

A War on Kidney Cancer

A decade after President Richard Nixon's 1971 declaration of war on cancer, urological surgeon W. Marston Linehan, M.D., at CCR, declared his own battle against kidney cancer. The disease afflicts nearly 40,000 adults in the U.S. annually, a third of whom will die from the illness or its complications. Then, as now, the incidence of kidney and similar renal pelvic cancers was rising by 2 percent each year.

Linehan saw the devastation firsthand while caring for patients at Duke University Medical Center. Thus, by the time he arrived at NCI in 1982, he was already compelled to take action.

"I looked at these people with kidney cancer, dying even after we had tried 300 different kinds of chemotherapy agents," said Linehan, who is now Chief of CCR's Urologic Oncology Branch (UOB). "We had to come up with a better approach."

And so began the 25-year story of a group of CCR investigators who dedicated their careers to doing just that.

All Kidney Cancers Are Not Equal

All kidney tumors are not created equal. Clinicians only began to understand this concept when genetic analysis was applied to kidney cancer. This new knowledge gave scientists a map with which to track the genetic roots for human disease. Whereas oncologists used to classify cancers by the tissue within which they first emerged, the emphasis on genes revealed the relative naiveté of this organ-based scheme.

Tumors arise from once-normal cells that begin dividing uncontrollably because of alterations in their genes. Many genes play a role in normal cell division. For example, certain genes in a skin

follicle cell prompt cell division in order for a person to grow hair. But that same follicle cell has a restraining system to stop cell division when inappropriate. (This is why eyelashes stop growing at a certain length, for example.)

Every cell has many genes at its disposal, each capable of prompting division or restraining it. Mutations in any single gene or set of them can cause cells to go renegade. And because different genes might become mutated in different, even adjacent,

All kidney tumors are not created equal.

cells, different tumors within the same tissue, and even different cells within the same tumor, can be genetically different from one another. Therefore, treatments that target one person's kidney cancer may not affect that of another.

Unraveling Kidney Cancer Gene-by-Gene

Early on, Linehan's group did not know the extent of this genetic diversity. Thus, they treated all kidney cancer as similar. But, after partnering with Berton Zbar, M.D., Chief of

the Laboratory of Immunobiology, in 1983, Linehan realized that the genetic knowledge could now be transformed into a groundbreaking new weapon against cancer.

"We had hoped that if we understood the genes that when mutated cause these cancers," Linehan said, "we might understand how they provide the foundation for the development of targeted therapies."

And the CCR researchers developed a new logic: if one wants to find the genes that cause kidney cancers, one must look for cancers that seem to be genetically inherited. Although these malignancies may occur rarely, one can extrapolate the information gleaned from the rare cases to all patients with more common forms of kidney cancer.

Embracing that strategy, Linehan's team began looking at patients with an illness called von Hippel-Lindau syndrome (VHL). It predisposes individuals to nearly 600 types of tumors in multiple organs, including a form of kidney cancer called clear cell renal cell carcinoma (CCRCC). This form of cancer accounts for 80 percent of people with renal cell carcinomas, which in turn represents more than 90 percent of all malignant kidney tumors. The hope was that any breakthrough in von Hippel-Lindau would apply to CCRCC, which is not inherited.

After nearly ten years of performing genetic analyses, the researchers in 1993

W. Marston Linehan, M.D., Chief of CCR's Urologic Oncology Branch, has spent 25 years crafting better approaches to treating kidney cancers.

Unfortunately, the CCR team soon learned that the faulty *VHL* genes, while clearly the cause of von Hippel-Lindau and most cases of CCRCC, are not the instigators of all types of kidney cancer.

The researchers needed to broaden their focus.

More Cancers, More Genes

Individuals with hereditary papillary renal cell carcinoma (HPRCC) are plagued with multiple tumors in both kidneys. Papillary kidney cancer represents 5 percent to 10 percent of all cases of renal carcinoma but, more importantly, can run in families. Thus, the Linehan team again conducted a thorough genetic analysis, this time in families affected by HPRCC.

In 1997, after seven years of research with Zbar and Urologic Oncology staff scientist Laura Schmidt, Ph.D., the culprit emerged: the *c-Met* oncogene. This gene does the opposite of *VHL*—it triggers cells to divide.

The discovery prompted a thorough biochemical search for the molecules that *c-Met* and its protein product interact with in order to accomplish its pro-cell division function. Today, investigators are now engaged in “a very intense effort” to develop agents that block the oncogene, said Linehan. Clinicians and scientists are searching for an agent like imatinib mesylate (Gleevec®)—a Novartis drug first developed as a treatment for one form of leukemia and later soft tissue cancer—characterized by

finally narrowed their search to one area of chromosome 3. Later, the team pinpointed within that region a mutant gene, called *VHL*, which normally functions as a tumor suppressor, a molecular brake on cell division.

VHL was the sixth gene discovered with links to human cancer, but the first associated specifically with kidney cancer. When *VHL* is mutated, it cannot do its normal job of holding cellular reproduction in check, including in the cells that line the tubules within the kidney (from which carcinomas arise). This finding allowed the CCR researchers, and many others working around the world, to work out the biochemical details of how the *VHL* gene encodes a protein that forms a complex that keeps normal cells from dividing.

The finding also held clinical benefit. Drug developers later targeted the *VHL* pathway and came up with agents such as

sunitinib maleate (Sutent®; Pfizer) that appear to work in keeping tumor growth at bay. Linehan's own laboratory recently launched a Phase II clinical trial of an anti-biotic derivative called 17-AAG (pioneered by Leonard Neckers, Ph.D., Senior Investigator in the UOB). It is hoped that this type of therapy will advance the treatment of kidney tumors in patients with *VHL* and, in the future, help others such as Jeanne McCoy and Alice Coday (see “Patients: Part of the Team”) who, thanks to early detection and treatment, are currently cancer-free, despite having the associated *VHL* mutation.

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mutation in the gene *c-Kit*—that can target the *c-Met* gene pathway. An agent like this should have therapeutic benefit for individuals with papillary kidney cancer as well as HPRCC.

One More Time

Individuals with Birt-Hogg-Dubé syndrome (BHD) have tumors in many tissues, including benign skin bumps arising from the hair follicles. While that manifestation might seem relatively trivial, 35 percent of these individuals also develop kidney cancer. And since BHD runs in families, it provided an opportunity for researchers at CCR to apply their strategy of gene discovery yet a third time.

In 2002, after seven years of scrutiny, Linehan, Zbar, Schmidt, and colleagues discovered what they called the *BHD* gene. It makes a protein that the team dubbed folliculin, a reference back to the benign hair follicle tumors.

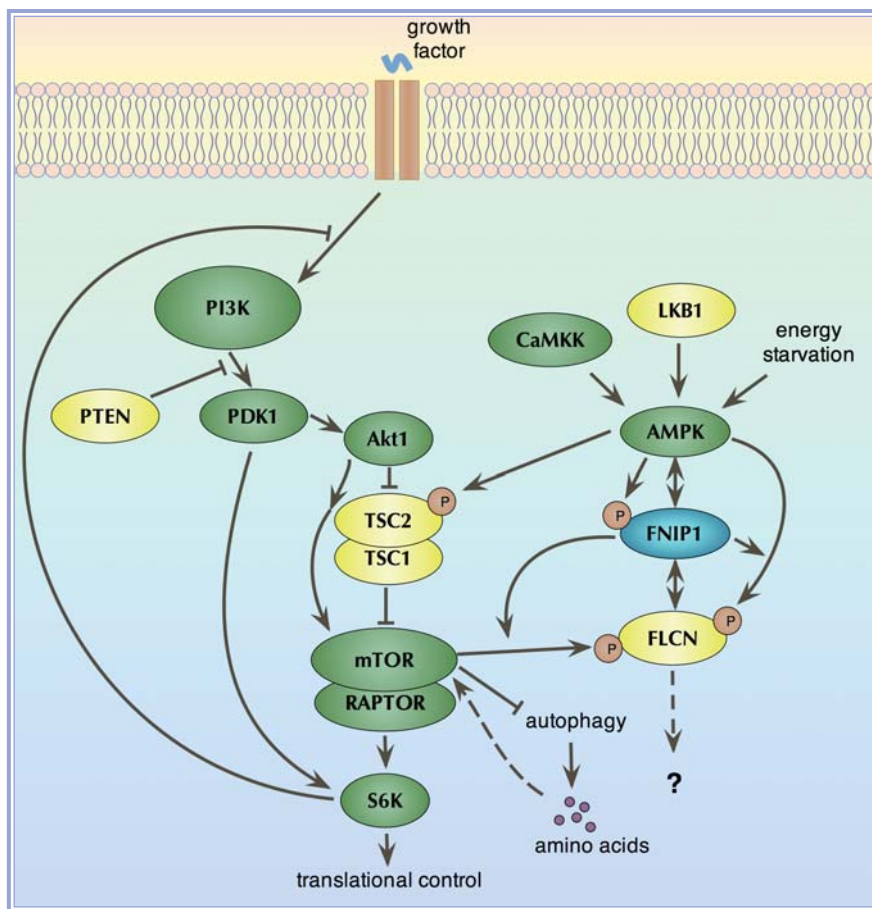
The gene was novel, with no known function. Linehan’s team set out to discover that function, hoping it would lead to more clues and ultimately therapies for BHD syndrome and the cancers linked to it. Using a mix of state-of-the-art genomic technologies and traditional biochemistry, the CCR team has unraveled the folliculin pathway and, with that knowledge, identified potential drugs (see “The BHD Mystery Solved”).

Collaboration

And still the kidney cancer story continues. The fourth chapter, and probably not the last, involves collaboration, a trademark of the environment at CCR.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a hamartoma syndrome, a condition in which affected individuals readily develop skin bumps called myomas. In women, these can also appear in the uterus as fibroids. About 20 percent of HLRCC patients develop a very aggressive type of kidney cancer.

In 2002, Ian P. Tomlinson, Ph.D., and colleagues at Cancer Research UK in



(Graphic: Feinstein-Kean Healthcare)

Possible placement of FNIP1 in the AMPK and mTOR growth signal transduction pathways. Proteins indicated in yellow are those that are known to be defective in hamartoma syndromes. The dotted lines indicate functional interactions that are not yet clarified. FNIP1 (blue) is regulated by AMPK through phosphorylation (P). FNIP1 is believed to facilitate folliculin (FLCN) phosphorylation by mTOR and AMPK signaling. The target(s) of FLCN remains unknown.

London showed that HLRCC is caused by mutations in an enzyme called fumarate hydratase, which normally acts in a fundamental metabolic pathway called the Krebs cycle. Although Tomlinson’s group knew that the enzyme could function as a tumor suppressor, they did not know how.

Linehan, working with senior investigator Maria Merino, M.D., of CCR’s Laboratory of Pathology, showed that the tumors taken from patients with HLRCC produce a very high level of a protein called hypoxia inducible factor (HIF). The protein normally senses low oxygen in tissues and, in response, spurs new blood vessel growth, an increase in glucose transport, and the secretion of growth factors. Linehan surmised that, in tumors, excess HIF might pro-

mote growth and sustenance, making the molecule or its activity a good target for an anti-cancer drug.

Still, Linehan did not have an answer to the question: what does a mutation in a Krebs cycle enzyme have to do with an upsurge in HIF? In a late-night conversation in the hallway outside his office, Linehan turned to Neckers. With the help of a chemistry fellow in his lab, Neckers offered the idea that excess fumarate might compete with the co-enzyme of another molecule, prolyl hydroxylase, which is critical for binding and regulating HIF. This was an “ah-ha” moment for Linehan, because his team had already implicated the VHL/HIF pathway in CCRCC. Since one of VHL’s functions is to

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A Team with a Patient Focus

Although Linehan's research team in the Urologic Oncology Branch is diverse, they share two common motivations: patients and a desire for performing in-depth basic research. At CCR, while people with kidney cancer seek help at the NIH Clinical Center, researchers are cloning, mapping, and probing DNA two floors below. This close juxtaposition of clinical and basic research is rare and exactly the reason that lab members have traveled to work here from around the world.

Donald P. Bottaro, Ph.D., Senior Scientist

Before joining the Linehan lab, cell biologist Don Bottaro's world fit on a microscopic slide. He spent two fruitful decades at NCI's Laboratory of Cellular and Molecular Biology, studying how molecules such as hepatic growth factor and a gene called *MET* signaled to each other in cells. Then, in 1999, he felt compelled to expand his borders.

"I wanted to use what we do in basic science to help patients with cancer," Bottaro said.

Thus, he left NCI for a two-year stint at a biotech company called Entremed, dedicated to discovering anti-cancer drugs. Bottaro then returned to join Linehan's multitiered, clinical and research-focused team. For three years, Bottaro attended surgeries and grand rounds each week, in addition to working long hours in the lab.

His heroic efforts to bridge "the very big gap" between basic research and clinical treatment is paying off. He has used the tumors from the patients seen upstairs to decipher how the *VHL* gene might cause the renegade invasiveness of cancer cells.

Masaya Baba, M.D., Ph.D., Postdoctoral Fellow

On his first day as a urologic surgeon in Japan, Masaya Baba developed a passion for kidney cancer. He examined a mother of three who had the disease, and who died within five years of diagnosis.

"That motivated me," Baba said. "I saw that many patients survive through surgery. But I thought that more research was necessary."

Thus, after establishing a clinical practice and later studying von Hippel-Lindau syndrome during graduate school in Japan, Baba wanted to push the frontier of cancer treatment still further. He wanted to work with a group that had molecular biology and genetic technology expertise, as well as access to many patients with rare, inherited forms of kidney cancers.

Enter the Linehan team, which Baba joined in 2003. Already he has uncovered the molecular underpinnings of folliculin.

Sunil Sudarshan, M.D., Clinical Fellow

Synergy drew Sunil Sudarshan to the Linehan lab in 2005. From the first day he studied biochemistry as an undergraduate to his residency in urology at the Medical University of South Carolina in Charleston, Sudarshan wanted to work both with patients and with their genes.

Sudarshan walked through the doors of the Linehan lab just as the researchers, in collaboration with Len Neckers, had made a breakthrough connection between the rare hereditary leiomyomatosis and renal cell cancer (HLRCC) and fumarate hydratase, which normally functions in the Krebs cycle.

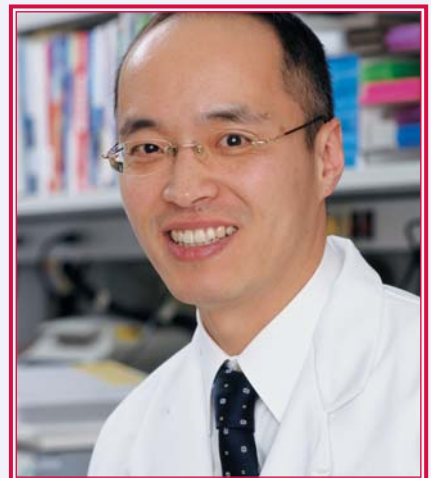
Immediately, Sudarshan jumped on board. He now applies this molecular knowledge directly in patients with HLRCC, seeing them in the clinic as he studies their DNA in the laboratory.

"That is what CCR is set up for," Sudarshan said. "It is rare that a researcher gets to do both clinical and basic research on essentially the same thing."



(Photo: R. Baer)

Donald Bottaro, Ph.D.



(Photo: R. Baer)

Masaya Baba, M.D., Ph.D.



(Photo: R. Baer)

Sunil Sudarshan, M.D.

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degrade HIF after oxygen concentrations reach adequate levels, the idea provided a possible connection between the two seemingly disparate forms of cancer through one biochemical pathway.

The next step was to prove the connection, which Linehan and Neckers both did. The researchers published the connection between fumarate hydratase and VHL in the August, 2005, issue of *Cancer Cell*. With the assistance of urologic surgeon Sunil Sudarshan, M.D., (see “A Team with a Patient Focus”) these investigators are working to

turn these findings into a targeted therapeutic approach for patients with HLRCC-associated kidney cancer. For example, Linehan is planning a trial to evaluate whether or not targeting HIF will have an effect on HLRCC as well. A drug called Avastin® (bevacizumab), which can inhibit new blood vessel growth, might reverse some of the damage caused by excess HIF.

The CCR researchers are in a perfect position to investigate the promise of Avastin and other therapies on patients because of the close association of lab

and clinic in CCR—the laboratories and clinic are housed two floors apart in the NIH Clinical Center. Researchers join clinicians on the Urologic Oncology Branch’s grand rounds, while clinicians work as fellows in the labs. It is a unique collaborative environment destined to advance the innovative basic research that translates to better therapies.

“After working on the complexities of biochemical mechanisms, it really brings us to back to earth to see these patients,” Linehan said. “We never lose that focus.”

The BHD Mystery Solved?

Individuals with Birt-Hogg-Dubé syndrome (BHD) have a mysterious condition, characterized by tumors in follicle cells, as well as those of the kidney, in some cases. To unravel the mystery, W. Marston Linehan, M.D., Laura Schmidt, Ph.D., and colleagues employed cutting-edge genetic mapping and linkage studies, thereby discovering the previously unknown gene folliculin.

But in order to find out how that genetic defect can lead to cancer, the CCR team had to map folliculin’s molecular interactions. The investigators used a technique called co-immunoprecipitation, in which one protein linked to a bead or antibody is used to fish out another from a mix of cellular contents. With that, the CCR team pulled out folliculin’s partner, a protein that functions in an energy/nutrition sensing pathway in the cell.

What Does Energy Regulation Have to Do with Follicle Tumors?

It turns out that folliculin-interacting protein-1, (FNIP1), as the CCR team named it, binds to

folliculin and so puts the brakes on cell division. Thus, this protein pair functions in a tumor suppressing pathway that appears to be controlled by the loss or gain of a phosphate group. And that group associates with an enzyme called 5’-AMP-activated protein kinase (AMP-K, a key energy sensing molecule within the cell).

Because the discovery of the folliculin-FNIP1-AMP-K relationship is so new—it was just published in 2006—Linehan, Schmidt, and colleagues can only speculate on how problems with an energy sensing pathway might be linked to renegade cell division. It could be that an energy deficit or stress triggers AMP-K, which works through another pathway involving a protein called mTOR. The kinase, when activated, appears to add a phosphate group to both FNIP1 and folliculin and so could potentially trigger a braking action within cells. This system likely evolved as a mechanism to keep cells from reproducing in times of energy scarcity.

Cells should release this brake when energy is plentiful, for example letting follicle cells divide and hair grow. But individuals with BHD make faulty folliculin proteins and so may lack this particular braking system. Thus, the follicle cells divide regardless of whether energy levels are high or low, fueling the benign skin bumps. These same mechanisms may allow the kidney cells in BHD patients to grow, although with more malignant consequences.

While further delving into how that putative pathway might work, Linehan’s team has already begun searching for clinical agents that block this pathway. Promising leads involve other drugs known to target the mTOR pathway. Linehan says investigators are studying this lead “very aggressively,” but cautions that the studies are preliminary.

Patients: Part of the Team

It ran in the family. Jeanne McCoy's grandmother and mother both suffered from von Hippel-Lindau syndrome (VHL), which manifests as a combination of one or more of nearly 600 types of tumors in multiple organs.

For McCoy's grandmother, the tumors appeared first on her retina (she went blind at the age of 30) and also on her brain stem. Eventually the cancer invaded her kidneys. Meanwhile McCoy's mother, who was told at a young age that she did not have the same diagnosis, was shocked to learn that she had already progressed to late-stage kidney cancer. The double diagnosis—combined with her family history—led McCoy to search the Internet, learn of the then recently-identified *VHL* gene, and seek out the NIH and Linehan.

Because of the late stage of McCoy's mother's cancer, she was quickly accepted into the clinical program at the NIH. There, she tested positive for the *VHL* mutation. Several months later, McCoy sent in her own blood sample and records to the NIH—just in case. The results confirmed McCoy's suspicions: she too, carried the *VHL* mutation, as did her grandmother.

But McCoy had no symptoms of kidney cancer. In fact, at age 34, she had just given birth only a year previously to the youngest of her three children. Except for faint back pain, something all new mothers experience, McCoy felt fine.

But she also knew that the *VHL* mutation foretold her fate. And she already had a benign tumor on the endolymphatic sac of her inner ear—a common symptom of VHL syndrome. So McCoy consulted her local oncologist, who advised her to undergo an ultrasound and CT-scan in a hospital near her Greenville, South Carolina, home. Within hours, she learned that both her kidneys were riddled with cysts and tumors that, if untreated, could eventually kill her.

Panicked, she called the NIH. Within two weeks, she was headed for Bethesda for more testing and consultation. There, she embarked upon an odyssey of surgeries, first to remove the tumor in her ear and then those in her kidneys. It was during the second of her kidney surgeries, just before Christmas 2003, that

McCoy first met Linehan, who came to see her in the surgical intensive care unit at the NIH.

"He sat down and talked to me and my husband," she recalled. "And suddenly, he put a real personal face on the research; that it wasn't these scientists lost in a lab; that this was about people, and this was about early diagnosis and detection, and this was about finding a cure."

In that interaction, and all of the subsequent ones over the next four years, McCoy moved from what she calls "survival mode" toward a more altruistic focus. She is part of "something larger," research that might help others today, as well as in the future.

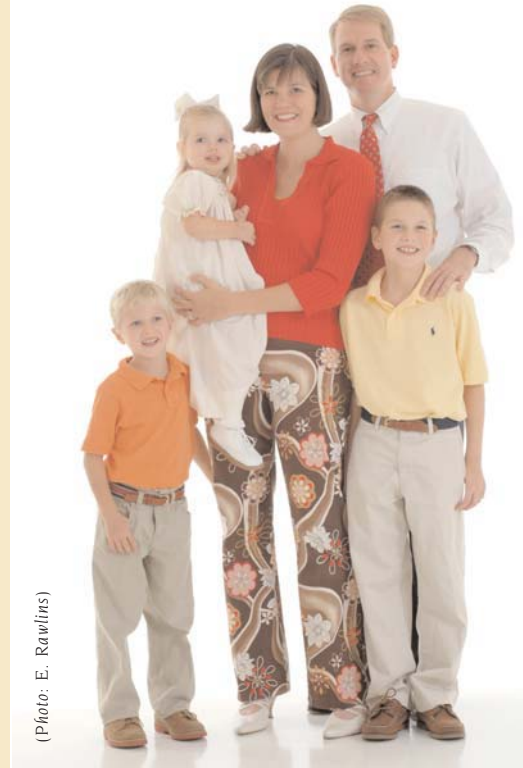
The same epiphany occurred for Alice Coday. The 52-year-old watched as physicians diagnosed her father, brother, and sister with VHL. All three had tumors on their brain stems. Coday did not have that kind of tumor, convincing her she had been spared the mutation.

But she had been diagnosed instead with Ménière's disease, characterized by tumors on the endolymphatic duct of the inner ear (just like McCoy). She suffered severe vertigo and, in 1988, lost hearing in her right ear. But she experienced nothing of the brain or kidney tumors that eventually plagued her family members.

Not until Coday had a happenstance conversation with a member of the Von Hippel-Lindau Family Alliance did she suspect a possible mistake in her diagnosis. She contacted the NIH and traveled from her home in Seattle for testing. Only then did she learn that she not only carried the *VHL* mutation but also had developed tumors in both of her kidneys and cysts in her pancreas.

In 1997, surgeons from CCR removed part of her left kidney and a year later, the right. She has been traveling to the NIH at least twice a year ever since.

She knows that if she had had the surgeries locally, she would have lost both of her kidneys entirely and probably would have needed dialysis. She knows that she could have died if the tumors had remained undetected. But she does not come to the NIH today for either of these reasons. Coday is cancer-free thanks to the discovery of the *VHL*



(Photo: E. Rawlins)

Patients like Jeanne McCoy (above with son Ford, daughter Mary Ellison, husband Ellison, and son Brooks) and Alice Coday (below, with her dog Clancy) are also Linehan's partners in kidney cancer research.



(Photo: A. Davison)

gene and the efforts of the Linehan group and others to foster early detection. She now comes to the NIH because she sees her larger purpose.

"When you go to a traditional doctor, you think of a doctor-patient relationship," she said. "The NIH is something more. You feel like you are participating. Like you are part of a team, making history."