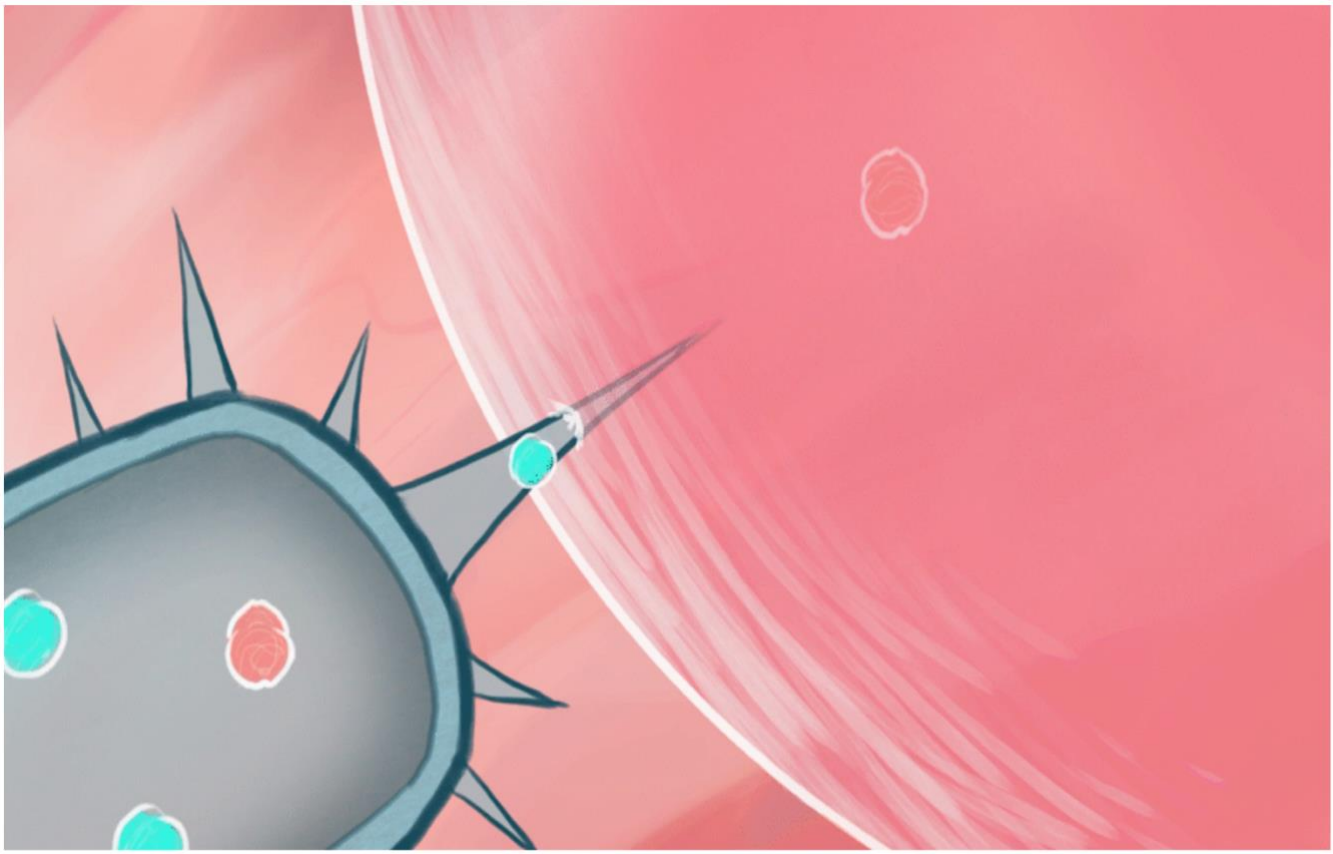
Fighting solid tumors with genetically modified bacteria



Kelian Buro

Kira Buomberger

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Preface

Cancer is one of the most common yet challenging diseases to treat due to its multitude of variations. Despite affecting a substantial portion of the population, conventional therapies like chemotherapy still cause numerous unwanted and unhealthy side effects, like the loss of hair or throwing up. So, when we learned about T3pharma's goal to eliminate some, if not all, of these side effects, our interest was naturally peaked.

What we found particularly intriguing is T3pharma's approach to fighting solid tumours. Unlike chemotherapy, which relies on powerful chemicals and in the process damages all cells. T3pharma is currently testing a method that uses genetically modified pathogen bacteria. The secretion system of these bacteria has been changed in a way that allows them to secret pre-determined protein directly into the targets cancer cell without damaging any healthy tissue. We had never heard of a treatment method that involves live bacteria, which made this startup even more interesting to us.

Of course, this innovative idea raised several questions, such as: How do these bacteria combat the tumour? Why don't the bacteria get targeted by the human immune system? And how advanced is the development of this technology? The aim of this paper is to answer these questions.

Introduction

Unfortunately, T3pharma's technology is not currently available on the market, because the development of such therapies takes time and extensive testing. But there has been an ongoing clinical trial for the past two years. While the results of these trials remain confidential, we can conclude that considerable progress has been made since the bacteria is now being tested on humans. Moreover, the start-up continues to explore new proteins that can potentially be used to harm the tumour or make it "visible" to our immune system.

As mentioned earlier, the technology is not yet in widespread use. However, once implemented, the technology could provide significant improvements compared to other cancer treatments such as surgery, chemotherapy, stem cell transplants, or radiotherapy.

If successful, the bacteria would be far less invasive than surgery or stem cell transplants, have fewer side effects than chemotherapy since it only targets cancer cells, and would not involve radiation exposure like radiotherapy.

Once on the market, this technology could potentially revolutionize cancer treatment and significantly improve the lives of countless cancer patients.

Type 3 secretion system

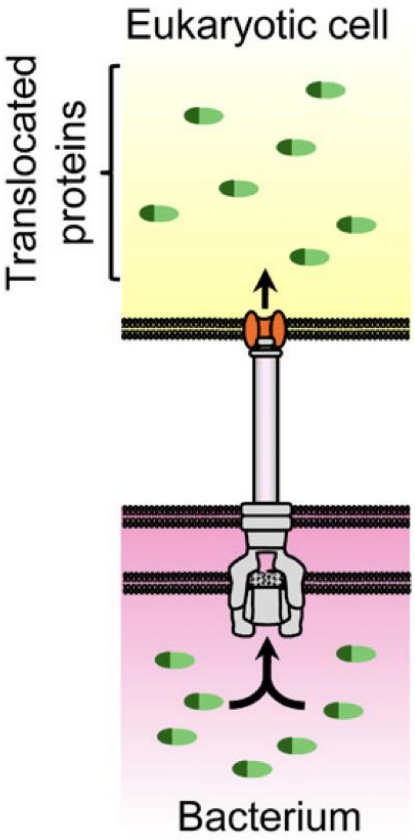


Figure 1depiction of the T3SS

Bacteria have developed sophisticated nanomachines enabling them to inject virulence proteins into eukaryotic cells. The type 3 secretion system (T3SS) consists of a protein resembling a small needle going from the cytoplasm of the bacteria through the bacterial cell membrane and cell wall to deliver bacterial effector proteins directly into the cytoplasm of the eukaryotic host cell. The T3SS is closely related to the bacteria's flagellum, so it is suspected that the T3SS evolved from the flagellum.

During the transportation through the T3SS proteins are unfolded to fit through the nano-needle and refolded once in the cytoplasm of the host cell to regain their function. In the host cell these refolded proteins can exert their virulence activity by interacting with various proteins and cellular machinery inside the infected host cell. These proteins vary greatly among different T3SS pathogens, but common functions of these proteins emerge. They can either cause interference with the host cell cytoskeleton to promote attachment and invasion, interference with cellular trafficking processes, cytotoxicity and barrier dysfunction, and immune system subversion.

Fighting solid tumors

Solid tumors are abnormal masses that usually do not contain cysts or liquid areas. Furthermore, these tumor cells locally downregulate the immune system to remain undetected by the immune system. Cells experience a certain number of mutations each day, but only the cells that gain the ability to downregulate the immune system through their mutations get the chance to develop into a tumor, because if they do not, they get killed by our immune system immediately. This is why the characteristic of downregulate the immune system is universal across almost all types of tumors T3Pharma uses this characteristic to directly target the tumor.

They genetically modified bacteria to make them lose most of their virulence factors and thereby most of their resistance to our immune system. Thereby all the bacteria that aren't located on the tumor get killed by our immune system and only the tumor gets colonized. Once on the tumor these genetically engineered bacteria can directly translocate researched proteins into the cancer cells to treat the tumor.

This allows for a very targeted treatment with almost no side effects.

Engineering techniques

Homologous recombination is a natural process used by cells to repair DNA and exchange genetic material. It involves the exchange of DNA strands between two similar or identical DNA molecules. We can use this to engineer organisms by replacing a section of their DNA (in our case the virulence factors) with a more useful DNA sequence, called a construct.

The first step is to create the construct. It contains the desired gene, which in our case is a therapeutic protein, which will ultimately be delivered into the target cell. It also contains an antibiotic resistance gene to be able to select the bacteria which have successfully been altered. And at last, we need flanking sequences which are necessary for the homologous recombination to work. The flanking sequences in the DNA construct are designed to be identical or very similar to the sequences on either side of the target region in the organism's genome. This similarity is crucial for the cell's DNA repair machinery to recognize and facilitate the exchange of genetic material.

Next, this construct must be inserted into the cell. T3Pharam does this by electroporation.

Once inside the cell, the DNA repair machinery recognizes the similarity between the construct and the target DNA thanks to the flanking sequences. The cell then swaps the target DNA with the new sequence from the construct, making the cell lose its virulence factors and gain the antibiotic resistance and the wanted protein.

The last step is the selection and testing of these newly engineered bacteria. Cells that have successfully been altered will also have the antibiotic resistance gene, which allows scientists to select these bacteria and either cultivate them in bioreactors or conduct various experiments.

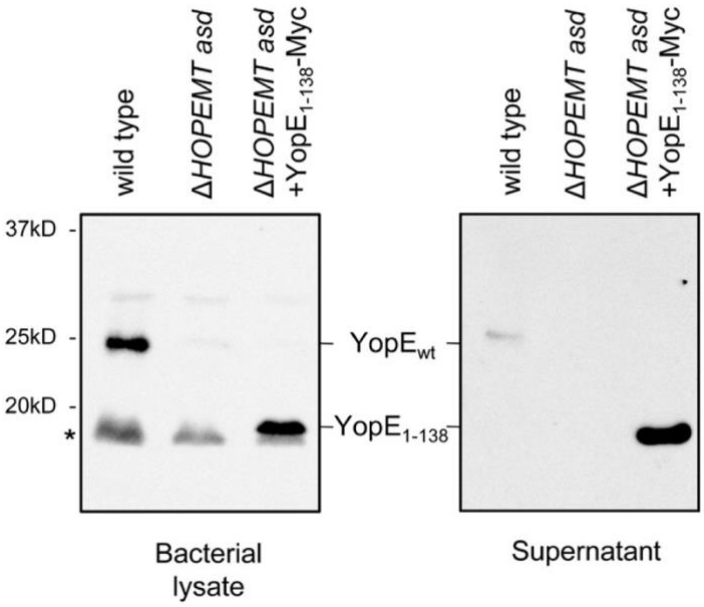
Discussion

There still is a lot of research to do for this technology to become commercially available. One of the problems T3Pharma is facing is the research of therapeutic proteins. There are a lot of different places where the delivery of these protein can fail. The first one being the delivery of the protein through the nano needle. For some reason, still unknown to T3Pharma, some proteins just do not get secreted properly or at all. For the moment there is no way of predicting if the secretion of a protein through the T3SS will work. Every Protein potentially useful protein must be tested with a simple experiment.

Western Blot

To understand this analytic method, we need to explain a few terms first:

- **Western Blot:** It is a method of separating a mixture of proteins by their molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein.
- **Bacterial lysate:** A preparation made by lysing bacterial cells. Lysing a cell is when you rupture the cells membrane by a physical, chemical, or enzymatic agent, which frees the inside of the cell, making a solution out of it. This allows the isolation of a certain protein inside the cell, or in our case to make an analysis of the proteins inside of the bacterial cell.
- **Supernatant**: In this case it is a solution of the proteins that were secreted by the T3SS.



The Image on the left shows how a western blot reveals which protein can get secreted through the T3SS (on the right) compared to all the tested proteins (on the left).

Figure 2 Image of a western blot experiment

Folding and unfolding of protein

Proteins that get secreted by the T3SS get unfolded to fit through the nano needle and then refold in the host cytoplasm. Sometimes proteins do not refold correctly and thus lose their therapeutic function. This is problematic, because this malfunction cannot be predicted and can only be detected in the mouse testing phase, which is quite late on in the research process.

Immune System

Another Problem which T3Pharma is working on is that the bacteria get detected and killed by the immune system before they even got the chance to colonize the targeted tumour. This can be fixed by adjusting the virulence factors. They need to find a way to make the bacteria resistant enough to get the chance to colonize the tumour, but also weak enough so that the bacteria only infect the tumour and not any other part of the body.

Ethical question

We do not think that there is any ethical problem with this technology. All the used methods for this treatment have been around for a long time. The only new part of this technology is how all these methods are combined to create a cancer treatment. The only possible ethical question to us is what would happen if these bacteria got free, but this will never be a problem because in this laboratory these bacteria are weakened and thus less dangerous than the wild type, which to begin with is not a dangerous bacterium.

Summary

This paper explains the technology behind T3Pharmas project. The Startup situated in Allschwil tries to revolutionize cancer treatment with the help of genetically modified pathogenic bacteria, that secrete proteins to target cancer cells selectively. Gene manipulation in the bacteria is achieved through homologous recombination and electroporation, allowing for precise gene replacement, deletion, or insertion.

Unlike conventional chemotherapy, this approach ensures that the therapeutic protein is delivered only to the desired location. This is possible because the bacteria used has had their key virulence factors removed, allowing the immune system to eliminate the bacteria easily. Tumours however locally suppress the human immune system, which makes their surroundings optimal for bacteria colonization, because they are not getting killed there.

The technique that is being tested relies on the type III secretion system (T3SS), which allows the bacteria to inject therapeutic proteins directly into the cancer cells.

The challenges that the start-up is currently facing are the premature detection of the bacteria by our immune system, alterations in protein structure and function during secretion through the T3SS and the spontaneous non release of certain proteins. To overcome these, they are constantly testing new proteins until suitable candidates are found.

Sources

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Interview with Malenka, from T3Pharma