Molecular genetics of kidney cancer: implications for the physician

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CME

CME, Part 2 of 3

Target audience: Urologists, oncologists, internists

Learning objectives:

1. To understand cancer genes.
2. To understand the genes for cancer of the kidney.
3. To understand the different types of cancer of the kidney.
4. To understand the different strategies for management of hereditary kidney cancer.

Faculty credentials/disclosure:

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Before beginning this activity, please read the instructions for CME on p. 446. This page also provides important information on method of physician participation, estimated time to complete the educational activity, medium used for instruction, and date of release and expiration. The quiz, evaluation form, and certification appear on pp. 446–448.

This year it is estimated that 30,000 Americans will be diagnosed with kidney cancer and 12,500 will die of the disease. Both the incidence rate and the mortality rate are increasing; because of the rapid rise, kidney cancer is on the National Cancer Institute's (NCI's) watch list. The increases cannot be explained by better detection methods. In fact, despite earlier diagnosis, mortality rates aren't improving. Fortunately, if we are able to see patients with localized disease, we can often give them a very good 10-year survival. We've had less of an impact on patients with locally advanced disease, and despite some treatment innovations and advancements, most patients with metastatic kidney cancer still die of their cancer.

There are 4 basic types of renal tumors: clear cell, which comprises 75% to 80% of all kidney cancers; papillary, which comprises 10% to 15%; chromophobe, which comprises 5%; and oncocytoma, which comprises 5%. There are 2 types of papillary tumors: type 1 and type 2.

Like most other cancers, kidney cancer comes in a hereditary as well as nonhereditary or sporadic form. While the sporadic form tends to be solitary and usually occurs in patients in their 40s, 50s, and 60s, the inherited form tends to be multifocal and bilateral and often has an early onset (1).

In this article, I review several types of kidney cancers and describe how their genes were identified. I also discuss practical implications of these findings. Before moving to the discussion of clear cell cancer, it is useful to summarize some basic principles of genetics.

ONCOLOGIC GENETICS

Our genetic material consists of 23 different chromosomes: 22 sets of autosomes and 1 set of sex chromosomes. We inherit 1 set from our mother and 1 set from our father. The chromosomes are numbered sequentially, with the largest called 1. The short arm of the chromosome is called the *p* arm (French for *petit*). The next letter in the alphabet, *q*, is used for the long arm. Within the chromosomes are genes, segments of DNA that code for a protein.

A cancer gene is simply a normal gene which, when it becomes damaged or mutated, leads to cellular proliferation. An oncogene is the simplest example: it is activated after a single change in the gene. With a tumor suppressor gene, both copies of the gene are damaged or lost.

Conventional wisdom among the experts is that cancer is a multigenetic process. Potentially a single gene leads to the start of a cancer, and other events may occur later that then lead to more aggressive cancer. That's the best model we have. For cancer of the kidney, we estimate that the first genetic change occurs 25 years before the disease manifests clinically. We are working to develop tools so that we can learn what happens in that 25-year period that causes the cancer, whether it's additional genetic changes or just the passage of time.

HEREDITARY CLEAR CELL CARCINOMA

In the early 1980s, in collaboration with Dr. Berton Zbar, we set out to find an abnormality on chromosome 3 that might be a kidney cancer tumor suppressor gene. We took tumors from patients with sporadic kidney cancer and evaluated the DNA, focusing particularly on this chromosome (2). The task proved quite large and complex. Dr. Al Knudson, considered the father of cancer gene study, suggested focusing on the hereditary form

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of the disease, which could prove to have the same gene as the sporadic form. This is what led to the NCI's concentration on a less common form of inherited kidney cancer called von Hippel-Lindau (VHL) (3).

Characteristics of VHL disease

VHL is a disease in which patients inherit a predisposition to develop tumors in a number of different locations, including the kidneys, adrenal gland, pancreas, cerebellum, retina, and spinal cord. The tumors are extremely angiogenic. VHL is inherited in an autosomal-dominant pattern; each child has a 50/50 chance of carrying the gene and developing these cancers, and early onset of cancer is common. VHL patients develop as many as 600 tumors and 1100 cysts in the kidneys. The number varies remarkably by the type of mutation in the gene. All of the kidney tumors in VHL patients are clear cell, and all are malignant. Historically, 35% to 45% of these patients will die of kidney cancer if they are not diagnosed and treated early.

Some of the earlier misconceptions about this disease may be related to lead-time bias. In other words, if you notice a tumor early with a computed tomography scan, it will look like it's growing slowly. However, the growth rate is the same that occurs with sporadic kidney cancers; we just don't detect those and watch them to the same degree. At the NCI, we manage VHL patients very aggressively with intraoperative ultrasound. We follow the patients until the tumors grow to a certain size—in our hands, it's 3 cm—and then we recommend nephron-sparing surgery. We're also conducting a pilot study in 30 patients to see what effect radiofrequency ablation treatment may have in the management of these patients.

We've learned a lot more about VHL from our patients. In fact, the strength of our program has been from going to the bedside to the laboratory, rather than vice versa. For example, one lady told me about her child with hearing problems and asked if that could be VHL. While I thought that hearing problems weren't associated with the disease, I learned otherwise. Upon further evaluation, we found that 12% of our VHL patients develop malignant papillary tumors in the inner ear.

The search for the VHL gene

To identify the gene for this hereditary cancer, we screened a group of gracious and brave families who came to the National Institutes of Health. Once we determined who was affected and who was not, we conducted a genetic linkage analysis to localize the gene. As a result, we were able to focus on a small region of chromosome 3p. Since the human genome project had not begun when we were working on this project, we had to decipher the code and develop our own long-range genetic map with sophisticated technology. A fellow working on the project discovered a common hole in the DNA in 3 of our families. By determining the DNA in that hole, we identified 2 candidate genes. One of them was G7 (4).

The G7 gene has 3 exons or coding regions, which code for what becomes protein. Through polymerase chain reaction, we were able to find specific mutations in the G7 gene in the germline in the blood of individuals affected with the disease but not in that of individuals not affected with the disease. This, then, was the gene for VHL and has been renamed the VHL gene.

We also noticed a striking correlation between the genotype and the phenotype, or the tumors we saw in our patients. Some of the first 76 families tested showed mutations at exon 1, some at exon 2, and many more at exon 3. One group of families with a mutation in a similar area all had pheochromocytomas. Additionally, they all had missense mutations. We continue to learn more details that will help us specifically identify each family's disease and its aggressiveness.

Clinical applications

Now that we know the gene for VHL, we recommend genetic testing of young children. Children as young as 1 year have been known to begin losing their visual fields, and we've heard of 9-year-old children dying with VHL. With early detection, we can tell parents which of their children will need to be screened and followed and will potentially need early intervention. Of course, once we know the hereditary prostate cancer gene, we will not recommend screening of anyone <18 years of age. This cancer does not develop in children, so most people consider it inappropriate to give the genetic information to a minor.

Knowledge of the VHL syndrome and its genes can also allow more accurate diagnoses and help us predict which organs are at risk. For example, we saw a boy with a right adrenal gland tumor. The child's mother had had a pheochromocytoma when she was 9 years old, and her brother had died of an unsuspected pheochromocytoma when he was 8 years old. This family was diagnosed years earlier but not known to have VHL.

When we screened the mother, we found an unsuspected solid pancreatic mass. When we ran the VHL blood test, we found a mutation in the VHL gene. This family, then, had VHL, not multiple endocrine neoplasia type 2, so we went ahead with genetic screening of the entire family. The phenotype in this family is predominantly pheochromocytoma. The knowledge that this was VHL also affected our thinking about treatment of the child.

SPORADIC CLEAR CELL KIDNEY CANCER

As Dr. Knudson foresaw, we came to learn more about the sporadic form of clear cell kidney cancer after investigating the hereditary form. A woman from a known VHL family presented to the clinical center at the National Institutes of Health. At that time, we hadn't yet identified the VHL gene; we were still doing the linkage analysis. When we screened her we found an abnormality in the left kidney. Our group said that she must have VHL. I had never seen a patient with VHL who had kidney cancer alone, so I wasn't sure how to manage the case. At the time, if it were kidney cancer, most might have recommended a nephrectomy. For VHL patients, on the other hand, it is important to save the nephrons. We ended up taking her to the operating room and doing a partial nephrectomy.

We found that this woman did not have a VHL mutation in her germline; she did have a mutation in her clear cell kidney tumor. As you would predict, the mutation was different than the mutation in her family. This patient does not have VHL and will not pass the disease on to her children. This is an example of a phenocopy, a person who develops a tumor in an organ at risk in hereditary cancer syndrome and does not have hereditary cancer syndrome.

We tested other tumors from patients with sporadic clear cell kidney cancer, looking for a single change (oncogene) or a double change (tumor suppressor gene) at the VHL gene site. We have found an abnormality of the VHL gene, as well as loss of the other allele, in a very high percentage of tumors from patients with clear cell kidney cancer.

Laboratory data have confirmed our theory that a tumor suppressor gene is associated with the development of sporadic clear cell kidney cancer. When scientists took a kidney cancer cell line with a mutated VHL gene and put it in a mouse, the cancer grew in the mouse. When we corrected the genetic defect and put the corrected gene in the tumor, the mouse developed no tumor or a very small tumor. This supports the hypothesis of a loss-of-function gene. When you replace the function, the cancer loses its ability to form tumors in mice.

Clinical applications

With knowledge of the gene for sporadic clear cell kidney cancer, one can now test aspirates from tumors to determine whether or not the malignancy is a VHL gene–associated clear cell kidney cancer. One can also test metastases to determine the primary site of the cancer. Clear cell and papillary kidney cancers are very different diseases caused by different genes and requiring different treatment. For example, clear cell kidney cancer makes vascular endothelial growth factor (VEGF), spreads early, and responds to immunotherapy. Papillary cancer tends to be more indolent, does not make VEGF, and does not tend to respond to immunotherapy.

THE VHL GENE

When we found the VHL gene in 1993, there was nothing like it in the databases. We've been studying this gene intensively for the past 7 years and have learned that the VHL gene works in the cytoplasm as well as in the nucleus. However, a mutation of the gene may alter its trafficking. A mutation in the first exon means that the protein may not be able to move into the nucleus. A mutation in exon 3, which is associated with pheochromocytomas, may mean the protein cannot leave the nucleus to go to the cytoplasm.

As an approach to understand the pathway for the VHL gene, we looked at proteins that bound the VHL protein. We initially found 2 proteins—elongin B and elongin C—that had just been described by a team at the Oklahoma Medical Research Foundation, Drs. Ron and Joan Conoway (5).

These 2 proteins were our first clue about how the VHL protein might work. What we know currently is that the VHL protein works in a complex of proteins that are transported from the cytoplasm to the nucleus. When the VHL gene is damaged, the VHL protein can no longer bind to elongin B and C. This is especially pronounced in patients with more aggressive cancer.

Clinical applications

Based on knowledge about the VHL gene and protein, 2 targeted therapies have been developed. One is an antibody targeted against the VEGF receptor. The second strategy involves small molecules to block the receptor of VEGF.

HEREDITARY PAPILLARY RENAL CARCINOMA

With hereditary papillary renal carcinoma, onset is later than with VHL, but the disease is highly penetrant—i.e., if a patient lives to be old enough, say 80 years of age, it's nearly certain that he or she will develop papillary kidney cancer. After doing genetic linkage analysis among families with this disease, we were able to localize the gene to chromosome 7 and identify a known oncogene, *met*, as a candidate. We found mutations of *met* in the germline of these individuals. *Met* is the receptor on the cell surface for a ligand called hepatocyte growth factor (6, 7).

In another study, we examined trisomy—3 copies of a certain chromosome—in tumors. We hypothesized that it would be the mutated gene that would be duplicated in the cancers. That is what we found in papillary kidney cancer: 3 copies of chromosome 7, a nonrandom duplication of the mutant allele. If 1 copy is good for growth, 2 copies are better.

We've also gained an understanding of the pathway of *met*, and we have an ongoing effort looking at drugs to block the pathway. Several candidate drugs totally block this pathway in the laboratory but must still be tested in humans.

HEREDITARY KIDNEY CANCER OF VARYING HISTOLOGY

We initially saw a number of kindreds with bilateral, multifocal, sometimes unifocal kidney cancer that differed from both VHL and hereditary papillary cancers because the tumors had varying histologies. We noticed that many of the patients also had tiny skin bumps, primarily on their face and neck, which turned out to be fibrofollicular tumors. This has been called Birt-Hogg-Dubé syndrome, and it is the most recent form of hereditary kidney cancer identified (8).

OTHER NEW CLINICAL APPROACHES IN KIDNEY CANCER

Dr. Richard Childs is directing a minitransplant protocol in patients with advanced kidney cancer who have a sibling with an HLA match. The protocol involves preparing the patient with an immunoablative regimen, taking lymphocytes from a sibling treated with granulocyte-macrophage colony-stimulating factor, transferring the lymphocytes to the patient, and then giving the patient cyclosporine. We wait until all the lymphocytes are from the donor and then back off on the immunosuppression, accepting some graft-vs-host disease and hoping to see the graft attack the tumor. The responses can take many weeks to occur.

A small number of patients have been treated, and the follow-up period is short. However, we are encouraged by the progress in the studies. We don't know what the donor lymphocytes are recognizing—whether it's some antigen on the cell surface or the VHL gene peptide. We're looking at 2 different strategies for vaccines in these patients, but we're very optimistic and encouraged by the early results in this pilot trial.

CONCLUSION

Kidney cancer is a heterogeneous disease. As we study it more closely, we're learning more about it, and we are hopeful that understanding the genes that cause these cancers will lead to better methods for early diagnosis, prevention, and treatment of these cancers.

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