PROGRAMMING GERMS TO FIGHT DISEASES

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While watching the documentary "Cooked" on Netflix recently, I was captivated by the part where writer Michael Pollan talks about eating a wedge of cheese grown with special bacteria and getting protected from disease. Wow! Visions of being vaccinated with Parmesan shavings popped into my mind. The truth is that we aren't far from the day when these scenarios could become reality, and the research to make it so is happening right here at NUS Medicine.

Applying design and production principles from synthetic biology, Associate Professor Matthew Chang from the Department of Biochemistry and the NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI) is leading a new research programme to modify microbial genes to produce safe and effective therapies for a range of important diseases. The programme is one of the NUHS's Summit Research Programmes, which bring together basic and clinician scientists from different disciplines to drive academic excellence, accelerate research outcomes, and train new clinician scientists.

The microbes in question are the 10 to 100 trillion bacteria and yeasts living in our bodies (mostly in the gut), which together comprise the microbiota.[1] Put together all of the genes of these trillions of organisms and you get the microbiome. The SRP team is figuring out the role of the microbiota and microbiome in infectious disease, metabolic disease and cancer, as well as tweaking some of our gut bacteria to create therapies for these diseases. Such engineered bacteria lend themselves well to oral therapies since they thrive in the gut environment. They also stay in the body for a long time, thus could be taken less frequently. However, long-term safety becomes very important — this could be ensured by measures such as changing the genes that control the production of toxic substances.

ENGINEERED MICROBES IN INFECTIOUS DISEASE

Pseudomonas bacteria are a major cause of hospital-acquired infections such as respiratory infections that start in the gut. The worrying thing about these infections is that they tend to be resistant to multiple antibiotics, which leads to a high rate of mortality from the bacteria in the blood and all over the body (sepsis). Dr In-Young Hwang, lead of the Mammalian Synthetic Biology progamme and co-lead of the Therapeutic Cell programme in Assoc Prof Chang's lab, headed the work in engineering "good" *E. coli* bacteria to sense and kill the harmful *Pseudomonas* bacteria. In animals, the engineered bacteria could clear established infections as well as prevent infections from gaining a foothold.[2]

The advantages of this system are: 1) its specificity - the engineered bacteria only release their toxins when they detect *Pseudomonas* bacteria in the vicinity; and 2) its ability to break down and prevent new biofilms, which are layers of bacteria within which the bacteria can hide from the action of antibiotics.

The team, which includes clinician scientists Dr David Ong and Dr Louis Chai, is also targeting *Clostridium difficile* bacteria, which also have a strong tendency to become antibiotic resistant, to make them more susceptible to the drugs. Engineered bacteria thus represent a novel addition to the limited treatment options for multidrug-resistant bacterial infections.

ENGINEERED MICROBES IN METABOLIC DISEASE

Assoc Prof Chang is working with clinicians Associate Professor Lee Yung Seng, Associate Professor Dan Yock Young, Dr Yvonne Lim, Dr James Huang and Dr Jonathan Lee to identify the types of bacteria that are associated with good health, eg, low risk of obesity and diabetes, as well as bacteria that are linked to undesirable conditions. Once these health-promoting bacteria are identified, the team aims to selectively enrich such bacteria through successive life cycles, and pinpoint the proteins responsible for these beneficial effects. The researchers can also engineer other microbes, such as "good" *E. coli*, to produce these particular proteins. Eventually, people could just consume the engineered microbes in the form of a pill (or a piece of cheese!) The microbes would make their way to the gut without much digestion by stomach acid, and enhance the production of favourable hormones and control glucose absorption.

The team is now identifying bacteria in the gut that are linked to good metabolic health. Previous studies found that favourable microbiota are specific to populations. For example, microbiota that have been linked to good metabolic health in Western populations are not beneficial in Asian populations. Profs Lee and Sanjay Swarup at the Singapore Institute of Clinical Studies (SICS) and Singapore Centre on Environmental Life Sciences Engineering (SCELSE) respectively, are studying the insulin responses and stool microbiota of 25 Indian, 25 Chinese and 25 Malay young men, after consumption of different types of food. The work is still in progress, but ethnic differences are emerging in the types of bacteria that are important for metabolising specific foods.

ENGINEERED MICROBES IN CANCER

Despite significant advances in cancer treatment during this period, current therapies still come with a range of side effects such as nausea and low blood cell counts. Another problem is the inability of cancer therapies to completely eliminate cancer cells, which can result in cancer recurrence and a high risk of death. To help overcome these issues, researchers are exploring new treatment options, one of which is the use of engineered microbes. Although the idea of using bacteria to fight cancer has been around for more than a century, the advent of synthetic biology is now making it a reality.

Using bacteria to fight cancer is appealing for several reasons. Firstly, cancer cells produce substances that suppress the immune response, creating a friendly environment for bacteria to grow. Secondly, many solid tumours are oxygen deprived, which certain bacteria called anaerobic bacteria actually prefer. Some of these anaerobic bacteria multiply better in solid tumours than in healthy tissue, giving them an inbuilt specificity for tumor tissue.

For example, researchers have engineered *Bifidobacterium* bacteria (a common probiotic supplement) to express anticancer substances such as the thymidine kinase enzyme produced by the *Herpes simplex* virus. Thymidine kinase converts a compound called ganciclovir into a toxic

Left: Dr Chun-Loong Ho ; Right: Dr In-Young Hwang

substance that kills the tumours.[3] Even the much maligned *Salmonella* bacteria can be used to detect tumors or to destroy them by delivering a payload of cancer-killing substances. These *Salmonella* organisms are either harmless strains or altered bacteria that do not cause food poisoning. Engineered probiotics have been shown to be effective against a range of cancer types, including cancers of the colon, liver, stomach, breast and skin.

Dr Chun-Loong Ho, who co-leads the Therapeutic Cell programme in Assoc Prof Chang's lab, is engineering probiotics to specifically target colorectal cancer cells. They engineered *E. coli* Nissle to be a probiotic that attaches to the surface of colorectal cancer cells and secretes an enzyme that converts dietary substances found in vegetables into anticancer agents. The engineered probiotics plus a vegetable extract result in the killing of more than 95% of the cancer cells in a dish. In mice with colorectal cancer, the engineered probiotics caused, on average, a three-quarter reduction in the number of tumours and a 3-fold decrease in tumour size than in untreated mice. He and Dr Yong Wei Peng, a colon cancer specialist, envision using these probiotics as tools to help clean up the cancer cells remaining after the larger tumors have been surgically removed. They are also working on converting waste substances into anti-tumour agents.

TESTING THEM IN HUMANS

So far, most of the infectious disease and cancer research has been in animals, but the team plans to test the more promising therapies in humans soon. As Prof Lee says, "The strength of the SRP is that it involves both scientists and clinicians from the beginning. Unlike most clinical and basic science collaborations, the clinicians in this programme feel that they have ownership and can shape the experiments from an early stage, based on their clinical knowledge. The scientists have the know-how to make it work."

REFERENCES

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