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Letter to the Editor

Enhanced Transformation of Mouse Embryo Cells by SV40 Virus Following Treatment with Fibroblast Growth Factor

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HORMONES have been shown to play key roles in modulating tumor growth *in vivo* and to influence cell transformation *in vitro* [1, 2]. Recently, a number of polypeptide hormones, termed growth factors, have been isolated and characterized. These include epidermal growth factor, fibroblast growth factor, platelet-derived growth factor and several more [3]. They all share the property of stimulating DNA synthesis in particular cell types, causing significant stimulation of cellular growth in culture and, possibly, *in vivo*. Epidermal growth factor (EGF) has recently been reported [4] to enhance adenovirus and radiation-induced transformation of rat cells and, on this basis, a role for EGF in transformation enhancement similar to that of tumor promoters has been suggested.

On the other hand, it is also well-known that cells actively synthesizing DNA or undergoing DNA repair as a result of chemical or physical injury are more susceptible to transformation by oncogenic viruses [5–7]. Therefore, it is conceivable that hormones with mitogenic effects may increase the frequency of transformation of their target cells by oncogenic viruses through stimulating their DNA synthesis. We here demonstrate that one such hormone, fibroblast growth factor (FGF), enhances the frequency of transformation by SV40 virus in mouse embryo cells pretreated with this polypeptide.

Cultures of logarithmically growing mouse embryo (ME) cells were infected with Simian Virus-40, (SV40, courtesy of Dr. I. Prasad of this institution) at various multiplicities of infection after a 16-hr period of pretreatment with various concentrations of FGF. The number of transformed foci was determined following a two-week incubation at 37°C and 10% CO_2 (Table 1). Another set of ME cell cultures was used to determine [^{3}H]-thymidine incorporation after a similar treatment with FGF. A third set of FGF-treated cultures was infected with SV40 at 100 PFU/cell and used 24 hr later for SV40-T-antigen immunofluorescent analysis utilizing hamster anti-SV40-T-antigen antiserum (courtesy of Dr. B. Carol, New York University School of Medicine, NY) (Table 2).

Based on the number of transformed foci appearing in monolayers of non-transformed cells, our experiments showed a greater than 100 percent increase in the frequency of transformation in cultures exposed to increasing concentrations of FGF as compared to those grown in unsupplemented tissue culture medium. At a concentration of 10 ng/ml this increase was highly significant ($P < 0.01$). The number of SV40-T-antigen-positive cells was also twice as high in the treated cultures one day after infection. The transformed character of the foci was further confirmed by show-

Table 1. Effect of FGF on the number of transformed foci produced by SV40-virus

SV40 (PFU/cell)	FGF (ng/ml) 0	10	50	100	200
10	7	12	14	14	14
1	6	11	13	14	13
0.1	5	9	10	13	13
0.01	3	7	8	7	8

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Table 2. Incorporation of [^{3}H]-thymidine and T-antigen analysis following FGF stimulation

FGF (ng/ml)	[^{3}H]-Thymidine (cpm/10^4 cells)	T-antigen-positive cells/ 100 cells examined
0	4666	4
10	8107	8.5
50	9186	10
100	8317	9.5
200	8411	10

ing that cells from them could form colonies in soft agar [8]. Similar results were obtained when growth in soft agar, instead of focus formation, was used to select for transformants in the infected population.

The rate of DNA synthesis, as assayed by [^{3}H]-thymidine incorporation, had approximately doubled as a result of 16-hr treatment with FGF prior to transformation. There was, however, no significant increase in the effect of FGF on DNA synthesis or on the enhancement of transformation frequency when its concentration was raised above 50 ng/ml. This, apparently, is the concentration at which the proliferative response is saturated in this system. By the same reasoning, it can be predicted that if quiescent cultures were compared with mitogen-stimulated ones, the apparent increase in efficiency of transformation might be much more dramatic.

If the cells in a mixed ME population vary in their sensitivity to FGF, then the enhancement of transformation by FGF in our experiments would affect mostly the more FGF-sensitive cells. Thus, such transformants would not be randomly derived from the total population. In fact, if the transformants reflected the characteristics of the subpopulation from which they were derived, they would appear more sensitive to the growth factor than the untransformed bulk population.

The correlation between the number of transformed foci and the increase in DNA synthesis in FGF-treated cultures supports the assumption that mitogenic hormones may enhance the frequency of transformation by increasing the number of cells synthesizing DNA, thus facilitating viral-DNA integration into the host cell genome.

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