

DECEMBER 21, 2003

Researchers Identify Molecular Cause of Drug-Resistant Prostate Cancer

Howard Hughes Medical Institute (HHMI) researchers have discovered a surprisingly straightforward mechanism that causes prostate cancer cells to develop resistance to cancer-fighting drugs. The studies also point to specific ways to improve drugs to prevent the problem of drug resistance in prostate tumors.

The researchers describe the molecular mechanism of resistance to anti-androgen therapy for prostate cancer in an advance online publication in the December 21, 2003, issue of the journal *Nature Medicine*.

"While drug therapy works in almost everyone for a period of time — usually measured in years — it stops working, despite the fact that patients continue to take the drugs."

— Charles L. Sawyers

HHMI investigator Charles L. Sawyers at the Jonsson Comprehensive Cancer Center at the University of California, Los Angeles, led the research. Sawyers collaborated on the studies with HHMI investigator Michael G. Rosenfeld at the University of California, San Diego. Co-lead authors were Charlie Chen and Derek Welsbie of Sawyers' laboratory. Another co-author on the paper is from the University of Washington in Seattle.

Sawyers and his colleagues were trying to understand why drug therapy for prostate cancer often fails despite early success. The current "gold standard" for treatment of prostate cancer consists of a drug regimen that lowers testosterone levels administered with "anti-androgen" drugs. These drugs compete for the binding site on the testosterone receptor proteins located in prostate-cancer cells. When testosterone activates these receptors, they, in turn, switch on internal cellular machinery that drives the growth of the tumors.

"While drug therapy works in almost everyone for a period of time — usually measured in years — it stops working, despite the fact that patients continue to take the drugs," said Sawyers. "And that is why men die of this disease."

According to Sawyers, the tumor cells become "hormone-refractory," meaning they somehow "learn" to continue to proliferate even in the absence of the hormone, androgen, which is their normal growth signal.

The scientists reasoned that one of the ways to get at the molecular signals driving drug resistance was to use DNA microarray technology to look for specific genes that are switched on only in drug-resistant prostate cancer cells. DNA microarrays measure the relative levels of gene expression in cells.

Before the researchers could look for genes that are switched on in drug-resistant cells, they first used a technique called xenografting to establish hormone-sensitive human prostate cancers in mice. After the human tumors were established in the mice, the scientists treated the mice so they had lower androgen levels, to cause the cancers to progress to a drug-resistant state.

"This process mimics what happens in patients," said Sawyers. "And the advantage for gene-profiling studies is that we end up with hormone-refractory cells that are directly derived from hormone-sensitive cells. So, they are otherwise genetically matched."

When the researchers used DNA microarrays, or "gene chips" to compare gene expression in seven different sets of the two genetically matched types of prostate cancers, they found a surprise.

"The microarray data pointed us to just one consistent change among all the xenografts," said Sawyers. "And that was a change in the expression of the gene for the androgen receptor itself." According to Sawyers, the identification of a single difference between hormone-sensitive and hormone-refractory cancers was entirely unexpected.

"We never really required that there even be one consistent change," said Sawyers. "We were fully prepared to find a signature of expression differences in some of the xenografts and another signature in others."

The researchers then performed additional experiments in which they established that the alteration in the androgen receptor gene produced functional changes in the cancer cells. Those studies showed that when the scientists engineered hormone-sensitive cells to overexpress genes for the androgen receptor, the cells behaved like hormone-refractory cells. Conversely, when they reduced receptor gene expression in hormone-refractory cells, those cells began to behave like hormone-sensitive cells.

They also established that the over-expressed hormone receptor still required binding by the ligand androgen in order to become hormone-refractory. "This finding is perhaps the most important," said Sawyers. "Everyone, including me, would have thought that you did not need to have ligand binding if you overexpressed the receptor. The fact that it is still required is a surprise, and it is very important for drug discovery. The current anti-androgen drugs work

by competing for this ligand-binding site, and this finding means that the site is still a key target for improved drugs."

According to the researchers, the fact that the receptors still require their hormone ligands means that receptor-overexpressing tumor cells are likely activated by even low levels of androgen in patients who are already being treated to reduce their testosterone levels. Other experiments by the researchers revealed that the normal cell-signaling machinery was still involved in the cancer cells' response to androgens.

The studies also revealed that higher levels of receptors somehow convert anti-androgen drugs into "agonist" drugs that activate receptors. "It's counterintuitive, and therefore quite surprising," said Rosenfeld. "We now have only a few clues about how this conversion occurs. Our guess is that as the level of androgen receptors increases, they can no longer recruit the co-repressor apparatus to a DNA-bound receptor in the cell that the antagonists recruit to prevent the actions of agonists. The exquisite sensitivity of prostatic cells to androgen that drives their proliferation may explain the uniform selection of high levels of androgen receptor in `resistance.'"

Sawyers and Rosenfeld are now collaborating on studies to determine how this conversion arises. Their hope is that they can use information gleaned from their research to identify a new generation of androgen antagonists that will be more effective while hopefully avoiding the phenomenon of conversion.