Commentary

How can we prevent cancer?

Carlo M. Croce*

Kimmel Cancer Center, Thomas Jefferson University, 233 South 10th Street, 1050 Bluemle Life Sciences Building, Philadelphia, PA 19107

Cancer is a genetic disease caused by a multistep process involving activation of oncogenes, loss of function of tumor suppressor genes, and alterations of modifier genes, for example, genes involved in DNA repair and genomic stability. Thus, most human tumors carry alterations in multiple genes affecting cell proliferation, cell survival, and genomic stability. In malignant diseases of the hematopoietic system and in soft tissue sarcomas, the process often is initiated by the activation of an oncogene, for example c-MYC in Burkitt lymphoma (1) or BCL2 in follicular lymphoma (2). In solid tumors the process often is initiated by the loss of function of a tumor suppressor gene (3).

Because tumorigenicity is a multistep process, it seems logical to postulate that if we are able to induce apopotosis of cells that have accumulated only few genetic changes and have just initiated the journey that will lead to malignancy, it will be possible to prevent the development of cancer.

Epidemiological studies indicate that vegetables and fruits can prevent a variety of human cancers (4) through the action of antioxidants such as carotenoids, vitamin E, etc., suggesting that such agents may be able to protect cells from the mutagenic action of reactive oxygen species (5). The pioneering work of Wattenberg (6), Talalay's group (7), and Conney's group (8) has shown that dietary chemicals can prevent chemical carcinogenesis in the laboratory and experimental animals.

Recent studies demonstrate that plants are rich in compounds that induce programmed cell death of premalignant and malignant human cells (9). Haridas *et al.* (10) extracted avicins, triterpenoid saponins, from the ground plant *Acacia victoriae,* and have shown that they can induce apoptosis of human leukemic cells by affecting mitochondrial function (10).

The question is, are avicins capable of inhibiting the processes that cause mutations or inducing apoptotic death of precancerous cells carrying mutations?

In this issue of PNAS, papers by Hanausek *et al.* (11) and Haridas *et al.* (12) show that avicins suppress the occurrence of H-ras mutations and aneuploidy in a murine 7,12-dimethylbenz[α]antracene (DMBA)-induced skin carcinogenesis model and inhibit activation of NFκB, a transcription factor involved in immune and inflammatory pathways.

Hanausek *et al.* have used two different protocols, one consisting of the administration of a single dose or repeated but smaller doses of DMBA, a powerful carcinogen also present in tobacco smoke (complete carcinogenesis model), and another consisting of the administration of DMBA (initiation) followed by repeated administration of 12-*O*-tetradecanoyl-phorphol-13-acetate (TPA) (promoter) to induce skin cancer in SENCAR mice (initiation/promotion model). The administration of avicins before that of DMBA or of DMBA and TPA, respectively, resulted in a significant decrease of the number of mice with papillomas (>70%) and the number of papillomas per mouse (>90%) The authors have used H-ras mutation at codon 61, DNA-modified base formation (8-OH-dG), and aneuploidy as early biomarkers to assess the ability of avicins to prevent the development of tumors and have shown that avicins suppress the occurrence of H-ras mutation and aneuploidy. These studies indicate that avicins could develop as important chemopreventive agents in many conditions where chronic inflammation and oxidative and nitrosative stress may lead to tumorigenicity.

Two radically different approaches can be used to develop a cancer prevention strategy. One discussed above and in the two papers published in this issue of PNAS, where natural products, shown to be able to prevent the development of tumors either by epidemiological studies or in animals, are exploited to investigate the molecular events associated with tumor prevention (Table 1).

The other is based on the genetic alterations present in precancerous and cancerous cells and replacing the tumor suppressor function lost in premalignant cells to eliminate cells already initiated for malignant transformation (13).

These two approaches are not mutually exclusive and they could complement each other, leading to the identification of novel ways to interfere with the multistep process of carcinogenesis. In ref. 11 significant reduction of the num-

Table 1. Two approaches to cancer prevention

I	II
Natural products	Cancer
[Epidemiological and experimental studies]	↓
↓	Gene identification
Extracts	[initiation/progression]
↓	↓
Purified compounds	Mechanisms of action
↓	↓
Cancer prevention	Gene targeted drug discovery
↓	↓
Mechanisms of action	Apoptosis of precancerous and cancerous cell
↓	
Drug development	
↓	
Apoptosis and precancerous and cancerous cells	

See companion articles on pages 11551 and 11557.

*E-mail: Carlo.Croce@mail.tju.edu.

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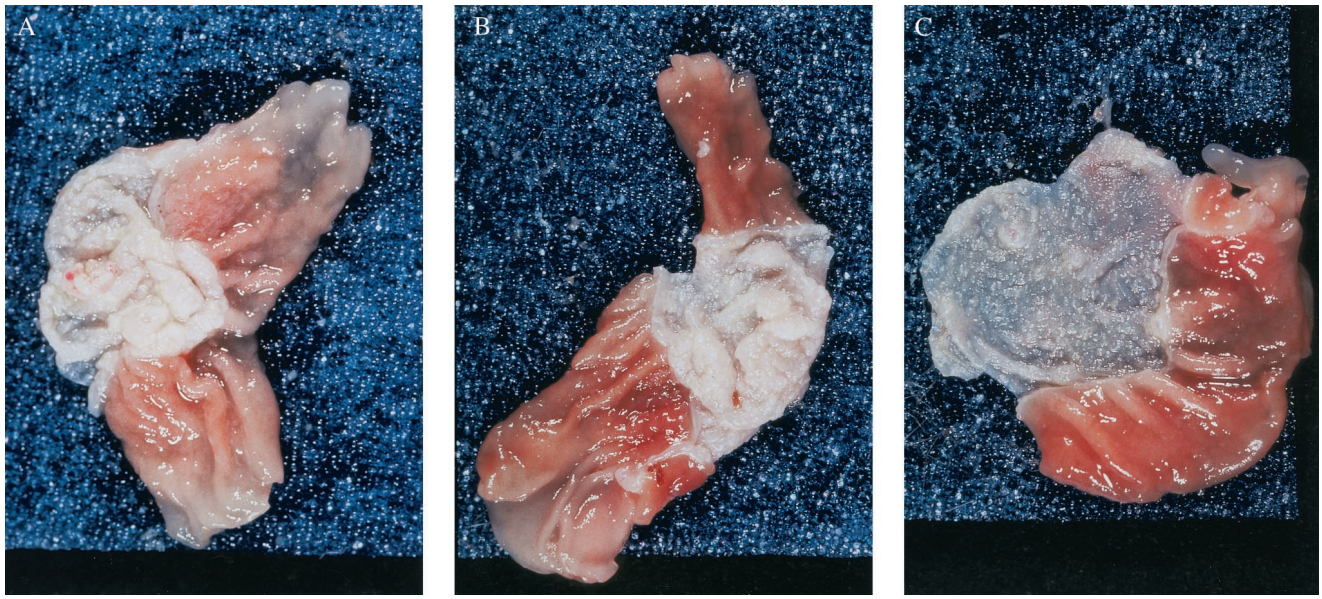

Fig. 1. Gene therapy for cancer prevention. *Fhit* +/− mice were treated intragastrically with NMBA and then exposed 48 h later to nothing (*A*) or an adenovirus vector containing the gene for green florescent protein (GFP) (*B*) or an adenovirus vector containing the *FHIT* gene (*C*). (*A*) The forestomach of the mouse not treated with the virus showed multiple tumors including ulcerated tumors that histologically were found to consist of papillomas and invasive carcinomas. (*B*) The forestomach of the mouse treated with the control virus containing the GFP gene showed multiple tumors consisting of papillomas and invasive carcinomas. (*C*) The forestomach of the mouse treated with the adeno *FHIT* virus showed a dramatic reduction of the number of tumors. (Magnification: ×20.)

ber of mice with papillomas and the number of papillomas per mouse was achieved by pretreating the skin of the mice with mixtures of triterpenoid saponins or with avicins, and Hanausek *et al*. have suggested possible mechanisms involved in tumor prevention. The question is, are avicins capable of inhibiting the processes that cause mutations or inducing apoptotic death of precancerous cells carrying mutations in specific cancer causing genes or both? The two papers appearing in this issue of PNAS and another also published in PNAS (10) suggest that avicins may have both effects: inhibition of oxidative and nitrosative stress and induction of apoptosis.

Fig. 1 shows the effect of *FHIT* gene therapy to prevent the occurrence of tumors in a chemically induced mouse tumor model. We have previously shown that the *FHIT* gene is knocked out at high frequency in a large number of human tumors, particularly those induced by environmental carcinogens such as tobacco smoke (14). We also have shown that *Fhit* +/− and *Fhit* −/− mice are more susceptible to spontaneous and chemically induced tumors (14, 15). Fig. 1*A* shows the forestomach of a *Fhit* +/− mouse treated with the carcinogen *N*-nitrosomethylbenzylamine (NMBA) intragastrically 12 weeks after treatment. As shown in Fig. 1, multiple tumors (that include papillomas and invasive carcinomas) are present in the forestomach of the mouse. Fig. 1*B* shows the forestomach of a *Fhit* +/− mouse treated with NMBA and then, 48 h later, with a adenovirus vector carrying the green fluorescent protein gene (AdGFP) intragastrically, Twelve weeks after treatment with NMBA this forestomach contained multiple tumors consisting of papillomas and invasive carcinomas. Thus, injection with the viral vector does not cause tumor prevention. Fig. 1*C* shows the forestomach of a *Fhit* +/− mouse treated with NMBA and 48 h later with an adeno *FHIT* viral vector intragastrically 12 weeks after treatment with the carcinogen. As shown in Fig. 1 the infection with the *FHIT* viral vector resulted in tumor prevention. Results of this experiment indicate that if we know the early genetic steps involved in tumor development we can prevent tumor formation by gene replacement.

Success and failure have accompanied treatment of human cancer. The failure is mostly caused by the ability of cancer cells to escape apoptotic death induced by various therapeutic modalities. Prevention is a more efficient and rewarding approach to cancer control. We can prevent cancer by eliminating substances that cause cancer, such as tobacco smoke, from the environment or avoiding exposure to radiation or viruses associated with cancer such as liver or cervical carcinomas. We also can exploit natural products and chemicals that can prevent oxidative and nitrosative stresses as those described in the PNAS articles cited here. Some of these products also may be able to produce apoptotic death of the precancerous and cancer cells. Finally, we can exploit the knowledge derived from the genetic dissection of the process of malignant transformation to define the earliest genetic steps involving carcinogenesis and devise gene-targeted specific therapeutic agents to eliminate precancerous cells. This approach will be particularly beneficial to tobacco smokers and former smokers who carry thousands and thousands of cells already initiated for malignant transformation.

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