# New Tumor Factor May Disrupt Calcium Levels

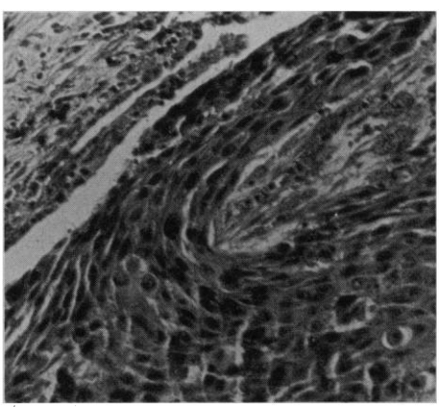
*Three research groups have identified a factor produced by certain tumors that may induce the high blood levels of calcium that complicate some forms of cancer*

A long-sought factor produced by certain kinds of tumors may be responsible for inducing dangerously high concentrations of calcium ions in the serum of some patients with cancer. Whether the newly identified protein acts alone to produce this condition, called hypercalcemia, is still uncertain. Three different groups of researchers simultaneously announced their identification of the factor at the recent American Society for Bone and Mineral Research meeting in Indianapolis.*

"The most striking thing is the realization that the substance produced by these tumors has a structure similar to that of the parathyroid hormone molecule," says T. John Martin of the University of Melbourne in Victoria, Australia. All three groups, including Martin's, find that about two-thirds of the first 15 amino acids of the factor are the same as those of parathyroid hormone, but their data differ somewhat on the size of the factor. Parathyroid hormone (PTH) is a normal product of the parathyroid glands that lie just behind the thyroid. The hormone stimulates both bone resorption and formation in vivo and is the body's principal short-term regulator of calcium levels.

Patients with hypercalcemia have increased bone resorption without normal bone formation, but how or whether the new PTH-like peptide contributes to this imbalance between the two processes is unknown. The new factor resembles PTH in its ability to stimulate the enzyme adenylate cyclase in cultures of bone or kidney cells, resulting in increased levels of cyclic adenosine monophosphate (cAMP), and it also appears to enhance bone resorption in vitro. If the PTH-like tumor factor has similar actions in patients with cancer, which has yet to be shown, its overall effect could be to release abnormally high amounts of calcium into the blood, which would cause damage to organ systems throughout the body.

Hypercalcemia occurs in 25 to 35% of patients with solid tumors of the lung and breast, is also frequent in patients with myeloma or head and neck tumors, and occurs less frequently in people with lymphoma, kidney, or ovarian cancer. Its symptoms include nausea, drowsiness, depression, muscle weakness, high blood pressure, and excess urine output. The underlying causes of hypercalcemia in different cancers probably vary, however. For example, tumors that develop in the flat cells lining air passageways in the lung, termed squamous epithelial cells, are probably more likely to secrete the PTH-like factor than are most breast cancers.



**Lung tumor tissue** *from a patient with squamous cell cancer and hypercalcemia that produces a PTH-like factor.* [*E. C. Abramson* et al., Calcif. Tissue Int. *36, 563 (1984)*]

"The hypercalcemia in breast cancer or myeloma is different, I think, from the hypercalcemia associated with lung cancer," says Gregory Mundy of the University of Texas Health Science Center in San Antonio. "In breast cancer, hypercalcemia occurs very late in the disease, is accompanied by extensive bone metastases, and is not normally associated with an increase in cAMP." In contrast, lung cancer patients may develop hypercalcemia early in the course of their disease, do not necessarily have accompanying bone tumors, and usually do have elevated cAMP levels. As a result of these and other differences, researchers generally agree that the newly defined PTH-like factor is likely to play a role in the hypercalcemia of only some cancers and of a very small percentage of breast cancers.

"We realized that such a factor existed for some time, but thought that it was quite different from parathyroid hormone," says Martin. "Antibodies against PTH failed to recognize it, but we knew that it bound to the PTH receptor." Martin, Jane Moseley, and Larry Suva, also of the University of Melbourne, and their colleagues recently collaborated with William Wood and Hugh Niall of Genentech in South San Francisco to clone and sequence the approximately 17-kilodalton protein factor that they initially isolated from a line of lung tumor cells. Although the first part of the molecule strongly resembles PTH, the remaining major portion of the factor is completely unlike PTH, and the entire molecule is about twice the size of PTH.

Working independently, Robert Nissenson and Gordon Strewler, both of the Veterans Administration Medical Center and the University of California in San Francisco discovered a similar factor in a line of cancer cells from human kidney. "The protein we have isolated appears to be smaller than that found by the other two groups," says Nissenson. The significance of this, he speculates, may be that only a small part of the larger molecule—perhaps the 6-kilodalton protein his group has isolated—is required for biological activity.

"We gave the factor to Paula Stern of Northwestern University in Chicago, and she found that it has bone-resorbing activity in cultured fetal rat bone," says Strewler. In addition, the San Francisco researchers find that the PTH-like factor stimulates adenylate cyclase activity in cultured rat bone tumor cells, and it inhibits the transport of inorganic phosphate in a line of opossum kidney epithelial cells. The latter preliminary data are similar to those reported at the recent meeting by Jean-Phillippe Bonjour, Lana Pizurki, and their colleagues of University Hospital in Geneva, Switzerland.

A third research group, Andrew Stewart and William Burtis both of the Yale University School of Medicine in New Haven and the Veterans Administration Medical Center in West Haven, Connecticut, Arthur Broadus and Marguerite Mangin of Yale, Andrew Webb of Wellesley College in Massachusetts, and James Posillico of the Brigham and Women's Hospital in Boston, described a similar factor initially obtained from human breast cancer cells. "When you look at the sequence of the new factor, what jumps out at you is that the final 2 amino acids of the leader sequence and the first 13 amino

*The annual meeting of the American Society for Bone and Mineral Research was held 6 to 9 June in Indianapolis, Indiana.

acids of the mature protein have a 67% homology to parathyroid hormone," says Broadus.

Since isolating the factor from breast cancer cells, the Connecticut group has also isolated an identical protein from lung tumor cells. "The PTH-like peptide exists in two forms," says Stewart. "Each of the tumors has a 17-kilodalton form and the lung has larger amounts of a smaller 7- to 9-kilodalton form. One of the big questions now is whether the 17-kilodalton form represents a larger precursor protein." Perhaps after selective enzymatic digestion the precursor is converted into a smaller biologically active molecule, he says.

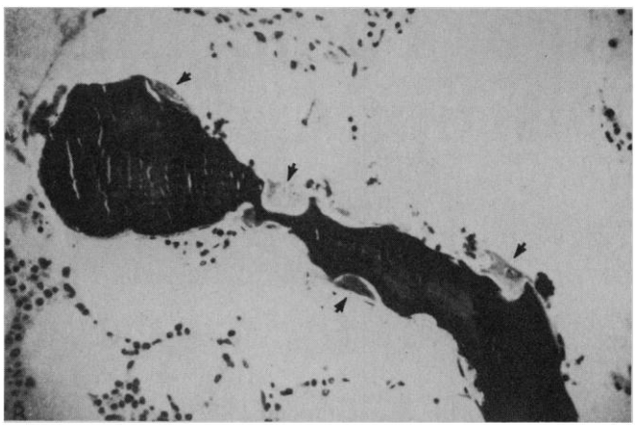
Several years ago, Broadus and Stewart, in collaboration with Leonard Milstone also of the Veteran's Hospital and Yale and Jack Merendino of Yale, reported that keratinocytes, a population of skin cells, also produce a factor that in many ways is similar to the newly identified PTH-like factor. Keratinocytes are the normal cellular counterparts of the squamous epithelial cells that form the kind of tumors most likely to make the PTH-like peptide. Because the keratinocyte factor and the tumor peptide have similar biological activities, Broadus and Stewart propose that they may be one and the same. "I think we're talking about a normal peptide that may be overexpressed by a number of tumors," says Broadus. He speculates that normal cells, such as keratinocytes in skin, may produce small amounts of the factor locally that have no overall effect on calcium levels, but that a lung tumor may release large amounts of the factor into the bloodstream, thus upsetting calcium metabolism throughout the body.

Although no one seriously doubts that such events occur in some cancer patients, researchers have yet to show that the PTH-like peptide actually circulates in the blood of these patients—a procedure that could be done more easily if an antibody was available. No effective antibody exists yet, principally because no one has had sufficient peptide to generate it. This situtation should change soon because both the Australian and Connecticut groups have cloned and sequenced the factor and can generate synthetic peptide in large quantities.

Researchers hail the identification of the new PTH-like factor as a significant advance in understanding a possible cause of hypercalcemia in patients with cancer, but few are ready to claim that the new factor is solely responsible. An ongoing subject of debate is whether a combination of factors is required to disrupt calcium balance.

"Why make simple things complicated?" asks Broadus. "Here we have something with a parathyroid hormone–like sequence, so why does anyone need to postulate anything else?" Mundy, in contrast, says that "tumors that make the PTH-like factor also produce other peptides that stimulate bone resorption—transforming growth factor alpha and interleukin-1, for example." At present he sees no evidence that the newly identified protein is a more potent stimulator of bone resorption than these other tumor-produced peptides. Martin adopts a middle position. "I think the PTH-like factor contributes substantially to the hypercalcemia of malignancy," he says. "But it is conceivable that it acts together with one or more other factors."

**Abnormal bone resorption**



*Bone tissue (dark structure) from a patient with cancer and hypercalcemia, showing an abnormally high number of areas of bone resorption (arrows) with no visible bone formation.* [*A. F. Stewart* et al., J. Clin. Endocrinol. Metab. *55, 219 (1982)*]

Several other questions about the new PTH-like factor also remain unanswered. Parathyroid hormone itself stimulates bone resorption and the PTH-like factor appears to have a similar action in vitro, but the cellular target for these compounds is unclear. "PTH does not have receptors on osteoclasts [cells that cause bone resorption], but it may act indirectly via osteoblasts [cells that cause bone formation]," says Broadus. "Not only do patients with cancer and hypercalcemia have bone resorption, but they have rip-roaring resorption, with little if any bone formation." Why the PTH-like factor does not stimulate the normal processes of bone turnover—both resorption and formation—as parathyroid hormone does, is yet to be determined (*Science*, 22 May, p. 914).

A related issue concerns the nature of the receptors for the PTH-like factor and whether they are identical to receptors for parathyroid hormone. Stewart proposes that the new peptide has its own receptors and also that multiple receptor subtypes for PTH exist. As evidence, he cites his observation that PTH and the PTH-like peptide have somewhat different effects in stimulating adenylate cyclase activity in different tissues. For example, parathyroid hormone stimulates the kidney enzyme more strongly, and the PTH-like factor has more potent effects in bone, he says.

Nissenson and Strewler cite evidence that PTH and the related peptide share at least some of the same receptors. The PTH-like factor competes with parathyroid hormone for binding to kidney cell membranes and the opposite also occurs; PTH displaces the factor. "The original reason that this material was called parathyroid hormone–like was because PTH antagonists could block its activity to stimulate cAMP formation in kidney and bone," Nissenson says. "The current data indicate that the factor can bind to and activate the classical PTH receptors in bone and kidney," he says and speculates that the new factor may act at other receptors as well.

Now that researchers have identified a new factor that is associated with cancers that are often complicated by hypercalcemia, it may be possible to design diagnostic tests or even therapies based on the new PTH-like peptide. "One thought we've had is that because this factor is made by squamous cell cancers, it may be useful to screen patients for the factor in order to measure tumor development or responses to therapy," says Stewart. Both he and Nissenson think that knowing the factor's identity and structure may make it possible to design drugs that specifically block its activity, perhaps slowing or preventing hypercalcemia. But the efficacy of such a treatment depends, at least in part, on whether the PTH-like peptide acts alone or in concert with other factors to cause the hypercalcemia of malignancy. ■

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ADDITIONAL READING


J. M. Moseley *et al.*, "Parathyroid hormone-related protein purified from a human lung cancer cell line," *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5048 (1987).

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A. F. Stewart *et al.*, "The relative potency of a human tumor-derived PTH-like adenylate cyclase-stimulating preparation in three bioassays," *J. Bone Min. Res.* **2**, 37 (1987).