

# Understanding the roles of growth factors in carcinogenesis: modulation of autocrine growth control by differentiation

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**ABSTRACT** It is widely believed that abnormal proliferation of tumor cells is due, at least in part, to the production of autocrine growth factors that are not produced by their normal counterparts. However, direct support for this belief is seriously lacking. The normal counterparts of the vast majority of tumor cells have not been identified adequately and, thus, the growth factors produced by the normal counterparts of tumor cells have not been described. This review summarizes the remarkable similarity in the types of growth factors and growth factor receptors produced by early mouse embryos and by mouse embryonal carcinoma cells, the stem cells of teratocarcinomas. Based on these similarities and the likelihood that embryonal carcinoma cells are derived from the totipotent cells of the mammalian embryo, it is argued that there is reason to suspect that ectopic production of growth factors is not a major cause of tumor induction and tumor growth. It is further proposed that differentiation under normal conditions can limit autonomous cell proliferation by shifting cells from a population that produces growth stimulatory factors to cell types that produce growth inhibitory factors instead. Consequently, blocks in differentiation may lead to tumor growth by maintaining the production of growth stimulatory autocrine factors.

**KEY WORDS:** *autocrine growth control, growth factors, differentiation, embryonal carcinoma cells, mammalian embryos*

## Introduction

During the 1970's, it became evident that transformed cells can release a variety of factors, including those that promote the overgrowth of non-transformed cells (Rubin, 1970), induce cell migration (Bürk, 1973), enhance transformation (Krycève *et al.*, 1976), and/or induce anchorage-independent growth of non-transformed cells (De Larco and Todaro, 1978). In recognition of these findings, Sporn and Todaro (1980) formally proposed that secretion of autocrine growth factors could help account for aberrant proliferation of tumor cells. Specifically, it was proposed that tumor cells are capable of producing the very growth factors required for their proliferation. During the 1980's, the autocrine growth control model received strong support from the finding that virtually all tumor cells produce growth factors and, more importantly, many of these growth factors were found to stimulate proliferation of the cells that produce them (Sporn *et al.*, 1986; Goustin *et al.*, 1986). In addition, many of the growth factors produced by tumor cells have been shown to induce non-transformed cells to exhibit a tumor phenotype under experimental conditions. Although the latter growth factors were initially thought to belong to a highly specific class of growth factors, it is now clear that these growth factors belong to many growth factor families, including the epidermal

growth factor (EGF) family (De Larco *et al.*, 1980), the transforming growth factor- $\beta$  (TGF- $\beta$ ) family (Roberts *et al.*, 1980), the platelet-derived growth factor (PDGF) family (Rizzino *et al.*, 1986), and the fibroblast growth factor (FGF) family (Rizzino and Ruff, 1986).

An important aspect of the autocrine growth control model is the contention that tumor cells, unlike their normal counterparts, produce growth factors that stimulate their own proliferation. However, it has been extremely difficult to prove that tumor cells ectopically (i.e. inappropriately) produce growth factors, since little is known about the normal counterparts of tumor cells. A major stumbling block has been the failure to identify with sufficient precision the normal counterparts of nearly all tumor stem cells. Furthermore, systematic attempts to study normal stem cells involved in tissue renewal, the very cells thought to be the targets of carcinogenesis, have been hampered severely by the difficulty of isolating and maintaining normal stem cells in tissue culture as

*Abbreviations used in this paper:* EC, embryonal carcinoma; ES, embryonic stem; EGF, epidermal growth factor; FGF, fibroblast growth factor; ICM, inner cell mass; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF- $\beta$ , transforming growth factor- $\beta$ .

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homogeneous populations. Equally perplexing is the recent realization by many investigators that the targets for carcinogenesis represent a very small fraction of all cells in any given organ. Consequently, simple comparisons between tumor cells and unfractionated, complex organs will yield little useful information about the growth factors produced by tumor cells and their normal counterparts.

### Embryonal carcinoma cells as a model system

Since the late 1970s, embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas, have been used as a model system to identify growth factors that are likely to be produced during early mammalian development (reviewed in Rizzino, 1989). EC cells provide a useful model system because they can be induced to differentiate into specific early embryonic cell types and, in so doing, closely mimic important stages of mammalian embryogenesis (Strickland and Mahdavi, 1978). Equally important, EC cells can be maintained in tissue culture as virtually homogeneous populations of undifferentiated cells.

A major premise of this article is that EC cells also provide a powerful model system to examine the question of ectopic growth factor production by tumor cells. Although it remains to be proven rigorously, there are many reasons for believing that the normal counterparts of EC cells are the totipotent cells of the early embryo. At the blastocyst stage, these cells are collectively referred to as the inner cell mass (ICM). As the embryo develops to the egg cylinder stages, the totipotent cells are believed to be restricted to embryonic ectoderm. During the past 20 years, numerous similarities between EC cells and the cells of the ICM have been described. EC cells are strikingly similar to the cells of the ICM both morphologically and biochemically (Solter and Damjanov, 1979; Martin, 1980). EC cell lines can be induced to differentiate into the early embryonic cell types that are known to be derived from the ICM (Martin, 1980), and multipotent EC cells have been shown to be developmentally equivalent to the cells of the ICM (Brinster, 1975; Mintz and Illmensee, 1975). In the latter studies, EC cells were shown to give rise to tumor-free chimeric mice and, in some cases, EC cells were found to give rise to the germ line of fertile chimeric mice (Dewey *et al.*, 1977; Dewey and Mintz, 1978; Mintz and Cronmiller, 1978). Although early studies suggested that EC cells are derived from primordial germ cells, there are many reasons for rejecting this view (reviewed in Graham, 1977) and direct experimental evidence argues that EC cells can form from embryonic ectoderm of the early egg cylinder (Damjanov *et al.*, 1971; Solter and Damjanov, 1979). In the next two sections, the expression of growth factors and growth factor receptors by EC cells and mouse blastocysts is described and they are shown to be remarkably similar.

### Expression of growth factors and their receptors by EC cells

Early studies established that the growth of EC cells in medium lacking serum is density-dependent and this suggested that EC cells produce autocrine growth factors (Rizzino and Crowley, 1980; Rizzino *et al.*, 1980). Initial efforts to identify the growth factors involved led to the finding that EC cells produce growth factors with the biological properties of transforming growth factors (Rizzino *et al.*, 1983). Further study established that EC cells produce PDGF (Rizzino and Bowen-Pope, 1985) — most probably as a homodimer of the PDGF A-chain (Tiesman *et al.*, 1988). In addition, it was

determined that the production of the PDGF-like factor is repressed when EC cells differentiate (Rizzino and Bowen-Pope, 1985). Subsequently, it was determined that the differentiation of EC cells also represses the production of k-FGF, a member of the FGF family (Rizzino *et al.*, 1988; Tiesman and Rizzino, 1989), and TGF- $\alpha$ , a member of the EGF family (Dmitrovsky *et al.*, 1990). Interestingly, differentiation does not decrease the production of all growth factors. Differentiation of EC cells increases the expression of several members of the TGF- $\beta$  family, including vgr-1 and TGF- $\beta$ 2 (Lyons *et al.*, 1989; Kelly *et al.*, 1990).

Although the mechanisms that regulate expression of these growth factor genes are only beginning to be understood, it is evident that the transcription of at least two of these growth factor genes changes dramatically after EC cells differentiate. In the case of the k-FGF gene, transcription decreases after EC cells differentiate (Curatola and Basilico, 1990; Ma *et al.*, 1992). Thus far, the 5' flanking region and the third exon of the gene appear to contain several cis-regulatory elements that control the transcription of the k-FGF gene in EC cells. In addition, it appears that repression of the k-FGF gene is due in part to decreased activity and/or production of octamer binding proteins when EC cells undergo differentiation (Ma *et al.*, 1992). Conversely, transcription of the TGF- $\beta$ 2 gene increases after EC cells differentiate. Thus far, three cis-regulatory elements have been identified that are likely to control expression of this gene in EC-derived differentiated cells (Kelly *et al.*, 1992). Interestingly, negative cis-regulatory elements appear to play important roles in the expression of both the k-FGF gene and the TGF- $\beta$ 2 gene (Kelly *et al.*, 1992; Ma *et al.*, 1992).

Differentiation of EC cells also exerts dramatic effects on the expression of growth factor receptors. Rees *et al.* (1979) were the first to show that differentiation increases the number of EGF receptors expressed on the cell surface of EC-derived differentiated cells. Subsequently, it was determined that this is also true for PDGF receptors (Rizzino and Bowen-Pope, 1985), FGF receptors (Rizzino *et al.*, 1988) and TGF- $\beta$  receptors (Rizzino, 1987). The effect of differentiation on the expression of TGF- $\beta$  receptors is of particular interest because EC cells were the first cells to be shown to lack detectable TGF- $\beta$  receptors. Furthermore, the differentiated cells, unlike the parental EC cells, respond to TGF- $\beta$ . TGF- $\beta$  not only inhibits the growth of the differentiated cells, it also alters their morphology and decreases their production of laminin (Rizzino, 1987; Kelly and Rizzino, 1989). Currently, the mechanisms that regulate the expression of EGF, PDGF, FGF and TGF- $\beta$  receptors by EC cells and by their differentiated cells are poorly understood. However, it appears that the expression of cell surface EGF receptors by EC-derived differentiated cells is due to multiple mechanisms, including increases in the steady-state levels of EGF-receptor mRNA. (Joh *et al.*, 1992).

### Expression of growth factors and receptors by preimplantation mouse embryos

The studies from several laboratories indicate that there are remarkable similarities in the types of growth factors and growth factor receptors expressed by mouse EC cells and by early mouse embryos at the blastocyst stage. Recently, reverse transcription-polymerase chain reaction (RT-PCR) was used to examine the expression of several genes that code for growth factors and their receptors (Rappolee *et al.*, 1988, 1990). It was determined that mouse blastocysts express transcripts for k-FGF, PDGF-A chain,

TGF- $\alpha$ , TGF- $\beta$ 1, insulin-like growth factor-II (IGF-II), as well as transcripts for insulin receptors, IGF-I receptors and IGF-II receptors. In contrast, no expression of EGF, insulin, basic FGF or granulocyte-colony-stimulating factor was detected in mouse blastocysts. Significantly, the same pattern of mRNA expression for each of these growth factors and receptors was observed in F9 EC cells (Rappolee *et al.*, 1988,1990). More recently, RT-PCR was used to examine the expression of FGF receptor genes and it was determined that EC cells and mouse blastocysts both express FGF receptors encoded by the *flg* FGF receptor gene (Campbell *et al.*, 1992). Lastly, it has been shown that EC cells produce little if any TGF- $\beta$ 2 (Kelly *et al.*, 1990) and the same appears to be the case for the ICM (Slager *et al.*, 1991).

Although there is remarkable similarity between mouse EC cells and mouse blastocysts with respect to the expression of growth factors and growth factor receptors, there are two possible differences. EC cells, but not mouse blastocysts, have been shown to express transcripts for IGF-I (Rappolee *et al.*, 1990). However, in the case of blastocysts, this observation is likely to be a false negative due to suboptimal RT-PCR conditions. In this regard, preimplantation bovine embryos have been shown to express IGF-I mRNA (Watson *et al.*, 1992). Similarly, expression of the FGF receptor gene *bek* has been detected in F9 EC cells, but not in mouse blastocysts (Campbell *et al.*, 1992). Again, this difference is likely to be due to less-than-optimal RT-PCR conditions.

Overall, the findings described above suggest that EC cells and the early embryo at the blastocyst stage produce the same repertoire of growth factors and receptors. While it remains to be determined whether the cells of the ICM specifically produce the same growth factors and growth factor receptors as EC cells, the current evidence indicates that this is the case for two of the growth factors that have been examined more closely: k-FGF (Tiesman and Rizzino, 1989; Niswander and Martin, 1992) and TGF- $\beta$ 1 (Rappolee *et al.*, 1988; Kelly *et al.*, 1990). Similarly, it remains to be shown whether the growth factors produced by EC cells and early embryos exert autocrine functions, but evidence for this argument already exists in the case of TGF- $\alpha$  (Dmitrovsky *et al.*, 1990; Paria *et al.*, 1991). Clearly, these findings question the view that growth factors are produced ectopically by EC cells.

### Revised model for autocrine growth control: an important role for differentiation

The striking similarities in the growth factors and growth factor receptors expressed by mouse EC cells and mouse blastocysts make it reasonable to ask whether this is typical of tumor cells and their normal counterparts. While it could be argued that only early embryonic cells produce their own autocrine growth factors, this seems unlikely given the recent reports of autocrine growth control in many other systems. Thus, it seems likely that in many, but probably not all, cases, tumor cells and their normal counterparts will be found to produce most, if not all, of the same autocrine growth factors.

If tumor cells and their normal counterparts both produce autocrine growth factors, how is the growth of normal cells kept in check? A likely answer is suggested by the studies with EC cells described above. These studies strongly support the hypothesis that EC cells are tumorigenic because they fail to differentiate in response to appropriate developmental signals and thus fail to turn off their production of growth stimulatory factors (e.g. k-FGF) and fail

to activate signal transduction pathways (e.g. appearance of TGF- $\beta$  receptors) used by growth inhibitors (e.g. TGF- $\beta$ ) that are produced by the differentiated cells. It is also reasonable to speculate that differentiation in other systems can block autonomous growth by shifting cells from a population of cells that produce growth stimulatory factors to cell types that produce and respond to growth inhibitory factors. If this is the case, one would predict that differentiation should restrict the ability of normal stem cells to become tumorigenic. In the case of EC cells (Pierce, 1967) and early mouse embryos (Solter and Damjanov, 1979), this is a well established fact. In this regard, teratocarcinomas can be induced by transferring embryos (up to the middle of the seventh day of gestation) to extrauterine sites. These tumors are transplantable because they contain EC cells. However, only benign, non-transplantable teratomas form when embryos at the ninth day of gestation are transferred to extrauterine sites.

The arguments put forth in this review provide a mechanistic and conceptual link between the autocrine growth control model for the proliferation of tumor cells and the long-held view that many, if not most, cancers are due to defects in differentiation of stem cells. The latter view has been held strongly and promoted actively by Barry Pierce for over three decades (Pierce and Dixon, 1959; Pierce, 1967, 1974, 1983). According to this view, defects in differentiation can block normal regulation of growth factor production and, as a result, allow continuous production of autocrine growth factors that are able to stimulate the proliferation of the cells that can not differentiate. If this hypothesis is correct then production of autocrine growth factors by tumor cells should not be viewed as a cause of carcinogenesis, but rather as the consequence of a failure of stem cell differentiation to discontinue the production of autocrine growth factors.

### Future perspectives

The widely held belief that tumor cells produce growth factors ectopically suggests that it may be possible to block the growth of tumors by blocking the production of their autocrine growth factors. For this reason alone, it will be important to determine whether the production of autocrine growth factors by tumor cells is in fact due to aberrant expression of growth factor genes. To answer this question and provide clinically useful information will require precise identification and detailed characterization of the normal counterparts of many different tumor stem cells. Unfortunately, for too many tumors only rough guesses can be made about the identity of their normal counterparts. Consequently, this is an area of cancer cell biology that warrants an effort equal to that of the present human genome project. In the case of teratocarcinomas and their normal counterparts, the best comparison is likely to be between the cells of the ICM and embryonic stem (ES) cells, the embryo-derived counterparts of EC cells. ES cells form teratocarcinomas when placed in an extrauterine site and they can form from isolated ICMs (Martin, 1981). Furthermore, early passage ES cells are a better choice than virtually all EC cell lines, which have been in culture for many generations and, thus, are likely to have undergone secondary changes that are unrelated to tumor formation. Lastly, EC cells and ES cells appear to produce the same growth factors (Mummery *et al.*, 1990).

On the other hand, if most tumor cells and their normal counterparts produce and require the same growth factors, then it will be necessary to selectively block growth factor production by the tumor

cells without blocking production of growth factors by their normal stem cell counterparts. This could be very difficult if growth factor production by tumor cells does not involve aberrant regulation. In this situation, methods for stemming the production of growth factors by tumor cells are more likely to be found by understanding how to override defects in differentiation. Therefore, one of the keys to understanding cancer is to obtain a better understanding of how differentiation influences the production of key growth regulatory molecules, including growth factors and receptors. More specifically, by obtaining a mechanistic understanding of how differentiation regulates the expression of key regulatory genes, we are likely to identify novel targets for new, more effective cancer therapies.

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Barry Pierce has made innumerable scientific contributions to our understanding of the relationships between cancer and differentiation. In doing so, he has influenced the thinking and the research of numerous investigators in oncology and in developmental biology. This is certainly true for this investigator, and many of the arguments put forth in this review are outgrowths of the work published by Barry Pierce during the past 30 years and conversations with him over the past fourteen years. Specifically, three aspects of Barry Pierce's work have been very influential on the focus of this investigator's research: the demonstration that EC cells are the stem cells of teratocarcinomas; the argument that tumors are derived from embryonic stem cells and not from adult cells by dedifferentiation; and the demonstration in many systems that differentiation can suppress the ability of cells to form tumors. From the latter studies, Barry Pierce argued that new and more effective cancer therapies could result from understanding how tumor cells could be induced to differentiate into harmless non-proliferating cells.

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