Megan, maybe we can start off with you telling us a little bit about yourself and how you got involved in this research project to begin with. Yeah, so it’s very interesting thinking back to what drew me towards science. I’m from a family of engineers, actually including both of my parents, but I always gravitated towards science, and in particular as a high school student, I took anatomy and physiology, and it was actually the
section of my textbook on cancer that really provided for me, I think the first kind of window into how a scientist could have a positive impact on human health in a way that was different from becoming a medical doctor, which I think all of us are a little bit more familiar with, certainly as children. And so I’ve been reflecting on that recently because it’s been a bit of a circuitous route that’s brought me back to Cancer Research. I really trended towards very fundamental kind of basic science. Questions for my initial training as an undergraduate and graduate student and even into my postdoc period where one typically is defining the kind of areas of research that they will pursue, and in their independent laboratory. But I discovered a connection between the cell biology of the nucleus, which is something that I had been training with Gunter Blobel at Rockefeller University in Genome Integrity, so that is the mechanisms that maintain the DNA blueprint as it should be and that was
really just something that I had not focused on before but it really changed the direction of my research and I became very interested in how aspects of how a cell works, are able to contribute to the mechanisms that maintain that genetic code. So tell us more about that. I think some of us can remember back to junior high biology where we kind of know what a cell is. And we know what a nucleus is and housed within that nucleus is the DNA which is responsible for that genetic blueprint as you say. So tell us more about the connection that you found between how a cell functions and genomic integrity. Yeah, so I was also fascinated with this idea of the the nucleus which is the organelle that houses the DNA, being kind of the brain. Having all of the kind of control and that plan for the cell, but I think one of the things that maybe isn’t always captured when we kind of make that diorama during school is that actually it’s not just a big ball of yarn, but actually the DNA has lots of different
regions and these regions are important for different aspects of that blueprint. And they’re not all created equal. There are specific regions of the DNA that are far more prone to damage.

And there are also mechanisms to repair that damage that may be quite specific, so if you have a leak in a pipe you may need a plumber, right? But if you’re siding has gone downhill, maybe you need someone who is more like a Carpenter. Or for any new paint you’re going to have a different kind of approach depending on what the issue is.

And it turns out for cells, that’s similar. There are actually different DNA repair mechanisms and you really need to use the right mechanism for the right kind of damage, and it turns out that much of that is actually dictated by these different flavors of the regions of DNA and physically where those different regions of the DNA blueprint are organized inside the nucleus, because it’s a much more compartmentalized kind of network.
than when we just again think of this string that has all of that coding material, so it’s not just where the break occurs in the DNA or what kind of a break it is, whether it’s a single strand break or a double strand break, but where exactly it’s located within the nucleus.

We think about two components. One exactly as you say physically, where is that DNA break in the nucleus? And then there’s also the other attributes of the DNA. So DNA doesn’t live on its own. It’s actually wrapped up and packaged around proteins that are called histones and this packaging is really important for whether a particular gene may be expressed or not. It turns out that a DNA break in a region of the genome that is coding for a protein, so it’s going to be transcribed into the messenger RNA and then translated into a protein. Those regions of the genome are a bit different than regions of the genome that may be silent, and so that also just leads to both some
challenges for DNA repair mechanisms and also some activities that may actually make it more prone to the accumulation of DNA damage. And so we think of both where the break is physically, and also where it is in context of what else is happening in that region of the DNA.

So we know that DNA can incur various forms of damage that can be in coding regions or in non-coding regions. How does that then evolve into your research with cancer? So initially as I mentioned our interest was the idea that these different locations in the nucleus might be important for making sure that those breaks are repaired by the right process and in order to study that we really need to be able to watch DNA repair in a cell that’s living while it’s happening and that as it turns out is actually quite a difficult problem, and so over the past ten years or so, one of the things that my group has invested in, is building so called assays where we can actually watch a single DNA break, which we actually control.
So we induce the break to occur in exactly the place where we want it to, and then we actually follow the repair of that break in real time and once we built this system, we became very interested in how we might leverage it to answer some important questions that were really arising in the field of cancer treatments. And really, I was driven towards those questions through my interactions with my fantastic colleagues here at the School of Medicine and at Yale Cancer Center who really brought a way of connecting the kind of questions I had become interested in, again as a postdoc and kind of just looking through the microscope to where we had a real need to understand specific questions in the field of DNA repair, and particularly those that were relevant to the kind of therapies that might be used in the context where patients have defects in DNA repair within their tumors. So first question, how exactly do you watch DNA being repaired in real time? I’m kind of blown away by that concept.
I remember back in junior high biology looking down a microscope at a cell and looking at the nucleus. And sometimes you could even see DNA separating into mitotic figures and so on. But to actually see DNA being repaired? I mean presumably that occurs at a base pair level and that’s just fascinating to me.

So how exactly do you do that and what kind of magnification would you need even to see that? Yeah, that’s a great question and honestly, this is why I’m a cell biologist at the end of the day because we love to just look. If we have a way we can look at something happening in real time that’s always the best thing in the world. However, as you say, it’s not easy and so our work is built on really critical discoveries that have driven cell biology, in particular, and I’ll just tell you about two of those that are critical for the assays that we’ve built. The first is the advent of these fluorescent proteins. Green fluorescent proteins and red fluorescent proteins. Now we have an entire rainbow of these fluorescent proteins, and so these are proteins that...
fold up and they’re able to make what’s called a chromophore and we can actually follow that specific molecule in a microscope. And what we do is we basically stitch that fluorescent protein onto a protein that we’re interested in, and now we can follow our favorite protein of interest in a live cell on a fluorescence microscope that can specifically detect that fluorescent protein, and so that’s one technology that’s absolutely critical. The other, I think this really speaks to the importance of kind of basic science discoveries and what really has impacts on human health these days is that we use tricks to insert a region that’s actually taken from a bacteria, so it’s not native to the cells we are modifying, and we essentially take that little sequence, and we put it into the place in the genome we’re interested in and then we have a protein that can bind to that very specific DNA sequence, and so we can monitor any kind of region of the genome that we want.
0:10:20.021 → 0:10:22.87 just by doing a little bit of editing
0:10:22.87 → 0:10:25.114 to that genome and putting these
0:10:25.114 → 0:10:26.862 in bacterial gene sequences
0:10:26.862 → 0:10:28.234 into our eukaryotic cell,
0:10:28.24 → 0:10:30.976 because that’s what we want to be studying.
0:10:30.98 → 0:10:33.038 In terms of the magnification,
0:10:33.04 → 0:10:34.129 you’re absolutely right.
0:10:34.129 → 0:10:37.197 We are able to do a pretty good
0:10:37.197 → 0:10:39.017 job following these events
0:10:39.02 → 0:10:40.656 even with a magnification,
0:10:40.656 → 0:10:43.504 usually between 100 and 1000 fold over
0:10:43.504 → 0:10:46.104 what you could see with the naked eye.
0:10:46.7 → 0:10:49.493 Wow, so essentially you can clip the
0:10:49.493 → 0:10:52.448 DNA where you want to make a break.
0:10:52.45 → 0:10:54.742 Insert a bacterial strand of genetic
0:10:54.742 → 0:10:56.85 material, flag it with a particular
0:10:56.85 → 0:11:00.228 flag so you know where the break is and
0:11:00.228 → 0:11:02.598 then have these chromophores which can
0:11:02.67 → 0:11:05.47 light up when they approach that break.
0:11:08.77 → 0:11:10.642 That’s right, so another critical aspect is
0:11:10.642 → 0:11:13.337 we have to know a lot about DNA repair,
0:11:13.34 → 0:11:14.548 and fortunately,
0:11:14.548 → 0:11:16.723 DNA repair has been a really rich
0:11:16.723 → 0:11:18.529 area of research for many decades,
0:11:18.53 → 0:11:20.679 and so building again on the knowledge
0:11:20.679 → 0:11:22.779 of many others we know pretty well
0:11:22.779 → 0:11:25.152 about the kind of timing and the events
0:11:25.152 → 0:11:27.066 that are taking place and repair.
0:11:27.07 → 0:11:29.198 So protein X shows up,
0:11:29.2 → 0:11:31.64 and it always shows up before protein Y.
0:11:31.64 → 0:11:32.864 And as you said,
we want to know what’s happening at the base pair level, like the smallest unit of DNA. We can’t really see something small in this assay, so we’re using proxies of factors that we know will show up at different points and that allows us to essentially monitor distinct events, because if we build up our library of these different flags that indicate different times and repair them more able to monitor those events, and we’re also able to monitor them in single, and it’s turned out that that’s really important. Because if we look at a million cells doing something they all kind of do it on a little bit different time over a little bit different time, then the cell next door and so by actually watching these events in single cells, that really gives us a resolution that’s really important for being able to make very mechanistic conclusions from the data. So we understand that you’ve got DNA that can get injured and it can
get injured in a variety of ways at a variety of places, each of which requires a specific mechanism to repair it. And we now understand that you’ve built this model to kind of see how DNA repairs itself over time, so tell us more about how this gets into cancer and into therapeutics And we’ll have to do that as soon as we take a break for a medical minute. So please stay tuned to learn more about DNA repair and cancer with my guest Doctor Megan King. Fund for Yale Cancer Answers comes from AstraZeneca, working to eliminate cancer as a cause of death. Learn more at astrazeneca-us.com. Breast cancer is one of the most common cancers in women. In Connecticut alone, approximately 3500 women will be diagnosed with breast cancer this year, but there is hope, thanks to earlier detection, noninvasive treatments and the development of novel therapies to fight breast cancer. Women should schedule a baseline mammogram beginning at age 40 or earlier if they have risk factors associated with the disease. With screening, early detection,
and a healthy lifestyle, breast cancer can be defeated. Clinical trials are currently underway at federally designated Comprehensive cancer centers such as Yale Cancer Center and Smilow. Digital breast tomosynthesis, or 3D mammography is also transforming breast cancer screening by significantly reducing unnecessary procedures while picking up more cancers. More information is available at yalecancercenter.org. You’re listening to Connecticut Public Radio.

Welcome back to Yale Cancer Answers. This is doctor Anees Chagpar and I’m joined tonight by my guest doctor Megan King. We’re talking about DNA repair and cancer, and right before the break we had gotten to the point in the story where we were talking about the fact that DNA gets injured and it can get damaged in a variety of places. And each of these breaks may be specific and may require a specific mechanism to repair it and we also talked about the fact that Doctor King’s laboratory had figured out a way to
actually watch how DNA gets repaired.
right under a microscope,
which was just fascinating.
But now Megan,
maybe you can help us to understand
how this really evolves into
understanding a little bit more
about cancer and therapeutics.
We built the capability now of
monitoring DNA repair and these single cells.
And now we get to the point
in a basic scientist life where you
think about, I've built this assay,
it took us many years to do it.
What do we want to study?
And it’s about this time that I had
been interacting increasingly
with members of Yale Cancer
Center and hearing about their
work in the clinic and their work
that is more translational.
So that’s when we kind of apply basic
science and fundamental principles,
directly to new treatments.
And through these interactions we became
very interested in how we might use this
assay to answer a question that has arisen
that was clearly critical to the treatment
of breast and ovarian cancer that is
tied to this familial cancer susceptibility
genes BRCA one and 2.
I always have a soft spot in my heart for BRCA 1 because it was discovered by Mary Claire King. No relation but we have the same initials and last name and in fact over the years I’ve gotten emails intended for Mary Claire King. So we’ve struck up already a kind of back and forth just because of people getting us mixed up. And so BRCA one really had become a success story of an approach called synthetic lethality. And so the idea is that BRCA one is very important, particularly in a type of DNA repair called homologous or combination and in individuals who have a loss of function and BRCA one, this leads to an increased susceptibility to breast and ovarian cancer in women. And so you are probably quite familiar with this because it’s become very well known. And it’s also well known even on the scientific front because of the advent of a therapy which is called PARP inhibitor therapies that specifically kill tumor cells that are defective in the functions of BRCA one or two, and actually more broadly in DNA.
repair through this mechanism called homologous recombination. And so this is fantastic. What does that mean for a patient? It means that all of their normal tissues can tolerate these drugs. They really only attack the cells that don’t have functional DNA repair. So DNA repair is this kind of double edged sword, on the one hand, a defect in DNA repair can lead an individual to be vulnerable to developing a cancer. But if the cancer is defective in DNA repair, it also opens up a window for therapies and PARP Inhibitors were something that could kind of fit into that window, so this was really a very exciting time and continues to be a really new approach to treating cancers that are tied to homologous or combination defects which we now know include a number of contexts that do not involve just BRCA 1 and 2. However, we also knew quite early on that these patients would often have acquired resistance to the PARP inhibitors.
They would initially respond very well, but the response would not be as durable as they and their physicians would like it to be, and investigators had gone in to try to ask how is it that these tumors are evolving, essentially, to become resistant to PARP inhibitors, and particularly in the case of BRCA 1 they found that there seemed to be secondary loss of other repair factors that were involved and we became excited about the potential of our assay to maybe provide some insight into how is it that these tumors are getting around this therapy, and even more importantly, might there be ways that we could actually target these cells again? So kind of re-sensitize them to PARP inhibitors, and so we modeled these mutations, so that cells no longer express a number of other factors called 53BP1 on a complex called shieldin. And somehow this allows cells that don’t have functional BRCA one to still survive in the presence of PARP inhibitors, and so we investigated those using...
this assay and we discovered that the loss of these factors that drove this PARP inhibitor to no longer work were affecting DNA repair in a very specific way by unleashing a DNA repair factor that really shouldn’t be functioning and this is a protein called the bloom’s helicase and it was able to kind of step in for BRCA one when these other factors are silenced and take over and so in a sense that seems like a bad thing, some other protein can come in and take the place of BRCA one, but it turns out one of the things we learned in our experiments was that this activation of this bloom’s helicase brought along, and it’s actually now this angle that we’re targeting, with the idea that there will be new combination therapies that will re sensitize these tumors to PARP inhibitors in combination with either inhibitors of the bloom helicase itself, but also some other additional treatments that have already been being pushed forward. Things like the DNA damage checkpoint, which is something that acts
downstream of unresolved DNA damage, so we’re pretty excited that these kind of very fundamental insights from this assay that I’ve described are really leading us to consider new combinations of drugs that may allow for not necessarily to make the PARP inhibitor but be a good therapy on its own for longer, but how we might use combinations that will allow for a very durable response for these patients. Let me make sure that we’ve got that straight for all of our listeners. So normally everybody has functional BRCA but when you have a mutation in that no longer becomes effective and the function of that BRCA gene is really to repair DNA because DNA we have in all of our cells and sometimes it can just get damaged and BRCA actually forms is a very important gene that can help us to repair that DNA, but when that’s defective we get cancers. But these PARP inhibitors are very effective against tumors that have DNA damage that is not being repaired by BRCA. But then you’ve got this bloom helicase which can step in for BRCA. It’s almost like a fail
safe kind of belt and suspenders
where if one repair mechanism doesn’t work,
then another repair mechanism can work,
but in cancer cells you really don’t want it to work.
So what you’re now doing is trying to find inhibitors to that secondary repair mechanism to ensure that the PARP inhibitors can kill off those cancer cells.
Yes, that’s exactly right, and it had been known for a while that there might be these two kind of parallel mechanisms to carry out a specific step in homologous recombination and indeed, it was known already that these two mechanisms existed, but actually we didn’t know very much about how a cell could decide to use one mechanism that would be this kind of BRCA one mechanism which works with this bloom’s helicase pathway, which as you said is kind of a backup mechanism.
One of the things we’ve discovered is that we think that the bloom’s helicase mechanism, although it’s a backup, it’s really not supposed to be working in normal cells, and that’s why there are a number of factors that keep it off and that
includes these proteins, the loss of which can drive PARP inhibitor resistance. So we think that actually there’s kind of a gain. We would call it a gain of function of the bloom’s helicase that underlies the PARP inhibitor resistance. Why might cells not want to be using this bloom’s helicase all the time? We think that it’s because actually it’s not a very well controlled enzyme, so its activity in the repair process kind of goes wild a bit. Even though this allows the cells to get around the PARP inhibitor, it actually may make them susceptible to additional targets that are being developed, and so we think just like a DNA repair defect opens up a therapeutic window, we think this kind of rewiring from BRCA one to the bloom’s helicase may also open up new ways that we could go about treating these tumors. So then the next question is, is there a way for us to figure out either upfront before we give any therapy whether a particular patient is going to have
0:24:46.572 -> 0:24:48.718 this bloom’s helicase turned on or not, 
0:24:48.72 -> 0:24:51.233 so that upfront we can decide whether 
0:24:51.233 -> 0:24:53.908 we should just give up our PARP inhibitor, 
0:24:53.908 -> 0:24:56.22 or whether we need to give dual 
0:24:56.22 -> 0:24:58.374 therapy or in a productive manner 
0:24:58.374 -> 0:25:00.83 where we can say, well, 
0:25:00.83 -> 0:25:02.714 if somebody hasn’t responded to the 
0:25:02.714 -> 0:25:04.99 PARP inhibitor as we would anticipate, 
0:25:04.99 -> 0:25:07.951 is there a way for us to figure out 
0:25:07.951 -> 0:25:11.048 if this is the mechanism by which 
0:25:11.05 -> 0:25:13.234 the cell is getting around that 
0:25:13.234 -> 0:25:14.878 PARP inhibitor and developing resistance 
0:25:14.878 -> 0:25:17.462 so that we can add in another drug. 
0:25:17.47 -> 0:25:19.717 Do we have those kinds of diagnostics? 
0:25:21.07 -> 0:25:22.072 You’re absolutely right, 
0:25:22.072 -> 0:25:24.919 this is exactly what we would like to have, 
0:25:24.92 -> 0:25:26.846 but we don’t have it yet, 
0:25:26.85 -> 0:25:29.418 so we would like to be able to take a 
0:25:31.562 -> 0:25:33.589 tumor sample and ask the question, 
0:25:33.59 -> 0:25:36.479 what is happening in this tumor? 
0:25:36.48 -> 0:25:38.085 Is this patient likely to 
0:25:38.085 -> 0:25:39.69 respond to the PARP inhibitor? 
0:25:39.69 -> 0:25:41.46 We know that if they have 
0:25:41.46 -> 0:25:43.22 a defect in DNA repair, 
0:25:43.22 -> 0:25:44.504 they’re likely to respond. 
0:25:44.504 -> 0:25:46.908 We know, as I told you, this bloom’s 
0:25:46.908 -> 0:25:49.32 helicase tends to go kind of overboard, 
0:25:49.32 -> 0:25:51.665 and we think that we can design 
0:25:51.67 -> 0:25:53.395 what we would call a 
0:25:53.395 -> 0:25:54.775 biomarker of that activity, 
0:25:54.78 -> 0:25:57.104 because it generates far too much of
0:25:57.104 → 0:25:58.79 this single stranded DNA generating
0:25:58.79 → 0:26:00.988 single strand of DNA is a critical
0:26:00.988 → 0:26:03.057 part of homologous or combination,
0:26:03.06 → 0:26:03.734 but again,
0:26:03.734 → 0:26:06.85 bloom’s helicase seems to do too much of this,
0:26:06.85 → 0:26:08.852 and we think that we might be
0:26:08.852 → 0:26:11.008 able to use proteins that bind
0:26:11.008 → 0:26:13.053 to that single stranded DNA,
0:26:13.06 → 0:26:14.059 kind of quantitatively,
0:26:14.059 → 0:26:16.39 and that may be an indication
0:26:16.452 → 0:26:18.706 that this is the mechanism by which
0:26:18.706 → 0:26:20.65 these cells elevated PARP inhibitors.
0:26:20.65 → 0:26:21.853 Another major mechanism
0:26:21.853 → 0:26:23.056 of PARP inhibitor resistance
0:26:23.06 → 0:26:24.69 are so called reversion mutations.
0:26:24.69 → 0:26:26.556 This is where there’s actually a
0:26:26.556 → 0:26:28.599 second mutation in the BRCA gene,
0:26:28.6 → 0:26:29.9 which essentially can reconstitute
0:26:29.9 → 0:26:30.875 its normal function.
0:26:30.88 → 0:26:31.762 In this case,
0:26:31.762 → 0:26:33.526 the tumor no longer has
0:26:33.526 → 0:26:35.119 a DNA repair defect,
0:26:35.12 → 0:26:36.704 and so we’d really like to
0:26:36.704 → 0:26:39.039 be able to tell is there a
0:26:39.039 → 0:26:40.655 reconstitution of normal repair.
0:26:40.66 → 0:26:42.568 But maybe that repair still has
0:26:42.568 → 0:26:44.58 some defects that we can target,
0:26:44.58 → 0:26:46.855 or is repair kind of totally normal,
0:26:46.86 → 0:26:48.883 in which case we know we’re going
0:26:48.883 → 0:26:50.916 to have to think about another
0:26:50.916 → 0:26:53.106 type of therapy to treat that patient.
So these are in development and this is something we’re really interested in, particularly again with our colleagues here and at Yale Cancer Center. To continue to push forward by partnering with those clinicians who are running clinical trials in this space. In patients with BRCA or other homologous recombination defects who have been enrolled on PARP inhibitors and looking at those resistance mechanisms. And if we can develop these types of biomarkers. I mean it’s so fascinating thinking about the fact that when we started this conversation, we started by saying that you know DNA can be damaged in different ways and each requires a specific repair mechanism. But now thinking about how you’re actually taking your science and in a way kind of again, moving towards personalized medicine, figuring out, well, if somebody develops resistance, how exactly is that resistance? And how can we get around it?
Absolutely, and I want to highlight we can do this really efficiently in cells in a laboratory that’s never going to tell us about what is happening in individual patients. So really, this discovery requires the commitment of patients who’ve been enrolled on these clinical trials. That’s not an easy thing to ask of patients in this case. For example, they’ve signed up for serial biopsies of their tumor, but that is absolutely essential for us to continue to discover the mechanisms that are at play and for us to come up with better treatments.

Doctor Megan King is an associate professor of cell biology and of molecular, cellular, and developmental biology at the Yale School of Medicine. If you have questions, the address is cancer answers at yale.edu and past editions of the program are available in audio and written form at yalecancercenter.org. We hope you’ll join us next week to learn more about the fight against cancer here on Connecticut Public Radio. Funding for Yale Cancer Answers is provided by Smilow Cancer Hospital and AstraZeneca.