FROM THE BENCH

Tung-Tien Sun, Ph.D., Rudolph L. Baer Professor of Dermatology and Professor of Pharmacology and Urology, has devoted his career to the study of epithelial cells.
A CONNOISSEUR OF EPITHELIA

BY MARJORIE SHAFFER

On the surface, the research of Tung-Tien Sun, Ph.D., is an amalgam of seemingly disparate disciplines. Over an academic career spanning 25 years, he has made important contributions to cell biology, dermatology, ophthalmology and urology and has published some 130 papers. How could one man contribute to so many fields of medicine in such a relatively short period of time? The answer lies in the epithelium, the cellular blanket that covers all the body organs, including the skin, eyes and bladder. Epithelial cells first fascinated Dr. Sun when he was a young biochemist doing his postdoctoral work at the Massachusetts Institute of Technology, and they continue to propel his work today at NYU School of Medicine, where he is Rudolf L. Baer Professor of Dermatology and Professor of Pharmacology and Urology.

Ostensibly, Dr. Sun has always studied the biology of epithelial cells. But his research has had an impact beyond the boundaries of the laboratory, in clinical areas ranging from corneal transplantation to urinary tract infections and cancer.

"His approach to science and to life are similar—he wants to experience lots of things and he enjoys looking at issues from a broad perspective," says longtime collaborator Robert Lherker, Ph.D., professor of Dermatology at the University of Pennsylvania School of Medicine in Philadelphia. "He isn’t afraid to go into areas that he isn’t necessarily grounded in, and his inquisitiveness has led him into completely different disciplines. He is a modern renaissance man."

INITIAL RESEARCH ON KERATIN

After receiving his undergraduate degree in agricultural chemistry from National Taiwan University in Taipei, Tung-Tien Sun obtained his Ph.D. in biochemistry from the University of California, Davis, specializing in protein chemistry. But the newly minted Ph.D. wasn’t especially happy as a protein chemist; he wanted to expand his interests. "I felt that protein chemistry was fun but a bit narrow for my taste," Dr. Sun recalls. "I was eager to broaden my horizons and work on mammalian cells. I wanted to learn cell biology."

So Dr. Sun went to the East Coast in 1974 for his postdoctoral training at MIT in the laboratory of Howard Green, Ph.D., a pioneering cell biologist and one of the country’s pre-eminent biologists. (NYU School of Medicine figures prominently in Dr. Green’s life, too—he was Chairman of the Department of Cell Biology at NYU before moving to MIT. Today, Dr. Green is George Higgenson Professor of Cell Biology at Harvard Medical School.)

In Dr. Green’s laboratory, Dr. Sun got the opportunity to study mammalian cells. At the time, Dr. Green had just made a breakthrough by growing epithelial cells in culture using irradiated 3T3 fibroblasts as feeder cells, which produce certain nutrients required for epithelial cell growth that aren’t available in standard culture medium. Up until then, no one had been able to grow the cells in culture. Now it was up to Dr. Sun to characterize the keratin produced by cultured epithelial cells.

Keratin is an intermediate filament, one of three classes of fibers lodged in a cell’s cytoplasm that form part of its mechanical support system. When Dr. Sun started his research, keratin was thought of as a kind of tough, fiber-like protein constituting the hair and nails. Almost everyone thought keratin was composed of only one type of protein and was produced only in certain parts of the body like the hair and nails.

Thanks largely to Dr. Sun’s research, it is now known that there are many tissue-specific forms of keratin produced by epithelial cells to form part of their cytoskeleton. Moreover, each epithelial cell can make a variety of keratins, depending on the cell’s location in an organ and its degree of differentiation or specialization.

Initially, Dr. Sun sought a way to detect keratin literally with his own hand—he clipped some calloused skin from his palm, ground up the skin in a buffered solution and plucked out keratin. He subsequently developed a system for looking at the structure of keratin by developing antibodies to it, which could be applied to fixed epidermal cells and then viewed under a microscope through immunofluorescent staining. It was then
possible for the first time to actually visualize the long, strand-like threads of keratin filaments.

Dr. Sun also used his anti-keratin antibody on frozen sections of tissue from human cadavers and found, to his astonishment, that the protein wasn’t some provincial cousin languishing in the hair and nails. Instead, keratin was a cosmopolitan protein present in the epithelial cells lining all of the body’s internal organs including the intestines, stomach and bladder. “There was this beautiful staining of epithelia all over the body,” he recalls. “It was totally unexpected.”

The unanticipated finding, like others in Dr. Sun’s career, led to new insights about the biology of epithelial cells. His initial discovery sparked a veritable stampede by other researchers to characterize the body’s keratins. Indeed, many groups would discover new keratins and when the dust had settled more than 20 tissue-specific types of keratins would be described.

During the time when keratins were being identified, Dr. Sun began to link the discoveries to another field entirely—oncology. He reasoned that since epithelial neoplasms comprise over 90 percent of all human cancers, it would be possible to use some of the keratins as markers for a particular type of neoplasm. In other words, the type of keratin could be used to determine the epithelial tissue in which the tumor originated.

In 1978, Dr. Sun went to Johns Hopkins, where he worked out a system for distinguishing epithelial neoplasms by developing monoclonal antibodies based on keratins. These monoclonal antibodies are widely used in cancer diagnostics today. He stayed at Hopkins only four years, but during this period he also systematically classified the keratins according to their location in an organ’s lining and degree of cellular differentiation, and identified two subfamilies for all human epithelial keratins.

Dr. Sun had moved south to Baltimore at the invitation of Irwin M. Freedberg, M.D., who had just left Harvard to become the first Chairman of the newly inaugurated Department of Dermatology at Hopkins. But their relationship extended beyond Baltimore. In 1981, Dr. Freedberg relocated to New York City to become Chairman of the Ronald O. Perelman Department of Dermatology at NYU School of Medicine and he once again asked Dr. Sun to join him. Dr. Sun accepted.

Finding Stem Cells in the Cornea

At NYU, Dr. Sun’s career took a different course when he expanded his research to yet another discipline—ophthalmology. His interest in the field stemmed from the fact that the cornea, like other structures, is covered by epithelium. While at Hopkins, he had already established a technique using 3T3 feeder cells to grow corneal and conjunctival epithelial cells, two cell types that are closely related embryologically but that diverge from one another later in development.

Now Dr. Sun wanted to characterize the keratins produced by these types of cells. He and his associates soon made a surprising discovery: a keratin called K3—a more specialized, differentiated type of keratin—was uniformly distributed throughout all the cell layers of the central corneal epithelium, an area that supposedly housed stem cells. Stem cells, present in all self-renewing tissues of the body, including the hair and the stomach lining, are primitive progenitor cells that supply the epithelial cells needed for replenishment. These cells, which are generally considered undifferentiated or “unspecialized,” produce an unspecialized type of keratin.

The fact that the central cornea and its lowest layer, the basal layer, which is supposed to be “undifferentiated,” did produce specialized K3 keratin prompted Dr. Sun to speculate that the stem cells might actually lie in a different area called the limbus on the periphery of the cornea. Subsequent research by Dr. Sun and Dr. Lavker of the University of Pennsylvania demonstrated that the limbus was indeed where the cornea’s stem cells resided, a finding that has had enormous reverberations in the field of ophthalmology.

Each year in the United States, thousands of people suffer from chemical burns, trauma or other mishaps that severely damage corneal epithelium, leading to blindness. If the corneal epithelium is scraped, it will regenerate.
as long as there is a bank of stem cells in the cornea. But the cornea will not regenerate and neither will a corneal transplant survive if the entire limbus is damaged. By transplanting pieces of the limbus from the patient’s good eye or from related donors—a procedure called “limbal stem cell transplantation”—ophthalmologists have been able to successfully restore sight in patients suffering severe corneal damage.

The Hair’s Stem Cells

The search for corneal epithelial stem cells whetted Dr. Sun’s and Dr. Lavker’s appetites for another prize—the stem cells of hair follicles. Each follicle gives rise to a new strand of hair, another of the body’s renewable, keratin-rich tissues. Once again, the researchers would make a surprising observation that would lead to new insights about the biology of epithelial cells. Most researchers had assumed that hair follicle stem cells lurked in the bottom of the follicle in a structure called the bulb, but Dr. Sun and his associates couldn’t find the elusive stem cells there. This unexpected finding led them to explore an area called the bulge; in this area just underneath the scalp they hit pay dirt. In an important paper published in the journal Cell in 1990, the researchers reported that they had found hair follicle stem cells in the bulge, a well-protected harbor that remains a permanent fixture of the follicle.

In fact, the biology of the hair follicle is much more complex than investigators believed. To form a new hair, stem cells must migrate from the bulge down toward the dermal papilla, a region in the bulb. When the stem cells and dermal papilla interact, substances are released from the papilla that promote hair growth. Hence, “hair grows down and then grows up,” says Dr. Sun.

This new model of hair growth has provided a fresh approach to developing novel therapeutic agents that could promote hair growth and to a better understanding of the regeneration of the skin. For example, after a severe burn the epidermis is regenerated from cells crawling out of the hair follicle, says Dr. Sun. Moreover, some researchers believe these cells may be the progenitors of the epithelial cells of the entire epidermis. If hair stem cells actually prove to be the source of all epidermal cells, it could revolutionize the understanding and treatment of many skin diseases, including skin cancer.

A human hair follicle.
The hair-specific keratin is green.

The Bladder

In recent years, Dr. Sun has become interested in the bladder, an organ that has a remarkable ability to stretch enormously and then shrink and stretch again without tearing or losing its function as an extremely impermeable sac. “No other organ in the body can pull this off,” says Dr. Sun.

Not surprisingly, epithelium also plays a rather large role in his current research on this organ. The bladder’s ability to stretch, yet remain leakproof, lies within the urothelium, the epithelial cells that line the bladder. Dr. Sun discovered that the urothelial surface is covered by numerous, rigid plaques, which form a barrier that prevents noxious material in the urine from spilling into the bladder’s interior cells. It is possible that the breaching of this barrier leads to various diseases.

Dr. Sun and Xue-Ru Wu, M.D., Assistant Professor of Urology and Microbiology at NYU School of Medicine, have found that the urothelial plaques are made up of only four proteins, which they have dubbed “uroplakins.” Their research has led to the first transgenic animal model for bladder cancer and the first
blood test for metastatic bladder cancer.

The researchers developed the animal model by constructing a short piece of DNA, called a promoter, that is derived from the gene for one of the uroplakin proteins. The gene is expressed only in the uppermost tissue layers of the mouse bladder. They then attached a known cancer-promoting gene to the promoter and injected the construct into mouse embryos. The embryos were subsequently implanted in donor mice primed for pregnancy to establish lines of transgenic mice. Last year, Dr. Wu and Dr. Sun reported at the annual meeting of the American Urology Association that they had successfully introduced an oncogene into the bladders of mice.

Transgenic mice can be used to assess whether other genes cause bladder cancer. For example, any candidate cancer-promoting gene can be attached to the promoter. The uroplakin genes also provide a means to develop a blood test for metastatic bladder cancer because uroplakin proteins detected in the blood indicate that a bladder cancer has spread beyond the bladder.

Dr. Sun and his collaborators have also found that they can use the uroplakin genes to make bladder epithelial cells of transgenic animals synthesize pharmacologically important molecules, creating a so-called bioreactor. In a paper published last year in the journal Nature Biotechnology, Dr. Sun’s group reported that they coaxed transgenic mice to produce human growth hormone in their urine by attaching the gene for the hormone to the uroplakin-derived promoter. It was the first report of a transgenic animal secreting a foreign protein into its urine, and may open an entirely new avenue of generating rare, medicinal human proteins.

The group also has found that uroplakins may be receptors for some bacteria that cause urinary tract infections, which affect millions of women. The proteins may provide an anchor for disease-causing bacteria, and severing this tie may provide a unique means of treating urinary tract infections.

Once again, Dr. Sun’s interest in a particular field has opened up a vast area of research. In fact, he recently received one of the largest grants ever awarded by the National Institutes of Health to study the bladder, and he has now assembled a multidisciplinary group of researchers to tackle the many facets of bladder biology. At NYU, for example, Gert Kreibich, Ph.D., Professor of Cell Biology, is focusing on the synthesis, processing and targeting of uroplakin membrane proteins, and Angel Pellicer, M.D., Ph.D., Professor of Pathology, is analyzing the roles of oncogenes and tumor suppressor genes in transgenic animal models of bladder tumor formation. And Xiangpeng Kong, Ph.D., Assistant Professor of Biochemistry, is analyzing the molecular structure of urothelial plaques, using electron crystallographic techniques.

A Keen Intellect

Dr. Sun’s keen intellect and curiosity have propelled his research into many fields, but, fundamentally, he is a devoted problem solver. “He is what a biologist should be,” says Dr. Freedberg. “He confronts important problems and seeks ways to understand and correct them. He has done spectacularly well.”

Dr. Sun has received a number of prestigious awards and prizes. Last year, for instance, he presented the kihei Anioku Memorial Lecture at the International Congress of Investigative Dermatology in Cologne, Germany. He won the Alcon Award for outstanding contributions in vision research in 1993, and he was the Susan Swerling Lecturer at the Dana-Farber Cancer Institute in Boston in 1991.

It is a sure bet that Dr. Sun will continue to grapple with fundamental questions of biology. It is an equally sure thing that this renaissance scientist will enrich whatever area he chooses to study.