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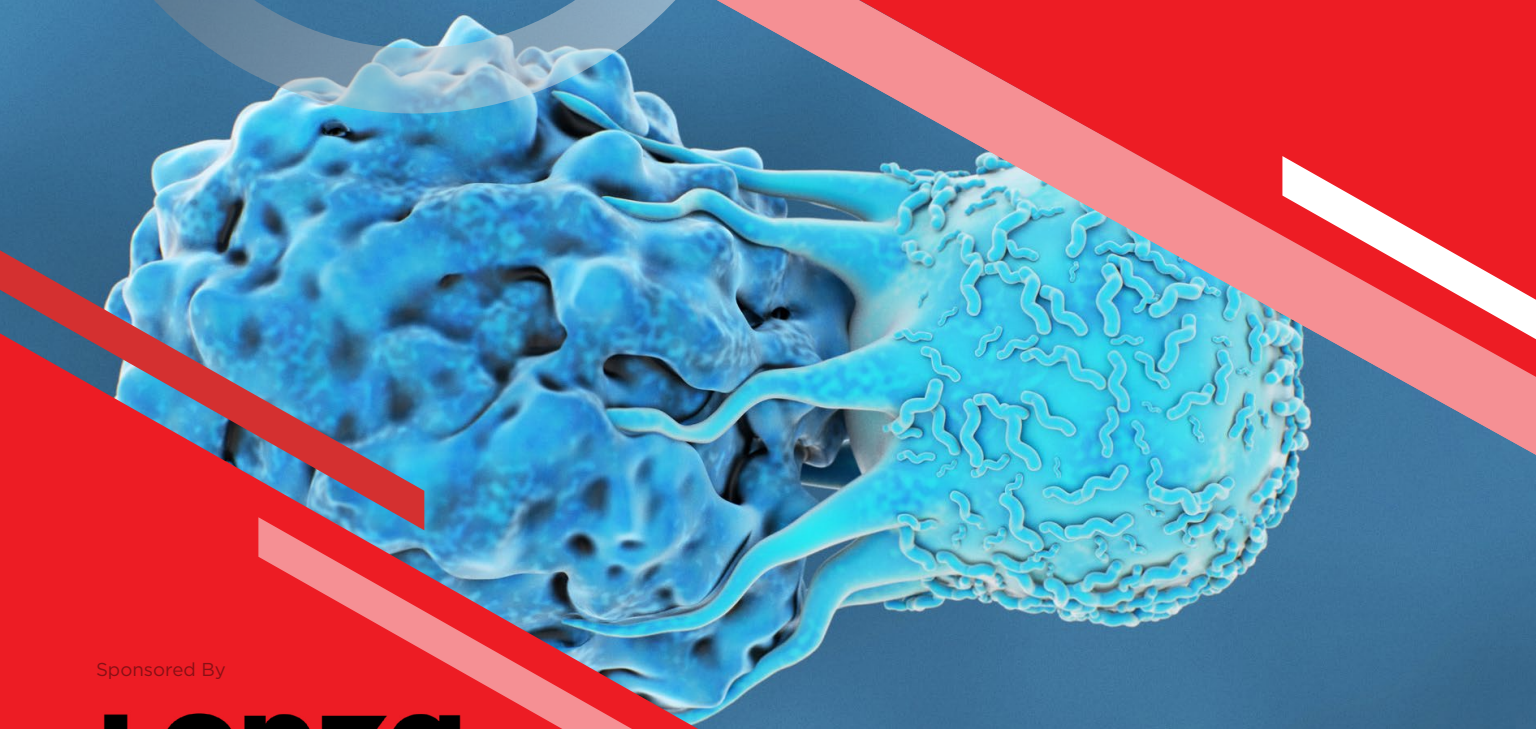

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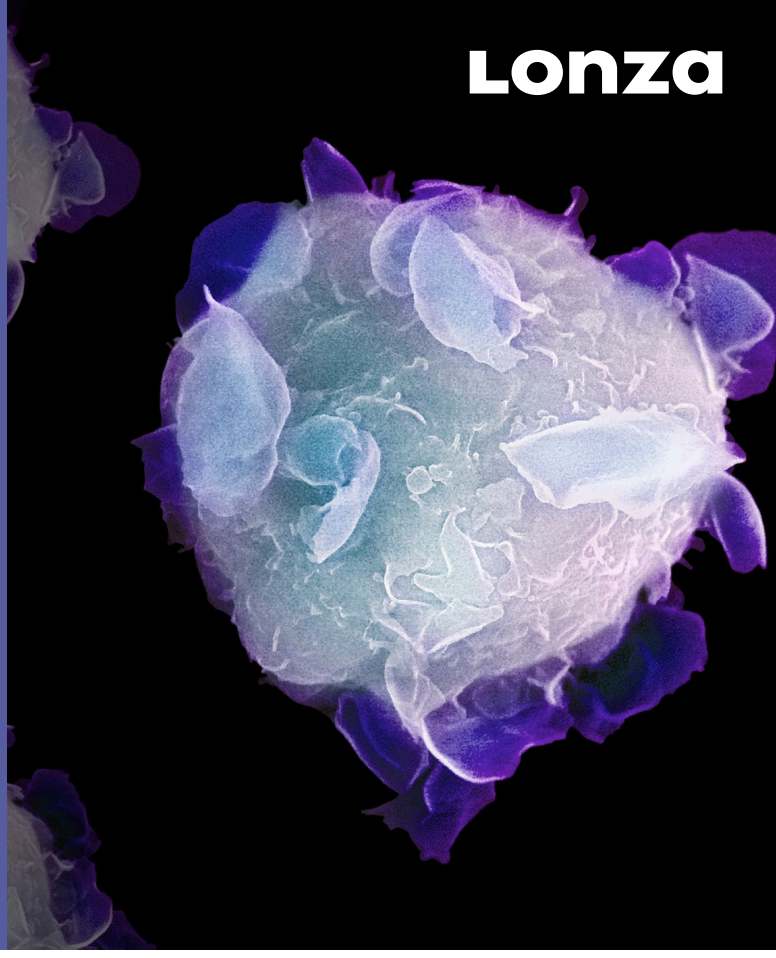

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BIOBANK STUDY UNCOVERS WHY SOME SMOKERS ARE MORE LIKELY TO GET CANCER

More genetic diversity in certain immune system genes can reduce the risk of lung cancer in smokers.

By Jennifer Zieba, PhD

The connection between smoking and lung cancer is well established.[1] However, not everyone who is a smoker ends up developing lung cancer. "I'm fascinated by those outliers that started smoking early on when they are in their teenage years and they never develop cancer," said Diego Chowell, an oncology researcher at the Icahn School of Medicine Mount Sinai. Predicting lung cancer risk is notoriously difficult.[2] Most studies focus on genes known to cause many forms of cancer such as *TP53*, which when turned off allows cancer cells to multiply and form larger tumors.[3] But Chowell thinks of cancer outliers differently. "I think there's something particular in the immune system of these individuals that is restraining the tumor," said Chowell.

In a recent paper published in *Science*, Chowell, a senior author of the study, and his colleagues sought to uncover more about the role of the immune system in lung cancer risk and why some smokers develop lung cancer while others do not.[4] Using large data biobanks, the researchers found that the genetic diversity in a certain part of the immune system plays a major role in whether a smoker will develop lung cancer. This is a novel mechanism and could open a new area of study in lung cancer diagnosis and treatment.

"[The study] broadly highlights the importance of considering this interplay between the cancer and the immune system," said Nicholas Mcgranahan, a cancer genetics researcher at the University College London who was not involved in the study.

The role of the immune system in tumors is well researched.[5] However, immune surveillance is understudied, which is the idea that the immune system not only patrols the body for pathogens but also cancer cells.[6] This is accomplished by the expression of human leukocyte antigen (HLA) genes which help the immune system recognize foreign pathogens and tissues by producing cell-surface antigen proteins that are unique to an individual. Whereas HLA Class I (HLA-I) genes are expressed in almost every cell, HLA Class II (HLA-II) genes are mainly expressed in immune cells such as macrophages and B cells. The more genetically diverse HLA genes an individual has, the better their immune system can identify a variety of foreign invaders.[7] The immune system also has a better chance of identifying mutation laden cancer cells before they become tumors.

"[The study] broadly highlights the importance of considering this interplay between the cancer and the immune system." Nicholas Mcgranahan, University College London

According to Chowell, genes that are highly genetically diverse can be difficult to study. Harnessing the power of numbers, Chowell and his colleagues used the UK Biobank and FinnGen databases, which contain clinical, genetic, environmental, and longitudinal data from over 400,000 people. Knowing that HLA genes are essential to immune surveillance, the researchers isolated the individual HLA genotypes and performed an association analysis with lung cancer incidence and smoking status. In their analysis, they found that current and former smokers with greater genetic diversity in HLA-II genes had a significantly lower risk of developing lung cancer. This is the first time researchers correlated HLA-II genes to lung cancer risk. "We were shocked, honestly," said Robert Samstein, a cancer immunology researcher at Mount Sinai and a senior author of the study. HLA-I genes are known to associate with cancer and the immune system, but the clear genetic signal found in HLA-II genes introduces another arm of the immune system that can be used to assess risk in patients.[8]

"This is something I see as novel and exciting … they did a great job using big data sets of lung cancer patients from independent cohorts … so they could validate the correlation," said Israel Cañadas, a researcher of nuclear dynamics and cancer at the Fox Chase Cancer who was not involved in the study. "[This is] really exciting and I see it really changing the field."

In the future, the researchers will study this immune-cancer connection across cancer types while also considering how this mechanism could be used in the clinic. "In the right setting, we can design immune prevention strategies, and we're trying to take that pretty rapidly in terms of thinking about clinical trials," said Samstein.

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SOX17 DRIVES COLON CANCER IMMUNE EVASION

Judith Agudo investigates what mechanisms determine early cancer immune detection and evasion.

Interviewed by Nathan Ni, PhD



Above image: Courtesy of John Deputy, Dana-Farber Cancer Institute

Immunologist Judith Agudo studies why the immune system detects and destroys certain cells while ignoring others—a phenomenon instrumental to tumor formation, growth, and metastasis. Working at the Dana-Farber Cancer Institute, Agudo and her colleagues recently discovered that the transcription factor SOX17 drives immune evasion in early colorectal adenomas and cancers, a finding published in *Nature*.[1]

Q. Why are you interested in early stage colon cancer?

From a basic research perspective, early cancer means cancer that cannot yet be detected using current tools and methods. If we can investigate this, we can understand how cancer starts and why the immune system fails to get rid of this cancer. If we are able to do that, we could potentially go beyond curing cancer and figure out how to prevent it. As for colon cancer, as an immunologist, I find that the intestine is a very interesting organ with many conditions where the immune system plays a role—autoimmune diseases, colitis, inflammatory bowel disease, food intolerance, celiac disease, and of course, cancers. The interesting question for me is, why does the intestine's robust immune system fail during colon cancer?

Q. What are the advantages of using organoids for studying cancer?

Organoids, as the name implies, are pseudo-organs that we can engineer and experiment with in vitro. In contrast to cell lines, which feel more artificial, organoids let us take primary colon cells—from either mice or humans—and put them in culture and grow either mini colons or mini colon cancers. The main advantage is that we can manipulate them. We can modulate a gene and see if it is important for growth patterns. The goal is always to build something that most resembles the patient, and for this, I think organoids are the best approach we currently have to ask questions about how and why colon cells become cancerous.

Q. In this study, you knocked out three genes to mimic a common gene profile found in colorectal cancers. Why is this profile important?

Colon cancer starts as a non-malignant polyp and then something causes it to become more aggressive and become a tumor. While things like diet are a factor, this process is supposed to be stochastic—random. But right now, there is an epidemic of cancer happening in younger people. We do not know why. It could be because this gene profile is occurring more often, and if so, what is causing these three genes to become mutated more often?

Other studies have reported—in many different organs—phenotypically healthy cells that possess oncogenic mutations. These cells, despite having cancer-associated mutations, do not grow into cancer and the immune system just ignores them. The thing is, these cells may eventually begin to present cancer phenotypes. At that point, will the immune system continue to ignore them? That is the question we are asking in this study: What dictates whether the immune system kills these cells or not?

Q. This study highlighted SOX17 as an enabler of immuno-evasion. What do we know about SOX family transcription factors and their involvement in cancer?

Before this study, we knew nothing about SOX17's role in colon cancer. That said, other SOX factors have been described in other cancers, and a colleague found that SOX in breast cancer drives immunotherapy resistance. SOX transcription factors are often related to stemness, where cells rewire back to a more fetal state and have more plasticity. They are essential for fetal development, where immune evasion helps protect the fetus from maternal immunity. However, they then generally switch off during adulthood. Is there a physiological reason for SOX activation in adults? I do not know, but I cannot imagine that the mechanism has evolved just to trigger oncogenesis.

Q. What are the next aims for this project?

The no-brainer is seeing if we can target SOX17. Unfortunately, it is not a low-hanging fruit because targeting transcription factors is really hard, so we are trying to address how SOX17 expression is induced, with the hope of finding something upstream that is easier to target. We are also working backwards and trying to find what is alerting the immune system to activate against cancer threats, with the possibility of boosting those mechanisms.

This interview has been condensed and edited for clarity.

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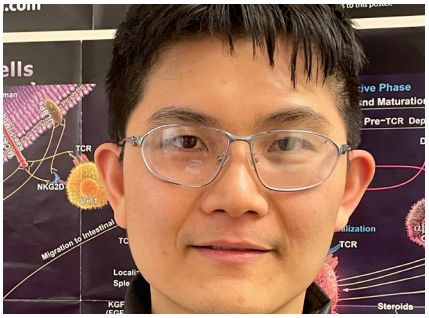
PD-1 PLASTICITY POWERS EXHAUSTION-RESISTANT T CELLS

Yin Wu studies gamma-delta T cells and their role in cancer immune tolerance to improve existing treatments and develop new adoptive cell therapies.

Interviewed by Deanna MacNeil, PhD



Above image: Courtesy of Maggie Joseph, King's College London

As a clinician scientist at King's College London and Guy's Hospital, Yin Wu predominantly treats patients with skin cancer, but his laboratory research is cancer agnostic. Wu investigates a rare subset of tissue-resident immune cells called gamma-delta (γδ) T cells and the self-tolerance mechanisms that different cancers exploit to evade immune and targeted therapies, including those mediated by the immunotherapy targeted T and B cell receptor called programmed cell death protein 1 (PD-1).[1]

Q. How did you become interested in γδ T cells?

In my preclinical medical studies, I focused on influenza virus immunology and began to look at γδ T cells, which were recognized as really potent antigen presenting cells. When I finished my studies, I ended up in Adrian Hayday's laboratory at King's College London, who first cloned these cells in the 1980s. The fundamental question of why some cancers are tolerized whereas others are rejected by the immune system really resonated with me.

Q. How are γδ T cells involved in immune tolerance?

γδ T cells are often enriched and resident within epithelial tissue, which is where most solid cancers develop. They are similar to conventional αβ T cells in that they mature in the thymus and have a T cell receptor (TCR) generated through somatic rearrangement. Once stimulated, the γδ T cells do not recognize classical peptide-MHC complexes that are often downregulated by cancers. Instead, γδ T cell receptors recognize innate and conserved molecules expressed in steady state and stressed tissues. They are central to tolerance mechanisms because they sit within these tissues and can be both immune inhibitory and activating. They are well placed to monitor the health of tissues and provide an immediate early response to eliminate cancer cells independent of γδ T cells, but equally, we think their activity is complimentary with αβ T cell functions.

Q. What is the relationship between PD-1 signaling and γδ T cells?

In the context of constant TCR signaling, PD-1 expression often indicates T cell exhaustion. We asked whether PD-1 has any unique biology within γδ T cells versus $CD8^+$ αβ T cells destined for exhaustion. We sorted cells based on PD-1 status and found that PD-1 positive γδ T cells resembled their PD-1 negative counterparts and we found that only two genes that were significantly differentially expressed. In contrast, several dozen genes differed between PD-1 positive and negative $CD8^+$ cells. PD-1 positive γδ T cells also remained functional, producing just as many effector cytokine and cytolytic transcripts as PD-1 negative cells, whereas $CD8^+$ cells showed a clear drop. This suggests that PD-1 is a more plastic marker in γδ T cells. When $CD8^+$ cells experience activation and PD-1 expression goes up, they differentiate and go down a pathway which they cannot come back from, but PD-1 positive γδ T cells probably shift between states.

Q. Why might γδ T cells be resistant to exhaustion?

In mice, these cells seed into specific tissues during development. Without this seeding, there are no tissue-specific γδ T cells; they do not replenish from the circulation. This tells us that they stay in the tissue long term without becoming exhausted too quickly. Equally, they are seeded in tissues that constantly experience environmental stress and activating stimuli: the skin, gut, and lungs. If αβ T cells constantly experience activation, they become exhausted. Because γδ T cells are more resistant to exhaustion, they need to maintain tolerance within the resident tissue, otherwise they would release pro-inflammatory cytokines that kill normal epithelial cells and lead to autoimmunity. That probably has some roots in how these cells are epigenetically regulated, which is what we are investigating now.

Q. Do these cells have therapeutic potential?

If these cells are inherently more resistant to exhaustion, they may be a little bit better for adoptive cell therapy than αβ T cells, which are chronically activated in the tumor environment. Understanding exactly which switches control these differences may be quite important, potentially allowing reprogramming of other therapeutically suitable immune cells to be more resistant to exhaustion.

This interview has been condensed and edited for clarity.

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A STRESSFUL SIDE OF CANCER

Xue-Yan He's research shows that stress can make tumors more dangerous.

Interviewed by Aparna Nathan, PhD



Above image: Courtesy of Xue-Yan He, Washington University in St. Louis

Stress isn't just in the brain; its physical symptoms cascade through the body. People with cancer are under extraordinary levels of stress, but studies show that this can make cancer more likely to grow and spread to other parts of the body through a process called metastasis.[1] Understanding the molecular consequences of stress can help inform better treatments. Xue-Yan He, currently a cancer biologist at Washington University in St. Louis, recently dissected one particular way that stress hormones promote metastasis by recruiting immune cells to wreak havoc on organs.[2]

Q. How did you first get interested in studying the mechanisms of stress in cancer?

As a postdoctoral researcher in Mikala Egeblad's laboratory, I came upon this interesting question by accident. I always ordered mice from a company that delivered the animals to us, so the mice experienced a distressing 10 hours of traveling. Usually researchers let the mice calm down before doing experiments, but I did not know this, so I performed an experiment immediately. I found that when the mice were under stress, their tumors grew very quickly.

Q. How did you experimentally stress out the mice?

We used different breast tumor models and mimicked stress with a physical restraint model, as well as a chronic unpredictable stress model where we randomly gave the mice two minor stressors every day, such as food or water deprivation or pinching their tail. We also used a metastasis model where we injected tumor cells into the mammary gland, let the tumor grow to a certain size, and then performed surgery to remove the tumor. This mimics what cancer patients undergo. After the surgery, we stressed the mice and found that two to four-fold more metastases developed in their lung tissue.

Q. What did you do to trace these stresses back to a molecular mechanism?

We first looked at the microenvironment of the lung—the site of metastasis—and found that stress had already changed it. There were fewer T cells, which can control tumor growth. But making the T cells insensitive to the stress did not reduce metastasis, so we concluded that the T cells were not to blame. We also found there were more neutrophils infiltrating the lung tissue. When we depleted all the neutrophils, the stressed mice developed the same amount of metastases as the controls, so we concluded that the neutrophils drive the metastasis.

We further found that glucocorticoids, a type of stress hormone, can bind to receptors on neutrophils. When neutrophils are activated by this hormone, they can form structures called neutrophil extracellular traps, or NETs, which are DNA scaffolds released by the neutrophils. This modifies the whole microenvironment of the lung.

Q. How can you use this knowledge to slow cancer progression or prevent metastases?

We tried two pharmaceutical or genetic interventions to prevent stress-induced metastases in mice. First, we depleted the glucocorticoid receptor on the neutrophils so that they do not respond to the stress hormone, and the stressed mice no longer developed more metastases. The second method we tried is using an enzyme called DNase I to digest the DNA in the NETs. We found that this also led to a large reduction of lung metastases.

Q. Are there any lessons here for the importance of managing stress in cancer patients?

We know that stress is bad and that we need to use behavioral interventions for cancer patients. Interestingly, when we analyzed lung tissue from stressed mice after removing their primary breast tumor, we found that the microenvironment was already changing. The stress is actually supporting future tumor growth through what we call a pre-metastatic niche. It is surprising and also scary.

Q. How do you plan to continue your research?

There are still a lot of unanswered questions. We are now working on understanding the other systemic effects of stress, such as how the stress affects the sympathetic nervous system and how this can affect the tumor. I am also expanding my research to other organs because they all have different microenvironments and respond differently to stress. For example, we know the gut environment is very different from the breast, which may be important for understanding how stress affects colorectal cancer.

This interview has been condensed and edited for clarity.

See references on page 8

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