Resistence Revisited: Looking Back at 10 Years of Multidrug Resistance Research

By Charlie Schmidt

This is part of an occasional series that recalls some of the stories reported 10 years ago in the News section of the Journal.

In 1998, JNCI published a two-part feature about efforts to overcome multidrug resistance in cancer, which at the time was thought to be governed chiefly by a superfamily of molecular transporters, known as ATP-binding cassette (ABC) proteins. ABC transporters act as efflux pumps, which expel toxins and drugs from a cell. By reversing those transporters in cancer cells—particularly P-glycoprotein (Pgp), which was, and still is, the best-characterized among them—researchers hoped to overcome drug resistance, a primary cause of treatment failure.

A decade later, efforts to reverse Pgp have proven futile, in part because the transport protein is also expressed by healthy tissues—leading to unacceptable side effects when its activity is knocked out. Furthermore, dozens of other ABC transport proteins identified within the last 10 years can take over for Pgp when its activity is reversed in cancer cells. Scientists are still studying clinical opportunities with ABC transporters, but drug resistance in cancer remains as much a problem now as it ever was.

Even so, scientists have new leads to follow. Advances in molecular biology, driven by genomics and related fields, have revealed new resistance mechanisms and broadened opportunities to overcome it, said Michael M. Gottesman, M.D., head of the National Cancer Institute’s molecular cell genetics section. “We just have to apply the mechanistic knowledge we’re gaining in the laboratory towards progress in the clinic.”

A New Front

Today, those efforts are proceeding on dual fronts. Whereas scientists 10 years ago were concerned only with multidrug resistance against chemotherapy, those working today must also contend with single-drug resistance to newer, targeted therapies such as imatinib (Gleevec), a drug for chronic myelogenous leukemia (CML) and other cancers that inactivates a cancer-inducing protein called Bcr-Abl. Unlike chemotherapy—which kills rapidly dividing cells regardless of whether they’re cancerous—targeted therapies interfere with specific molecules involved in cancer and tumor growth.

The new era of targeted therapy was supposed to leave chemotherapy behind and multidrug resistance behind with it. That’s because researchers associated such resistance almost exclusively with efflux pumps, which act against a broad array of natural and synthetic toxins. By using nontoxic compounds targeted against specific molecules in carcinogenesis, scientists hoped that they could avoid resistance. But those expectations were dashed when clinicians found that although patients typically respond well to imatinib—among the first targeted drugs to reach the market—many also relapse within 3–5 years.

Confronted with that distressing problem, scientists were reluctant to blame mechanisms like efflux, which were generally attributed to multidrug resistance and chemotherapy, for imatinib resistance. Instead, their explanations veered toward gene mutations that, by chance, might allow CML cells to survive imatinib exposure. In that scenario, most CML cells are killed by the drug, but those with mutations that favor survival multiply over time, leading to relapse. Scientists call this type of drug resistance “acquired.”

To an extent, subsequent research has borne out this hypothesis, which was proposed when imatinib resistance was first observed, within a few years of the drug’s approval by the U.S. Food and Drug Administration in 2001. More than a dozen gene mutations have since been implicated, of which the most effective is T315I, according to Susan Bates, M.D., director of the NCI’s molecular therapeutics section. This simple variation alters Bcr-Abl’s three-dimensional binding site, thus blocking imatinib’s access to the target protein.

But Bates argues that mutations alone can’t account entirely for imatinib resistance. Patients whose leukemic cells have identical mutations can vary with respect to the degree of resistance, she said, which suggests that other factors—including mechanisms linked to multidrug resistance—are also at play. “For example, interindividual variation has been found in the amount of Gleevec that winds up in the bloodstream, which could reflect differences in cellular absorption of the drug,” Bates explained. “We can’t rule out that ABC transporters might be involved, given that Gleevec is a substrate for at least two: Pgp and breast cancer resistance protein. In CML cells, those transporters reduce the amount of Gleevec that reaches the protein target, and that fosters resistance.” (However, variation in resistance among patients could also be due in part to behavioral factors, such as adherence to the drug regimen [see JNCI 2008; 100:912–3].)
New Approaches
While researchers work to tease out resistance mechanisms against targeted therapies, efforts to overcome multidrug resistance to chemotherapy are making headway. Researchers in Gottesman’s lab at NCI, who are among the leaders in this area, approach the problem by using three general strategies. Gottesman’s research team collaborates with pharmaceutical companies to develop drugs that circumvent known resistance mechanisms. Compounds that evade ABC transporters—of which there are 48, according to current estimates—fall into that general category. Cytoskeletons, for example, a new class of cytotoxic molecules identified as potential chemotherapy agents, aren’t recognized by Pgp. “This provides proof of concept that new classes of anticancer agents that don’t interact with multidrug transporters can be developed,” he said. This approach is currently in early developmental stages, with no compounds in clinical trials.

Applying an alternate approach, the team strives to block resistance by inhibiting or reversing it. Clinical applications based on this approach would involve drug combinations: one drug to inhibit the resistance mechanism and another to kill the cancer cell. This strategy already has a long and unsuccessful history, exemplified by compounds such as PSC-833, a Pgp-reversing agent tested often in clinical trials. PSC-833 failed in part because of unpredictable pharmacokinetic interactions, leading to underdosing in some patients and overdosing in others. Scientists now hope to overcome this problem with third- and fourth-generation inhibitors designed for low pharmacokinetic interactions, as well as tight binding with target proteins. According to researchers in Gottesman’s laboratory, promising candidates include tarquidar, a compound that offers extended Pgp inhibition, now in phase III clinical trials, and CBT-1, a plant alkaloid that inhibits Pgp in addition to an ABC transporter known as MRP1.

The third strategy exploits certain unique features of multidrug-resistant cells, such as high surface expression of Pgp or a paradoxical hypersensitivity to a range of compounds. These features make it possible to target these cells directly, Gottesman said. For instance, thiosemicarbazones—a class of compounds with known antiviral, antimicrobial, and antitumor activity—kill resistant cells through Pgp-related mechanisms. This approach has not yet produced compounds for clinical trials.

Scientists have also focused on the influx mechanisms that cells use to absorb certain compounds, including cancer drugs, instead of the efflux pumps that they use to expel them. Solute-carrier (SLC) protein transporters, recognized as the largest superfamilies of membrane proteins, participate in these influx processes. Jeffrey Moscow, M.D., chief of pediatric hematology/oncology at the University of Kentucky Medical Center in Lexington, is now working to identify SLCs expressed uniquely by cancer cells. By harnessing these proteins, he hopes to pump drugs into a cancer cell faster than efflux transporters can pump them out. He’s already identified an SLC expressed uniquely by lung and gastrointestinal malignancies, known as OATB1B3, and another expressed by leukemia, known as SLC22A16. “In these cases, the specificity of therapy would be determined by the expression of the solute carriers,” he said.

Tackling Single-Drug Resistance
Efforts to overcome single-drug resistance to targeted therapies differ from those applied to multidrug resistance. Instead of circumventing, inhibiting, or targeting mechanisms that cancer cells use to avoid poisons, scientists try to augment their treatment options with additional molecular targets, said Jeffrey Settleman, Ph.D., a professor at Harvard Medical School and scientific director of the Massachusetts General Hospital Cancer Center. Settleman’s laboratory has accumulated the largest collection of human cancer cell lines in existence—more than 1,000 in all, representing all the major tissue types. With automated screening technology, he and his colleagues test putative targeted therapies until they find a positive hit in a sensitive cell line. Those lines are treated with the drug until only the resistant cells remain. By culturing those cells, Settleman’s team can look for mutations that confer resistance, as well as for new targets to which the cells might be sensitive.

The clinical strategy for managing resistance in targeted therapy, Settleman said, entails drug combinations to inhibit several targets sequentially. “The scenario could be that we convert cancer to a chronic disease by giving drugs in succession; as resistance develops to one drug, we shift to another,” he said. This approach is already being used now. For instance, CML patients who become resistant to imatinib can be treated with dasatinib (Sprycel).

In a melanoma cell line, Settleman’s laboratory recently modeled the acquisition of resistance to a candidate Raf kinase inhibitor. They found that resistant cells merely switched their metabolic dependency from Raf to another related kinase. “Raf kinase inhibitors are being tested clinically now,” Settleman said. “It’s early days for these compounds, and we’re not sure they’re going to work. But if they do, we may have zeroed in on the resistance mechanism in advance.”

The ability to switch from one kinase to another shows how adaptable cancer cells are in the face of drug pressure. But fortunately, it appears that cancer cells have at most three to four resistance mechanisms that they can direct against any particular compound, Settleman said. “And that shows we’re ultimately dealing with a manageable problem,” he said. “It indicates how important it is to tackle resistance from more than one angle. We’re faced with the same resistance mechanisms that infectious microbes use to evolve and mutate their way around a drug. We treat [human immunodeficiency virus] with multidrug cocktails, and we’ll be doing more of the same with cancer; our aim is to cut off the cells’ options to adapt.”

So, although the previous 10 years revealed new mechanisms in cancer cell biology, including roles played by cancer stem cells that could offer the best therapeutic targets of all, accelerated research during the coming decade might bring the problem of resistance under better control. But doing that won’t be easy, Settleman admitted. “Cancer cells are crafty organisms in their own right,” he said. “And resistance is still the final frontier in treatment.”

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