Do Tumor Promoters Affect DNA After All?

Tumor promoters may contribute to cancer development by generating activated oxygen compounds that damage DNA

Within the past year or two investigators have begun to question the conventional wisdom that tumor promoters foster cancer development without acting on DNA. Promotion, which is studied as an experimental model for the prolonged, multistep development of natural cancers, usually includes two steps. In initiation, a low dose of a true carcinogen is applied to the target, most often mouse skin. This is followed by repeated applications of the promoter, a chemical which is not carcinogenic by itself but which enhances the production of malignant tumors in mouse skin and other cells that have undergone initiation.

The prevailing view has been that initiating carcinogens act on DNA but promoters do not. The best studied promoters, the phorbol esters, do not bind to DNA and they are negative in the Ames mutagenicity assay, whereas initiating carcinogens usually meet one or both of these criteria.

The current work shows that tumor promoters can damage DNA without reacting with it directly. "Tumor promoters unexpectedly produce vast amounts of chromosome damage," says Peter Cerutti of the Swiss Institute for Experimental Cancer Research in Lausanne. They apparently do this by stimulating cells to produce activated forms of oxygen, including superoxide anion radicals and peroxides, agents that are free radicals or generators of free radicals. These reactive substances can attack DNA and have been implicated as mediators of the mutagenic effects of gamma radiation.

Nancy Colburn of the National Cancer Institute remarks, "It is a new development for people to show that TPA can cause DNA damage." (TPA is the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate.) The postulated effect of promoters on DNA is especially intriguing when viewed in conjunction with recent research suggesting that cellular oncogenes (*onc* genes) are activated as a result of the chromosomal rearrangements associated with certain cancers (*Science*, 3 December, p. 983).

An early indication that tumor promoters could generate DNA-damaging materials was obtained in Walter Troll's laboratory at New York University Medical Center. "We started by accident," Troll says, while investigating the activation of polymorphonuclear leukocytes (PMN's), a type of phagocytic immune cell. When PMN's and other phagocytes are activated, they undergo an oxidative burst. Their oxygen consumption increases markedly and they produce superoxide radicals and hydrogen peroxide, which help them perform their cell-killing and scavenging duties.

Troll and Bernard Goldstein, who is now at Rutgers University Medical School, found that treatment of PMN's with TPA produces an oxidative burst about 40 seconds later. In general, the degree of stimulation of the oxidative burst by TPA and other promoters is correlated with their promoting efficacy. "All tumor promoters cause free oxygen radical release in PMN," Troll says, "and that release is inhibited by antipromoters."

For example, the Troll group has shown that retinoids, which inhibit promotion of mouse skin tumors, prevent activated oxygen production by PMN's in response to promoters. That effect of TPA is also blocked by protease inhibitors. Protease production is an early response to tumor promoters and protease inhibitors block promotion in the mouse skin system.

Further evidence implicating free radicals in tumor promotion comes from Thomas Slaga, who recently moved from Oak Ridge National Laboratory to the University of Texas System Cancer Center in Smithsville. Slaga showed that benzoyl peroxide and other compounds that generate free radicals, but are chemically unrelated to the phorbol esters, are promoters of mouse skin cancers.

Although free radicals may be intermediaries in tumor promotion, they do not necessarily have to act by attacking DNA. These radicals are very catholic in their choice of targets. They might instead attack membrane or other cellular components.

However, there is evidence that promoters damage DNA and that tumor development depends on their radical-generating capabilities. Chaim Birnboim of Atomic Energy of Canada Limited in Chalk River, Ontario, has shown that TPA induces strand breaks in the DNA of human white blood cells. "We saw a lot of chromosome damage, quantitatively equivalent to that caused by 500 to 1000 rads of gamma radiation," Birnboim explains.

Other promoters, including benzoyl peroxide, also cause strand breaks. Just as there is a correlation between a compound's tumor-promoting efficacy and its stimulation of the oxidative burst, Birnboim finds a correlation between tumor-promoting ability and the amount of DNA damage. In contrast, compounds that block promotion inhibit the induction of strand breaks by TPA, which suggests that promoter-induced DNA damage somehow contributes to the eventual development of cancer.

The DNA damage is also prevented by compounds that block the formation of superoxide radicals; by superoxide dismutase, an enzyme that destroys superoxide anions; and by catalase, which breaks down hydrogen peroxide. Inhibitors of the enzymes increase the cells' sensitivity to TPA-stimulated DNA damage. Moreover, white blood cells that are genetically incapable of producing superoxide anions are very resistant to the damage.

Birnboim suggests that activated oxygen release by phagocytes in response to tumor promoters might contribute to the development of cancers in mouse skin and possibly in other tissues. He has shown that white blood cells, after stimulation with TPA, can induce DNA damage in other cells.

Phorbol esters and other promoters in the mouse skin system cause inflammatory changes in the treated area, including inward migration of phagocytes. Consequently, large numbers of these cells are present in the region where tumors eventually develop. Birnboim speculates, "Phorbol esters may be so effective because white blood cells are amplifying the damage."

Although normally white blood cells rapidly repair the DNA strand breaks caused by gamma radiation, repair of the breaks caused by phorbol esters proceeds much more slowly, according to Birnboim. Eventually, he postulates, a change might occur that would permit an initiated cell to express its malignant potential.

Phorbol esters resemble hormones in that they must bind to specific membrane receptors before promotion can occur. Cerutti has proposed a model to

explain how membrane-active agents such as promoters might produce chromosomal damage. This also depends on the production of activated forms of oxygen, which participate in the generation of a "clastogenic factor" that may serve as a signal for transmitting changes induced in the cell membrane by TPA to the genome of the original target cell and to other cells. (A clastogen is a substance that causes chromosomal abnormalities.)

Cerutti and Ingrid Emerit of the Université Pierre et Marie Curie in Paris have found that TPA can induce chromosomal abnormalities in human PMN's and lymphocytes that have been stimulated to divide. Again, superoxide dismutase inhibits this effect on DNA.

Cells that have been treated with TPA secrete into the incubating medium a clastogenic material that induces the chromosomal abnormalities in untreated cells. "The signal molecule," Cerutti told participants in a recent workshop,* "is not only responsible for damage in the stimulated cell itself but also communicates the damage to neighboring cells." Superoxide dismutase, in addition to inhibiting TPA induction of chromosomal abnormalities in the original cells, blocks the formation of the clastogenic factor and the factor's effects in secondary target cells. These findings indicate that superoxide radicals are involved at all three stages.

One of the early membrane effects of the phorbol esters is stimulation of arachidonic acid release from phospholipids. According to Cerutti and Emerit, oxidation of arachidonic acid may play a key role in transmitting the effects of promoters from the membrane to the genetic material. The researchers found that compounds such as indomethacin and ETYA (5,8,11,14-eicosatetraynoic acid), which inhibit the conversion of arachidonic acid to prostaglandins, thromboxanes, and leukotrienes, block the clastogenic action of TPA. The same compounds are also effective antipromoters. "The inhibitor effects clearly implicate the arachidonic acid cascade as a signal for the chromosomal damage induced by TPA," Cerutti maintains. The arachidonic acid oxidation pathway includes short-lived lipid peroxides that release active oxygen, the potential cause of the damage, as they are converted to more stable derivatives. Cerutti and Emerit suggest that the arachidonic acid pathway is important for promotion in some types of cells, but that in others, such as PMN's and macrophages, direct stimulation of the production of activated oxygen, as proposed by Birnboim and Troll, may predominate.

Cerutti and Emerit are attempting to isolate and characterize the clastogenic factor. They know that its molecular weight is less than 10,000, that it is relatively unstable, and that it is not a protein. Chromatographic analysis of TPA-treated lymphocytes shows little increase in the concentrations of prostaglandins, thromboxanes, and hydroxyarachidonic acids, compared to those in untreated cells. But the treated cells do contain elevated levels of arachidonic acid and an unidentified, highly polar material. The investigators speculate that the clastogenic factor consists of arachidonic acid, lipid hydroperoxides, and aldehydic compounds.

So far, the evidence that promoters foster cancer development by increasing the production of activated oxygen and thus damaging DNA is largely circumstantial. There have been no direct demonstrations that effects such as these cause cancer in the living animal. But Cerutti and Emerit obtained another piece of circumstantial evidence from studies of cells from patients with Bloom's syndrome, a genetic disease characterized by an increased incidence of chromosomal defects and also of cancers, particularly cancers of the blood cells.

Although there have been suggestions that Bloom's syndrome is caused by defective DNA repair, there is little evidence for defective repair in the cells of the patients. Cerutti and Emerit postulate that the chromosomal defects of Bloom's syndrome are caused by DNA damage that exceeds the capacity of the normal repair system. They found that cells from the patients produce a clastogenic factor that induces chromosomal damage in normal white blood cells and that is also present in serum from the patients.

The action of the clastogenic factor from Bloom's syndrome cells is inhibited by superoxide dismutase—a finding reminiscent of the results with tumor promoters. Cerutti says, "The results are parallel to what we have just demonstrated with TPA." The Bloom's syndrome cells may either produce too much activated oxygen or be deficient in their ability to destroy it.

The manner in which tumor promoters, by inducing DNA damage, might contribute to the development of cancer is not known, although there are several possibilities. The DNA changes might, for example, cause amplification or activation of cellular *onc* genes, perhaps leading to increased production of the gene product or formation of an altered product. It was recently shown that the major chromosomal abnormality of Burkitt's lymphoma involves translocation of the *myc* gene, apparently leading to its abnormal activation. It will be interesting to see whether treatment with tumor promoters affects cellular *onc* genes in any way.

Meanwhile, not everyone agrees that the indirect effects of promoters on DNA are necessary for promotion. For example, I. Bernard Weinstein of Columbia University's College of Physicians and Surgeons points out that, within minutes or hours of exposure to a promoter, the entire exposed population of cells responds, acquiring many of the properties of cells that have been malignantly transformed. It is unlikely that these rapid changes could be caused by damage to DNA as there would not be sufficient time for a particular change to spread through the population.

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The reversibility of these early changes, when the promoter is removed, also weighs against the possibility that they depend on DNA damage. Nevertheless, the alterations eventually become permanent and an autonomous tumor that no longer depends on exposure to a promoter develops.

These late stages may or may not require effects on DNA. Weinstein and others have suggested that the changes in cells that have undergone initiation and promotion may give the cells a competitive advantage over other cells, ultimately allowing the treated cells to produce an autonomous tumor. Nevertheless, DNA effects might be an alternative explanation of the progression to autonomy.

Tumor progression and malignancy are poorly understood. They are also characterized, as Weinstein notes, by frequent chromosomal abnormalities. "If the indirect action of promoters on DNA is important, it is probably important in the later stages," Weinstein concludes. "Then everybody could have their way."—**JEAN L. MARX**

*The workshop, Free Radicals in Promotion, was sponsored by the National Cancer Institute and held in Bethesda, Maryland, on 26 October 1982.