ENDOCRINOLOGY

Drug's Link to Genes Reveals Estrogen's Many Sides

Depending on the health concern, the hormone estrogen is either a villain or a savior. On the one hand, estrogen is thought to contribute to the development of breast and uterine cancers. But on the other, it seems to protect women against heart attacks and other cardiovascular problems; osteoporosis, a condition in which the bones become brittle and break easily; and possibly Alzheimer's disease.

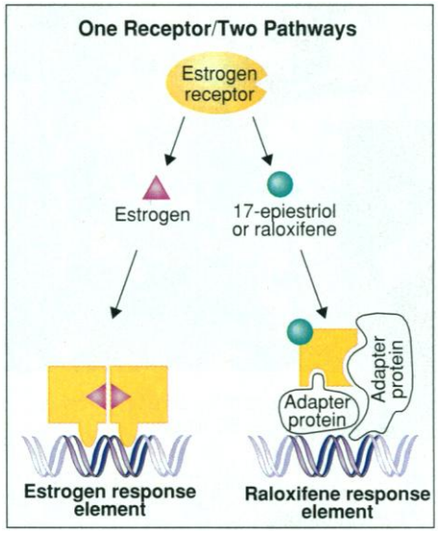
But so far, drugs developed to block estrogen's bad effects, or mimic its good ones, have proved to be as contradictory as the natural molecule. Take tamoxifen, which is widely used to treat or prevent breast cancer. In breast tissue, tamoxifen blocks estrogen's cancer-promoting effects, but it mimics them in uterine tissue, where it increases the risk of uterine cancer.

Now new findings, described on page 1222 by a team from Lilly Research Labs in Indianapolis, may help clarify estrogen's contradictory personality and open the way to designing drugs that selectively block estrogen's unwanted effects—or mimic its beneficial ones. In studies conducted with raloxifene, a drug that counters estrogen action in the breast while mimicking it in bone, the Lilly team, led by molecular biologist Na N. Yang, has discovered a possible explanation for how the same drug may cause different effects, depending on which tissue it is acting in—and how the natural hormone itself manages to adopt so many different guises.

The answer, it seems, lies deep in the molecular pathways that convey signals from a cell's estrogen receptor to its genes. The researchers found that raloxifene sends its bone-preserving signals to the genes by a new route, different from the one traced for estrogens in breast and other reproductive tissues. Coupled with other hints of a diversity of gene-activating pathways for estrogen, the discovery suggests that "in each cell type, you may have different pathways that act on different genes to create the specific biology [of that cell type]," says Bert O'Malley, a molecular endocrinologist at Baylor College of Medicine in Houston. That realization, he adds, could have "a lot of pharmacological relevance." By making drugs that tweak a single pathway—say, the one involved in bone preservation—without affecting others, unwanted side effects might be avoided.

Early in raloxifene's development, Lilly researchers realized that this drug might display some of that kind of specificity. Like tamoxifen, it counters estrogen's effects in breast while acting like estrogen in bone. But raloxifene does not promote excess growth of uterine tissue, implying that it might combat osteoporosis without increasing a woman's chances of getting cancer. "That [finding] was so intriguing," Yang says, "that we had to figure out the underlying molecular mechanism of raloxifene." They also wanted to clarify the mechanism by which estrogen and raloxifene preserve bone. That was a puzzle because both agents activate the gene encoding transforming growth factor-β3 (TGF-β3), a protein considered key to keeping bones strong, in living animals, but only raloxifene has a strong effect on the gene in cultured cells.

The biological responses to estrogen, raloxifene, and tamoxifen are all believed to start in the same way. As lipid-based molecules, they can slip through the cell's fatty outer membrane unaided and into the cell nucleus, where they bind the estrogen receptor. Estrogen then spurs the complex to bind to a DNA sequence called the estrogen response element and activate the appropriate target genes. But raloxifene doesn't work that way, it turns out.



SOURCE: NA N. YANG *ET AL.*

Alternate route. The estrogen receptor recognizes a different response element with raloxifene than with estrogen itself.

To pin down what enables raloxifene to activate the TGF-β3 gene, Yang and her colleagues began dissecting the estrogen receptor to see which parts are necessary for raloxifene's action. Surprisingly, when the team removed the DNA-binding domain, they found that the receptor-raloxifene complex still activated the gene, even without any obvious way to link with the DNA.

Further work suggested that one or more additional proteins were needed to link the complex to the gene, because activation occurred only in the presence of cellular extracts. And furthermore, the adapter protein, which has not been identified, does not recognize the usual estrogen response element. Yang's team systematically deleted small segments of DNA in the TGF-β3 gene's regulatory regions until they found a deletion that prohibited raloxifene from activating the gene. The deleted sequence, which Yang's group calls the raloxifene response element, is different from the estrogen response element.

But it is activated, the Lilly group found, by 17-epiestriol, an intermediate formed as the body prepares estrogen for excretion. This may explain why estrogen activates the TGF-β3 gene in living animals, but not in cultured bone cells, which cannot produce the 17-epiestriol themselves. Other estrogen byproducts may similarly take estrogen's place in turning on target genes through alternate pathways, especially in nonreproductive tissues, Yang says.

Scientists had already known about yet another response element, called the AP1 site, involved in gene regulation by estrogen. Peter Kushner and Paul Webb of the University of California, San Francisco, found that both estrogen and tamoxifen act through the AP1 site in uterine tissue. They now have unpublished data indicating that raloxifene does not interact with the AP1 site, Kushner says. This difference may explain why raloxifene does not have the estrogenic effect in uterus that tamoxifen has, he adds.

Moreover, the finding of estrogen activity at the AP1 site and now of the raloxifene response element "lends credence to the notion that these nonclassical elements may mediate the majority of steroid responses," Kushner says. And while it is still too early to tell just how many alternate paths there are, other recent results are further undermining the idea that there is a single main pathway for estrogen action. For example, the discovery of a second estrogen receptor, reported by George Kuiper of the Karolinska Institute in Huddinge, Sweden, in the 11 June *Proceedings of the National Academy of Sciences* "clearly adds another layer of complexity to estrogen regulation," Yang says.

For those hoping to beat osteoporosis, breast cancer, and heart disease without incurring other health risks, this complexity can only be good news. By targeting new drugs to specific pathways, it may be possible to " develop drugs that have some of the good properties of estrogen but not the awful ones," Kushner points out.

–Elizabeth Pennisi