

Researchers developing technology to outsmart metastasized cancers

WEST LAFAYETTE, Ind. — News that a malignant tumor has spread to other parts of the body seems like a death knell to the ears of many cancer patients. But Endocyte Inc. and Purdue University researchers are developing treatment methods that may cure some cancers, even those that have reached an advanced stage.

Many types of cancer cells have a great affinity for folate — a form of water-soluble B vitamin because they need the nutrient in order to grow and divide. In fact, cancer cells have evolved a mechanism to capture folate more effectively than normal cells. Because of this selectivity, researchers have developed a way to trick cancer cells into attracting and even ingesting anticancer agents that are attached to folate molecules. As a result, these chemotherapeutic agents can be delivered more specifically to cancer cells while leaving the surrounding normal tissue unharmed.

One form of this "Trojan Horse" therapeutic approach (folate-targeted immunotherapy) was used by a joint Purdue-Endocyte research team to successfully treat more than 200 mice with late-stage metastatic disease. The results of these studies were published in the May Journal of Cancer Immunology and Immunotherapy.

"It's using cancer's nutritional needs against itself," said Philip Low, Purdue's Joseph F. Foster Distinguished Professor of Chemistry who led the research team that discovered this diagnostic and treatment method. "We are essentially slipping medicine in with cancer's favorite food." The discovery has thus far yielded two different but complementary treatment methods that involve attaching various markers (folate-targeted immunotherapy) or anticancer agents (folate-targeted chemotherapy) to the vitamin.

The treatment method that "marks" cancer will be tested in Phase I Food and Drug Administration-regulated human clinical trials beginning in November. The objective of this method is to force the body's immune system to fight the disease, said Christopher Leamon, Endocyte's vice president of research.

"There's no better drug than your own immune system, which consequently is capable of getting rid of every last bacterium, every last virus or every last fungus in the body. Today's drugs can't do that," Leamon said. "Unfortunately, many cancers develop ways to evade immune surveillance. But we've found a way to redirect a patient's immune system to kill those resistant cancer cells by using our folate-targeted approach."

Researchers have found that certain cancers — among them ovarian cancer and renal cell carcinoma — have high levels of folate receptors. These receptors are located on the surfaces of cancer cells and are responsible for binding folate in a very specific manner, similar to the way a key is inserted into a lock. Cancer cells that are receptive to folate often express more folate receptors in secondary (metastasized) sites formed once the cancer becomes widely disseminated throughout the body.

Researchers hoped to bypass normal, healthy cells by delivering folate linked to an anticancer agent directly into the cancer. They discovered, though, that cancer cells don't take in all of the folate at once, but rather wear the excess folate on their cell surfaces, storing it for future use. Some of the drugs they attach to folate, consequently, also are stored on the cancer cell surfaces.

Taking advantage of this discovery, Purdue researchers and Endocyte scientists devised an alternative way to fight the cancer cells. One of their strategies involves priming the body's immune system to fight a certain substance or "beacon," and then attaching that beacon to the cancer using folate. The beacon would enable the immune system to "see" the cancer and begin to remove it.

"We essentially decorated the cancer with an inert chemical the body was programmed by our vaccine to fight," Leamon said. "By retargeting the immune system, its natural killer (NK) cells and macrophages were alerted to intruders and eliminated the cancer along with them."

To retarget the immune system, scientists first inoculated mice to raise antibodies against a hapten (a small, inert organic chemical) called fluorescein. Once high levels of the antibodies were circulating throughout the rodents' systems, cultured cancer cells were injected. Tumors were allowed to establish themselves and spread throughout their hosts. Normally this would cause death in 21 days, however, the

mice that were injected with the folate-fluorescein conjugate eight days after the injection of cancer cells lived well beyond 120 days (a point at which the animals were considered to be cured).

Another part of Endocyte's technology stems first from its ability to diagnose cancer. In order to determine which cancers have folate receptors and, therefore, are receptive to a folate-related treatment, cancer must be detected by using a folate-related diagnostic procedure Endocyte developed called FolateScan. This diagnostic method now is being tested in Phase I/II FDA-regulated human clinical trials.

To diagnose cancer, another type of "beacon" — this time a radioactive imaging agent — is attached to the folate. FolateScan is administered intravenously to patients. When nuclear imaging of the patient shows that the radio-labeled folate conjugate has concentrated in an unknown mass, it tells researchers that the mass has folate receptors and that the tissue is malignant. More importantly, it means that the cancer detected is the type that may be receptive to a folate-related therapy.

Researchers initially used the radioactive agent indium to detect folate-receiving cancers, but then developed a new diagnostic approach with technetium. Technetium decays more rapidly and decreases the patient's exposure time to the radioactive agent from one month to two days. The shorter half-life of technetium also allows researchers to more safely inject patients with larger quantities of radioactive agents in order to improve the quality of the scanned image. Preclinical results of this research are expected to be published this fall.

Founded in 1996, Endocyte is located in the Purdue Research Park. Purdue has licensed the drug delivery technology to Endocyte under an exclusive commercial arrangement. The company employs 24 people.

Related Web sites:

Endocyte: <http://www.endocyte.com>

Purdue Research Park: <http://www.adpc.purdue.edu/PRF/Welcome>

PHOTO CAPTIONS:

Graphic illustrates a Purdue University-licensed immunotherapy treatment method, which forces the immune system to fight cancer by inoculating the body against an inert hapten (fluorescein), and then marking the cancer cells with that hapten. Endocyte scientists developing the treatment can mark the cancer by attaching that hapten to one of cancer's favorite foods (folate). (Illustration by Endocyte Inc.)

A publication-quality photograph is available at

<ftp://ftp.purdue.edu/pub/uns/endocyte.metastasized.jpeg>

Christopher Leamon, Endocyte's vice president of research, works to develop Purdue University-licensed technology that involves attaching various markers to the vitamin folate to enable the body's immune system to "see" cancer and eradicate it without damage to surrounding tissues. (Purdue News Service Photo by David Umberger)

A publication-quality photograph is available at <ftp://ftp.purdue.edu/pub/uns/endocyte.leamon.jpeg>.

ABSTRACT

Folate targeting of haptens to cancer cell surfaces mediates immunotherapy of syngeneic murine tumors

Authors - Yingjuan Lu and Philip S. Low

A variety of human cancers over-express a cell surface receptor with high affinity for the vitamin, folic acid ($K_d \sim 10^{-10}$ M). Covalent attachment of folic acid to virtually any molecule or particle has been shown to yield a conjugate/complex that can bind to and enter folate receptor-bearing cells via the same endocytic pathway followed by free folic acid. Here we report on the use of folic acid to target covalently attached imaging and immunotherapeutic agents to cancer tissues *in vivo*. Using both optical and radio imaging agents (with the aid of laser and gamma scintigraphic instruments, respectively), we first demonstrate that folate conjugates concentrate in a variety of tumors in both humans and mouse tumor models. We then demonstrate that this tumor-specific delivery can be utilized to convert poorly immunogenic tumors into highly immunogenic target tissues. By linking folic acid to a novel hapten, we have been able to decorate folate receptor-expressing cancer cell surfaces with $>10^6$ haptens/cell *in vivo*.

Following marking of such cells with haptens, the cells are observed to become opsonized with autologous anti-hapten antibodies, which in turn mediate the cells' removal via antibody-dependent cellular cytotoxicity (ADCC). Supplemental administration of low levels of ADCC-activating cytokines (e.g. IL-2 and IFN-) is shown to synergize with the folate-targeted immunotherapy. Thus, using M109 syngeneic lung cancer cells injected intraperitoneally into Balb/c mice that were previously immunized against fluorescein, a significant extension of lifespan is observed following treatment with folate-fluorescein conjugates, and complete cures are observed upon supplementation with moderate levels of IL-2 and IFN-. Because control tumor-bearing mice treated with the same cytokines, but with non-targeted fluorescein, show minor extension of lifespan, we conclude that tumor-specific opsonization is an essential step in the immunotherapy. Finally, because the anti-fluorescein antibodies are unable to access the folate receptors on the apical membranes of the kidney proximal tubules, no kidney or other normal tissue cytotoxicity is observed. These data suggest that retargeting of haptens to folate receptor-expressing cancers might constitute a method for mobilizing the immune system specifically against poorly immunogenic tumors.

ABSTRACT**Folate Synthesis and biological evaluation of EC20: A new folate-derived, ^{99m}Tc-based radiopharmaceutical**

Authors - Christopher P. Leamon, Matthew A. Parker, Iontcho R. Vlahov, Le-Cun Xu, Joeseeph A. Reddy, Marilyn Vetzal, and Nikki Douglas*

A new peptide derivative of folic acid was designed to efficiently coordinate ^{99m}Tc. This new chelate, referred to as EC20, was found to bind cultured folate receptor (FR)-positive tumor cells in both a time- and concentration-dependent manner with very high affinity ($K_D \sim 3$ nM). Using an *in vitro* relative affinity assay, EC20 was also found to effectively compete with ³H-folic acid for cell binding when presented either alone or as a formulated metal chelate. Following intravenous injection into Balb/c mice, ^{99m}Tc-EC20 was rapidly removed from circulation (plasma $t_{1/2} \sim 4$ min) and excreted into the urine in a non-metabolized form. Data from gamma scintigraphic and quantitative biodistribution studies performed in M109 tumor-bearing Balb/c mice confirmed that ^{99m}Tc-EC20 predominantly accumulates in FR-positive tumor and kidney tissues. These results suggest that ^{99m}Tc-EC20 may be clinically useful as a non-invasive radiodiagnostic imaging agent for the detection of FR-positive human cancers.