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Plant Compound Kills Brain Tumor Cells

A chemical isolated from a weed that grows in mountain meadows in the western United States kills the cells of an aggressive brain cancer that affects some children. The compound, cyclopamine, blocks a signaling pathway that appears to be important for the survival of medulloblastoma, a form of cancer for which there is no effective treatment.

In an article published in the August 30, 2002, issue of the journal *Science*, a research team led by Howard Hughes Medical Institute investigator Philip A. Beachy reported that cyclopamine effectively killed cultured mouse medulloblastoma cells and tumors implanted in animals, as well as medulloblastoma cells extracted from human tumors.

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— Philip A. Beachy

It will be difficult to obtain sufficient quantities of cyclopamine, since it must be extracted and purified from the plant source, *Veratrum californicum*, the corn lily, said Beachy, who is at The Johns Hopkins University School of Medicine. However, we believe that with this study, the evidence is in place to justify an effort to develop a supply so that it can be tested in humans. Beachy and his colleagues at Johns Hopkins collaborated with researchers from the Fred Hutchinson Cancer Research Center and the University of Washington/Childrens Hospital in Seattle.

Beachy said there are some parallels between cyclopamine and taxol, a drug used to treat breast cancer drug that was initially in short supply because it had to be isolated from the bark of the Pacific yew tree. However, as taxol proved clinically effective, researchers developed an alternate method of partial synthesis of the compound from a more plentiful precursor in the needles that made the drug available in sufficient supply.

Beachy and his colleagues began to explore whether cyclopamine would be effective against medulloblastoma after studies by several groups, including HHMI investigator Matthew Scott and his colleagues at Stanford University,

showed that both animals and humans developed tumors, including medulloblastomas, when the Hedgehog signaling pathway was activated. The pathway — named for its key regulatory protein Hedgehog — has long been known to be critical for the growth and differentiation of cells during embryonic development. Scott and his colleagues showed that the tumors they studied consisted of cells that had most likely reverted to a highly proliferative embryonic state, due to a mutation that enabled the activation of the Hedgehog pathway.

The earlier studies showed that the Hedgehog pathway was switched on when the function of one of the pathways key genes, called *Patched*, was lost because of mutation. The protein produced by *Patched* normally represses a downstream member of the Hedgehog pathway — a protein called Smoothed. Loss of *Patched* activates the Smoothed protein, turning on the Hedgehog pathway and leading to malignancy.

Beachy and his colleagues had shown previously that cyclopamine blocks the Hedgehog pathway in mouse embryos by inhibiting the activity of Smoothed. Reasoning that the drug might also prevent activation of the Hedgehog pathway in tumors, the scientists tested cyclopamine's effects on a mouse model developed in Scott's laboratory, in which one copy of the *Patched* gene had been disrupted. In these mice, Beachy and his colleagues also removed the gene for p53, a protein that normally triggers the death of cells with damaging mutations. The mice developed medulloblastomas at a young age when expression of the second normal *Patched* gene was inactivated.

The researchers cultured medulloblastoma cells from the mice and introduced those tumor cells into other mice that had compromised immune systems. We showed that in these tumor cells we could readily suppress the Hedgehog pathway by treating the tumors with cyclopamine, said Beachy. We next decided to see whether the drug would affect tumors that had already been established in mice by injecting the tumor cells. We were pleased to see that cyclopamine could not only block the Hedgehog pathway, but could also stop the growth of these tumors and even cause them to regress, he said.

The next logical step, said Beachy, was to determine whether cyclopamine was effective in human medulloblastomas. For these studies, co-author James Olson and colleagues at the Fred Hutchinson Cancer Research Center supplied medulloblastoma cells from patients who had undergone surgery to remove the tumors.

When we treated dispersed cells from these tumors with cyclopamine, they died very quickly, and in fact, the drug appeared to be killing the cells faster than any drug Jim Olson and his colleagues had yet tested, said Beachy. Also promising, said Beachy, was that in the animal studies, the drug produced no discernible side effects. Of course, we can't be sure that there are no side effects at this point, since we can't ask a mouse how it's feeling, said Beachy. But we saw no obvious adverse effects from the treatment.

One promising result from the studies of human tumor cells, said Beachy, was that all seven of the human medulloblastomas the scientists tested responded dramatically to cyclopamine. Genetic studies have shown that only perhaps twenty percent of such sporadic tumors can be assigned to mutations that specifically activate the Hedgehog pathway, he said. So, this finding suggests that perhaps activation of the Hedgehog pathway is essential to tumorigenesis, even when it is not specifically switched on by mutation.

Although Beachy advocates immediate pre-clinical and clinical trials of cyclopamine for medulloblastoma, he cautioned that supply is a critical problem. Right now, cyclopamine must be purified from the corn lily, and it is unclear how much could be harvested or cultivated, said Beachy. Synthesizing cyclopamine might be possible, but it would be very difficult.