

COMMEMORATIVE LECTURE

Cancer research in the 21st century

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In 1961, I went to the University of Pennsylvania in Philadelphia, in the United States, to begin my first year as a graduate student and to train in science. I had two goals at that time which I've kept with me for my 40 years in research: The first goal was to work with viruses, because viruses were the simplest of all living organisms. The second was to work with viruses that cause cancer in animals. I was hopeful that this research would elucidate the origins of cancer in animals. We would then be able to take that knowledge and apply it to the origins of cancer in human beings.

Reflecting back, we see that in 1961, we did not know the right questions to ask. We couldn't even begin to phrase the questions that would elucidate the origins of cancer in human beings. Even if we had the right questions, we did not have the technological ability which would allow us to arrive at these insights. We didn't have the experimental tools or the information. Developing both novel techniques and new information was a great quest and the right path for the past 40 years – from 1961 to the year 2000.

Now, we live in extraordinary – even revolutionary – times in science. And today, as we start our new century, we understand for the first time what the causes of cancer are in human beings. We have identified many of the genes that humans have, and the changes in those genes that can heighten the risk of developing cancer. So I want to tell you the story of how that happened. I want to review with you the experiments that have contributed to shedding light on the causes of cancer in humans – from the beginning of the 20th century until today.

This story begins in the year 1911 in New York City, at The Rockefeller University, which at the time was called the Rockefeller Medical Research Institute. This is the same institute which I joined two and a half years ago, as a professor and president. A young man who went to Johns Hopkins Medical School in Baltimore,

and who trained as a microbiologist in 1908 and 1909, came to The Rockefeller Medical Research Institute as an assistant professor. His name was Peyton Rous. Upon his arrival, the director of the institute, Simon Flexner, said to Peyton Rous: "I want you to work on cancer, and what causes cancer." Peyton Rous was terrified by this idea, because no one knew in 1911 what cancer was – the cause of it, how it forms in humans. But Peyton Rous was a good scientist and started his research on cancer.

Within the first year of working at The Rockefeller Medical Research Institute, a farmer from New Jersey walked into his laboratory carrying a chicken. This farmer's chicken had a tumor in its breast – in the muscles of the breast, to be more specific. The chicken had what we now call a sarcoma, a tumor of the muscle tissue of the breast. Peyton Rous listened carefully as the farmer told him the story of how this chicken got a tumor, along with other chickens in the flock. The possibility that whatever caused cancer in chickens was infectious in nature occurred to Peyton Rous for the first time. Maybe because chickens live so close together in chicken coops, one chicken might give the next chicken the agent that carries the information that causes a tumor. It also occurred to Peyton Rous that the agent could be a virus.

Peyton Rous decided to test this hypothesis: could a virus cause the chicken to develop a tumor? To test his theory, he chose one of the more innovative methods available at the time – the experiment aided by the work of Dr. Kitasato, the first dean of Keio Medical School. This is an experiment where the tumor is removed from the chicken and the tumor tissues are ground up so that no cells survive. All of the debris is then removed to leave a clear supernatant, which is then put it through a Kitasato filter or a Chamberland filter. If the agent that causes the tumor passes through the filter, it must be smaller than any bacteria and, by

definition, must be a virus. So Peyton Rous took the clear, filtered fluid and re-inoculated a second chicken. The second chicken developed a tumor. He repeated this same experiment numerous times over two years. And every time, in the end, he was able to transmit the tumor in the clear filtrates that penetrated the filter. Therefore, he concluded that some cancers – a sarcoma in chickens, at least – could be caused by a virus. In 1911 he published his paper and for the first time the field understood that, in chickens, cancer could be caused by viruses.

Peyton Rous' discovery was one of the cornerstones of the foundation upon which we now understand cancer, and we did not have to wait very long for the second cornerstone to be laid. We now move from New York City in 1911 when Peyton Rous published his paper, to Tokyo, Japan in 1914, when Yamagiwa and Ichikawa did a very important experiment at the University of Tokyo.

Drs. Yamagiwa and Ichikawa took the chemical components from coal tar – found in the soot from chimney smoke or in places with concentrated cigarette smoke – extracted the chemical components, and painted those chemicals on the backs of mice. And right where they painted the chemicals, the mice developed tumors. Through this experiment, they realized for the first time that there were certain chemicals that could cause tumors. They had published their results by 1914 – that chemicals could cause cancer as well as viruses. What the relationship was between the viruses and the chemicals, no one yet understood.

By the 1930's, at the University of Wisconsin, Jim and Betty Miller had been able to repeat Ichikawa and Yamagiwa's experiments. The Millers' experiments were repeated with a larger number of chemicals that were shown to be able to cause cancer. These chemicals were called carcinogens – or chemicals that cause cancer.

Also in the 1930's, scientists found inherited cancers in mice. Shortly after that, human geneticists began to appreciate that cancer could occur in some families. In these families with high occurrences of cancer, they were able to track the genetic information that could cause the cancer. This focus allowed them to narrow in for the first time on the fact that certain altered genes or mutant genes that travel in some families could be the cause of cancer in human beings. By the year 1950, we had many examples of an inherited basis of cancer. And now we had three parts to the cornerstone of the foundation that's being built here:

- 1) Cancer can be caused by viruses;
- 2) Cancer can be caused by chemicals; and
- 3) Cancer can be caused by genes.

Of course, from the perspective of the 1950's, we didn't know how these three elements related to each other,

and we still could not begin to understand how cancer formed in human beings.

By 1960, a fourth group of people came to play in the cancer field: epidemiologists. Epidemiologists are scientists who study how relationships between various factors in a person's life relate to his susceptibility to specific disease: whether gender is important, whether age is important, whether your environment is important, whether your socioeconomic group is important, or whether the job you have is important. The epidemiologists found, by the 1960's, that cancer was, by and large, a disease of the elderly. Very few young people got cancer.

If one looks at the rate or the incidence of cancer – how many people acquire cancer at a particular age – very few 20-year-olds acquire cancer. At 30, still very few people acquire cancer. At 45, just a few more people acquire cancer. But at 55, the curve starts rising very rapidly. By 65, it rises very, very rapidly. And by 75, the incidence is very high. That is an exponential curve. It takes a lifetime to accumulate something – whatever it is – that gives rise to cancer. So an individual becomes more likely to develop cancer as he ages.

In 1961, as I started my graduate career, I knew those four facts – the four cornerstones of the structure upon which we now understand cancer.

- 1) Cancer can be caused by viruses.
- 2) Cancer can be caused by chemicals.
- 3) Cancer can be caused by genes.
- 4) Cancer can be caused by aging.

In fact, there is a thousand-fold increase in the rate or incidence of cancer when comparing a 20-year-old to a 75-year-old. If you smoke, there is a hundred-fold increase. If you have the viruses hepatitis B or hepatitis C, there is an eighty-fold increase. But aging is a thousand-fold increase, so age is clearly one of the four cornerstones for understanding cancer.

As a first-year graduate student who wanted to know what caused cancer in human beings, I had these four, disparate observations with which to work. And everybody in science was arguing with each other, convinced that one or the other of these observations was the most important in understanding the origins of cancer in human beings. But no one could put them together.

With that as a background, we enter the decade of the 1970's with the extraordinary development of technology that has come to be called the recombinant DNA revolution. This is the ability, for the first time, of scientists and laboratories to go into the chromosomes of any animal or human being, and to pull out from those chromosomes genes – genetic elements – which contain information about how to build the whole organism.

In the 1970's, we enter the laboratories of Harold Varmus and Mike Bishop, at the University of Califor-

nia at San Francisco. Drs. Varmus and Bishop, who subsequently won the Nobel Prize for their observations, began their research with, of all things, the Rous sarcoma virus: the same virus found in chickens that Rous had isolated in 1911. Upon examining the Rous sarcoma virus, they found that the virus contained a gene – genetic information – which caused the cancer in chickens. They called this the “sarcoma gene” because the tumor was a tumor of the muscle tissue, or a sarcoma (*src*), and the *src* gene caused the tumor. They named the gene an “oncogene” – or cancer-causing gene.

This discovery united two of the cornerstones of our understanding of cancer for the first time. Viruses could cause cancer, and genes could cause cancer. And now we knew that viruses carried genes that could cause cancer. It related these two elements for the first time. But perhaps much more remarkably – and why Varmus and Bishop were awarded the Nobel Prize – was that they found that there was a very close relative of the oncogene in normal chickens. Chickens possessed a gene which was almost identical to the *src* gene in the virus. The only difference was that the viral *src* gene carried some mutations. It carried some genetic changes or mistakes that made the normal gene (*c-src*), an oncogene (*v-src*) – a cancer-causing gene that was in the virus. Mutations converted the normal gene to a cancer-causing gene, with the virus picking up the cancer-causing gene, resulting in cancers in these chickens. Mutations are also caused by chemical carcinogens, just like the coal tar and the cigarette smoke that were found to be carcinogens in 1914. This related three of the four early observations: genes, chemicals, and viruses were all brought together. By 1980, we understood for the first time the relationship between the cornerstones:

- 1) Chemicals can cause genetic mutations;
- 2) Mutated genes cause cancer;
- 3) Some viruses can acquire mutated cellular genes, like *src*, and cause cancer.

This did not yet explain, however, why it was that aging was important in cancer. If we really were to understand that these oncogenes caused cancer, and that they could be picked up by a virus and be mutated, why is it that cancer is a disease of the elderly? And the reason for that is that there isn't just one oncogene that causes cancer; there are whole groups of other genes that have to suffer mutations when cancer arises, and these genes were discovered in 1979, when *p53* was first discovered. My laboratory at Princeton University was one of the research groups that discovered *p53* and showed it to be not an oncogene at all, but something called a tumor suppressor gene – a gene that prevents cancer. Oncogenes cause cancer when they're mutated. Tumor suppressor genes normally prevent cancer and, when mutant, they fail to prevent cancer.

So here, we had a situation of two different kinds of genes: the oncogenes, which cause cancer, are very much like the accelerator of an automobile. If the accelerator of an automobile breaks and is stuck in the “on” position so that the automobile keeps going, then you have a mutated oncogene. The tumor suppressor gene, on the other hand, prevents cancer by acting like a brake of an automobile. If the brake is broken by a mutation *and* you have an accelerator that's stuck in the “on” position, then you have two mutations – one in a tumor suppressor gene and one in an oncogene: and together, they greatly increase your susceptibility to cancer. It's not sufficient to have just a faulty oncogene. You have to have a bad accelerator *and* you have to have a faulty brake, and then cancer begins to develop in human beings.

Now, what kind of “brake” is the *p53* tumor suppressor gene that we found in Princeton in 1979? It is a kind of brake that helps us make corrections when we make a mistake. For example, when we get on our computer in the morning and type a few paragraphs, only to see that we've made some spelling mistakes, we press the “spell check” key. The spell check key is linked to a dictionary and it replaces misspelled words with correct ones. *p53* is our spell check. If our chromosomes make a mistake when they replicate, they carry a mutation from that point on, which can create and oncogene that helps cause cancer. *p53* notices when the DNA is damaged and a genetic mistake – or mutation – is going to be made, and activates a very, very strong correction device: it kills the mutant cell. So it wipes out the entire paragraph that contains a spelling mistake. And that's how a tumor suppressor gene works – it acts as a genetic spell check which eliminates cells that have damaged genetic information and carry mutations in oncogenes, and helps to prevent the development of cancer.

But what happens when your spell check in the computer breaks because it has a mutation in the *p53* gene? The error frequency increases greatly. We make mistakes that can't be corrected. Every time an oncogene has a mutation, there is no correctional device, and other mutations occur in other oncogenes and other tumor suppressor genes. The combination of these numerous mutations eventually leads to cancer.

This understanding, for the first time, explained why aging was important in cancer. We have in our body some 60 or 70 genes that, if they suffer mutations, become oncogenes. And there are perhaps 25 or so tumor suppressor genes, and if they suffer mutations these can increase one's susceptibility to various kinds of cancer. And you have to have multiple mutations in oncogenes and tumor suppressor genes for cancer to develop.

So what happens over a lifetime is that, at the age of

20, you may suffer a mutation in an oncogene for the first time. Although one mutation is not sufficient to cause the cancer, it sits there for life. And at the age of 35, you may suffer another mutation in an oncogene – your second. And at the age of 45 or 50, one of your tumor suppressor genes may get a mutation. And if you have a tumor suppressor gene with a mutation and two oncogenes with a mutation, and they happen to be all in the same cell, you're on your way to developing cancer. Then, at 60 or 65, the fourth mutation may occur in the same cell, in a tumor suppressor gene, and now the combination of these mutations is sufficient to initiate a cancer. Suddenly, we are able to understand how genes and chemicals and viruses and aging all come together to elucidate the understanding of the origins of cancer in human beings.

As we complete the 20th century, we now have a clearer picture of the causes of cancer. We now have identified some 100 genes in our chromosomes which, when altered via mutation can, in certain combinations, contribute to cancer formation.

We can now fairly ask the question, understanding the origins of cancer in human beings, "What can be done?" And in response to this question, I can end with an optimistic story that takes us into the 21st century. But in order to tell you that story, I must go back in time again, and focus upon a specific cancer. We're going back to 1961 – my first year as a graduate student, at the University of Pennsylvania.

Just down the hall from where I was working on viruses and cancer in my own laboratory, there was a pathologist named Peter Nowell. Peter was particularly interested in a disease called chronic myelogenous leukemia, a disease of white blood cells termed CML. And what Peter had found, in every leukemic cell, were parts of each of two chromosomes came together, fusing to make one. This creates a translocation, or a fusion of two chromosomes – a type of mutation in the genes. So he discovered a very close association of this chromosome fusion and the disease, chronic myelogenous leukemia. Since a translocation was found in every patient with chronic myelogenous leukemia, most people thought that Peter had found that this particular mutation caused the disease. This type of chromosome translocation came to be called the Philadelphia chromosome, because it was found in Philadelphia. This happened in 1965.

In the 1970's, as additional oncogenes were discovered and it was shown that an oncogene called *abl* was localized at the junction of the translocation on the Philadelphia chromosome. The *abl* oncogene suffered a mutation because of the chromosome translocation, just as Varmus and Bishop had found that the *src* oncogene suffered a mutation and caused the cancer. In David Baltimore's laboratory at MIT, they took the trans-

located and mutated *abl* oncogene and put it into a mouse. The mouse developed chronic myelogenous leukemia, proving that the translocation was the cause of the disease.

In a very short time, Owen Witte and David Baltimore were able to show that the mutated oncogene called *abl* produced the protein – an enzyme called a protein kinase. This protein kinase was important in keeping the cancer cells alive and causing the disease. The 1980's ended with our understanding that the translocated gene caused cancer, and that this enzyme – protein kinase – that sustains mutations or changes, was driving the disease in some way. The protein kinase was causing the survival of the cancer cells.

Now, as we entered the 1990's, we began to realize that oncogenes caused cancer. A large number of people began to appreciate that if we could develop drugs, if we could find small molecules, chemicals, that would inhibit these abnormal oncogene enzymes that were called protein kinases, that for the first time we would have a rational basis for trying to cure cancer. A young man in Basel, Switzerland named Alex Matter, who was the head of oncology at a pharmaceutical company called Ciba Geigy (now Novartis), appreciated that if it were possible to inhibit the *abl* oncogene protein kinase, you could stop chronic myelogenous leukemia. His research group spent a number of years developing a small chemical that fit into the site in the protein kinase, and blocked its ability to function. And they put it into their first patient which was a mouse; the mouse that David Baltimore had made, that contained the *abl* oncogene and developed chronic myelogenous leukemia. Within a month they were able to cure the mouse by inhibiting the *abl* oncogene product on which the cancer depended for its survival.

Brian Drucker then started clinical trials with humans and was astonished to see that the first group of people, who had chronic myelogenous leukemia and who received this drug, almost all went into remission. We now realize that from the benefit of 90 years of science – from 1911, as Peyton Rous isolated the sarcoma virus, all the way to our understanding of oncogenes that cause cancer – we really do understand the origins of cancer in human beings. We have the ability, for the first time, to rationally develop drugs against the series of oncogenes, or to reactivate the series of tumor suppressor genes, to turn off the accelerator and fix the brakes.

So we now have one example of a drug that was developed as a rational response to our knowledge about the origins of cancer. It's a small triumph. In the United States, 4,000 people a year develop chronic myelogenous leukemia. Soon, every one of them will receive four pills a day that they will take for life, which will reverse the disease without harsh side effects. The

horrific side-effects of chemotherapy and radiation become obsolete if we are able to develop drugs that have rational targets. The name of this particular drug, which I'm sure will change as soon as it becomes commercially available, is STI-571. What is most important about STI-571 is not that it cures a leukemia that 4,000 people

have in the United States each year, but that it is, in fact, the first generation of many drugs that will be designed to inactivate oncogenes and to activate tumor suppressor genes. As we understand the origins of cancer in humans, we can, for the first time, design drugs to fight this disease.