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Stanford researchers first to turn normal cells into 3-D cancers in tissue culture dishes

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Researchers at the Stanford University School of Medicine have successfully transformed normal human tissue into three-dimensional cancers in a tissue culture dish for the first time. Watching how the cells behave as they divide and invade surrounding tissue will help physicians better understand how human cancers act in the body. The new technique also provides a way to quickly and cheaply test anti-cancer drugs without requiring laboratory animals.

"Studies of this type, which used to take months in animal models, can now occur on a time scale of days," said Paul Khavari, MD, PhD, the Carl J. Herzog Professor and chair of dermatology at Stanford. The researchers focused on epithelial cells, which line the surfaces and cavities of the body. Cancers of epithelial cells make up approximately 90 percent of all human cancers.

The study of three-dimensional tumors also avoids the use of cancer cell lines, which are typically grown in single layers and may have accumulated genetic changes that don't accurately reflect what happens in humans.

Khavari, who is also a member of the Stanford Cancer Center and serves as dermatology service chief at the Veterans Affairs Palo Alto Health Care System, is the senior author of the research, which will be published online Nov. 21 in *Nature Medicine*. Todd Ridky, MD, PhD, a former postdoctoral scholar in Khavari's laboratory, is the first author. Ridky is now an assistant professor at the University of Pennsylvania.

The researchers worked with normal human epithelial cells gathered from surgical samples from skin, cervix, esophagus and throat. Unlike cancer cell lines, some of which have been grown in laboratories around the world for years, these primary cells were minimally cultured.

To make these normal cells cancerous, the researchers used viruses to tweak just two genetic pathways known to be involved in uncontrolled growth. One drives cells forward in the cell cycle while the other disables an internal checkpoint that normally blocks abnormal proliferation. Many naturally occurring human cancers display identical genetic changes, and the researchers found that simultaneously altering the two pathways is highly effective at transforming normal cells.

Khavari and Ridky then added the altered, pre-cancerous epithelial cells to a tissue culture dish containing other components of human skin. Epithelial cells normally sit on a thin partition called the basement membrane that separates them from a lower layer of skin called the stroma. They found that at first the cells nestled down on the basement membrane and formed what looked like a normal, three-dimensional cross-section of skin. But within about six days, the cells started to behave more ominously — punching through the membrane and invading the stromal tissue below.

"This reflects what we see happening in spontaneous human tumors," said Khavari. "Cells go from a pre-malignant state to invasive cancers, often over the course of years. Only in this intact, human-tissue model it occurs much more quickly." In contrast, unaltered cells remained obediently on their side of the basement membrane.

When the researchers examined the patterns of gene expression in the newly cancerous cells, they found that the patterns closely matched the genetic profiles of spontaneously occurring human cancers. But when the cells were grown in a single layer, without the basement membrane,

stroma and normal three-dimensional tissue structure, their gene expression profiles were markedly different.

"This tells us that conclusions drawn from studying cells grown in two-dimensional culture need to be correlated with other findings to help ensure clinical relevance," said Khavari.

The researchers took advantage of their new "tumor-in-a-dish" model to test 20 new experimental anti-cancer drugs. Many of these drugs cannot be easily tested in animals because they are difficult to administer and may be toxic in their current form. But Khavari and Ridky were able to quickly home in on three promising candidates that stopped the altered epithelial cells from invading through the membrane. While the drugs will still have to be optimized for testing in animals, this type of pre-screening allows researchers to narrow down the possibilities.

The three-dimensional culture system also indicated that the stromal cells themselves somehow encourage the invasion of the altered epithelial cells, and that the cells don't need to be dividing wildly in order to be able to invade.

"These things had never been directly tested before in human tissue," said Khavari, who pointed out that the new model still doesn't incorporate many other biological players, such as the immune system and an active metabolism. And yet "now that we can create human tumors from multiple different human tissues, we have a new way to assess what might be going on in spontaneous human tumors."

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