Fluorescent Sensors for Systems Biology

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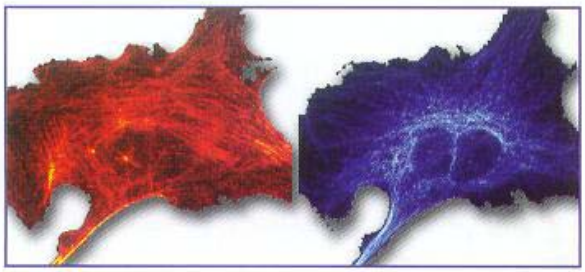
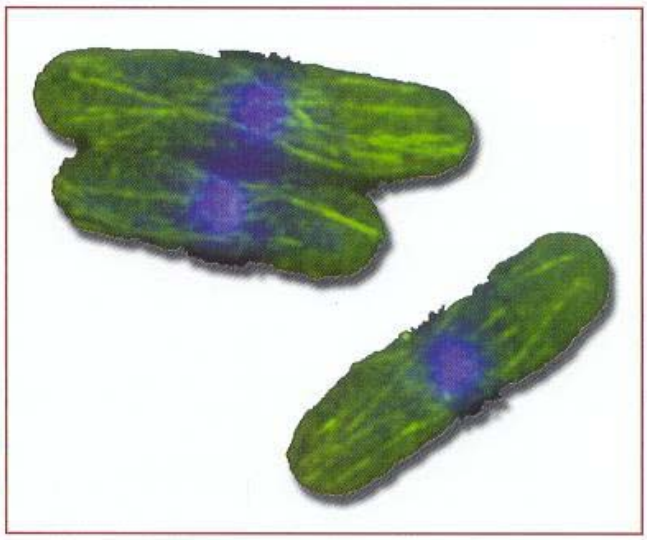
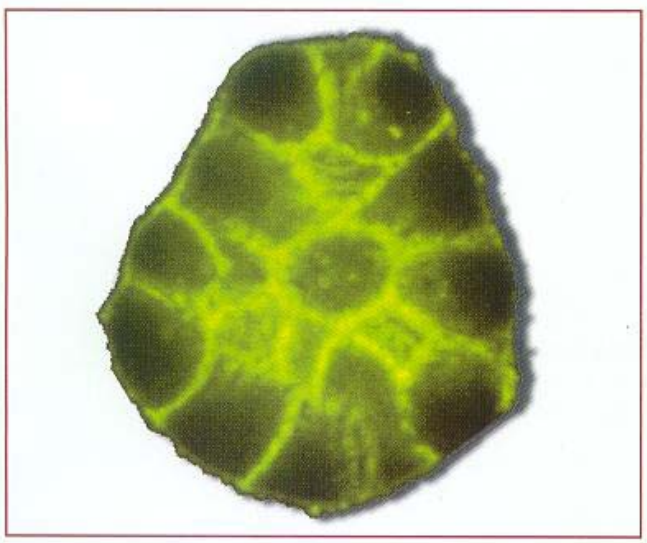
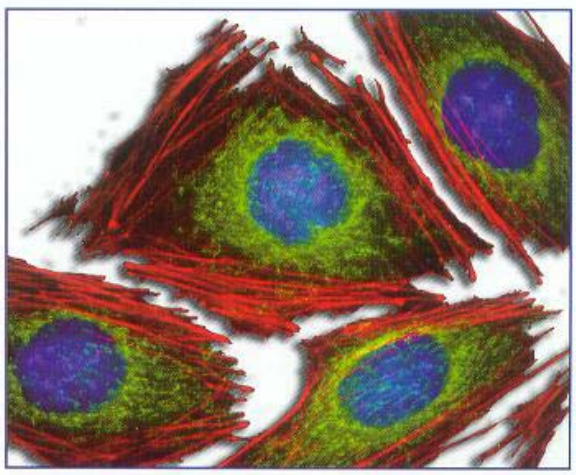




Which Camera Is Right for You?





LEDs Improve Analytical Instruments

Dual-color fluorescence imaging distinguishes tumor from host

Study reveals that host cells cooperate with tumor cells

Researchers from AntiCancer Inc., a San Diego-based cancer research company, and from the University of California, San Diego, and Massachusetts Institute of Technology (MIT) in Cambridge have developed a method for probing the interaction between host tissue and metastatic cancer. The group has drawn on multiple colors of fluorescent protein to label both the host tissue and the cancer. Their work with numerous types of the disease clearly demonstrates the potential of the new technique for further probing the points at which cancer and host tissues cooperate.

***To study how host tissue interacts with growing cancer cells, researchers at AntiCancer Inc. labeled human colon cancer cells with red fluorescent protein and inserted them into transgenic mice labeled with GFP. Images reprinted with permission of* PNAS.**

In 1889, *The Lancet* published an article that used agricultural terms to describe metastasis. "The seed and soil hypothesis" states that a tumor distributes "seeds" throughout the body via the bloodstream, but that the cancer only grows in tissues that support it. Since that time, research has continued to uncover the crucial role that a receptive host tissue plays in metastasis.

Typically, a tumor somehow coaxes the host tissue to grow blood vessels to nourish it. As the tumor grows, so does its blood supply.

"The earliest paper by Judah Folkman [on tumor angiogenesis] was published in 1971," explained Sheldon Penman of the department of biology at MIT. "However, it took many years for the significance of this work to be widely appreciated."

In the past several years, oncology researchers worldwide have turned new attention to these blood vessels as therapeutic targets. The rationale is simple: Kill the tumor by choking off its blood supply or by preventing the blood supply from growing in the first place. The result has been a host of drugs specifically designed to kill rapidly growing cells, such as these new blood vessels. What hasn't been studied fully is the interaction between the tumor seeds and the host cells that accept and eventually nourish them.

The principal investigator, Robert M. Hoffman from the University of California and founder of AntiCancer, along with Penman and a team of other AntiCancer researchers set out to explore this area further. To tackle the problem of how to observe the different tissues as they grow and change, they turned to GFP and to DsRed2, a red fluorescent protein (RFP). Using genetic engineering, researchers can make virtually any living cell express either of the two proteins, which then fluoresces red or green when exposed to ultraviolet light. Because the cell's DNA makes the protein, any cell division that occurs carries the fluorescent protein's code so that its cellular offspring also fluoresce.

They obtained mice that had been genetically engineered to contain GFP and crossed them with a nude variety to produce nude transgenic mice for the study. The GFP mice expressed the protein in almost every tissue in their bodies. Then the scientists genetically engineered four types of tumor, representing the major types of human cancer — prostate, breast, cutaneous melanoma and orthoptic colon cancers — to contain red fluorescent protein.

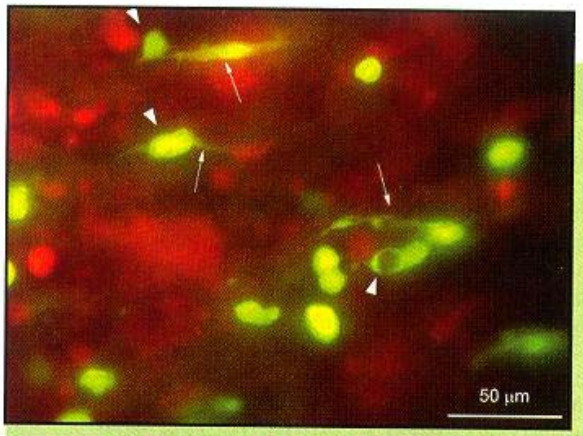
The scientists introduced the RFP tumors into the GFP mice and, over time, the two colors allowed them to see the interaction between the tumors and mouse tissue. At intervals from three days to four weeks after the cancer cells were introduced, the team obtained biopsies from the tumors and conducted whole-body imaging studies.

Hoffman said that the main challenges to this work were the sensitivity, resolution and depth required for whole-body imaging. The group needed a camera sensitive enough to detect low levels of fluorescence and to distinguish the desired fluorescence from tissue autofluorescence. It also needed to image deep into the mouse with a resolution high enough to resolve single cells. To ensure that the cancer cells placed in the mice would fluoresce brightly, the team cultured them in

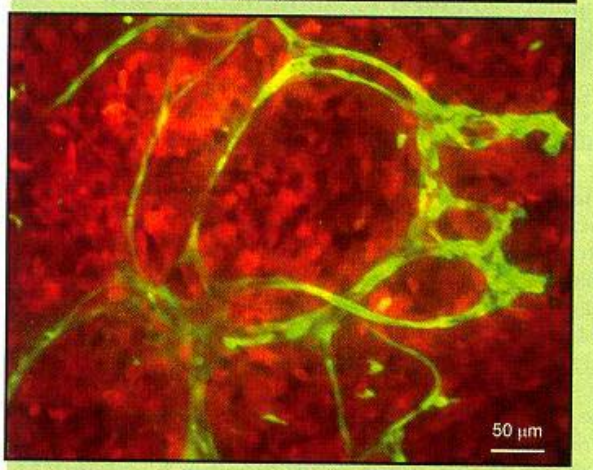
vitro and isolated the brightly fluorescent cells, which were introduced into the mice.

To conduct the whole-body imaging, the scientists used a fluorescent light box illuminated with 470-nm light from a mercury lamp coupled to a fiber optic lighting system from Lightools Research of Encinitas, Calif. To capture the whole-body fluorescence, they used a long-pass filter from Chroma Technology of Brattleboro, Vt., and a Hamamatsu C5810 three-chip, cooled, color CCD camera from Hamamatsu Photonics of Bridgewater, N.J. Hoffman said that many other types of CCD cameras could be used for the imaging.

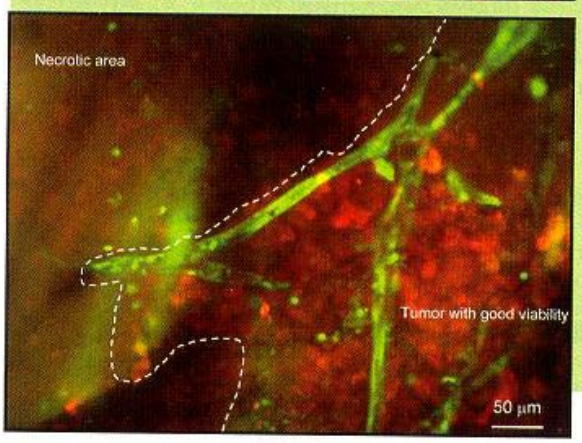
To study the biopsies, the researchers used an Olympus BH2-RFCA fluorescence microscope with a 100-W mercury lamp for sample excitation and illumination. To capture both red and green fluorescence, they passed the excitation light through a D425/60 bandpass filter and a 470 DCXR dichroic mirror. Then they captured the image using the same camera and a long-pass filter.



Labeling cancer cells with red fluorescent protein and host cells with GFP allowed researchers to see the onset of angiogenesis in host epithelial cells (arrowheads) and fibroblasts (arrows).



The GFP clearly labels host-derived blood vessels feeding mouse melanoma cells labeled with red fluorescent protein.



In the part of a tumor containing necrotic tissue, the tumor blood vessels — developed from GFP-expressing host tissue — can be easily identified, unlike the necrotic area where only fragments of the vessels can be seen.

Cooperating cells

In the November issue of *PNAS*, they report that they saw, in short, the cooperation of host cells with tumor cells to start angiogenesis. Images from their work clearly show green fibroblasts and endothelial cells from the host tissue starting to form tiny blood vessels inside the tumor mass. They captured images of green host dendritic cells contacting tumor cells with their dendrites. They also used the model to see immune system responses, such as green lymphocytes attacking a red breast tumor mass and green macrophages engulfing red prostate cancer cells.

Although there are no direct clinical applications of this method, Hoffman said that the research has valuable clinical implications, such as the ability to develop both antitumor therapy and therapy that targets cells that support the tumor, such as the endothelial cells forming the tumor blood vessels. This latter approach promises to be a major innovation in cancer chemotherapy because it offers an entirely new target that is distinct from normal host tissue, he explained.

The group plans to continue applying the method to better understand the interactions between host and tumor cells. It has developed more than 100 distinct lines of fluorescent tumors, of which only about 15 have been studied. □

Kevin Robinson