CONCOMITANT SYNTHESIS OF GROWTH FACTORS AND THEIR RECEPTORS—AN ASPECT OF MALIGNANT TRANSFORMATION*

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Abstract—Sporn and Todaro [1] have suggested that malignant transformation may involve autocrine stimulation by transforming growth factors. In an extension of this hypothesis it is proposed that the malignant phenotype may be characterized by the simultaneous expression of a growth factor and its appropriate receptor, either one of which, but not both can be expressed by normal cells.

Cells only divide when instructed by specific signals

Two major and mutually exclusive hypotheses have been proposed to account for the mechanisms of proliferation control in mammalian cells. The oldest idea, now largely discredited, proposed that the proliferation is the natural state for all well nourished cells and that *in vivo* this mitotic exuberance is suppressed by some form of negative feedback (e.g. by chalones). Cancer was consequently regarded as a state in which control over proliferation was lost; an absence of, or unresponsiveness to, hypothetical suppression mechanisms. In recent years overwhelming evidence has accumulated for the alternative view that states that normal cells proliferate as an adaptive response to a sequence of external signals. Cancer can no longer be regarded as a loss of function, but rather as a gain; namely the ability to undergo division without the need for triggering stimuli from the environment.

The best studied of these mitogenic signals is a group of peptide hormones, the activity of which is mediated by binding to high affinity specific receptors on the cell surface. The available data are consistent with a model in which at least two sequential signals are needed; the first set of polypeptide growth factors—and the ones to which this discussion addresses itself—renders cells competent to divide in response to the second class of growth factors which allow progression into division [2]. The latter are insulin-like and include the somatomedins. They have a very broad target specificity and tissue distribution and are present in plasma at concentrations sufficient to induce mitosis. The "first signal" growth factors which render cells capable of responding to the insulin-like growth factors have a restricted target specificity and act predominantly in the locality in which they are produced, unlike the systemically active insulin-like factors. The biological information for

these growth factors appears to reside in the receptor which is modified by interaction with the hormone; the hormones do not require internalisation for activity [3]. This raises ideas about how cell division might be susceptible to control by antagonists which bind to the receptor but do not activate it.

The major growth factor present in serum which confers proliferative competence on normal mesenchymal cells such as fibroblasts, and which does so at nanomolar concentrations is platelet derived growth factor (PDGF) [2]. Present in the α granules of platelets this exceedingly stable 30 Kd peptide is released during the clotting process and is thought to play a role in wound healing and may even play a part in the pathogenesis of atheroma [4]. Many other types of growth factor with broad target tissue specificities have been described. Epidermal growth factor (EGF) found in murine submaxillary glands and in Brunner's glands in the small intestine, may play several distinct roles since it is mitogenic for a wide range of target cells (not exclusively epidermal) and is also a very potent inhibitor of gastric acid production (in which latter role it is better known as urogastrone). Current speculation concerns its potential role as a growth regulator of small intestinal epithelium. Relatively well characterised growth factors have been described in the lymphoid system (the interleukins, $1L_1$, $1L_2$, $1L_3$ etc.) and in the bone marrow (the ever-expanding family of colony-stimulating factors).

Growth factor requirements of cancer cells

Malignant transformation of cells such as fibroblasts abolishes their requirements for specific growth factors such as PDGF [5, 6]. Indeed, loss of the requirement for exogeneous growth factors appears to be an important, if not the paramount, feature defining the malignant phenotype. Density-dependent growth inhibition of normal cells *in vitro* is due to depletion of growth factors from the medium [7]. Absence of such inhibition shown by transformed cells is a consequence of their loss of the requirement for growth factors provided by the culture medium. One interpretation of such findings would be that malignant cells do not require a trigger-

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ing first signal in order to divide in response to the insulin-like growth factors. Such an hypothesis seems to be untenable in view of the evidence that in some cases the medium in which human as well as animal cancer cells (both virally and chemically induced) are grown has the capacity to render normal cells competent to progress into division in response to insulin-like growth factors, i.e. has PDGF-like activity.

While malignant transformation appears, from current evidence, to be a common cellular destination reached by many different routes, conceptually, the simplest explanation for autonomous proliferation in the absence of exogenous growth factor stimulation is "autocrine secretion". Sporn and Todaro [1] have suggested that a malignant cell synthesis and releases growth factor-like peptides which bind to and activate autologous receptors. These tumour-derived growth factors, whether isolated from culture medium or by extraction from tumour tissue [8], appear to be chemically similar in being acid and heat stable polypeptides, some of which bind to EGF receptors and show close amino-acid sequence homology with EGF [9], others which resemble PDGF and bind to the appropriate receptors. A further group, less well defined, which bind to receptors which do not bind PDGF or EGF has been described. The role of these polypeptides as an autocrine stimulus to proliferation remains to be proven but the circumstantial evidence is persuasive.

These cancer-derived growth factors were claimed by De Larco and Todaro [10] to differ qualitatively from the EGF or PDGF type of growth factor in that they stimulated normal cells not only to divide but to grow in vitro with the morphology of transformed cells and to exhibit other phenotypes of transformation such as overgrowth (i.e. lack of density dependent growth inhibition) and ability to form colonies in soft agar. Accordingly, these heat and acid stable polypeptides were referred to as transforming growth factors (TGF). Upon removal of TGF normal cells resumed normal growth patterns and the permanence of the transformed phenotype in malignant cells was attributed to their inherited capacity to produce TGF. Inherent in this hypothesis is the assumption that EGF-PDGF growth factors lack the capacity to induce a malignant phenotype. The different TGF's have been classified as either α TGF's, which bind to EGF receptors and whose effects are not potentiated by EGF or β TGF's, which do not bind EGF receptors and whose effects (e.g. anchorage-independent growth) are strongly potentiated by EGF.

It has often been assumed that TGF's are qualitatively abnormal peptides, whose abnormalities are responsible for their activity in the reversible induction of the malignant phenotype in suitably sensitive untransformed target cells. Some TGF's such as the first of the class to be studied, sarcoma growth factor (SGF), may well be abnormal peptides but there is no convincing evidence that such abnormalities are responsible or necessary for their transforming activity. Firstly, TGF's extracted from normal tissues such as placenta or kidney are effective at inducing reversible anchorage-independent growth in susceptible untransformed cells and secondly the auth-

entic growth factors such as EGF and PDGF can, under some circumstances, confer upon normal cells many of the features associated with the transformed phenotype. Moreover, TGF's from different sources (normal and malignant) differ from one another chemically and in their capacity to trigger different types of target cells [8].

Kaplan and Ozanne [11] have recently shown that the ability of growth factors to induce anchorage-independent growth in clones of rat fibroblasts is not a unique property of TGF's but may be exhibited by EGF, PDGF or even whole serum. The target cell clones employed varied in their responsiveness to growth-factor-induced anchorage-independent growth. They also showed that cells resistant to this effect of growth factors are also resistant to transformation by a variety of oncogenic viruses, a finding which implies that cellular responsiveness to growth factors in some way plays a central role in the expression of the malignant phenotype.

Autoreceptors on cancer cells for normal growth factors

The malignant phenotype does not therefore appear to depend upon the synthesis by cancer cells of abnormal growth factors which cause cells to adopt an in vitro growth pattern associated with cancer. We are attracted by the hypothesis that in cancer cells neither the growth factor they constitutively synthesize nor the receptor for this factor need be qualitatively different from the factor or receptor in normal cells. It is their simultaneous presence which may distinguish malignant from normal. Thus many types of normal cell make growth factors, which when tested in vitro may produce the transformed pattern of growth. However, normal cells presumably do not have receptors for the growth factor which they produce and these growth factors act on other cells so as to achieve regulated cell proliferation in development, in maintenance of the adult organism and in wound healing. We propose that the essential characteristic of malignancy does not lie in producing a TGF but is a consequence of a cell making at the same time a growth factor and its receptor. A component of malignancy is the simultaneous expression of two genes, one for a TGF and the second for its receptor. Malignant transformation could therefore arise in three ways: (a) in cells having a receptor by activating the appropriate TGF gene; (b) in cells making a TGF by activating the appropriate receptor gene; (c) by activating both the TGF and the receptor gene.

How could such events be brought about? Firstly, known transforming peptides in oncogenic viruses may show homology with known mitogens. For example, the middle T peptide which is responsible for malignant transformation by polyoma virus shows close homology with gastrin, a known mitogen [12]. The B-lym oncogene detected in chicken bursal lymphomas induced by the avian myelocytomatosis virus (although it does not appear to be a retroviral oncogene) encodes a peptide with close homology to the amino terminus of transferrin [13]. Furthermore some retrovirus oncogenes (v-onc) or the corresponding cellular proto oncogenes (c-onc) may encode peptides with growth factor activity and Water-

field *et al.* [14] and Doolittle *et al.* [15] demonstrated that the p28^{sis} product of the simian sarcoma virus sis oncogene had marked homology with a sequence of at least 90 amino acids of platelet derived growth factor. On the other hand many oncogene products such as those encoded by the ras gene family do not encode for growth factors but cells transformed by these genes express the appropriate p21^{ras} oncogene product as well as an apparently unrelated growth factor. Further candidates for transforming growth factors may be found in the recent isolation, cloning and sequencing of the EGF mRNA [16]. Detected by cDNA sequencing this message encodes a protein of 1217 amino acids (with a molecular weight of 133,000). The EGF moiety in this message comprises only 53 amino acids which are flanked by extensive unidentified peptides. The amino terminal segment of this precursor contains seven distinct peptides with sequences similar to, but not identical, with authentic EGF. So, we are faced with seven intriguing peptides in search of a function: suitable candidate molecules for TFF activity.

A connection between the induction of receptors for growth factor and the malignant transformation is indicated by the finding that the src oncogene of avian sarcoma virus encodes a phosphoprotein with potent tyrosine phosphorylating activity. This pp60^{src} protein kinase is intimately associated with the EGF receptor and may phosphorylate important cytoskeletal proteins.

The recent demonstrations of sequential oncogene activation in the *in vitro* generation of the malignant phenotype is intriguing [17, 18]. The apparent existence of two distinct complementation groups of oncogenes, one for "establishment" or "immortalization" functions and the other for "transformation" functions encourages speculation about the sequential activation of cellular oncogenes which may encode growth factor peptides and/or receptor-associated proteins.

Retroviral oncogenes and their cellular homologues may therefore be involved on the one hand in inappropriate growth factor production or on the other, inappropriate receptor activation.

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