Scientists reprogram cancer cells with low doses of epigenetic drugs

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Experimenting with cells in culture, researchers at the Johns Hopkins Kimmel Cancer Center have breathed possible new life into two drugs once considered too toxic for human cancer treatment. The drugs, azacitidine (AZA) and decitabine (DAC), are epigenetic-targeted drugs and work to correct cancer-causing alterations that modify DNA.

The researchers said the drugs also were found to take aim at a small but dangerous subpopulation of self-renewing cells, sometimes referred to as cancer stem cells, which evade most cancer drugs and cause recurrence and spread.

In a report published in the March 20, 2012, issue of *Cancer Cell*, the Johns Hopkins team said their study provides evidence that low doses of the drugs tested on cell cultures cause antitumor responses in breast, lung, and colon cancers.

Conventional chemotherapy agents work by indiscriminately poisoning and killing rapidly-dividing cells, including cancer cells, by damaging cellular machinery and DNA. "In contrast, low doses of AZA and DAC may re-activate genes that stop cancer growth without causing immediate cell-killing or DNA damage," says Stephen Baylin, M.D., Ludwig Professor of Oncology and deputy director of the Johns Hopkins Kimmel Cancer Center.

Many cancer experts had abandoned AZA and DAC for the treatment of common cancers, according to the researchers, because they are toxic to

normal cells at standard high doses, and there was little research showing how they might work for cancer in general. Baylin and his colleague Cynthia Zahnow, Ph.D., decided to take another look at the drugs after low doses of the drugs showed a benefit in patients with a pre-leukemic disorder called myelodysplastic syndrome (MDS). Johns Hopkins investigators also showed benefit of low doses of the drugs in tests with a small number of advanced lung cancer patients. "This is contrary to the way we usually do things in cancer research," says Baylin, noting that "typically, we start in the laboratory and progress to clinical trials. In this case, we saw results in clinical trials that made us go back to the laboratory to figure out how to move the therapy forward."

For the research, Baylin and Zahnow's team worked with leukemia, breast, and other cancer cell lines and human tumor samples using the lowest possible doses that were effective against the cancers. In all, the investigators studied six leukemia cell lines, seven leukemia patient samples, three breast cancer cell lines, seven breast tumor samples (including four samples of tumors that had spread to the lung), one lung cancer tumor sample, and one colon cancer tumor sample. The team treated cell lines and tumor cells with low-dose AZA and DAC in culture for three days and allowed the drug-treated cells to rest for a week. Treated cells and tumor samples were then transplanted into mice where the researchers observed continued antitumor responses for up to 20 weeks. This extended response was in line with observations in some MDS patients who continued to have anticancer effects long after stopping the drug.

The low-dose therapy reversed cancer cell gene pathways, including those controlling cell cycle, cell repair, cell maturation, cell differentiation, immune cell interaction, and cell death. Effects varied among individual tumor cells, but the scientists generally saw that cancer cells reverted to a more normal state and eventually died. These results were caused, in part, by alteration of the epigenetic, or chemical

environment, of DNA. Epigenetic activities turn on certain genes and block others, says Zahnow, assistant professor of oncology and the Evelyn Grolman Glick Scholar at Johns Hopkins.

The research team also tested AZA and DAC's effect on a type of metastatic breast cancer cell thought to drive cancer growth and resist standard therapies. Metastatic cells are difficult to study in standard laboratory tumor models, because they tend to break away from the original tumor and float around in blood and lymph fluids. The Johns Hopkins team re-created the metastatic stem cells' environment, allowing them to grow as floating spheres. "These cells were growing well as spheres in suspension, but when we treated the cells with AZA, both the size and number of spheres were dramatically reduced," says Zahnow.

The precise mechanism of how the drugs work is the focus of ongoing studies by Baylin and his team. "Our findings match evidence from recent clinical trials suggesting that the drugs shrink tumors more slowly over time as they repair altered mechanisms in cells and genes return to normal function and the cells may eventually die," says Baylin.

The results of clinical trials in lung cancer, led by Johns Hopkins' Charles Rudin, M.D., and published late last year in Cancer Discovery, also indicate that the drugs make tumors more responsive to standard anticancer drug treatment. This means, they say, that the drugs could become part of a combined treatment approach rather than a stand-alone therapy and as part of personalized approaches in patients whose cancers fit specific epigenetic and genetic profiles.

Low doses of both drugs are approved by the U.S. Food and Drug Administration for the treatment of MDS and chronic myelomonocytic leukemia (CMML). Clinical trials in breast and lung cancer have begun in patients with advanced disease, and trials in colon cancer are planned.

In addition to Baylin and Zahnow, other investigators participating in this study include Hsing-Chen Tsai, Huili Li, Leander Van Neste, Yi Cai, Carine Robert, Feyruz V. Rassool, James J. Shin, Kirsten M. Harbom, Robert Beaty, Emmanouil Pappou, James Harris, Ray-Whay Chiu Yen, Nita Ahuja, Malcolm V. Brock, Vered Stearns, David Feller-Kopman, Lonny B. Yarmus, Yi-Chun Lin, Alana L. Welm, Jean-Pierre Issa, Il Minn, William Matsui, Yoon-Young Jang, and Saul J. Sharkis.

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