NANOMEDICINE

Dodging Drug-Resistant Cancer with Diamonds

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When treating metastatic tumors, chemoresistance can cause problems. A report in this issue of *Science Translational Medicine* demonstrates the potential of nanodiamond carriers (2 to 8 nanometers) for treating cancers with drug-efflux–based chemoresistance. Nanodiamond-mediated delivery of the chemotherapeutic doxorubicin (Dox) allowed for prolonged activity and increased apoptosis with decreased toxicity when compared with free Dox in liver cancer cells in culture as well as in vivo in mouse liver tumors. This finding may represent a broadly applicable strategy for overcoming adenosine 5′-triphosphate (ATP)–binding cassette (ABC) drug transporter–mediated resistance during cancer chemotherapy.

CHEMORESISTANCE

Taken together, all forms of cancer were projected to account for more than 1500 deaths per day in the United States in 2010 (*1*). Chemotherapeutics are crucial in combating this deadly disease; however, drug resistance represents a major factor that limits the efficacy of chemotherapeutics, and treatments that are initially effective in mediating tumor growth often result in relapses over time. Tumors can be intrinsically resistant to a given therapy, but even initially responsive tumors can become chemoresistant owing to the proliferation of a small population of therapy-resistant cells that may have survived an earlier treatment regime (*2*). This acquired resistance often imparts a cross-resistance to other drugs with different mechanisms of action, an effect termed multidrug resistance (MDR) (*3*). Resistance to chemotherapeutics is believed to be responsible for treatment failure in more than 90% of patients with metastatic cancer (*3*), and the vast majority of cancer deaths are attributed to such treatment failure (*4*). Overcoming drug resistance therefore represents a substantial hurdle to increasing cancer patient survival. In this issue of *Science Translational Medicine*, Chow *et al.* propose a nanoparticle-based method that not only increases the response of resistant tumors to the common chemotherapeutic doxorubicin (Dox) but might represent a platform for overcoming multiple mechanisms for such resistance (*5*).

Cellular resistance to drugs can develop from a variety of mechanisms (*6*, *7*). Resistance to a particular drug, or class of drugs with similar mechanisms of action, might arise from an alteration in the drug's cellular target or by an increase in the repair of drug-induced damage, often to DNA. Rapid enzyme-mediated degradation of the drug, cellular avoidance of apoptotic pathways, and intracellular drug sequestration represent other common pathways for chemoresistance. However, the most common mechanism of resistance involves the efflux of drugs from the cell by one or more adenosine 5′-triphosphate (ATP)–binding cassette (ABC) transporters. In healthy cells, ABC transporter proteins fulfill a variety of roles, including the regulation of local permeability in the nervous and reproductive systems and excretion of toxins in the liver, kidneys, and gastrointestinal tract. In cancer cells, the ABC transporters work to eject chemotherapeutics from the cell to nontoxic concentrations, thus decreasing their therapeutic effects.

Of the 48 membrane proteins that comprise the ABC transporter family, 15 have been associated with drug resistance (*7*). Although much progress has been made to elucidate the molecular mechanism of these MDR-conferring ABC transporters, this knowledge has not yet been translated to clinical relevance. The most widely studied MDR protein is P-glycoprotein, also called

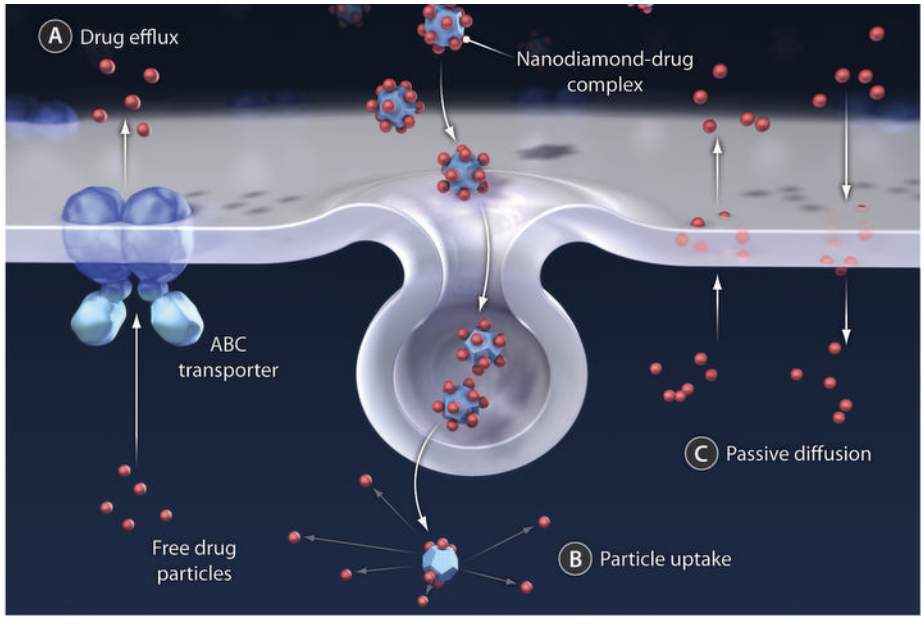

Fig. 1. NDX avoids efflux. A schematic representation of a proposed mechanism for ND-drug conjugates in efflux-transporter–expressing cells. (**A**) ABC transporter proteins efflux drug out of the cell. (**B**) Endocytosis of ND-drug conjugates. (**C**) Diffusion of free Dox across the cell membrane. NDX is more difficult to remove from the cell than free Dox, which is rapidly eliminated. Measured release of drug from these NDX conjugates helps to maintain a steady, lethal dose of drug in the cell versus free Dox.

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Pgp, MDR1, or ABCB1. Extensive attention also has been given to the roles played by ABCC1 and ABCG2 in MDR breast cancer, but the exact role of these proteins remains difficult to decipher (*7*). A major difficulty in this approach is that ABC transporters other than the one singled out for study may play a role in MDR, which is a possible explanation for the lack of success in bringing antagonists of this class of proteins to the clinic (*8*–*10*). Indeed, therapies that target a single ABC transporter might result in a selective enhancement of cancer cells with other resistance mechanisms, including alternate ABC transporters. In light of this difficulty, nanocarriers—such as the nanodiamonds studied by Chow *et al.*—appear increasingly promising as a method to bypass drug-efflux–based chemoresistance (*11*).

CANCER MEETS NANO

In their study, Chow *et al.* evaluated nanodiamonds (NDs) for their potential as a carrier of Dox in the treatment of chemoresistant cancers (*5*). NDs, which are carbon particles 2 to 8 nm in diameter, have several characteristics that identify them as promising candidates for clinical translation, including high biocompatibility, low toxicity, and "cargo" versatility (that is, they can be modified to carry various types of drugs). By treating a sample of oxidized NDs with base (sodium hydroxide), the authors were able to electrostatically bind Dox to the ND surfaces, resulting in the formation of 80-nm complexes they termed NDX. The mechanism of NDX formation was probably a result of deprotonated carboxylic acid moieties on the NDs interacting reversibly with protonated amines on the Dox molecules. The NDX released the bound drug over time in a functional form, which resulted in a statistically significant increase in cell death versus free Dox in both the LT2M (LT2-Myc) mouse liver tumor cell line and the 4T1 mouse mammary tumor cell line, each of which has shown some resistance to the free drug. Addition of verapamil, a drug transporter inhibitor, increased the efficacy of Dox in these cells, whereas response to NDX was unchanged, thus demonstrating that the NDX complexes were insensitive to ABC transporter–based efflux. To evaluate the enhanced levels of Dox remaining in resistant cells after efflux, drug retention was measured in MDCK cells, which overexpress the MDR1 drug transporter. After an hour of exposure to NDX or Dox, followed by 4 hours to allow the cells to efflux the drug, the authors found that NDX treatment resulted in a 10-fold increase in retained Dox versus the free drug.

The NDX was also effective in vivo in treating both the 4T1 and LT2-Myc tumors in a mouse model, in which acute apoptotic response was increased versus Dox treatment in the tumor tissue. In long-term tumor growth studies evaluated 21 days after the start of treatment, weekly doses of NDX improved growth inhibition and increased survival probability when compared with Dox. Although the exact mechanism is unknown, the key to the efficacy of the NDX in these resistant tumors seems to be in the sustained release of Dox from the NDX complexes that had been taken up by tumor cells. Effective chemotherapy requires both reasonably high concentrations and a prolonged exposure time (*12*). NDX complexes, once internalized by a cancer cell, are difficult to eject via drug transporters (Fig. 1). Measured release of Dox from NDX particles must maintain high enough intracellular Dox concentrations over a prolonged period to provide therapeutic effect, despite efflux. Passive permeation of free Dox into the cell might also play an important role in maintaining a therapeutically relevant intracellular Dox concentration (*12*), with a decrease in the net outward diffusion of Dox resulting from the presence of NDX (Fig. 1). Indeed, NDX treatment resulted in an increase of 1.5 μg Dox per milligram of tumor compared with unmodified Dox when measured 7 days after treatment.

Even at high ND doses, Chow *et al.* found the NDs to be biocompatible—a necessary trait for clinical relevance (*13*). Dosing with NDs resulted in neither an inflammatory response, as measured by the absence of elevated sera interleukin-6 (IL-6) concentrations, nor altered liver function, as indicated by normal serum alanine transferase amounts (*5*). Histological analysis of tissues from ND-dosed mice also showed good biocompatibility, as evidenced by no substantial changes in multiple tissues as compared with undosed samples. Previous studies on ND particles have also demonstrated good biocompatibility. For instance, incubation of RAW 264.7 mouse macrophages with NDs resulted in no change in expression of IL-6, inducible nitric oxide synthase, or tumor necrosis factor–α, thus indicating a lack of inflammatory response. The same study also found no decrease in expression of Bcl-x, an apoptotic regulatory protein, which would have suggested potential cytotoxicity (*13*).

NDs have been used for several in vivo applications, including MRI imaging (*14*), fluorescent imaging (*15*, *16*), and as drug carriers (*13*, *17*, *18*), partly owing to their low toxicity. Nitrogen defects in NDs can lead to strong fluorescence that does not photobleach, leading to use as biological imaging agents (*16*). Surface modification of ND particles allows for the conjugation of biomolecules or organics, with covalent or noncovalent attachments possible (*18*). The ability to bind to a variety of substrates via either reversible or covalent attachment implies great potential for NDs as generic nanocarriers, although ND complexes with other chemotherapeutics, such as paclitaxel (*18*) or 10-hydroxycamptothecin (*17*), will need to be evaluated to determine activity against chemoresistant tumors. The question of the exact mechanism of imparted chemosensitivity remains a crucial one, both for extending these results to other therapeutics and for translating them to clinical relevancy. A key to unlocking such chemosensitivity might be in determining the necessary rate of drug release from the ND carrier. For example, slower drug release would cause efflux to maintain drug concentration below toxic levels, whereas more rapid release might result in insufficient exposure times for cytotoxic effect.

FUTURE PROSPECTS

Although the authors' findings imply great potential for nanoparticle-based drug carriers, a variety of different nanocarriers have shown promise for avoiding efflux-based chemoresistance. A polymersome that consists of two Pluronic block copolymers—ones based on ethylene oxide and propylene oxide—and Dox, named SP1049C, has demonstrated antitumor activity for some chemoresistant tumors, as well as activity in a mouse model of ABCB1-resistant leukemia (*19*). Folate-targeted liposomal carriers of Dox have also proven insensitive to Pgp-mediated drug efflux in vitro in mouse lung carcinoma multidrug-resistant cells (M109R-HiFR) and in a mouse model, in contrast with free Dox (*20*). Other carriers with promise in overcoming chemoresistence in cell lines or mouse tumor models include polymeric nanoparticles (*12*, *21*), polymer-drug conjugates, lipid nanocapsules (*22*), and micelles (*23*, *24*). Passively targeting efflux pumps with nanocarriers might prove to be a more successful and reliable approach than direct inhibition. Specific inhibition of the efflux process has

produced mixed results, with limited efficacy or toxicity restricting the treatment (*8*–*10*), perhaps in part because of nanocarrier specificity toward a single ABC transporter protein. Nanocarriers such as NDX appear to have a less-specific mode of action and may be generally effective against multiple ABC transporters.

Are ND-chemotherapeutic conjugates the answer to efflux-based chemoresistance? As Chow *et al.* demonstrated, NDs possess superior biocompatibility and show decreased toxic side effects as compared with free Dox. The lack of toxic myelosuppression response, a dose-limiting side effect of free Dox, to NDX allowed for the use of a higher dose than is possible with free Dox (up to 200 μg of Dox) (*5*). Perhaps the most promising trait of ND carriers is their potential versatility. NDs can be outfitted with a variety of different surface functionalities, including amine, alcohol, or carboxyl groups, which allow for conjugation to a range of drug substrates. It is likely that a combination approach that involves active targeting of cancer-specific or overexpressed membrane proteins, inhibition of ABC transporters, and encapsulation with nanocarriers will be most effective in treating MDR cancers. To this end, it will be interesting to see whether ND carriers can be loaded with a combination of drugs, targeting ligands, and efflux inhibitors and whether these multiply conjugated particles have greater efficacy than NDX alone.

Despite these advances in the field of nanomedicine, substantial translational questions remain regarding the efficacy of NDX on metastasized cancers and in vivo in human patients. However, FDA approval of nanoparticle-based drugs, such as doxil and abraxane, might "grease the wheels" of progress for nanomedicine, making way for their application to chemoresistant cancers. Nanocarrier-based approaches will soon represent a crucial and much-needed tool in the ongoing fight against cancer.

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