

## Chemotherapy: Too Much of a Good Thing?

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Recent studies in Robert Gatenby's laboratory at the Moffitt Cancer Center used computational models that were tested in cancer cells cultured *in vitro*, to provide support for the concept of "adaptive therapy" [1]. The idea for this type of therapy stems from a desire to capitalize on the extra metabolic strain that is placed on chemoresistant cancer cell populations, relative to their chemosensitive counterparts. Cancer chemotherapy drugs are normally given at the maximum tolerated dose (MTD), so that they have the greatest effect in killing cancer cells. But, this frequently leads to development of resistance to the chemotherapy. Adaptive therapy exploits a vulnerability of the chemo resistant cancer cell by providing a dose of drug that is less than the MTD, and instead, providing the minimum drug dose to maintain a stable tumor burden.

Two breast cancer cell lines were used in this study: MCF-7 and a cell line derived from MCF-7, MCF-7/Dox, which has acquired resistance to doxorubicin. The latter cell line has increased expression of the P-glycoprotein (PGP) membrane pump, which mediates active efflux of chemotherapeutic agents, including doxorubicin from the cytoplasm. An increased presence of PGP in the membrane results in decreased retention of a number of chemotherapeutic agents, leading to increased resistance to multiple other drugs or multidrug resistance (MDR). One tactic that has been somewhat effective in combating MDR is to treat with non-chemotherapy drugs, such as verapamil or cyclosporin A, which are substrates of PGP that can compete with the chemotherapy drug to slow down its efflux. But as pointed out by the authors, the amount of non-chemotherapy drug needed for effective competition is too toxic to be feasible, in the absence of other factors that limit development of chemoresistance.

They first found that when equal numbers of the two breast cancer cell lines were plated for 24 hr in culture media with a normal glucose concentration (2 g/L), and then switched to media with normal glucose, low glucose (0.5 g/L) or no glucose, the wild-type MCF-7 cells grew well in all three media. However, beginning at about 100 hr after exposure to the 3 levels of glucose, the MCF-7/Dox cells grown in low- or no-glucose media became much less viable than the wild-type MCF-7 breast cancer cells.

Why the MCF-7/Dox cells did show decreased proliferation? The authors proposed that glucose restriction might place the MCF-7/Dox cells at a disadvantage, because they require more energy to maintain the PGP membrane pump. They tested this idea by growing co-cultures of the cells in the presence of 2-deoxyglucose, a competitor for glucose transporters, and additionally low doses of the non-chemotherapy drug, verapamil. Glucose restriction with 2-deoxyglucose increased the doubling time of both cell lines to about the same degree, but whereas addition of verapamil plus 2-deoxyglucose to the media had little additional effect on MCF-7 cell proliferation; this combination increased the doubling time two-fold in MCF-7/Dox cells. Thus, when energy was restricted in the MCF-7/Dox cells that over express PGP, and these cells were additionally subjected to the presence of a decoy molecule that increased the metabolic cost of drug resistance, the cells became less fit and growth rates slowed. In the presence of doxorubicin and high glucose, the MCF-7/Dox cells survived better than MCF-7,

but either verapamil or low glucose decreased survival of the MCF-7/Dox cells.

The requirement for extra glucose in the PGP-over expressing MCF-7/Dox cells was illustrated by the finding that the rate of glucose uptake by MCF-7/Dox cells was 4 times higher than the wild-type MCF-7 cells. The authors measured acid production and oxygen consumption rates of both cell lines, and made several assumptions related to oxygen consumption and ATP production to arrive at an estimate of energy production. Their results indicated that when cells were moved from low- to high-glucose conditions, the PGP mutant MCF-7/Dox cells switched to an anaerobic metabolism to a much greater extent than the parental MCF-7 cells. Under these conditions, MCF-7/Dox cells produced most of their energy by glycolysis, and the addition of oligomycin demonstrated that the maximum glycolytic potential of these doxorubicin-resistant cells is roughly three times higher than that of the parent cells. A take-home message of these studies is that the cell lines overexpressing PGP consume more energy than the parent breast cancer cells, which has also been shown in other studies using PGP mutant cell lines [2,3], and that the extra energy requirements are met primarily by anaerobic glycolysis.

One of the appealing aspects of this study is the computational model that was built to simulate the response of breast cancer patients to the glycolytic anti-metabolite and PGP substrate used in this study. Their model contained one sub-population of chemo sensitive and another of chemo resistant cancer cells, with one variable being the percentage of PGP-positive cells, initially present. The model incorporated the effects of optimum and energy-restricted conditions, with and without verapamil exposure on growth rates and drug sensitivity, and was based on their *in vitro* data using MCF-7 and MCF-7/Dox cell lines. Their model incorporated simulations of: 1) MTD; 2) MTD combined with chronic verapamil and 2-deoxyglucose; 3) adaptive therapy with an initial dose of half of the MTD and modification of the dose intensity by  $\pm 20\%$ , to correct for tumor growth or reduction, respectively; and 4) AT combined with chronic verapamil and 2-deoxyglucose. They set  $10^9$  cells as the detection level for a tumor and  $10^{12}$  cells as a lethal tumor burden. Their model confirmed the cell culture results that showed the important role of anaerobic glycolysis in supplying energy to the MCF-7/Dox cells.

Their model also indicated that when PGP-positive MCF-7/Dox

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cells made up less than 10% of the initial cell population, the combination of adaptive therapy with both 2-deoxyglucose and verapamil led to increased survival, and a stabilization of tumor burden for a period longer than cells exposed to the MTD of doxorubicin. Adaptive therapy led to a two- to three-fold increase in survival, and the results were more impressive, with the combination of adaptive therapy plus the anti-metabolite of glucose plus the alternative substrate of PGP. In this case, there was a four-fold increase in progression-free survival using their simulations. When the percentage of MCF-7/Dox cells was lowered to 5%, adaptive therapy combined with 2-deoxyglucose and verapamil increased patient survival to over 500 days, compared to a survival of 122 days for patients treated with the MTD of drug. In addition, their simulation suggested that survival was four-fold increased by adaptive therapy in the sub-population containing 5% MCF-7/Dox cells, and by ten-fold in this sub-population undergoing adaptive therapy with 2-deoxyglucose plus verapamil.

The work by this research group provides additional evidence that adaptive therapy has benefits in preventing or slowing the development of a doxorubicin-resistant population of cancer cells. Although only one drug was studied in one breast cancer cell line, a proof of principle was established that it may be possible to take advantage of a vulnerability in resistant cancer cells that relates to their greater requirement for cellular resources. In this case, it was a greater requirement for energy, but additional liabilities of drug-resistant cells may be uncovered, that would allow this approach to be expanded to control proliferation of chemo resistant cancer cell populations that form as a result of exposure to a variety of other chemotherapeutic agents. Under conditions where chemo resistant cells are vulnerable during periods where there is no exposure to the chemotherapy drug, the chemo sensitive cells would

become competitive, leading to decreased proliferation of the chemo resistant population. The authors make the point that adaptive therapy is in many cases applied to a cancer patient inadvertently, when a dose of drug is toxic or not well tolerated, and during these drug-free periods chemo sensitive cells would be able to compete with the more susceptible chemo resistant sub-population and keep it suppressed.

A question that arises from this approach is whether use of the minimum drug intensity needed to maintain a stable tumor burden would permit metastasis, since treatment with the MTD of the drug may initially greatly lower the tumor burden, whereas this may not be the case with adaptive therapy. However, a recent study indicates that tumor chemoresistance goes hand-in-hand with increased metastatic propensity via a signaling network linking chemo resistance and metastasis [4]. Thus, the presence of a tumor that is largely chemo sensitive which would occur with adaptive therapy, might very well not promote metastasis.

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