

Yale CANCER CENTER *answers*

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The Human Genome and Cancer

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Yale Cancer Center Answers

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Welcome to Yale Cancer Center Answers with your hosts doctors Francine Foss and Anees Chagpar. Dr. Foss is a Professor of Medicine in the Section of Medical Oncology at the Yale Cancer Center and she is an internationally recognized clinician and clinical researcher. Dr. Chagpar is Associate Professor of Surgical Oncology and Director of the Breast Center at Smilow Cancer Hospital at Yale-New Haven. Yale Cancer Center Answers features weekly conversations about the most recent advances in the research diagnosis and treatment of cancer and if you would like to join the conversation, you can submit questions and comments to canceranswers@yale.edu or you can leave a voicemail message at 888-234-4YCC. This week you will hear a conversation about Human Genome Research with Dr. Narendra Wajapeyee. Dr. Wajapeyee is an Assistant Professor of Pathology at the Yale School of Medicine. Here is Anees Chagpar.

Chagpar Narendra, we hear a lot about cancer and we hear a lot about the genome, help us to understand what these are and how they are connected?

Wajapeyee As some of you will know, each human cell has 23 pairs of chromosomes, all the chromosomes look the same between male and female except there is a difference between X and Y chromosomes. Females have two X chromosomes and males have one X and one Y chromosome, other than that everything is same. In cancer cells, there are specific changes that actually cause deletion of certain genes or amplification of certain genes in various cancers and that can actually change a lot in terms of what happens to the cell. For example, there is a class of genes called tumor suppressor genes and when they are deleted or their expression is lost the cell has no control in how long it can divide. Normally, a human cell will divide for certain numbers and after that they will stop growing, but cancer cells when they lose control through loss of tumor suppressor genes they can divide indefinitely and that is one of the issues, how the normal cells become cancer, that is one of the alterations. Another driving force for cancer cells is activation of oncogenes which is contrary to tumor suppressor gene as they actually are growth promoting genes, and oncogenes can be activated or increased in their activity by various methods, one is acquiring changes in its DNA which is called mutations and those mutations make them constitutively active, which means that they can function without any signal. Usually in a normal cell oncogenes will require some certain signal to function, but in cancer cells when they acquire mutation they, without any signal, can constitutively function and drive growth of cancer cells and oncogenes are very, very interesting in terms of therapeutic targets in several different cancer types like lung cancer where they have identified a mutation or several different mutations in a gene called EGFR, which is epidermal growth factor receptor gene. The mutation in this EGFR gene can be targeted in terms of pharmacological drugs so you can have a drug which will selectively bind to the receptor and block signaling and kill the cell selectively, so it provides an opportunity to personalize therapy to lung cancer patients and similarly this kind of treatment has been applied to other cancer types, for example, leukemia, specifically chronic myelogenous leukemia, or ALL, where they have acquired a translocation which is a fusion between two chromosomes which leads to a protein called BCR-ABL. It is based on the two genes which come together after chromosomal translocation and there are several drugs which have been very effective in killing cells which have BCR-ABL translocation, it does work very well in chronic myelogenous leukemia,

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it has limited success in acute lymphoblastic leukemias and also, other than these changes in genome there is something called epigenome. So it is not necessarily that all the time your DNA has to change, there are occasions where you can have no change in DNA, but the structure of the DNA changes because of what proteins it binds to or the proteins which are histone, they sometimes get modified and it will either compress or loosen the DNA which will allow expression of certain genes which are not usually present in normal cells and that also plays a role in cancer initiation and progression and those changes are called epigenetic alterations and those are also now being targeted using various therapeutic approaches.

Chagpar Let me make sure I have got this straight. I have often heard of cancers being thought of like a car that has an accelerator and it has got a brake, so the tumor suppressor genes are kind of like the brakes, right?

Wajapeyee Yes, that is right.

Chagpar If you do not have a brake, you keep driving and you drive right off the cliff.

Wajapeyee That is right.

Chagpar And if you've got an oncogene, the oncogenes are like the accelerator pedal and so if the oncogene is on and you have got a stuck accelerator you are going to keep driving off the cliff. I get that, but explain to me how people figure out what are the oncogenes, what are the tumor suppressor genes, and where they are located, how many genes do people have?

Wajapeyee Based on a rough estimate humans have 23,000 reference genes.

Chagpar And so 23,000 genes, how exactly are you going to figure out which ones turn cancer on, which ones keep cancer off and how they are affected?

Wajapeyee Initially when all the studies for cancer started, everything started with karyotype, taking the cells of cancer cells and checking what is the karyotype for them. Most of the time they are still deployed or semi-deployed and there are some alterations in each of the chromosome, but in many cases you see sometimes multiple chromosomes, for example, rather than two, you have four, so this kind of polyploidy which is called a polyploidy phenotype is also found in cancer. This initially led to some identification of some cancerous specific alterations, whether they have polyploidy or if there are certain alterations which are very specific to cancer, for example, for CML. Whenever they analyze chronic myelogenous leukemia they always find this chromosomal translocation between chromosome 9 and 21, which was very obvious in all the cases and they identified it and called it a Philadelphia chromosome and when they mapped what actually comes together by this, is the fusion between a gene called BCR-ABL, ABL is a proto oncogene and BCR after fusing, can constitutively drive the activity of the ABL gene and once they identify that there is a specific chromosomal translocation they have mapped the fusions and once they identify

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where there is a chromosomal translocation and the chromosome break, they clone that particular region and once you have that you can actually test using various method using mouse models, using cell culture methods, you can now add it on the cells and see if it actually promotes leukemia formation, for example. So, if it is an oncogene, it promotes leukemia formation, there are several mouse models where you can take blood cells, which is hematopoietic stem cells specifically, and then introduced the BCR-ABL gene and then lethally irradiate the mice by which you can remove completely the bone marrow from the mice and then reimplant these cells with BCR-ABL and look for whether they found leukemia or not and it is found that when you have BCR-ABL in hematopoietic stem cell which are injected into the mice, similar to humans, they can form leukemia so that was a confirmatory evidence that is actually how most of the oncogenes are identified. The second approach is people have taken simple genomic DNA isolates and pregenomic DNA from tumors and then take cells, which are not cancers, but not normal either, so those are called immortalized, so this is a stage in between primary and cancer cells and using these immortalized cells when they add genomic DNA some of them actually become cancer-like, so now they isolate those cells and try to sequence what actually got into those cells what made them cancerous and that is how HER2 which is a breast cancer associated oncogene was identified.

Chagpar So, karyotyping which was the first way that you talked about is just looking at the chromosomes because the chromosomes all look different so you can tell which one is 9 and which one is 21 and when you have got a chromosome that looks like it is half number 9 and half number 21, you can say, well how come it is half and half, most people do not and that is how they were able to look at that fusion area and clone that and figure out that an activator turned on a gene from one chromosome to the proto oncogene on the other chromosome which causes cancer and that made it constitutively active.

Wajapeyee That is right.

Chagpar Explain to me these immortalized cells that are in between normal cells and cancers cell where do you find those? What exactly are these cells, where do you find them, and how do you then figure out how to make them immortal, because it sounds to me like what you are trying to do is you are trying to take a normal cell, but the next stage up is this immortal cell, and you are trying to make an immortal cell a cancer cell. How do you figure out what is an immortal cell?

Wajapeyee Let me step back a little bit and tell you what the normal carcinogenesis process is. First you have a normal cell which will become immortalized, then cancerous and then it spreads which is a process called metastasis. What happens is if you look at the end of the chromosomes there is a structure called telomere, these are long structures at the end of chromosomes that protects them from any bad effects on the chromosomes. If you take a mouse cell versus a human cell, mouse cells have very long telomeres at their ends, but humans are very short. So what happens is every time the cell divides because of a replication defect, the telomere length shortens every cycle so after a few generations, 60 or 70 normal cells actually lose their telomeres completely and that leads to a process, a stage called replicative senescence which is also aging. So it is a normal process for every cell to occur.

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Chagpar Are you telling me then that mice live longer than humans?

Wajapeyee Mice cells do not go through a replicative senescence, their cells do not age the way humans cells age by telomere maturation, it is a much more long drawn out process so it takes several generations, for example, mice usually telomere shortening takes six generations compared to human cells and that is why it is easier in cell culture or in a laboratory setting to make a cancer cell out of a mouse cell versus a human cell and it is also clear when you analyze human tumors, if you see a lung cancer you have at least 16 different genetic changes compared to a normal cell, before a normal cell can become a cancer cell there are lots of alterations. For a normal cell to become a cancer cell it has to bypass a process called replicative senescence, which is the growth inhibitory effect of telomere shortening. So, what happens in that context is most of the cancer cells, actually 90% of cancer cells activate an enzyme called telomerase, which is required for maintaining the telomere length. It is only present in the normal human setting in stem cells like hematopoietic stem cells and other stem cells, but not in normal cells, but when it is at the verge of becoming a cancerous cell, it acquires the activity of this enzyme called telomerase and due to that, now it can grow forever so it is an acquired phenotype which is called an immortal phenotype and can divide forever in the cell culture, but it still not cancerous, it is in between, it has acquired more changes, for example, benign nevus. These are immortalized cells that have divided to a certain extent, but due to some other changes they have not become, for example, melanoma. So it requires some other additional genetic and epigenetic change for them to become from melanocyte to nevus, to full blown melanoma, so it is an in between stage and most cancers actually have an in between stage.

Chagpar Getting back to the question of how you figure out where these genetic mutations are, you take these cells that are immortal because they have activated this telomerase so they can grow forever, but they have not turned into a cancer and then you find a gene that you think may be related to cancer and you put that gene into this immortal cell and you see whether or not this becomes a cancer cell?

Wajapeyee That is right, this can especially be done in the context of oncogenes. An oncogene, by definition, is defined as a gene that can transfer many immortalized cell lines and also it is done in the context of a specific cancer type, so if you identify a breast cancer specific oncogene, you have to test it as an immortalized breast epithelial cell and if you are finding a melanoma specific oncogene then you have to test it in context to myeloma specific.

Chagpar So it depends on the cancer we are looking at. We are going to pick up on this conversation about how we get from normal cells to cancer cells and how all of this has to do with the genome and epigenome right after we take a short break for a medical minute.

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*Medical
Minute*

Genetic testing can be useful for people with certain types of cancer that seem to run in their families. Genetic counseling is a process that includes collecting a detailed personal and family history, a risk assessment and a discussion of genetic testing options. Only about 5% to 10% of all cancers are inherited and genetic testing is not recommended for everyone. Resources for genetic counseling and testing are available at federally designated comprehensive cancer centers such as Yale Cancer Center and at Smilow Cancer Hospital at Yale-New Haven. The Yale Cancer Center Cancer Genetic Counseling Program is a new frontier in the fight against cancer. The program provides genetic counseling and testing to people at increased risk for hereditary cancer and helps them to make informed medical decisions based on their own personal risk assessment. This has been a medical minute brought you as a public service by Yale Cancer Center and Smilow Cancer Hospital at Yale-New Haven, more information is available at yalecancercenter.org.

Chagpar

Welcome back to Yale Cancer Center Answers. This is Dr. Anees Chagpar and I am joined today by my guest, Dr. Wajapeyee and we are talking about cancer research, but really cancer research at its most fundamental and fascinating level. We were talking a little bit about how cancer biologists and researchers figure out what the oncogenes are that turn cancers on or what are the tumor suppressor genes that need to be inhibited to make cancer grow as well and we talked about a few ways that is done. You mentioned another way that cancers can be turned on or off, that has nothing to do with DNA itself, it is epigenetics, tell me more about that.

Wajapeyee

As I said, in epigenetic alterations you do not have to have a change in the DNA sequence itself, what happens is if some of the DNA basis get modified by a chemical modification, for example, cytosine in the context of CPG in DNA can be methylated and that methylation which is CH₃ group on cytosine in the DNA can cause a lot of alteration, first and foremost, what is associated with cytosine methylation and DNA is gene repression. So, if any gene requires cytosine methylation it is shown to acquire silent stage, it will not be expressed in the cell. So one of the ways cancer cells inactivate tumor suppressor gene is by putting more methylated marks on their promoter sequence, by which now these tumor suppressor genes cannot be expressed. There are some contexts in which there is activation of oncogenes also, in which all normal cells oncogenes have methylated DNA, and in cancer cells they lose that. So now the genes which were not expressed in normal cells are going to be expressed in cancer cells. In addition to that, DNAs in chromosomes are actually wrapped around a group of proteins called histone proteins and these histone proteins are known to be post-transcriptionally modified and by that I mean when the protein is made, the proteins can be chemically modified differently at different residues and that also can either activate or repress a gene. So, in combination with post translational modification histone along with the methylation of DNA the cancer cells can determine which genes they want to turn on and which gene they want to turn off and that effects a lot in terms of cancer initiation. It has been shown if you affect the epigenetic stage of cancer cells it actually prevents their ability to form a tumor. So it is as important as epigenetic change in cancer cells.

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- Chagpar So, it is not like these methyl groups that are added in the process of methylation really change the DNA itself, it is still a cytosine residue. It is just that you have got a methyl group stuck to it which is the same DNA code but now it is turned off, essentially?
- Wajapeyee That is right, so it actually compresses the DNA in such a way that the proteins that are required for expression of these genes will not be able to access the DNA and there is no gene expression and there is no protein made.
- Chagpar Help me to understand how it is that you guys figure out all of this in the lab. We talked a little bit about if you stick an oncogene into an immortal cell and it turns into a cancer cell then you figured out that this is the gene that is making this immortal cell a cancer cell, it must be pretty difficult to figure that out if the genes all look the same.
- Wajapeyee That is right, there are specific methods now which can determine epigenetic alterations also, so there is a method called bisulfite sequencing which actually converts unmethylated cytosine to uracil and then it is read using PCR as a D which if it is methylated still stays the C so you can really treat the genomic DNA with bisulfite and then sequence and then see how many of each remains C, that means those Cs were methylated Cs. So that way you can discriminate between a normal cell and a cancer cell, and then you can see if it correlates with gene expression or not.
- Chagpar How do you even figure out which genes are of interest, do you say, well I just happened to think that this particular gene number you know 2196 out of the 2300 genes that you have might be the one.
- Wajapeyee That actually is dependent a lot on the cancer genome sequencing projects or human genome sequencing projects, so the nucleotide structure of all the genes are known. What we can do is we can bioinformatically using a computational program can analyze the promoters of different genes to look for something called CpG islands. So DNA methylation in that leads to transcription repression, it only happens in the context of CpG islands in most cases, and it is shown that many of these tumor suppressor genes which are epigenetically silenced by promoter DNA hypermethylation have those CpG islands. So, we can take the sequence of a promoter of a tumor suppressor and put it in a computer based program and it will tell whether it has a CpG island or not. So that is the starting point. After that, actually there is a specific chemical called decitabine which is also used for myelodysplastic syndrome and you can add it on the cells and see if the expression of gene has gone up or down, if the expression of the gene goes up, that means it does definitely have that CpG methylation phenotype. Once you have done these two easy experiments then we do bisulfite sequencing to confirm whether these were really associated with methylation or not. But the initial setup is to find the CpG islands and then go from there. Now there are high throughput methods where they have actually identified whole human genes which are associated with CpG islands and they have developed chips and you can probe the whole genome for methylation alteration. You can also do high throughput deep sequencing where you can sequence all the methylated DNA and find where the methylation is.

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- Chagpar If you have all of this information, if we have the human genome project, we know what all the genes are, we have all the fancy-schmancy stuff to figure out where the CpG islands are, are you telling me that we kind of know all the genes that turn on and turn off cancer, so how come we do not have a cure for cancer yet?
- Wajapeyee Because cancer is a moving target. The issue is when you treat a particular cancer with a drug they acquire resistance because they are very flexible in terms of how they regulate gene expression within the cells. There are several of these drugs including EGFR inhibitor, BCR-ABL inhibitors, and they work very well initially and then after that the resistance arises. So, you cannot consider cancer as a single disease and/or stable disease, first of all it is heterogeneous, there are several difference cancer cell types within a single cancer. So if you are targeting one cell type by your therapy, maybe the second cell type which is not responding will enrich and create its own cancer, so now you have to deal with that and also when you treat with drugs especially in chemotherapeutic drugs they cause DNA damage because that is exactly what happens to cause mutations and that DNA damage causes mutations and makes even more changes in the DNA so they become even harder to treat. That is why chemotherapies and even the targeted therapies for that matter, work only to a certain extent. Now there are new classes of therapies which are actually harnessing the immune system, host immune system, to treat cancer which are working very well because you do not have to really do anything other than telling the human immune system that there is a cancer cell and you have to kill it and it has worked very well in the case of melanoma. It has increased the overall survival and disease free progression over 18 months. It has worked in some other cancer types like lung cancer. It has not had success in cancers like pancreatic cancers so there is lot to be learned in the context of different cancers but it looks like the immunotherapies will be next generation therapies for now because they are very durable and the mechanisms of resistance are not as diverse as you will see with targeted therapies and chemotherapies and also the side effects are no different than any other therapy and they are manageable.
- Chagpar If these cancer cells are so smart that they can figure out how to get around targeted chemotherapies, can they figure out how to get around the immune system?
- Wajapeyee The reason is they do actually. For example, in the case of Acquired Immuno Deficiency Syndrome (AIDS) patients one of the things they see is cancer. So it is very-very clear that the immune response has various important roles in controlling cancer initiation and progression and so cancer cells have acquired many-many mechanisms to evade human response because they acquire new genes, new proteins and now the body is going to view them as a foreign body and then will essentially, like when somebody get someone else's kidney which is not a match, it should reject it, but what the cancer cells have done is developed mechanisms called immunomodulatory or immune-editing mechanisms, or it is called immune evasion mechanisms and they are very good with that. What this new chemotherapy does is kind of suppress that phenotype in cancer cells and now activate the immune response. Now when the immune

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response acts, the immune response is a strong agent, not just a small chemical, it simply eradicates all the cells. So the residual tumor is none or zero. So there is less chance of developing a resistance tumor compared to where you are giving a chemotherapy or targeted therapy which is kind of shrinking the tumor size and letting it stay there at that size, because if you have even a few cells left, they have a chance to grow back.

Chagpar That makes sense that if you have got a really vague immune response and you reduce the immune evasion of cancer cells the immune system can wipe out the remaining cancer and you are cured. But potentially if you leave some cancer cells there or somebody does not have as robust an immune response, is it possible that cancer cells could evade that immune system response yet again and that we would be back into finding a secondary immunotherapy?

Wajapeyee Yeah, that is true and that is what people are trying now, trying to see, trying to activate immune response by different ways and rather than using a single approach to activate immune response and it has worked, there are these antibodies called anti-CTLA-4 antibodies and anti-PDL1 antibodies which are now being combined for melanoma treatment and they are seeing much better effects, even now the effects are so good, people do not like to talk about biomarkers in the context of immunotherapies because they think everyone who has access to these antibodies or this therapy, should be put on trials for treatment.

Chagpar Are these available on clinical trial or is this something you can get at your local oncologist?

Wajapeyee Some of these are clinically FDA approved now for metastatic melanomas and if FDA approved, you can use off-label for other cancer types and there was a huge study in The New England Journal of Medicine where they have tested immunotherapies against several different cancer types. So as I said, it worked in most of the cancers except for pancreatic cancer.

Chagpar What was the deal there?

Wajapeyee It has been essentially going back to the basics. Initially, people thought that all cancers are different, there should be personalized therapies for each different cancer based on their genetic thing but there is one very common feature of all cancers, they evade immune response and that is true for all the cancers and that is coming back to the basic hallmark of cancer cells that they evade immune response and that is being tried now. For example, angiogenesis therapies where they block blood formation and tumor formation that is also common across tumor types but does not really work that well in most of the cancers other than the renal cancers.

Chagpar What is up with the pancreas? Do pancreatic cancers not evade the immune system like everyone else and did the cancer cells in the pancreas not read the book?

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Wajapeyee Pancreatic cancer cells are very different in the context that it has something called desmoplastic environment. So the cancer cells are very few but they actually cause proliferation of something called fibroblast all around them. So even treating them by chemotherapy is difficult because it is not possible for the drug to reach the tumor. It has been shown in some cases where they treated the tumors and it shrank and only the fibroblast shrank but the cancer cells were still there. So there is this new approach where they have used a signaling pathway inhibitor which is called Sonic Hedgehog inhibitor which removes the desmoplastic environment or compartment and allows the cells to acquire chemotherapeutic agents when you inject them and then trace to see if that works better. It is being tried, it did not work out very well but I think the desmoplastic environment also has to do with why pancreatic cancer cells do not respond very well.

Dr. Narendra Wajapeyee is an Assistant Professor of Pathology at the Yale School of Medicine. We invite you to share your questions and comments with Dr. Foss and Dr. Chagpar. You can send them to canceranswers@yale.edu or leave a voicemail message at 888-234-4YCC. As an additional resource archived programs from 2006 through the present are available in both audio and written versions at yalecancercenter.org. I am Bruce Barber hoping you will join us again next Sunday evening at 6:00 for another addition of Yale Cancer Center Answers here on WNPR Connecticut's Public Media Source for news and ideas.