

Yale CANCER CENTER *answers*

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Hosts

Anees Chagpar MD

Associate Professor of
Surgical Oncology

Francine Foss MD

Professor of Hematology

Steven Gore MD

Director of
Hematological
Malignancies

Targeting Metastatic Breast Cancer

Guest Expert:

Qin Yan, PhD

*Associate Professor of Pathology, Yale
School of Medicine*

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Welcome to Yale Cancer Center Answers with your hosts doctors Francine Foss, Anees Chagpar and Steven Gore. Dr. Foss is a Professor of Medicine in the Section of Medical Oncology at the Yale Cancer Center. Dr. Chagpar is Associate Professor of Surgical Oncology and Director of the Breast Center at Smilow Cancer Hospital and Dr. Gore is Director of Hematological Malignancies at Smilow. Yale Cancer Center Answers features weekly conversations about the research diagnosis and treatment of cancer and if you would like to join the conversation, you could submit questions and comments to canceranswers@yale.edu or you can leave a voicemail message at 888-234-4YCC. This week it is a conversation about research into metastatic breast cancer focusing on epigenetic mechanisms with Dr. Qin Yan. Dr. Yan is Associate Professor of Pathology at Yale School of Medicine. Here is Dr. Steven Gore.

- Gore So epigenetics, this is not something that the common public knows a lot about. It sounds like gobbledygoo. Could you tell us what epigenetics refers to?
- Yan Epigenetics is a word outside of genetics, especially if you cannot explain something with genetics, then you can explain it with epigenetics. In some way, it is a study of heritable traits and gene expressions that do not involve changes of underlying DNA sequences.
- Gore A heritable trait, so is this passed from generation to generation or a heritable trait from one cell to another, how does that work?
- Yan I would say both. In both occasions, you have this from generation to generation as well as from different cell traits and one cell will carry the epigenetic trait and they can pass on the donor cells. That is also called epigenetic phenomenon.
- Gore I see, but this does not have to do with mutations, is that right?
- Yan This has nothing to do with mutations. You do not have to have changes of genetic material itself.
- Gore Can you give a certain example of something that might be an epigenetic trait?
- Yan One of the examples that you can think about, if you think about a caterpillar and a butterfly, they have the same genetic materials; however, they look very different as you can see.
- Gore Yeah, so that is like magic, right, is not that just magic?
- Yan Well, yes, sure it is.
- Gore How is that epigenetic? How do the epigenetics impact the change or the metamorphosis from the caterpillar larva to the butterfly?

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Yan The epigenetics will affect not only gene expression changes and that will be refractive to the cell trait and what kind of cell you can see and what kind of organism you will see when you have different epigenetic states.

Gore Got it, so you are telling me that the genome is the same for the caterpillar and the butterfly of course, but by changing the epigenetic regulation of the genes, you go from having caterpillar genes to butterfly genes.

Yan That is correct.

Gore Something like that.

Yan Yes.

Gore I like that allusion too and what the audience does not know is that my research in leukemia has to do with epigenetics as well, so I am playing kind of a fool here, but that is because I think it is important for everyone to understand that this is a rather complicated field. I do not know if this is true or not, but I was once at this zoo in Florida and they have two animals that are bred between tigers and lions, one is a liger and one is a tigon, depending on which one is the mother and apparently how the animal looks at the end, this is supposedly epigenetically determined I guess in terms of whether it is more lion genes or tiger genes that get activated or inactivated, I did not go to prove that but that is what it said on the sign. I thought it was interesting that at a zoo they were talking about epigenetics.

Yan That is an interesting observation and although genes can be determined by whether this is from the father or the mother, this is the problem with epigenetic mechanism, some of the genes will be expressed if it is inherited from the mother and some of the genes will be expressed if it is inherited from the father, so in some cases, this is called imprinting and this has many fundamental contributions to what we see in everyday life, I would say.

Gore In terms of whether we have our father's gene expressed or mother's gene expressed.

Yan Yeah, that is true.

Gore What does any of this have to do with breast cancer?

Yan This has to do with many different cancer types, not only breast cancer. What we are most interested in now is to understand what epigenetic aberrations that will cause cancer and how to find ways to treat those epigenetic aberrations. What we study is actually tumor metastasis and if you think about the metastasis, the tumor cells have to get all the way from the primary organ to the distal organ and if you compare the genetic information from the breast versus the distal

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organs, you actually do not see a lot of changes, genetic mutations of those cells, so when we form the hypothesis is that those tumor cells will have to undergo a lot of gene expression changes caused by epigenetic state switches.

Gore Let me get this straight before you go on, so what you are saying is that you can sample a primary tumor that starts in the breast and you can study the metastasis that arises from that tumor either in the liver or the bone or wherever it is and that the DNA, the genes, are fundamentally the same but the reason you are speculating they are different is because obviously the cells behave differently and one has stayed in the breast and one has set up shop in the distant organ, and maybe that one is more aggressive, so they act differently?

Yan Not only that but also metastasis is a multistage process, the cells have to change themselves and on the way, they have to get all over the breast and then get into circulation, not only survive there but they will need also to get out of circulation to the distal organs and during this process, they have these changes over the phenotype, again back to more tumor like epigenotype phenotype when they get into the distal organ. They have to not only get out of there, but they also have to adapt to that foreign environment to survive there. It is not easy for them to adapt to those changes.

Gore So your hypothesis was that in order to break away to survive in the bloodstream or the lymphatic system and get to the environment that might seem hostile like the liver and adapt to it, make it a nice happy home for the tumor, that this has been driven by changes in gene expressions that have been influenced by these epigenetic changes, is that right?

Yan Yes that is correct.

Gore And were you right, did you find any such epigenetic changes?

Yan We did, and recently we studied this hypothesis and then what we studied was patient material and we used the integrated computation analysis. We actually identified a potential driver of breast cancer metastasis.

Gore What is an integrated computational analysis?

Yan Basically we use different algorithms to identify what genes were associated with the instance of metastasis, to put it simply, but it requires a lot of computational power to mine screw all of the patient samples to arrive at this kind of conclusion.

Gore Just walk us through what is involved, are you comparing the primary tumor to the metastasis tumor or are you looking for common themes between different patient's primary tumors?

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Yan In this initial study, we focus on the primary tumors that will metastasize and those that will not metastasize, so we are comparing the different choice between those primary tumors. We were able to identify the one side that correlated with the instance of metastasis.

Gore Because this is archival tissue.

Yan That is right, yeah.

Gore And you know what has happened to the patient from reading the charts.

Yan Yeah that is true. Breast cancer takes a long time. We will need to get to those archival tissues.

Gore Got it and so that is why if you are a patient and you are approached by doctors about donating your tissue for future research, it is so important and that is how we learn this stuff. So you have all these different tumors and you know that some people eventually developed metastasis unfortunately and some did not and what do you do with the tissue, do you sequence the DNA?

Yan In this case, we just look at the expression of many of the genes, many of them are actually involved in metastasis progression, so that we can look at how those genes are changing and in this case we focused on epigenetic regulators which are the regulator of the epigenetic states.

Gore You are getting too fast for me now. I am still trying to get the integrated part, so you are looking at a gene expression across a lot of genes, is that right?

Yan Yeah.

Gore How many genes do you look at in a particular tissue?

Yan For those we can look at all the genes, but in this case, we just focused on the ones we are interested in, meaning the regulators of epigenetic states.

Gore How many genes is that?

Yan That is about 300 genes.

Gore Wow, so you are looking at this set of 300 genes that you think are going to dictate epigenetic changes and you will look at this across a bunch of tumors, right?

Yan Right.

Gore And how many tumors are involved?

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Yan This was 2000.

Gore 2000 tumors.

Yan Yeah.

Gore 300 genes each right?

Yan Right, that is true.

Gore That is where you get into this integration thing because I guess if you are doing this on a calculator, on the spreadsheet, it is going to get kind of tedious.

Yan That is kind of difficult to do.

Gore So you feed all this information, 300 genes, 2000 tumors into Cal the computer and what does Cal do, what does the computer do?

Yan We can look at and separate the patients based on their propensity to metastasize.

Gore So you are going to teach the computer which ones are the ones that are metastasized?

Yan Yeah that is true.

Gore That is something called a supervised analysis, right?

Yan Yeah.

Gore So you give it the information that here are the ones that had metastasis eventually and here are the ones that did not? Do you also tell ones had what kind of metastasis, like which went to the bone?

Yan We will also do that. For breast cancer, the major site of metastases are the brain, bone and the liver, so those are the major categories. We also classify those based on the site of metastasis.

Gore So not just lymph nodes.

Yan Not just lymph node.

Gore So you have Cal the computer, it has got all the information on the 300 genes, I am really a simple person so you have got to take me slow, you've got 300 genes, 2000 tumors, that metastasized eventually, what did Cal tell you at the end?

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Yan At the end, it will give me a curve showing whether the expression of a particular gene will be able to stratify the patients based on whether they will metastasize or not.

Gore And did you find such genes?

Yan We did find several genes that were interesting and then we actually published one of those studies.

Gore So in the genes, first of all, how many such genes seemed to be selected by Cal the computer?

Yan We actually used multiple analyses and at the end we narrowed it down to about 10 genes.

Gore 10 genes and are these 10 genes up in all of the people who are going to metastasize or different sets?

Yan Actually not, only a subset of patients.

Gore And do some of them tend to go with each other, like A, B, C go together and D, E and F go together or it is just a grab bag?

Yan Some of them do go together but some of them do not, some of them will have strong association but some of them do not.

Gore This is complicated but so very interesting and we are definitely going to follow up on what happened to your 10 genes and your 2000 patients after the break, but right now, we are going to take a short break for a medical minute. Please stay tuned to learn more information about epigenetics in breast cancer with Dr. Qin Yan.

*Medical
Minute*

There are over 13 million cancer survivors in the United States and over 100,000 here in Connecticut. Completing treatment is an exciting milestone but cancer and its treatment can be a life changing experience. Following treatment, cancer survivors can face several long-term side effects of cancer including heart problems, osteoporosis, fertility issues and an increased risk of second cancers. Resources for cancer survivors are available at federally designated comprehensive cancer centers to help keep cancer survivors focused on healthy living. The Survivorship Clinic at Yale Cancer Center focuses on providing guidance and direction to empower survivors to maximize their health, quality of life and longevity. This has been a medical minute brought to you as a public service by Yale Cancer Center and Smilow Cancer Hospital at Yale-New Haven. More information is available at yalecancercenter.org. You are listening to WNPR, Connecticut's Public Media Source for news and ideas.

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Gore Welcome back to Yale Cancer Center Answers. This is Dr. Steven Gore and I am joined today by my guest Dr. Qin Yan. We are discussing research involving the regulation of breast cancer behavior based on what is called epigenetics. Qin, before the break we were talking about your work which was trying to understand, and tell me if I am wrong, whether there is a difference in expression of genes which impact epigenetic changes or the epigenome in patients with primary breast cancer who are going to eventually metastasize.

Yan That is correct.

Gore And you told me that you studied 2000 tumors, which is huge, seems huge to me, and something like 300 genes which might influence epigenetics in each tumor and Cal the computer picked 10 that seemed to be higher regulation, are more expressed.

Yan They are more expressed than in patient tumors that will metastasize.

Gore So what do we do with that next? Sometimes in this kind of study, you have to validate this gene set in a whole separate database, just to make sure it was not a statistical artifact, is that something you do?

Yan We did do that, we used a separate cohort of patients and validated all analysis and moreover we used more traditional research nav based studies to validate the function using cell based assay and using mouse models.

Gore Let's take that one at a time, so you looked at different tumors besides the 2000?

Yan Yeah, that is true.

Gore How many more?

Yan About 300 patients.

Gore 300 extra.

Yan Yeah.

Gore And they also seemed to have this pattern, the ones that metastasize?

Yan Yeah, very similar.

Gore Very similar, okay and then you said you validated it in classic cell models, what does that mean?

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Yan In the research, we used some cancer cell lines, in this case, breast cancer cell lines, to study the ability of those breast cancer associated genes in breast cancer progression.

Gore So you have your breast cancer cell line and I think there is one called MCF-7 if I am not mistaken, right?

Yan Yes.

Gore It is just the one I know, and you have got these genes, some of these 10 genes, and you show that they are also up-regulators?

Yan Up-regulators, they are more aggressive cell lines also.

Gore Then do you knock the expression of the gene down to see what happens?

Yan Yes that is what we do actually. We basically knock down the expression basically decreasing the expression of those genes in the aggressive breast cancer cell lines.

Gore And what do they do?

Yan We show that those breast cancer cells express much lower expression over the genes related to metastasis, meaning that they control this gene expression program and that is involved in metastasis and at the same time we look at those cells and they have decreased ability to invade and migrate.

Gore So are you watching them move around the dish?

Yan Yeah we look at them moving through the dish, actually moving through the membrane on the dish.

Gore I think the audience might enjoy hearing about this. This is one of those things where you have got two different containers connected by a membrane. Is that right?

Yan Yeah, that is correct.

Gore And you have got the cells on one side?

Yan The cells on one side and then we have some attractant and we attract those cells down there. And the metastatic breast cancer cells like to move down there and if we take all this gene the others do not.

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Gore They do not want to move down there. They want to stay at the home base?

Yan Yeah, that is correct.

Gore That is so cool. That is called the Matrigel right.

Yan That is the Matrigel membrane.

Gore Very cool. So you found that when you knock down some of these epigenetic regulators, the cells no longer invaded through the Matrigel which is your model for the metastasis. Then you said you have some mouse models, what is that about?

Yan We actually use two different kinds of mouse models, one of the mouse models we use is that we use those human breast cancer cells that I mentioned, MDA-MB-231 cells and this is the cell line, a human cell derived from human patients.

Gore Some people have probably read the book about Henrietta Lacks and how her cancer gave rise to HeLa cell lines, and this was one of the best sellers a couple of years ago. This is a great story at John Hopkins. So, this is one of the cell lines that the scientist had made from tumors, from patients with breast cancer that originally came from a patient and now they live in a test tube.

Yan Correct.

Gore So you have got your aggressive cell line and you put this into mice?

Yan We inject those into the mice and see how they can grow in distal organs, in this case it is the lung.

Gore Do you inject the cells into the breast or do you inject them into the blood?

Yan This one we inject into the blood through the tail vein.

Gore And it circulates around and the cells set up shop to make tumors?

Yan Yeah, and then they will set up the shop and then make tumors there.

Gore So you have your cells, you have your mice and you have this model where you establish metastases in these unfortunate animals and so how do you manipulate your epigenetic genes?

Yan What we did is we exchanged the expression of the genes and cell lines before we inject into the mice.

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Gore Can you turn them off permanently?

Yan We can turn them off permanently. We can turn them transiently too. We can do both.

Gore Which you are doing, you are doing both, you are doing the permanent and the transient.

Yan In the previous public study, we did the permanent turn off. And right now we are doing the transient turn off experiments.

Gore You turn off these genes permanently and then you inject them back into a separate set of mice, and are there fewer metastases?

Yan There is much less metastasis.

Gore That is pretty cool. What will you do when you can transiently turn them on and off?

Yan We can use a special system where we can put a drug into the food of the mice and that will induce the expression or induce, in this case down regulate the expression of the genes.

Gore So you let the metastasis be set up and then turn off those genes?

Yan Yeah, we are trying to see whether this has treatment potential, that is correct.

Gore And you do not have any data to share with us about that, right?

Yan We do not have data on that yet.

Gore That sounds super exciting. Do you know whether this kind of mechanism is operating in other cancers that metastasizes, have you had a chance to look at any other cancers that metastasize?

Yan Yes, actually we looked at this in lung cancer, it had a similar phenotype and in addition, we showed that the activity of this enzyme is actually very important for the lung cancer cells to metastasize.

Gore And that is one particular enzyme you are talking about now?

Yan Yeah, this is one particular epigenetic regulator that is called RBP2 or retinoblastoma binding protein 2.

Gore What does that do?

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Yan It is a histone demethylase. It can remove those methyl groups from the histones and histones are those proteins found next to the DNA and any changes on those protein will have fundamental changes over the gene expression.

Gore For the audience, the histones are proteins as Dr. Qin explained, and they come in groups of eight, eight different ones and DNA wraps around the what?

Yan Nucleus.

Gore The eight histones makes the nucleus, and the DNA wraps around it 2.5 times and it looks like little beads on a string, right.

Yan Yeah that is correct.

Gore And then depending on how the histone proteins are modified, the DNA is stuck to it more tightly or more loosely. And when it is really tight, the gene is turned off, right?

Yan Yes that is correct.

Gore Okay, got it, so the RBP2 protein modifies these histone tails by taking away methyl groups. Does that make the gene more likely to be activated or less likely?

Yan Less likely to be activated.

Gore It turns the gene off. So when you have got the RBP2 protein, it turns the gene expression off.

Yan Yeah, so if we inhibit its activity, we can activate these targeted genes.

Gore And you are saying that this particular protein is involved in the metastasis?

Yan Yes, both in breast cancer and lung cancer, two of the major cancer types that you always hear about.

Gore So you would like to turn it off. You want to turn this gene off.

Yan We want to turn this off and with a small amount of kinase inhibitors. Actually, we have done some experiments and we have identified the lead compound of those inhibitors.

Gore Where do you find compounds to test against your RBP2 protein? If nobody knows if there is any particular drug, do you invent the chemicals or do you screen chemicals, what do you do?

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Yan We did the screening initially, but right now, we are also making new compounds to see whether the newer compounds have better effects on inhibition of this enzyme. So this was done initially with the Yale Center for Molecular Discovery and we set up an assay that can analyze the activity of this enzyme biochemically and we can grind through 10,000 compounds and we can identify several compounds that have some ability to inhibit this enzyme.

Gore Where do you get these compounds from? You cannot go to the shop and order them right.

Yan We cannot. We can get it from a commercial library and we can get it from National Cancer Institute and actually Yale has a compound library too.

Gore So these are resources of all sorts of chemicals that are available for screening.

Yan Yeah that is for screening, but right now actually in this project, it is recently supported by the NCI, and the Developmental Therapeutics Program, which has the ability to translate the findings all the way to the clinic and actually they have 18 phase II and phase III clinical trials right now, so what we are doing over there is to not only screen bigger libraries but also we are designing new molecules to target this enzyme using the information we know.

Gore How do you do that? Are you working with the lead compound that you have already identified and are you modifying it, or are you actually looking at the structure of the protein and inventing something geometrically that looks right?

Yan We are doing both, we have a set of compounds that we know and we are modifying those compounds to see whether it can inhibit the enzymes better and based on those studies, generally the structure of this enzyme, we are talking of different kinds of compounds on this enzyme, to see whether we can find even better or more selective compounds.

Gore This is done on the computer?

Yan This is all done on a super computer, this is Cal again.

Gore And it actually has a picture of what the protein looks like in three dimensions and it takes these compounds and it tries, when you say docking, it is like trying to get a lock in a key, right?

Yan That is correct and we try to just rotate the molecules and then try to fit different kinds of molecules to see which one would fit the best.

Gore What about all those other lonely genes, those other 9 genes you found, are you going to leave them alone?

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Yan No, we are also looking at other ones. We are running through a similar pipeline and we are trying to go through them one by one.

Gore Wow, that sounds like a lot of work.

Yan It is.

Gore It keeps your computers busy as well as your postdoctoral fellows.

Yan Postdoctoral and the graduate students.

Gore What about the tumors once they have metastasized? Do you continue to express this RBP2 or any of these other genes or do they get turned off once they have done their job?

Yan In this case, they still express RBP2, but for some other ones, they could be turned off and because metastasis is such a complicated process, in certain cases, some of the genes have to be turned off for one stage and the cancer cells will not need it for the next stage, so this is actually very important in terms of therapeutics because we want to hit them at the right time.

Gore I guess theoretically if there was a gene that was important early in the process of established metastasis, you might give the one drug but then once there were metastases you would need a different drug.

Yan Yeah that is true.

Gore And so far is anything promising happening in your mice, have you gotten rid of some of those metastases?

Yan We just got compounds from a company and we are trying to test whether they can suppress the metastasis in the mouse model.

Gore And have you come up with any names for your new drug, like qinomycin or something like that?

Yan We do not have these fancy names, for the initial compound we have just named it as YUJ1, J2, J3, YU means Yale University.

Gore You need to get some marketing people involved, you're gonna be famous.

Yan Yeah we need to do that for sure.

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Dr. Qin Yan is Associate Professor of Pathology at Yale School of Medicine. We invite you to share your questions and comments, you can send them to canceranswers@yale.edu or you can leave a voicemail message at 888-234-4YCC and as an additional resource, archived programs are available in both audio and written format at yalecancercenter.org. I am Bruce Barber hoping you will join us again next Sunday evening at 6:00 for another edition of Yale Cancer Center Answers here on WNPR, Connecticut's Public Media Source for news and ideas.