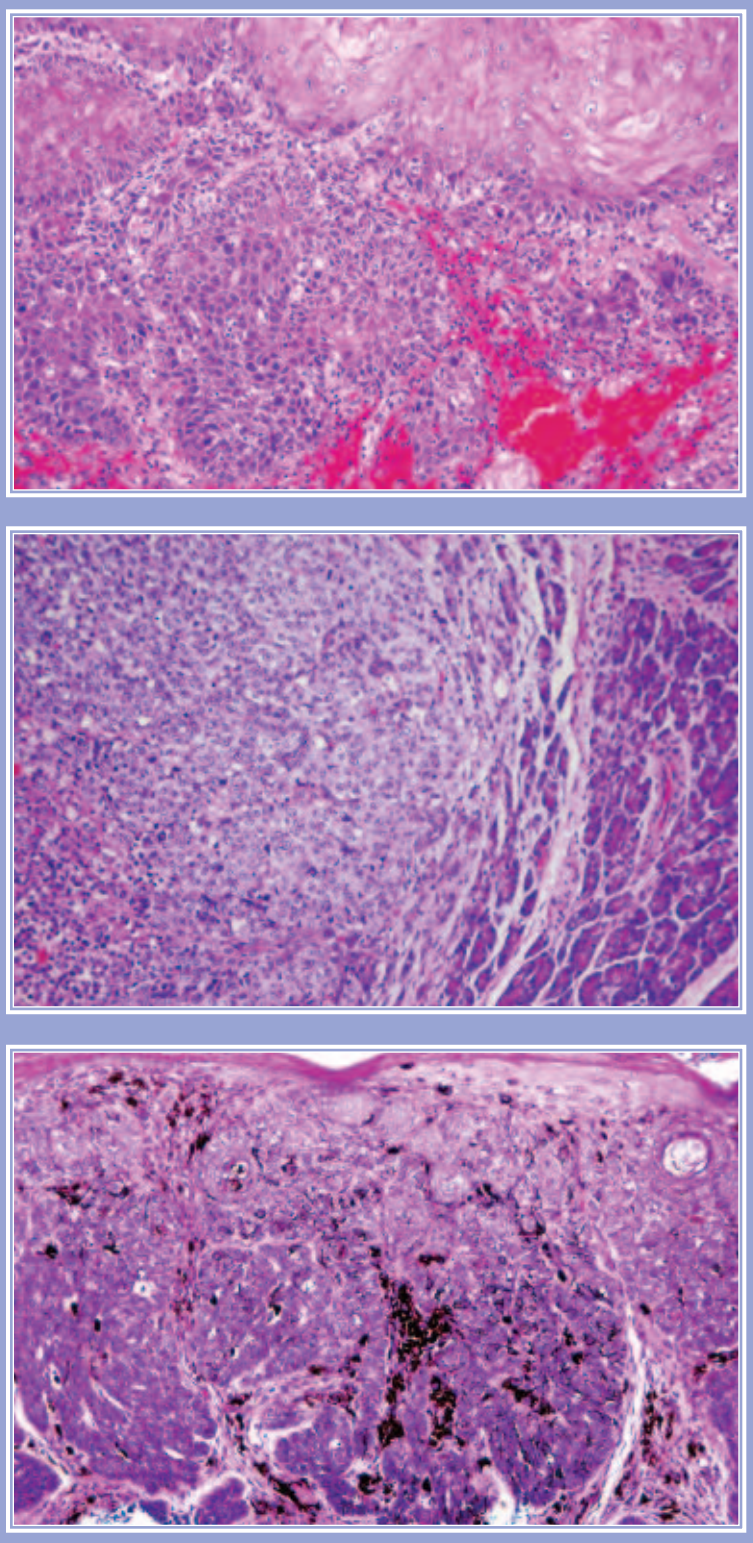


(Photos: C.R. Lee, CCR)

Squamous cell carcinoma (top) is a relatively aggressive skin cancer, and it shares many of the features of lethal solid tumors of the internal organs. The Yuspa laboratory's efforts to model the biology of skin have given great insight into the genesis and treatment of this and other major skin malignancies, such as basal cell carcinoma (middle), and malignant melanoma (bottom).

No Longer Skimming the Surface of Skin Cancer

When **Stuart H. Yuspa, M.D.**, *began studying the skin in the late* 1960s, *scientists understood some of the basic biology of skin cancer. They knew what was required to produce and diagnose benign and malignant tumors—but not much more. "We've made remarkable leaps since then," says the Co-Chief of CCR's* Laboratory of Cancer Biology and Genetics (*LCBG*).

(Photo: E. Branson)

Stuart H. Yuspa, M.D.

"Now we know the genetic changes associated with each stage of cancer development and how those genetic changes translate to biochemistry in each stage," Yuspa, a specialist in squamous cell skin cancers, explained. "And we have markers to recognize where we are in the progression from normal to malignant."

Yuspa added, "We also know a lot more about normal skin. We know how skin homeostasis is controlled and the pathways that regulate it. That's extremely important because you have to understand normal to understand abnormal." To generate this understanding, over the last 36 years the Yuspa laboratory has developed *in vitro* models that recapitulate the normal growth and differentiation of skin epithelial cells called keratinocytes—precursors to the squamous cells that give squamous cell skin cancer its name. The models also reproduce each stage of carcinogenesis as it occurs in mouse skin cells.

These model systems have been "really important for understanding mechanisms of cancer," said Adam Glick, Ph.D., a former Postdoctoral Fellow and Principal Investigator who still collaborates with Yuspa.

And the work is moving from discovery to application. Yuspa, Glick, and their colleagues recently identified genetic markers that distinguish low-risk benign skin tumors from high-risk tumors in mice and opened possibilities for targeted therapies that block early tumors from progressing to invasive lesions.

Yuspa's models are making inroads for other cancers as well, since similar epithelial cells are involved in cancers of many internal organs, such as the lungs, head and neck, esophagus, colon, and stomach.

A Good Decision

Yuspa first came to NCI in 1967 to work in the lab of Richard R. Bates, Ph.D., who was working on skin carcinogenesis. "There were two of us and a few technicians," Yuspa recalled. "We did some very nice work looking at carcinogen binding to DNA and how that caused mutations. I loved it! I stayed for the two years of my United States Public Health Service Commissioned Corps obligation, then asked to stay a third." The young M.D. then left to pursue his clinical training to see what aspect of medicine he liked most—research or clinical work.

"Dick said he'd hold the spot for me, which would be impossible today, but it was the early days of the War on Cancer," Yuspa continued. After finishing his medical residency training—and realizing that the lab was where he wanted to be—Yuspa returned to the Bates lab to work on the skin model. He became a Senior Investigator at NCI in 1972.

The Place to Be

"Stu's lab was a place that people came to from all over the world—and still do—to learn how to culture the skin," according to Molly Kulesz-Martin, Ph.D., who was a Postdoctoral Fellow in Yuspa's lab from 1979 to 1981 and is now Director of Research and Professor in the Department of Dermatology at Oregon Health & Science University (OHSU).

When Kulesz-Martin joined the Yuspa lab, very few people were working on epithelial cells, even though most human cancers arise from them. "Everybody was working on cells that were easy to grow, but there was no way to assure they were like some particular organ in the body. And they weren't," she recalled.

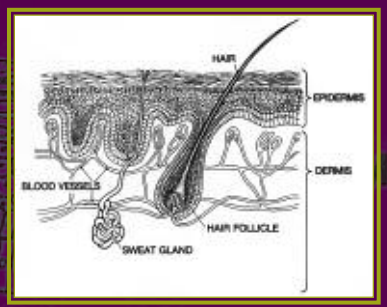
"Stu was working out ways to grow epithelial cells so they behaved as they do in the body. We had to find a way to make culture conditions good for growing epithelial cells," said Kulesz-Martin. Her work built on earlier laboratory observations that reducing calcium levels enabled cells to grow for much longer than two weeks. From that, she created a transformation assay to quantify the strength of various carcinogens.

Kulesz-Martin's experiences in Yuspa's lab set the path for her future as a researcher. At OHSU, she continues to work with mouse and human cells to determine the initial changes and later insults that push a cell from normal to malignant to metastatic. "I didn't fall far from the tree," she admitted. "I still want to study cancer, still study skin as a model, and I'm still

What Kind of Skin Cancer?

Each type of skin cancer—melanoma, basal cell carcinoma, and squamous cell carcinoma—arises in different cells within the skin. Melanoma forms in melanocytes (skin cells that make pigment) and is the most dangerous form of skin cancer. Basal cell carcinoma forms in cells found in the hair follicles. Basal cell cancers are the most frequent human tumor in Caucasians, but they grow slowly and rarely spread to other parts of the body.

Squamous cell carcinoma, the focus of the work of Stuart Yuspa, M.D., begins in squamous cells (flat cells that arise from keratinocytes and form the surface epidermis of the skin). They are not the most frequent skin cancers, but they share many characteristics—genetically and biochemically—with highly lethal cancers that arise in internal organs like the lung, head and neck, esophagus, and stomach.



(Graphic: NCI)

The skin's epidermis harbors the keratinocytes and melanocytes that give rise to skin cancer. The Yuspa laboratory's efforts to model the biology of skin have given great insight into the genesis and treatment of skin cancers.

learning new things about cancer genes that tell us about skin cancer and other kinds of epithelial cancers, such as prostate, liver, and kidney cancers."

"Many of the events that Stu has identified in the mouse have been confirmed as also being causal genetic alterations that result in human tumors," said Dennis R. Roop, Ph.D., who worked with Yuspa at NCI in the 1980s and is now Chair of Regenerative Medicine and Stem Cell Biology at the University of Colorado Health Sciences Center. "His early studies in the mouse provided the groundwork for understanding that after a stem cell is initiated, it can sit for a long time, then get an accumulation of other genetic events to lead that initial epidermal stem cell to result in a tumor."

The epidermis, where the keratinocytes live, is a hotbed of cell turnover, Roop explained. This outer layer of our skin replaces itself every three to four weeks in humans. "Yet many of us develop skin cancer late in life. Only the stem cells that reside for a lifetime are around to accumulate enough genetic damage to lead to cancer."

Roop added, "Stu was also really the first to identify mutations in the Harvey *ras* gene [a proto-oncogene known for its connections to skin and bladder cancers] as a complete initiating event by showing that just a single Harvey *ras* mutant gene in a keratinocyte [that is grafted onto an animal model] would result in formation of a tumor."

"All of us have learned so much from the work Stu started and others who've further developed the model," Kulesz-Martin added.

A Physician's Dream

"It's everyone's dream," said Yuspa, "particularly if you're a physician who's done basic science for the last 30 years, to translate your discoveries into treatments for patients." To do that, Yuspa's team is focusing on two relatively new discoveries. Using a mouse model of induced skin inflammation, they are studying how inflammation influences the frequency with which skin cancers develop. In this model, skin cancer is much more frequent. The group identified a receptor that seems to be essential for tumors to develop. He hopes that it could be a novel target for preventing or treating skin cancer.

The second discovery builds on an insight made nearly 10 years ago. They have shown that inactivation of the tumor suppressor gene *p53* is important for malignant

A Generous Spirit

(Photo: Courtesy of M. Kulesz-Martin)

Molly Kulesz-Martin, Ph.D.

(Photo: Courtesy of A. Glick)

Adam Glick, Ph.D.

Colleagues and former students of Stuart Yuspa, M.D., unanimously point out his generosity with his ideas and time—and his love of science.

He has built a dynamic community of researchers exploring skin cancer. He sees mentoring as a happy obligation. "The only legacy that will be remembered is the people you've trained," Yuspa said. "Part of my job as a scientist, mentor, and member of the NIH is to help anybody I can to succeed."

About 36 postdocs have moved through his lab. "I couldn't be more proud of the people who've trained here. They've been uniformly successful and most [30] have stayed in the same field," Yuspa beamed.

"The reason that many of us have continued to work in science is that his enthusiasm for science is infectious," said Dennis Roop, Ph.D. "When we trained with him, we could see how much he enjoyed science. He hasn't lost that. He's still like a little kid when he starts talking about science."

Adam Glick, Ph.D., called Yuspa the "grandfather of the field." Go to any of the big meetings, he noted, and everyone is somehow connected to Yuspa, either as collaborators, postdocs, or postdocs of his postdocs.

Replying wryly that he'd rather be called the "*father* of skin carcinogenesis," Yuspa agreed that his proudest moments in science occur at meetings when he sees his protégés and theirs presenting important and interesting information. "It feels very good."

"Once you go to Stu's lab, you become part of a huge network of people from around the world who are doing top-rate science," said Molly Kulesz-Martin, Ph.D. "We're all excited about the skin and the science of the skin."

And these "Yuspa graduates" open their arms to the new people who continue to come through the lab. "We share with anybody who asks. It's a trait of Stu's that we learned at his knee," Kulesz-Martin continued. "I remember talking to Stu about how competitive science is and how people don't want to share. He said, 'I've always shared data. Maybe sometimes you get burned, but I always learn something new when I share.'"

Dennis Roop, Ph.D. (left), presents Stuart Yuspa, M.D. (right), with the Stephen Rothman Memorial Award for Investigative Dermatology in 2004. The Rothman Award is the highest award given by the Society to members who have made outstanding contributions to investigative cutaneous biology.

conversion, a late event in carcinogenesis in the skin. Using keratinocytes from *p53*-null mice, they discovered one of *p53*'s transcriptional targets, a gene called chloride intracellular channel 4 (*CLIC4*). *CLIC4*'s gene product, CLIC4, was originally described as a chloride channel protein but, as it turns out, is multifunctional, being involved in cellular responses to stressors such as DNA damage, metabolic inhibition, and senescence. Yuspa's team found that, in mouse skin cancer and most major human cancers, CLIC4 is lost from tumor cells. The protein is essential for cell death induced by *p53*. They are trying to reactivate the expression of *CLIC4* in tumor cells—not an easy feat. "We know the gene is there; it's not lost, just silenced in some way," said Yuspa. They are hopeful they can unsilence the gene, reactivate *CLIC4* expression, and have some influence on tumor cell growth.

Other pathways are standing out as significant players as well. Yuspa's group has identified a pathway through protein kinase C delta (PKCdelta)—a regulator of many different signaling pathways studied in numerous solid and hematologic cancers—that is important to the development of skin cancer in mice. In an animal model, Yuspa has been able to stimulate PKCdelta in tumor cells and cause the tumor cells to regress, activating a cell death (or apoptosis) pathway.

An Australian company named Peplin is developing a natural product drug that works through a similar mechanism. In clinical trials, this drug appears to do the same thing to PKCdelta in humans that the Yuspa lab has done in animals. NCI has a cooperative research agreement with the company to sort out the pathways involved in the death of the tumor cells.

Looking More than Skin Deep

Yuspa's group continues to make exciting discoveries today. A recent study with Adam Glick, now an Associate Professor in the Center for Molecular Toxicology and Carcinogenesis at Pennsylvania State University, has ramifications for early detection and prevention of skin and other cancers.

In May 2007, Yuspa, Glick, and colleagues from the American University of Beirut reported that they had found an 87-gene expression pattern, or genetic signature, in mice that distinguishes benign tumors with a high or low risk of becoming malignant. In particular, the high-risk lesions had reduced expression of immune function genes compared with low-risk lesions, a finding Yuspa calls a "surprising, important component of the study. High-risk tumors may in some way be protected from surveillance by the immune system."

Now they will have to determine how those findings can apply to human cancer—and moving from mouse to human is neither easy nor automatic. Human skin cancers are induced by ultraviolet light, while the tumors in Yuspa's animal models are induced by chemical carcinogens or oncogenes. As a result, the genetics may differ. "But," Yuspa added, "we feel that the pathways that are downstream from the inducing agents are very similar."

Knowing that skin cancer takes different forms, Yuspa has also started using his models to investigate different cells. He has initiated a collaboration with Glenn Merlino, Ph.D., his Co-Chief at the LCBG, who studies melanoma. Since both melanomas and squamous cell cancers occur in the skin, and there is an intimate relationship between keratinocytes and melanocytes in the progression of melanoma, "bringing the two models together will give us great insight into melanoma's induction," said Yuspa.

He has also pulled in partners to make a difference in internal organ cancers as well. "I've convinced [LCBG Adjunct Investigator] Jonathan Wiest, Ph.D., to look at skin as a model for lung cancer," Yuspa said. With Adam Glick, he's hoping to look at head and neck cancers.

"These are all areas where we think we can go beyond boundaries," he said.

> "It's everyone's dream," said Yuspa, "particularly if you're a physician who's done basic science for the last 30 years, to translate your discoveries into treatments for patients."