Estrogen in a New Kind of Bind

Newly discovered estrogen receptor sheds light on cancer and its treatment.

BY MARGIE PATLAK

ESTROGEN IS A paradoxical hormone—essential to women's health but also implicated in several forms of cancer. Now the discovery of a new type of estrogen receptor provides further clues to the complex functioning of estrogen and may even lead to improved therapies for breast cancer and other estrogen-related disorders. Studies of the new receptor also may help to explain why patients do not always respond as expected to "anti-estrogen" drugs like tamoxifen.

"Our findings open up a whole new area in estrogen biology that requires a re-evaluation of some of the assumptions made in the past," says Eric Prossnitz, a professor of cell biology and physiology at the University of New Mexico Health Sciences Center in Albuquerque. Using state-of-the-art instruments and technologies funded by NCRR, Prossnitz and his colleagues showed that the protein known as GPR30 functions as a novel estrogen receptor that triggers distinctive signaling pathways.

Estrogen receptors are of particular interest because the hormone is thought to fuel a number of cancers, including breast, ovarian, uterine, and possibly prostate cancer. When selecting appropriate therapies for breast cancer patients, physicians first determine whether the tumor cells are positive or negative for estrogen receptors. Receptor-positive tumors often are treated with compounds—like tamoxifen—that block estrogen from binding to its receptors, thereby hindering tumor growth. Tamoxifen has been shown to slash the risk of breast cancer recurrence by 30 percent to 50 percent in some—but not all—women whose tumor cells are positive for estrogen receptors. The newly discovered estrogen receptor may help to explain why some receptor-responsive tumors appear unaffected by the drug.

Prossnitz and his colleagues found that GPR30 responds to tamoxifen differently than the other two estrogen receptors known in humans. Specifically, tamoxifen essentially pulls the trigger of the GPR30 receptor rather than blocking its actions. Therefore, the growth of a breast tumor that has both standard estrogen receptors and GPR30 might not be stopped as effectively by tamoxifen as tumor cells that have only the standard receptors.

The discovery of the new estrogen receptor builds on the hunt for new receptors that began in earnest in the 1990s. Much of that search focused on finding G protein-coupled receptors (GPCRs), a large family of receptors that help to regulate most physiological processes in the body. "Regardless of the disease you are looking at, there's probably a GPCR that plays an important role," says Prossnitz.

In the late 1990s, four independent research groups reported that they had discovered a new GPCR—GPR30—the function of which remained a mystery. Other scientists later found that cultured breast cancer cells that had GPR30 but lacked standard estrogen receptors tended to react biochemically to estrogen. This suggested that the mysterious GPR30 might bind to estrogen.

To explore this possibility, Prossnitz and his colleagues created a fluorescent version of GPR30 and then used a confocal fluorescence microscope to see where the receptor

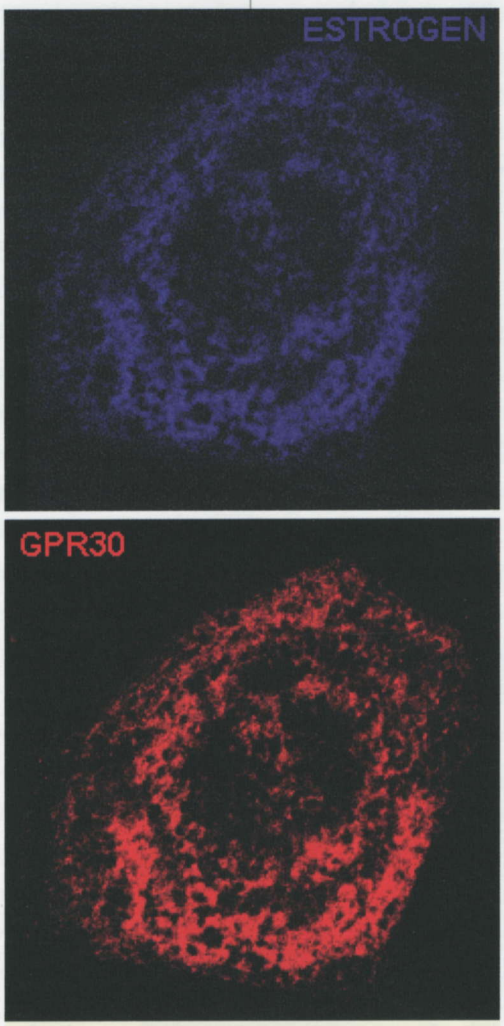
appeared as a brilliant color in cultured cells. The scientists then added, to the same cells, stains for various cellular components, such as the mitochondria, the outer cell membrane, the nuclear membrane, and a tubular network known as the endoplasmic reticulum.

When the researchers compared the location of fluorescent GPR30 with that of the specific stains, they were surprised to discover that GPR30 congregates in the membrane of the endoplasmic reticulum, inside the cell. In contrast, other known GPCRs inhabit the outer cell membrane, where they can bind to compounds incapable of traversing the membrane. Because GPR30 lodges within the cell, the receptor must bind to a compound—like estrogen—that can pass through the cell's outer membrane.

To confirm that GPR30 binds to estrogen, the researchers created a fluorescent version of the hormone and added it to the cultured cells. The resulting fluorescent pattern was identical to that seen when fluorescent GPR30 was added alone to the cells, suggesting that estrogen must bind to GPR30 (see images at right).

Key to this research was the laser-scanning confocal fluorescence microscope purchased with an NCRR Shared Instrumentation Grant (SIG). (For more information about the SIG Program, see the *NCRR Reporter,* Fall 2004, pages 8-9.) "A standard fluorescence microscope, which visualizes the entire thickness of the cell, has trouble distinguishing two structures in the cell that are piled atop one another," Prossnitz says. "A mitochondrion might be above the endoplasmic reticulum, but you can't distinguish the two."

It is impossible to cut thin slices of living cells to examine under the microscope, but a laser-scanning confocal fluorescence microscope uses optical techniques to create visual "slices" through a cell. Because the microscope collects light only from a very thin layer of the cell, everything above and below that layer is ignored. "It allowed us to get very high spatial resolution images, especially when using multiple colors as we did. We could look at the very precise location of these colors in the cell," says Prossnitz.



New Mexico researchers used fluorescent stains in a breast cancer cell to show that estrogen (blue) appears in the same location as GPR30 (red). This strongly suggests that GPR30 binds to estrogen.

Additional NCRR funding, to create a Center of Biomedical Research Excellence (COBRE) at the University of New Mexico Health Sciences Center, enabled the purchase of more cutting-edge instruments for flow cytometry and microscopy, which Prossnitz and his colleagues used daily for their investigations. (For more information about COBRE, see the *NCRR Reporter,* Winter 2004, pages 4-7). "Without the NCRR funding, it would have been very difficult to do our research," Prossnitz says.

Once they established that GPR30 binds to estrogen, Prossnitz and his colleagues showed that the binding triggered a unique series of biochemical pathways that differed from the effects seen when estrogen binds to traditional estrogen receptors. These novel pathways also are triggered when tamoxifen binds to GPR30. The researchers then looked at a few cultured breast cancer cell lines and found that the more aggressive cancers tended to have more GPR30 receptors than the less aggressive ones.

"But much work has to be done to figure out precisely where GPR30 fits into the estrogen picture," Prossnitz says. "Our findings suggest that the signaling mechanisms used by estrogen are more complicated than previously expected and that, to fully understand how estrogen mediates its effects, we must cast a wider net and consider new estrogen receptors when we study estrogen and disease." Many of those studies are already underway in Prossnitz's lab, including research aimed at finding which tissues and cancers have GPR30 and what compounds selectively inhibit the receptor. ■

The research described in this article is supported in part by funding from NCRR, the National Institute of Allergy and Infectious Diseases, the National Institute of Biomedical Imaging and Bioengineering, the National Cancer Institute, and the National Science Foundation.

ADDITIONAL READING

■ Revankar, C. M., Cimino, D. F., Sklar, L. A., et al., A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 307:1625-1630, 2005.

IMAGES COURTESY OF ERIC PROSSNITZ, UNIVERSITY OF NEW MEXICO