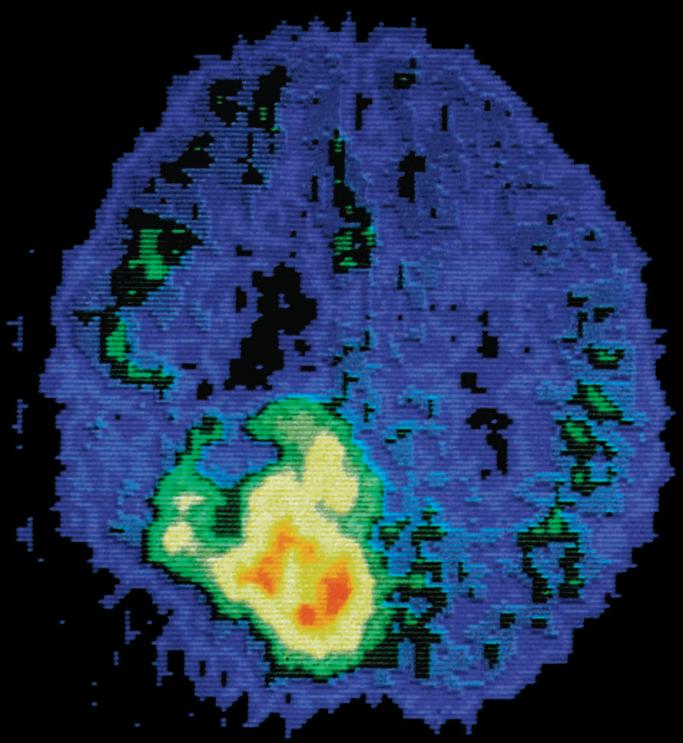

Unraveling the Obesity-Cancer Connection

A growing body of research shows that insulin and a related hormone play a key role in fueling tumors. They also may be a link between obesity, diabetes, and cancer

GROWING BREAST CANCER CELLS IN THE lab has been a revelation to Vuk Stambolic. The protocol he follows is decades old and widely used, but there's a puzzle at its core. The recipe calls for a large dose of glucose, a growth factor called EGF, and insulin. Add these to tissue culture, and tumor cells will be fruitful and multiply. A curious thing happens if you try to wean the tumor cells off insulin, however: They "drop off and they die," says Stambolic, a cancer researcher at the University of Toronto in Canada. "They're addicted to [insulin]."

What makes this so "bizarre," Stambolic says, is that this behavior is totally unlike that of the healthy breast cells from which these tumor cells are derived. Normal cells are not sensitive to insulin—or at least not nearly to the same degree. They don't have insulin receptors, and they lack key elements of the insulin signaling pathway necessary to make insulin outside the cell immediately relevant to what goes on inside. Indeed, normal cells thrive without insulin. By contrast, the tumor cells in culture can't live without it.

This observation, although not original, is one of the insights that drew Stambolic to investigate the tumor-promoting effects of insulin. It has led him to spend the past decade studying a signaling pathway that is activated by insulin in healthy muscle, fat, and liver cells. Named for one of its key components—the PI3 kinase pathway—it also happens to be among the most frequently mutated pathways in human cancers.

Peculiar dependency. Vuk Stambolic says that breast tumor cells seem "addicted" to insulin.

Insulin, a hormone produced in the pancreas, is more commonly known for its role in diabetes. But its reputation may be changing. Insulin and a related hormone known as insulin-like growth factor (IGF) are now at the center of a growing wave of research around the world aimed at elucidating what many scientists consider to be their critical role in fueling a wide range of cancers. Elevated levels of insulin and IGF are also the leading candidates to explain a significant correlation in epidemiology that has gained attention over the past 30 years: Obese and diabetic individuals have a far higher risk than lean healthy people of getting cancer, and when they do get it, their risk of dying from it is greater. And now that obesity and diabetes rates are skyrocketing, the need to understand this link has become far more urgent.

High burn. A PET scan lights up the brain where cancer cells are consuming glucose at a rapid rate.

Online
sciencemag.org
Podcast interview with author Gary Taubes.

The correlation between obesity and cancer can be found in the medical literature going back for several decades. But it wasn't until 2004 that two cancer epidemiologists put it all together, says Robert Weinberg, a cancer researcher at the Massachusetts Institute of Technology (MIT) in Cambridge. An article that year in *Nature Reviews Cancer* by Rudolf Kaaks, then of the International Agency for Research on Cancer, and the late Eugenia

Continued on page 30

CREDITS (TOP TO BOTTOM): SCOTT CAMAZINE/NEWSCOM; SASHA PURIC

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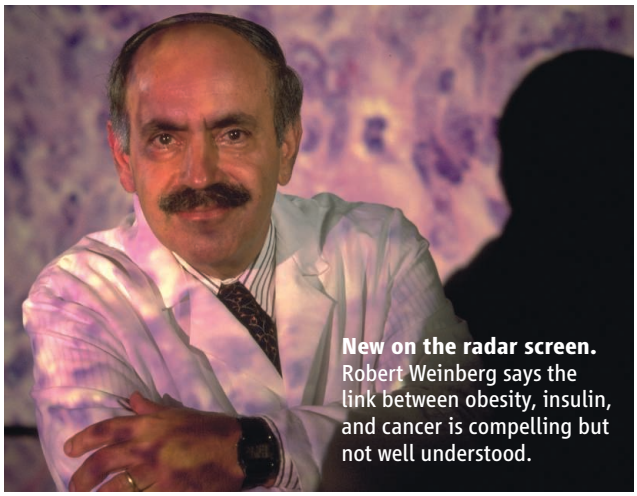
Calle of the American Cancer Society "laid down a challenge to the rest of us … to determine why obesity is such an important determinant of cancer risk," Weinberg says.

The message of this research is straightforward, Kaaks says: Excess body fat seems to account for between one-quarter and one-half of the occurrence of many frequent cancer types—breast, colorectal, endometrial, renal cell, and adenocarcinoma in the esophagus, in particular. Kaaks adds, "The list is growing."



New on the radar screen. Robert Weinberg says the link between obesity, insulin, and cancer is compelling but not well understood.

"The magnitude of the effect is huge," in large part because obesity and diabetes are now so common, says Michael Pollak, an oncologist at McGill University in Montreal, Canada. It seems that cancer "loves the metabolic environment of the obese person," Pollak says. Epidemiologic studies have also found that not only is type 2 diabetes associated with increased cancer incidence and mortality but so are circulating levels of insulin and IGF.

Recent drug studies have sharpened the picture: Type 2 diabetics who get insulin therapy or drugs to stimulate insulin secretion have a significantly higher incidence of cancer than those who get metformin, a drug that works to lower insulin levels (see sidebar on metformin, p. 29). There's a large and growing body of evidence implicating insulin and IGF in cancer, Pollak says, "and it's causing a lot of people to stay up at night thinking about it."

Parallel worlds

Researchers have recently upped their interest in the idea that insulin and IGF drive cancer in part because other hypotheses of cancer causation have failed to pan out. W. Robert Bruce, for instance, a cancer researcher at the University of Toronto, embarked in the late 1970s on what he described as a lengthy and fruitless search for mutagens in the diet and environment that might be responsible for colon cancer. "About a ton of feces later," he says, he had found nothing.

Now many cancer researchers, including Bruce, have come to believe that, whatever the carcinogenic substances or factors are, they mostly work not by directly damaging DNA but by promoting tumor development through a change in the hormonal environment around incipient tumor cells, increasing, for instance, insulin and IGF levels in the circulation. "There is a change in the endocrine and growth factor environment of cells," Kaaks says, "that pushes cells to proliferate further and grow more easily" and to evade built-in programs that cause normal cells to die.

To learn about the research on insulin, IGF, and cancer, Bruce says he had to read the diabetes literature—and what he found was a parallel universe. "It was a complete edifice of research in and of itself, not linked by any papers with the edifice of research in the cancer field—two big towers."

A few bridges have been built between these two parallel worlds, however. One connects cancer to diet and obesity. It was not obesity's harmful effects that first drew cancer researchers' attention but the flip side: the observation that tumor growth in animals is inhibited if not prevented entirely if the animals are semistarved. Peyton Rous, who would later win the Nobel Prize for his discovery of tumor-causing viruses, was the first to make the observation. It was confirmed in 1942 by Albert Tannenbaum, a Chicago pathologist, who demonstrated that feeding rats a diet just sufficient to keep them alive markedly increased their life span, in part by inhibiting tumors.

Bad signals. Lewis Cantley suspects that a dysfunctional PI3K pathway may be behind many cancers.

Tannenbaum suggested that a likely mechanism was a phenomenon known as the Warburg effect, in which cancers adopt an inefficient type of metabolism commonly used by bacteria, known as aerobic glycolysis (see sidebar, p. 31). It goes along with a significant increase in the use of glucose for fuel by the cancer cells. In semistarved, growth-stunted animals, Tannenbaum proposed, the tumors could not obtain the huge amounts of blood sugar they need to fuel mitosis, division of the nucleus, and continue proliferating.

In the decades since, researchers have debated whether the amount of blood sugar available to the tumor could be a driving or limiting factor in tumor development. But positron emission tomography scans of patients given fluorodeoxyglucose, a traceable analog of glucose, show that tumors continue to burn high amounts of glucose even if the blood glucose levels in the patients themselves are relatively low. "There's always plenty of glucose around," says Chi Dang, a cancer researcher at Johns Hopkins University (JHU) in Baltimore, Maryland, "so it's got to be something else" fueling the tumors.

Cancer accelerant

That insulin and IGF may be the relevant "something else" that fuels cancer is a relatively new idea. But the evidence, as Bruce points out, has been accumulating for decades. In the mid-1960s, researchers demonstrated that insulin acts as a promoter of growth and proliferation in both healthy and malignant tissues. By the late 1970s, C. Kent Osborne, then at the National Cancer Institute, and his colleagues reported that a line of particularly aggressive breast cancer cells were "exquisitely sensitive to insulin" and that breast cancer cells express insulin receptors, even though the cells from which the tumors derive do not.

"You find the highest level of insulin receptors in liver, muscle, and fat tissue naturally," says Lewis Cantley, director of the Beth Israel Deaconess Medical Center at Harvard Medical School in Boston. Cantley originally trained as a biophysical chemist and found himself working on the link between obesity, diabetes, and cancer when he started studying

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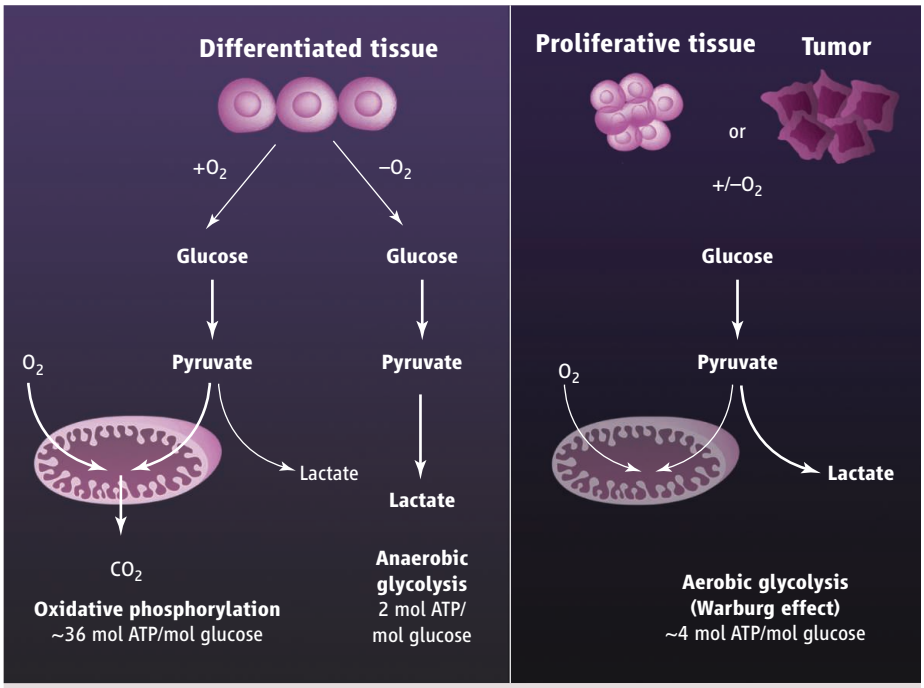

Warburg effect. Healthy tissues (*left*) get energy through the efficient process known as oxidative phosphorylation. Tumors (*right*) use aerobic glycolysis, the so-called Warburg effect, which is inefficient but seems to enable proliferation.

how hormones and growth factors regulate cell metabolism. Low levels of insulin receptors, he says, can be found in half a dozen other healthy tissues as well, but that's it. So the abundant presence of insulin receptors in prostate cancer, colorectal cancer, and breast cancer cells, among other cancers, is significant, Cantley says: "They must be there for a reason; they must be helping to grow the tumor. And one thing they're doing is giving cancer cells the ability to take up glucose at a higher rate."

The second suspect in this scenario, the hormone IGF, was discovered in the late 1950s; its designation "insulin-like" wasn't made for another 20 years after that. IGF's structure is similar to that of insulin, and its effects can mimic those of insulin. But its secretion is stimulated by growth hormone, says Derek LeRoith, a diabetologist who now runs the Metabolism Institute at the Mount Sinai Medical Center in New York City.

In the early 1980s, researchers discovered that tumor cells typically have two to three times as many IGF receptors as healthy cells, making them much more responsive to the IGF in their immediate environment. In rodents, functioning IGF receptors appear to be a virtual necessity for cancer growth, according to Renato Baserga of Thomas Jefferson University in Philadelphia, Pennsylvania, who "stumbled" upon the discovery in the late 1980s. Shutting down the IGF receptor in mice leads to what Baserga calls "strong inhibition, if not total suppression of [tumor] growth"; it is particularly lethal to tumors that have already metastasized from a primary site elsewhere in the body.

LeRoith has genetically engineered mice so that their livers do not secrete IGF, resulting in one-quarter of the IGF concentration in their circulation compared with normal mice. When colon or mammary tumors are transplanted into these mice, according to LeRoith, both tumor growth and metastasis are significantly slower than when identical tumors are implanted in normal mice with normal IGF levels. When IGF is injected into these genetically engineered mice, tumor growth and metastasis accelerate.

The consensus among those researchers studying the role of insulin and IGF in cancer is that these hormones supply both the fuel necessary for tumors to divide and multiply and the signals to continue doing it.

CREDIT: ADAPTED FROM M. G. VANDER HEIDEN *ET AL.*, *SCIENCE* **324** (22 MAY 2009)

Ravenous for Glucose

The focus on obesity, cancer, and hormones has kindled a wide interest in the metabolism of cancer cells and particularly in work done in the 1920s by the German biochemist and later Nobel laureate Otto Warburg. Warburg observed that tumor cells can survive without oxygen and generate energy by a relatively inefficient process known as aerobic glycolysis. This conversion of cancer cell metabolism to aerobic glycolysis has been known as the Warburg effect ever since. It is akin to how bacteria generate energy in the absence of oxygen, although cancer cells do it even when oxygen is present (hence "aerobic"). Rather than converting glucose to pyruvate and burning that with oxygen in the cells' mitochondria, the pyruvate is converted to lactate in the cells' cytoplasm outside the mitochondria, and no oxygen is used. The process yields only one-ninth the energy, four ATP molecules instead of 36, from each molecule of glucose.

One result is that cancer cells have to burn enormous amounts of glucose to thrive and multiply. This abnormally high glucose consumption is what's detected by the imaging technology known as FDG PET when it's used to identify where tumors might have spread in the body. Warburg hypothesized that the high-glucose metabolism is what drives cancer. But there has always been, and still is, significant controversy about why cancer cells use it: What's in it for them, if it's such an inefficient means of supplying energy? And how can we tell whether it is a byproduct of the cancer or a cause? Most researchers studying the Warburg effect now believe that the signaling pathways driving it are the insulin and insulin-like growth factor pathways. The question they're still hoping to answer is which comes first: the metabolism change or the cancer? **–G.T.**

A third conspirator

An additional player has been identified as a key member of this particular network that influences metabolism, growth, and cancer: the enzyme PI3 kinase, discovered by Cantley and his colleagues in the mid-1980s. PI3K lies in the insulin signaling pathway and is activated by both insulin and IGF. Through its effect on other molecules, PI3K effectively regulates a cell's sensitivity to insulin. When PI3K is activated, insulin is more effective at stimulating the transport of glucose into cells.

PI3K also turns out to play a major role in cancer—a discovery that came in a series of steps in the late 1990s. The finding that "put PI3K on everybody's radar screen as something important in human cancer," Cantley says, was the realization that it is linked with a tumor suppressor gene called *PTEN*. Identified in 1997, *PTEN* is "the most frequently deleted gene in a whole host of advanced human cancers," Cantley says.

When researchers set out to elucidate what exactly the intact *PTEN* was doing to suppress tumors, they learned that it counteracts the work of PI3K. It removes a phosphorus atom from the fat molecule that PI3K makes, an effect equivalent to decreasing the influ-

ence of PI3K itself. And it turns out, as Victor Velculescu, a geneticist at JHU, has demonstrated, PI3K itself is commonly mutated—in a way that bypasses normal mechanisms for turning it off—in colon cancer and a host of other cancers as well, including breast, lung, brain, and ovarian cancers.

The point, Cantley says, is that researchers have identified two general ways that work to step up activation of the PI3K pathway: by mutations such as those that alter *PTEN* or by abnormally elevated levels of insulin and IGF in the circulation. Most obese individuals have elevated insulin and IGF levels, as do type 2 diabetics. When PI3K signaling is increased, cells take up more glucose and may convert to a high-glucose metabolism—the aerobic glycolysis described by Warburg. It may be an inefficient means of generating energy, Cantley says, but it doesn't matter to the cell because the insulin makes sure it has considerable glucose to burn.

So what's in it for the cancer cells? The answer, according to Cantley, appears to be that the carbon backbones of the glucose molecules are shunted aside during aerobic glycolysis rather than burned for fuel, and these carbon backbones can then be used to make new fatty acids.

Normal fat cells do the same thing when they burn glucose: They preserve the carbon backbone for storing fatty acids as triglycerides, Cantley says. In cancer cells, the fatty acids are used to build new membranes for daughter cells. The glucose is also used to make new DNA and protein for the cells. So the cancer cells are effectively trading off an inefficient means of producing energy for a means of obtaining the resources necessary to create new cancer cells. It's a tradeoff they can easily afford because there's so much glucose now pouring in. "Remember, cancer cells have to duplicate themselves," JHU's Dang says. "So this way you see the interplay between energy production and at the same time providing the skeletons, the building blocks for the cancer cells."

These researchers now suggest that it may make sense to divide tumors into two types, like diabetes: insulin-dependent and insulin-nondependent. If there are no mutations enhancing the activity of the PI3K pathway, Pollak says, then the cancer process will be dependent on the insulin and IGF in the circulation. "But if PI3K is mutated," he says, "that cell is going to be highly proliferative, highly aggressive, and it couldn't give a damn about the insulin environment."

Recent evidence of the power of this signaling pathway comes from work by David Sabatini of MIT's Whitehead Institute and Nada Kalaany, who's now at Children's Hospital Boston. In 2009, they showed that PI3K appears to determine whether a tumor responds to calorie restriction. When they induced different types of human cancers in mice and then put the mice on semistarvation diets, some of the tumors shrank in response and some didn't. Tumors grew less in the mice with low PI3K pathway activity, more in those with high activity.

Full circle. By tweaking insulin signaling, Craig Thompson and others have induced high-glucose metabolism in mice.

For cancers with one of the mutations that activates the PI3K pathway, Sabatini says, calorie restriction has little to no effect because the insulin signaling is turned on anyway. These cancers are resistant to changes in insulin levels. "In obesity," Sabatini says, "there are many things going on, but one of them is hyperinsulinemia [high circulating levels of insulin], and that is going to be an important driver of tumor genesis in animals or people. It's like mimicking the hyperactivation of PI3K. Instead of doing it by mutation, you do it by having tons of insulin around."

The picture that's emerging now, Dang says—one that's "clearly simplified and needs to be tweaked"—is that many common cancer genes when activated may increase the uptake of glucose and convert the cell to the Warburg-type of metabolism.

Cause or consequence?

This still leaves open the question of what comes first: the Warburg effect or the mutations that drive a cell to adopt it. Craig Thompson, now president of Memorial Sloan-Kettering Cancer Center in New York City, has been working on this problem for a decade. He believes, as does Cantley, that the likely first step in the progression to cancer is the increase in insulin signaling, which then induces the Warburg effect. Genetic defects follow. Thompson and his colleagues have shown that they can induce the Warburg effect in the cells of healthy mice, or in cells associated with cancer, just by activating PI3K and increasing insulin signaling. "If you put in components of the insulin pathway into these cells," Thompson says, "you get the Warburg effect."

Once this happens and cells have increased their glucose metabolism 10- to 20-fold, Thompson says, one result is a significant increase in the generation of reactive oxygen species—free radicals—that can induce mutations in the genome. Cantley describes it as a vicious cycle. "The faster you do glucose metabolism," he says, "the more likely you are to get free radicals that can damage DNA. … If the mutations happen to be in *PTEN* or PI3K, that could make the whole system rev up even further, making more free radicals, causing more DNA damage. So you're getting this feed-forward acceleration of tumor growth."

This hypothesis still has plenty of critics—MIT's Weinberg being the most prominent. Insulin and IGF may be the "most attractive mechanisms" to explain the obesity-cancer link, Weinberg says. But he argues that their primary role is not to turn on the Warburg effect or promote proliferation but to suppress cell-suicide mechanisms. "One of the mechanisms," he says, "by which the body protects itself from cancer is by inducing incipient cancer cells to kill themselves by a variety of mechanisms. One of those mechanisms is apoptosis, and insulin and IGF activate an enzyme that in turn emits a series of antiapoptotic signals. A minimal amount of IGF is required just to protect normal cells from killing themselves. They're always poised on the brink."

Still, as Weinberg says, the role of insulin and IGF in cancer only "recently came on the radar screen" of most cancer researchers. "The epidemiology connecting obesity with cancer is very compelling," he says. But "our understanding of the mechanism is still pretty soft." **–GARY TAUBES**

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