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Transforming Growth Factors (TGFs): Properties and Possible Mechanisms of Action

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Transforming growth factors (TGFs) are growth-promoting polypeptides that cause phenotypic transformation and anchorage-independent growth of normal cells. They have been isolated from several human and animal carcinoma and sarcoma cells. One TGF is sarcoma growth factor (SGF) which is released by murine sarcoma virus-transformed cells. The TGFs interact with epidermal growth factor (EGF) cell membrane receptors. TGFs are not detectable in culture fluids from cells which contain high numbers of free EGF cell membrane receptors. SGF acts as a tumor promoter in cell culture systems and its effect on the transformed phenotype is blocked by retinoids (vitamin A and synthetic analogs). The production of TGFs by transformed cells and the responses of normal cells to the addition of TGFs to the culture medium raise the possibility that cells "autostimulate" their own growth by releasing factors that rebind at the cell surface. The term "autocrine secretion" has been proposed for this type of situation where a cell secretes a hormone-like substance for which it has external cell membrane receptors. The autocrine concept may provide a partial explanation for some aspects of tumor cell progression.

Key words: **transforming growth factor, sarcoma growth factor, epidermal growth factor, membrane receptor, tumor promoter, retinoid, growth factors, transformation**

A growth-promoting transforming polypeptide is characterized by the following properties: It is a strong mitogen which causes loss of density-dependent inhibition of cell growth in monolayer culture; it causes morphologic transformation of normal cells and anchorage-independent growth (a property in cell culture that correlates best with tumorigenicity in vivo) [1, 2]. Polypeptides that cause phenotypic transformation of indicator cells and meet the above criteria for a transforming protein have been isolated from a number of human and animal carcinoma and sarcoma cells. These polypeptides have been termed transforming growth factors (TGFs) [3]. The first TGF to be recognized as such was sarcoma growth factor (SGF) [4].

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It was observed that murine sarcoma virus (MSV)-transformed cells are characterized by a loss of measurable cell surface receptors for the growth-stimulating polypeptide epidermal growth factor (EGF) [5, 6]. The apparent loss of cell surface receptors occurs in both fibroblastic and epithelioid cells transformed by MSV and can be demonstrated with cells derived from various species [6, 7]. The effect is seen with transforming RNA viruses but not with DNA virus transformation nor with most chemical carcinogen-induced transformation. Over the years we have accumulated cells transformed by a variety of agents, including DNA viruses such as simian virus 40 (SV40) and polyoma, RNA viruses such as murine and avian sarcoma viruses, chemical carcinogens, and radiation, as well as cells which have become transformed spontaneously during passage in cell culture. These have been obtained from Swiss/3T3, Balb/3T3, and other mouse and rat cell systems. In collaboration with Stanley Cohen, these transformed cells were tested for their ability to bind ^{125}I-labeled EGF [5]. Of 47 independently isolated, chemically transformed cells, five show a pattern like the MSV-transformed cells, ie, almost complete loss of EGF receptors with normal levels of other receptors maintained. The chemically transformed cells without detectable EGF receptors have not yet been further characterized for the growth factors they may be producing. They represent a minority of chemically transformed cells that, with respect to this phenotype, behave like the MSV-transformed cells. The basis for this finding appears to be the production by the sarcoma virus-transformed cells of a family of growth factors called "sarcoma growth factors" (SGFs) [4]. Sufficient quantities are released into serum-free medium of Moloney MSV-infected mouse 3T3 cells to allow for their partial purification and characterization [4].

The growth factors that are produced by the sarcoma virus-transformed cells are a family of heat- and acid-stable transforming polypeptides. Addition of these SGFs to the culture medium of normal cells results in rapid and reversible changes. They cause normal rat fibroblasts to grow and form large colonies in soft agar (induction of anchorage-independent cell growth). They also have a pronounced morphologic effect on normal fibroblasts, converting them to transformed cells that pile up and are virtually indistinguishable from those genetically transformed by sarcoma viruses (Fig. 1). Thus, these polypeptides have the property of reversibly conferring the transformed phenotype on normal cells in vitro, and, in this sense, can tentatively be considered proximate effectors of the malignant phenotype [4]. The SGFs are specific for murine or feline sarcoma virus-transformed cells in that supernatants from untransformed cells or DNA tumor virus-transformed cells do not contain detectable quantities of these factors [4].

One of these SGFs has been further purified and shown to specifically bind to EGF membrane receptors [8]. The ability to bind to and be eluted from EGF receptors provides an important purification step in the isolation and characterization of EGF-like growth factors. SGF binding to EGF receptors can be completely blocked by mouse salivary gland EGF. The chemical properties of radiolabeled SGF that has been purified using this method give further support to the idea that SGF and EGF are distinctly different molecules. The SGFs have been shown to compete with EGF for available membrane receptors, yet they do not crossreact with antibodies to EGF, and their biological activity is distinct from that of EGF. Cells lacking EGF receptors are unable to respond to the growth-stimulating effects of this partially purified SGF. We concluded, therefore, that SGF released by MSV-transformed cells elicits its biologic effects via specific interaction with EGF membrane receptors.

Polypeptides which are characterized by their ability to confer a transformed phenotype on an untransformed indicator cell have also been isolated directly from tumor cells

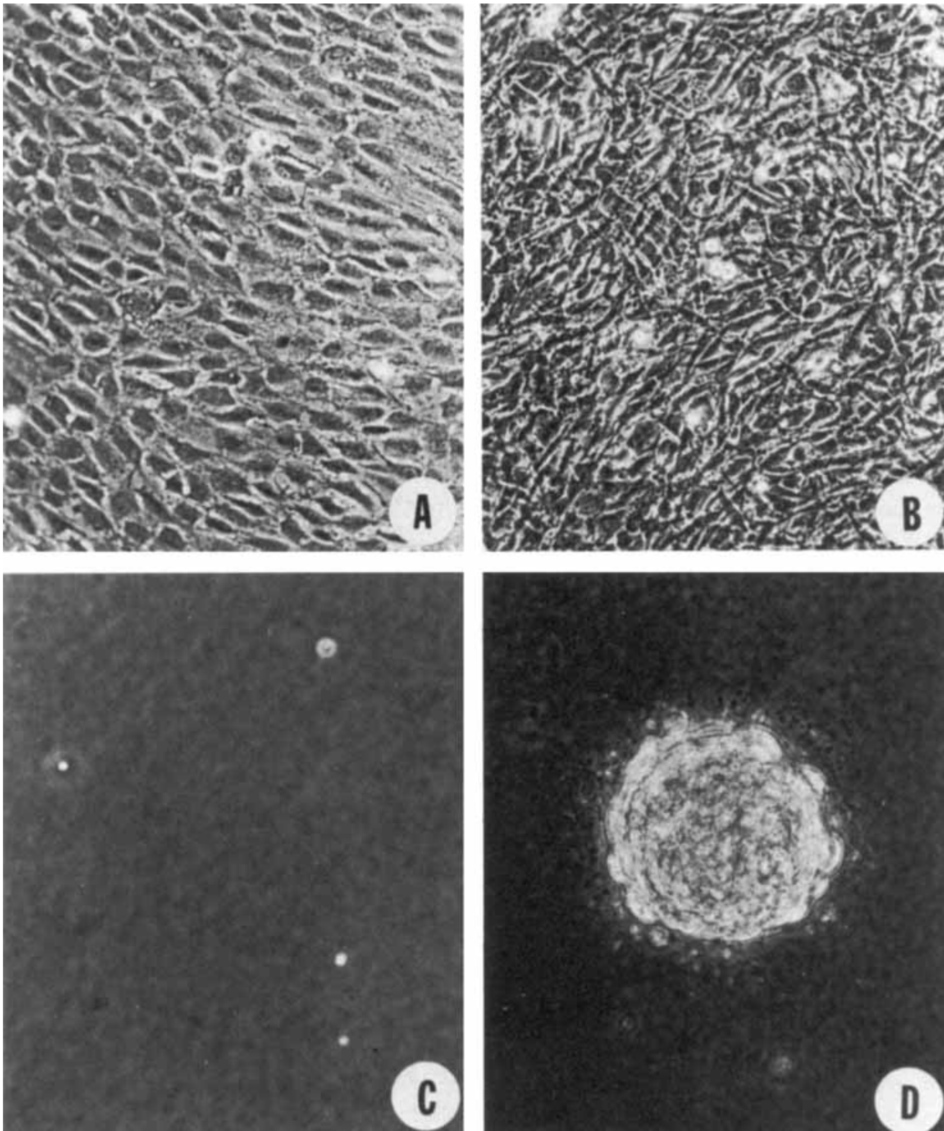

Fig. 1. A) Untreated NRK cells. B) NRK cells treated with an aliquot of SGF at 10 μg/ml and photographed six days later. The cells have grown to considerably higher cell density and display a morphology similar to that of virus-transformed cells. Magnification: A and B, 125X. C) Untreated NRK cells plated in 0.3% soft agar. D) NRK cells plated in 0.3% soft agar, treated with an aliquot of SGF at 10 μg/ml and photographed two weeks after treatment. The untreated cultures show primarily single cells with two or three cell colonies, but none of larger size. In the treated cultures, many colonies contained well over 500 cells. Magnification: C and D, 250X.

growing both in culture and in the animal using an acid-ethanol extraction procedure [9]. The properties of these intracellular polypeptides from both virally and chemically transformed cells are similar to those described for the SGFs isolated from the conditioned medium of sarcoma virus-transformed mouse 3T3 cells, suggesting the definition of a new class of transforming growth factors common to tumor cells of different origin. Thus, the TGFs represent a new class of polypeptides common to cells transformed either by chemicals or by sarcoma viruses and possess biological activity distinct from that of EGF.

Murine sarcoma virus-transformed cells lack available receptors for EGF. We have shown that this altered phenotype is the result of the endogenous production of growth factors by the MSV-transformed cells themselves. There is no evidence that SGF acts as a complete carcinogen itself, producing permanent cell transformation; its properties resemble classical chemical promoters of carcinogenesis, like 12-O-tetradecanoylphorbol-13-acetate (TPA) [10–12], the highly active component of croton oil. While TPA is an exogenous plant derivative acting on an animal or a cell, SGF is an endogenous, virally induced growth promoter.

Retinoids (vitamin A and synthetic analogs) [13] block the action in vivo of exogenous and endogenous promoters, preventing carcinogens from producing new tumors, but do not reverse the growth of many established tumors [13–16]. Retinoids prevent cancer of the lung [13, 17], skin [15], bladder [18], and mammary gland [19] in experimental animals, block cell transformation induced by chemicals [20] and radiation [20, 21] in culture, and reverse the anchorage-independent growth of transformed mouse fibroblasts [22]. If SGF is part of the natural tumor-promoting system and retinoids are part of the natural defense against that system, then one should be able to demonstrate a direct antagonism in cell culture.

We have used a subclone (536-7) of a rat fibroblast cell clone (NRK 49F) [6] that showed pronounced morphologic transformation and anchorage-independent growth when treated with SGF, forming multiple cell layers and crisscrossing each other in an apparently random fashion. Although the effects are all reversible, these treated cells resemble MSV-transformed cells in their phenotype [4]. The cells that were treated with both SGF and retinoids did not have a disordered growth pattern. Retinyl acetate, at 6 ng/ml, almost abolished the growth-stimulatory effect of SGF, as determined by the final cell density reached by the monolayer cultures ten days after the experiment began. The effect of retinoids on SGF-induced morphologic alterations was evident within a few days after treatment.

The retinoid concentrations ($1-2 \times 10^{-8}$ M) neither reversed the phenotype of virally transformed cells, nor blocked cell transformation produced by transforming viruses, such as the Moloney strain of MSV or SV40. Mouse 3T3 cells and rat fibroblasts were tested for susceptibility to transformation by MSV and by SV40. Neither retinyl acetate nor retinoic acid, up to 2×10^{-6} M, could be demonstrated to block either the initiation or the maintenance of virally induced transformation when efficient transforming viruses, like MSV or SV40, were used. In the same experiment, however, the SGF-induced morphologic transformation was inhibited. Retinyl acetate did not inhibit normal cell growth or the cloning efficiency of the rat fibroblast cell clones in petri dishes, but did have a pronounced effect on the final cell ("saturation") density of cells treated with SGF. SGF-induced cell growth was blocked and normal growth properties were essentially retained.

Table I shows that at concentrations well below those that show any evidence of toxicity, retinoids prevent SGF-induced colony formation in soft agar. Colonies that did form were smaller and contained fewer cells than those treated with SGF alone. Retinoic

TABLE I. Effect of SGF and Various Retinoids on the Colony-Forming Ability of Rat Fibroblasts Plated in Soft Agar

Treatment	Colonies/plate		
	Expt. 1	Expt. 2	Expt. 3
Untreated controls	0	0	0
+ Retinyl acetate (1.9×10^{-8} M)	0	0	0
+ Retinoic acid (2.0×10^{-8} M)	NT[a]	0	NT
+ Retinylidene dimedone (1.5×10^{-8} M)	NT	NT	0
+ Retinyl methyl ether (2.0×10^{-8} M)	NT	NT	0
SGF-treated (10 μg/ml)	44.5	39.0	49.5
+ Retinyl acetate (1.9×10^{-8} M)	2.5	1.5	8.0
+ Retinoic acid (2.0×10^{-8} M)	NT	3.2	NT
+ Retinylidene dimedone (1.5×10^{-8} M)	NT	NT	0.5
+ Retinyl methyl ether (2.0×10^{-8} M)	NT	NT	14.5

On day 0, 1×10^5 rat fibroblast cells, clone 536-7, were treated in monolayer cultures using DMEM with 1% fetal calf serum. On day 2, they were seeded at 1×10^4 cells per plate in 0.3% soft agar containing the additions shown as previously described [8]. All cells not treated with SGF (whether treated with retinoid or not) remained as single cells with occasional (<10%) small colonies of 2–4 cells. Colonies with greater than 20 cells after two weeks in agar were scored as positive.
[a]NT = Not tested.

acid, retinyl acetate, retinyl methyl ether, and retinylidene dimedone were all effective. Cells not treated with SGF and plated in agar remained as single cells with occasional 2–4 cell colonies. The clone used (536-7) has a spontaneous transformation rate, as determined by agar colony growth, of less than 1 in 10^6 cells plated. The preparation of SGF used, at 10 μg/ml, produced 40–50 large colonies per 10^4 treated cells and many smaller colonies with between four and 20 cells as well. The inhibiting effect of the retinoids was less evident or absent when more active SGF preparations, or higher concentrations of SGF were used. Retinylidene dimedone, of the compounds tested, was the most efficient inhibitor of SGF-induced phenotypic transformation (Table I, experiment 3). As a control against selective toxicity of retinoids to transformed cells, as compared to normal cells, MSV-transformed mouse and rat cells that grow well in agar without adding SGF were plated in soft agar in the presence of retinyl acetate at 2×10^{-6} M. No reduction in colony-forming ability was seen.

These experiments establish that, in the system used here, retinoids block the transforming effect of the polypeptide hormone, SGF. Only one concentration of each retinoid, well below the level that shows any cell toxicity, was used, and both the growth promoter and the antagonists were added to the cells at the same time. This system is now available for further studies where the concentrations, duration of treatment, and the nature of the interaction between each of the three components (promoter, antagonist, and responding cell clone) can be varied in a systematic manner.

The general transformation model we are proposing has these features: Viruses and chemical carcinogens act by inducing cells to produce normally repressed or inactive growth-promoting factors. These factors, which may be endogenous or exogenous to given cells, could be important in embryonic development, but if inappropriately expressed later in life could lead to transformation. Tumor viruses either provide transforming genes directly or activate cellular genes; chemical carcinogens do only the latter. These growth-promoting and transforming factors may be produced during early embryogenesis and then "switched off." The endogenous viruses, with their capacity to recombine with cellular genes, have the ability to transfer information between cells and presumably within a cell, like bacterial insertion sequences. They may well be vehicles that allow expression of the endogenous growth-promoter structural genes. In this model, the promoters, be they endogenous (SGF) or exogenous (TPA), act as proximal effectors of transformation.

The virogene-oncogene hypothesis [23] points out the possibly erroneous assumption that virally induced tumors would have to arise through external infection by emphasizing that virus-coded or virus-associated genes are already present in several animal species. These genes, rather than the environmentally transmissible agents, are more likely to be involved in the origin of natural cancers. The tumor viruses, although unnatural in that they had often been selected for producing rapid disease, have provided extremely powerful tools to dissect out and understand the molecular mechanisms involved. Genetically transmitted viral genes and transforming genes are now accepted as being part of the normal genetic makeup of many organisms and of being activated by agents such as chemical carcinogens, hormones, and radiation [24, 25]. In parallel with this is the frequently made assertion that chemical carcinogenesis and environmental carcinogenesis, or even industrial carcinogenesis, are almost interchangeable with one another. The finding that SGF, produced by animal cells themselves, is an extremely potent promoter in cell culture systems, suggests that endogenous growth promoters may be significant factors in naturally occurring cancers.

Since it was established that mouse sarcoma virus-transformed cells produced TGFs, we decided to screen human tumor cells for similar endogenous factors related to EGF and SGF. The human tumor cells tested for production of factors analogous to SGF were chosen for study because they had no apparent EGF receptors and readily form colonies in soft agar. Normal embryonal lung fibroblasts, unable to grow in soft agar, and A431 epidermoid carcinoma cells, which have a very high number of EGF receptors and grow poorly in soft agar, were used as controls.

Figure 2 shows the results of experiments comparing the five cultures for their ability to form colonies in soft agar. The cells were grown in monolayer cultures, harvested and seeded at varying densities into medium with 0.3% agar. Colonies were scored at five and 10 days. Colonies with more than 10 cells were counted as positive. The results shown in Figure 2 were obtained at five days; the later reading showed no additional positive cells. The cell line 9812 (a bronchogenic carcinoma) formed progressively growing colonies even when relatively low numbers of cells were seeded. A431 cells only showed colony growth when high cell inocula were used. This suggests that a critical concentration of diffusible factors from these cells is required for anchorage-independent growth.

Cells which are potential producers of factors that stimulate growth in soft agar (eg, human tumor cells) were seeded in one layer of agar at 1×10^6 cells per plate and overlaid with indicator cells (eg, rat fibroblasts) at 1×10^4 cells per plate. The indicator cells formed colonies when certain human tumor cells were seeded in the other layer. A673 (human rhabdomyosarcoma), 9812 and A2058 (human metastatic melanoma) cells elicited the

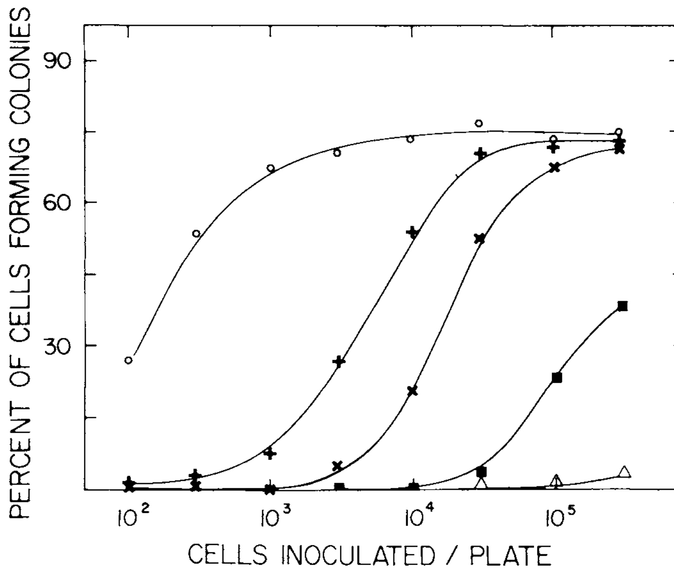

Fig. 2. Soft agar colony formation as a function of cell density. Soft agar assays were set up in 60-mm tissue culture dishes (Falcon #3002) by applying a base layer of 0.5% soft agar (Difco, Noble) and a 2-ml layer of 0.3% agar containing the appropriate cell number. HEL 299 (△); A431 (▪); 9812 (○); A673 (+); A2058 (X).

greatest response and released as much agar growth-stimulating activity as did a comparable number of MSV-transformed mouse 3T3 cells.

Figure 3 shows the results of experiments in which serum-free supernates from A673 cells were collected, concentrated, and run over a Bio-Gel P-100 column in 1 M acetic acid. Individual fractions were tested for protein concentration, ability to stimulate cells to form colonies in soft agar, and ability to compete with ^{125}I-labeled EGF [26]. The majority of the protein is in the void volume of the column. A major peak of soft agar growth-stimulating activity was found in the included volume with maximal activity in fraction 54. When the same fractions were tested for competition with ^{125}I-EGF binding, one major peak was again found, with maximal activity also in fraction 54. Aliquots were tested for stimulation of cell division in serum-depleted cultures of mouse 3T3 cells, rat NRK cells, and human skin fibroblasts; in all cases, the major growth-stimulating activity was found in fraction 54. Fractions 51 to 57 were pooled, concentrated by lyophilization, and used for further studies.

The identical procedure was used to test for growth-stimulating factors and EGF-competing peptides from the supernates of four other human cell cultures. Figure 4 shows that the two highly transformed tumor cell lines, 9812 and A2058, release a growth-stimulating and EGF-competing activity with an apparent molecular weight of 20,000–23,000 daltons (Fig. 4B,C). A2058 cells release a second factor with an apparent molecular weight of 6,000–7,000 daltons. Figure 4A shows that the supernate from normal human fibroblast cells did not release a detectable growth-stimulating activity and had no significant

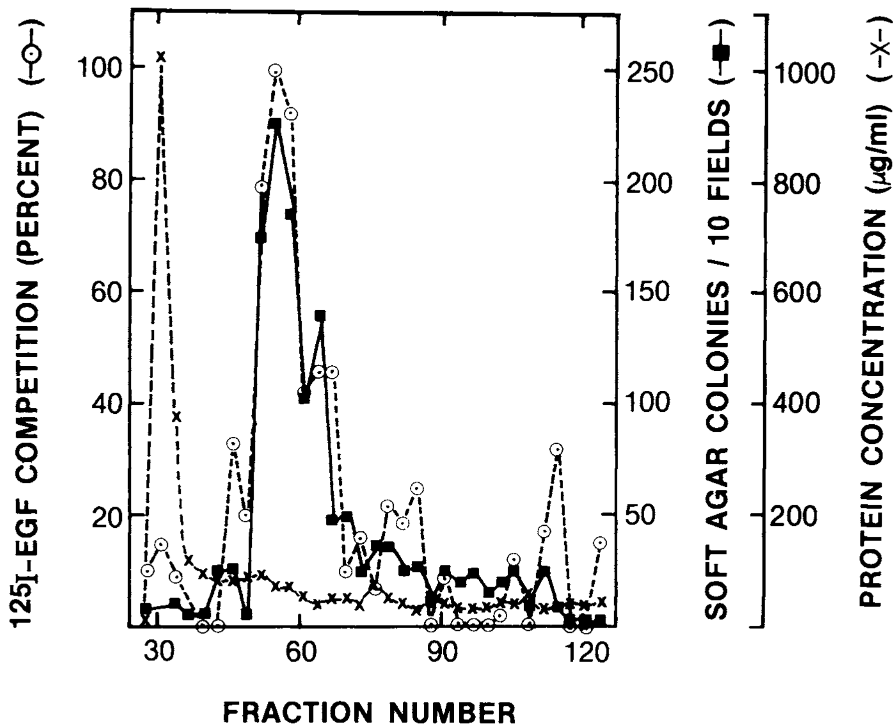

Fig. 3. Biological activity and protein determination of P-100 column fractions of concentrated conditioned media from A673 cells. EGF competition was performed as described. Nonspecific binding, determined by an addition of a 500-fold excess of unlabeled EGF, was approximately 200 counts per minute (cpm). Specific binding was approximately 1,200 cpm. Percent competition was determined after correcting for nonspecific binding. Soft agar assays were performed as described. Protein concentration was determined by the method of Lowry et al [48].

EGF-competing activity. A431 cells showed a smaller peak of growth-stimulating activity with an apparent molecular weight of 21,000 daltons; no EGF-competing activity was found.

Figure 5A shows a dose-response curve measuring soft agar growth as a function of protein concentration. The pooled, peak fractions from A673 cells are compared with those from normal human fibroblasts. There was a 50- to 100-fold difference in soft agar growth-stimulating activity.

The relative sensitivities of three different assays for growth-stimulating activity are compared in Figure 5B. The data are presented as the percentage of the maximal response. Induction of DNA synthesis as tested with serum-depleted rat fibroblast monolayer cultures was slightly more sensitive than the soft agar growth assay; EGF-competing ability was the least sensitive. The latter two assays were used in further studies, since they have greater specificity. Each of the TGF activities was destroyed by trypsin or dithiothreitol but was stable at 100°C for two minutes and to repeated lyophilization from 1 M acetic acid.

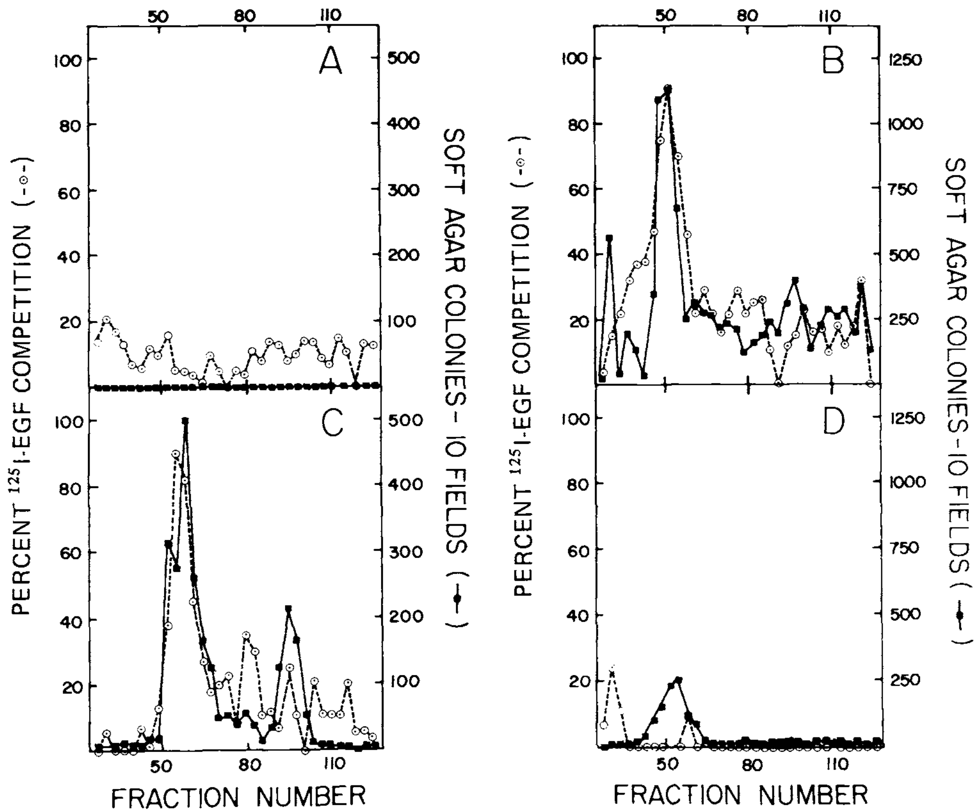

Fig. 4. Biological activity in P-100 column fractions of serum-free conditioned media from four human cell lines in culture. EGF competitions and soft agar assays were performed as described. A) HEL 299; B) 9812; C) A2058; D) A431.

Table II shows that the growth-stimulatory factor(s) released by the human tumor cells induce anchorage-independent growth of normal human fibroblasts. Two cell strains were tested; passage eight of HEL 299 (a human embryonic lung cell line) and the fourteenth passage of HsF (a skin strain from a normal human adult). A673 cells were tested at 10 μg/ml and 1 μg/ml. 1×10^4 cells were seeded per plate and 1,000 single cells were followed for two weeks. Those that grew to colonies containing 10 cells were scored as positive. The percentages of HEL 299 and HsF single cells that gave rise to colonies were 4.2% and 3.1%, respectively, using 10 μg/ml of P-100 purified TGF. In contrast, 23.6% of the rat fibroblast cells showed a pronounced response even at 1 μg/ml. TGF also induced soft agar growth of a mouse epithelial cell line MMC-1 [27] (data not shown).

In order to test whether human tumor cell lines could also respond to TGFs, cells such as A431, that untreated could not form colonies in agar unless inoculated at high density, were used. Carcinoma cell growth in agar also depends on "conditioning" factors, such as TGFs, which partially replace the requirement for high cell density. The results

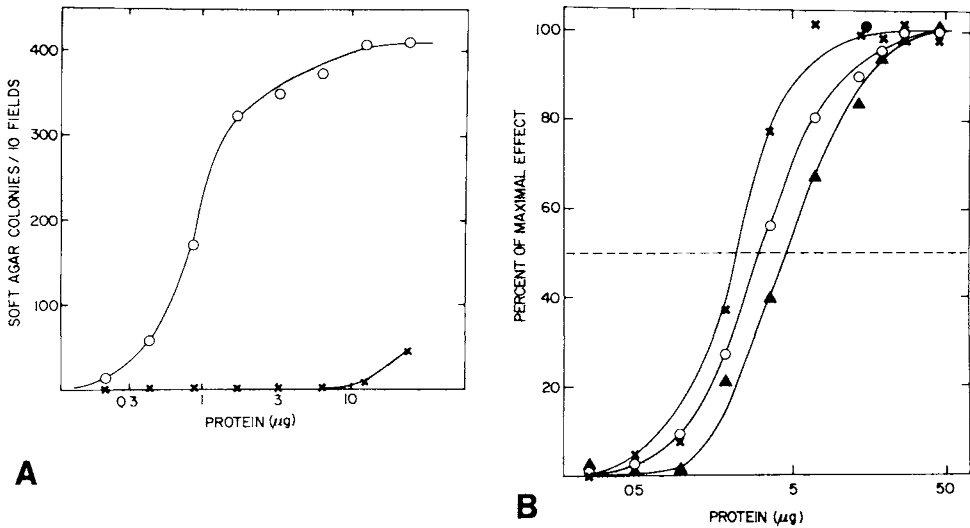

Fig. 5. A) Soft agar colony formation as a function of protein concentration. Bio-Gel P-100 column fractions from the 20,000–23,000 dalton region were pooled and lyophilized. Aliquots in 0.1 M acetic acid were added with the cells in the soft agar overlay. A673 (⊙); HEL 299 (X). B) Plot of the percent of the maximal effect as a function of protein. ^{3}H-Thymidine incorporation, EGF competition, and soft agar growth assays were performed as described. Maximal response was seen at 25–50 μg of protein; 73,500 cpm and 1,800 cpm, respectively, were incorporated for the ^{3}H-thymidine assays and control plates; 430 colonies per 10 fields where the control plates had none for the soft agar growth assay; 96% inhibition of ^{125}I-EGF binding.

TABLE II. Stimulation of Growth in Agar of Human Diploid Fibroblasts and Human Tumor Cells by TGF

Cell	Type	Colonies >10 cells/1,000 cells		
		Control	+ TGF (10 μg/ml)	+ TGF (1 μg/ml)
HEL299	Embryonic lung fibroblast	1	42	3
HsF	Adult skin fibroblast	2	31	2
A431	Epidermoid carcinoma	3	31	8
TE85	Osteosarcoma	1	75	14
NRK (clone 49F)	Rat kidney fibroblasts	0	236	37

were more striking when the human osteosarcoma line TE85, which can be further transformed by MSV and certain chemical carcinogens [28], was used as an indicator cell. These results demonstrate that normal and tumor cells respond to TGFs in the same manner as rat fibroblasts. The results, then, are not dependent on an unusual property of a particular indicator cell.

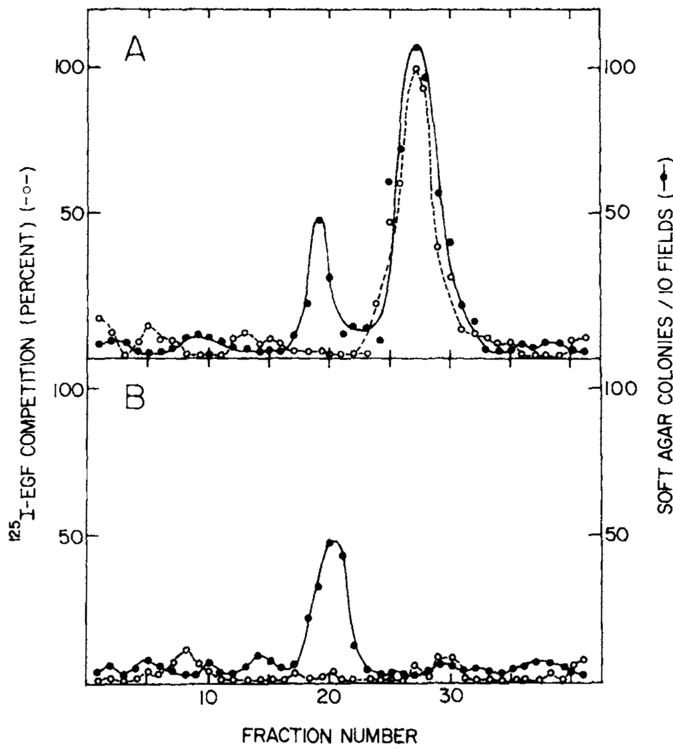

Fig. 6. Chromatography of biological activities in the peak region of Bio-Gel P-100 columns rechromatographed on a carboxymethyl cellulose column. A) A673; B) A431.

The active fractions from P-100 columns of A673 and A431 cells were pooled, concentrated, and applied to carboxymethyl cellulose columns. Two peaks of agar growth-stimulating activity were obtained from A673 cells; only the major activity was associated with the peak of EGF-competing activity. Dose-response curves from each peak show an activity detectable when concentrations of 10 to 20 ng/ml are added to soft agar. The comparable fraction from supernates of cultures of the normal human fibroblast showed no activity. Fractions derived from A431 cells showed (Fig. 6) only the less active, earlier eluting peak which is not associated with EGF-competing activity. We conclude that A431 cells which grow poorly in agar and have a high level of EGF receptors produce a factor capable of stimulating anchorage-independent growth of cells through a mechanism independent of the EGF receptor system. The highly transformed A673 cells, however, make at least two different factors. One interacts with the EGF receptor system and accounts for over 90% of the total activity in the fraction. The other is independent of the EGF receptor system and may be analogous to the factor produced by the A431 cells.

These results demonstrate that human tumor cells produce a growth factor(s) capable of inducing transformation in normal indicator cells. It has many properties in common with the factor from mouse and rat sarcoma virus-transformed cells. The major activity,

although considerably larger than SGF, is closely associated with EGF-competing activity. We have found that a chemically transformed mouse 3T3 cell line produces growth-stimulating factor(s) active in the soft agar growth assay (unpublished experiments). Production of these factors then, is not restricted to RNA tumor virus-transformed cells, sarcoma cells or rodent cells but, rather, may be a more general expression of the transformed phenotype. In assays comparing growth stimulation of mouse, rat, and human fibroblasts in monolayer cultures there is no evidence for species specificity of the factors produced by human cells. Conclusions as to whether the carcinoma, sarcoma, and melanoma cells are producing an identical factor(s) await further chemical purification. The present experiments show that anchorage-independent growth of tumor and normal cells is stimulated by these growth factors. Their production by transformed cells and the responses of their normal counterparts raise the possibility that cells "auto-stimulate" their growth by releasing factors that rebind at the cell surface [29]. Experiments demonstrating that growth in soft agar of tumor cells depends on the number of cells seeded per unit area argue that diffusible substances released by cells stimulate neighboring cells. Those cells that grow best in soft agar are the most efficient producers of transforming peptides. Additional cell lines will have to be tested under different conditions before conclusions can be drawn as to the significance of this association.

Roberts et al [9] described a procedure for purifying TGFs. The peptides are stable in acidic 70% alcohol. Intracellular growth factors have been extracted from cultured MSV-transformed mouse cells and from tumor cells in athymic mice. The major peptide with soft agar growth-stimulating activity has an apparent molecular weight of 6,700 daltons. The peak of EGF-competing activity is in the same fraction. A transplantable, transitional cell, mouse bladder carcinoma had agar growth-stimulating activity for rat fibroblasts. Ozanne et al [30] described a transforming factor from Kirsten sarcoma virus-transformed rat fibroblasts with properties like SGF and TGFs and report a similar activity in a spontaneously transformed rat cell line. The effect of the transforming factor on morphologic transformation can be blocked by actinomycin D early after treatment, suggesting that new RNA is produced prior to the change in phenotype of the indicator cells. Inhibitors of protein synthesis also produce a rapid reversion in the phenotype of the treated cells [30].

If release of the factor and rebinding to EGF receptors is essential for growth stimulation, tumor cell growth could be interrupted by exogenous agents, perhaps analogues that interact with the receptors but do not confer the ability to proliferate under anchorage-independent conditions [3]. Anchorage-independent growth is a cell culture property closely associated with the transformed state in vivo [31, 32]. These peptides, then, are potent proximal effectors of cell transformation. Their continued production appears to play a role in maintaining the transformed phenotype. This can be directly demonstrated in temperature-sensitive mutant transformants of rodent cells [30, 33], but has not yet been shown for factors produced by human tumor cells. The approach described here offers a sensitive assay for growth-stimulatory factors associated with maintaining the transformed state. Purification of such factors may lead to the development of specific immunologic assays for their production by tumor cells and their presence in body fluids. The factors may be analogous to peptide growth factors expressed early in normal embryonic development [33]. This is supported by experiments by Nexo et al [34]. In the mouse embryo (days 11 to 18) there is 5–10 times more EGF-like material than mouse EGF. Why the factors produced by transformed cells are so potent in stimulating anchorage-independent growth while EGF is not effective is unclear but suggests the possibility that there may be more "transforming" variants of the normally expressed growth factors produced in adult life.

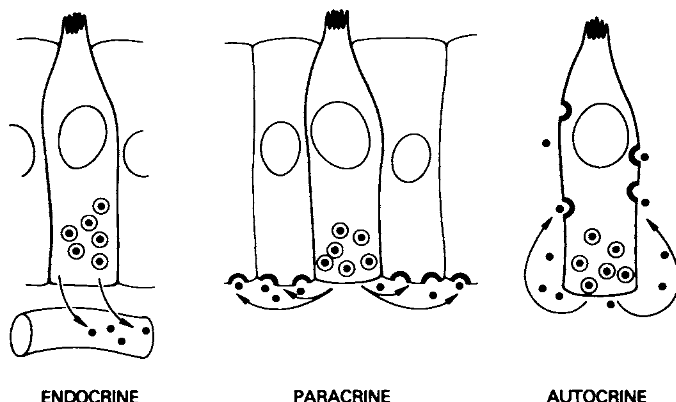

Fig. 7. Diagrammatic representation of autocrine, paracrine, and endocrine secretion (adapted from Dockray [21]). Regulatory chemical messengers are shown in latent form within the cell. The thickened, semicircular regions of the cell membrane represent receptor sites.

We suggest that these factors, like SGFs, are EGF-related peptides as insulin and somatomedins are related [35] and appear to have evolved from common ancestral proteins [36]. Further purification of these and other growth factors from human tumor cells is needed to define their relationship to other biologically active peptides that cells produce. We are also testing the possibility that certain tumor cells may also produce factors related to the phorbol ester family of growth promoters.

The growth of normal cells is largely controlled by the interplay between several polypeptide hormones and hormone-like growth factors that are present in tissue fluids [37]. Many new polypeptide growth factors have recently been identified in blood, serum, tissue fluids, and cellular extracts [38, 39]. Malignant cells, however, are not subject to all the same growth controls as are normal cells. In general, malignant cells require less of these exogenous growth factors than do their normal counterparts for optimal growth and multiplication, and it has been suggested that "transformed or malignant cells escape from normal growth controls by requiring less of [such] hormones or growth factors" [37]. For example, chick fibroblasts transformed by Rous sarcoma virus require less of an insulin-like growth factor for cell multiplication than do normal chick fibroblasts [40], and murine 3T3 cells transformed by SV40 virus require much less serum for multiplication and growth than do their nontransformed, contact-inhibited counterparts [41].

Furthermore, to account for the previously mentioned "lesser requirements of transformed cells for exogenous growth factors" [40, 41], one might suggest two additional properties: The transforming polypeptide should be produced by the putative transformed cell itself [29], and the putative transformed cell should have its own functional cellular receptors for this polypeptide, allowing phenotypic expression of the peptide by the same cell that produced it. The term "autocrine secretion" has been proposed [42] for this type of self-stimulation, whereby a cell secretes a hormone-like substance for which the cell itself has functional external receptors (Fig. 7). With this model of autocrine secretion, the classic "lesser requirement of malignant cells for exogenous growth factors" can be simply

explained: The endogenous production of growth-promoting polypeptides by the transformed cells lessens its own requirement for an exogenous supply of similar growth factors [29, 43].

The autocrine concept that we have outlined provides a simple conceptual model for certain aspects of malignant transformation, in suggesting that one of the ways in which cells become transformed is by endogenous production of growth factors for which they have their own receptors and to which they are capable of responding [29]. This internal production of growth-promoting polypeptides would serve as a constant stimulus for continued cell division, thereby releasing the peptide-producing cells from some of their normal exogenous physiologic controls. This molecular and cellular concept of malignant transformation may be placed in the broader context of developments in both neuroendocrinology and gastrointestinal endocrinology, in which the concept of "peptide humoral regulation" [44] is assuming increasing importance. As Grossman has noted, "We are coming to recognize that the substances that had been called gastrointestinal hormones are members of a broader group of regulatory chemical messengers that are produced by neural, endocrine, and paracrine cells in many parts of the body" [45]. Increasing attention is now being given to the study of paracrine control mechanisms, which involve the local diffusion of a peptide or other regulatory molecule to its target through the extracellular space but not via the bloodstream. In primitive organisms, such as coelenterates, that have no circulatory or glandular endocrine system, paracrine secretion is a principal form of humoral regulation of cells [46]. This type of paracrine humoral regulation of growth and differentition must also be important in the very early premammalian vertebrate embryo, which also has no circulatory or glandular endocrine system to sustain it.

The idea that malignant cells have some relation to early embryonic cells is an old one. Study of primitive mechanisms of "peptide humoral regulation" has already been shown to be of major importance for understanding comparative aspects of neuroendocrinology and gastrointestinal endocrinology [44]. On a purely deductive basis, autocrine secretion should be viewed as an even more primitive use of "regulatory chemical messengers" than either endocrine or paracrine secretion. Autocrine mechanisms for self-stimulation would confer obvious selective growth advantages on very early embryonic cells and could help to account for the explosive growth and multiplication of cells that occur during the earliest stages of embryogenesis, when a critical mass of cells that will survive as an organism must be established very rapidly. For example, a functioning circulatory system is established in the developing chick embryo within 48 hours of the start of incubation, long before the development of many aspects of endocrine function. It is also obvious that autocrine mechanisms are potentially very dangerous to the survival of the organism if they are not closely regulated as soon as they are no longer needed. We are suggesting that malignant transformation of cells may result from inappropriate later expression of autocrine growth factors that were required by cells during normal early embryogenesis [3, 29, 47]. The recent isolation and characterization of defined polypeptide transforming growth factors, which appear to function by such autocrine mechanisms, suggests that malignant transformation may be controlled some time in the future by means of specific inhibitors of the action of these peptides.

REFERENCES

1. Kahn P, Shin S-I: J Cell Biol 82:1, 1979.
2. Cifone MA, Fidler IJ: Proc Natl Acad Sci USA 77:1039, 1980.

3. Sporn MB, Newton DL, Roberts AB, De Larco JE, Todaro GJ: In Sartorelli AC, Bertino JR, Lazo JS (eds): "Molecular Actions and Targets for Cancer Chemotherapeutic Agents." New York: Academic (in press).
4. De Larco JE, Todaro GJ: Proc Natl Acad Sci USA 75:4001, 1978.
5. Todaro GJ, De Larco JE, Cohen S: Nature 264:26, 1976.
6. De Larco JE, Todaro GJ: J Cell Physiol 94:335, 1978.
7. De Larco JE, Todaro GJ: Cell 8:365, 1976.
8. De Larco JE, Todaro GJ: Symp Quant Biol 44:643, 1980.
9. Roberts AB, Lamb LC, Newton DL, Sporn MB, De Larco JE, Todaro GJ: Proc Natl Acad Sci USA 77:3494, 1980.
10. Boutwell RK: Crit Revs Toxicol 2:419, 1974.
11. Slaga TJ, Sivak A, Boutwell RK (eds): "Mechanisms of Tumor Promotion and Carcinogenesis." New York: Raven, 1978.
12. Weinstein IB, Wigler M: Nature 270:659, 1977.
13. Sporn MB, Dunlop NM, Newton DL, Smith JM: Fed Proc 35:1332, 1976.
14. Bollag W: Cancer Chemother Rep 55:53, 1971.
15. Bollag W: Eur J Cancer 8:689, 1972.
16. Verma AK, Boutwell RK: Cancer Res 37:2196, 1977.
17. Saffiotti U, Montesano R, Sellakumar AR, Borg SA: Cancer 20:857, 1967.
18. Sporn MB, Squire RA, Brown CC, Smith JM, Wenk ML, Springer S: Science 195:487, 1977.
19. Moon RC, Grubbs CJ, Sporn MB, Goodman DG: Nature 267:620, 1977.
20. Merriman RL, Bertram JS: Cancer Res 39:1661, 1979.
21. Harisiadis L, Miller RC, Hall EJ, Borek C: Nature 274:486, 1978.
22. Dion LD, Blalock JE, Gifford GE: Exp Cell Res 117:15, 1978.
23. Huebner RJ, Todaro GJ: Proc Natl Acad Sci USA 64:1087, 1969.
24. Aaronson SA, Stephenson JR: Biochim Biophys Acta 458:323, 1976.
25. Todaro GJ, Callahan R, Sherr CJ, Benveniste RE, De Larco JE: In Stevens JG, Todaro GJ, Fox CF (eds): "Persistent Viruses." ICN–UCLA Symposia on Molecular Biology. New York: Academic, vol 11, 1978, pp 133–145.
26. De Larco JE, Reynolds R, Carlberg K, Engle C, Todaro GJ: J Biol Chem 255:3685, 1980.
27. Keski-Oja J, De Larco JE, Rapp UR, Todaro GJ: J Cell Physiol 104:41, 1980.
28. Cho HY, Rhim JS: Science 205:691, 1979.
29. Todaro GJ, De Larco JE: Cancer Res 38:4147, 1978.
30. Ozanne B, Fulton J, Kaplan PL: J Cell Physiol 105:163, 1980.
31. Shin S, Freedman VH, Risser R, Pollack R: Proc Natl Acad Sci USA 72:4435, 1975.
32. Montesano R, Drevon C, Kuroki T, Saint Vincent L, Handelman S, Sanford KK, DeFeo D, Weinsten IB: J Natl Cancer Inst 59:1651, 1977.
33. Todaro GJ, De Larco JE: In Jimenez de Asua L, Levi-Montalcini R, Shields R, Iacobelli S (eds): "Control Mechanisms in Animal Cells: Specific Growth Factors." New York: Raven, vol 1, 1980, pp 223–243.
34. Nexo E, Hollenberg MD, Figueroa A, Pratt RM: Proc Natl Acad Sci USA 77:2782, 1980.
35. Rinderknecht E, Humbel RE: J Biol Chem 253:2769, 1978.
36. Niall HD: In Goodman G, Meienhofer J (ed): "Peptides: Proceedings of the Fifth American Peptide Symposium." New York: Halsted, 1977, pp 127–135.
37. Holley RW: Nature 258:487, 1975.
38. Sato G, Ross R (eds): "Cold Spring Harbor Conference on Cell Proliferation." New York: Cold Spring Harbor Press, vol 6, 1979.
39. Gospodarowicz D, Moran JS: Ann Rev Biochem 45:531, 1976.
40. Temin HM: J Cell Physiol 69:377, 1967.
41. Holley RW, Kiernan JA: Proc Natl Acad Sci USA 60:300, 1968.
42. Sporn MB, Todaro GJ: N Engl J Med 303:878, 1980.
43. Pastan I: Adv Metab Dis 8:7, 1975.
44. Zimmerman EG: Fed Proc 38:2286, 1979.
45. Grossman MI: Fed Proc 38:2341, 1979.
46. Dockray GJ: Fed Proc 38:2295, 1979.
47. Todaro GJ, Heubner RJ: Proc Natl Acad Sci USA 69:1009, 1972.
48. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951