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Welcome to Yale Cancer Answers with doctors Howard Hochster, Anees Chagpar and Steven Gore. I am Bruce Barber. Yale Cancer Answers is our way of providing you with the most up-to-date information on cancer care by welcoming oncologists and specialists who are on the forefront of the battle to fight cancer. This week Dr. Hochster is joined by Dr. Ryan Jensen for a conversation about DNA repair and the role it plays in cancer risk. Dr. Jensen is an Associate Professor of Radiology at the Yale School of Medicine, and Dr. Hochster is a Professor of Medicine and Medical Oncology and Associate Director for Clinical Sciences at Yale Cancer Center.

Hochster I guess a good place to start is like about DNA, what is DNA? Now, when I learned about DNA, it was kind of the static double helix that had just been discovered and it was kind of the master molecule, but we thought it was pretty static. That's not the way it is, right?

Jensen That is not necessarily true, yeah. So, I too learned about DNA as being the blueprint for life and it codes all the instructions to make all the proteins that are necessary to do all the work in the cells. But what I learned through my training and getting interested in this whole field of DNA repair is that the genome is not static and that it is actually malleable, and that our DNA is constantly getting attacked by the cellular environment, all these chemicals, it is getting twisted and contorted by DNA replication, transcription, and then all...

Hochster Not to mention things like radiation also.

Jensen Radiation, free radicals from the oxygen we are breathing, food that we eat can create metabolic byproducts that damage the DNA.

Hochster All kinds of bad stuff for the DNA.

Jensen Lots of bad things going on. And so, luckily our cells have evolved this ability to repair all this damaged DNA through these DNA repair pathways, and there are different proteins that have jobs for repairing specific types of DNA damage, and so my lab has really been focused on this DNA repair gene called BRCA-2. So, you probably have heard of BRCA-1 and BRCA-2. Angelina Jolie had this famous article in The New York Times - she had a BRCA-1 mutation. And so, these genes are really important for specific type of repair pathway, DNA repair pathway called homologous recombination. And that particular repair pathway is important for repairing DNA double-strand breaks. So, that is where the DNA actually gets broken in half and the BRCA-2 gene will actually migrate to that site of DNA damage in the nucleus, recruit other proteins important for our DNA repair and repair and basically ligate that DNA back together in a way that is hopefully beneficial for the cell.

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Hochster Yeah. I would like to come back to BRCA in a little bit, but I mean the basic concept I think that you already articulated is that the DNA is a flexible molecule, it is kind of wound up in a ball, we have ways of unraveling it, copying the DNA which is necessary for protein synthesis and then kind of rewinding it and that kind of falls apart sometimes and then there are other things that can be external influences that cause DNA damage, DNA breaks, and there are ways to fix that too. And then, there is this whole thing with familial syndrome that happens to involve DNA repair. So, can you just give us any idea of the kind of the whole scope of the DNA repair thing, just beyond BRCA, like how often is this happening in the cells, is it like does this happens every minute, every day, what do we know about the overall process of DNA damage and repair.

Jensen Right. So, it is happening on an enormous scale, which is a little bit scary to some of us, especially those of us who studied DNA repair. So, it is happening in every cell in our body, a hundred to a thousand times a day our DNA is getting damaged, and all of these different repair pathways have evolved over time to repair these specific types of damage. One type of DNA damage externally from the environment people think about is UV damage from the sun. So, that will cause...

Hochster We talked about that last week...

Jensen Right. So, it is important for melanoma and other types of cancers.

Hochster Skin cancers...

Jensen Yes. So, that is why a lot of dermatologists recommend stay out of the sun completely from 10 a.m. to 3 p.m. during the day, wear sunblock, wear sun protection clothing because what happens is the UV actually creates what are called pyrimidine dimers between the DNA, and if these are not repaired correctly, what can happen is that these can result in mutations, and the mutations are bad, right? So, if you get mutations in the right set of genes that control for instance let us say the ability of the cell to divide and grow, so these can lead to a normal cell going down this path to becoming a tumor cell because the wiring sort of gets crossed in the cell and it is the tumor cell starts to proliferate out of control, it starts ignoring its other cellular neighbors and ignoring signals to stop growing and this is what forms the tumor.

Hochster So, that is like one of the key concepts that the DNA is always getting damaged and repairs itself, but sometimes the wrong kind of damage can lead to cancer.

Jensen Exactly. If there is some failure in the DNA repair system; for example, if you carry a mutant copy of the BRCA-2 allele, then you are somewhat compromised for that repair pathway and eventually over time, we think that this leads to the mutations that accumulate in our genome leading to cancer.

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Hochster So, we talked about skin cancer, that is one example. I guess with the BRCA genes, you could land up with breast cancer, pancreatic cancer, ovarian cancer, many different kinds of things. What are some of the other DNA repair conditions that lead to cancer that you can tell us about.

Jensen Right. So, one thing I have always found interesting about DNA repair pathways and the proteins and the genes that make up each of these pathways is that a lot of these sort of rare, inherited predispositions to cancer are caused by mutations in these various DNA repair genes, and it turns out a lot of them are specific for certain organs and tissues, like BRCA-1 and BRCA-2, why is there this preponderance of breast and ovarian cancer. I mean, you can get other cancers like pancreatic and prostate, but there seems to be a lot of selectivity for those particular organs and tissues. Another repair system called the mismatch repair system, which happens when the wrong bases get paired together. These proteins called mismatch repair will recognize that and then basically excise the damage and put in the correct DNA base, and if you have a mutation and a mismatch repair gene, that can predispose you to for instance hereditary colon cancer. And there is a lot of these other rare genetic diseases and various nucleotide excision repair for repairing the damage from the sun, there are these rare genetic diseases like Li-Fraumeni syndrome and xeroderma pigmentosa, where actually these kids, especially in South America who cannot even go into the sun, like they cannot go out during the day because they have a such high risk for melanoma and other skin cancers, they are called children of the night.

Hochster So, probably the most common thing around here would be either the mismatch repair or BRCA. And the mismatch repair enzymes problem really came to light because of research on families that had early development of colon cancer and some geneticist, Henry Lynch at Creighton University in Omaha began to describe these families that had colon cancer in multiple generations and people would get it younger than age 50. So, that eventually lead to describing the family but the biology showed the problem was mismatch repair enzymes, which we know are very active in the intestines, so somehow those protect your GI cells from developing cancer, and we know when you have a break in that, you actually land up with little pieces of DNA. They can actually test the DNA for the size of the DNA in the cells and when you have this defect, you get something called microsatellites, which are tiny pieces of DNA, so that is an example that is kind of similar to the BRCA where biology and the genetics kind of met up to describe the DNA repair problem. So, this is something that is probably, these 2 are the most common cancer-causing familial syndrome in our part of the world at this time?

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Jensen Yeah, that is exactly how the BRCA genes were discovered. So, we work on BRCA-2, obviously there is also a BRCA-1, and the way BRCA-1 was discovered back in 1990 actually was Mary-Claire King was an investigator at UC Berkeley and she was looking at all of these families, so women in what is called pedigree analysis, where all these women who are related and families coming down with breast or ovarian cancer, and she was trying to track down the location of the BRCA-1 gene to a specific region on our chromosome, and she eventually found it, and at the time, I think a lot of cancer researchers thought that it was a little bit crazy. They thought, well cancer is probably caused by multiple genetic mutations, cannot be caused by just a single mutation. But what they did not realize at that time is that BRCA-1 and also BRCA-2 which was discovered a couple of years later, they are sort of master control genes, so we call them caretaker genes because they are actually controlling the rate of genome stability of these mutations throughout the whole genome. So, that is why if you carry mutation in one copy of the BRCA-1 or BRCA-2 genes, you start to accumulate these mutations, eventually leading to this high risk for cancer.

Hochster So, people at that time did not really know that it was going to involve DNA repair.

Jensen They did not know. At the time the gene was cloned and eventually sequenced, they had no idea BRCA-1 and BRCA-2 were involved in the DNA repair. So, it took a lot of basic research, working in the lab, you know lots of late nights, weekends for many years to actually understand that BRCA-1 and BRCA-2 are involved in this specific repair pathway called homologous recombination, which we can talk about more later and then that is really what these genes are involved in. But at that time, they had no clue.

Hochster It is not like just one mutation in the BRCA, there are a lots of mutations, there are some very older ones, they call founder mutations, what does that mean?

Jensen Right. So, the mutations, there is no what we call particular hotspots, so there is no one specific site that is always mutated, that always results in this high risk for cancer. The mutations in the DNA that code for BRCA-1 and BRCA-2 are spread throughout the whole gene. So, there are particular founder mutations, like you talked about, what you find in certain ancestries like Ashkenazi Jewish have a particular mutation in BRCA-2 that is very common and there are some tests that are for instance less expensive than sequencing the entire BRCA-1 or BRCA-2 genes and look for that particular mutation that has been passed down from generation to generation. And actually that brings up an interesting point because one of the interesting things we are studying in my lab is what are called these variants of uncertain significance. And so, these are really a result of all of the sequencing that we have been doing for the past 15-20 years of the BRCA-1 and BRCA-2 genes.

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Jensen We have been sequencing these 2 genes probably more than any other genes in the human genome, and while a lot of the genes, the mutations we find in BRCA-1 and BRCA-2 are some of these common founder mutations, there are a lot of these variants of unknown or uncertain significance where we do not really know what the impact of that variant is on that person's particular future cancer risk or if we find it in their tumor, are they going to have a defect in homologous recombination, which we might talk about later PARP inhibitors as a therapeutic strategy for treating their tumors.

Hochster So, when a geneticist or a physician see somebody they think has BRCA, if they order the simple test, they are just going to look for the common mutations and that might come back negative if you have a different kind of mutation that is the common ones. So, these tests are not comprehensive then, they have to request sequencing.

Jensen Right. So I think that is sort of the danger of some of these companies like 23andMe who for a while were just testing 1 or 2 or a few mutations in the BRCA genes, which could give you a false negative, sort of sense of security that "oh! I don't have a BRCA-1 or BRCA-2 mutation according to 23andMe," but if you are actually to sequence base by base pair of the entire gene, you might find the mutation that actually is what we call deleterious or pathogenic, which could result in your risk for cancer.

Hochster So, I mean just for people out there who are getting tested, the basic test is kind of like looking for common misspellings. It is kind of like really cheap spellchecker that looks through every page for when you reverse the I and the E kind of thing and you are just looking for that, but if you really want to know, if you still think somebody has got a BRCA mutation and they do not have that, then you need to like go through letter by letter for the whole book and look for every kind of misspelling error, and that would be called the sequencing of the whole DNA in that gene.

Jensen Exactly. And that is what some of these companies like Myriad Genetics offer to patients to sequence the entire BRCA-1 and BRCA-2 genes.

Hochster But even with Myriad, the standard test, they do not go automatically to full sequencing. They kind of screen it first.

Jensen Yes, that is my understanding.

Hochster Okay, well, thanks for that very interesting discussion, Ryan. We are going to take a short break for a medical minute. Please stay tuned to learn more information about DNA repair and BRCA genes with Dr. Ryan Jensen.

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targeted oncology medicine that address unmet needs. More information at astrazeneca-us.com. Genetic testing can be useful for people with certain types of cancer that seem to run in their families. Patients that are considered at risk for a familial or a hereditary cancer receive genetic counseling and testing, so informed medical decisions can be based on their own personal risk assessment. Resources for genetic counseling and testing are available at federally designated comprehensive cancer centers such as Yale Cancer Center and at Smilow Cancer Hospital. Smilow's Genetics and Prevention Program is comprised of an interdisciplinary team that includes geneticists, genetic counselors, physicians and nurses who work together with the goal of providing cancer risk assessment and taking steps to prevent the development of cancer. More information is available at YaleCancerCenter.org. You are listening to WNPR, Connecticut's public media source for news and ideas.

Hochster Welcome back to Yale Cancer Answers. This is Dr. Howard Hochster and I am joined tonight by my guest Dr. Ryan Jensen, and we are discussing DNA repair and cancer. So, Ryan, this seems like kind of a very interesting work but kind of a very particular kind of area to focus in on so closely. Can you tell us a little bit about your background and how you got interested in research on BRCA and DNA repair.

Jensen Yeah, sure. So, I began my research career as an undergrad. Actually, my senior year at Berkeley and I finally got into a lab doing independent research in Steve Martin's lab, not the comedian but the cell biologist.

Hochster Right, I was going to say that, "Laugh a minute."

Jensen So, Steve Martin, he was actually one of the pioneers in understanding the SRC gene, and the SRC is this funny oncogene that actually Harold Varmus and Michael Bishop won the Nobel Prize across the bay at UCSF. And it was actually they discovered it was a chicken virus gene that caused a particular type of cancer and Steve Martin discovered a particular mutation where he had actually induced it by shifting cells from a lower temperature to a higher temperature and found that the cells became transformed or became cancer-like in vitro, so in a petri dish. My first project was to examine the SRC gene, try to understand how it transforms normal cells into tumor cells. So, I was kind of in this whole field of what is called oncogenes, they are called kinases, so these proteins phosphorylate other proteins and they tend to drive cells to grow and proliferate in the absence of exogenous signals.

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Hochster So, oncogenes are genes that make proteins that turn on other genes and sometimes they are stuck in the on position which can cause cancer.

Jensen Exactly. So, you know people use the example where is accelerator on a car, it is like the oncogene and basically push down the accelerator, go through all the red lights and just push forward cells, keeps proliferating the tumor, keeps

proliferating, or on the flipside there are these tumor-suppressor genes which are thought to restrict cell growth that put on the brakes. And so you can get mutations in both tumor-suppressor genes and oncogenes that can drive tumor cells to keep getting growing and larger and larger. And I stayed in that field for a couple more years, actually I worked at a biotech company in San Francisco area called Sugen working on kinases. But then, one day actually I started reading about DNA repair and getting more interested and my wife actually found a job for me at Stanford as a tech and I knew an assistant professor's lab who was studying a funny disease called ATM, which is not automatic teller machine, and it turns out it is ataxia telangiectasia mutated and it is one of these rare genetic predisposition diseases where the patients eventually get a whole slew of different types of cancers.

Hochster It's kids, the pediatric disease.

Jensen Yeah, and it is very rare, and it turns out ATM, even though the disease is very rare, like only 1 in 100,000 people may come down with this disease in the US; however, it turns out the ATM gene itself is actually mutated in a lot of what we call sporadic cancers, so cancers that are not inherited, caused by a random assortment of mutations in these oncogenes and tumor suppressor genes. So, what was interesting is we actually learned a lot overall about cancer from some of these rare inherited predisposition genes that are involved in DNA repair.

Hochster So, what you are saying is that sometimes people have an inherited defect in a gene that leads to cancer, like you say ATM things, but much more commonly you just get it by living every day and something goes wrong with your gene and that is what we call an acquired gene mutation, and we see that a lot in many kinds of cancers.

Jensen Exactly. So, all these environmental insults – breathing the oxygen we breathe, eating the tasty red meats that get barbecued too much and all these byproducts, they damage our DNA. These things just start to accumulate. And we know that cancer is really a disease of age, right so the elder you are, the higher your risk goes up, and so having mutations in these DNA repair genes just really increases that rate of mutation and increases your risk of eventual cancer. So, that is how I got interested. And then, I came to Yale actually for graduate school, working in Peter Glazer's lab, working on another sort of complex repair pathway called non-homologous end-joining and cisplatin, which is a very common chemotherapy drug for a variety of tumors in fact, Lance Armstrong was cured by using cisplatin. And so, I was trying to understand the mechanism of how cisplatin works because a lot of these chemotherapy drugs have been around for decades, but we still do not fully understand mechanistically how do they actually work on a cell, and it turns out cisplatin damages DNA and a lot of chemotherapy drugs and radiation therapy work because they damage DNA and that kills the tumor cells.

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Hochster So, it is a little unusual that you are working here in a department of therapeutic radiology, which is our radiation oncology department, people who work on DNA repair are usually in many other different kinds of departments like molecular biology, cell biology, whatever, you can probably articulate a few more, but that is a little unusual that we have a big focus on that here.

Jensen Yeah, I think one of Yale's strengths is actually DNA repair. So, our department, a lot of investigators in therapeutic radiology are studying different DNA repair mechanisms, how do they play out in cells, how can we develop novel strategies to create more targeted therapies, how can we identify patients who may be more amenable to some of these new synthetic lethal strategies like PARP inhibitors, so we are kind of studying it at every level from the most basic level, basic science all the way to translational studies to how can we come up with new biomarkers and new therapeutic strategies for targeting cancer.

Hochster And I should add just for everybody's understanding that one of the reasons that radiation oncologists are interested in this area is because that is basically how radiation works, it is high energy photons that interact with DNA and cause various kinds of DNA breakage and damage. So, this is an underlying mechanism of radiation therapy. Anyway, so, getting back to BRCA, so now we kind of understand that involves this protein that controls homologous repair and there is a drug for it.

Jensen So, one way to understand what a gene like BRCA does is to actually isolate the protein, so purify that single protein out of all the other thousands of proteins in the cell, isolate it in a test tube and do what is called biochemistry to understand exactly what is the mechanism, what is it that the BRCA-2 does, does it bind specifically to damage DNA, does it recruit other DNA repair proteins to the site of DNA damage and then those proteins repair the DNA, what exactly is it doing. And it turned out to be pretty difficult.

Hochster So, purifying it was a little bit like hunting for the needle in a haystack?

Jensen It was. So, pulling the single protein out of all the other thousands of proteins in the cell was much more difficult than I had anticipated. So, I had not had a lot of experience in purifying protein, so probably be naïve, I thought "oh! this is definitely doable" during my post doc work. And so, I set out to do this and it took about 4-5 years to purify the BRCA-2 protein, and I spent most of that time in what we call a cold room which is at 4 degrees Celsius because once you break open the cells, proteins like to be cold. It sort of slows down their degradation, it slows down all the processes that breakdown proteins, and so, most of the work is done in this cold room which was in California was good training for moving back to New England.

Hochster I imagine, you could have gone all the way to further north, Canada.

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Jensen Yeah exactly. So, eventually after spending enough time in the cold room and running these various what we call column chromatography, different columns with different chemistries that bind to the protein, I came up with a strategy to purify the human BRCA-2 protein. And once I was successful in doing that, I could then do all these biochemical assays with purified DNA substrates and other purified proteins to basically work out exactly what it is that BRCA-2 does and that is what we succeeded in doing. My lab here at Yale continues to work on BRCA-2 to figure all its mysteries.

Hochster And what is the connection with this enzyme called PARP.

Jensen Right. So, the PARP inhibitors were discovered actually back in 2004 and what was discovered was that they selectively kill tumors in patients who have BRCA-1 or BRCA-2 mutations, and now that we know more about how they work, it actually turns out any tumor that has a defect in this pathway called homologous recombination for repair of DNA damage are sensitive to these PARP inhibitors. It is a little bit complex, and the term that we like to throw around as biologists, we call it synthetic lethality. And so what a PARP inhibitor actually does is it blocks another repair pathway called base-excision repair, at least that is what we think about it how it works now. And in these tumor cells, they are actually crippled for repair by homologous recombination because they have these mutation of BRCA-1 or BRCA-2, and by the PARP inhibitor knocking out this other pathway, this base-excision repair pathway, the tumor cell basically has no recourse for repairing that DNA damage and eventually it dies.

Hochster So, like if I95 has a traffic accident, you got to take US1, that is the PARP pathway, and the inhibitors block US1 also and the cell dies then.

Jensen Exactly. In other way I think about it is, it is like hanging onto a cliff, right, and so if you are the bad guy in a movie and it is the end of the movie and the good guy, he stomps on one hand and you are hanging on with one hand to the cliff and then he stomps on the other hand, and that is basically PARP inhibitor, that is the 1-2 punch, and basically, it turns out that tumor cells are actually pretty stressed out and they are crippled for some of these repair pathways and just by knocking out this other pathway, you can basically push them over the cliff and kill them.

Hochster So, really it is not directly involving BRCA-2, it is involving another pathway that the BRCA mutation makes more essential. Jensen Right. It is like a backup or a redundancy pathway.

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Hochster And so these drugs are actually now approved. There is one that is actually approved, Lynparza or olaparib for ovarian cancer for I think 2 years already and now just for breast cancer if you have a BRCA mutation, we are actually doing a study here at Yale for pancreatic cancer, very similar to the

other ones, what they found was that if you get chemotherapy for ovarian cancer and then after a few cycles, you take this drug, compared to not continuing chemotherapy, people live 3-4 times as long and it is much less toxic than chemotherapy, it is really pretty amazing for those patients.

Jensen That is the other benefit I think of these PARP inhibitors as going forward, we are trying to come up with more targeted selective therapies that have less of these side effects of these chemotherapy drugs and they have been around for decades.

Hochster So, what kind of new things are you working on related to DNA repair.

Jensen Right. So, we are still heavily focused on BRCA-2 and we want to understand more about what the protein looks like, so there is not a whole lot of structural information as to what the entire BRCA-2 protein looks like, so we are working towards that goal, so we need to purify a lot more of the protein to get what is called an x-ray crystallography picture of the protein or perhaps Cryo-M which is we have a new Cryo-M facility at the West Campus here at Yale. So, that is one avenue that we are pursuing. We are also interested in trying to understand if we can develop certain biomarkers to identify some of these homologous recombination deficient tumors, and I think to really get those biomarkers which are going to be important going forward for deciding how to treat patients, we really need to understand the basic mechanisms of homologous recombination and all the proteins and pathways that are involved could be potential biomarkers.

Hochster So, like this ATM thing we talked about before, there may be other kind of minor deficits in DNA repair that we can identify and pick out the best treatment for patients.

Jensen Exactly, and something that is amenable to taking like a tumor biopsy and you can grind it up and then identify some protein or some metabolite that says "Oh, yeah that cell is definitely deficient for homologous recombination repair pathway, so let's treat that patient with a PARP inhibitor."

Dr. Ryan Jensen is an Associate Professor of Radiology at the Yale School of Medicine. If you have questions, the address is canceranswers@yale.edu and past editions of the program are available in audio and written form at YaleCancerCenter.org. I am Bruce Barber reminding you to tune in each week to learn more about the fight against cancer. You are on WNPR, Connecticut's public media source for news and ideas.