Breast cancer drug fulvestrant appears more effective in the presence of CK8 and CK18

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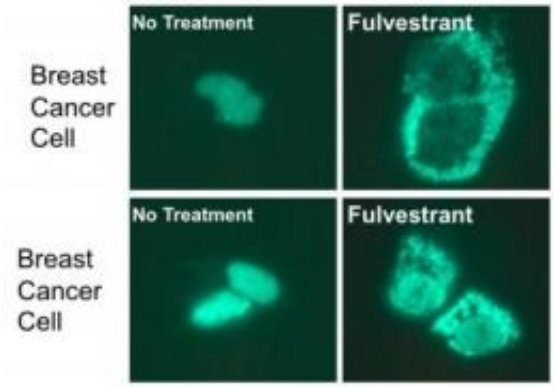

The figures show that fulvestrant-mediated cytoplasmic localization of ER is associated with intermediate filament proteins CK8 and CK18. After treatment of MCF-7 and T47D cells (breast cancer cells) with fulvestrant, dramatic cytoplasmic localization of ER was observed. The results indicate that the presence of CK8 and CK18 is necessary for fulvestrant-induced cytoplasmic localization of ER, which keeps the receptor away from the nucleus where it could activate growth promoting genes. Credit: Kenneth Nephew

Women's responsiveness to the second-line breast cancer drug fulvestrant may depend on whether the cancer cells are expressing two key proteins, Indiana University Bloomington scientists report in this month's *Cancer Biology & Therapy*.

Fulvestrant appeared to exert maximum anti-cancer effects in vitro when cells produced normal or elevated quantities of the cytokeratins CK8 and

CK18, structural proteins that help give the nucleus its shape.

For fulvestrant to work well, the cells must also be responsive to estrogen, and producing the estrogen receptor ER-alpha. ER-alpha's importance to fulvestrant's anti-estrogenic action had been established in previous reports. The present study confirms fulvestrant's binding relationship to ER-alpha, while also showing two other proteins, cytokeratins 8 and 18, can strongly enhance fulvestrant's anti-estrogenic activity. Testing for the presence of these three proteins, and perhaps many others, could help doctors decide whether fulvestrant should be prescribed to their patients.

"We need an effective panel of markers that inform physicians about what treatment options will be most beneficial to patients," said Medical Sciences Program Bloomington cancer biologist Kenneth Nephew, who led the study. "These three gene products should be investigated further to determine whether they should be included in that panel."

Medical Sciences Program Bloomington is a division of the IU School of Medicine. Nephew is a professor of cellular and integrative physiology, and obstetrics and gynecology.

"Normal" breast cancer cells can grow faster in the presence of estrogen, a hormone. Estrogen attaches to receptors embedded in the cancer cell, such as ER-alpha in the cytoplasm and nucleus. The estrogen-ER complex can then act to turn on genes or amplify their expression. Not all cancer cells are responsive to estrogen, however, or to fulvestrant, which counteracts estrogen's effects.

Although fulvestrant has been used to treat cancer since the late 1980s, and is now commonly prescribed as a second-line defense against metastatic cancer cells, how the drug works is still not completely understood. Nephew said one of the aims of the research is to clarify

fulvestrant's biochemistry, and understand why cancer cells eventually become unresponsive to the drug.

Second-line breast cancer therapies are employed when first-line approaches (tamoxifen, for example) don't work or stop working.

After conducting analyses of different cell lines and assaying gene and protein activity, Nephew, Xinghua Long (now a faculty member at Jiangnan University), and Meiyun Fan (now an assistant professor at the University of Tennessee-Memphis) believe they are able to present a compelling model for fulvestrant's action. The scientists believe that when fulvestrant encounters ER-alpha and binds to the receptor, the receptor forms a two-protein complex either with another ER-alpha -- or with ER-beta, a related but different estrogen receptor. The alpha-alpha or alpha-beta "dimer" is then removed to the nuclear matrix, where it binds to CK8 and CK18. It's the binding of ER-alpha to the nuclear matrix that would seem to signal protein-killing proteases to destroy ER-alpha. As the number of available estrogen receptors plummets, the connection between estrogen and cancer-related gene activity is weakened, and estrogen can no longer contribute to the growth of cancer cells.

Because many drug treatments can have a severely negative impact on quality of life, Nephew said fulvestrant and other cancer drugs should only be prescribed when their use is associated with a reasonable chance of successful outcomes. However, compared to frequently prescribed endocrine treatments for advanced disease like tamoxifen, anastrozole, letrozole and exemestane, fulvestrant is well tolerated. If biopsied cancer cells can be shown beforehand to be resistant or unresponsive to fulvestrant, the doctor may prevent some of the commonly reported side effects seen with the drug.

Nephew said that it wouldn't be easy for physicians to simply order a

separate test that analyzes biopsied tissue for the presence of CK8 and CK18.

"It would require a few things a typical hospital doesn't have on hand," Nephew said. "But we're currently investigating how to do that. We also need to be able to show that the expression of the two cytokeratins can be prognostic of fulvestrant's effectiveness. To that end we are talking with George Sledge at the Indianapolis campus about the feasibility of clinical studies. That would be the next step."

George Sledge Jr. is the Ballve-Lantero Professor of Hematology/Oncology at the IU School of Medicine's Melvin and Bren Simon Cancer Center.

When the study was conducted, report coauthors Xinghua Long and Meiyun Fan were at IU Bloomington as a Ph.D. student and a postdoctoral fellow, respectively. The research was funded with grants from the National Cancer Institute's Integrative Cancer Biology Program, the Walther Cancer Foundation, and the National Natural Science Foundation of China.

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