Nadav Ahituv: "The CRISPR-Cas9 technology has revolutionized the way we can now modify or modulate DNA"

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Dr. Nadav Ahituv's lab investigates gene regulatory elements and their relationship to human diversity and disease.

Dr. Nadav Ahituv is a geneticist / genomicist who uses advanced computational and genomic tools to characterize how variation in gene regulatory elements leads to human disease. In his laboratory of Bioengineering and Therapeutic Sciences of the Institute for Human Genetics of the University of California in San Francisco-UCSF (USA) he uses different genomic technologies, such as RNA-seq, ChIP-seq, ATAC-seq and CRISPR / Cas9, to characterize the gene regulatory elements. To functionally

determine these elements, he works in various model systems, like the **zebrafish**, mice and cell cultures. In addition, he has created and is developing technologies that allow mass analysis, in parallel, of thousands of sequences. His main lines of research focus, among others, on gene regulatory mutations and their relationship with human limb malformations, obesity, mental illness and response to medication.

- ***What is your current area of research?***

My work focuses on the search for regulatory elements; that is, sequences that tell genes when and when not to activate. Basically we want to find mutations in the genes that provoke diseases in humans or that cause differences between different species, since diseases are related to these regulators that are responsible for turning the genes on and off.

- ***How did you become interested in genetics and its relation to human illnesses?***

It has a lot to do with my own illness. At age 13 I was diagnosed with severe scoliosis. My first treatment was a corset for my spine, something that, for a teenager, is not exactly pleasant. But it didn't work, since my curvature was greater than 50º, so I had to have surgery when I was 16 years old. My younger sister also had this problem. Hence my interest in genes and genetics, genetic diseases, etc.

- ***Do you have any research projects about this disease?***

Although I had never worked on scoliosis until now, three years ago we started a line of research on this disease because we think that many of the mutations that lead to scoliosis are caused by regulatory elements that turn the genes on and off. Now we have a big project in this field.

- ***What is the current reality of genetics and what are the actual treatment possibilities offered by the modern techniques of genetic modification?***

There are many diseases in which the problem is caused by the dose of the gene; that is, the gene is not expressed at the necessary levels and that generates a series of events that provoke an illness. We work to use these regulatory elements with the aim of reversing this situation and promoting the expression of a gene, by increasing or reducing its expression. We have studies, some recent, in which we have activated these regulatory elements to overcome a deficit of expression in the gene and, thus, increase the expression of RNA or proteins.

I am very optimistic and I think we are going to enter the golden age of gene therapy

- ***There’s much talk about genetic modification and its possible risks. What are they?***

At first genetic therapy generated a lot of expectations among the scientific community, and in society as well, but over time these expectations were deflated by issues related to their safety or efficiency. But the situation has changed a lot. Call me optimistic if you´d like, but with the current tools, such as CRISPR-Cas9 technology, and even considering that there will be some setbacks, we will be able to move faster and in a safer way.

- ***You currently work in many areas: human limb malformations, obesity, autism, etc. Which is the most promising?***

We all know that the transition from the laboratory to the clinic can take years. But fortunately, nowadays we have at our disposal a series of technologies that make this process less slow. For

example, gene therapy has benefited from innovative routes of administration through viruses, which are currently much safer than some years ago. CRISPR-Cas9 technology has revolutionized the way we can now modify or modulate DNA, which is what we do at our laboratory. I believe that thanks to all these developments, the current waiting time between the findings in the laboratory and its clinical application is shorter. Because if we look back, for example, years ago we started talking about the modification of RNA, but 20 years passed before the first RNA therapy in the clinic was approved. Things take time, but just by looking at what is currently happening with gene therapy we can see that this time is getting shorter. For example, the FDA has just approved a gene therapy for the treatment of spinal muscular atrophy.

- ***Your team has recently published a study in Science that describes the use of a modified version of the CRISPR gene edition to prevent severe obesity in mice. Could you explain what it is about?***

This is the proof of concept that confirms that our therapeutic approach really works. As I said, there are many human diseases that are caused by abnormalities in the expression or dose of the genes and, probably, the most classic are those caused by a deficiency in one of the copies of the gene. It is called haploinsufficiency, and it occurs because a single copy of the normal gene is unable to produce protein in sufficient quantity or quality to ensure normal function. That is, of the two copies of a gene that we inherit, from our mother and our father, one of them does not work, so 50% functionality is produced. And sometimes, a 50% protein production is not enough, and so a disease is generated. Our job is to make the normal copy of the gene increase its expression and produce more protein and RNA. This way we can treat the disease that causes this insufficiency. Our proof of concept was done with obesity, because it is very easy to see if the therapeutic approach works. You see it very easily: the mouse gets fat or not. The idea is to target obesity-related genes in which only one copy works and increase its expression. And we have seen that, intervening in two different genes, SIM1 and MC4R, both involved in the processes of appetite and satiety regulation, obesity is prevented in these animals. The idea is to move towards people with morbid obesity, who die before the age of 50 usually due to heart problems, diabetes, etc. Obesity in these people is not a consequence of their lifestyle, but of a malfunction of the genes, which makes them never feel satiated and eat without control. Once we have proven that it works, the next step is to address other serious diseases with a high mortality rate in children.

20 years have passed for the first RNA therapy to be approved in the clinic

- ***What is that modified version of CRISPR like?***

Our CRISPR technique uses the dead Cas9, which does not cut DNA or make any changes in the DNA. Like the conventional **CRISPR-Cas9 technology**, our modification also specifically targets a specific DNA sequence, but once there it doesn’t cut anything, but rather enhances the expression of a particular gene. Dead Cas9 has a mutation that eliminates its ability to cut DNA, but not to join it. In this way we get it to work as a distributor, which leads a regulator to where we want it to and tells the gene when, where and at what levels it should be activated. In the case of CRISPRa, this regulator is a protein that provides ‘extra transcriptional machinery’, so that more messenger RNA is produced, which is the intermediary that translates the information of the protein-encoding DNA. Thus, greater activity of the gene that works correctly is achieved, compensating for the lack of the defective one. By not carrying new copies of the genes possible side effects are avoided.

- ***And when can this research be applied on humans?***

That is our goal! If we are able to use this technique on just one person to treat them, I would be the happiest person in the world. But as I said there are many aspects to resolve.

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- ***What are the biggest challenges in the use of gene modification to treat human illnesses?***

Of course we have to take into account the ethical aspect. It is most likely that the first approvals will be given for those very serious diseases in which there is no other hope, such as spinal muscular atrophy. But we must not forget other aspects, such as the immugeneicity of CRISPR, which we do not know much about, and must worry about it, and increase our knowledge on it. The administration of therapy is also a challenge, that is, the use of viruses to reach the specific target and not adjacent tissues. But, in any case, as I said before, I am very optimistic and I think we will enter the golden age of gene therapy. The technological tools we currently have, together with the significant investment in gene therapy that the biotechnology industry is carrying out, aware of the value of these techniques and their potential, make me believe that we are very close.

- ***Yes, but it seems it will be a therapy accessible only to very few people, after the FDA approved a therapy that costs 1,5 million dollars.***

That is another important challenge. We have to make these treatments accessible to most of the population that needs them. But it is complicated; on one hand, there are pharmaceutical companies that invest billions of dollars in the development of these innovative therapies and expect an economic return on their investment. We cannot forget that these are single-use treatments. It is a complicated situation. In my opinion, it is more likely that, as this field advances, therapies will become more affordable, although of course it will be much more difficult for people living in less developed countries.

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- ***However, if we consider that it is just a single dose, then we would save the expenses derived from the illness.***

That is exactly what the pharmaceutical companies argue, that in the long term it saves costs. And indeed it is true and it is possible that it is even more economical. But, even agreeing with this point, we’re talking about millions for a treatment ... I don't know ... it seems excessive.

- ***Where will we be in 30 or 40 years?***

I hope and wish that these techniques will have already reached the clinic.

- ***What do you love most about your job?***

Exploring and discovering new things. That is what fascinates me most about my work. I would be happy, as I said, if it serves to treat patients. But we also work on other topics that I love, such as why bats develop wings. That fascinates me too.

- ***How important is mentoring in the career of a researcher?***

I try, at least. We are the teachers of great scientists and we have a big responsibility. In addition, nobody teaches us how to be a good mentor, you learn on the go and, especially, my case in particular, from mistakes. I try to maintain permanent contact with my researchers and it is my duty to meet with them once a week, for an hour, to talk, not only about their work, but also about aspects more related to their life, their goals, etc.

- ***Is it your first visit to the CNIC?***

Although I know many people at the CNIC, it is the first time I have come and it is really impressive. I have collaborated with Miguel Manzanares and currently one of the students from his group is in my laboratory in San Francisco.

- Dr. Nadav Ahituv, gave a seminar at the CNIC entitled "Functional characterization and therapeutic targeting of gene regulatory elements" invited by Dr. Miguel Manzanares.
