Organic Chemistry in Biology and Drug Development
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Lecture - 58
Anti-cancer Drugs (Contd.)

In the last session, we are discussing about the alkylating agents- single alkylating agents, double alkylating agent. We have found that guanine is preferentially alkylating. That causes double alkylation *i.e.* inter molecular or intra molecular. Final outcome is that the transcription process or the replication process is stopped because the DNA cannot be unwind in that position.

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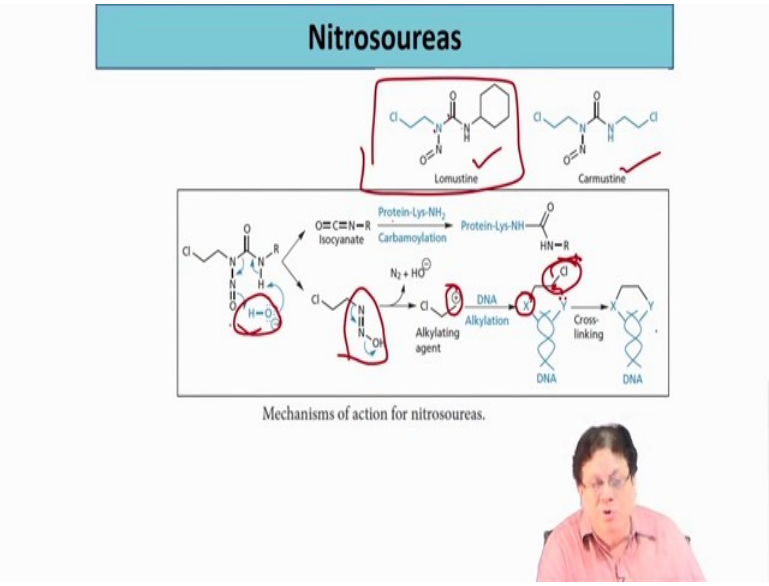

Mechanisms of action for nitrosoureas.

Either you started with the dihalo compounds or there could be aziridine compounds. Aziridines are not the unstable. It will be unstable when it is attached to positively charged aziridinium ion. There could be electron withdrawing groups attached to it.

So, I gave you some examples quinone and imine type of a triazine compound where the aziridine ring is more susceptible to ring opening. Another important drug is cyclophosphamide which is a pro drug where the nitrogen lone pair is locked with the phosphate group. Once that phosphate is released the nitrogen lone pair is free and that can do the alkylation of the DNA.

There are other different types of alkylating agents possible like nitrosoureas. They are derivatives of urea. So, one is lomustine and another is carmustine.

One of the method of preparation of diazomethane is addition of base to nitroso in nitrosomethyl urea. In this cases also, very similar type of reaction happens. If it is little bit basic the base abstracts this hydrogen which is pretty acidic. That bond goes here, this comes here and that takes the hydrogen. So, that will form this diazo hydroxide.

This will generate this diazonium ion by removing the OH and these are aliphatic diazonium compounds. So, they will lose nitrogen. Once there is a plus charge one of the guanine base can attack this plus. It undergoes alkylation. In fact, all diazonium salts are very good alkylating agents and in addition you have this chloride.

It attack the chloride. So, you get a cross linked DNA. Same fate will happen to this DNA that it cannot do the proper transcription. So, this is the nitrosoureas.

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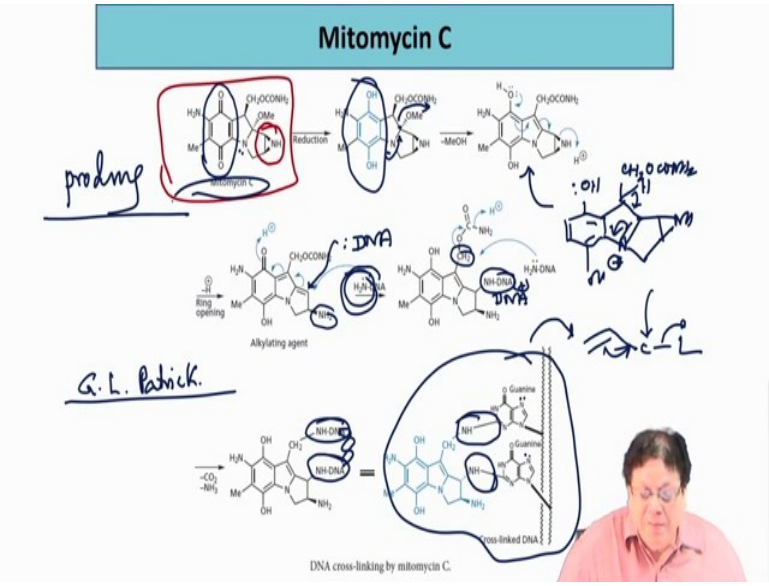

Then you have these natural products which are called mitomycins. In mitomycins, the quinine is here, then a five membered ring and then bis five membered ring which is nothing but pyrrolidine know. On the end there is a aziridinium ring. Quinones are electron withdrawing. If quinine is reduced in the biological system you have lot of reducing agents like cyclophosphamide deoxidized hydroxylated and the product is converted to a drug.

In the first step, Mitomycin C is reduced by NADH or NADPH mediated enzyme. So, it will become a quinol now. There is a vast difference between the electronic character of quinol and quinone. Quinone is electron sink and on the other hand quinol is electron source. Electrons can start flowing in this direction because now it is electron rich.

As soon as it sees that there is a nitrogen lone pair it is also feeling the influence of this quinone part as an electron sink. So, the lone pair is kind of locked there. As soon as it becomes quinol the pair is now free. The lone pair will now can kick out this methoxy. Methoxy is present at this carbon the ring junction. Once it forms the plus the lone pair now can start migrating from here.

There is one step which is missing. So, this is the OH, that is OH and you have this is nitrogen and then there is a aziridine ring. This nitrogen is plus and there is CH_2O $CONH_2$. Here another hydrogen shift will take place, this hydrogen will come back. I will just erase this, this is yet to show electron source character.

The double bond is here and the nitrogen is neutral. There is a good electron relay system to open up the aziridine ring.

When the aziridine ring opens up this will be NH_2 and there is a double bond and this is now a carbonyl. So, it is become an electron sink. If it is guanine or cytosine it will be the ring nitrogen. DNA with a lone pair adds here and it flows back.

So, that becomes quinol again. The ring opening occurs, then the DNA adds. So, you can write here DNA. This is also become allylic. Allylic carbons are very susceptible to nucleophilic attack. It could be by S_N2' reaction where it can attack the double bond and then subsequently it can leave or it can be just direct S_N2 attack. In this case, it is believed that there is a direct S_N2 reaction. So, that another base from the DNA same DNA attacks the methylene and this goes off resulting the formation of this type of molecule here. They have used the guanine amine nitrogen. This is guanine amine nitrogen. In many of the other text books, it will be that this nitrogen which attacks. As it is written in the book we have to go by the book of GL Patrick.

In that book, important point is that the DNA is cross linked whether it is through this guanine nitrogen or this is the amine nitrogen. That could be debatable but the overall effect remains the same like this is attached to the DNA and that is also attached to the

DNA. So, basically that is a bis alkylation but with a different kind of molecule. Now we need so much architecture in the molecule just to give some specificity to the DNA itself and this molecule does not attack other molecules. Mytoycin C must be a prodrug because that needs to be activated by reduction and then it shows alkylating power.

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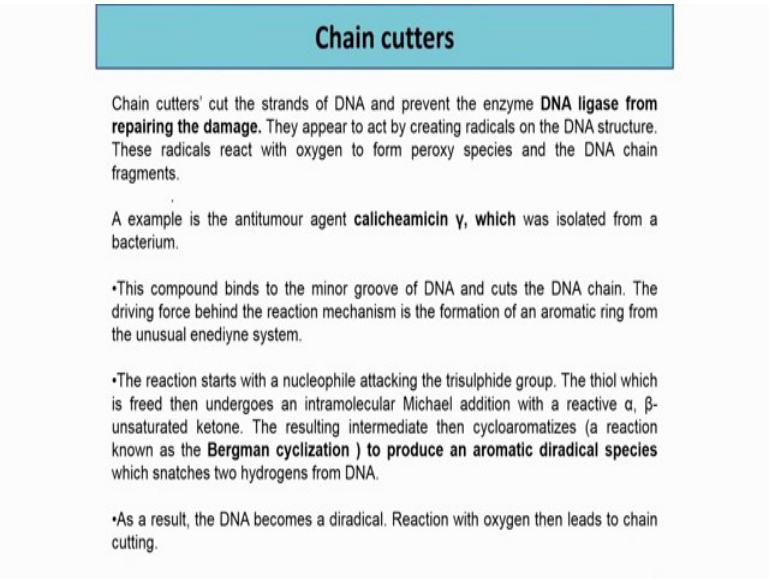

Now, let us come to the chain cutters or the artificial scissors. Artificial scissors will directly cut the DNA into pieces. That is the most direct approach to destroy a cell. Now what type of molecules can cut a DNA?

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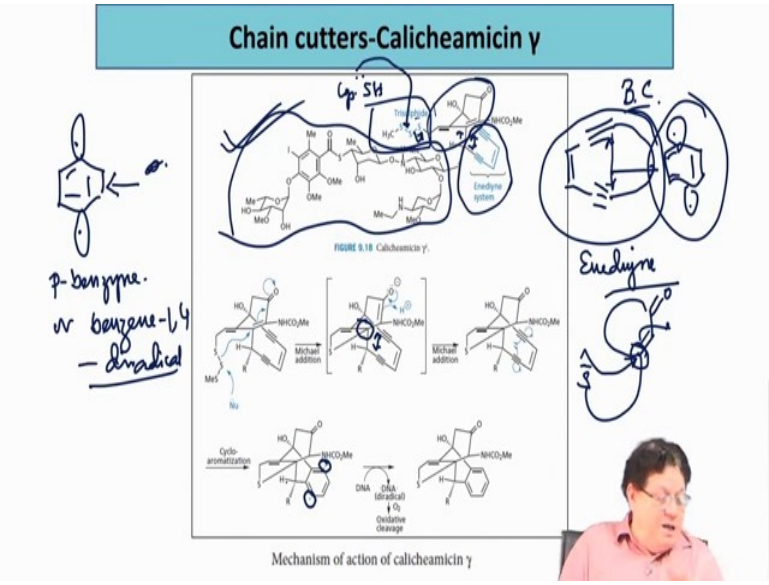

There are various types of molecules. In the last 20 years, there are certain molecules which can produce under the biological condition. That are called para benzyne or benzyne 1,4 diradical. It will abstract hydrogen from hydrogen donors.

If this diradical is formed right in inside the cell and right in front of the DNA it tries to abstract the hydrogen. If it is right in front of the DNA it will abstract hydrogen from the DNA. Now this is formed from a system which will be which is called maybe I can write here, which looks like this. If you read this system it will go to the diradical benzyne diradical and then abstract the hydrogen and form the benzyne.

The driving force is obviously the formation of the aromatic system. If it is confined as a cyclic system this reaction only occurs at room temperature. The natural product was isolated from soil organisms. It looks like a big structure but it is enediynes framework. Then it has got this enone part, then a trisulphide, and then this complex carbohydrate system.

Basically we can say that we can identify 3 important parts- one is enediyne, another that forms the diradical and the trisulphide. This is a very bizarre kind of molecule because is a I do not know any other natural product where trisulphides are present but this is certainly one. The complex carbohydrate gives binding to the DNA. That becomes selective to cut the DNA only.

This trisulphide sulphur are very labile like the peroxide O-O bond. So, a biological nucleophile like cysteine sulfur of glutathione can attack the middle sulfur. A thiolate is released by attack of the cysteine sulfur possibly in the form of glutathione. Then it attacks this double bond by conjugate addition means Michael addition.

Once there is a chain of hybridization it is extremely difficult and very complicated. This s minus attacks here and that results in the conversion of the sp^2 carbon into an sp^3.

Once it forms a sp^3 carbon this connection between the 2 terminal acetylenes is called a Bergman cyclization BC. This will form diradical provided they are sufficiently close enough.

So, this diradical species now abstracts hydrogen because the whole thing is already placed on the DNA via these complex carbohydrate molecules. If you take out the

carbohydrate molecule it will lose the specificity. If you take out the trisulphide it will be very unstable and it will show the Bergman cyclization from the very beginning. So, all the 3 parts are very important.

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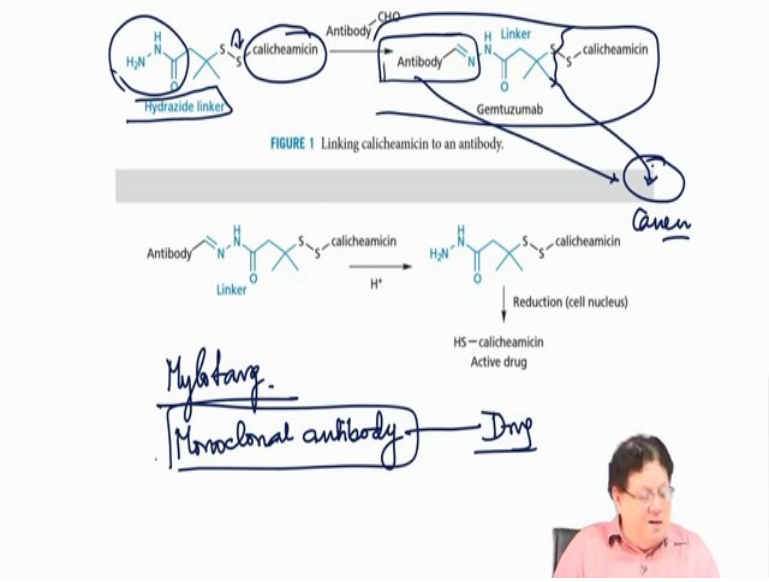

The natural product calicheamicin is extremely cytotoxic. It works in pico gram to kill cell. Although it binds to the DNA but it can not a become a anti-cancer drug due to its toxicity.

Alkylation stops the cancerous cell from dividing *i.e.* from copying/ transcription /replication. What will happen to the normal cell?

In recent years, drugs are made with tailored specificity to the end to the cancerous cell and not the normal cell. The cancerous cells are rapidly growing. If you add something which destroys the cells the rapidly growing cells they will be affected first and the slow growing cells will be affected last. So, that is the principle of designing the anti-cancer drugs. If you see that somebody is taking chemotherapy with these kinds of drugs he or she also loses hair because the hair cells grow very fast.

However, if you give the dose for sufficiently long time it will ultimately kill the normal cells also and that is one of the major drawbacks of chemotherapy.

Apart from the cancerous cells it also hit the normal cells and then kills the normal cells or cause mutation in the normal cells. So, that is major concern about the chemotherapy.

Nowadays the cancer drug like calicheamicin is attached by this disulfide bond. It becomes a thiolate and the thiolate triggers formation of the diradical.

These thiolate is attached to a hydrazide linker $CONHNH_2$. Disulfide is attached to the thiolate by a hydrazide linker. Antibodies are basically specific to cells. You can some antibodies which are very specific for the cancer cell.

You take the cancer cell from the person who is suffering and then inject it to I think an animal get the antibody. But an animal antibody cannot be injected that into the human. There is a process called humanize antibody. We have to do some tinkering on the antibody to make it acceptable by the human body.

If you attach that antibody to the drug antibody recognizes the cancer cell and the drug is released by breakage of the disulfide bond. So, the drug that has been made is called Mylotarg. This is nothing but a monoclonal antibody which is specific for the cancerous cell.

Human antibody is attached to the drug calicheamicin. There are many drugs to be available which are extremely specific because these drugs are attached to the antibody that recognizes the cancer cell and delivers the drug only to the cancer cell and not to the normal cell. However, this drug are extremely expensive because of this monoclonal antibody production and then attachment. Lots of chemistry and biochemistry involved here.

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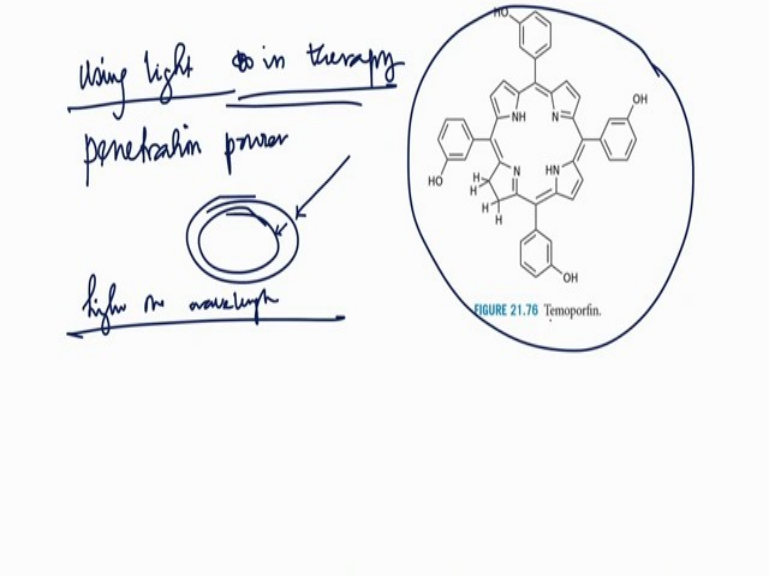

The other way of targeting the DNA is called the photo dynamic therapy. Photo dynamic therapy is basically you take a compound which absorbs red light (not in the UV range not in the infrared).

If you shine light to destroy a tumor this is actually photo dynamic therapy. Photodynamic therapy can be used in cancer.

If you shine light to activate a drug how far this slide will goes. This molecule absorbs light of higher wavelength. Higher the wavelength the greater is the penetration power.

This is a kind of porphyrin molecules tetra pyrrole molecule in a cyclic framework. It absorbs the light and goes into the photo excited state. Then it transfers the energy to oxygen, which is in the triplet stage. The triplet state it is very unreactive and then it goes to the singlet.

So, basically it is an energy transfer. This porphyrin goes to the higher excited state, transfer, comes back, and releases energy which excites the oxygen. So, oxygen becomes the singlet oxygen and singlet oxygen is very dangerous for the cell. So, it now destroys whatever cell components are there.

That is one of the examples of a photo dynamic therapy. Remember one important point, first of all you have to use a drug which absorbs light and generates singlet oxygen. This singlet oxygen generation can be done by many molecules but this is a special one

because this absorbs at higher wavelength. Higher wavelength means greater penetrations. You have to kill the whole tumor. Remember ultimately after chemotherapy, if only a single cancer cell is present that can again cause cancer.

So, you have to get rid of all cancer cells in the body because your immunity is not working. Cell cannot be taken care by the immunity. So, you have to destroy all the cells.

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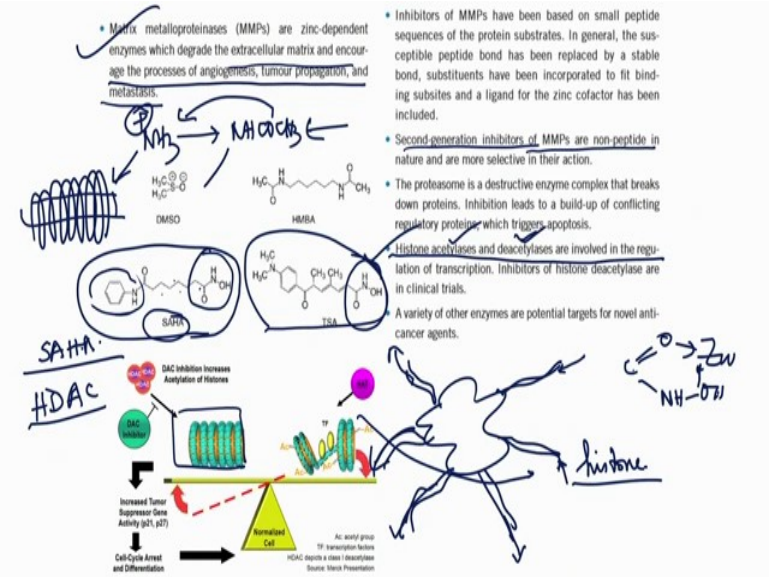

We have not touched many of the targets because of the shortage of time but I will just point out some of the other targets which one is called matrix metalloproteinases. These are zinc dependent enzymes which degrade the extracellular matrix and encourage the process of angiogenesis tumor progression and metastasis. Matrix metalloproteinases are important because they encourage this process of angiogenesis tumor propagation.

Cell has to grow in the blood vessels in different directions. The cancer needs more nutrients than the normal cell because it has to grow very rapidly. So, it has to grow a number of blood vessels capillaries and this process is called angiogenesis.

An angiogenesis is supported by this matrix metalloproteinases. If you can develop inhibitors of matrix metalloproteinases it can be good and anti-cancer agent. In fact, some of the non-peptide inhibitors of matrix metalloproteinases are available as anti-cancer agent.

I will also talk about this histone acetylase and deacetylases enzyme. This is one aspect which we have not told you in the biochemistry class. The first section is that the DNA the length of the DNA has quite very long piece, but the cell dimension is much less. How the DNA is packaged in the cell. The packaging is done by like our threads put on the ribbons.

So, basically there are a ribbons and the ribbons are made up of the DNA. Threads are packed on a ribbon. In inside, there is this protein called histone. So, histones are nothing but kind of a ribbon like shape or solenoid. So, you can pack actually a lengthy piece. You can go by with turns. You can package the long DNA into the cell around a protein ribbon or a spindle.

It will bind because the charge of the DNA is negative and the histone proteins are must be positively charged.

Arginine and lysine are positively charged at the biological pH because they will be NH_3^+ or arginine will be also plus charge. So, the histone proteins have lot of lysines. So the protein will be positively charged and that makes a very good force between the histone protein versus the DNA.

At the time of replication or transcription what will happen? You have to detach the DNA from the histone protein. The enzyme does acetylation of the lysine. If you do acetylation of the lysine it loses the positive charge. It can detach for the time being so that your replication process and transcription takes place. Once it is done there is another enzyme which brings it back to the amine form.

Histone acetylase and histone deacetylase both are required. Histone acetylase basically detaches the DNA and allow the DNA to detach from the histone protein. Histone deacetylase attach it again to the histone protein. This is continuously going acetylation deacetylation. So, this is directly related to the cell growth cell division.

If you can find ways to disturb this enzymes by having inhibitors you can get anti-cancer drugs. In the market, this histone deacetylation enzymes have a zinc in their active sign. They are zinc dependent enzymes. Deacetylation means a zinc mediated hydrolysis and lot of molecules have been made. I will tell you a simple molecule what is called suberic acid. So, this is a suberic acid based hydroxamic acid.

On the other side, there is hydroxamic acid. This is suberinyl aniline and that is your hydroxamic acid. If you abbreviate that is called SAHA. SAHA is the one of the latest anti-cancer drug which works on the deacetylase enzyme and abbreviated as HDAC. HDAC is histone deactylation.

This CONHOH actually chelates to the zinc. You have to chelate the zinc surprisingly. These are simple molecules which are approved in the market and they work by histone deacetylase inhibition.

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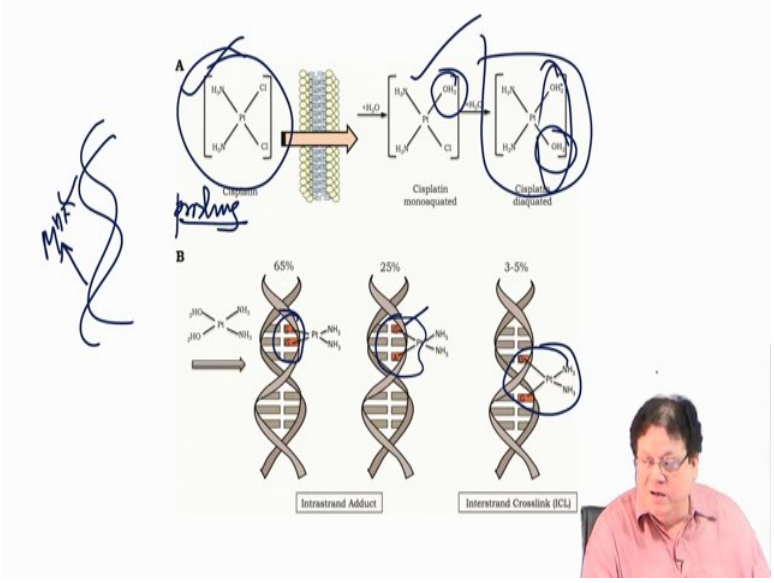

Cisplatin is an anti-cancer drug. Bis metalation means 2 bases of DNA have coordinated to the metals.

This is nothing but a kind of bis alkylation. The alkylating agent is the metal. In Cisplatin, this chlorines are *cis* here and transplatin does not work. Cisplatin crosses the their cell membrane and then this chlorines are very labile. So, one chlorine is replaced by the water followed by chlorine. So, this is the actual active drug. So, this is nothing, but a prodrug again.

Prodrug has to be activated. It goes inside the cell and it gets activated into this form. Now this hydroxy ligands are replaced by the ligands of the 2 guanines. They showed intra molecular or this could be inter molecular.

This is inter molecular and this is intra molecular. If there are 2 Gs one after another, then that could be the structure. If there are one G here and then followed by a different base added it also can participate. Remember guanine is a preferential side but that does not rule out adenine or cytosine to chelate to the metal ion. So, that is the working principle of cisplatin.

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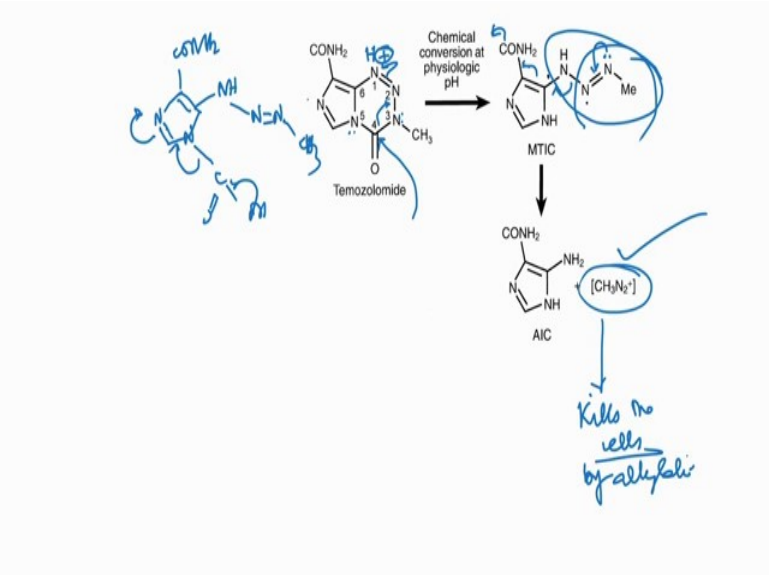

Another recently developed anti-cancer drug is temozolomide. Temozolomide works on the principle of generation of diazomethane. Diazomethane is a very strong alkylating agent. So, it alkylates the DNA. But it has to be produced inside the cancer cell. pH of cancer cell is much less than the normal cells. The reason for production of lactic acid is that cancer cell wants lot of energy to grow. So, cancer cells are more acidic than the normal cells and that is exploited by this molecule. This is temozolomide. This carbonyl, this nitrogen cannot enter into resonance with the azide N double bond N. Similarly this nitrogen cannot enter into resonance with this imidazole nitrogen because of this carbonyl. The cancer cells are acidic. It will be preferentially protonated in the cancer cell than the normal cell. Water will come here, attacks and this becomes a species like this. What will happen to the carboxy because the carboxy will now be a beta immuno system? This is $CONH_2$, this will be NH, then N double bond N and methyl.

The carboxy will decarboxylate a beta keto acid. Once you have this species this lone pair comes here. This N minus is in conjugation with this double bond. There is a amide

here. Amide carbonyl becomes O minus which is the driving force for release of this. This methyl diazonium salt kills the cells by alkylation.

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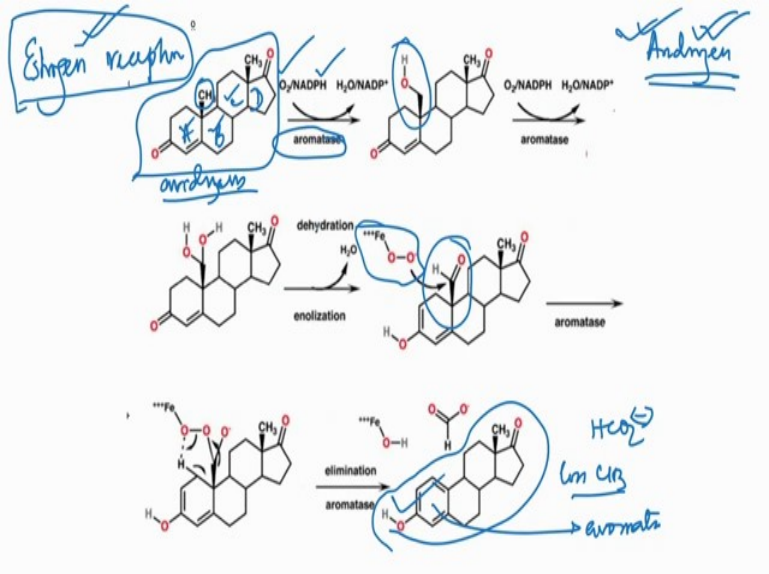

Other difference of cancer cell from normal cell is that many of the receptors work by attachment of neuro transmitters or by hormones.

Hormones go through the whole body. So, there are receptors that can be over expressed. So, you need to express the receptors for more growth factors because the cancer cells are growing more rapidly. Growth factors are over expressed in cancer cells.

One of the receptor is estrogen receptor and another is androgen receptor. Androgen is the is the male hormone mainly and the estrogen is the female hormone. Many of these cancers are related to mainly to women like the breast cancer. You can exploit in those cases because their estrogen receptors are over expressed.

So, you have to develop antagonists of estrogen receptors. Similarly for male, androgen receptors are over expressed which can be a causative factor for prostate cancer. So, you have to utilize what are called anti androgens. Here you have to use anti estrogen.

It is one thing that you can develop your anti estrogen to stop the estrogen receptor. To stop the estrogen from binding is one approach. The other approach is to check the bio synthesis of estrogen. If estrogen is not there then how the receptor will be activated? So, there are 2 ways- either you add an antagonist to the estrogen receptor or you stop the bio

synthesis of estrogen. We are talking here the bio synthesis of estrogen. Androgens are basically steroid molecule steroids. These have 3 rings called perhydro phenanthrene rings and this is a five membered ring -that is the typical skeleton of a steroid.

This is the structure of estrogen. In this reaction, the methyl is gone and the ring becomes aromatic. This is aromatic and the methyl is also gone and they found that the methyl is actually coming out as formate. The methyl has been oxidized first to alcohol, then aldehyde and then followed by formate.

So, they have started looking at the mechanism of this compound how these androgens are converted into the estrogen. Finally, they found out that it is the enzyme which is called aromatase because you are going to aromatize the A ring. This is by the way the A ring of steroid, this is B, this is C and this is D.

You have to aromatize A ring. So, the enzyme is called aromatase- it needs oxygen and it needs a NADPH. So, that is actually the electron donor agents and itself becomes oxidized to NADP plus. First it goes to CH_2OH and then it goes to CHO. It is an iron dependent oxidation.

Now aromatase inhibitors are available in the market. We will discuss chemistry that are associated with this aromatase in the next session.