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Growth factors in melanoma

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Abstract

Human melanoma cells in culture are the source of a wide variety of polypeptide growth factors. Melanoma-derived basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF)-A and PDGF-B chains, transforming growth factor (TGF)-α and TGF-β, interleukin (IL)-1α and IL-1β, and melanoma growth stimulatory activity (MGSA) have similar biochemical and functional properties when compared to their counterparts produced by untransformed cells. In contrast to melanoma cells, normal melanocytes, even under optimal growth conditions, express only TGF-β1 and MGSA at detectable levels suggesting that production of the other growth factors is a tumor-associated phenomenon. Recent evidence suggests that at least two of the growth factors, bFGF and MGSA, contribute to autocrine growth stimulation of melanoma cells. Whether PDGF, TGF-α, IL-1, and TGF-β act in an autocrine mode is unclear at present. However, these four growth factors are among those secreted by melanoma cells and, therefore, can be expected to interact with normal cells of the tumor stroma *in vivo*. Such paracrine effects include not only growth modulation in the context of angiogenesis and stroma formation, but also tissue degradation by proteolytic enzymes, the modification of extracellular matrix composition, and expression of adhesion receptors.

Introduction

In the last decade, malignant melanoma has been the subject of a number of studies addressing tumor-associated changes in growth regulatory pathways. This activity was initially stimulated by the observation that virus-transformed fibroblasts produce growth factors that contribute to their own proliferation [1], which provided the basis for the concept of autocrine growth stimulation of malignant cells. According to this idea, the constitutive, endogenous production of growth factors by malignant cells provides one mechanism of escape from exogenous regulatory mechanisms which control the proliferation of normal cells. Unrestrained growth allows the spread of malignant cells either locally or at distant sites, where they destroy normal tissues. In addition, perturbations in the signal transduction pathways, at the level of growth factor receptors or intracellular second messengers, may contribute to the functional independence of malignant cells from exogenous control. In this review, we focus on the relevance of different growth factors and growth factor receptor systems to melanocyte growth regulation. Particular attention is given to the constitutive production of growth factors by malignant melanocytes and to their potential as autocrine factors.

Human melanoma as a model for tumor growth regulation

Human melanoma has emerged as an experimental system well-suited to address tumor-associated alterations in growth control. Pioneering clinical and histopathological work identified five sequential steps in the progression from precursor lesions to

malignant melanoma [2, 3]. The first two steps of melanoma progression are characterized by focal proliferation of structurally normal melanocytes to lesions with architectural and structural abnormalities (common acquired and dysplastic nevi). The last three stages are represented by the radial growth phase melanoma with no competence for metastatic spread, the vertical growth phase melanoma capable of metastasizing and, finally, metastatic melanoma. Advances in culture techniques allow the establishment of not only metastatic melanoma cell cultures at a high rate of success but also cultures from precursor lesions and, most importantly, cultures from normal epidermal melanocytes [4–6]. Subsequent studies have shown that normal human melanocytes in culture remain untransformed since they are non-tumorigenic in nude mice, do not grow anchorage-independently, have a finite life span, and show no chromosomal abnormalities (see [7, 8] for review). In addition, neither spontaneous transformation nor immortalization has been reported for these cells *in vitro,* an observation that allows the comparative investigation of growth regulatory pathways in normal versus malignant melanocytes.

The development of defined culture media has led to the identification of exogenous growth factors necessary to support the *in vitro* proliferation of melanocytes at various stages of progression (see [9, 10] for review). Successive stages of melanoma progression are characterized by a progressive loss of growth factor requirements. Whereas *in vitro* growth of normal melanocytes depends on a set of four exogenous mitogens including a phorbol ester, basic fibroblast growth factor (bFGF), insulin or insulin-like growth factor (IGF)-I, and α-melanocyte stimulating hormone (MSH), the majority of metastatic melanoma cells (> 90% of cultures tested) proliferate in the absence of any exogenous protein or phorbol ester. Intermediate stages of progression show a progressive decline in exogenous growth factor requirements. These findings are consistent with the prediction of the autocrine concept that tumor-derived factors which stimulate proliferation of the producing cells may replace exogenous growth factor requirements.

Growth factor production by melanoma cells

In the following, we describe endogenous growth factor production by human melanoma cells in culture and we focus on those factors that have been studied in greater detail. Particular emphasis is given to the constitutive expression and production of these growth factors in melanoma cells as compared to normal melanocytes. Evidence is reviewed with regard to potential autocrine roles for each of the known melanoma-derived factors. In addition, we will give a brief overview of as yet hypothetical paracrine functions of melanoma-derived growth factors (MDGF).

For a particular factor to be considered as autocrine the requirements are as follows: 1) the growth factor protein is produced; 2) the growth factor receptor is expressed; and 3) suitable growth factor antagonists inhibit proliferation of the growth factor-producing cell. Growth factor antagonists represent a wide range of agents with more or less specificity for individual growth factors. We confine this discussion to antagonists with well defined specificities, notably neutralizing antibodies to growth factors, antibodies that bind to and inhibit activation of growth factor receptors, and antisense oligonucleotides which presumably interrupt translation of growth factor genes in a sequence-specific manner.

An additional potential requirement for autocrine growth factors is that the cells producing a factor for autocrine stimulation also respond to this factor after it is added to the medium. However, tumor cells may respond variably to exogenous growth factors depending on the media used prior to growth experiments. Furthermore, tumor cells may produce growth factors in amounts already sufficient to induce maximum growth stimulation. For these reasons, growth stimulation by exogenous factors in culture is not a necessary requirement for a tumor-derived growth factor to be considered autocrine.

Transforming growth factors (TGFs)

TGFs were initially identified in mouse sarcoma

virus-transformed 3T3 fibroblasts [1, 11] and were found to reversibly induce transformation of cultured mammalian cells. Subsequently, TGFs were divided into two classes, TGF-α and TGF-β, which represent two different polypeptides recognizing separate receptors and having widely different effects on the proliferation of target cells including melanocytes.

TGF-α

TGF-α belongs to the epidermal growth factor (EGF) family of mitogens [12]. EGF and TGF-α have closely related tertiary structures based on the homologous spacing of cysteine residues that form 3 intracellular disulfide bonds. TGF-α competes with EGF for binding to the EGF-receptor. Although TGF-α-like proteins of high molecular weight appear to be expressed and/or secreted by various transformed cells, the fully processed, mature form of TGF-α (5 600 M_r) is a monomeric 50aa protein encoded by an mRNA of approximately 4,800 bases [13].

TGF-α was one of the first MDGFs to be reported [14, 15]. Melanoma cells often produce and secrete large quantities of TGF-α-like factors as compared to other tumor cell lines [16–18]. Low molecular weight [19, 20] and high molecular weight [19] species of TGF-α-like activities have been detected in the urine of melanoma patients. It remains to be determined whether TGF-α in the urine of melanoma patients is tumor- or host-derived. In this respect, Hudgins *et al.* [21] detected rat EGF but not human EGF or TGF-α in the urine of nude rats xenotransplanted with human lung carcinoma or chondrosarcoma cells. In a recent survey, four of five melanoma cell lines constitutively expressed TGF-α transcripts (Table 1). This finding is in accord with other reports describing expression of the TGF-α gene in fresh melanoma tissue specimens [22] and in melanoma cell lines [23]. No TGF-α transcripts have been detected in normal melanocyte RNA (U. Rodeck, unpublished) nor do normal melanocytes *in vitro* produce detectable quantities of TGF-α unless stimulated by ultraviolet light irradiation [24]. These results suggest that TGF-α production is a tumor-associated phenomenon in human melanoma.

Expression of the EGF/TGF-α receptor in cultured melanoma cells, as determined by the binding of an EGF receptor-specific monoclonal antibody (MAb), appears to correlate with an increased dosage of chromosome 7 [25]. *In situ*, expression of the EGF receptor correlates with tumor progression [26, 27]. The EGF receptor is not detected on normal melanocytes and common acquired nevi, is expressed in approximately 20% of dysplastic nevi and radial growth phase primary melanomas, and is highly expressed in vertical growth phase primary (89%) and metastatic melanoma (80%) lesions [27]. *In vivo,* TGF-α and EGF-receptor mRNA transcripts appear to be coordinately expressed within the same lesion [22]. Exogenous EGF moderately stimulates the growth of some [28], but not all, melanoma cells [29], suggesting that the receptor is functional. The mitogenic effects of EGF *in vitro* appear to depend at least in part on culture conditions. Normal melanocytes and melanoma cells at early passages respond to EGF, whereas established melanocyte cultures do not [30, 31].

Although TGF-α and immunoreactive EGF/TGF-α receptor are co-expressed by some melanoma cells, no direct evidence supporting an autocrine role for melanoma-derived TGF-α is available. We have used EGF receptor-reactive MAb 425, which is an EGF/TGF-α antagonist, to demonstrate the autocrine function of TGF-α for carcinoma cells [32]. In defined culture media that do not contain exogenous EGF/TGF-α, Mab 425 has no reproducible inhibitory effect on growth of melanoma cells that coordinately express TGF-α and EGF receptors. This finding is in agreement with an earlier report that described lack of growth inhibition of melanoma cells by an EGF-antagonistic MAb to the intact EGF receptor [33]. Based on these findings, we conclude that secreted, melanoma-derived TGF-α is not essential for melanoma cell proliferation *in vitro*.

TGF-β

TGF-β is structurally and functionally distinct from TGF-α. Mature and bioactive TGF-β (25 000 M_r) consists of two disulfide-bonded subunits of 112aa each (see [34] for review). Five closely related isoforms numbered from one to five have been cloned. Mature TGF-β is the product of complex proteolytic processing of much larger precursor molecules. Pro-TGF-β is a non-covalently-linked complex, consisting of the precursor peptide and the mature TGF-β dimer. Further processing is required to release the biologically-active mature TGF-β polypeptide. Bioactive TGF-β binds to high-affinity cell surface receptors that are as yet poorly characterized. TGF-β exerts pleiotropic effects in tissue development, wound healing, and growth regulation.

Active TGF-β appears to have a negative growth regulatory role in the majority of normal and malignant epithelial cells [35, 36]. It also inhibits the monolayer growth of normal melanocytes [37] and melanoma cells in culture (U. Rodeck, unpublished). TGF-β1-induced inhibition of anchorage-independent growth of B16 murine melanoma cells is apparently potentiated by the presence of polyunsaturated fatty acids in the culture medium [38]. The effect of polyunsaturated fatty acids on human melanoma cell proliferation has not yet been characterized. The majority of melanoma lines in culture express TGF-β1 mRNA constitutively [23, 39; Table 1]. DeLarco *et al.* [16] have shown that TGF-β also is secreted by melanoma cells in culture. Interestingly, with the exception of melanoma growth stimulatory activity (MGSA), TGF-β1 is the only growth factor known that is expressed in culture by both normal melanocytes and malignant melanoma cells (U. Rodeck, unpublished). One could speculate that TGF-β is a negative autocrine growth regulator in melanocytes. However, using a polyclonal antiserum that neutralizes TGF-β1 activity on TGF-β1-expressing melanoma cells, no growth-modulatory effect was observed, suggesting that TGF-β might be secreted by melanoma cells in its latent form. We need more detailed studies to determine whether melanocytes and melanoma cells secrete the latent (inactive) or the biologically-active form of TGF-β. TGF-β receptors expressed on melanocytic cells have not been characterized.

Platelet-derived growth factor (PDGF)

Human PDGF is a dimeric molecule (28 000 M_r–31 000 M_r) consisting of PDGF-A and/or PDGF-B subunits which are encoded by different genes (see [40] for review). Both homodimers (PDGF-AA and PDGF-BB), as well as the heterodimer, PDGF-AB, are biologically-active and bind with different affinities to two different receptors, the PDGF-α and -β receptors, except that PDGF-AA homodimers do not bind to the PDGF-β receptor. The v-*sis* oncogene of simian sarcoma virus encodes a protein that is homologous to the PDGF-B chain [41, 42], binds to the PDGF receptors [43], and has been shown to act in an autocrine fashion in simian sarcoma virus-transformed cells [44].

The expression of either one or both PDGF isoforms and secretion of a mitogen which appears to be homodimeric PDGF-A [45] has been observed in human melanoma cells derived from one primary and two metastatic lesions of the same patient, but not in normal melanocytes in culture. Subsequent studies have shown PDGF-A mRNA expression in either 3/6 [23], 6/16 [46], or 4/5 melan-

Table 1. Constitutive expression of growth factor genes by melanoma cells

Growth factor	Cell line				
	WM 983-B	WM 164	WM 239-A	WM 852	WM 35
PDGF-A	+[a]	–	+	+	+
PDGF-B	–	–	–	+	–
TGF-β	+	–	+	+	+
TGF-α	–	+	+	+	+
bFGF	+	+	+	+	+
MGSA	+	–	–	–	–
IL-1α	–	–	–	+	–
IL-1β	+	–	–	+	–
IL-3	–	–	–	–	–
Total	5/9	2/9	4/9	7/9	4/9

[a] + = positive expression of mRNA and/or protein; – = no expression.

oma cell lines (Table 1). In summary, these results indicate expression of PDGF-A by about 50% of cultured melanoma lines, whereas expression of PDGF-B appears to be less frequent. Most melanoma cells do not express detectable PDGF receptors at the cell surface in ligand binding assays (B. Westermark, personal communication). However, radiolabeled exogenous PDGF appears to be translocated to the nucleus of melanoma cells [47, 48], suggesting the expression of receptor proteins at low levels. Harsh *et al.* [49] reported the expression of PDGF-receptor genes in 3/8 melanoma cell lines tested. We have observed weak expression of the PDGF-β-receptor mRNA in one of four melanoma lines tested. This cell line also expresses PDGF-A and PDGF-B transcripts. It is not known whether the lack of PDGF-receptor expression on the membrane is due to down-regulation of the receptor by endogenous ligand. Exogenous PDGF is not mitogenic for melanoma cells in short-term [^{3}H]thymidine incorporation, nor in long-term cell proliferation assays. Neutralizing polyclonal antisera, specific for PDGF, do not inhibit proliferation of PDGF-producing melanoma cells. Although it cannot be excluded at present that endogenous PDGF might stimulate PDGF receptors at an intracellular location inaccessible to blocking antibodies [50], no evidence for an autocrine role of this factor is available at present.

bFGF

bFGF, belonging to the class of heparin-binding growth factors, is mitogenic for a broad spectrum of cells, and has angiogenic properties (see [51] for review). It is a member of a gene superfamily including the acidic FGF gene, the *hst* oncogene (K-fgf), the FGF5 and FGF6 genes, and the *int*-2 gene. Basic FGF is a 155aa protein of 16 000 M_r–18 000 M_r which lacks a signal peptide characteristic of secretory proteins.

The proliferation of normal melanocytes is stimulated by exogenous bFGF [6, 31, 52]. Melanoma cells in culture are only weakly stimulated by bFGF and produce FGF protein [53]. The bFGF gene is expressed by most, if not all, cultured malignant melanoma cells, but not by normal melanocytes [52; Table 1]. Injection of neutralizing anti-bFGF antibodies into bFGF-producing melanoma cells leads to significant inhibition of melanoma cell growth [52]. Endogenous bFGF apparently acts at an intracellular location since addition of neutralizing antibodies to the culture media does not result in melanoma growth inhibition. Antisense oligonucleotides targeted against human bFGF sequences similarly inhibit melanoma proliferation [54]. It should, however, be noted that growth inhibition observed in antibody- and oligonucleotide-treated cells was not complete. It is not known whether this finding is due to insufficient intracellular concentrations of the respective bFGF antagonist or to a subpopulation of bFGF-independent melanoma cells. Both normal melanocytes and melanoma cells express a 145 000 M_r bFGF-membrane receptor and express a 3.5 kb message hybridizing to a cDNA probe of the FGF receptor (D. Becker, unpublished).

Thus, bFGF fulfills the criteria for an autocrine MDGF. Melanoma cells constitutively produce bFGF, express FGF receptors, and bFGF antagonists inhibit proliferation of melanoma cells. In addition, normal melanocytes, which require exogenous bFGF for proliferation, do not produce it at detectable levels. It should be noted that murine melanocytes infected with a bovine FGF recombinant retrovirus lose their requirement for exogenous bFGF, lending further support to the autocrine role of bFGF human melanoma [55]. Interestingly, murine melanocytes infected with the bFGF recombinant retrovirus achieved independence from exogenous growth factors, but were not tumorigenic in nude mice indicating that, in the melanocytic system, growth autonomy alone does not signal a fully transformed phenotype.

MGSA

MGSA was first isolated from conditioned media of the human melanoma cell line Hs294T and is identical to the *gro* gene product [56]. MGSA bioactivity is found primarily in polypeptides ranging from 14 000 M_r–28 000 M_r [57]. The fully pro-

cessed mature form of MGSA appears to be maximally 73aa long. The deduced amino acid sequence of MGSA reveals extensive similarities to the precursor of β- thromboglobulin and connective tissue-activating protein, heparin-binding platelet factor-4, the interferon-γ inducible gene gIP-10, and IL8/NAP-3/MIP-2 [58, 59]. In addition to its mitogenic activity, MGSA appears to be a mediator of chemotaxis and inflammation.

MGSA binds to the surface of, and is a mitogen for, the producing Hs294T melanoma cells [60, 61]. An MAb that binds to MGSA inhibited growth of Hs294 cells, indicating that in this cell line MGSA has an autocrine function [62] although effects of the MAb on the proliferation of other melanoma cell lines have not been reported. Expression of the MGSA gene in Hs294T cells is weak or absent in Northern blot analysis of total RNA preparations when these cells are grown in serum-free media [63]. In another study, only one out of five melanoma cell lines expressed a weak MGSA transcript constitutively when grown in culture medium free of exogenous growth factors (U. Rodeck, unpublished). Chevenix-Trent *et al.* [46] have detected MGSA transcripts not only in the majority of melanoma cells tested, but also in normal melanocytes. These discrepancies may be explained by different culture conditions since MGSA production is inducible by exogenous mitogens such as PDGF and MGSA added to the culture medium [63].

The receptor for MGSA on melanoma cells has not been described. It remains to be determined whether it is related to the recently characterized IL-8 receptor on lymphopoietic cells [64]. In summary, MGSA appears to be an autocrine growth factor for Hs294T melanoma cells. Further studies will show whether MGSA is an important growth regulator for other melanoma cells.

Interleukin (IL-1)

IL-1 was originally purified from monocytes [65]; however, it is produced by a variety of cell types developmentally as diverse as lymphoid cells, endothelial cells, fibroblasts and keratinocytes (see [66] for review). Two distinct forms of IL-1, IL-1α and IL-1β, encoded by separate genes have been identified. These isoforms share the same molecular weight (18 000) and a common cell surface receptor.

Both IL-1α and IL-1β present in conditioned media of monocytes are strong inhibitors of the proliferation of A375 human melanoma cells [67, 68]. In a more recent study, IL-1β inhibited [^{3}H]thymidine incorporation of 3/9 melanoma cell lines significantly [69]. By contrast, coinjection of IL-1 and a variant of A375 melanoma cells enhanced the formation of metastatic foci in the lungs of recipient athymic mice [70].

Cultured human melanoma cells are not only the target, but also a source of both isoforms of bioactive IL-1 [71, 72]. IL-1 is produced by 16 of 27 metastatic melanoma cell lines tested (J. Bennicelli, unpublished). Secreted and cell-associated forms have been identified. The secreted form is functionally very similar to monocyte IL-1 but has a higher molecular weight. It is unclear at present whether melanoma-derived IL-1 modulates growth of the producing cells. However, it appears possible that IL-1 *in vivo* acts primarily in a paracrine fashion. Rice and Bevilacqua [73] have shown that IL-1 induces an adhesion molecule on the surface of cultured endothelial cells (INCAM-110) which mediates the attachment of melanoma cells to activated endothelium. Thus, cell-associated melanoma-derived IL-1 may facilitate metastatic spread by enhancing tumor cell/endothelial cell adhesion.

Other growth factors

IGFs

IGF-1 and insulin are potent exogenous growth factors for cultured melanoma cells [28]. Melanoma cells express the IGF-1 receptor and the mitogenic effects of both insulin and IGF-1 are significantly inhibited by an MAb to the IGF-1 receptor that inhibits binding of these ligands. Of a panel six human melanoma cell lines tested, none produced IGF-1 or IGF-2 proteins (R. Furlanetto, personal communication), nor did any express detectable mRNA transcripts for these two factors (unpub-

lished). Thus, IGFs are not likely to serve an autocrine role in human melanoma.

Nerve growth factor (NGF)

The receptor for NGF is expressed on melanoma cells in culture [74, 75], and *in situ* [27]. However, no mitogenic effects of NGF on cultured melanoma cells were observed (unpublished). No evidence for the expression or endogenous production of NGF by melanoma cells is available.

α-MSH

MSH and the related melanotropins are pituitary-derived peptides that have widely divergent effects on the growth of non-human melanocytes (see [76] for review). A subset of melanoma cell lines and melanoma lesions express melanotropin receptors [77–79]. Recently, it was reported that metastatic melanoma lesions simultaneously express MSH receptors and contain immunoreactive α-MSH protein [80]. These findings suggest a potential autocrine role for this factor. However, Ellem and Kay [81] reported that exogenous α-MSH did not affect proliferation of human melanoma cells MM96. MSH is involved in the induction of differentiated traits in melanocytes, namely pigmentation [82, 83]. It remains to be studied whether it can serve additional growth regulatory functions.

Melanoma-derived growth inhibitory factors

Three MDGFs have been documented to inhibit growth of some melanoma cells when added exogenously to *in vitro* cultures. Growth inhibitory effects of exogenous bioactive TGF-β and IL-1 have been described above. Recently, a melanoma-inhibiting activity (MIAII) was described [84, 85] that was purified from the conditioned medium of a human metastatic melanoma cell line (HTZ 19-dM) and, after purification, inhibited proliferation of the producing cells. This factor (8000 M_r) is apparently unrelated to TGF-β and IL-1, but the structure of the protein and the coding sequence have not been determined yet. The biological significance of tumor growth inhibition by autocrine factors is not known.

Co-expression of growth factor genes in melanoma cells

Several recent studies have measured the expression of multiple growth factor genes in individual melanoma cell lines on the protein [86] or the mRNA level [23, 46; Table 1]. Pichon and Lagarde [86] reported the simultaneous production by a variant of MeWo human melanoma cells of proteins that are related to PDGF, bFGF, and MGSA. Chenevix-Trent *et al.* [46] described co-expression of TGF-α, PDGF, and MGSA transcripts in various cell lines, whereas Lizonova *et al.* [23], showed co-expression of the PDGF-A chain, TGF-α, and TGF-β by individual lines. Results of our recent survey of growth factor gene expression by five melanoma cell lines are summarized in Table 1. In contrast to the studies by Chenevix-Trent *et al.* [46] and Lizonova *et al.* [23] we propagated these cells for at least two days prior to RNA extraction in culture media free of exogenous polypeptides. Therefore, we have minimized the effects of exogenous growth factors on gene induction and consider expression of these genes to be constitutive.

These studies suggest that melanoma cells may employ different growth factor/growth factor-receptor pathways to achieve growth autonomy. The limited data presented in Table 1 and in the other two reports do not support the idea of coordinate regulation of growth factor gene expression in this system. However, the sample size available is too small to answer this question definitively. It is unclear whether some of the MDGFs co-expressed by individual cell lines can replace each other with regard to their autocrine capacity. The lack of growth inhibition observed in melanoma cells treated with either PDGF- or EGF/TGF-α antagonists is consistent with the idea that the secreted forms of these two growth factors are not critical to melanoma cell proliferation *in vitro*. It cannot be excluded at this point, however, that intracellular

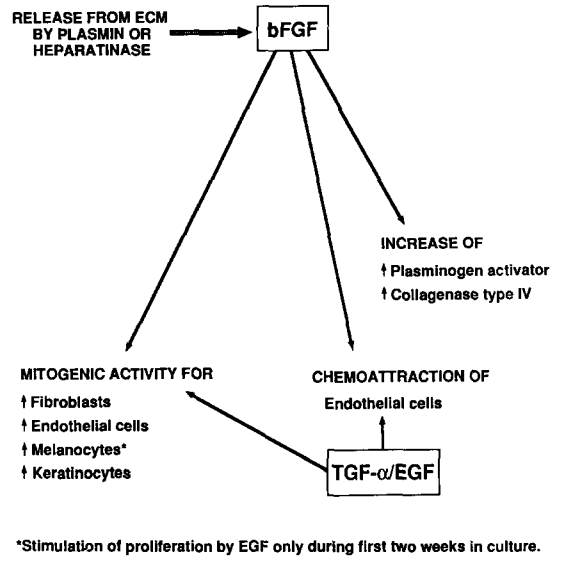

Fig. 1. Hypothetical biological activities of bFGF and EGF/TGF-α in melanoma lesions. The ligands may be produced by melanoma cells (except EGF) or, after appropriate activation, by surrounding normal cells such as fibroblasts and endothelial cells.

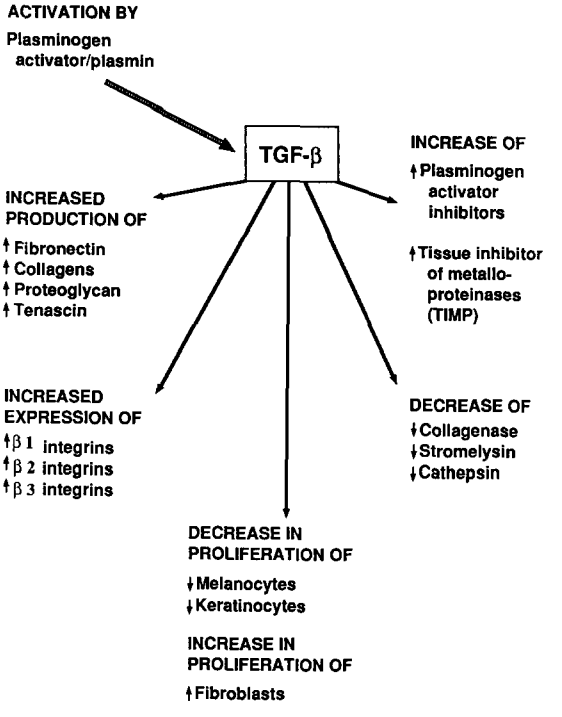

Fig. 2. Hypothetical regulatory functions of TGF-β produced by either melanoma cells or surrounding normal cells.

activation of receptors by these two endogenous factors renders antagonistic antibodies inefficient for growth inhibition studies; inhibitory effects of such antibodies may be limited to extracellular ligands and cell surface receptors only. The use of suitable antisense oligonucleotides that suppress growth-factor protein synthesis has been successful in the case of bFGF [54], and may provide an alternative approach.

It should be noted that, with few exceptions, growth factor expression data were collected on cultured melanoma cells, but not from tumor cells *in situ*. Although the phenotypic characteristics of melanocytes derived from lesions representing different stages of tumor progression are remarkably stable [7], it is not known which of the melanoma-associated growth factors expressed *in vitro* actually play a role *in vivo*. However, the fact that most MDGF genes are expressed constitutively *in vitro* suggests that at least some of these factors are also relevant *in vivo*.

Paracrine effects of MDGFs

Irrespective of their autocrine potential, secreted tumor-derived growth factors can be expected to bind to, and modulate the function of host tissue surrounding the producing cells (see [87] for review). Media conditioned by melanoma cells are a source for TGF-α and TGF-β, PDGF-AA homodimers, MGSA, and IL-1. Other factors such as bFGF, although not found in conditioned media of melanoma cells, may be recovered from the extracellular matrix produced by melanoma cells. The secretory nature of these factors, taken together with the wide distribution of their receptors on normal cells, suggests that these factors are likely to act on stromal cells in the microenvironment of melanoma lesions. Tumor stroma consists mainly of two cellular components, endothelial cells and fibroblasts, as well as an extracellular matrix (ECM) produced by normal and tumor cells. It is beyond the scope of this paper to review in detail the possible role of paracrine growth factors in the

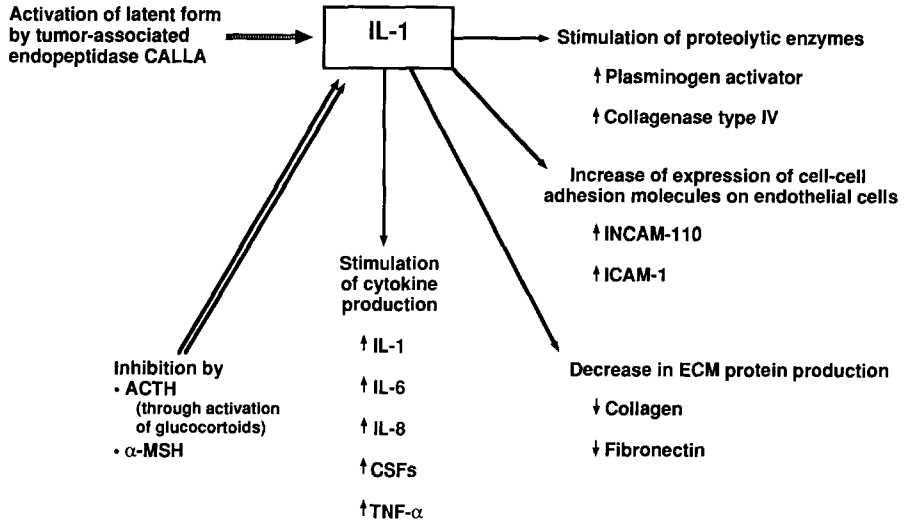

Fig. 3. Biological activities of normal cell- or tumor-derived IL-1.

complex interaction of melanoma cells with support structures. In addition, very little information on such interactions with regard to melanoma cells is available. The complex, yet largely hypothetical effects of tumor-derived growth factors and cytokines on growth, tissue degradation, motility, and attachment/adhesion are summarized in Figs 1–3.

Basic FGF is released from the ECM by the proteolytic enzymes heparitinase (heparanase) and plasmin (Fig. 1). In addition to its angiogenic properties, bFGF stimulates the production of plasminogen activators and collagenase type-IV and is a mitogen for fibroblasts, endothelial cells, melanocytes, and keratinocytes. TGF-α and EGF are also mitogens for these normal cell types and stimulate angiogenesis.

TGF-β is a highly pleiotropic growth factor. Melanoma-derived TGF-β has, hypothetically, a variety of effects on the melanoma cells themselves, as well as on normal cells in the microenvironment of the tumor (Fig. 2). Activation of the latent form of TGF-β may occur through plasminogen activators and plasmin. Bioactive TGF-β increases the activity of inhibitors for proteolytic enzymes, the production of ECM proteins and the expression of integrins [88–92]. On the other hand, TGF-β decreases the production of proteolytic enzymes and the proliferation of melanocytes and keratinocytes.

IL-1 also exerts diverse biological activities; it induces production of proteolytic enzymes, stimulates cytokine production in fibroblasts and endothelial cells, enhances expression of adhesion molecules by endothelial cells, and down-regulates production of ECM proteins (Fig. 3). Whether any of these effects can be triggered by melanoma-derived IL-1 is unclear at present. Latent IL-1 may be activated by the endopeptidase CALLA which is expressed on melanoma cells. A role of IL-1 and TNF-α in the adhesion of melanoma cells to endothelia has recently been demonstrated by Rice and Bevilacqua [73].

It is apparent that the assessment of the function of particular growth factors beyond their autocrine role awaits the investigation of complex networks of interdependent growth factors and their interactions with ECM/adhesion molecules. A case that illustrates this problem is IL-1 which when added to culture media inhibits growth of A375 cells, yet enhances the formation of metastatic foci when coinjected with A375 cells into nude mice.

Conclusions

Different stages in the development of malignant melanoma have been characterized with regard to their exogenous growth factor requirements and the endogenous production of growth factors. Ba-

sic FGF has emerged as the main autocrine growth factor for melanoma cells, whereas the wider role of MGSA for autocrine growth stimulation remains to be investigated. The growth regulatory roles of PDGF, TGF-α, TGF-β, MSH and IL-1 in melanoma remains largely undefined. The constitutive production of PDGF-A and -B chains, bFGF, and TGF-α appears to be limited to malignant melanocytes, whereas the expression of TGF-β1 and MGSA is shared by normal melanocytes and melanoma cells.

In addition to autocrine effects, MDGFs are often secreted and, therefore, can be expected to have paracrine effects. Paracrine effects are not restricted to growth modulation of stromal cells but encompass the production of extracellular matrix proteins, the expression of adhesion molecules, and the production of proteolytic enzymes by normal and tumor cells. These effects need to be taken into account in future studies on the biological role of growth factors produced by melanoma cells.

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