

Profile of Susan Band Horwitz

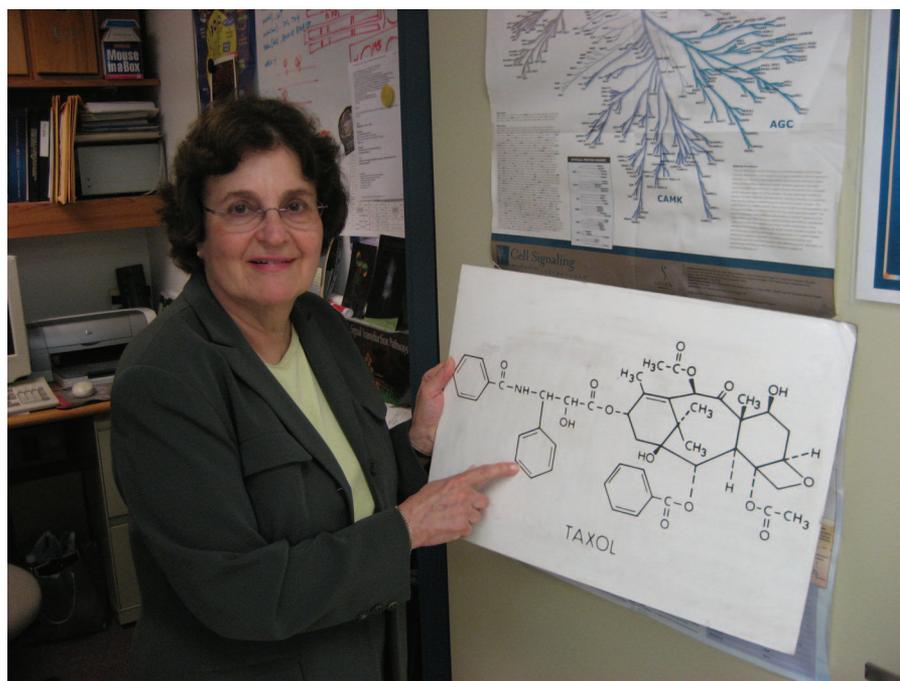
Taxol is one of the world's most successful cancer drugs, but it has not always held the spotlight. Up until 30 years ago, little was known about how Taxol (generic name, paclitaxel) exerted its anti-tumor effects. But in the 1970s, molecular pharmacologist Susan Band Horwitz investigated and explained Taxol's mechanism of action, work that was of interest to cell biologists and pharmacologists but almost completely ignored by the medical community and pharmaceutical industry at the time. Her research eventually led to the widespread clinical use of Taxol and its application in cancer therapeutics worldwide.

Elected to the National Academy of Sciences in 2005, Horwitz is Distinguished University Professor and Rose C. Falkenstein Professor of Cancer Research at Albert Einstein College of Medicine (Bronx, NY). She continues to study Taxol, the molecule whose unique and elegant structure first intrigued her 30 years ago. Taxol's antitumor activity is based on its ability to stabilize microtubules in tumor cells, promoting mitotic arrest and cell death. In her Inaugural Article in this issue of PNAS (1), Horwitz and her colleagues analyzed the structural changes in both β - and α -tubulin upon microtubule stabilization by Taxol. The work clearly demonstrates that this methodology can aid in the study of conformational effects induced by small molecules, such as Taxol, on microtubules.

History to Science

Horwitz was born in 1937 in Cambridge, MA. Growing up, she "was quite interested in history," Horwitz says, and did not think of science as a career option. She first began to develop an interest in science at Bryn Mawr College (Bryn Mawr, PA), which she entered in 1954 with the intent to major in history. Having attended a small high school in a suburb of Boston, Horwitz found that Bryn Mawr "opened up a whole new world for me," she says. After taking her first science requirement, a freshman biology class, Horwitz discovered that she enjoyed science and went on to major in biology. Learning how scientists think and formulate a hypothesis appealed to her. "I found the scientific method very attractive," she says.

Upon graduating with her bachelor's degree in biology in 1958, Horwitz applied for various science positions in the Boston area. But job prospects for graduates with a B.A. in biology were bleak. Horwitz recalls one particularly distaste-



Susan Band Horwitz

ful cosmetics-testing job that would have required her to insert dyes into the eyes of rabbits—a prospect that helped motivate her to apply to graduate school instead.

"At that time, there were few graduate schools that were very receptive to women," she recalls. "Women were not very prominent on the faculty or in the student body." One university stood out from the others, however. Brandeis University (Waltham, MA) had just started its graduate program in biochemistry. "Brandeis was a new and exciting place, and the people there wanted it to succeed," says Horwitz, "yet it also had a relaxed atmosphere that was really perfect for me."

Enzymes to Pharmacology

Horwitz joined the laboratory of the then-new department's chairman, Nathan O. Kaplan. With Kaplan, Horwitz studied hexitol dehydrogenases from the bacteria *Bacillus subtilis* and *Aerobacta aerogenes*, studying enzyme catalysis and kinetics (2). "I had every intention of going on in the area of enzyme mechanisms," she says. But, after her first year, Horwitz met her future husband, Marshall Horwitz, a medical student in Boston who had a summer fellowship to do research in biochemistry at Brandeis. They married in 1960, and 3 years later, a month before Hor-

witz received her Ph.D. in biochemistry, she gave birth to twin boys.

"Although I had arranged a postdoctoral position in enzyme kinetics, I realized that I could not go to a high-powered laboratory," she says. Horwitz asked for Kaplan's help in finding a 3-day-per-week position, to accommodate her family schedule. He spoke with a friend at Tufts University Medical School (Boston, MA), Maurice Friedkin, who was chairman of the Department of Pharmacology, and Horwitz soon joined Tufts' pharmacology department as a part-time postdoctoral fellow with Roy Kisliuk. Her project involved studying the antifolate properties of new compounds by using bacterial assays (3, 4). "I had known virtually nothing about pharmacology," Horwitz says, but she soon found that she liked it. "I loved the idea that small molecules could do great things," she says, "and I knew that I really wanted to stay in that field."

New York, Einstein, and the Letter

Upon leaving Tufts in 1965, Horwitz and her family moved to Atlanta, GA, where she worked as a part-time postdoctoral fellow at Emory University School of Medicine. In 1967, she moved

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 10166.

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Horwitz in the laboratory.

to New York for what was to be a temporary stay for her and her husband at the Albert Einstein College of Medicine. “Of course,” she notes, “we’ve never left.”

At Einstein, Horwitz continued as a research associate in the laboratory of Arthur Grollman, who shared her interest in small molecules. Horwitz was one of the first to study the anticancer agent camptothecin’s effects on DNA degradation in both cells and viruses (5, 6). When her twin sons entered first grade, Horwitz decided to work full time, and in 1970 became an Assistant Professor in the Department of Pharmacology.

In 1976, Horwitz received a letter that set her on a research path that she still follows today. “I received a letter from the National Cancer Institute in which they asked me to study Taxol, which I’d never heard of. There was only a single paper in the literature on Taxol (7),” she says. The drug had been isolated from the bark of the Pacific yew tree, *Taxus brevifolia*, and its chemical structure intrigued Horwitz. “It’s the kind of structure that only a tree would make,” she says. Horwitz published her first paper on Taxol in 1979 in *Nature* (8). “We were able to show a mechanism of action for this drug that had never been described before,” she says.

Using HeLa cells, Horwitz and then-doctoral student Peter Schiff found that Taxol stabilized microtubules. Microtubules are responsible for separating chromosomes during mitosis. Horwitz and Schiff found that Taxol caused the

cells to develop “a paralyzed cytoskeleton,” as Horwitz describes it, and the cells were unable to divide normally. Horwitz incubated the cells with Taxol and observed the effects using electron microscopy. As a reaction to the drug’s stabilization of the microtubules, the cells produced more tubulin, which itself also became stabilized. The cells appeared jam-packed with microtubules. “We knew we had something very special,” Horwitz says. “It was one of the most exciting times in my life.”

“We suggested that [Taxol] was a prototype for a new class of drugs,” she says, but no drug would be forthcoming for over 15 years. Horwitz explains that at the time, in the late 1970s, “there was very little interest in what I was doing.” She did not mind the lack of attention, however, because she believed that what she was doing was important and enjoyed doing the work for the National Cancer Institute and American Cancer Society. Although the funds to do the research existed for Horwitz, the expertise with organic chemistry was lacking for a molecule as complex as Taxol. When Horwitz needed assistance with some chemical aspect, such as photolabeling the molecule, she found pharmaceutical companies unwilling to lend a hand. Still, in the early 1980s, Taxol entered its first clinical trial.

Taxol is a highly hydrophobic molecule. “You cannot just dissolve it in saline and give it to patients,” Horwitz says. Therefore, the drug is administered with the solubilizing agent Cremo-

phor, which presents its own problems. “Taxol is by no means a cure,” cautions Horwitz. “Therefore, we’re interested in determining if there are novel drugs we can use in combination with Taxol.” Most of the choices for combinations with Taxol have been intuitively derived in the past, but Horwitz explains that there is now a push to combine Taxol with newer, targeted cancer drugs that alter defective signaling pathways. Together with colleague Hayley McDaid, Horwitz is investigating what she calls “rational combinations,” drugs that potentiate each other’s antitumor effects (9, 10). With the mouse as a model system, Horwitz can look at tumor growth and excise tumors during and after treatment to assess drug effect on various signaling pathways.

Fitting into a Pocket

In the 1990s, Horwitz began a project to determine the nature of the interaction of tubulin with Taxol, using photoaffinity analogs of the drug. Although Taxol clearly interacted with microtubules, a covalent bond was not being formed, and analyzing the binding of Taxol and tubulin was difficult. Photoaffinity labeling essentially forces the formation of covalent bonds by using light. For Horwitz, this multidisciplinary approach involved collaboration with a colleague at Einstein, George Orr. She continued to collaborate with Orr until his death in 2005. “He was a very close colleague and friend of mine,” Horwitz says.

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Together, Horwitz and Orr studied three different photoaffinity analogs of Taxol, each with a photolabel in a different place on the molecule. In addition, they added a radiolabel close to where they expected the Taxol–tubulin bond to occur. Labeled Taxol was incubated with tubulin and subjected to light to stimulate bonding, and the tubulin bound to Taxol was purified and sequenced. In this way, Horwitz and Orr narrowed down the points of interaction between Taxol and tubulin. “We now knew what parts of the microtubule were interacting with the drug,” she says (11–13). Concurrently, Eva Nogales and Ken Downing at the Lawrence Berkeley National Laboratory (Berkeley, CA) were using electron crystallography to study the structure of the tubulin dimer

with Taxol (14), and the data from both groups were complementary.

The work showed that the α - β tubulin dimer has a binding pocket for Taxol, but “you don’t get a dynamic picture of what was occurring,” Horwitz says. However, her PNAS Inaugural Article (1) provides “a dynamic picture of the conformational changes that occur in the protein in the presence of the drug,” she says. The technique used to elucidate the structural changes, hydrogen/deuterium exchange (HDX), was Orr’s idea, says Horwitz. In HDX, amide bonds are exchanged for deuterium. HDX was applied to cell microtubules both alone and in the presence of Taxol. Deuterium incorporation of the microtubules was then measured, providing a picture of the conformational changes between free tubulin and Taxol-bound tubulin. Taxol was found to modify tubulin conformation not just at the binding sites but at allosteric sites as well (1). The results indicated that, whereas in the absence of Taxol the tubulin dimers can adopt a curved conformation associated with the destabilization of microtubules, in the drug’s presence the dimer is locked into a straight conformation promoting assembly of microtubules and stabilization. Horwitz is proud of the work in her Inaugural Article, saying, “Frankly, I think the results are very exciting.” This finding also explained the puzzling mutations Horwitz and Chia-Ping Yang had found in α -tubulin (15) that conferred resistance to Taxol even though Taxol binds to β -tubulin.

This unique strategy “opens up new avenues of work that we can do with this technology,” says Horwitz. “With small molecules you can only go as far as the biochemistry has been developed.” Taxol has become a blockbuster cancer drug, and several drugs with similar mechanisms are currently in clinical

trials. Horwitz has worked on characterizing two of these novel drugs to see whether they have properties distinct from Taxol. “These new drugs are totally different in structure,” she says. One of these compounds, discodermolide, comes from the marine sponge *Discodermia dissoluta*. Horwitz has found that Taxol and discodermolide are synergistic, “which is surprising because drugs that work by the same mechanism are not usually synergistic,” she says (16, 17). Currently Horwitz wants to investigate whether the conformational changes that the two drugs induce in tubulin are distinct.

Flip Side

One of Horwitz’s long-term goals is to understand how tumors become resistant to drugs. She is curious about what she calls the “flip side” of antitumor drugs—when cancerous cells begin to evade the drugs’ cytotoxic effects. “Most cancers eventually develop resistance to chemotherapy. There are many alterations on a molecular level that occur in a drug-resistant cell,” she says. Overcoming drug resistance is difficult and complex, and Horwitz’s laboratory continues to study mechanisms of resistance, including the actions of *p*-glycoprotein, an ATP efflux pump that essentially keeps Taxol out of cells (18), and mutations in tubulin that make tubulin resistant to stabilization by the drug (15).

In addition to Taxol, Horwitz has studied the cytoskeleton of cells. “You want to work with small molecules, but you also have to work with the target so that you can understand the interactions better,” she explains. In particular, Horwitz has studied the isotypes of tubulin, of which there are six α and seven β isotypes. “Why are there all of these isotypes? Why are there so many post-translational modifications in tubulin? Does Taxol interact with all isotypes?”

she asks. With her colleague Pascal Verdier-Pinard, Horwitz is developing mass spectroscopy techniques to determine the isotypes and posttranslational modifications in tumors from individual patients (19).

Horwitz is proud of the high quality of work that her laboratory has produced. She tries to maintain a relaxed but intellectually stimulating environment, and a culture of transparency for research findings, in her laboratory. “I think that it’s important that my students and fellows enjoy coming to the laboratory everyday,” she says. Horwitz is also proud of her part in the Taxol story. “I didn’t discover Taxol or isolate it, but I tried to understand how it worked,” she says. “I feel that I have made some small contribution to the development of this drug.” Others feel the same way, as Horwitz has received countless honors. From 2002 to 2003, she led the American Association for Cancer Research as president, and in 2004, she received the Mayor’s Award for Science & Technology. Most recently, in 2006, Horwitz earned the Bristol-Myers Squibb Cancer Distinguished Achievement Award.

In addition to its use in cancer, Taxol is now used in coronary stents to prevent restenosis and is also being considered as a treatment for some neurodegenerative diseases. Horwitz points out what Taxol represents in its natural habitat. “All of the compounds that stabilize microtubules are from natural products,” she says, as well as from organisms that are sessile, suggesting that these molecules play a role in protecting the organism. She believes that the compounds have evolved along with the organisms and that is what helps make Taxol such a successful and effective therapeutic agent.

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