INTERVIEW WITH AN EXPERT: DR. GEORGE CALIN

Whether it is destiny or just plain luck, life encounters with the right people, in the right places at the right times can have a major impact on society. Dr. George Calin always had a natural interest in genetics and molecular biology. The path he chose and decisions he made are impacting cancer research and science in general.

Dr. Calin acquired his MD and PhD in his native country of Romania. After he graduated, he ventured to Italy to further study under Dr. Massimo Negrini, a well known geneticist. Once his apprenticeship was complete, he returned to Romania where he then specialized in gastroenterology and emergency medical care. During this time, he also worked closely with the Romanian police in the molecular genetics laboratory for the National Forensic Institute. His continued interest in genetics and a lack of resources in Romania brought him to America where he worked under Dr. Carlo Croce, first at Thomas Jefferson University and then at Ohio State University. It was in Dr. Croce's lab that Dr. Calin made a discovery that is changing biology textbooks around the world.

WHAT INITIALLY INTERESTED YOU IN GENETICS?

Young people always want to do that which is not allowed. This is also true in a communist country. Growing up in Romania, genetics was seen as a secondary science, as a non-truth. Scientific publications were not readily available, and the teachers had difficulty teaching genetics because they did not fully understand the material. It was much more advantageous to study chemistry or mathematics as opposed to genetics.

HOW DID YOU LEARN ABOUT GENETICS WHEN YOU WERE YOUNGER?

I learned by myself through the limited scientific literature available, and had a very good teacher, Dr. Dragos Stefanescu, a scientist in a cytogenetics laboratory at the Carol Davila University in Bucharest. This is the way in which I started learning and working.

WE UNDERSTAND YOU MADE A DEDICATED EFFORT TO LEARN ABOUT MOLECULAR GENETICS. WHAT WAS THE OUTCOME?

Between 1996 and 1997, I sent 120 letters to scientists around the world, including Carlo Croce in Philadelphia, stating something like, "I would like to learn, but I have no background." I received

about 10 answers, and it was because of these letters that I went to Italy. Massimo Negrini was working in Carlo Croce's lab when my letter was received. He was returning to Italy where I had also written to his new boss. So Negrini discovered from two different places that there is a crazy guy who is sending letters. He ultimately invited me to work in his lab and gave me a chance.

DID YOU COME INTO CLL BECAUSE OF CYTOGENETICS?

Yes, CLL is a very good disease for researching cytogenetics because of the chromosomal abnormalities. Now thinking in hindsight, it was a mix of things because I was in Croce's lab where CLL was the project, and also because I have a cytogenetics background. Coming here [to MD Anderson], the CLL story is much stronger than other places. It is the best place to study CLL, so my path makes sense. I am working here and can study any type of cancer, but I still work with CLL because of the nature of my research. It is also good luck because I was in the right moment at the right place. This is a good point to make- that by chance Croce and I, we were both working with chromosomes in different locations, both of us realizing that cytogenetics is important. I went to work in his lab at the recommendation of Massimo Negrini, and this is when everything happened.

HOW DID YOU DISCOVER MICRORNAS?

It's all luck. Had I not gone to Carlo Croce's lab, I would not make these discoveries. He gave me the "worst" project (newcomers always get the worst projects), which was cloning genes in CLL. I had never worked in leukemia before. They were trying to figure out the genes involved in the 13q- abnormality, but no real progress had been made for more than one decade before I arrived in his lab in 2000. You must also understand that Croce was never telling us point-by-point what to do, but gave us the "liberty" to discover. There are laboratories where the PI [principle investigator] does not allow you to jump into other types of research. If I worked in a place like that, I would do what the PI was asking me and I never would have found microRNAs.

YOU STRAYED FROM THE PROJECT YOU WERE GIVEN?

Yes, because the project was not successful. We were trying to discover a new type of gene. This was the point. I started reading anything and everything. When I read about these new genes originally found in worms, then flies and mice, I decided to clone them in humans and this was it. In 2002 we published our work. We were 2 years in advance of the rest of the world, which is very difficult to do these days because so many minds around the world are reading the same literature and performing the same experiments. We were ahead because we picked up on all of these new genes while everybody was thinking it was trash. It is important to know that normal genes are much

larger than microRNAs, so no one was looking for something so small in humans, especially something responsible for cancer.

DID YOU KNOW THAT MICRORNAS WERE THERE?

We initially didn't know what microRNAs were because nobody was reading stuff that was published in 1993 about worms. There are a lot of genetic variations from species to species, but 3 papers were published around the same time stating that the small genes found in worms had now been found in mice. Of course, I read these signs and realized that this may be the important genes missing in the 13q- region where nobody was finding any classical genes. Sure enough, we found these genes. Ambros and the other guys who discovered microRNAs will probably get Nobel prizes because they have a good part in what we have done. They discovered the class of the microRNAs. We found only the link with cancer, but still it is an interesting story.

WAS DR. CROCE OPEN TO YOU FURTHER INVESTIGATING MICRORNAS AFTER THE INITIAL DISCOVERY?

Yes, we first discovered the same region - a very, very small region - missing in two patients. This warranted further investigation. If fact, Croce understood immediately when I talked with him that this was going to be big. This discovery by me and Croce was the first link between non-coding RNAs and human disease. There are now 3 or 4 papers a day on microRNAs in cancer, immunity, schizophrenia, lupus, all types of diseases. It's everywhere.

WERE OTHER RESEARCHERS SKEPTICAL TO THE IDEA OF MICRORNAS HAVING SIGNIFICANCE IN CANCER?

Yes, 99% of them. A lot people were laughing initially. The belief at the time was that microRNAs could not be important. Also, we used classic genetic approaches in good samples from patients while others were spending a lot of money performing high level experiments without success.

TEN YEARS AGO THERE WAS NO INFORMATION ABOUT MICRORNAS. TODAY THERE ARE COMPANIES DEDICATED SPECIFICALLY TO EXPLOITING MICRORNAS. WHAT IS IT LIKE TO KNOW YOU ARE RESPONSIBLE?

I know I have to do something different.

DO YOU WORK WITH OTHER CANCERS?

Yes I am working with any type of cancer.

DO YOU THINK THAT MICRORNAS ARE GOING TO BE THE CURE FOR CANCER?

Yes, but it depends on the type of cancer you are working on because the genome has so many other genes. In CLL microRNAs are very important. By targeting miR-15 and miR-16 [the microRNAs discovered in 13q-] great advances can be made for patients therapeutically speaking. But, for example, in colon cancer where there are so many abnormalities and so many genes, microRNAs will probably not have an immediate, huge impact in therapy. I think they do have a huge impact in identifying biomarkers in all cancers.

ARE YOU FOCUSING PRIMARILY ON BIOMARKERS FIRST?

Primarily, I am working on discovering new non-coding RNAs because I think you must initially have a good list of genes to work with. You must first know who the players are and then start to put the players in appropriate places. We have at least a couple of new types of non-coding genes. We are working on biomarker discovery and then we work on function.

ARE MIR-15 AND MIR-16 SIGNIFICANT IN ALL CLL PATIENTS?

I do not believe they are important in 100% of cases because CLL is a very diverse disease. We think these are the genes of indolent CLL. For the aggressive CLL, I do not know for sure how important they are. We are looking for microRNAs and other non-coding RNAs in other types of CLL. We are currently trying to find the microRNAs and other non-coding RNAs associated with 6q- and 17p-. Other non-coding RNAs can be much longer than microRNAs, but they still do not code for proteins. We have moved on to researching the different types of non-coding RNAs, the majority of which are probably not known at this moment. Very few people in the world are working on this.

WILL ANY OF THE PROJECTS YOU ARE WORKING ON NOW BE BENEFICIAL TO PATIENTS SOON?

We have a project on plasma microRNAs [recently funded by CLL Global] which I think will help us find biomarkers to predict a patient's response to therapy. This can be applied universally in five years. Gene therapy is difficult because first you have to get approval, followed by laboratory experiments and phase I and phase II trials. This is a lot of work and the earliest this will be applicable is 5 to 10 years.

Also, we are studying hypoxia which is when not enough oxygen reaches the tissues. This can be found in tumors, but it is found also when the heart is not working properly or even when you go to

high altitudes. We took samples and found that in cells affected by hypoxia a lot of non-coding RNAs were also affected. The concept behind our research is that in cancer, or in myocardial infection, hypoxia is only in the affected cells, not in normal cells. By using gene therapy on the affected microRNAs, the hypoxic effects can be reversed in cancer cells or in myocardial cells. Of course if the cancer is far advanced or the heart is far affected you can do nothing. So we are trying to deliver this type of gene therapy to specific cells.

YOU HAVE YOUR MD, SO WHY HAVE YOU CHOSEN TO DEDICATE 100% TO RESEARCH?

I like research a lot, but I like both. I sometimes think about taking the exams needed to go back to seeing patients. I very much enjoyed working in emergency care. I also enjoy science because every day is different - we have projects to work on, manuscripts to write, grant applications to submit. I travel and give several talks and I interact with different people every day. If you make important discoveries it is difficult to leave research, as research is the only healthy addiction – the more you do the more you want!

YOU HAVE LEVERAGED SEVERAL GRANTS RELATED TO YOUR CLL GLOBAL PROJECTS. WHAT IS YOUR MOTIVATING FACTOR?

I don't have lots of grants. I don't have enough grants. I need many more. Being a scientist, I apply for as many grants as I can because I need so much money in order to be able to shift my science in real time.

WHAT ARE SOME OF YOUR HOBBIES AND NON-RESEARCH INTERESTS?

Science is my hobby. I like science; I *get* science. So my job and my hobby are the same thing. I am lucky in that way, but I do other things. I like sports and I love being with my family.

DO YOU HAVE ANY INSPIRATIONS?

It is difficult to answer because from the time I was young I was reading one scientific article a day. I have learned from a lot of people. I have a lot of mentors…Usually I am speaking a lot, but I am also very careful to learn from the people around me. This is why I have so many collaborations, because I learn new ideas and approaches to science.

DO YOU HAVE ANY DESIRE TO TAKE YOUR KNOWLEDGE BACK TO ROMANIA TO INSPIRE THE NEXT GEORGE CALIN?

I go back to Romania every opportunity I have because I like to teach the people. But if you are asking me if I would go back and live in Romania, I do not think my wife would let me. She initially wanted to remain in Romania. Now she doesn't want to leave America. Also, it is important that we live in a place where my kids have the best opportunity.

I am currently working with Romanian scientists. We have a paper on microRNA levels in plasma of septic shock patients. 60% of these patients die because it is a very critical condition. If a person has an aggressive type of septic shock they have almost no chance of survival regardless of what hospital the person is at or who they are in society. So we are trying to find out if microRNA plasma can predict how aggressive the septic shock is from the first day when the patient goes to ICU. We will see what we can do because the treatment can then be more or less aggressive according to the parameters, but this concept is far from reality at this point. So I have publications and I am trying to write grants with the people from Romania.