

# How Cancer Spreads

*Tumor cells roam the body by evading the controls that keep normal cells in place. That fact offers clues to fighting cancer*

by Erkki Ruoslahti

Our body is a community of cells, in which each cell occupies a place appropriate for its tasks on behalf of the whole. With the exception of white blood cells, which patrol the body for microbial invaders and tissue damage, normal cells stay in the tissue of which they are part. Cancer cells, however, are rogues that trespass aggressively into other tissues.

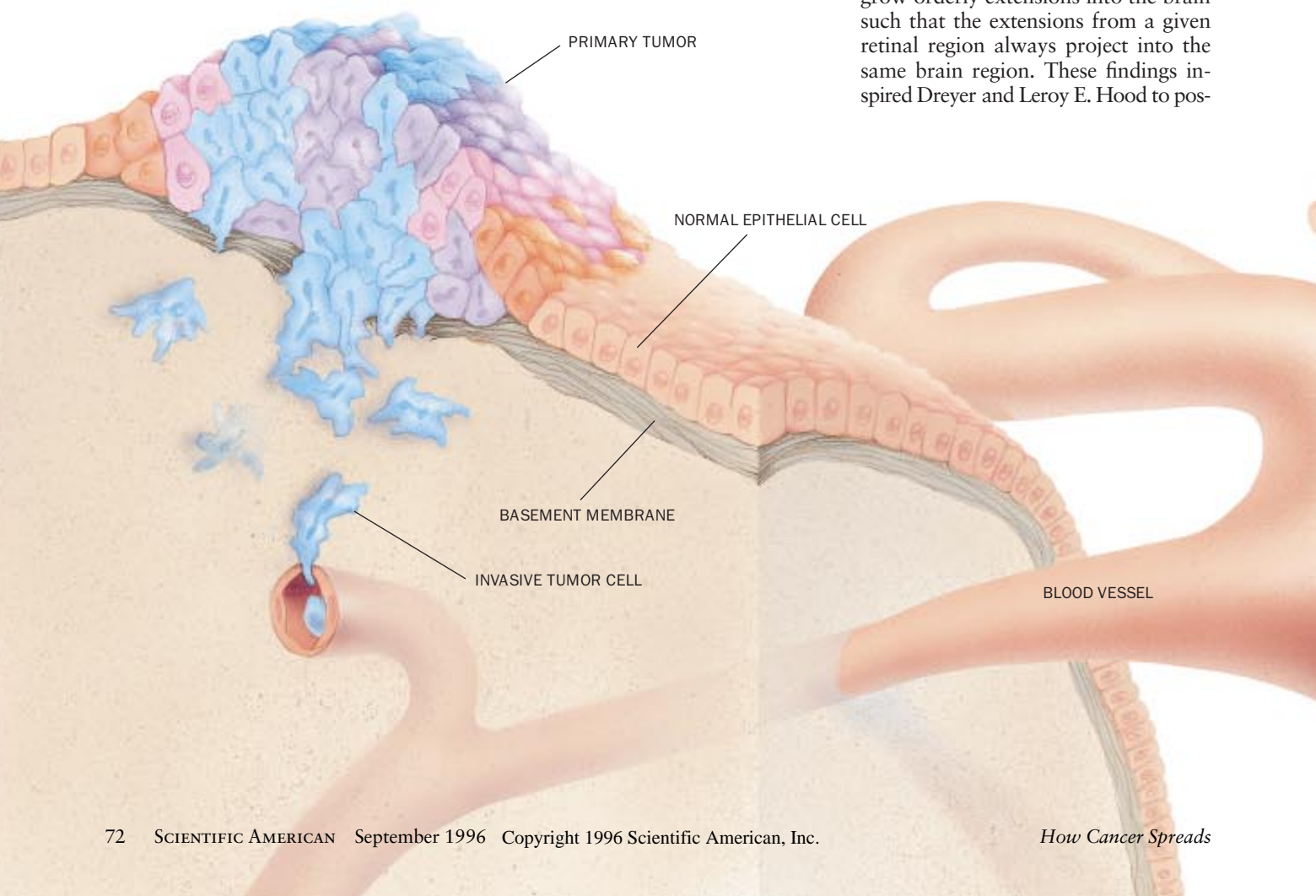
Metastasis, the spread of cancer to

distant sites in the body, is in fact what makes cancer so lethal. A surgeon can remove a primary tumor relatively easily, but a cancer that has metastasized usually reaches so many places that cure by surgery alone becomes impossible. For that reason, metastasis and the invasion of normal tissue by cancer cells are the hallmarks of malignancy. In countries where health care is primitive, one sometimes sees people who live with tumors

as big as a soccer ball; the cells that make up these so-called benign tumors obviously overproliferate, but unlike malignant cancer cells, they do not invade or metastasize.

Acquiring the capabilities needed to emigrate to another tissue is therefore a key event in the development of a cancer. To metastasize successfully, cancer cells have to detach from their original location, invade a blood or lymphatic vessel, travel in the circulation to a distant site and establish a new cellular colony. At every one of these steps, they must escape many controls that, in effect, keep normal cells in place.

A fruitful way of understanding how tumor cells evade these controls has consequently been to study the signals that normally direct cells to their place in the body and keep them there during adulthood. When I was a postdoctoral fellow at the California Institute of Technology from 1968 to 1970, my mentor, William J. Dreyer, had become interested in those questions. Roger W. Sperry, also at Caltech, had found that the light-sensing nerve cells in the retina of the eye grow orderly extensions into the brain such that the extensions from a given retinal region always project into the same brain region. These findings inspired Dreyer and Leroy E. Hood to pos-



tulate their “area code” hypothesis, that a cell has on its surface an address system—written in one set of molecules and readable by molecules on other cells—that identifies where the cell should be.

It seemed to me at the time that if a molecular address system existed, something had to be wrong with it in cancer, because cancer cells did not stay put. I decided to try to find such molecules. As the work of many laboratories eventually showed, area code molecules do exist. They mediate cell adhesion, the anchoring of cells to adjacent structures.

In normal tissues, cells adhere both to one another and to an insoluble meshwork of protein filling the space between them, known as extracellular matrix. (This arrangement is particularly descriptive of the epithelia, which are the cell layers that form the outer surface of the skin and the lining of the gut, lungs and some other organs, and from which most cancer originates.) The two kinds of adhesion play different critical roles during tissue invasion and metastasis.

Cell-cell adhesion molecules appear to help keep cells in place; these molecules seem to be missing or compromised in cancer cells. For example, various kinds of cancers lose some or all of an inter-

cellular adhesion molecule called E-cadherin. By manipulating this molecule in cultured cancer cells, one can change the cells’ ability to invade tissues and form tumors. Walter Birchmeier, now at the Max Delbrück Center in Berlin, first showed that blocking the function of E-cadherin can turn a cultured lineage of cells from noninvasive to invasive. Conversely, restoring E-cadherin to cancer cells that lack it can negate their ability to form tumors when they are injected into mice. Thus, loosening of the adhesive restraint between cells is likely to be an important early step in cancer invasion.

### The Need for Adhesion

Adhesion to extracellular matrix, on the other hand, allows cells to survive and proliferate. As researchers have known for many years, cultured cells cannot reproduce until they attach to a surface, a phenomenon called anchorage dependence. This attachment is mediated by cell-surface molecules known as integrins that bind to the extracellular matrix. As Steven Frisch of the Burnham Institute in La Jolla, Calif., Martin A. Schwartz of the Scripps Research Institute, also in La Jolla, Calif., and Mina

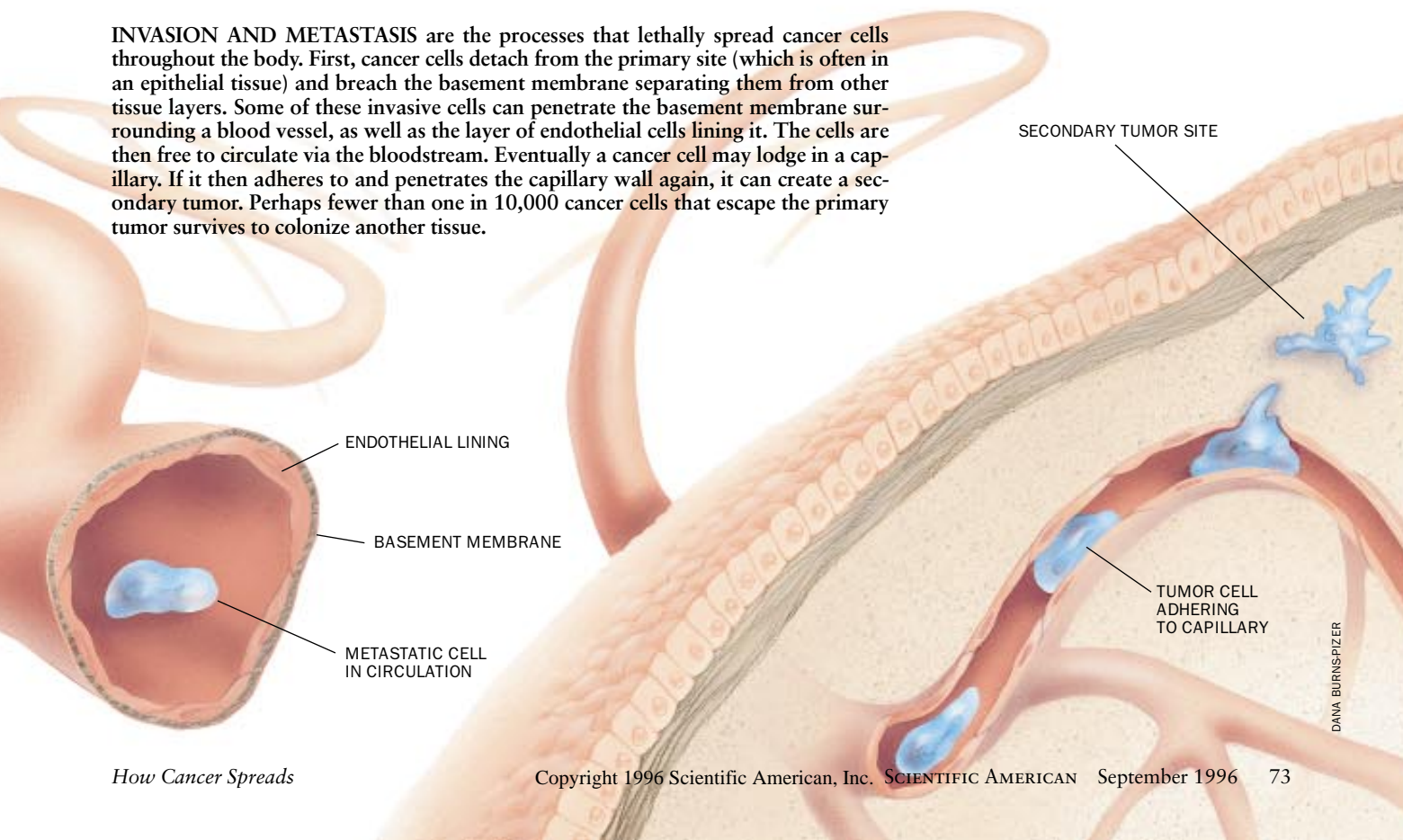
J. Bissell of the University of California at Berkeley have shown, only attachments involving integrins can satisfy the requirements of anchorage dependence.

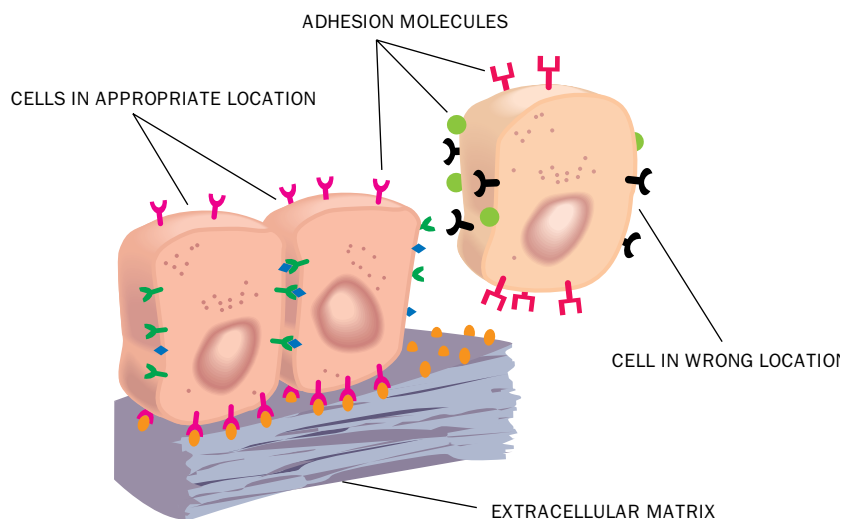
My laboratory at the Burnham Institute, together with Tony Hunter of the Salk Institute for Biological Studies in San Diego, Calif., has recently shown that unattached cells stop growing because one of the nuclear proteins (known as the cyclin E-CDK2 complex) that regulates the growth and division of cells becomes less active. Inhibitory substances in the nuclei of these cells seem to shut down this protein.

As Frisch, Schwartz and Bissell also discovered, when many types of cells are denied anchorage, they not only stop proliferating but commit suicide. That is, they spontaneously undergo specific changes that lead to their own death. This kind of cell death, in which the cell is an active participant, has been termed apoptosis.

My group has demonstrated that for cells to survive, the extracellular matrix to which they adhere must bear the right “area code,” one that is probably found only in the extracellular matrix of select tissues. Moreover, they have to use the appropriate integrin to attach to the ma-

**INVASION AND METASTASIS** are the processes that lethally spread cancer cells throughout the body. First, cancer cells detach from the primary site (which is often in an epithelial tissue) and breach the basement membrane separating them from other tissue layers. Some of these invasive cells can penetrate the basement membrane surrounding a blood vessel, as well as the layer of endothelial cells lining it. The cells are then free to circulate via the bloodstream. Eventually a cancer cell may lodge in a capillary. If it then adheres to and penetrates the capillary wall again, it can create a secondary tumor. Perhaps fewer than one in 10,000 cancer cells that escape the primary tumor survives to colonize another tissue.





DANA BURNS-PIZER

**“AREA CODES” FOR CELLS** take the form of specific surface adhesion molecules and receptors. During development, a normal cell recognizes its proper place in the body by fitting its adhesion molecules to those on other cells and on the extracellular matrix. In cancer, something goes wrong with this address system.

trix. As all these results show, a molecular explanation for anchorage dependence is beginning to take shape, although much more critical detail still needs to be filled in.

Cellular suicide from lack of anchorage or from inappropriate anchorage is likely to be one of the safeguards that maintain the integrity of tissues. Cells usually cannot just float away from their tissue and establish themselves somewhere else, because they will die on the way. Yet cancer cells get around this requirement; they are anchorage independent. The cyclin E-CDK2 complex in such cells stays active whether the cells are attached or not.

How cancer cells accomplish this trick is not fully understood, but it seems that oncogenes can be blamed. (Oncogenes are mutated versions of normal genes called proto-oncogenes; these mutations can turn normal cells into malignant ones; see “How Cancer Arises,” by Robert A. Weinberg, on page 62.) In effect, as various experiments have shown, proteins made by these oncogenes convey a false message to the nucleus that the cell is properly attached when it is not, thereby stopping the cell from arresting its own growth and dying through apoptosis.

Anchorage dependence is only one of the constraints that a cancer cell must overcome to roam around the body. Epithelial cells, the most common sources of cancers, are separated from the rest

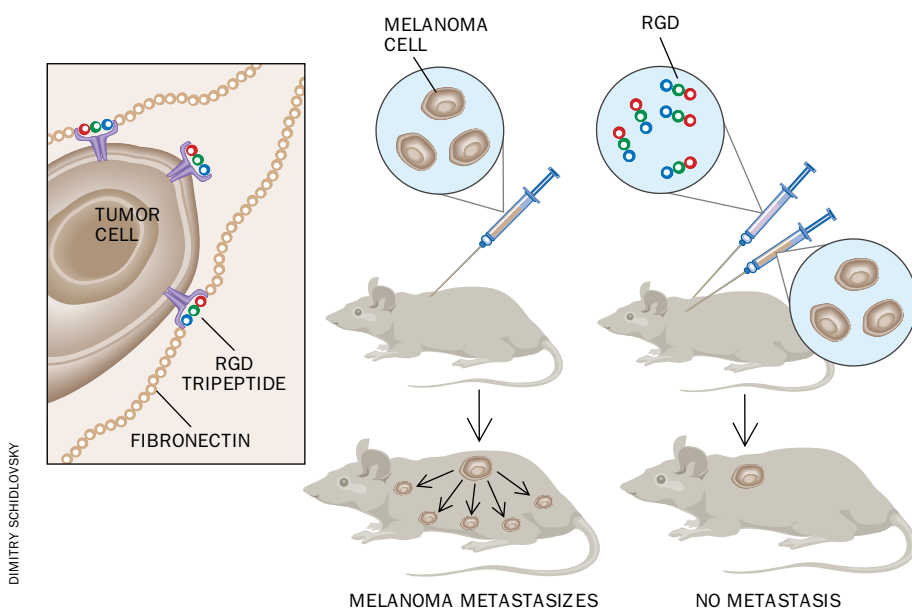
of the body by a basement membrane, a thin layer of specialized extracellular matrix. Basement membranes form a barrier that most normal cells cannot breach, but cancer cells can [see “Cancer Cell Invasion and Metastasis,” by Lance A. Liotta; *SCIENTIFIC AMERICAN*, February 1992].

This fact can be strikingly demonstrated by giving cells in a test tube an op-

portunity to invade through a natural or reconstructed basement membrane: cancer cells will penetrate it; normal ones will not. Furthermore, in this experiment, cells from metastatic cancers generally invade faster than those from nonmetastatic tumors. White blood cells, in keeping with their role as security patrol, are an exception to the rule that normal cells do not invade—they, too, are adept at penetrating tissues, including basement membranes. Cancer cells and white blood cells do so by releasing enzymes, called metalloproteinases, that dissolve basement membranes and other extracellular matrices. Other cells have less of these enzymes and more enzyme inhibitors.

After a cancer cell has passed through the basement membrane separating it from the rest of the tissue at its original site, it soon encounters another basement membrane, one surrounding a small blood vessel. (A blood vessel is usually nearby, because to sustain themselves successful tumors induce the growth of new blood vessels.) By penetrating this second basement membrane barrier and the layer of endothelial cells that form the vessel’s inner lining, the cancer cell gains access to the bloodstream and is carried elsewhere in the body.

New technology makes it possible to detect cancer cells in the blood of pa-



DIMITRY SCHIDLOVSKY

**INHIBITING METASTASIS** by interfering with cancer cell adhesion may someday be a therapeutic option. In mouse experiments, injections of RGD, a fragment of the protein fibronectin, discouraged melanoma cells from spreading to the lungs. Presumably, the RGD molecules blocked receptors that wandering cancer cells needed for binding to fibronectin in the extracellular matrix of tissues.



tients. Great strides have been made in identifying telltale marker molecules that distinguish a cell as having come from a specific tissue or type of tumor. At the same time, researchers have also developed ultrasensitive assays (based on such techniques as the polymerase chain reaction and monoclonal-antibody tagging) for detecting those molecules. From studies employing these methods, we know that malignant cells are often circulating even when a clinical examination cannot yet find evidence of the cancer's distant spread.

The further development of such tests may eventually improve therapies, by helping physicians determine whether they need to prescribe treatments beyond surgery for seemingly contained tumors. Detection of micrometastases in the blood and elsewhere in the body is a significant step forward in early diagnosis, and it is the vanguard of applied research on metastasis.

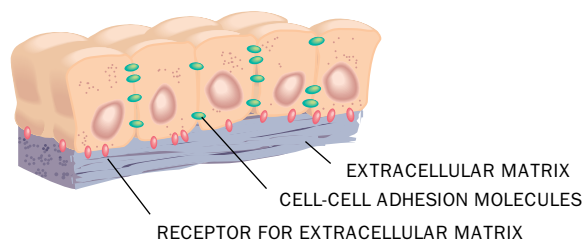
Some doctors have also wondered whether the manipulation of a tumor during its diagnosis or surgical removal might be enough to release cells into the circulation. The new testing methods should allow researchers to prove or disprove this ominous hypothesis, but to my knowledge, that has not yet been done. But even if the hypothesis proves to be correct, it is clear that the benefits of diagnostics and surgery far outweigh the possible risks from inaction.

### Vulnerable in the Blood

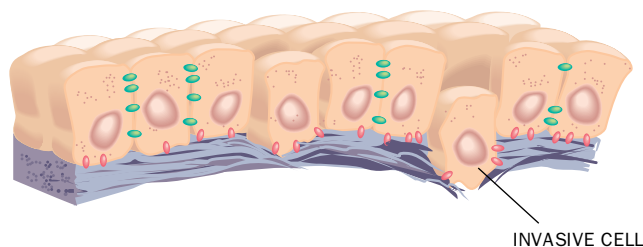
Fortunately, even when cancer cells do get into the circulation, the formation of secondary tumors is not inevitable. The circulating cell still faces several more hurdles: it must attach to the inner lining of a blood vessel, cross through it, penetrate the basement membrane at this new location, then invade the tissues beyond and begin multiplying. Each of these obstacles makes demands of the tumor cell that may go beyond those it faced in its home tissue. Furthermore, it may also be that many cancers cannot entirely overcome the defense mechanisms that keep our cells in the right places—another hindrance to metastasis.

Probably fewer than one in 10,000 of the cancer cells that reach the circulation survive to found a new tumor at a distant site. The reasons for this apparent vulnerability while in the blood are not

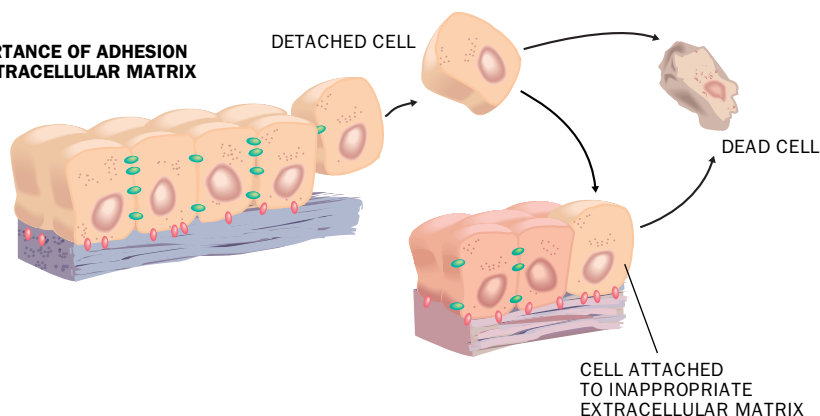
### CELLULAR ADHESION



### IMPORTANCE OF CELL-CELL ADHESION



### IMPORTANCE OF ADHESION TO EXTRACELLULAR MATRIX



DANA BURNSPIZER

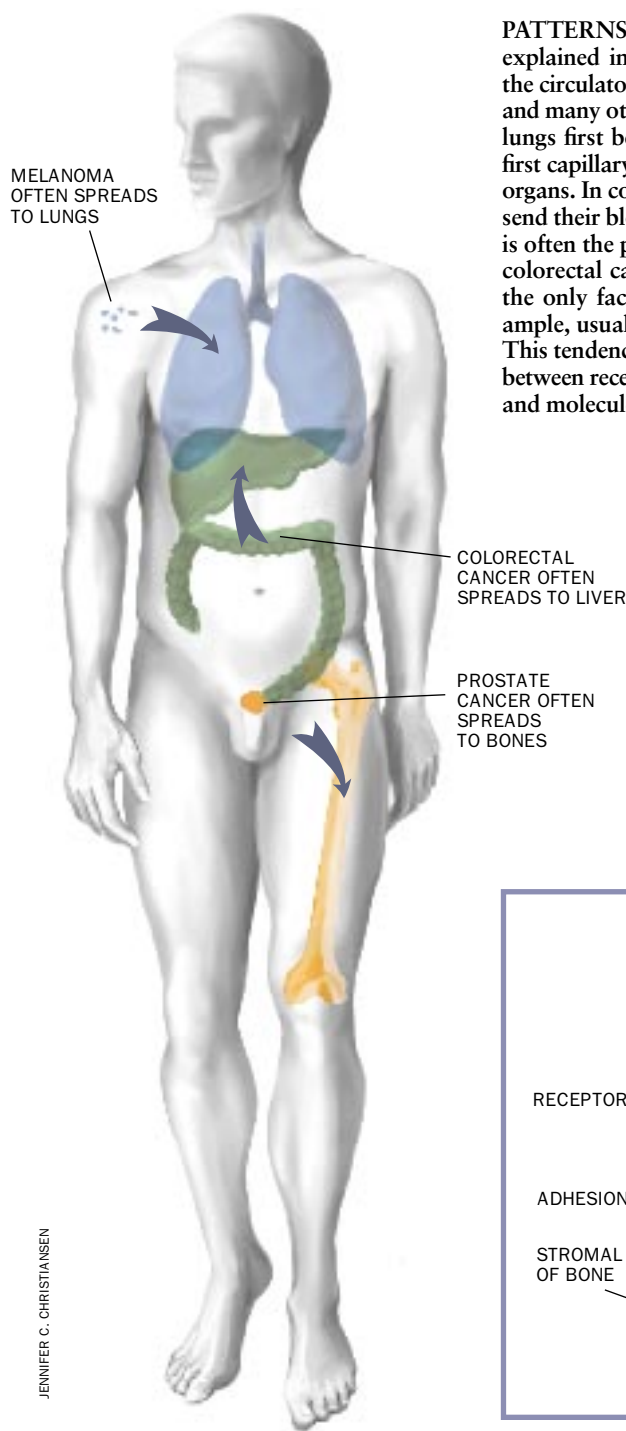
**CELLULAR ADHESION** is a vital brake on the migration of normal cells. Two types apply to most body cells: cell-cell adhesion and adhesion to the extracellular matrix (*top*). If a cell cannot adhere to other cells, it may become more invasive and migrate through the matrix (*middle*). If a cell lacks adhesion to the extracellular matrix, it can detach from its native tissue (*bottom*). Usually, if a cell fails to reattach to the extracellular matrix or if it attaches to the wrong type of matrix, it dies through apoptosis (cellular suicide). Cancer cells, however, can survive without this adhesion.

well understood—perhaps the anchorage independence of the tumor cells is not complete, and they sometimes die through apoptosis after all. In any case, researchers believe the cells need to attach fairly promptly to the inner lining of a small blood vessel.

Blood circulation explains much about why various metastatic cancers spread preferentially to certain tissues. Circulating tumor cells usually get trapped in the first vascular bed (or network of capillaries, the finest blood vessels) that they encounter “downstream” of their

origin. The first vascular bed encountered by blood leaving most organs is in the lungs; only the intestines send their blood to the liver first. Accordingly, the lungs are the most common site of metastasis, followed by the liver.

In part, cancer cells lodge in small blood vessels because these cells tend to be large. Also, some cancers produce chemical factors that cause platelets, the tiny blood cells that initiate blood clotting, to aggregate around them. These aggregates effectively make the cancer cells even larger and stickier. (It is also



**PATTERNS OF METASTASIS** can be explained in part by the architecture of the circulatory system. Tumors in the skin and many other tissues often colonize the lungs first because the lungs contain the first capillary bed “downstream” of most organs. In contrast, because the intestines send their blood to the liver first, the liver is often the primary site of metastasis for colorectal cancers. Yet circulation is not the only factor: prostate cancer, for example, usually metastasizes to the bones. This tendency may result from an affinity between receptors on prostate tumor cells and molecules in bone tissues (*inset*).

sues could explain the predilection of the cells to migrate selectively. Different concentrations of growth-promoting factors and hormones in various tissues may also play a part.

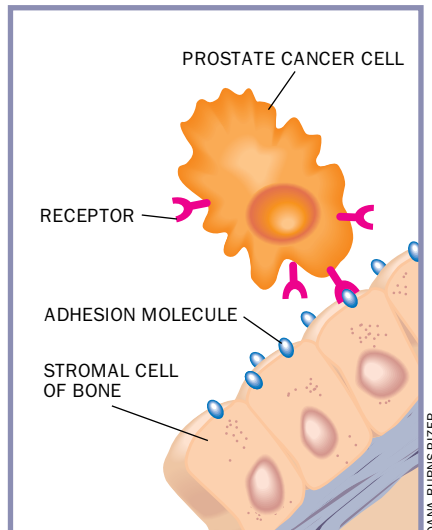
Recently, in an elegant piece of work, Ivan Stamenkovic of Harvard Medical School and his colleagues showed that he could direct the metastatic spread of tumor cells: he genetically engineered mice so that their livers displayed a target for an adhesion molecule found on certain tumor cells. As predicted, the tumor cells homed in on the liver. For these experiments, Stamenkovic borrowed receptors and targets from the molecular adhesion system used by white blood cells to leave the circulation and enter tissues. Although this system was artificial, it may be that cancers naturally mimic white blood cells in much this way—cancer cells do often manufacture certain molecules (called  $Le^x$ ) important to the mobility of white blood cells in the body.

### Finding the Body's Area Codes

If, as seems likely, there is much to be learned by identifying the molecular addresses that white blood cells and tumors use to find particular tissues, a method of doing so that Renata Pasqualini, a postdoctoral fellow in my laboratory, and I have devised should prove helpful. We adapted a technique for isolating biologically active molecules from huge collections, or “libraries,” of diverse compounds. The theory behind this approach is that if one screens a sufficiently large number of compounds, one can find a molecule for almost any purpose.

We use a large library of peptides (small pieces of protein) as the source of our compounds. During the 1980s, George Smith, now at the University of Missouri, devised a technique for building such a library that employs a phage, a type of virus that infects bacteria. If a short random piece of DNA is inserted into the phage's gene for a surface protein, the phage will thereafter display on its surface a corresponding random peptide. Applying Smith's method, one can create an entire library of phages carrying a billion different peptides, with each individual phage expressing only one peptide.

Our innovation was to test the affinities of peptides in this library by injecting the diverse viruses into a living ani-



noteworthy that platelets produce their own rich supply of growth factors, and these may help the cancer cells to which they bind survive in the blood. This may be why, in some experimental systems, drugs that interfere with platelet functions have anticancer effects.)

Physical trapping of cancer cells in the blood vessels at the site of metastasis is not the whole story, however. If it were, cancers would not spread so diversely

through the body. Indeed, some types of cancer show a striking preference for organs other than those that receive their venous blood—witness the tendency of metastatic prostate cancer to move into the bones. Once again, the explanation seems to rest with the molecular address system on cell surfaces. A specific affinity between the adhesion molecules on cancer cells and those on the inner linings of blood vessels in the preferred tis-

mal. Any phage that carried a peptide with an affinity for molecules on a particular tissue would stick there. We looked for and found phages that bound preferentially to blood vessels in a mouse's brain and kidney. That success suggests that specific addresses for other organs could also be discovered and tested for their involvement in tumor cell homing.

Knowledge of the addresses that tumor cells seek may eventually pay off in clinical benefits. Given the vulnerability of tumor cells in transit, anything we can do to make it more difficult for tumor cells to attach to tissues may be beneficial to patients.

Initial work in that direction has started. In 1984 Michael D. Pierschbacher, who was then a postdoctoral associate in my laboratory and is now at Telios Pharmaceuticals, and I showed that all cells attach to fibronectin and several other extracellular matrix proteins at a structure made up of just three amino acids. This result was surprising, given that fibronectin is a long chain of 2,500 amino acids. We went on to show that artificial peptides containing this critical tripeptide (arginine-glycine-aspartic acid, designated as RGD) can act like a decoy, binding to cells' receptors for fibronectin and blocking their attachment to the matrix.

Martin Humphries and Kenneth M. Yamada, who were then at the National Cancer Institute, and Kenneth Olden, then at Howard University, subsequently showed that if they injected mice with cells from melanomas (lethal skin cancers), RGD peptides could prevent the cells from colonizing the animals' lungs. Such peptides can even prevent metastasis from melanoma tumors grown under the skin of mice—an experimental

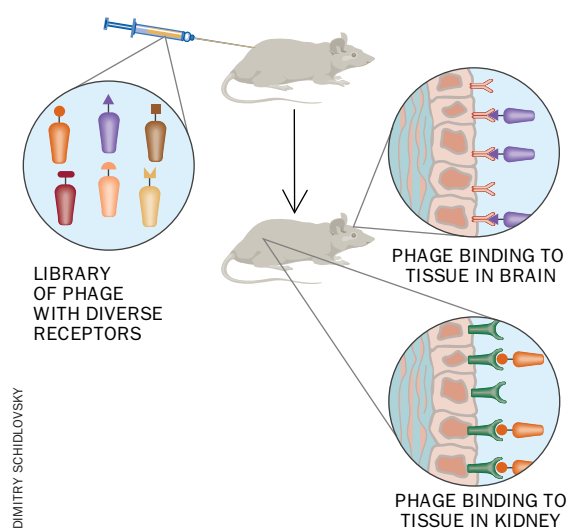
system that more closely resembles the human disease. David A. Cheresh of the Scripps Research Institute has shown that RGD compounds can also prevent the formation of new blood vessels that nurture tumors. Related compounds therefore may someday augment physicians' anticancer arsenal, but much work will have to be done first so that these peptides can be taken orally and will act longer.

### Understanding Invasion

Disappointingly little is as yet understood in molecular detail about the mechanisms that turn a cancer from a locally growing tumor into a metastatic killer. Some of the same genetic changes that allow cancer cells to escape growth control and avoid apoptosis are clearly important in the early stages of metastatic spread, because they enable cells to survive without anchorage. What then turns on the programs that make the cancer invasive and metastatic, however, is not really known.

Genetic approaches similar to those used in the discovery of oncogenes and tumor suppressor genes have produced some candidates for genes with a specific role in metastasis. Further genetic comparisons of local and metastatic tumors may well explain their differences, but it is also possible that entirely new thinking is needed.

My own bias is that studying resistance to cancer invasion at both the tis-



**PHAGE LIBRARY**, consisting of billions of viruses sporting diverse receptor molecules, can help identify the area codes of tissues to which cancer cells home. In one experiment, a phage library was injected into a mouse. Some of the viruses bound uniquely in either the brain or the kidney.

sue and genetic levels may provide important answers. For example, some tissues are not invaded by cancer: cartilage and, to an extent, the brain. Cancers originating elsewhere in the body can metastasize to the brain, but they do not truly invade the brain tissue—they just grow bigger within and near the blood vessels. Something about brain tissue seems to repel otherwise invasive tumor cells. Some species of animals also appear to be unusually resistant to developing cancers. I suspect that much could be learned if the molecular bases for these and other phenomena were understood. The fact that metastasis is the deadliest aspect of cancer adds the utmost urgency to our quest for this knowledge. SA

### The Author

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### Further Reading

**MOLECULAR MEDIATORS OF INTERACTIONS WITH EXTRACELLULAR MATRIX COMPONENTS IN METASTASIS AND ANGIOGENESIS.** L. R. Bernstein and L. A. Liotta in *Current Opinion in Oncology*, Vol. 6, No. 1, pages 106–113; January 1994.

**ANCHORAGE DEPENDENCE, INTEGRINS, AND APOPTOSIS.** Erkki Ruoslahti and John C. Reed in *Cell*, Vol. 77, No. 4, pages 477–478; May 20, 1994.

**EVOLUTIONS: METASTASIS.** David I. Lewin in *Journal of NIH Research*, Vol. 8, pages 82–87; June 1996.

**FIBRONECTIN AND INTEGRINS IN INVASION AND METASTASIS.** S. K. Akiyama, K. Olden and K. M. Yamada in *Cancer and Metastasis Reviews*, Vol. 14, No. 3, pages 173–189; 1996.

