Minicells overcome tumor drug-resistance

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Bacterially derived minicells loaded with siRNA reverse drug resistance in tumor xenografts.

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The emergence of drug resistance after prolonged monotherapy undermines many promising anti-cancer drugs. To address this problem, cancer researchers have devoted considerable effort to developing combination therapies that both inhibit drug resistance and deliver cytotoxic drugs[1]. In this issue, MacDiarmid *et al.*[2] describe a new approach to combination therapy based on bacterially derived minicells that are targeted to tumors with antibodies (**Fig. 1**). First, the minicells deliver small interfering RNA (siRNA) or plasmid-encoded short hairpin RNA (shRNA) directed against a multidrug-resistance transporter to suppress drug resistance in the tumor; seven days later, a second injection of minicells delivers the cytotoxic drug to which the tumor was previously resistant. The authors provide compelling evidence that this strategy inhibits the growth of drug-resistant tumor xenografts for up to four months in athymic (*nu*/*nu*) mice. This nanoparticle-based combinatorial approach thus provides a promising example of an engineering strategy that could address tumor drug resistance in the clinic.

When tumor cells are exposed to a chemotherapeutic agent, genetic and epigenetic changes can render them resistant to the drug and to closely related molecules[3]. A recent example is the case of imatinib mesylate (Gleevec), a kinase inhibitor administered to patients with chronic myeloid leukemia. Some patients who had a promising initial response to Gleevec relapsed quite dramatically after prolonged exposure to the drug[3]. The mutations associated with drug resistance, which often affect the gene encoding the drug target, can alter the sensitivity, functionality, uptake, metabolism or export of drugs from cancer cells. A common mechanism for multidrug resistance involves overexpression of ATP-dependent efflux pumps belonging to the family of ATP-binding cassette (ABC) transporters[4]. A prototypical member of the family is P-glycoprotein (PGP)[4], which extravasates a broad range of drugs from the intracellular compartment, thereby reducing their cytotoxicity.

The efficacy of chemotherapeutic drugs is also influenced by their ability to penetrate tumor tissue, which is often characterized by an unstructured, unstable, fenestrated and poorly functioning vascular network[5]. This abnormal vasculature, combined with the tumor's compromised lymphatic drainage system, can provide

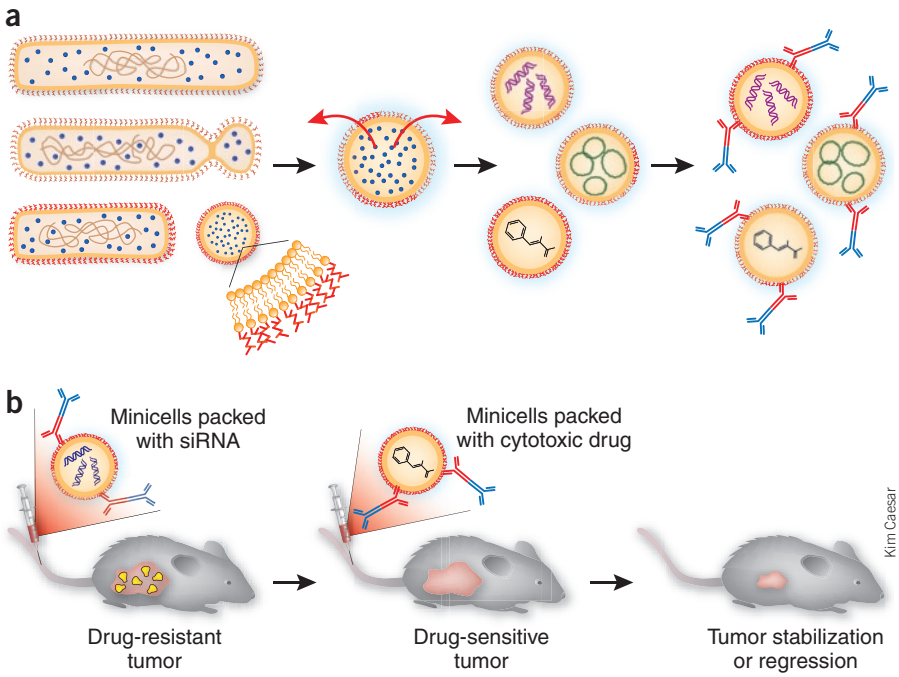

Figure 1 Bacterially derived minicells can deliver siRNA and plasmid-encoded shRNA to reverse tumor drug resistance *in vivo*. (**a**) Minicells are achromosomal particles produced during the division of mutant *Salmonella typhimurium*. Their lipid bilayers are decorated with polysaccharides (red). Once emptied of their contents, minicells can be loaded with siRNAs (purple) or chemotherapeutics (black) by incubation in the appropriate solution. Plasmids encoding shRNA (green) can be introduced by transforming the mutant bacteria that generate the minicells. Loaded minicells are then functionalized via bispecific antibody conjugates, with one arm specific for sugars on the minicell surface (red) and the other specific for a targeting antigen or receptor (blue). (**b**) When injected intravenously into mice bearing drug-resistant tumors, minicells carrying siRNA against a drug-resistance gene bind the tumor antigen (yellow), are endocytosed and release the siRNA into the cytoplasm, silencing the drug-resistance gene. Subsequent delivery of minicells containing a cytotoxic drug suppresses tumor growth.

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for nonspecific, passive targeting of nanometer-sized formulations into tumors—a mechanism known as the enhanced permeability and retention effect. Macromolecular formulations with this passive tumor targeting entered clinical trials almost 20 years ago, and demonstrated improved side-effect profiles relative to systemic administration of chemotherapeutics[6]. Alternatively, tumor targeting systems have been developed using ligands that specifically bind molecules expressed on the surface of target cells. These moieties include antibodies and peptides that can bind to antigens or receptors aberrantly expressed on cancer cells or the tumor endothelium[7].

A relatively new approach for delivering chemotherapeutics, described in an earlier study by MacDiarmid and colleagues[8], uses bacterially derived particles known as minicells. Minicells are achromosomal bacterial cells, ~400 nm in diameter, produced from mutants in which division is uncoupled from chromosomal replication (**Fig. 1a**). MacDiarmid *et al.*[8] showed that minicells can be emptied of their protein and RNA contents, filled with cytotoxic drugs and targeted to tumors by means of bispecific antibody conjugates. Compared with liposomes or other nanoparticles, minicells can be readily modified with antibodies owing to the long O-polysaccharide chains on their outer membranes. Targeting is mediated by bispecific antibody conjugates that recognize both O-polysaccharide and a tumor antigen. The authors previously reported[9] *in vivo* efficacy with minicells as targeting vehicles for an array of chemotherapeutic drugs with variable physical, chemical and structural characteristics. These drugs include 5-fluoracil, carboplatin, cisplatin, doxorubicin, irinotecan, paclitaxel, and vinblastine. The ability of minicells to encapsulate a large number of drug molecules (1–10 million per minicell), as well as a convenient drug-loading process that requires only one-step co-incubation of a solution of the drug with the vehicle, makes minicells a potentially attractive alternative to other macromolecule-based drug formulations.

In their latest study, MacDiarmid *et al.*[2] demonstrate the feasibility of using minicells for siRNA-mediated treatment of mouse tumor xenografts by two different approaches. The first is a monotherapy involving siRNA against cell-cycle proteins to induce cell-cycle arrest and apoptosis. The second is a combinatorial strategy that uses initial suppression of the PGP transporter in multidrug-resistant colon, breast and uterine tumor xenografts and subsequent delivery of chemothera peutic drugs.

> "This nanoparticle-based combinatorial approach provides a promising example of an engineering strategy that could address tumor drug-resistance in the clinic."

Despite the fact that minicells, like other cells, are bounded by a lipid-based plasma membrane, the authors show that they can be loaded with double-stranded siRNA to an estimated density of ~12,000 molecules per minicell. Although the loading mechanism remains obscure, MacDiarmid *et al.*[2] provide evidence that siRNA-containing minicells targeted to the epidermal growth factor receptor (EGFR) are internalized by EGFR-mediated endocytosis and release siRNA into the cytoplasm. Similar results are shown for minicells containing ~100 copies of an shRNA-encoding plasmid, derived from bacteria transformed with the plasmid.

The authors first use minicells carrying siRNA or plasmids encoding shRNAs specific for polo-like kinase, kinesin spindle protein and cyclin-dependent kinase 1 to inhibit tumor-cell proliferation both *in vitro* and *in vivo*. In the second, combinatorial therapy, designed to suppress drug resistance, they administer EGFR-targeted minicells carrying plasmid encoding shRNA against PGP followed by EGFR-targeted minicells loaded with the cytotoxic drugs doxorubicin, paclitaxel and irinotecan, to which the tumors were initially resistant. Using an optimized system, the authors report complete reversal of drug resistance and 100% survival of mice for up to 110 days after tumor implantation.

Delivery of siRNA across the plasma membrane of cells is challenging given the negative charge, hydrophilicity and large size of these molecules, and various nanoparticulate formulations have been developed to address this issue[11]. Aouadi *et al.*[12] recently reported a similar siRNA delivery system involving β1,3-D-glucan particles purified from baker's yeast to target macrophages. Porous 2–4-μm particles were derived from yeast cells and treated with alkali and solvent to hydrolyze the outer cell wall. The particles were packed with polyethylenimine-complexed siRNA against mitogen-activated protein 4 kinase 4, a mediator of cytokine expression. After oral administration, the particles were shown to inhibit proinflammatory cytokines *in vivo*. However, the size of the particles used in this study may restrict their use primarily to siRNA delivery to monocytes and macrophages, and the lack of stability of polyethylenimine *in vivo* may prevent their use in humans.

The work by MacDiarmid *et al.*[2] is an important example of a rationally designed, nanoscale delivery system for overcoming tumor drug resistance—a key strategy for improving success rates of cancer chemotherapies. Future work evaluating the efficacy of minicells as drug vehicles for siRNA in large animals, as well as more detailed analysis of the immune response to these bacterially derived particles, will be needed for clinical translation of this technology.

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