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00:06 Sarah Crespi: Welcome to the Science Podcast for October 19, 2018. I'm Sarah Crespi.

00:11 Meagan Cantwell: And I'm Meagan Cantwell.

00:12 SC: On this week's show, I talk with Phil Jones about his work on finding non-cancer causing mutations in the human body. How many do we have? And what are they doing?

00:23 MC: And I talk with Praveen Vemula about developing a gel to protect farmers from harmful pesticides.

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00:33 SC: In our lifetimes, we're constantly replacing cells all over the body: Our organs, skin, blood, things like that. Each time these cells arise, the genome is copied from their parents and mistakes can be made. But whether these changes, these changes in the genomes are harmful or benign, these cells are just not quite like their parents. And we don't really understand how common these changes are, these mutations are, or their impact on the body as a whole. Phil Jones and colleagues looked for these slight genetic variations in the cells lining the esophagus, and we'll talk about why the esophagus in a minute, and they found a hidden world of mutation in people that don't have disease in their esophagus. He's here to tell us about these findings in more detail and what they might mean for our understanding of cancer and aging. Hi, Phil.

01:23 Phil Jones: Hello, hi.

01:24 SC: That was a bit of a long intro, but I think everybody's just dying to know, why did you look at the esophagus when you wanted to find out how common mutations were in cells?

01:35 PJ: Well, we've done a study a few years ago where we looked at normal human skin and we found quite a lot of mutations. The thing with skin is, we all go out in the sun, and the sun is very good at changing the letters in DNA code, and it's not really a surprise if you find lots of mutations in the skin. But now we wanted to go and turn our attention, if you like, to a place where the sun doesn't shine, where we wouldn't expect to see perhaps so many mutations. And I think we expected not to find very much if I'm honest, which is why the results that we had in completely normal healthy people's esophagus were a real shock.

02:14 SC: And these are the same type of cells that you see on the outside of the body?

02:17 PJ: They're first cousins, if you like, the one's on the outside. So there are some differences, but essentially they work the same way.

02:24 SC: How many people did you look at? And how much of their esophagus did you look at?

02:28 PJ: We were very privileged to get the esophagus from people who had died suddenly and unexpectedly, whose organs were being taken for transplanting to other people. We're very grateful to the relatives of these people and they signed a consent and let us take the middle third of the esophagus, this tube that connects your mouth and your stomach, that's about 5-10 cm in length. We cut the lining into little squares and then we applied a sequencing method to read the letter changes in those squares. And that essentially was the project. We had nine people, the youngest was in their 20s, the oldest was 75. And all of them have no history of disease of the esophagus, the esophagus we took a great deal of pains to show it was normal as far as we could say.

03:15 SC: Yeah.

03:15 PJ: So what we were doing is trying to get a snapshot of normal people's esophagus.

03:20 SC: And when you looked at the cells of the esophagus, you did see an unexpectedly large number of mutation in the genomes of those cells.

03:29 PJ: What we were really looking in detecting is not mutations in individual cells, but groups of cells that had picked up a letter change and then passed it on to their daughters and their daughters and their daughters and so on, to generate a large clump of cells carrying the same single letter change in the DNA. And what we found was that we were seeing hundreds of those. Each cell seems to carry a couple of thousand mutations over its genome, but what matters are the ones that triggered the cell to alter its behavior to make a really big clump of cells.

04:00 SC: Right, and that's this idea of competitiveness, so that these cells are...

04:04 PJ: Yeah, but they're so many... These clones got these... And a clone is just a bunch of cells, a family if you like.

04:09 SC: Yeah.

04:09 PJ: A family tree having the same letter change. They get so big that they start to grow, and they're beating up the normal cells, and eventually they hit each other. My postdoc calls it, Game of Clones. We got a Game of Clones because you've got different families competing for the same size bit of territory, and that is the basis of something that's very parallel we think, to what happens in species evolution, we get competition and survival of the fittest, and the fittest in this case are the altered genes that make the cells best able to compete.

04:42 SC: And they were taking over big swaths of the tissue that you examined.

04:46 PJ: Yeah. So, basically by the time you're 40, well over half of your esophagus by area is mutant. This is a bit of a shock.

04:56 SC: Yeah.

04:56 PJ: And in fact, we looked at 74 of the usual suspect genes, and we chose genes that are linked to cancer because they're ones that have to have hang out in normal tissues for a long time to turn into a cancer. I don't think our findings are necessarily directly relevant to cancer, but that's what we show. But only 12 of those turned out to be the kind of super competitors that would win out in this clone wars that we were seeing in the normal tissue.

05:21 SC: Huh! So, there's some link to cancer here. One of the ideas about how cancer grows or progresses is, it's better at cloning itself and making more of itself than the surrounding tissues, and that it grows out of control, right?

05:35 PJ: Yeah, the big health warning to that idea is that this is completely normal tissues.

05:39 SC: Right.

05:39 PJ: If you compare the number of mutant cells in normal tissues with the number of cancers, cancer's like a one in a billion, one in a trillion event. So what we're looking at, you could almost call normal getting older physiology-like, looking more wrinkly when you look in the mirror. Well, if you could see the mutations in your esophagus, you can basically read off your age from the pattern that you see.

06:01 SC: And do you see that increase as your samples were from older people, you saw...

06:06 PJ: Absolutely, there's about fivefold increase in the number of these big clumps of cells carrying the same mutation as you go from in your 20s to in your 70s. And then you do see some flavors, some particular genes winning out. The strongest winner, which was really interesting to us, was a gene called NOTCH1, which has been found in cancers, but in fact we found it was 5-10 times more common than normal than it was in cancer. And that tentatively raises hints at, I don't want to overclaim it, that mutation in this gene might be protecting you against developing cancer.

06:42 SC: Does research like this help us pick out who are the bad guys?

06:46 PJ: At one level, it's very helpful to compare it with the cancer. So, there's long been a very good idea that the mutations that are in cancer are the ones that keep showing up, but we've done that without knowing what was in the normal tissue. So like people have said, "Well, NOTCH1 must cause cancer 'cause it does show up 33% of cancers," but actually it's much more common than the normal. The interpretation of that could change entirely.

07:09 SC: When we talked about the skin and UV light and how likely that is to change the DNA, did you see any markers of environmental effects on these mutations?

07:20 PJ: We very strikingly did not see markers of environmental effects, and this was a real surprise. I wouldn't wanna to overclaim it. Hey, it's nine people. Does that represent the whole world's population? No. We had four smokers and five non-smokers, but there was no difference in the... We call them "mutational signatures." So it's a bit like an artist signs that work on the DNA

and you can read off what causes some of the letter changes from the pattern and get a kind of signature out of it for what might be causing those letter changes.

07:52 PJ: And essentially what we saw are ones which are just essentially linked to getting older. What we're saying is that these things will just happen to you, whether or not you're kind of have a clean lifestyle or a bad lifestyle. Now, I absolutely would stress, this is not giving you an excuse to say, "Oh, well, it's just bad luck," because what we think is that things that promote cancer, alcohol for example, cigarettes, whatever, they're gonna work between these kind of mutated cells than normal, and the cancer will now know the steps. There are several steps you have to cross, hurdles you have to cross, to get the cancer from these mutant clones than normal.

08:28 SC: We've connected this with cancer and environmental concerns. But in your paper, you also talk about the relationship between the changes that you see in the esophagus and aging, particularly this idea of these expansions of these little families. How does that relate to aging? How do you see that fitting together?

08:46 PJ: If these mutations didn't change the way cells behaved in aging, they'd kind of appear and then they disappear spontaneously, and they would never really fill up the tissue. The fact that they have this kind of Darwinian advantage, they're selected for, it means that you could have just a few of them and over many, many cell divisions they'll relentlessly colonize the tissue, which is what we see in the esophagus.

09:09 PJ: Now, if that was true, I don't know if it is, but if it was true much more broadly across the body, I would say we are relentlessly accumulating, as we get older, clones which have a competitive advantage. Now, it might be that they, like the esophagus, leave your tissue looking completely normal, normally, but imagine if you stressed the tissue, maybe those mutant cells would do less well and maybe the reason, certainly when I look in the mirror I get depressed 'cause I'm not as young-looking as I was five years ago, is because not only my skin but also elsewhere, my body is not working quite so well because it's more and more mutant.

09:43 SC: Right. And those competitive advantages of these cells that are taking over, it's not, "We're making your body healthier and stronger," it's, "We're really good at growing. That's what we do."

09:53 PJ: Well, they're good at taking over the normal tissue and not misbehaving that badly; it's not giving you cancer. And we genuinely don't know how many of these there are across the whole genome, across the tissues, and that's a really big objective for our institution or for other people around the world.

10:09 SC: So is that the next step to build up a library of mutation? What's likely in different tissues? How they change with age?

10:18 PJ: We're very focused on the skin in the esophagus. We're basically getting a lot more old people now. Once you're over 65, your risk of this sort of cancer goes up a lot, and in fact most common cancers are like that. What would be really bad, if you have cancer risk, is not if you have

just one letter change, but suppose you had four or five different changes in critical genes. And the best place to find evidence of those clones with multiple letter changes is gonna be in the oldest people. And we're modifying the technology now to try and drill down and find, as we enter that, the cells which are most at risk of changing.

10:53 PJ: The other thing we're doing is we're exploring transgenic mouse models where we can introduce these mutations. We can also mutate the mice randomly a bit, recreate the clone wars, and just see whether we can test ideas to alter the competition process. 'Cause we know in evolution, if the environment changes, then one species does really well and another species does really badly. Suppose you could take the bad mutations and make it harder for them to win in the war.

11:20 SC: I think it's really interesting, this idea that our cells are going about their business, but their business is not always in our best interest. And it's not that it's cancer, it's just that they have different priorities. [chuckle]

11:31 PJ: Well, in a way, they're blind. So it's just like the blind watchmaker idea. The mutant cell neither knows nor cares about how the tissue works when it's colonized half of it. It certainly doesn't care about you as an individual. And all it needs is to have an advantage. And it's likely, for these normal tissue mutations, they're not causing a huge problem because the kind of tissue looks normal. You literally can't tell down a microscope where one of these clones ends and another begins. It's more that when you add them up or when you get another aging-related process that things might start to go wrong. But this is gonna be a very exciting period as we start to unravel what's hidden in there.

12:14 SC: Well, thank you so much, Phil.

12:14 PJ: Thanks a lot.

12:15 SC: Phil Jones is a Professor of Cancer Development at the University of Cambridge. You can find a link to this research at sciencemag.org/podcast. Stay tuned for Meagan's interview with Praveen Vemula on developing a protective gel for agricultural workers at risk of pesticide exposure.

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13:49 MC: Around the world, many crops are protected from pests by organophosphate pesticides. These chemicals attack an important enzyme in animals, acetylcholinesterase, which results in a build-up of the neurotransmitter, acetylcholine, too much of which prevents muscles from being able to relax. They're not harmful in low doses like on the fruits and vegetables we eat. Organophosphates can be dangerous in high doses, like when farmers apply these pesticides without protective gear. I'm here with Praveen Vemula to talk about a gel he and other researchers created to protect farmers from exposure to these types of pesticides. Hey, Praveen.

14:27 Praveen Vemula: Hi, Meagan. How are you doing?

14:29 MC: Good, good. So what type of health problems does pesticide exposure usually lead to?

14:35 PV: Typically, these pesticides are highly neurotoxic chemicals. When you see a person gets acute toxicity, they start having this shaking, shivering tremors, they no longer can control their neuronal signaling that subsequently leads to respiratory arrest, respiratory dysfunction, muscle movement goes down, and they lose their stamina. So because of that they do have severe productivity loss.

15:03 MC: What is the timeline that people start to exhibit this after they've been exposed to this pesticide?

15:08 PV: When we thought of working on this problem, we used to think that people get exposed to over a period of several years and they have to chronically get exposed severely, then only they start seeing the symptoms. And in fact, that that experience was completely eye-opener for us because the day they spray, they typically spray from an hour to three hours depending upon the land area they are spraying, and end of an hour or a couple of hours, they start feeling sick immediately.

15:40 MC: So in the seasons that they're not applying these pesticides, does that mean they recover or once you're exposed, there's no coming back from that?

15:48 PV: Once these pesticides enter into the body, they go and they deactivate an enzyme called acetylcholinesterase, which plays a pivotal role in all your neuromuscular function, and when they get exposed, starts inhibiting this enzyme in an irreversible manner. But over a period of time this enzyme can regenerate in the body. That's why if we have very high dose, severe exposure you have, then you have severe acute toxicity. And for example, in the last season, in one district in India, 40 people lost their lives. But if it is sub-little dose every two weeks, they recover.

16:27 MC: Oh, okay.

16:28 PV: Yeah. So that's why whenever they spray and they have symptoms, severe symptoms for three days, from day four onwards, they slowly start getting recover and in a week also, they'll

completely recover. Whenever they spray again, they have the similar cycle.

16:43 MC: And these type of pesticides are used globally, but it just seems like the application is the problem in India particularly. Or is this also in other countries as well?

16:53 PV: If you see Western countries, most of the time, these are not the manually sprayed. Whereas, in developing countries, primarily these individual farmers, they go and spray manually, so that's why you have a much higher exposure in developing countries.

17:08 MC: Physical barrier creams aren't new to prevent against these things, and they've been tried in the past. What about your gel is different from past iterations?

17:17 PV: We designed the gel in such a way that it chemically deactivates any pesticide molecules that comes into contact, then the pesticide no longer has the ability to provide the toxicity. That's how it chemically deactivates rather than just physical barrier. I think that's the major reason why they're successful.

17:36 MC: Right, and how did you test this gel?

17:38 PV: We really didn't have any valid animal models to systematically study this exposure. So we have developed these new animal models where we can collect the blood at different time points and measure the activity of acetylcholinesterase enzyme. So the moment when they get exposed, we can see the completely drop-off of this enzyme activity. That's a direct biomarker to show what is the level of exposure. The same doses, when you apply in the presence of the gel, and then you measure the enzyme activity, we don't see any of such inhibition at all, which clearly suggests that there's a chemically deactivates and prevents them going through the skin.

18:21 MC: How often would people have to apply the gel for them to be fully protected?

18:25 PV: We have done very interesting experiment to show its catalytic activity. Day one, we applied once, a thin layer of the gel on the skin. On the same gel, almost four days everyday, a new amount of pesticide was added, but even then it could completely deactivate and protect 100% survival we observed in these animals. That clearly suggest that as long as you have a thin layer, it is sufficient to clear any amount of pesticide it comes into contact.

18:57 MC: You interviewed people that work in these agricultural fields, right? And what did you learn from their experiences that guided how you were gonna take a hold of this project and create a gel?

19:07 PV: All of them are aware that the side-effects they are getting is because of the exposure. One way to reduce this, is having this full suits they can wear with the plastic gowns, but unfortunately, tropical countries like here, where temperature can shoot up 45-50 degrees Centigrade, it's impossible for them to wear on the field and use it. They are very cognitive about the cost but if technology comes in an affordable manner, they're ready to adopt.

19:36 MC: You want to make sure that this gel was cost effective. So, did you have any problems finding components that were affordable?

19:44 PV: We are cognitive about right from the beginning to keep the cost lower, that's why we took one of the very abundantly available polymer. Simultaneously, we have been working to optimize in a much larger scale synthesis, which is essential when you go for high scale production.

20:03 MC: Yeah, so what is your timeline right now, for hopefully distributing this gel to farmers?

20:08 PV: There are two more steps needs to be done before it goes to the market. Right now, we are in the process of evaluating a detailed biocompatibility and long time exposure effect. And once that is done, we will be submitting for regulatory bodies to get the permission to start the human pilot studies. We are expecting at least 2-2 1/2 years from now to reach to the market. That's the timeline.

20:37 MC: I'm curious if there's any kind of collaboration with governments or cities to help subsidize the cost of this gel to provide it to farmers?

20:45 PV: I think that's absolutely critical for successful distribution of this compound. We are in dialogue with some of the agriculture ministers here. I think once we make a little more progress with the human data, we will definitely like to engage with the government where we can do the go distribute this technology in a subsidized manner to farmers.

21:08 MC: That's great. Alright, thank you so much.

21:10 PV: And thank you.

21:11 MC: Praveen Vemula is a research investigator at the Institute for Stem Cell Biology, and Regenerative Medicine in Bangalore, India. You can find a link to his research at sciencemag.org/podcasts.

21:24 SC: And that concludes this edition of the Science Podcast.

21:27 MC: If you have any comments or suggestions for the show, write to us at SciencePodcast@aaas.org.

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21:47 MC: This show is produced by Sarah Crespi and Meagan Cantwell, and edited by Podigy. Jeffrey Cook composed the music.

21:54 SC: On behalf of Science Magazine and its publisher, AAAS, thanks for joining us.