is damaged many times every day by UV radiation from the sun. What prevents us from developing cancer? The answer is DNA repair enzymes, and also every mutation that may get copied into a new molecule of DNA from a damaged strand does not necessarily result in cancer. A more in depth explanation of this and what ultimately does lead to cancer is written below:

Once a carcinogen has damaged DNA, several outcomes are possible. DNA repair enzymes, which are constantly checking for errors in our genetic code, can find the error and repair it, relinking the backbone in the case of strand scission and then replacing the nucleobase to match its counterpart on the complementary strand. However, if the DNA Polymerase enzyme reaches the error sight first, the error is copied into the new strand and the protein that it codes for will be changed. This change may either have no noticeable result in the protein, or it can fundamentally change the way the protein functions. If this misformed protein in turn is responsible for regulating a process as a component of mitosis, then the cell may not be able to regulate the rate of its division and can begin to replicate itself uncontrollably, cancer.

DNA Reactivity

Molecules that begin hydrophobic and then are oxidized into great electrophiles have the most potential to be carcinogenic, because electrophilicity gives a DNA nucleobase nitrogen nucleophile something to attack. However the most electrophilic molecules have no potential to be carcinogenic unless they interact with DNA. Usually these interactions entail two different pathways, intercalation and groove binding, which will both be discussed in depth shortly. Once either bound to or intercalated with DNA, carcinogens damage the DNA by either **1)** cleaving a nucleobase from the ribose-phosphate backbone or by **2)** alkylation. Carcinogenic molecules will interact with the DNA primarily at its most nucleophilic parts as seen below in the adenine example:

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Another great example of the reactive sites in DNA nucleobases is shown below, after an explanation:

There are no good sites of electrophilicity in the nucleobase of DNA. However, as evidenced by the diagram below, there are sites of nucleophilicity. These nucleophilic sites are liable to react with the electrophilic carcinogens. The main sites of nucleophilicity are the N3 and the N7 nitrogens, both of which are imine like, allowing the lone pair to be utilized in a nucleophilic attack of the carcinogen. This is due to the sp2 hybridization of the nitrogen, where the lone pair is held in the non-bonding sp2 orbital. The N3 nitrogen is used for minor groove binding and the N7 is used for major groove binding. The N1 of adenine looks like it would be nucleophilic, because it too is sp2, but since it is involved in Watson - Crick base pairing, it remains non-nucleophilic. This follows for any nucleophile involved in Watson - Crick base pairing. The N9 nitrogen is also very non-nucleophilic because its lone pair is involved in the aromatic pi system. The N on the primary amine, the 1 spot, is a medium nucleophile due to the sp3 hybridization.

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Carcinogens as Pro-drugs

Some carcinogens are a natural pro-drug, their deadly affects are not present in their natural form. These small organic molecules have a latent chemical reactivity that is activated by the human body in an attempt to process them.

First off, carcinogens tend to be small, hydrophobic molecules. Water solubility of a molecule is needed for it to be able to be excreted from the body through the urinary tract. This is where the liver functions. The liver employs an enzyme called cytochrome P450 to oxidize C=C bonds into epoxides. Epoxide hydrolase then hydrolyzes the epoxide, now rendering the molecule as hydrophilic with two hydroxyl groups. This oxidation can turn hydrophobic compounds into hydrophilic ones, as well as turning weak electrophiles into strong ones.

Below are the mechanisms employed by the liver to achieve this oxidation.

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Turning the hydrophobic compounds into hydrophilic ones causes these molecules to now be able to move around in the aqueous cellular environment. Also, by turning weak electrophiles into strong ones, the body is now making nucleophilic attacks on the molecule easier. This can cause serious problems if the molecules come into contact with the nucleophilic sites on DNA. These carcinogens cleave a nucleobase from the ribose-phosphate backbone, which in turn splits the backbone of the DNA strand.

Attaching to DNA">Attaching to DNA

There are two ways in which carcinogens bind to DNA, **intercalation** and **groove binding**.

Intercalation

Intercalation occurs with small, aromatic molecules that can insert between two sets of base pairs in the double helix of DNA. Size matching is a key factor that affects an intercalating agent's ability to squeeze itself between the phosphate backbone of DNA, and the best candidates for intercalation are often molecules that resemble the nucleobases themselves. Their aromatic profile allows them to participate in pi stacking with the aromatic systems of the nucleobases, which holds these molecules in place. This is made possible because the pi orbitals of both systems can align themselves and undergo slight electron delocalization.

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Notice the similarity in size of the DNA base pair and Ethidium Bromide, this is key for the molecule to be able to fit between the sugar phosphate backbone. Also the molecule is planar and aromatic, two requirements of a good intercalating agent.

Groove Binding

Groove binding is carried out by larger molecules which hydrogen bond to nucleobases oposite to where the base pairing occurs. Most carcinogens will bind with the minor groove in DNA as they are generally too small for the major groove. These molecules have a contour that follows the groove and have hydrogen bonding elements in the correct places to hold them in position. Groove binding molecules can effect DNA during replication by acting as a block for polymerases, thus inhibiting replication and transcription, or they can alter the DNA once bound to it.

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Altering DNA

After binding with DNA, 2 errors are likely to occur: **purine nucleobase cleavage (including abasic strand cleavage**), and **thymine alkylation**.

Epoxidation Reconsidered- Aflatoxin">Epoxidation Reconsidered- Aflatoxin

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Purine Nucleobase Cleavage

Purines readily undergo alkylation due to nucleophilic nitrogens on their nucleobase. This results in DNA strand cleavage, and can ultimately cause cancerous cells to grow depending the region in which the carcinogen reacts. Nucleophilic attacks occur by the purines on the carcinogens. The two nitrogens responsible for these attacks at the N_7 and N_3, which are located on the nucleobase.

Below are the two separate mechanisms that can occur when a nucleophilic attack occurs on an electrophilic carcinogen using a purine. Note that the N_7 guanine alkylation results in abasic site cleavage. This can continue to total strand cleavage. However, the N_3 guanine alkylation results in no reaction.

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Thymine Alkylation

When the lone pair on nitrogen collapses down, electron density is shuttled through the pi system and the carbonyl becomes a reasonably good nucleophile. Under these conditions, a Sn2 reaction lads to alkylation of the carbonyl.

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Small alkyl halides, like I-CH3, can slip into the DNA and alkylate the carbonyl. Unlike strand scission, the damage with alkylation is not imemediate and does not come until transcription.

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The alkylated thymine cannot undergo its normal base pairing with adenine and instead pairs with guanine. After transcription, the new DNA strand will have a cysteine-guanine base pair where it originally should have a thymine-adenine base pair.

Double Strand DNA Cleavage

An agent of chemical warfare along with cancer chemotherapeutics are two main functions of Nitrogen Mustard and similar gases. It is capable of causing an irreversible DNA double strand scission directly leading to apoptosis of the cell. It does this through the following mechanism:

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The nitrogen mustard lone pair attacks one of the electron depleted carbons which kicks off its chlorine leaving group, forming an electrophilic three member ring which the lone pair of a N7 DNA nucleobase readily attacks. This binds the mustard to that nucleobase.

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The nitrogen mustard lone pair now attacks the other electron depleted carbon, kicking off another chlorine, and forming another ring that is attacked by another nucleobase, this one on the opposite DNA strand from the first nucleobase. This will attach the mustard to both DNA strands.

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All of the steps that lead to strand cleavage occur and both strands are now cleaved. Now there is no way for the DNA to be repaired. It is occasionally possible for DNA repair enzymes to fix a single strand cleavage, but never double. The cell is doomed to die.

Also messing with DNA...

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Uracil is a DNA damaging agent because DNA does not recognize uracil in its replication process. Because it is not a naturally occurring base pair in DNA, a replicating strand of DNA will not copy out a new uracil. Instead, the process will be halted at that irregularity. Uracil has less fidelity than thymine. Therefore, it is acceptable to be in RNA (considerably more temporary) than DNA. DNA tags all of its thymines with a methyl group, so it can tell the 2 nucleobases apart. Cellular chemistry will transform the uracil, in the DNA, to cytosine (and not the needed thymine). This would leave an incomplete base pairing since adenine pairs with thymine or uracil, not cytosine.

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This sulfur mustard gas functions like the nitrogen mustard gas as a DNA damaging agent. The reactive epoxide intermediate (created after a lone pair from the sulfur attacks a carbon, kicking off a chlorine) is bound to the N7 of a nucleobase. Following the same mechanism as the above nitrogen gas alkylation, double stranded cleavage is inevitably the disastrous product. Sulfur mustard has been used as a chemical warfare agent, especially in World War II. It is a powerful blistering agent that can result in first or second degree burns, or even death.

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Ethidium bromide is used as a staining reagent for DNA. It works by intercalation. Interestingly, ethidium bromide (EtBr) gives rise to a visible florescence. It is this florescence that allows it to be used in identifying nucleobases in a laboratory. It is an aromatic, planar molecule, and thus is easily slipped between base pairs. The reason for ethidium bromide's fluorescent marking ability is because the hydrophobic interior between the base pairings in DNA forces the EtBr to shed any water molecules previously associated with it. Water is known to quench florescence, so a sudden absence of it results in the glowing property of the EtBr. Although this may not be counted as the most harmful of DNA agents (certainly not as bad as mustard gas), the insertion of EtBr within the DNA base pairing has the possibility of altering the natural biological processes of DNA, such as transcription and translation.

Inherent within the effectiveness of the nitrogen mustard gas is its ability to attack the carbon attached to the chloride to send it off as a leaving group. However, it should be noted that mustard gas reacts much too quickly with other physiological nucleophiles to survive long enough to attack DNA or even enter the nucleus. Cyclophosphamide (used in one leukemia cocktail treatment) is commonly used because it is less likely to react with other physiological nucleophiles due to the resonance stability of its lone pair.

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Answers

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Drill29-2.mov	Drill29-2.mp4
Drill29-3.mov	

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