Scientists shut down pump action to break breast cancer cells' drug resistance

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(PhysOrg.com) -- Breast cancer cells that mutate to resist drug treatment survive by establishing tiny pumps on their surface that reject the drugs as they penetrate the cell membrane – making the cancer insensitive to chemotherapy drugs even after repeated use.

Researchers have found a new way to break that resistance and shut off the pumps by genetically altering those breast cancer cells to forcibly activate a heat-shock protein called Hsp27. This protein regulates several others, including the protein that sets up the pumps that turn away the chemotherapeutics.

In experiments, the common chemotherapy drug Doxorubicin killed about 50 percent more drug-resistant breast cancer cells in which Hsp27 had been activated than it did in normal drug-resistant cells.

Though these results have been shown only in cell cultures in a lab, they suggest that there someday could be a clinical way to use this approach to reverse the drug resistance that can develop in breast cancer cells. The study was conducted in MCF-7/adr breast cancer cells, which resist the effects of Doxorubicin.

"These cells are actually resistant to multiple drugs, so the resistance will be there even if clinicians move on to other chemotherapeutics. It's a serious issue," said Govindasamy Ilangovan, associate professor of internal medicine at Ohio State University and senior author of the research. "The plausible way to circumvent this effect is to suppress the

resistance by shutting down the drug extrusion pump using molecular approaches. That is what we're trying to address."

The study appears in the Sept. 23 issue of the *Journal of Biological Chemistry*.

The researchers analyzed proteins in regular breast cancer cells from the MCF-7 cell line as well as multidrug resistant cells from the same line. The normal breast cancer cells are known to be sensitive to chemotherapy drugs.

The tests indicated that the normal cells contained HSF-1, the transcription factor that activates the heat shock protein Hsp27. However, in the drug-resistant cells, HSF-1 was extremely low and Hsp27 was present in only trace amounts.

Previous research has established that Hsp27 controls two other proteins, including the culprit protein that makes the cells drug resistant by establishing pumps to extrude chemotherapeutics out of cells. This pumping protein's level was clearly high in the drug-resistant cells, and nonexistent in the normal breast cancer cells.

"The existence of this pump-action protein, called P-gp, is bad because it is removing the drugs from the cells. The cells do not even feel the presence of the drug in the system. We need to lower the amount of P-gp so that the drug stays in the cells to cause the intended damage. So we decided to model how to achieve this by forcibly bringing back the Hsp27," said Ilangovan, also an investigator in Ohio State's Davis Heart and Lung Research Institute.

In these experiments on cells, the researchers performed gene transfer using a viral vector to deliver the Hsp27 gene into the drug-resistant cells. As expected, after the addition of this gene the Hsp27 protein was

abundantly induced and the drug-resistant protein p-gp went down in the cells.

"Then we tested the cells under this condition to determine whether we could sensitize these resistant cells to the drug," Ilangovan said.

After treatment with varying levels of Doxorubicin, about 50 percent more resistant cells in which Hsp27 had been forcefully activated died compared to normal drug-resistant cells. In addition, fluorescent staining of the cells showed that the expression of Hsp27 in these cells also caused them to absorb much more of the chemotherapy drug – a sign the drug was not being pumped away.

Additional experiments showed that these genetically altered cells died in the way they typically would in response to chemotherapy – by undergoing a programmed cell death process called apoptosis.

"We proved Doxorubicin does kill these cells in the way it is supposed to," Ilangovan said.

The findings represent a twist of sorts in molecular biology because heat-shock proteins typically are activated by stress. But in this case, the drug-resistant cells become reprogrammed over time – presumably, in response to the stress of being treated with drugs intended to kill them – and instead of being full of heat-shock protein activity, those stress-induced proteins are silenced.

"Because these proteins are involved, it looks at present like there could be a strong link between chronic stress and this drug-resistant mechanism evolving in the cells, but we need to carry out additional new studies to make any solid conclusion on chronic stress and drug resistance in cancer," Ilangovan said.

Translation of this technique in humans is years away, but would likely involve the delivery of the Hsp27 gene into drug-resistant cancer cells to induce activation of the Hsp27 protein, he said. The next step in this work involves refining the gene transfer process and demonstrating it in animal models.

More information: www.jbc.org/

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