tinually sending messages from one part of the cell to another. For example, many molecules bind to cell surface receptors and, without entering the cell, initiate a specific series of molecular interactions that result in gene activation in its nucleus.

The idea behind the Exelixis technique is that once a single protein in a pathway is known, the gene encoding the protein can be transferred to the fly. Then, as with Woodhouse's experiments, single mutations with P elements are made in the fly DNA until one or more interrupted genes is found that changes the effect of the original protein.

Potential Dividends

The company has already used the technique to identify two signaling pathways in fly eye development. This method also has the exciting potential of taking solitary genes such as BRCA1 and putting them in the context of a molecular process.

"Most of the biotech companies focus on a specific product, such as a growth factor or a family of similar genes. We're hedging our bets on a method rather than a molecule," said Remi Barbier, chief operating officer of the company, which raised $12.5 million this spring.

Whether the fly will yield the rich dividends that investors, patients, and researchers wish for remains unclear. But it does seem certain that the double life of the fly is secure for many more years.

— Nancy J. Nelson

New View of Metastasis Is Spreading

Researchers long assumed that metastasis was an extraordinarily perilous journey, because few of the cells that depart from the primary tumor form new colonies of malignancy. It was believed that most migrating tumor cells succumbed to the hydraulic pressures of the circulatory system, and that of the few that arrived intact within another organ, most failed to tunnel through the blood vessel walls into the tissues.

Ann Chambers, Ph.D., had expected to support this prevailing orthodoxy when she peered into the blood vessels of experimental animals to observe the tumor cells' passage. For the first time, using a new technique called intravital videomicroscopy, Chambers and her colleague Alan C Groom, Ph.D., coul watch the action in rea time, as it happened.

"Originally w thought we would use th procedure to confirn what everybody alread knew about how metasta sis happened," sai Chambers, who is hea of the Division of Experi mental Oncology at th London Regional Cance Centre of the Universit of Western Ontario Groom is professor o medical biophysic there.

But instead, "We kep running into discrepan cies that said maybe w didn't understand all that much about the basics of metastasis."

Bruce R. Zetter, Ph.D., professor of surgery and cell biology at Harvard Medical School, Boston, put it more strongly: "If [Chambers'] results hold up for a wide variety of tumors, it will turn some of the dogma in the field onto its ear."

Instead of succumbing to circulatory pressures, most metastatic cells she observed arrived intact at their destinations, Chambers said. Furthermore, most succeeded in burrowing out of the circulatory system and into the tissues.

"Over 80% of melanoma cells entering circulation survive and extravasate

Mammary carcinoma cell arrested in the vessel (sinusoid) of mouse liver.

(burrow through the blood vessel walls)," she said. "In the organs we've looked at, a population of cells has completed extravasation between 1 and 3 days after the population arrives in the organ." This puts the rate-limiting step for metastatic inefficiency at some step after the cells have gotten out of the blood vessels," said Chambers.

Other experiments have since supported this view. The old dogma held that enzymes called metalloproteinases were important to metastasis because tumor cells used them to break through the basement membrane of the vessels in the target organ.

Dr. Ann Chambers

A protein called tissue inhibitor of metalloproteinases, or TIMP-1, had been known to inhibit metastasis, and researchers assumed it did so by destroying a tumor cell's ability to cut through basement membrane, said Chambers. Melanoma cells that express excessive amounts of TIMP-1 metastasize poorly.

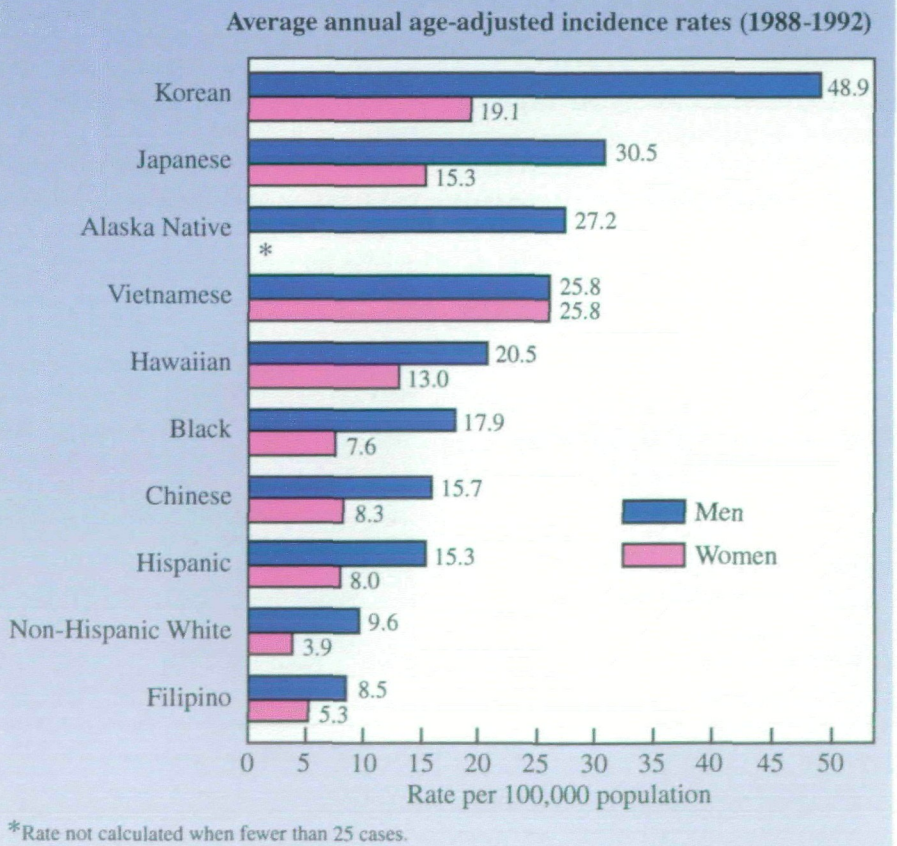
Time Bombs

But when Chambers' colleague Sahadia Koop, M.D., a Ph.D. student in both Chambers' and Groom's laboratories, turned the videomicroscope onto these cells, "To our great surprise, TIMP-1–up-regulated cells extravasated perfectly fine," said Chambers. But "they formed fewer and smaller metastases than normal tumor cells, implicating metalloprotei-

Stat Bite

Stomach Cancer Incidence by Race/Ethnicity in the U.S.

Stomach cancer was once the most common form of cancer in the world and is probably now only surpassed by lung cancer. Worldwide, stomach cancer rates are highest in Japan and Eastern Asia and are higher than average in Eastern Europe and parts of Latin America. Incidence among ethnic minorities in the United States reflects these trends. Risk factors for stomach cancer include infection with *Helicobacter pylori* and heavy consumption of salted, smoked, or pickled foods.



*Rate not calculated when fewer than 25 cases.

Source: *Racial/Ethnic Patterns of Cancer in the United States, 1988-1992* (SEER Monograph).

nases in regulating growth after extravasation."

This means, Chambers suggested, that "a lot of extravasated cells are sitting out there as potential time bombs, and it is going to be important to learn how to control these potentially activatable cells."

Dr. Bruce R. Zetter

She added that, "This fits with the dormancy in breast cancer. Ten to 20 years after the primary [tumor] is removed, a metastasis can appear. My guess is that they had already extravasated and were sitting as individual cells or micrometastases in the target tissue." These silent invaders, Chambers suggested, may present an important target for measures to combat metastasis.

Another Surprise

Still another surprise is that so-called adhesion molecules do not serve to anchor the tumor cells once they reach their destination. "Everyone in the field, myself included, has a slide of a big vessel and a little cancer cell, with the cancer cell sticking to the side," said Chambers. "That model comes from white cells." In fact, tumor cells get stuck in capillaries that are too small to allow their passage, and most then extravasate.

One researcher offered several minor caveats about Chambers' work. "Most of Ann's work has been on tumor cells entering the liver, and the vasculature in the liver is very loose," said Zetter. "It allows easy penetration of tumor cells across that barrier. So one question that will be very interesting for Ann to find out is whether the same ease of exit is found in other sites where the blood vessels have tighter junctions between them, as in brain and muscle and skin."

(The cancer that most commonly metastasizes to liver is colon cancer, and the three most common sites for metastatic cancers are lung, liver, and bone marrow, said Zetter.)

Chambers explained that one of her models, the chorioallantoic membrane (CAM) of the chick embryo, is functionally similar to lung in that it is the breathing organ for the embryo, and is structurally similar because it has a complete basement membrane and endothelial cell lining. "The behavior of the cells doesn't seem to be all that different in CAM versus liver. But it's an absolutely valid point. We're working on being able to do the lung."

Despite the caveats, every researcher queried for this article praised Chambers' work. Judah Folkman, M.D., professor of pediatric surgery and cell biology, Children's Hospital and Harvard Medical School, said Chambers' work is "outstanding just in the elegance of the experiments. It has changed our thinking about how tumor cells metastasize."

Dr. Judah Folkman

Folkman had assumed that the metastases lodged in the place where they had broken out of circulation. But Chambers' work suggests that they had "wandered through the desert of the organ prior to coming to rest on the venules," he said.

"It's amazing stuff," said Lynn Matrisian, Ph.D., professor of cell biology at Vanderbilt University. "Some of my own work has been leading in similar directions, suggesting that [metalloproteinases] affect the growth of [metastasized] tumor cells as opposed to the preconceived idea that they were poking holes in the basement membrane and allowing tumor cells to escape."

Robert M. Hoffman, Ph.D., president of AntiCancer, Inc., and professor of surgery at the University of California, Los Angeles, said Chambers' research is consistent with the results from his company, published last year in *Proceedings of the National Academy of Sciences*. Hoffman and colleagues found that both metastatic and nonmetastatic cells derived from human colon tumors had similar ability to invade the blood vessels of the mouse colon.

Critical Event

However, when cells were transplanted directly into the liver, the metastatic cells would grow, but the nonmetastatic tumor cells would not.

"Both Chambers' data and our data from very different approaches reached the same conclusion: the critical event is growth in the target organ," said Hoffman.

"Steven Paget said in 1889 that metastasis boils down to seed and soil," he added. "One hundred seven years later we're finding he's right. Ann Chambers has literally opened a new window on metastasis."

— *David Holzman*