A Tale of Two Cells: Discovering the Origin of Prostate Cancer

By Ken Garber

In July, researchers in the lab of Owen Witte, M.D., at the University of California, Los Angeles, reported in Science that human prostate basal cells, transformed and then transplanted into mice, could form tumors. But in September 2009, a different group reported in Nature that prostate cancer in mice originated from a rare population of luminal cells.

Basal versus luminal: irreconcilable? Apparently not.

"Neither of them contradicts each other at all," said Lynette Wilson, Ph.D., a professor in the departments of cell biology and urology at New York University. It’s possible that there are different prostate cancer cells of origin, she said. And the two papers are together a big step forward for a field badly lacking such basic knowledge.

That’s because the cellular origins of prostate cancer are still largely unknown. “The prostate cancer field is struggling because, unlike [for] the breast, there is not a clear consensus about prostatic cell lineage,” said Gail Risbridger, Ph.D., a professor in the department of anatomy and developmental biology at Monash University in Melbourne, Australia. In breast cancer, six different subtypes have been mapped out, each arising from a distinct population of cell biology and genetics. Both basal and luminal cells have now been identified as prostate cancer cells of origin, in human and mouse, respectively. "This is a common precursor cell. But we do not yet have such a map for prostate cancer. “People were very puzzled,” said Wilson. “Witte’s paper is so important because it shows very nicely that you can transform a basal cell and still end up with a luminal tumor.”

But luminal tumors can also arise from luminal cells, at least in mice—Shen’s equally convincing demonstration. For more than a decade, Shen’s group had studied Nkx3.1, a transcription factor that’s a key regulator of prostate epithelium. (Nkx3.1 works with the androgen receptor to direct epithelial differentiation during embryonic development.) To their surprise, they found a small population of Nkx3.1-positive luminal cells in mouse prostates after castration, contradicting previous studies.

In the experiments described in Nature, Shen’s group monitored these castration-resistant Nkx3.1-expressing cells (CARNs) in mice genetically engineered to individually display these rare cells and their descendants by using a reporter protein. (This method is called “lineage tracing.”) Luminal CARNs expanded dramatically during postcastration, androgen-driven regeneration of the prostate, yielding new prostatic tissue containing both basal and luminal cells. This finding showed that CARNs can regenerate normal prostate tissue.

Luminal, Basal or Both?

Controversy over the origin of prostate cancer is not new. The disease has two feasible cell types of origin: the luminal secretory cells that line the prostate epithelium and the basal cells underneath. Tumor tissue consists of luminal cells, not basal, so most pathologists have assumed that the disease has luminal origins. But in the normal prostate, basal cells have more regenerative potential, so most cell biologists suspected that the disease originated there.

That conclusion did not, however, explain how basal cells could transform into luminal cell cancer. “People were very puzzled,” said Wilson. “Witte’s paper is so important because it shows very nicely that you can transform a basal cell and still end up with a luminal tumor.”

Luminal versus basal: irreconcilable? Apparently not.

"Neither of them contradicts each other at all," said Lynette Wilson, Ph.D., a professor in the departments of cell biology and urology at New York University. It’s possible that there are different prostate cancer cells of origin, she said. And the two papers are together a big step forward for a field badly lacking such basic knowledge.

That’s because the cellular origins of prostate cancer are still largely unknown. “The prostate cancer field is struggling because, unlike [for] the breast, there is not a clear consensus about prostatic cell lineage,” said Gail Risbridger, Ph.D., a professor in the department of anatomy and developmental biology at Monash University in Melbourne, Australia. In breast cancer, six different subtypes have been mapped out, each arising from a distinct population of cell biology and genetics. Both basal and luminal cells have now been identified as prostate cancer cells of origin, in human and mouse, respectively. "This is a common precursor cell. But we do not yet have such a map for prostate cancer. “People were very puzzled,” said Wilson. “Witte’s paper is so important because it shows very nicely that you can transform a basal cell and still end up with a luminal tumor.”

But luminal tumors can also arise from luminal cells, at least in mice—Shen’s equally convincing demonstration. For more than a decade, Shen’s group had studied Nkx3.1, a transcription factor that’s a key regulator of prostate epithelium. (Nkx3.1 works with the androgen receptor to direct epithelial differentiation during embryonic development.) To their surprise, they found a small population of Nkx3.1-positive luminal cells in mouse prostates after castration, contradicting previous studies.

In the experiments described in Nature, Shen’s group monitored these castration-resistant Nkx3.1-expressing cells (CARNs) in mice genetically engineered to individually display these rare cells and their descendants by using a reporter protein. (This method is called “lineage tracing.”) Luminal CARNs expanded dramatically during postcastration, androgen-driven regeneration of the prostate, yielding new prostatic tissue containing both basal and luminal cells. This finding showed that CARNs can regenerate normal prostate tissue.

Luminal, Basal or Both?

Controversy over the origin of prostate cancer is not new. The disease has two feasible cell types of origin: the luminal secretory cells that line the prostate epithelium and the basal cells underneath. Tumor tissue consists of luminal cells, not basal, so most pathologists have assumed that the disease has luminal origins. But in the normal prostate, basal cells have more regenerative potential, so most cell biologists suspected that the disease originated there.

That conclusion did not, however, explain how basal cells could transform into luminal cell cancer. “People were very puzzled,” said Wilson. “Witte’s paper is so important because it shows very nicely that you can transform a basal cell and still end up with a luminal tumor.”

But luminal tumors can also arise from luminal cells, at least in mice—Shen’s equally convincing demonstration. For more than a decade, Shen’s group had studied Nkx3.1, a transcription factor that’s a key regulator of prostate epithelium. (Nkx3.1 works with the androgen receptor to direct epithelial differentiation during embryonic development.) To their surprise, they found a small population of Nkx3.1-positive luminal cells in mouse prostates after castration, contradicting previous studies.

In the experiments described in Nature, Shen’s group monitored these castration-resistant Nkx3.1-expressing cells (CARNs) in mice genetically engineered to individually display these rare cells and their descendants by using a reporter protein. (This method is called “lineage tracing.”) Luminal CARNs expanded dramatically during postcastration, androgen-driven regeneration of the prostate, yielding new prostatic tissue containing both basal and luminal cells. This finding showed that CARNs can regenerate normal prostate tissue.

Then, after deleting the PTEN tumor suppressor gene in CARNs in castrated mice, Shen’s group resupplied androgens and watched as prostate tumors developed and grew rapidly. The team concluded that CARNs are a luminal cell of origin for prostate cancer. Although Shen did not use human cells, “his evidence in [the] mouse is very convincing,” according to Risbridger.
Witte and colleagues did use human cells. They started with benign tissue from prostates removed from patients with bladder cancer, prostate cancer, or benign prostatic hypertrophy. Then they isolated separate basal and luminal cell populations on the basis of two surface protein markers. When the team injected the basal cells under the skin of immunodeficient mice, the cells grew into prostate tissue (having both basal and luminal cells), but the luminal cells did not grow. And only the basal cells formed tumors after researchers inserted oncogenes into their nuclei and injected the transformed cells into mice.

This study broke new ground in two ways: It was the first solid evidence for a basal-cell origin for human prostate cancer, and it yielded a system for transplanting specific types of benign human prostate cells into mice for later growth. Such separation and transplantation had been hard to do. “The advance that Witte made is to show that it is possible to do this work in human cells,” said Risbridger. “That in itself is a significant step forward.”

Witte did not prove that luminal cells were not prostate cancer cells of origin. That’s because, in his hands, normal luminal cells didn’t grow, either. That failure points to a technical limitation, not to a lack of tumorigenicity, said Risbridger. “It may be more to do with the technical aspects of xenografting luminal cells per se, which we’ve always known has been difficult to do.”

Witte acknowledged that his study does not exclude luminal cells. “Right now the most logical conclusion is that there’s at least one cell of origin for human prostate cancer, and we certainly haven’t ruled out that there may be others,” he said.

**Critical Questions**

So the next question is whether humans have CARNs and thus whether luminal as well as basal cells can give rise to human prostate cancer. Shen’s lab is working on this. He also points to recently published work from Donna Peehl’s lab at Stanford University, identifying Nkx3.1-positive cells in slices of human tissue after they had been transplanted into mice that underwent castration and androgen restoration. (The work appeared in the July issue of the *American Journal of Pathology.*) “It’s not the central point continued on page 1535
of their paper,” Shen said. “But their work does indeed indicate that there are human CARNs.”

Another question is whether Witte’s basal prostate cancer cells of origin are normal prostate stem cells. “He actually had a heterogeneous population of basal cells,” said Risbridger. The normal prostate stem cells and the cancer cells of origin may not be the same cell. The cancer cell of origin, said Risbridger, could be a differentiated cell that becomes malignant and acquires properties of “stemness.” Witte’s lab is now working on identifying the exact cell types that give rise to normal tissue and to cancer.

Yet another question is whether cancer stem cells exist in prostate tumors. These cells, which Risbridger prefers to call “cancer repopulating cells” to distinguish them from normal prostate stem cells, reportedly occur in several cancer types, most convincingly in leukemias. Prostate cancer repopulating cells, if they exist, represent the small reservoir of cancer cells that can form new tumors. Such cells are not necessarily the same as the cancer cell of origin.

“Two different questions,” said Wilson. Asking whether normal tissue has cells that could transform into cancer cells, she said, is not the same as asking whether cells with repopulating potential are part of a tumor that already exists. For example, the basal cell could be the cell of origin, but a luminal cell could be the cancer repopulating cell—a plausible scenario, because prostate tumors are luminal. CARNs are by definition androgen resistant and thus could, in theory, be the cell type that causes fatal relapse after androgen-deprivation therapy.

The possibilities don’t end there. More than one type of cancer repopulating cell could be in play. Or cancer repopulating cells may not even exist, because the “cancer stem cell model” may not apply to prostate cancer—or perhaps most prostate cancer cells can proliferate extensively. Or cancer repopulating cells may exist in certain prostate cancers but not others.

Answering these questions requires finding new markers to better separate these different cell populations in humans and test them in mice for stemlike properties, cancer-initiating ability, and tumor-repopulating potential. Witte’s lab is actively pursuing this endeavor. Shen, meanwhile, is trying to determine whether CARNs could become cancer stem cells.

Research for prostate cancer has lagged behind that for other cancer types in such experiments. For example, researchers have been isolating putative cancer repopulating cells (i.e., cancer stem cells) from human leukemias, melanomas, and brain and colon cancers for years and transplanting them into mice to form tumors—but not for prostate cancer.

“No one has yet published a tumor-initiating study with primary human prostate cancer cells,” noted Shen. (Witte’s experiments used benign tissue.) The likely reason is that human prostate cancer cells don’t graft easily into mice—or into culture, for that matter; only a few prostate cancer stem cell lines are in common use. And the reason for that? Risbridger thinks that the stroma—the fibrotic mass of connective tissue, fibroblasts, leukocytes, and blood vessels that surrounds and supports the tumor—is especially crucial for prostate cancer growth. So it’s hard for prostate cancer cells by themselves to proliferate outside of their microenvironment.

“The stroma is obviously critical,” said Risbridger. One sign of this is that both Witte and Shen cotransplanted their prostate cells with rodent connective tissue to enable grafting. Risbridger thinks the field should tackle cell lineage mapping in the prostate cancer stroma as well as in the tumor cells themselves.

“It’s [all] knowledge that we need, to be able to understand the disease etiology,” she said. “Absolutely fundamental information.”