more complicated because there are two sources of deep water: one originating within the the circumpolar ocean—that is, Antarctic Bottom Water (AABW)—and one in the north (NADW), each with distinctly different ^{14}C concentrations. The preanthropogenic $\Delta^{14}C$ value of NADW source water is estimated at -75 ± 5 per mil (*4*, *7*), whereas the deep water entering from the circumpolar ocean has a $\Delta^{14}C$ value of -158 per mil. The mass balance for the Atlantic Ocean (Table 1) is described in terms of four transport terms (N, C_a, C_a^r, and W_a). We assume the northern source input to be twice that of the southern source ($N = 2C_a$), as indicated by Broecker (*7*), on the basis of the NO_3^-, O_2, and SiO_2 characteristics of the deep water of the Atlantic Ocean.

Estimating the four transport terms for the deep water of the Atlantic Ocean requires that one of these be set. Earlier estimates of the NADW transport, based on either ^{14}C distribution or geostrophic calculations, range from 9 to 20 Sv (*4*, *7*–*9*). Here we have assigned a median value of 14 Sv for the NADW transport. Because $N = 2C_a$, the southern deepwater source is then fixed at 7 Sv, in good agreement with earlier geostrophic and ^{14}C calculations of AABW transport (*4*, *9*).

If we solve the mass and ^{14}C balances for $N = 14$ Sv, we obtain $C_a = 7$ Sv, $C_a^r = 11$ Sv, and $W_a = 10$ Sv (*10*). These transport calculations indicate a deepwater residence time for the Atlantic Ocean of 275 years, corresponding to a basin-wide upwelling rate of 4 m year^{-1}. There is also a net southward flow of deep water from the Atlantic to the circumpolar ocean ($C_a^r > C_a$) of 4 Sv, in contrast to the net northward flow from the circumpolar to the Indian and Pacific oceans.

Our transport calculations indicate that approximately 41 Sv of bottom water (> 1500 m) must be forming within the entire circumpolar ocean, resulting in an 85-year residence time for the deep circumpolar ocean. The ^{14}C balance in the circumpolar ocean (Table 1) implies that this "new" deep water has $\Delta^{14}C = -149$ per mil, in good agreement with earlier estimates of -148 to -152 per mil (*11*).

Deep-sea residence times are important for climate modeling and nuclear waste disposal. Whereas some parcels of water may have been isotopically isolated from the surface for up to 1700 years, the average replacement time of the abyssal waters in the oceans is much shorter. A parcel of bottom water formed near Antarctica will surface again after an average of 595 or 335 years, depending on its Pacific or Indian ocean choice. A parcel of deep water will reside in the Atlantic about 275 years. The overall replacement time for the entire deep ocean (which is influenced most by the residence time of the deep Pacific Ocean) averages about 500 years when the realities of abyssal circulation and ^{14}C atmosphere-ocean exchange are taken into account.

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References and Notes

1. The ^{14}C activity of the sample (A_s) was compared to the age-corrected ^{14}C activity of the National Bureau of Standards (NBS) oxalic acid standard (A_{ox} = 0.95 NBS activity), after appropriate normalization to a $\delta^{13}C$ value of -25 per mil for the samples and -19 per mil for the standard. The results are expressed as $\Delta^{14}C = (A_s/A_{ox} - 1)1000$ per mil. Because the comparison of A_s and A_{ox} is made for identical amounts of carbon, the quantity $(1 + \Delta^{14}C/1000)$ is a measure of the specific sample activity. The $^{14}C/C$ ratio of the oxalic acid standard is known [M. Stuiver, *Radiocarbon* **22**, 964 (1980)], and the absolute quantity ($\Sigma^{14}C$) of ^{14}C in a sample is given by

$$\Sigma^{14}C = 1.176 \times 10^{-12}(1 + \Delta^{14}C/1000)\Sigma CO_2$$

where both $\Sigma^{14}C$ and ΣCO_2 are expressed in identical units (for instance, micromoles per kilogram of seawater). The technical details of the measurements and calculation, and also the discussion of the age correction of the oxalic acid standard, are given in M. Stuiver, S. W. Robinson, H. G. Ostlund, H. G. Dorsey, *Earth Planet. Sci. Lett.* **23**, 65 (1974); M. Stuiver and S. W. Robinson, *ibid.*, p. 87.
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10. Extreme ranges of the model estimates can be obtained if one combines the limits of NADW transport estimates with the maximum errors in the NADW source $\Delta^{14}C$ value. A range of $N = 9$ to 20 Sv would result in a range of $C_a = 4.5$ to 10 Sv, $C_a^r = 10$ to 13 Sv, and $W_a = 2$ to 12 Sv. The corresponding range for Atlantic deepwater replacement time is 190 to 425 years. The upper limit increases to 480 years for $N = 3C_a$.
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Tumor Suppression with a Combination of α-Difluoromethyl Ornithine and Interferon

Abstract. *α-Difluoromethyl ornithine and mouse type 1 interferon, when administered simultaneously, were highly toxic to B16 melanoma cells in culture. Oral administration of α-difluoromethyl ornithine suppressed B16 melanoma development in mice 85 percent whereas interferon given subcutaneously inhibited tumor growth only 24 percent. Total or near total suppression of tumor growth was observed in mice receiving both treatments.*

It is thought that the naturally occurring polyamines putrescine, spermidine, and spermine play an important role in cell growth processes (*1*). Studies with α-difluoromethyl ornithine (DFMO), an irreversible inhibitor (*2*) of ornithine decarboxylase, the first step in polyamine biosynthesis, have demonstrated that DFMO retards the growth of a number of tumor cell lines both in vitro and in vivo (*3*, *4*). Interferons are a group of glycoproteins with antiviral, anticellular, antitumor, and immunoregulatory activities (*5*, *6*). It was recently reported that the decrease in the growth rate of cells treated with interferon is accompanied by a rapid loss in the activities of the polyamine biosynthetic enzymes ornithine decarboxylase and *S*-adenosylmethionine decarboxylase (*7*). Interferon retards a number of animal tumors (*8*) and some malignancies in man (*9*). DFMO treatment of mice bearing B16 melanomas inhibits tumor growth about 80 percent. These findings prompted us to examine the effects of DFMO and interferon, both separately and in combination, on the growth of B16 melanoma cells in vitro and in vivo. We found that the combination of the two agents markedly suppresses the tumor.

Melanoma cells (B16, line F_1) were grown as monolayers at 37°C in minimal essential medium supplemented with 10 percent fetal calf serum and antibiotics in an atmosphere containing 5 percent CO_2. Treatment with DFMO (2.5 m*M*) or mouse fibroblast interferon (type 1, 100 U/ml) inhibited cell growth 67 and 40 percent, respectively, after 5 days. How-

Table 1. Effect of DFMO and interferon on B16 melanoma growth and polyamine concentrations. B16 melanoma cells (1×10^5) were injected subcutaneously in the interscapular region of C57/BL mice. The animals were divided into four groups of ten. The first group was given normal drinking water and served as a control. The second group received mouse fibroblast interferon (1000 U per mouse, subcutaneously) starting on the second day after tumor inoculation and on alternate days thereafter for a total of ten doses. The third group received 2 percent DFMO in the drinking water (an oral dose of approximately 3 g/kg per day, based on fluid intake) starting 24 hours after tumor inoculation and continuing until the end of the experiment. The last group of animals received a combination of the DFMO and interferon treatments. After 3 weeks the animals were killed and their tumors were dissected out and weighed. A portion of the tumor tissue was used for polyamine determination by dansylation and thin-layer chromatography (*14*). Data are means ± standard deviations for two experiments done at different times. N.D., not detectable (< 20 pmole).

Treatment	Tumor weight (g)	Number of animals without detectable tumors*	Inhibition of tumor growth (%)	Polyamine concentration in tumor tissue (nmole/g; $N = 10$)		
				Putrescine	Spermidine	Spermine
Control	6.75 ± 1.82	0 of 20		24.5 ± 9.2	1139 ± 288	814 ± 149
DFMO	1.05 ± 0.49†	0 of 20	84	N.D.	47 ± 8	599 ± 125
Interferon	5.12 ± 1.94	0 of 20	24	35.1 ± 15.5	1129 ± 205	772 ± 125
DFMO plus interferon	0.24 ± 0.11†	4 of 20	96	N.D.	71 ± 14	840 ± 104

*As determined by visual inspection for pigmentation. †$P < .001$ (Newman-Keuls procedure).

ever, a combination of DFMO and interferon completely inhibited cell growth. DFMO alone rapidly depleted intracellular concentrations of putrescine and spermidine and caused a 30 percent reduction in the concentration of spermine. Interferon alone reduced intracellular putrescine and spermidine 93 and 41 percent, respectively, but had no effect on spermine. The combination of DFMO and interferon depleted intracellular polyamines to the same extent as DFMO alone. The pronounced cytotoxic effect of DFMO and interferon was confirmed by determining the cloning efficiency of tumor cells treated with increasing concentrations of interferon in the presence and absence of 2.5 m*M* DFMO (Fig. 1). Interferon alone, at the highest dose used (500 U/ml), killed 40 percent of the cells, while DFMO alone (2.5 m*M*) killed 30 percent. The combination of interferon and DFMO effected a much greater destruction of tumor cells (95 percent) than that which would be anticipated from an additive effect (58 percent).

To evaluate the effect of DFMO and interferon in vivo, B16 melanoma was maintained in C57/BL mice by serial transplantation of dissociated tumor cells. Inocula of 1×10^5 cells given subcutaneously formed solid tumors weighing about 7 g in 3 weeks. Treatment of the mice with 2 percent DFMO in drinking water inhibited tumor growth 84 percent compared to controls (Table 1), whereas interferon (1000 U/per mouse) given subcutaneously on alternate days for a total of ten doses inhibited tumor growth only 24 percent. No tumors were palpable in mice given both DFMO and interferon, although minute tumors (average weight, 0.24 g) were observed on autopsy in most cases. The combined treatment thus inhibited tumor growth 96 percent. Inhibition of tumor growth by DFMO was associated with a decrease in the concentration of putrescine and spermidine in the tumor tissue (Table 1). Interferon, whether given alone or with DFMO, had no effect on the concentration of polyamines in the tumor tissue. Indeed, spermine concentrations measured after the combined treatment were higher than the control values. A mock interferon preparation did not show any antitumor activity, nor did it enhance the activity of DFMO.

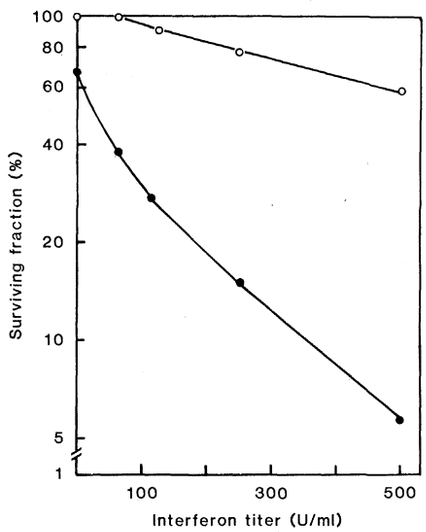

Fig. 1. Effect of DFMO and interferon on the cloning efficiency of B16 melanoma cells in culture. The cells were plated at a density of 1×10^5 cells per dish (diameter, 35 mm) and treated with DFMO (2.5 m*M*) and various concentrations of interferon. After 5 days of incubation, cells were collected by trypsinization, counted, plated (400 cells per dish), and incubated at 37°C for 10 days in an atmosphere containing 5 percent CO_2. After incubation the colonies were fixed with Formalin, stained with crystal violet, and counted under a dissecting microscope. We routinely obtain a plating efficiency of 50 percent for B16 melanoma cells. The number of colonies obtained in untreated control cultures was taken as 100 percent. Each concentration of interferon was tested in triplicate in each of two experiments; values are means for the two experiments. Symbols: (○) control and (●) DFMO-treated.

The mechanism underlying this suppression of B16 melanoma by combined DFMO and interferon is not clear. Possibilities include enhanced inhibition of polyamine biosynthesis, increased anticellular activity due to potentiation of protein synthesis inhibition by interferon in polyamine-depleted tumor cells, and enhancement of the host's immune response in the presence of interferon. The antitumor effect observed does not seem to be related to an enhanced inhibition of polyamine biosynthesis because interferon did not increase the polyamine depletion caused by DFMO. The apparent inhibition of polyamine biosynthetic enzymes by interferon observed by others (*7*) may be an indirect effect, since these enzymes have a short half-life and since inhibition of protein synthesis by interferon would affect the activities of these enzymes. Recent studies of the molecular mechanisms of interferon action indicate that interferon induces a specific protein kinase (*10*) that phosphorylates eukaryotic initiation factor 2 (eIF-2) (*11, 12*), slowing protein synthesis (*12, 13*). Furthermore, a novel protein kinase from adrenal cortex (protein kinase 380), which also phosphorylates eIF-2, is markedly inhibited by polyamines (*13*). Hence, depletion of intracellular polyamines by DFMO would enhance the phosphorylation of eIF-2 by interferon-induced protein kinase, resulting in increased inhibition of protein synthesis and cell death. Enhancement of host immune response (*6*) by interferon, along with its anticellular activities, could also account for its marked suppression of tumor growth in combination with DFMO.

It remains to be determined whether other tumor models are sensitive to

DFMO and interferon and, ultimately, whether these observations can be extended to human neoplasias. In preliminary studies we have obtained similar results with metastic Lewis lung tumor (3LL) in mice. However, in L1210 leukemia (a condition in which the tumor cells are rapidly dividing), the two agents did not show any striking antitumor activity. Hence, the effectiveness of DFMO and interferon may vary with tumor growth rate and type.

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The Human c-*ras*$_1^H$ Oncogene: A Mutation in Normal and Neoplastic Tissue from the Same Patient

Abstract. *The c-ras$_1^H$ oncogene can be distinguished from its normal cellular counterpart by the loss of a restriction endonuclease site. This sequence alteration is the basis of a rapid screening method for the presence of this oncogene. DNA's from 34 individuals were screened by this method, and all were homozygous for the normal allele. In contrast, DNA from a patient's bladder tumor, as well as DNA from his normal bladder and leukocytes, were heterozygous at that restriction endonuclease site. Further restriction enzyme mapping pinpointed the change in the mutant allele as being one of two nucleotides, either of which would change the 12th amino acid (glycine) in the normal c-ras$_1^H$ gene product. Point mutations in the codon for this amino acid have previously been described in a bladder tumor cell line and in the viral oncogene v-ras^H. These results indicate that the patient carried a c-ras$_1^H$ oncogene in his germ line, raising the possibility that the c-ras$_1^H$ oncogene confers a predisposition to neoplasia.*

DNA-mediated gene transfer (transfection) studies have shown that DNA from many human tumors—or from cell lines derived from tumors—transform NIH 3T3 mouse fibroblasts, whereas normal human DNA generally does not transform these cells (*1–3*). The DNA from transformed NIH 3T3 cells can be used for serial passage of the phenotype of transformation. This result led to the concept that a discrete genetic element, an oncogene, is responsible for initiation or maintenance (or both) of the transformed state.

One of these oncogenes, which was cloned from the related human bladder tumor cell lines EJ and T24 (*3, 4*), is closely related to the oncogene of the Harvey sarcoma virus (v-*ras*^{H}), as well as to a homologous genetic element present in normal human DNA (c-*ras*$_1^H$) (*5*). The biological differences between the bladder oncogene and its normal cellular counterpart were investigated by forming recombinants between segments of the viral and cellular genes and assaying these for the ability to transform cells (*6*). The segment harboring the transforming phenotype was thus mapped to a 351–base pair fragment extending from an Xma I site to a Kpn I site (Fig. 1). This fragment contains 26 nucleotides of a 5′ untranslated sequence, the first exon encoding 37 NH_2-terminal amino acids,

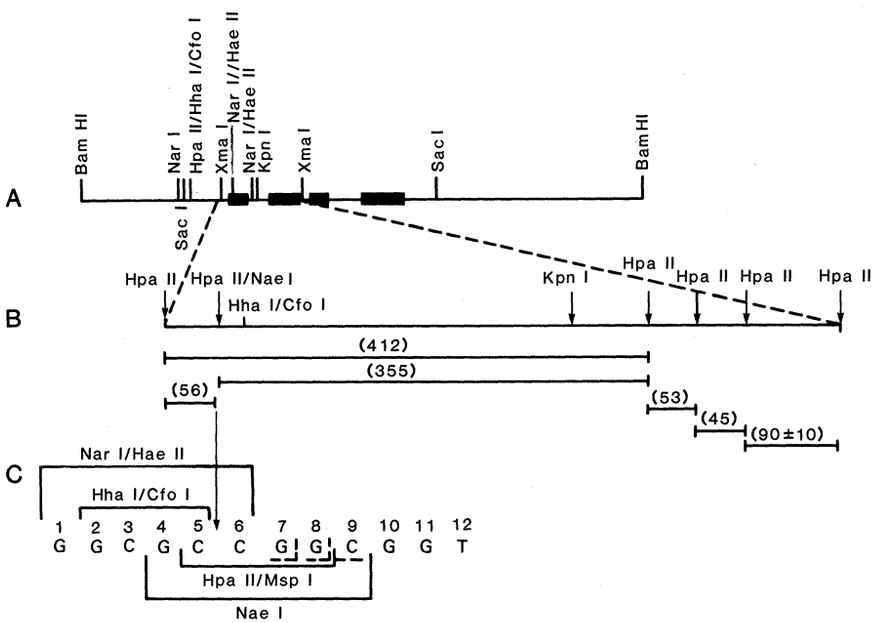

Fig. 1. Restriction map of the c-*ras*$_1^H$ gene. (A) The restriction map of the 6.6-kilobase Bam HI fragment of the c-*ras*$_1^H$ gene. The black boxes depict the coding exons. (B) The restriction enzyme map of the 600–base pair Xma I fragment. The lengths in base pairs of the various restriction fragments are shown in parentheses. The arrow from (B) to (C) extends through the Hpa II/Nae I site. (C) The nucleotide sequence surrounding that site. The numbers above the sequence have been arbitrarily assigned for reference in the text. The dotted line underscores GGC, the codon for glycine at amino acid 12. Brackets enclose the recognition sites for the indicated restriction enzymes. This figure was assembled from data in (*6*).