00:00:00:03 - 00:02:03:29
Dr. Robert Granger
Thank you, everyone, for being here. This is our third Porter lecture series. Bill Porter gave the first one. And he's here in the audience. So, we have Mr. Bill Porter to thank for this evening. Thank you, sir. Y'all can applaud now. There you go. Hey. Thank you. So, I want to introduce our speaker tonight, but we're doing a little bit of an experiment. I went online and found out everything I could about you, and I dumped it into Copilot and said, create an introduction from this. So, we'll see how it does. You can grade the AI and tell me if it if it did a good job. I left all the stuff with the sheriff's department off. All right. So, Doctor David Cortez, PhD, professor of biochemistry at Vanderbilt, holds a distinguished Richard Armstrong chair of innovation in biochemistry. He's chair of the biochemistry department, associate director for basic science research at the Vanderbilt Ingram Cancer Center. Doctor Cortez is a leading expert in genome maintenance and DNA damage response. His research focuses on understanding how cells preserve genome integrity during DNA replication and repair. A process challenged daily by thousands of lesions and replication stresses. The Cortez Lab employs a multidisciplinary approach spanning genetics, biochemistry, cell biology, proteomics, and structural biology to uncover mechanisms that safeguard DNA and preserve diseases not preserve present, prevent diseases such as cancer and premature aging. Current projects include identifying novel DNA repair proteins, studying replication fork protection, and developing therapeutic strategies targeting DNA damage pathways. Doctor Cortez's work provides critical insights into how manipulating these pathways can improve cancer treatment outcomes. How did the AI do? Very good. All right, very good. All right. Well, without further ado, I'm looking forward to hearing everything you have to say. And we, we are all yours.

00:02:04:01 - 00:41:01:02
Dr. David Cortez
Thank you. Thank you. Okay. So, can everybody hear me, okay? Yeah. Great. It's a real pleasure to be here and to give this distinguished lecture. I've enjoyed my day. I've learned a lot, actually, about The Citadel and about what's going on here. So, thank you for that. I'm going to try to teach you a little bit about what I care about. And when it gets too technical, just wait for a couple of slides. And I'll go back to concepts. And, if you really are getting lost and falling asleep, yell at me and tell me you want me to stop and tell you something more easy and to explain it a little bit more. I don't mind. So, this where I work. Actually, if this is working, this building here is where I'm spending most of my

day. And and what we what we try to understand is really these questions. Well, let me start with the numbers. So, in each of our cells, we have a lot of DNA, about 6.8 billion base pairs of DNA. And in a human lifetime, at least Google tells me it's 10,000 trillion cell divisions. I don't know if that's correct, but it's a lot of times you have to copy that DNA and segregate it into daughter cells. And every cell in your body, every day, is experiencing DNA damage. 10,000 or more DNA lesions. Your skin cells are experiencing a lot more than you go out in the sun and getting UV radiation, but even your cells in your brain are getting damaged all the time. And so, what we want to understand is how does this work, right? How does it if you think about this, I use this analogy. If you think about it, you try to copy a book with 6.8 billion letters in it, and you have to copy it tens of trillions of times, and somebody is constantly ripping up the pages and pouring coffee on it, how is that possible you can do that successfully? And yet we do a fairly successful job at that. But sometimes it doesn't happen correctly. And when it doesn't happen correctly, we get disease. And when we get diseases, we want to know, well, how do we fix that? And so, some of my laboratory is trying to understand how we can fix those, those problems. So, a lot of what I study in my lab revolves around DNA replication. DNA replication happens when the DNA is being, has to be opened. And and and copied. Right. And this is my diagram of DNA. So, it's not very accurate. This should be a double helix. It's not I can't draw a double helix. But this is the part of DNA, the black DNA. And then what's happening here is your copy of your opening it up with this enzyme here. It's called a human case. Opens the DNA. And then you have a nice, powerful, nice epsilon. And when I start that, I'm working to synthesize new daughter strands. And they do that in a coordinated fashion. And we call that leading strand replication here, lagging strand replication over there. But this is not happening in one location. Let's start with the chromosome and. Copy the other end. It would take forever. Instead, there's thousands of these replication forks is what we call them. They're active at the same time, completing the replication. And in the human cell, most of our cells, it takes about eight hours for it to complete replication. Okay. So, there's a lot of machinery going on here. And this is challenged all the time by problems in the DNA template. All this DNA damage. I talked about tens of thousands of DNA lesions. And there's all kinds of DNA damage. You can't base damage with just a, whatever the base is, is, is oxidized or methylated or something. You can have proteins. They across the DNA, all the DNA itself can get crossing through each other kind of chemically. You can have breaks in the DNA, in mismatches that form all kinds of problems. And so, the replication part and the machinery has to encounter these problems, recognize there's a problem, and deal with it. And the goal is to finish replication. Right. So, if a cell starts replicating, if it doesn't

finish, it's going to be dead. It has to finish replication. And then a secondary goal is to do it accurately. Not make a mistake, not make any mutations. And actually, the rate of rotation is very low, and very few mutations happen every cell division. So, our cells are very good at copying our DNA. Even though they're challenged by all these problems. So how does it do it? Let me give you an example of what the one thing that. Sorry, one way a cell, a few days a cell might encounter damage and fix it. So, here's my replication product. The DNA is being around that little green star. There was meant to be some type of base. Lesion, some kind of problem. Okay, usually, what base station will not stop it unwinding. The helix can still pull the DNA apart. And that's fine. But the polymerase, when it encounters that the emission it's looking for an AGCT. And if it's not, and you see what it says, I don't know what to do. So, it pauses. It stops. And that means that the helix keeps going a little bit. The place pauses, and you end up with this similar stranded DNA that's generated. And that's a signal to the cell that says, I have a problem, or we can do something. Now there's lots of options of what you do, okay. One thing you can do is what we call translesion synthesis. Essentially, that's taking this polymerize and exchanging it with a new one that doesn't care that there's damage there. And it's going to insert some random base and move on. So it's called a translesion bypass place. It makes a. Mutation. But that divides the cells. That can continue. Replication. And that's a word for a chance of getting it right, okay. That happens. The other thing that happens is the cell just says, well, I'll just do nothing for now. I'm going to bypass that. And restart replication and just get on with it. And then it has a gap. And the gap can then have to be fixed later by some other mechanism. That allows the cell to keep the replication moving. And going. Then there's this really weird thing called fork reversal, which makes no sense whatsoever. But what this does is back up the DNA. It just basically moves backwards instead of instead of unwinding it's rewinding the DNA. Now the balance of cell of that is it places that lesion that caused the damage back in the duplex DNA. And the story I'll tell you about today, we'll explore why being in duplex DNA is really important, primarily because that reason is repairable in duplex DNA, where it is not repairable here. And that's largely because if you think about it, how would you repair damage? The way to repair it is to cut it out. Okay. Well, if you cut it out there, that's a problem because you have nothing to hold things together. And then you do that as some other DNA strand on to tell you what to synthesize. As you do what's called repair synthesis. In this case, you could cut it out, use that other strand as a template, and do repair synthesis. So that's why a cell would do this. All this is really complicated, and it requires a lot of machinery. And one of the goals of my laboratory for a long time has been just to describe what the machinery is required to do all this

stuff. All right. So how do we study this? We figure we needed to understand what all the parts are. Now, the cadets and students in the room don't even know what that is, but the older people around will know this is a copy machine. We used to copy paper. Right. Now people don't copy it now. Nobody copies paper anymore. But a copy machine is sort of my analogy for the DNA copy machine. And to understand how that copies piece of paper, you need to understand what all the parts are. You need a parts list. And then once you have a parts list, you need to know how the parts go together. So, the goal that we've been working on is find the parts list and figure out how it goes together. And then, as I'll tell you about a little bit later, is how those parts go wrong in disease and how can we fix it? So how do we get parts list for what's happening here, especially when there's a DNA lesion that has to deal with? Just two main approaches scientists use. One is a genetics approach. We basically make mutations, and we figure out what goes wrong. Another one is a biochemical approach. And that's what my laboratory is used. And our biochemical approach is essentially to purify this structure under different conditions and ask, what are all the proteins that are there just just like like a protein list. But that requires to be able to purify this from a cell. So how do you purify that? Well, you need some kind of handle on it, something to be able to, to grab on to. And so, I'll describe a method that we invented to do that. Okay. And this method, this is a nucleoside that is different than an AGC or T. It's this thing. It's called EdU. Instead of a thidamy, which looks like thidamy, but it's got this weird chemical group out here called the alkyne. That's a C triple bond C. That's not a natural thing in our bodies, in ourselves. But this can be fed to cells, can be fed to people. And you can it will go into the cell, and then it will be incorporated through DNA anywhere a T should be. It will go in because it base pairs with A, okay. And when it does that, it leaves all these alkyne groups on the DNA. And what's special about that is that if you have an A side group or a chemical group here, you can make this react with that and make a chemical bond very easily just by adding copper. And it does it very efficiently. Okay. So why am I telling you this? Because we can hook up the A side to a group called biotin. Biotin is a naturally occurring molecule. It's a nice little handle that we can pull on to purify things because there are proteins. Called one. It's called stripped, and that binds biotin. And then you can use that as a handle to basically pull it out. So, what we do is we do this, we feed our cells, this nucleoside EdU that gets incorporated into DNA by the polymerase. We only do that for a few minutes. So, it's only incorporated right where the replication is happening. When we do this biotin conjugation reaction, we break up the DNA, and we pull on it, essentially purifying that DNA with this streptavidin DNA. So now we've purified all the DNA that's been labeled, but we also purify these proteins. And the associated

proteins are all the ones that will be doing the copying, because we only label the DNA that's right at the replication fork. And then we can analyze what those things are by mass spectrometry or other methods. And essentially this gives us a way to just say what are the proteins at this place where DNA synthesis is happening, where the copying is happening. And how do they change in abundance or modifications of those proteins if there's the DNA damage or a lesion or something odd going on? Right. So, this is this is what's called a Western blot. It's one way of looking at these proteins. Does it really matter how it works? But it gives us a proof of principle, essentially allows us to say if we do this method, do this purification, we call it click capture. If we do this purification, can we purify proteins, we know are at replication forks? This is for example, imagine that it wouldn't matter what it does. It's a protein that's required for replication. We can purify it, okay. So, the method works. I'm going to skip that. We usually don't do that method because that requires us to have an antibody if you don't like. So, it's very similar to that. You can only look at one protein at a time. Instead, we do mass spectrometry. Mass spectrometry is a method that allows you to look at whatever is in your test tube and look at all the things in there all at once, and you can do it quantitatively to get an idea of how much is in there. Right? So how much of the helicase subunit is there? How much of the polymerase is in there? And how does that change under two different conditions? If I take and damage, my DNA here, I've used a chemotherapeutic agent to damage the DNA. Okay. And I say, well, how does that change when I purify this fork-labeled proteins here, how does that change? I can see here that under this condition, I purify more of this protein called ATR, that's a sequence of that that I'm looking at here by the mass spectrometry. There's more of it in this sample than in that sample. Whereas this protein this is a histo. These are the proteins that your DNA is wound up on. That doesn't change with DNA damage. Doesn't really matter. So, this is just an introduction to tell you I can look at anywhere DNA synthesis happens is happening, and I can tell you what the proteins are there and how they change if I perturb that system. So, we know now from our work, there's actually 600 proteins involved in this process. 600 is a large number. There's only 20,000 genes in the genome, 20,000 proteins; essentially 600 of them are dedicated to doing this process of replicating your DNA. And that's because it's essential, right? You have to replicate it. You have to copy it. You have to copy it accurately to avoid disease. A lot of these proteins have what we call the core machine replisome. These are the proteins that are required that if you pull a test tube together, you can get copying to happen. But they have all kinds of other proteins. DNA repair proteins, other signaling proteins that are recruited when you have a problem. If I treat a patient with a chemotherapeutic agent that damages DNA or radiation, that damages

DNA, these proteins get recruited to this cancer cells in those locations to try to repair those problems. You'll also see proteins that are involved of duplicating. The chromatin, the histones that are there. Okay. So over here we've then looked at these lists of 600 proteins. And come to the lab, look at that list, and they'll say what's that protein. And there's nothing published on it. And they'll try to figure out what it does. What is that protein? And this is just listing some of the protein names. It really doesn't matter that they are that over the years, we've characterized, and we've tried to figure out. But what I want to do today is give you an example of how this works and how this discovery happens, and why it might be important. Okay, I'll come back to that why at the end. And I'm going to use this as an example. This is an example of a protein called HMCES. We didn't give it its name. The names we gave that one RADX, that one's easier to say than HMCES. It's difficult to say. So, if I rename it, it's easy this way. Somebody else had already named. But I'm going to give you an example of what we discovered. And why it might be important. And these are the people that did this work. This is Kareem. He's at the Mayo Clinic now. Jorge, Petria, and Houde were students in the laboratory. And I have a great collaborator at Vanderbilt, Brandt Eichman, and his student Katherine, helped us work on it. Okay. And I'm getting giving away what it is here. But I'm going to come back to explain what that is. Okay. So, this protein, HMCES, do we choose it of the 600 proteins to study? The reason we chose it is this. This is a sequence alignment of the protein. The top line is the human protein sequence. M G C T as you can't read it. It doesn't really matter what the sequence is. But these are all different organisms, and the sequence of the same protein in those organisms. You can't read this, but these organisms span the entire evolutionary tree. Every organism on the planet has one of these proteins. Every, you know, fish, worm, spider, plant, bacteria, archaebacteria, these funny things that live in the oceans and in vents of these hot thermal vents, they have one of these proteins in them. In fact, some some viruses encode this protein in their genome. And that to me said it's got to be important. Every organism on the planet encodes this protein in their genome. And that to me says it's got to be important. Right? It is surprising to us to every organism encode a protein that nobody had ever studied before, because lots of people have been studying DNA replication for a long time, but nobody ever studied this one. So, this is why we got interested in it. Okay? It has to be important because it's been around forever through evolutionary time. That was my argument. This is a structure of this protein. This is what it looks like in three-dimensional space. And that was important to us when we saw the structure, because it immediately suggested a functional thing that it did, because this structure that's color-coded here is the electrostatic sign. Blue means negative. I'm sorry, blue means positive. Blue means positive.

Okay. Red means negative. So, this has a very positive groove in it. And if we're studying DNA like I do, a positive groove in a protein like this immediately makes you think DNA-binding protein. That looks like where DNA could combine. Okay. And this is an acid; I'm not going to explain what it really means. That just showed us that it yes, it binds DNA, but it doesn't bind just normal duplex DNA. Most of the DNA in the cell is duplex DNA. This protein only binds to the single strand of DNA. Where the single strand of DNA is where it's being unwound at the replication fork. That's largely where single-stranded DNA is, and that's where it binds. Okay. So, we have a protein, we know it binds DNA, whether it's at replication forks. Great. It evolutionary conserved. Let's study it. What does it do? I'm a biochemist. I really want to understand that. Hey, what is this protein there for? Why is it? Why is it present? So, what we do we turn to genetics. We knocked it out. We deleted it from cells. But then here in organisms like this, in bacteria. But we also deleted it from human cells. When we do that, the cells keep growing. They don't look like they have any problem. But if we look carefully, we notice they're experiencing more mutations. So somehow this is suppressing mutations. The only thing it started doing is challenging these cells. We challenged them with ionizing radiation, X-rays, UV radiation. This is an agent. That left right means this is an environment. Or is it actually used to. Be incorporated in in in bakery products. Potassium bromate that oxidizes our DNA, all these things damage DNA in different ways. It channels the cells with all these things. And the cells that lacked HMCES, compared to, well, type cells died. They didn't like being challenged with these agents. So, somehow these proteins required for cells to resist these DNA-damaging agents? No problem. That was very interesting to us. The problem with it is, as I said, all of these agents caused different DNA damaging agents. And we know that these types of damage, like this is a double-strand break. It's repaired very differently than this lesion than that is by different pathways. So that because it is important in response to all of these things, it said it's not a base extension and nucleotide excision, or double-strand break repair protein. It can't be one of these things because it's involved in multiple things. So, I actually wasn't smart enough to figure this out. We sat there and scratched our head and said, well. What could this thing be doing? And then I'll talk to my colleague, a nucleic acid chemist at Vanderbilt. And he said, well, you know, yeah, those are all the canonical lesions, all these things cause, but either directly or indirectly, all of these agents will also cause a common lesion, the same lesion. And that is a abasic site. Okay, what's an eight basic site? It's exactly what it sounds like. It's a location of the genome without a base, the DNA backbones intact. But the A G C, or T has been lost. And it turns out that's the most common lesion in our cells. It happens most frequently. Tens of thousands of times a day. Very frequent. Okay.

And all of these agents will increase the frequency of them. So, he said well, why don't you look at whether this protein is involved in the repair or processing of these abasic sites. So yeah, they can come about, I should say they come about for all kinds of reasons. Spontaneously, you lose a base. This is just meant to be DNA with bases. You lose is base because the base damage that enzyme for the cells that remove bases; you have enzymes that actually do this on purpose to make you lose bases. But usually that's an antiviral response in our cells, okay. So, there's thousands of them per cell per day. So, the thing about this is when he suggested it to me, I thought, well, this is boring. It can't really be important for this because we already know how cells repair abasic sites. This is what they do. If there's a base missing, so this is one strand. That's the other strand of phosphate backbone is, and these squares are meant to be bases. One base is lost. This is repaired through excision by this enzyme. It cuts the backbone. And then a polymerase by a console it synthesizes. It uses this sequence to synthesize the right base. And then you get ligation, and you fix the DNA. And this happens very rapidly. It's almost instantaneous. It's very fast. It's base excision repair. And the Nobel Prize that I would have been given to somebody for studying this and discovering it 25, 30 years ago. So, I'm thinking this is. Can't be right. It's boring. How can we have something else involved? Well, we know everything about it, but my postdoc did this experiment. He took a piece of single-stranded DNA. Remember, I told you this protein only binds single-stranded DNA, and he put abasic site in it. He could just synthesize it with an abasic site. And you did a DNA binding assay. Now, this is a DNA-binding assay. The way it works is as a gel, you have p32 labeled DNA, and it went on the gel because, you know, it's getting pulled by electrophoresis the gel, it runs fast. If a DNA binds, if you add the protein to the DNA, it will shift that DNA because it only targets mobility on a negative gel. It's going to just bind it, and it will be bigger and won't go through. The gel so fast. So that is binding. This cell tells us this protein binds this DNA. That's fine. We already knew it bound DNA. But this was the interesting experiment, even he geled it he boiled it, before putting on a gel body. Boiling it should denature the DNA and make the protein fall off. But it didn't. The protein still was stuck to the DNA after boiling. And we got really good at this. And you could see that this protein kept sticking to the DNA even after it emerged through the protein. The protein completely unfolded, and it still stuck to the DNA. And it only does that if the DNA contains an abasic site. AP is another name for an abasic site. Okay. But if the DNA contains a family like to show the base, it's fine, not bind it under those conditions. If it is a like a mimic of an abasic site that is a hydrogen instead of a hydroxyl group, it won't do this. So, it really needs this type of ribose sugar can open like this. What normally and only this is what abasic site really looks like.

It's an equilibrium between these two forms. And when it's in these forms, this protein binds it and gets stuck to it. And when I say gets stuck, it's covalently linking itself to it. That's really weird, okay. Because a covalent linkage to DNA is actually a bad thing. You'd think it would be a problem. It's it's usually not what should ever happen. But in fact, this protein is programmed to do this. Within this basic surface where the DNA looks like it should bind, there are three conserved amino acids that are invariant. These are always variant in every organism. Okay. In fact, the critical one is this cysteine. And it's always have a little acid position to it. Every organism every every protein HMCES protein, whether it's in yeast, E.coli, flies, always has a cysteine amino acid number two, the first amino acids, methionine. Right. But the methionine is missing from the structure because it's removed, okay. This gets removed before it has the proteins folded, which gets removed. So really, the cystine is the terminus. And here's the cystine. It's always the end terminus of every protein. And we know that if we stuck something on the end of this protein and tried to put that in the cells, it would inactivate it. Okay. Any type of tagging, the protein inventiveness. The people often put fluorescent tags, GFP tags, to make proteins fluorescent. We tried to do that with this protein at the time I said inactivated. It can’t, it can't, it can't have anything at the terminus except the cysteine. So, what we thought might be happening is the following. We've got a protein that binds single-stranded DNA and an abasic site. Irt crosslinks to it. If abasic sites can do this, ring opening. And this is what the end terminus of this protein looks like. It's got an internal amino group. It could be doing an attack, creating a shift base. And then the sulfur group, because it's a cysteine. Remember, it's at the end. If there is positioned in to do another attack, they form a called a thiazolidine ring. And that would be a covalent bond. So that would be resistant to boiling. And that's exactly what it's doing. These people, Petria and Katherine, saw the structure of this protein with this DNA found with abasic site. But that that's the backbone, that it's missing the base. And then they zoom in on this this is the structure. It looks exactly like that. We've got that DNA base and the cystine, and this is ring just like we predicted. So. If you're falling asleep, you can reengage. I can summarize what I said without any data. But I'm telling you is this protein HMCES is that replication forks. It will bind a single-stranded DNA replication fork. And if there's a abasic site it will crosslink to it. So, like this. And when it does that it gets stuck on the DNA. And every organism on the planet has one of these proteins. And this is a very common thing that happens, a very successful. You start polymerizing, but the polymerase gets stopped by the abasic site foundational premise can't deal with it releases and this protein comes and gets stuck on it. So now you should be saying why? Why would cells have proteins to do this? Because after

all, this is a really bad thing. A DNA protein cross-link is very difficult to repair. It's not an easy repair mechanism like basic station repair. So why do cells do this? So, we come back to this abasic sites usually repaired. Abasic sites are used to repair your basic site. The first step is this, and the nuclease to cut the DNA. I remind you that HMCES is working not in duplex DNA but in a single-stranded DNA context. And if you cut that which you generate is a double-strand break. A double-strand breaks are really bad for cells. So what this protein is doing is it's recognizing the abasic site, and it's blocking base excision repair. It's an anti-repair protein. It's actually stopping the cells from repairing the DNA. And why. Because if you try to repair this, the first step is cutting that backbone, you end up breaking it. And that's really bad for the cell. So, it's an anti-repair protein. That is what it's doing. It also blocks this translation synthesis pathway that does mutations. These alternative polymerases can't work with HMCES. So normally this might synthesize across here, HMCES blocks that, okay. And I'm going to skip the data to show you that. But that's what it's doing. It's blocking both the double-strand blocks being generated. And there's polymerases that would otherwise create a mutation. And we have a bunch of data that's consistent with that. I'm not going to show you all that data, okay. And by doing that, it's preventing double-strand breaks and base mutations. Of course, at the expense of having a protein locked on the DNA, which is a problem. So now you should be thinking to yourself. Well, for those that really want to keep this protein stuck to it, to the DNA, so how does it get reversed? Well, it is removed, okay. And it actually can remove itself. It's got this special chemistry that it can do, but it only removes itself when there's duplex DNA available. So, let's say that a single strand DNA is stuck on there. But if I give it complementary DNA it will then self-reverse. And now the basic site comes back. But it can be repaired through basic system repair very accurately and efficiently. So, this protein is basically telling the cell ignore this abasic site, don't do anything. Wait till I have some kind of duplex DNA available so I can repair it accurately. And that's what it does. Now, all the specifics about how that works, we're still investigating. So, we don't have all the answers. But this is only one of the proteins, of course, that we've studied, and we studied many more. But I think that we should be asking is, why does any of this matter? It's kind of fun, you know, it's a it's a neat thing to think about. Biology is really cool. Cells have all kinds of weird pathways in them. But why should we care? Right now? I come back to this. What happens if this doesn't work properly? What can we do about it? What are the types of problems that would be created? If this doesn't happen? Okay, so this is one of the types of problems if this doesn't happen properly. So, this is a child here. He's the same age as his colleagues here on his baseball team. Obviously very small, right?

Short stature. He has a disease called Schimke immunosseous dysplasia. It's not in the HMCES gene itself. It's in another later gene that we've studied as well. And this mutation inherited through 2 bad copies. One from mom, one from dad. Okay. And unfortunately, not just growth is affected. These children end up having poor immune systems because they're not copying the DNA properly. And so that gives rise to problems during, you know, B and T cell generation. There are other problems in other organs, and many of them will get cancer because they're generating lots of mutations during their DNA replication process. We saw benefits very rarely see this. We saw two of them at Vanderbilt. Both of them ended up dying in their teens from from cancer. So, it's an awful disease. It's rare, fortunately. But many other diseases are caused by similar problems. Most notably, all cancers are caused by by alterations in our DNA. Increased mutations that activate either tumor suppressors or activate what we call oncogenes. And that's caused by mutations or misrepair or double-strand breaks that caused rearrangements in our genome. Okay, so when this protein doesn't work properly you have an increased risk of cancer. Now, what can you do about it? I'm going to give you an example. And then I come back to HMCES. So, here's a different example. And this is something that actually works in the cancer clinic. So instead of a abasic site, what if you had a nick in the DNA, okay. Well, if you have a nick in the DNA, you also repair it by base excision repair. But the first step of recognizing that nick is a little different than the abasic site. The first step of recognizing that nick is up is by a protein called poly ATP ribose polymerase. PARP. Don't worry about what it does. It doesn't really matter. It just sees this nick, and it tells the cell, hey, let me let come fix it. Okay. Now, if you don't fix that nick, if you look at this, this is very replication. What will happen? Well, if the helicase kept unwinding is this, eventually it would run right off, and you end up with this kind of structure, a double-strand break. Again, a problem. The reason HMCES deficient cells are alive is they do this all the time, but our cells can repair this through another mechanism. They repair through double-strand break repair. And hat double-strand break repair depends on two genes that some of you have heard of BRCA1 and BRCA2. When I say to blast of ovarian cancer susceptibility genes one and two. So, Angelina Jolie has a mutation in BRCA2, okay. She's a high-risk cancer. So, she had prophylactic surgery because of that. People with mutations in these genes will get cancer, men and women. Women more susceptible. But even men will get cancers because of these mutations. Okay. So, if you have intact BRCA1 and BRCA2 to this mix or HMCES deficiency. Doesn't really matter. But if you have a mutation in these genes, which you do, if you inherit a bad copy, then you can't repair this break. Why am I telling you this? Well, we can use that property to treat cancers. This is lost in many cancers, okay? We can't do this pathway. So that means you really

better do this pathway well. But we can drug that pathway. We can inhibit it. So, what we get is what we call synthetic lethality. If you have two wildtype copies, BRCA2 and PARP1 in part by functional fiber. So, if you BRCA2 and PARP1 cancer cells. Right. If we inhibit PARP1 in a normal cell that has BRCA2 wildtype, it's fine because we can always use the BRCA2 pathway. But if you have a cancer cell and you've lost that BRCA2 double-strand broke repair pathway, you can't fix the problem if you also inhibit Parp1. You have two pathways, both inactive. Those cells die. So, what drug companies did and actually academics first, is they come up with inhibitors themselves on PARP. And they work. They work in cancers that have BRCA1 or BRCA2 mutations. These are four drugs that are FDA given to patients. They got by different names when you when you get prescribed them. But you know when with certain types of breast cancer of all cancer, men with some types of prostate and some pancreatic cancers, they get prescribed these drugs, and they're all available. And not all chemotherapies. They're actually well tolerated. They don't cure all the time. They cure some of the time. Sometimes you get resistance mechanisms arising, just like for many drugs. But this is a way of treating a cancer that is quite powerful. You essentially find the vulnerability that the cancer cell has. The cancer cells lost this pathway, a double-strand break repair. Why did it lose that pathway? Because they're allowed to generate lots of mutations and genetic rearrangements. But that loss of ability to do repair made it susceptible to a different DNA repair inhibitor agent. So let's come back to HMCES. Could HMCES be a good drug target? And where would it be a good drug to target? So if you have inhibited HMCES, what you would do is you would generate more abasic sites that would degenerate into double-strand breaks during replication. So it might be quite useful in patients with BRCA1 and BRCA2 mutations. The other place that might be useful is any cancers that generate lots of abasic sites. And they're all cancers that do that. There's a whole group of proteins that our cells called apobec proteins. These are antiviral agents. Essentially, they sit in our cytoplasm. They recognize invading DNA or RNA from a virus, and they attack it by generating abasic site, DNA in the size type of things in there, and generating abasic sites. But sometimes they go into our nuclei, and they cause damage in their nucleus, and that's when they cause cancer. So, lots of cancers are caused by apobec that's been expressed apparently in our, in our nuclei. Well, those cancers should be generating lots of abasic sites. We know they do. They should be particularly sensitive to loss of HMCES or mass loss of Brca1 and Brca2. Or both would be really great. So maybe this would be a good drug target. And it's an activation be lethal when apobecs are expressed, and you can't do that double-strand break repair pathway. It's a speculation hypothesis. We don't know if it will work, but we'll try it right. And there's many other opportunities like this. And this is why

doing is basic research of understanding how do is the cell DNA of DNA damage during replication. How does that maintain its genome? You know, that's a very basic fundamental question. But every once in a while, you might come up with an answer that could be useful. Not only ten years before we know it, this is a pattern. More than that, before we know if this will be useful. And they require a lot smarter people than me because I don't know how to make drugs, so somebody else will have to come up and make the drug. We can test it and things like that. We'll see. So that's the end. I didn't do any of this research. I sit in the office, and I get to come and meet people and all of you and tell you about it, but these are the people who really worked on this. Kareem started this project. Petria and Jorge continued it. Houde is still in the lab working on this project, the aspects of it, and these great collaborators. And then I can't do any of this work without funding. Most of my funding comes from the federal government. The NIH pays my least half my salary and the people in my laboratory. So, I'm very thankful to the American taxpayer for that. And hope that that continues. And we get some funding from a private organization for breast cancer research. And thanks. I'll be happy to answer any questions you have.

00:41:01:02 - 00:41:09:01
Audience Member 1
Hi. Thank you so much. Fabulous talk. Thank you. Do you see any malignancies that you've looked at that have mutations in HMCES?

00:41:09:04 - 00:41:49:09
Dr. David Cortez
Yeah, we've looked. So, it turns out not so much. I mean, there are mutations; it actually is. It's not so easy to answer that question. If you look at sequencing, but lots of researchers around the world sequence lots of cancers. And if you look through those databases, every gene in our genomes are mutated in cancers because cancers have high mutation rates. The question. So HMCES. Yes, it's mutated in cancers. The question is do any of those look causative? Are they generating the disease? And we have very little evidence that they're generating any specific cancers at this point.

00:41:49:12 - 00:41:53:06
Audience Member 1
And is it inherited, or do you generally see that those mutations are random?

00:41:53:11 - 00:42:41:13
Dr. David Cortez
They usually somatic that come about. They're rare. It's not really seen as an inherited. Well, I don't know if there are if this. Is.

So if we make a mouse with that lack HMCES says it does, it does develop into a mouse, it has problems. I would imagine people could be born without this gene and develop. I don't know if they would have a disease. Nothing's been described yet. It's not unusual. Most of the genes we find will have been linked to some disease in the person, but they're often very rare syndromes because it's such a fundamental process of copying a genome and repairing it. Usually, it's a very deleterious thing to have lost those.

00:42:41:13 - 00:42:50:08
Audience Member 1
This is usually during S phase, right? So, you normally see either translesion synthesis or something like if there's a homologous, you know, structure. H.R.

00:42:50:14 - 00:42:59:08
Dr. David Cortez
Yes. Right. Well, when we lose HMCES, we need HR more, and we need TLS, the translesion synthesis more. Both of those things go. up when we lose HMCES.

00:42:59:14 - 00:43:00:19
Audience Member 1
Very cool. Thank
you.

00:43:00:19 - 00:43:10:06
Audience Member 2
Hey. So, I was just wanted to ask, you were talking about apobec proteins, but how are they come to be? They seem kind of bad overall.

00:43:10:06 - 00:43:52:02
Dr. David Cortez
Yeah, so apobec proteins are usually good for us because they are part of our sort of innate immune responses to invading organisms. That are usually confined to the cytoplasm. And within the cytoplasm, they can't do them into our nuclear genome. It's only

when they get persistently pushed into the nucleus which is an imaret process, where they start damaging our own DNA. And even then, we can repair most of that damage most of the time. We have very robust repair mechanisms in our cells. But it does yield an increased frequency of mutation, which over time yields in the increased frequency of things like cancer.

00:43:53:13 - 00:43:58:22
Audience Member 2
And is there like any specific cancers where that is more common to occur in the apobec protein?

00:43:58:23 - 00:44:10:15
Dr. David Cortez
I don't know the answer to that. I'm sure it's true that there must be, but I don't know the specific ones. The way people have studied this is by looking at these cancer genomes and looking at sequences. You can recognize the pattern of mutation caused by apobec. And that's how we diagnosis that that cancer must have had apobec-induced genesis. So, there are people that will be able to answer. The answer to your questions is known, but I don't know it.

00:44:23:06 - 00:44:24:20
Audience Member 2
Thank you for your time, sir.

00:44:24:29 -
00:44:25:20
Dr. David Cortez
Thank you.

00:44:25:23 - 00:44:37:13
Audience Member 3 (Cadet Boone Morgan)
Hello. My name is Cadet Boone Morgan. I'm a senior biology major here. My question is about the tears you were talking about earlier. So, is there anything we can do, I understand how you were saying how like our skin out in the sun from the radiation can face tears. Is there any, besides, like, external factors on our skin, is there any way we can limit these tears in our bodies? Or are they just going to naturally?

00:44:49:11 - 00:47:03:02
Dr. David Cortez
So, it turns out there is a way of reducing mutation rate and reducing aging in almost all organisms that have been tested. And that is to reduce caloric intake by a lot. So, if you take a mouse

and you put it on a very restricted diet, it will live longer. You put a yeast cell on a very restricted diet, it will live longer. The same is probably true for people, but you have to really cut down your metabolism a lot, because a lot of the byproducts of metabolism are often bad. They damage DNA, they also damage other proteins and lipids and things. So yes, and there are some anti-aging researchers. Or, you know, aging researchers who are actually self-administering things that are, that can do that and can mimic that. I think is a little scary to me to do that, but they do it. There's a whole pathway that basically senses, that controls metabolism, and that's what they're taking drugs that. I it that's the only I know. Theoretically. Okay. Actually, in some organisms, they do repair better. This is this is report of bowhead whales that live to like 250 years old. Now bowhead whales, huge, right, is a humongous organism. Lots more cells than we do lots more cell divisions. So why does that bowhead whale go live to 250 years and not get cancer? Turns out they have better repair mechanisms than we do. So, you can imagine if we can repair DNA better, theoretically, we can slow down aging and reduce the incidence of cancer. It's not a simple thing to do. If we figure out how the bowhead whale does it, well, we actually have some knowledge about how it does it, but I don't know how you can make that happen in a human being. But yeah, it's an interesting question. And there's lots of billionaires in, in San Francisco studying how to prevent aging, so maybe they have some suggestions on how to do it. Thanks for the question.

00:47:04:11 - 00:47:06:17
Cadet Boone Morgan
Thank you. It's a very interesting topic. Have a nice day.

00:47:06:18 - 00:47:23:27
Dr. David Cortez
The other organism that doesn't get cancer that's huge is elephants. Elephants don't get cancer. And they live a long time. And we actually know how they don't get cancer too. It's not through upregulating DNA repair. But it's upregulating the response to DNA damage to sort of cool.

00:47:24:00 - 00:47:42:21
Audience Member 4 (William Porter)
So, they've all cancers are caused by mutations in the DNA. What is common about it? All of those mutations that cause cells to replicate it without controls. Where is control?

00:47:42:23 - 00:50:31:26
Dr. David Cortez
There's there's two main places that are common. First, if you think about, what's telling the cell to grow. So, cancer cells basically

have too much signal to grow. That pathway is a signaling pathway that comes in from the exterior of cell cell senses its nutrient environment, its growth factors, and it signals into the cell until the nucleus to to say we have sufficient growth factors to grow. And then what happens in cancers, that pathway gets stuck on, and there's all kinds of genes in that pathway that can be mutated to make that stuck on it. So, it's it's not one gene, and it's not actually one pathway. It's multiple converging pathways. But we know how that works. So, there are things called wrasse and PI3 kinase that get stuck on by mutation. And it basically tells the cell it's like a gas pedal on your car; it's just stuck down. But that's not sufficient. The other thing you have to do is release the brake. In relation to brake is known too. There's two genes that are actually critical for them. And we know those genes. One's called P53, one's called RB, and those get mutated. P53 is mutated in half of all cancers. The thing is, is that those two mutations have to happen in the same cell to get cancer. And that's still not sufficient to get cancer because the cell has to be able to survive in a weird environment to metastasize and things like that. So, you need more mutations. So where are these mutations come from? Every cancer cell gets mutations, more mutations because they inactivate some kind of path that like a DNA repair pathway, so it can accumulate mutations faster. So almost all cancers have an active BRCA1 and BRCA2 mutations so they can generate mutations and p53 RB. These are the growth pathways. Pedal on and the break off. So, we do know a lot about that. And we can drug those on pathways, so in the cancer clinic people are getting drugs to those those pathways. It's just that resistance arises quite quickly to those things because you can get other mutations that just compensate, unfortunately. We're optimistic about cancer by the way. We know a lot, and we're doing a lot better. The survival for cancer is much better than it used to be. I mean, now most, not most, but yeah, actually most breast cancers are turned into a chronic disease that are treatable and and often times that's not that will will kill a woman. Whereas 30 years ago, breast cancer was very lethal. So, we're doing better. Just there's a lot a lot of ways to go.

00:50:31:28 - 00:50:59:15
William Porter
So, on a practical diagnostic aspect of it, the DNA damage, there's biochem biochem biomedical industry now where there's rapid sequencing of, of well, that's all a liquid biopsy. Yeah. They take a blood sample, and that circulating DNA is rapidly sequenced. Is any of that going on at Vanderbilt?

00:50:59:15 - 00:52:41:18
Dr. David Cortez

Yeah, we do that in specific instances. And you can see. So, what Bill was talking about here is that you cells shed DNA, and cancer cells shed DNA, and it goes into your bloodstream. And so, if you have a sensitive enough test, you can sequence the DNA in the bloodstream. And if it is from a cancer cell, you can recognize it because they'll have mutations. So, you can diagnose cancer through what's called a liquid biopsy by taking DNA, DNA from as a blood and sequencing it and looking for mutations. Where that's most useful, looking for recurrence, where you already know what to look for. You only know this person had cancer in the past. This was the type of cancer. This is where the mutations in the cancer. Let's look and see if it's going to come back by looking for the specific ones. That's where it's not most most useful so far. It's there's a lot of challenges to it. Still technically, but it is we have clinical trials using that. It's, and, and we then match up the patient to the drug based on what they get. Nobody gets a PARP inhibitor like a lapper if they don't have, you know, mutation in BRCA1, BRCA@, or a constellation of genes that give you all the names that are that are doing the same pathway of repair. So, we can target, give the right drug to the right person. Yeah. Well, thank you for your attention. I hope that was mostly understandable. But it's what I'm passionate about so I'm happy to talk about it, even if you weren't so if you if you didn't stand all of that. So, thank you.