# Master Molecular Switch May Prevent the Spread of Cancer Cells to Distant Sites in the Body

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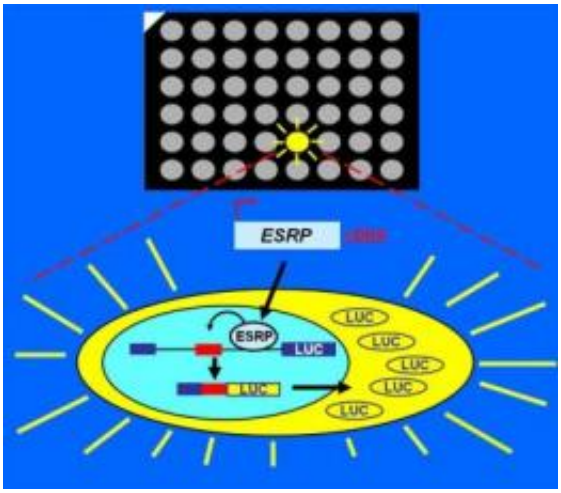


Identification of the Epithelial Splicing Regulatory Proteins (ESRPs) in a firefly luciferase-based high throughput cDNA expression screen. Tissue culture plates containing cDNAs placed separately in individual wells were used to screen cells containing the splicing reporter (top). cDNAs that entered the cells and caused the reporter to switch splicing produced firefly luciferase (Luc). One of the wells that "glowed" contained a cDNA for one of the ESRPs, leading to its identification. A blow up of this well is shown at the bottom with a schematic presentation of a cell containing the reporter. A cDNA for ESRP (boxed) entered the cell nucleus (light blue) and was transcribed into ESRP protein (oval). When ESRP enhanced the splicing of an exon (red), the resulting mRNA produced luciferase protein in the cytoplasm of the cell. Credit: Russ P. Carstens, M.D., University of Pennsylvania

Researchers at the University of Pennsylvania School of Medicine have identified a master switch that might prevent cancer cells from metastasizing from a primary tumor to other organs. The switch is a protein that, when in the "on" position, maintains the normal character of cells that line the surface of organs and body cavities. These epithelial cells are the type of cell from which most solid tumors arise. However, when the switch is turned "off" or absent, epithelial cells acquire characteristics of another cell type, called mesenchymal cells, and gain the ability to migrate and move away from the primary tumor. The researchers report their findings in this month's issue of *Molecular Cell*.

Understanding how this switch works may one day lead to a drug that controls cancer cell metastasis and tissue fibrosis.

This change in cell motility is called the epithelial to mesenchymal transition, or EMT, and is an important process during the development of embryos. But when the transition is aberrantly reactivated in adults it can have dire physiological consequences, leading to cancer metastasis as well as other disease processes such as tissue fibrosis. Fibrotic tissue is a hallmark of organ failure, as in liver cirrhosis or kidney failure.

The master-switch is called the Epithelial Splicing Regulatory Protein, and comes in two closely related versions, ESRP1 and ESRP2. These proteins are able to change how RNAs that are produced from genes are spliced together. This is achieved by splicing different exons -- the sequence of DNA that codes information for protein synthesis -- together in different ways so that there can be more than one messenger RNA (mRNA) produced from the same gene. These mRNAs then go on to make different proteins.

The mRNA for Fibroblast Growth Factor Receptor 2 (FGFR2) is the focus of the Molecular Cell study. FGFR2 mRNA has two forms, one called IIIb, which is expressed in epithelial cells and IIIc, which is

expressed in mesenchymal cells. The protein that is made from the IIIb form interacts with factors outside the cell that promote the epithelial cell behavior, that is to remain stationary. When the IIIc form is aberrantly produced in cancer cells derived from epithelial cells, the resulting FGFR2 protein type no longer promotes the epithelial cell identity, and switches to the mesenchymal cell type, which has the ability to detach from its primary site, invade local tissue and migrate, or metastasize to distant sites of the body.

"If we can find a way to maintain expression of ESRP1 and 2 in epithelial cells, then it might be possible to prevent metastasis or control fibrosis," notes corresponding author Russ P. Carstens, MD, Assistant Professor of Medicine. "ESRP1 and ESRP2 are necessary for splicing FGFR2 mRNA in the epithelial cell manner. This is one of few known splicing factors that operate in a clear cut cell-type-specific manner. Epithelial cells, which make up the lining of organs, are the only cells that produce ESRP1 and ESRP2."

To discover ESRP1 and ESRP2, the team used a high-throughput genetic screen for rare proteins developed by collaborator and co-author John B. Hogenesch, PhD, Associate Professor of Pharmacology, an innovator in the use of these types of screens. In addition, Claude Warzecha, a graduate student in the Carstens lab, played a key role in the completion of the screen.

The screen consists of about 15,000 different cDNAs (DNA that has been synthesized from messenger RNAs) that each express a different gene and are arrayed on plates so that each well of the plate expresses only one individual gene product. The Carstens lab developed a splicing "reporter" that makes cells express a firefly luciferase gene and "glow" when it is spliced in the epithelial cell pattern. Cells with this reporter were individually placed over wells containing each cDNA and cells that "glowed, indicated those cDNAs that produced proteins that promoted

the epithelial splicing program. It was from this screen that ESRP1 and ESRP2 emerged.

In ongoing work, the team found that ESRP1 and ESRP2 are critical for epithelial-specific splicing of many other genes in addition to FGFR2. Several of the proteins made from these RNAs also have different functions that either help cells to stay attached in place or to promote local invasion of cancer cells that are capable of traveling to distant sites. The team is also engineering mice in which the genes for ESRP1 and 2 can be selectively "knocked-out" so that they can further study the importance of these two proteins during development as well as in disease. In addition, studies are planned to use the same splicing reporter system to screen for drugs that might restore the epithelial pathway and interfere with metastasis and fibrosis.

Source: University of Pennsylvania School of Medicine (news : web)