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The winding road to the discovery of the SR/CR mice

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Introduction

On April 30 th 2003, our paper titled "Spontaneous regression of advanced cancer: Identification of a unique genetically determined, age-dependent trait in mice" went online at the *Proceedings of National Academy of Sciences USA* website and was later published in printed form in the May 27th issue (1). This paper describes a serendipitously discovered mouse that possessed a unique genetic trait, a very powerful resistance to a wide array of highly lethal, transplanted mouse cancer cells. The trait segregated as a single locus, in a dominant and non-gender linked manner. The cancer-resistant mice in the colony were named SR/CR mice due to two different displays of the phenotype. In the display of complete resistance (CR), the mice never developed ascites after cancer cell transplantation. In the display of spontaneous regression (SR), the mice first developed ascites that then suddenly regressed, leaving the mice cancer-free and healthy. The display of either CR or SR was determined by whether the mice were younger or older when they were first exposed to cancer cells. The SR/CR mice resist a great number of cancer cells repeatedly, a thousand or even a million times the lethal dose. Yet, this highly effective resistance has many surprises: (i) This genetically determined host resistance to very aggressive cancers exists only in a rarely found mouse and his offspring but not in the general population. (ii) Spontaneous regression of advanced malignancy, a great mystery in medicine, can occur in the SR/CR mice in a well-controlled and predictable manner in the laboratory and is merely a delayed onset of resistance mechanism. (iii) A profound, negative impact of aging on the cancer resistant mechanism begins as early as middle age. (iv) Cancer cells are not eradicated by the adaptive immune system, but rather by the cells of the innate immune system. (v) The rapid lytic destruction of cancer cells is highly selective without harming normal cells. The paper was coauthored by a team of investigators from Wake Forest University School of Medicine in Winston-Salem, North Carolina: Zheng Cui, Mark C. Willingham, Amy Hicks, Martha Alexander-Miller, Timothy Howard, Mark Miller, Holly Weir, Wei Du, and Cynthia DeLong.

A few days before the paper appeared online, both Wake Forest University and *PNAS* had prepared different news releases for the websites of embargoed news to publicize our upcoming paper. Mark Willingham and I were also summoned to receive media training. We practiced in mock interviews, and then joined a critique session of our own on-tape performance. Yikes, we seem to only remember how surprised we were to find ourselves with imperfections amplified so clearly on camera, and how difficult it was to use lay language to accurately convey a scientific message to the general public. We were thankful that the interviews weren't real. Despite ample preparation, we were still nervous and anxious since we had never done anything like this for a publication or for anything else. Our nerves were soothed a little when we were told that this should last, like many other news stories, for only a couple of days and life would quickly go back to normal. We never prepared for what eventually happened. When the news embargo was about to be lifted and in the ensuing weeks after the publication, our lives were suddenly overtaken by an unexpected media frenzy. The public was fascinated by knowing that advanced cancer can regress spontaneously and that this miraculous event could now be studied in the laboratory. People were also intrigued that this new line of research stemmed not from a brilliant idea but rather from a failed experiment intended for something else. We were inundated by countless requests for TV and phone interviews from the media and heart-wrenching pleas for help from cancer patients. We also heard cheers from people who claimed that they also had a similar gene, since their families had no cancer for many generations and some family members even lived cancer-free to very old ages despite being lifelong heavy smokers. It is not hard to see how cancer affects all of us: some of us have to deal with it and the rest of us fear it. But it is hard to believe that all the excitement and attention were caused by a little mouse; yes, a single little mouse. Many people wondered if this could finally be a most desired way to eradicate cancer cells from a host: no radiation, no anti-proliferation drugs, no hair loss and no damage to normal tissues, just a bunch of immune cells that really know what they are doing.

Since the publication, there have also been many questions about how the discovery was made, why unconventional decisions were made and who these investigators are. Dr. Lloyd Old invited me to give a personal reflection of the events leading to this discovery that has been full of surprises, twists and turns. My initial hesitation to this generous invitation was that it might be too early for me to write such an article. Such an honor is usually reserved for very accomplished scientists. On the other hand, Dr. Old's persistence led me to realize that this might be a good opportunity to relive one more time those roller-coaster days that went by so fast, since none of us have been able to pause and smell the flowers. And, more importantly, I could have a forum to recognize my friends and colleagues who helped this project in so many different ways. These mice have offered a hope to the world, but translation of a hope into effective therapies for cancer remains a formidable challenge. While our investigative team is growing bigger, I am confident that the mystery of this fascinating trait won't stay in the dark for too long.

The initial surprise: The failed induction of ascites

The whole thing began in late spring of 1999 when Liya Qin, a postdoctoral fellow who earned her PhD in my father's lab at Dalian Medical College in China and who was introduced to my lab by my father, came to my office. "I injected S180 into 16 BALB/c mice that I immunized with *TrpE*-CT fusion protein. Fifteen of them developed ascites and I am characterizing these samples of ascites looking for antibodies to CT. But one mouse never developed ascites. I injected him again, but still no ascites." "Are you sure that you injected the mouse?" "Yes, I thought I injected him the first time. But when he had no ascites, I wasn't sure anymore. So, I injected him again and I am 100% sure about the second injection." "Do you know if the S180 cells were good?" "Sure! Other mice with the same batch of S180 all developed ascites." "How many cells did you inject him with?" "First time I gave him 200,000 and the second time I doubled the dose just to make sure that he would get ascites. I need to know what you want me to do with him. Get rid of him?" "He may still have the CT antibody that we want. Could you give him another injection? This time, let's try 2 million. Oh, don't forget to inject a few more BALB/c mice as controls to make sure the cells are good." "Sure!" That was the end of a very casual conversation. Neither of us had either given a second thought or expected to have another conversation about this mouse. We both had experiences of occasionally failed injections of S180 into the peritoneal cavity but we always managed to get it right with second injections.

This project was Liya's effort to produce mouse antibody against a rat cytidylyltransferase (CT), an enzyme in the pathway for phosphatidylcholine synthesis. My lab studies phospholipid biosynthesis in mammalian cells. The technique that I learned in graduate school was very simple and straightforward. One can make an in-frame fusion construct between the yeast *TrpE* gene and the gene of interest, express the fusion protein in *E. coli*, briefly enrich the fusion protein and immunize several mice. When the antibody titer becomes detectable, mice are then given an injection of mouse S180 cells. Two weeks later, the injected mice would invariably develop ascites, a massive accumulation of body fluid in the peritoneal cavity. The ascites fluid can be collected and hopefully contains lots of antibodies of interest. The procedure to produce ascites is well-established and very reliable, and it had never failed in my hands over many years. Liya also used this same model when she was back in China to screen for anti-cancer drugs, in which the slightly changed survival times of S180 ascites-bearing mice were used to measure efficacy. Ascites production with S180 cells had not failed in her hands, either. But this notion was about to change.

A few weeks later, Liya came back with the same news: still no ascites in the mouse while all the other mice had ascites. I was in a defiant mood this time. "Let's do the injection together this time but with a lot more cells." I was definitely convinced that this mouse should contain the antibodies that we wanted, and I was not ready to give up easily, not when the ascites procedure was so simple. Liya prepared another batch of S180 cells. This time she injected 20 million of them into this mouse with me standing right next to her. I examined the mouse carefully for a few minutes after injection to make sure that the cells would not escape from the injection site. I also paid extra attention to how this mouse was marked: painted yellow on the right front leg and a clipped right ear. There was no way he could be mistaken for another mouse. Two weeks later, the news was still the same: no ascites. I really needed a sense of humor to deal with my growing frustration: "How about 200 million!" All we wanted was a terminal event so that we could collect antibody, and the experiment would be over. This was not too much to ask! Eventually, all our attempts at trying to induce ascites in this mouse failed. No other mouse had ever lasted more than 2 months in my lab. This mouse managed to stay in my lab for more than 8 months. The cost of buying this mouse from Charles River was less than $7. But the cost to keep this mouse in our facility had now grown to over $70. By then, my curiosity was finally piqued. "Could this mouse resist S180?" Normally, mice in any strain would succumb to as few as several thousand S180 cells injected into the peritoneum. The more S180 cells are injected, the faster ascites develops and the faster the mouse dies. To really appreciate how unusual this failure to grow ascites was, all one has to know is how aggressive S180 cells are.

S180 is an abbreviation for mouse sarcoma 180, a cell line of mouse cancer cells derived from a soft tissue tumor of a Swiss mouse. S180 is well known for its aggressiveness when transplanted into mice, since the cells grow everywhere in the mouse and invariably kill the host in a few weeks, if not months. It is also known for being non-specific, meaning that it can be transplanted into any mouse strain, inbred or outbred. This ability to grow indiscriminately is likely due to the absence of MHC on its surface, meaning that S180 shouldn't be rejected by the mechanism of transplant rejection. At the cellular level, S180 cells are extremely large, about 10 times larger (by volume) than normal mouse white blood cells, and these cells are highly polyploid. S180 is also known to secrete a large amount of abnormal cytokines known to be cytotoxic at high levels. S180 cells grow "like weeds" both *in vitro* and *in vivo*. S180 cells ravage through the body of the host leaving a trail of destruction, among the worst pathology of any cancer in mouse or human. No wonder it never failed to kill a host. In the 1970's, a few studies demonstrated that some Swiss mice could resist the transplantation of S180 nodules from one mouse to another, spurring the hope that S180-resistant mice might exist. But this hope was soon dashed since none of the mice that resisted S180 nodule transplantation could resist S180 induction of ascites. Transplanted solid tumor nodules require re-establishment of vascular networks after surgery to survive in a new host. A possible explanation for the resistance to S180 nodules could be failed re-establishment of a vascular network after surgical procedures, but not a true resistance.

On the other hand, intraperitoneal injection of S180 cells to induce ascites is a highly reliable model, because the cells are placed directly into a favorable environment in which they resume growth immediately and require no supply by blood vessels. Once taking hold in the peritoneal cavity, S180 cells double every 10 hours, invade neighboring tissues and destroy everything along the way. The lymphatic ducts, which mainly function as a drainage system for the peritoneal cavity, are quickly plugged by the fast-growing cells. Like a plugged sink, fluid backs up and accumulates to form ascites, which also serves as an ideal culture medium for more cell growth. This vicious cycle continues until a vital organ fails or blood vessels rupture, and the host finally succumbs to cancer. It is hard to believe how much grotesque damage can be inflicted by several *thousand* S180 cells in a host. It is even more inconceivable that any mouse could survive an injection with *millions* or even *billions* of these lethal cells. A search of the literature also failed to show that this kind of resistance had been reported before. After eliminating the possible trivial reasons of bad cells and failed injections, I began to accept the idea that this could be a cancer-resistant mouse.

The decision to breed and Mendel's smile

In the fall of 1999, I gave a presentation at the annually scheduled Cancer Center retreat. After presenting my normal stuff on lipids, I closed up by briefly mentioning that this mouse could be resistant to cancer, hoping to get some reactions from the audience about whether the direction of my thinking was right. During the presentation of my lipid stuff, the audience remained stoic and respectfully quiet, a familiar scene to me. When my presentation switched to the mouse, I spotted a sudden change in the air. Dr. David Priest of the Medical University of South Carolina, our only outside reviewer for the retreat, leaned forward in his chair in the front row with his eyes full of sparkles. I also sensed an unusual commotion in the audience. I was the last to present before the coffee break, and afterwards people rushed to me with curiosity. It was quite surprising to many people that this mouse had such an unusual power of cancer resistance. But the major problem was that I had only one mouse. There were so many conversations being tossed in front of me and across me. Suggestions started to flood my ears: preserving the DNA, preserving body parts, cloning the mouse, because some people from Hawaii had just published a paper on mouse cloning, etc. Suddenly a calm voice came from behind me: "Can you breed this mouse?" I turned around and saw that the voice was that of Mark Willingham, my collaborator on my first R01 grant to study the cell biology of *Bcl2*-induced polyploidy in a CT mutant.

Mark came to Wake Forest University (WFU) from Charleston, South Carolina in 1997, together with Dr. Jerry Garvin who became the chairman of the pathology department. To many WFU people who write grants, Mark is a God-sent gift from South Carolina. Mark was also trained as a cancer researcher at the NCI with Dr. Ira Pastan. During the NCI days, Mark managed to establish a reputation of being one of the top cell biologists in the world, with now over 300 peer-reviewed publications to his credit. Adding Mark's name to a grant would undoubtedly increase the appeal and fundability, let alone the many beautiful microscopy figures Mark normally generates. With so many credentials under his belt, no one expected to see him still working in his lab, doing all sorts of experiments with his hands. It was quite surprising to many people first seeing him working hands-on, but not at all surprising to people who know him well. He is always quiet about himself. But his friends are not very quiet about his stories, such as how he assembled an electron microscope from pieces, how he built his own electric car that he drove every day to work at NCI and how he graduated from medical school at age 23, etc. Like many other people at WFU, I also asked him to be on my grant. I must confess that I had the similar intent of trying to take advantage of his credentials to boost the appeal and fundability of my grant application. If I have to brag about one thing, it is that I was the first among many investigators at WFU to get funded with Mark on the grant. Moreover, I often felt being outclassed by some friends who bragged about not having to do bench work anymore because there are many students and postdocs in the lab. Later, I began to feel much more at ease to be hands-on again, with Mark as a role model, or even side-by-side with him at the lab bench. So, my collaboration with Mark started even before this unusual mouse came along.

After a few conversations with Mark in the ensuing weeks, I decided to breed the mouse. I quickly hit the book on mouse genetics by Lee Silver and soaked up all the necessary information about breeding. The first female partner was pregnant after a few weeks in the cage with the cancer-resistant mouse and gave birth to 2 male pups. The decision to screen or not to screen the next generation pups with S180 was difficult. If the founder carries two copies of a recessive gene, the next generation would be lost when being screened with S180 cells. If the founder mouse carries one copy of a dominant gene, only a half of the next generation pups would be resistant. Then if not screened, the breeder chosen from the F1 generation would have a 50% chance of not having the gene and the resistance would never show again in subsequent generations. Even worse, if there were more than one gene involved, the chance of the F2 pups being carriers would be 25%, 12.5% and 6.25% with each additional gene. But these genetic rationales were not the biggest threat to generating a line of cancer-resistance mice. What if the resistance to cancer cells was not due to genetic elements, but due to an infection, a food poison or other outside factors, etc.? When facing risks either way, rationales and logical thinking become useless. Heck, let's just do it and find out what happens! So, we bred the F1 again to normal BALB/c without knowing the phenotype. The first F2 litter had 7 pups. At 6 weeks of age, all mice in this litter were injected with 2 million S180 cells, a dose 2000 times higher than the lethal level. If we didn't have survivors, the story would be over by then because I would never have the courage to pursue a non-genetic event or a complex genetic trait, no matter how intriguing the resistance. I am, after all, a lipid biochemist. The only chance to have survivors would be that the genetics was very simple and we were extremely lucky. The odds in our favor for having survivors were 1 in 5 at best according to my estimation.

Possibly due to my fear of Murphy's Law or the fear of low odds of success, I didn't pay any attention to the mice for 2 weeks after the injection. I asked Liya about the mice and she said she had not looked at them either for 2 weeks. So, we went upstairs to the mouse room together. When the cage tops were opened, I was stunned immediately! Four of 7 mice had huge bellies and the other 3 were normal. It couldn't be true! I double-checked everything. The cage cards were correct and marks on the mice were correct for all having been injected. I was there. It's all true and against all odds! Not only were we extremely lucky, but also we had a mouse line and probably a viable project. We also had many questions answered. It was not any infection. It was not any food poison or any other outside factors. It was genetic and a germ line-transmissible element. It looked like a single gene, and a dominant, not sex-linked trait. The moment was, to me, better than a prayer answered. When I rushed to tell this to Mark, his face was full of amazement with a playful smile, "According to Murphy's Law, this shouldn't happen!" I also couldn't help feeling another smile coming from a distance, "It was just like the peas in my garden." It must be Mendel's smile.

This genetic experiment led to the first grant from the institutional intramural research committee. Our enthusiasm was boosted. Suddenly, in the spring of 2000, Liya decided to leave to work in another institution, citing the reason that she was more interested in research on Parkinson's disease than the projects in her hands. Apparently, even the promises of the cancer-resistant mice in her care failed to convince her otherwise. Her decision to depart couldn't have come at a worse time, when I was about to travel for a whole month outside of the country, leaving no one to care for the mice. At this difficult point, the one coming to the rescue was my good friend Mark Miller of Cancer Biology. Miller is an experienced mouse toxicologist/carcinogeneticist working on mouse transplacental carcinogenesis. He earned his PhD from Columbia University and is always articulate, with a great sense of humor and often with strong convictions. We hit it off when we first met a few years back. When Miller volunteered to help after knowing my crisis, another thought came to my mind: why not screen the colony double-blindly, because Miller had never seen those mice before. In the previous months, I had become more paranoid. How could I not be! The more I read the literature, the more I felt the phenotype might be "too good to be true". How could these mice resist such a great number of S180 cells repeatedly! I really needed someone else to tell me that what I saw was true. So before my trip, the mice were mixed intentionally with newly purchased control mice, numbered randomly and injected with S180. We then rolled the cage carts carrying the entire colony of already screened mice from my mouse room to Miller's mouse room. The key to the mouse identities was locked in my drawer. Mark assigned one of his technicians to document the ascites development or death of all the mice, without knowing the true identity of any mouse except the numerical markers. One month later when I returned, I was elated again to find out that all of the control mice were dead, and all the alive and healthy ones were our resistant mice, a 100% match. Miller was also amazed by what he saw and suggested that I should breed this mouse into different strains of mice for genetic studies, a great idea which proved to have multiple implications later on.

The first mouse had the code name "#6" when he first survived S180 injection. I felt compelled then to call him "Super" and gave him a new code name in the breeding colony "AM1". "A" stands for the first generation. "M" stands for gender and "1" is the numeric order of mice in a given generation. A few weeks after two graduate students and I had a big day of photo-ops with him, he passed away of old age in late March 2001, but not without leaving a fascinating legacy of a colony approaching 700 cancer-resistant mice, a paper that generated a buzz around the world, a wealth of knowledge that intrigued many scientists and a hope that we may be able, some day, to fight off cancers in humans, too. This mouse was later called by many people: "super mouse", "magical mouse", "mighty mouse" or "perfect mouse". We never set out to look for him. But he managed to come to us, to survive highly improbable odds against him and lived to tell his stories.

After Liya was gone in the early summer of 2000, I had to decide who was going to take care of the mouse colony. Who would be better than Yours Truly! By then I had read many mouse books and papers. I not only happened to enjoy the odor of mouse rooms, but also felt the necessity to become a hands-on mouse geneticist. Plus, nothing could be more important than seeing things with my own eyes. For nearly two years, I enjoyed making daily trips to the mouse room and spending entire Saturdays in the mouse room to observe, to think (I liked to feel that I could think better with the smell of mice in the air) and to do genetic experiments, till Amy Hicks and Holly Weir, two graduate students, finally took over for their own joys of classical mouse genetics.

Nude mice and T cells

Being a biochemist, I began my adventure in immunology with a love-hate relationship. For one, you can always find immunology experiments to argue for or against a claim. At the beginning, I was told by some immunologists that I should abandon this project: "If you had been born 20 years earlier, you would have known the big body of literature on transplant rejection of mouse tumors by T cells and by ADCC: this must be a transplant rejection." Without knowing all the history of immunology but knowing all the bad characteristics of S180, I was easily convinced otherwise that S180 cells cannot be rejected as a non-self entity by T cells. S180 cells don't have MHC on their surface and grow in all mouse strains and even in rats. Then, I found out that the majority of attention in the cancer immunology field had been paid to T cells as effector killer cells since the 1950's and 60's when Lewis Thomas and Mac Burnet, two intellectual giants in immunology, articulated their thoughts on the role of T cells in cancer immunity.

When I talked to different immunologists, I was convincingly led to believe that this must be a T-cell mediated immunity to cancer. If I could prove that this resistance was, indeed, based on T cells, I might be able to find out what antigen is on the surface of S180 cells that provoked such a strong T cells response. Then there might be an antibody-based therapy at least for mouse cancers. I was also trying to find a way out of this mess of conflicting concepts on T cells. After having learned some mouse immunology and genetics, I felt that I needed to deplete T cells by breeding the resistant mice to nude mice that carry two copies of a recessive mutation in the *foxn1* gene to block the development of the thymus, and thus the maturation of T cells. They are hairless and it would be easy to identify the trait.

But I was quite surprised again to find out from the literature that there had been a great debate in the field of cancer immunology about whether nude mice still have T cells, due to the mutation being "leaky". A prevailing argument for the still-present protection in the nude mice against cancer was that they might still have T cells due to the leakiness of the *foxn1* mutation. Other immune cells in the nude mice rarely got any credit for this protection. I brought my questions to Mark. He told me "Don't worry, who cares if you can find a few cells with CD4 and CD8 markers if the nude mice don't have thymus and take any transplant perfectly. No thymus, no T cells." Mark also showed me during mouse necropsy what a thymus looked like in a normal mouse and how it was missing from a nude mouse. Yes, "T" stands for thymus-derived lymphocytes. I had no intention to join the debate of how the nude mice are protected against cancer but I was easily convinced that, without a thymus, no cells should be called T cells. With this much more simplified frame of thinking, I set out to expect that the 40% germ line transmission rate in normal mice would disappear in the mice with the nude background that don't have a thymus and T cells, thus proving that T cells were the effector cells.

The breeding experiments were a lot of fun. Nude mice were not good mothers since they don't have fur to keep the pups warm. So, I used male nude mice to mate with cancer-resistant mothers. All the pups had fur but carry one copy of the *foxn1* mutation. The pups were screened with S180. The survivors should also carry one copy of the cancer resistant gene. The female survivors were bred again with the nude fathers. Magically, half of the pups had hair and the other half didn't. I began to hear Mendel's voice again in my head. "Aha!" I had 16 nude mice from a total 31 pups and all were given S180 cells. I expected that about 40% of the mice with hair would survive and that all of the nude mice would be dead. Three weeks later, I was stunned again. Six of 15 mice with hair had survived (not really surprising), but 6 of 16 nude mice had also survived. These nude mice were cancer-resistant without T cells. I was then completely confused. I even began to believe that if the "magic bullet" coined by Paul Ehrlich did indeed exist in the SR/CR mice, it could only exist as small diffusible molecules instead of effector cells. Yes, there are many other immune cells in the nude mice. But all those other cells were out of fashion anyway, sort of. I couldn't have been more wrong.

The magical disappearing act of S180: Spontaneous regression

No one believed that this could really happen, not in a million years. When Liya told me for the first time that one mouse developed ascites like the others and then that the ascites disappeared suddenly overnight, I was convinced that she had made a mistake in the mouse's identity. Then, she notified me a second time, and a third time. I simply refused to believe. She then painstakingly measured the daily body weight of every mouse that developed ascites and was clearly marked. Once I saw the well-documented curves of body weight changes, I had no choice but to believe it. Then, a few more spontaneous regressions happened even after I began to pay close attention to them. By now, it was clear that we had two different phenotypes. One was that of mice that never developed ascites (CR). The other was that of mice that first developed ascites followed by a rapid, complete regression (SR). The SR happened in the resistant mice only once with the first exposure to S180, but not with subsequent injections to which they were completely resistant.

I first thought that there might be another mutation. But then, genetic studies showed that CR and SR belonged to the same genetic element. Thus, the mechanistic difference between them could only be that the same resistance mechanism kicked in faster in the CR mice, and slower in the SR mice. It was hard to believe at first, but it was even harder to predict. I was completely baffled. I kept asking myself what could cause the slower onset of the resistance mechanism, but couldn't be satisfied with the answers I came up with. When Liya decided to leave and I went away for a month, there was a large population of pups left unscreened. I finally got around to injecting them with S180 cells 4-5 months after these mice were born. We never otherwise left so many mice unscreened for so long. Keeping them so long cost a lot of money unnecessarily. The longest time we normally waited before injection was 2 months. Due to so many other urgent things before and after my trip, I just couldn't inject them sooner. But this time, the outcome was very different.

Two weeks after injection, to my big disappointment, all of the injected mice developed ascites. My fear was that we might have lost the phenotype for good. My mind was full of worst-case scenarios. I was so disappointed that I didn't want to go to the mouse room for several days, until I finally felt I needed to get rid of these mice. When I returned to the mouse room this time, I was surprised again. One half of the mice that had already developed ascites underwent SR and became healthy. What happened? SR happened before, but only infrequently and very unpredictably. Now, all of the resistant mice underwent SR this time. I couldn't resist the urge to find out why. But everything at first glance seemed to be the same this time as other times. The mystery continued to haunt me for the next few days, until one morning I woke up screaming: "It's the age". Ignoring the mild protest of my startled wife, I got up, showered quickly and rushed back to my office to look at the lab notes. Here they were. The only difference between this time and the other times was the age at the first injection of S180.

Apparently, the older mice took a longer time to have their resistance mechanism kick in. One more test of this idea in another group of older mice confirmed the element of age at the time of the first injection.

Since resistance was a genetic element and the resistance lasted for life, I never suspected that aging during mid-life could have such a profound impact on the primary response of resistance to cancer, not at 6-months old. But by looking at the national data on mortality rates, I was surprised to find out that cancer becomes the number one cause of premature death (before age of 75) for the age groups of 46-55, 56-65 and 66-75, replacing accidental death as the number one cause for the age groups younger than 45. Heart and coronary diseases become the number 1 cause of death only for the age groups older than 76. Mice enter their adulthood at the age of 6-8 weeks. By age 5-6 months, they are apparently well into their midlife crisis. Then, completion of Figure 1F for the paper was only a matter of time. Figuring out the mystery of SR has been the effort apparently with the biggest emotional roller-coaster ride. I also began to believe that each thing, bad or good, happens for a reason. If it weren't for Liya's sudden departure that unintentionally delayed the injections, we may never have found out what SR was and why it happened. One day, an investigator from NIH visited me and was also amazed by the mice, but apparently had his own take home message: "Who really cares about giving mice some cancer cells and seeing nothing happens! But it is really something when cancer grows first and then completely disappears. That is amazing!"

Seeing is believing: The power of microscopes and time-lapse studies

Whole animal studies tell the bottom line story. Ascites either grow or not. Mice are either dead or alive. The only assay that one needs to do is to open the cage tops. However, once seeing a full-blown ascites disappear so quickly, an intense curiosity grew inside me to know what happens to the S180 cells. Were they dead? Or had they become dormant? Or were they transformed back to benign cells? Mark and I began to think about how to study the S180 cells inside resistant mice. We couldn't find anything in the literature. Another big problem was that we didn't want to do any experiment involving a terminal procedure, since we needed the mice to breed. We had many unsuccessful attempts. One day, I tapped some ascites fluid from two mice that had big bellies, placed the fluids into two separate 10-cm culture dishes with culture medium and placed them in a CO_2 incubator. This was the normal procedure for us to prepare a lot of cultured S180 cells in a hurry. The next day, I noticed that one of two mice underwent SR and became healthy. For some reason, I remembered the culture dish that contained the cells from this mouse. I took the dishes to the microscope in Mark's lab and was stunned again by what I saw: the culture from the SR mouse looked very different from the other one. I couldn't really figure out very fast what was going on. I ran into Mark's office, dragged him into the lab and said "Mark, you have got to have a look at this. I don't know what happened but it looks like a war zone." Looking through the microscope, Mark shook his head in amazement: a lot of cell debris, a lot of cell-cell aggregates and a lot of big fat macrophages, a sharp contrast to the happily growing S180 cells from the normal mouse. We both realized then that we might have a very good way to study the cellular events in the resistant mice. Cancer cells can be easily put into and washed out from the peritoneal cavity to study their fate and their interactions with host cells.

Once the door was open, new ideas and new experiments just kept running wild. One of the most memorable days was when we first used antibodies to stain the immune cells washed out from the peritoneal cavities at various time points after S180 injection. We began the day at 9 am. From preliminary studies we knew we were going to have a very good day. The mice had been injected with S180 in the preceding days. I washed out the peritoneal cavities of the mice, control and resistant. Mark took over from there and was busy and focused. I was anxious but I didn't want to bother him. So, I locked myself in my office to write grants. When there was a knock on my door, I realized that it was already past midnight. Standing at my office door, Mark's face was glowing with rarely seen excitement, "I think it's the NK cell that does the killing. I couldn't find any T cells." He waved the pictures in his hands. That moment was the beginning of our adventure into the innate immune system to find cancer-killing cells. We also began to understand why the nude mice were resistant to cancer when bred with the SR/CR mice. The other immune cells in the nude mice had finally unveiled their role as unsung heroes in silently defending against cancer cells.

James Watson once said that he couldn't get a single good night's sleep since his discovery of the double helix until 25 years later when the crystal structure of double-stranded DNA was finally solved. Well, the SR mice were certainly not at the same level as the double helix, but my lingering worry about what may happen to those magically disappearing S180 cells was not any less. Fortunately, my restless nights only lasted till Mark showed me the tape of time-lapse video microscopy capturing how the S180 cells were ruptured by the SR leukocytes in cell culture. As a biochemist, I was trained to see things in an abstract way. Proteins are bands on gels, metabolites are numbers in the scintillation counter, lipids are spots on TLC plates, etc. What we often do is to describe molecules laboriously with many words. With microscopes and time-lapse in hand, words became pictures and videos of moving cells doing their thing in front of our eyes. It was so refreshing that I felt like I was on intellectual steroids. If it weren't for the powerful microscopes and Mark's expertise in cell biology, the mystery of the S180 cells' disappearing act in the SR mice might still be a mystery.

Closing remarks

Due to space considerations , I want to limit this personal reflection to the stories of the early stages of this project. Since then and through this project, I have met so many new wonderful friends and colleagues. And, of course, there have been many new equally intriguing stories. Furthermore, the mice have never ceased to surprise us. I hope the future may bring me another opportunity to tell these stories, perhaps after we know more about the mice. The nature of this mouse project is drastically different from other projects with which I have been associated before. The latter ones are open-ended, meaning that one works on a molecule or a protein and tries to find out how it functions in cells or an animal model. The former one is to find out how a unique event happened, very much like a crime case investigation. Yet both types of research demand stringent mental exercise and rigorous intellectual workout. The mouse project has also offered us many lessons. The most important one is to keep an open mind and follow the clues provided by unbiased observations. Dr. Old once told me: "We can be thankful that you are not an immunologist. Otherwise, you would have definitely thrown away this mouse without hesitation." In this particular case, unintentional lack of knowledge in immunology might have led to an opportunity that would otherwise have been lost due to complacency. Yet, I enjoyed immensely my learning process in becoming familiar with immunology. As it turned out, the established knowledge in immunology has played critical roles in later stages of this project. We can be also thankful to the fact that this project is being conducted in the advent of great technological advances. Hopefully, we can eventually get to the heart of this great mystery. What is the gene? What is the mechanism that allows the innate immune cells to recognize cancer cells with such perfect specificity? What is the nature of the differences between cancer and normal cells? And, the most important question of all: "Can this mechanism be used to treat human cancers?"

I have two favorite personal gadgets. One is a laser range finder that measures the distance to the pin on the golf course so that I always know how far I have to go. The other is a portable GPS system that always takes me to the desired destination with precise and clear guidance. But neither of these gadgets may be of any use in my future endeavors with these mice. However, I can always count on my old and new friends and colleagues who share a similar fascination and a strong belief that God didn't have to take more unnecessary trouble to create mice and humans to be so different so that all the efforts in mice would be in vain for humans. Why not just simply change a few percent of the genome. Voila! You have a new species that even pays tribute back to you every Sunday! After all, both mice and humans are similarly at risk for cancer at old age. One species is here to provide clues to a mystery, and the other is here to use these clues wisely for solutions, for the benefit of both.

Abbreviations

CR, complete resistance; CT, cytidylyltransferase; SR, spontaneous regression

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