Journal of Cellular Biochemistry 53:280–287 (1993)

Activities of Growth Factors in Preimplantation Embryos

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Abstract The development of the mammalian preimplantation embryo in vitro occurs more slowly and less successfully compared to development in the uterus. The fact that it can occur at all in a defined protein-free medium suggests that the process is autonomous. Accumulated evidence indicates that a number of peptide growth factors contribute in an autocrine fashion to preimplantation development. Other growth factors are maternally derived and act in a paracrine manner on the embryo. Some of these factors such as insulin-related factors stimulate growth preferentially, but others such as epidermal growth factor (EGF) play more important roles in differentiation. Several cytokines appear to be implicated in peri-implantation events and in maternal-fetal interactions. At this stage, the data are mostly descriptive. Are all these different growth factors and receptors necessary for early development? Some implications of apparent redundancy of gene expression are discussed and future studies are predicted. © 1993 Wiley-Liss, Inc.

Key words: insulin-related factors, epidermal growth factors, cytokines, gene expression, growth factor receptors, platelet-derived growth factors

The preimplantation mouse embryo is able to develop from a fertilized egg into a hatched blastocyst of about 100 cells in vitro over a 4.5 day period without need for protein or growth factors in the culture medium. Even the development from full-sized oocyte to a fertilized egg can be simulated in culture and the egg can develop into a blastocyst subsequently. Embryos from each mammalian species, however, have a characteristic developmental capacity in vitro. The ability of preimplantation embryos to reproduce the developmental program in vitro has been the subject of interest to farm and veterinary technologists for the practical purposes of animal husbandry. In vitro fertilization of human eggs demands culture conditions that maintain the viability of the resulting embryos before transfer to the uterus. Genetic manipulation of eggs and embryos in vitro allows the production of transgenic animals that could be useful models of human diseases as well as concentrated sources of biosynthesized products secreted into milk. These are some of the practical reasons why this area of research is of interest.

For the developmental biologist, the dissection of the mechanism of autonomous developmental behavior is of great interest, and growth factors have naturally received the most attention. Some years ago it was noticed [Wiley et al., 1986] that embryos in culture do better in a small drop of culture medium compared to a large drop. This indicates that embryos can condition their own medium. This was the stimulus to identify growth factors that could stimulate development when added to embryos in culture. The supposition is that the preimplantation embryo produces its own growth factors, and this is why its development can be independent of the uterus. However, development in vitro is slower and less frequently successful compared to development in utera, and this clearly indicates that the uterine environment plays a role. This review covers only the preimplantation stage of embryonic development, and relevant observations have not progressed much from the descriptive stage of analysis with a few exceptions. The search for autocrine effects of growth factors has led to some interesting discoveries. Growth factors that are generally regarded as mitogens can play dual roles in this system. Several types of growth factors can influence preimplantation development in vitro, but this merely illustrates the presence of an active receptor rather than the necessity for the growth factor for development. Cytokines are involved in the latter stages of the pre- or peri-implantation embryo as the embryo meets the challenge of the mother's

Received April 9, 1993; accepted May 28, 1993.

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TABLE I. Growth Factors Produced by Embryos

Factor		Stage	Detection method	Reference
TGFα	mRNA	1-cell-bc	RT-PCR	Rappolee et al., 1988
	Protein	Blastocyst	Immunofluorescence	Rappolee et al., 1988
	Protein	ICM + polar trophect.	Immunoelectronmicro.	Dardik et al., 1992
EGF	mRNA	NO	RT-PCR	Rappolee et al., 1988
GM-CSF	mRNA	NO	RT-PCR	Rappolee et al., 1988
NGF	mRNA	NO	RT-PCR	Rappolee et al., 1988
kFGF	mRNA	4-cell-bc	RT-PCR	Rappolee et al., 1990
bFGF	mRNA	NO	RT-PCR	Rappolee et al., 1990
FGF and/or PDGF-like	Protein	Blastocyst	Soft agar growth of fibroblasts	Rizzino, 1985
INS		NO	RT-PCR	Rappolee et al., 1990
IGF-I		NO	RT-PCR	Rappolee et al., 1990
IGF-II	mRNA	2-cell-bc	RT-PCR	Rappolee et al., 1990
	Protein	Blastocyst	Immunofluorescence	Rappolee et al., 1992
TGFβ	mRNA	1-cell-bc	RT-PCR	Rappolee et al., 1988
	Protein	Blastocyst	Immunofluorescence	Rappolee et al., 1988
	Protein	1-cell-bc	Immunoperoxidase	Paria et al., 1992
PDGF-A	mRNA	2-cell-bc	In situ hybridization	Palmieri et al., 1992a
	Protein	2-cell-bc	Immunofluorescence	Palmieri et al., 1992a
PDGF-B	mRNA	NO	In situ hybridization	Palmieri et al., 1992a
LIF	mRNA	bc	RT-PCR	Murray et al., 1990
IL-6	mRNA	bc	RT-PCR	Murray et al., 1990

immune system. Studies such as these are the focus of this essay. Other more comprehensive reviews of gene expression in preimplantation embryos have recently been published [Mercola and Stiles, 1988; Schultz and Heyner, 1992].

EGF AND EGF-RECEPTOR FAMILIES OF GENES: THEIR ACTIVITIES IN EARLY DEVELOPMENT

The EGF-receptor is a glycoprotein of 170 kDa that is inserted into the cellular membrane of a wide range of cell types. The extracellular domain of the receptor has the function of binding EGF and its related ligands, and this leads to activation and to the generation of signals unique to each ligand. Activation of the receptor involves its dimerization, conformational change, and stimulation of the tyrosine kinase activity of the intracellular domain. Three other EGF-receptor related genes have been cloned, but nothing is known of their occurrence in the early embryo or the role of any in preimplantation development.

So far there are five known natural ligands that bind to the EGF-receptor. The best known is EGF itself. It is produced in small amounts by several tissues and stored in large amounts in the male murine salivary gland [Cohen, 1962]. The other major cellular product that has the ability to bind to the EGF-receptor is transforming growth factor-alpha (TGFα). Both are produced as membrane inserted precursors that could function in a juxtacrine fashion. The occurrence of other EGF-related growth factors and members of several other growth factor families that may play roles in preimplantation embryos is summarized in Table I. Although no EGF mRNA can be found at any stage in the mouse preimplantation embryo, it is found in the pig embryo [Vaughn et al., 1992]. The relevant ligand in mouse could be embryo-derived TGFα since the corresponding mRNA (like the EGF-receptor mRNA) is found in increasing concentrations which reach high levels at the blastocyst stage. The TGFα protein is also detectable by the blastocyst stage [Rappolee et al., 1988] and is located mainly on the ICM and polar trophectoderm cells [Dardik et al., 1992].

Since the ligand can create a developmental signal only after binding to the EGF-receptor, various assays have been used to demonstrate the occurrence of the receptor on the surface of embryo cells (Table II). Binding of [^{125}I]-EGF detected by autoradiography of the 8-cell embryo and later stages has been observed and confirmed by immunofluorescence assays with anti EGF-receptor antibodies that demonstrated receptor protein on late 4 cell embryos with strong increases on later embryos [Adamson,

TABLE II. Growth Factor Receptors on Embryos

Factor		Stage	Detection method	Reference
EGF-R	Protein	8-cell-bc	[^{125}-]-EGF binding	Paria and Dey, 1990
		Blastocyst	Immunofluorescence	Adamson, 1990
		8-cell-bc	Immunofluorescence	Wiley et al., 1992
	mRNA	1-cell-bc	RT-PCR	Wiley et al., 1992
	Active protein	ICM and TE of bc	EM for EGF and IEM for EGFR	Dardik et al., 1992
INS-R	Protein	8-cell-bc	[^{125}-I]-Ins binding	Mattson et al., 1988 Heyner et al., 1989 Rosenblum et al., 1986 Harvey and Kaye, 1991b
	mRNA	8-cell-bc	RT-PCR	Rappolee et al., 1990, 1992
IGF-I-R	mRNA	8-cell-bc	RT-PCR	Rappolee et al., 1990
IGF-II-R	mRNA	2-cell-bc	RT-PCR	Rappolee et al., 1990, 1992
IGF BP	mRNA	2-cell-bc	RT-PCR	Schultz et al., 1993
PDGF-Rα	mRNA	1-cell-bc	In situ hybrid	Schultz et al., 1993
	Protein	1-cell-bc	Immunofluorescence	Palmieri et al., 1992a
PDGF-Rβ			Not detected	
TGFβ-R		8-cell-bc	[^{125}-I]-TGFβ binding and cross-linking	Paria et al., 1992
CSF-1-R (c-*fms*)	mRNA	2-cell-bc trophect.	RT-PCR	Arceci et al., 1992
c-*kit*	mRNA	Blastocyst	RT-PCR	Arceci et al., 1992

1990; Wiley et al., 1992]. The EGF-receptor is active at the blastocyst stage of preimplantation embryos since EGF stimulates the synthesis of EGF-receptor in metabolically labeled embryos [Wiley et al., 1992]. EGF activation of EGF-receptors also elicits the induction of c-Fos protein [Adamson, 1990]. However, the receptor appears to be active as early as the 8-cell stage [Paria and Dey, 1990]. Further evidence is provided by the microinjection of antisense RNA into 2-cell embryos and by antisense deoxyoligonucleotide addition to the culture medium. The effects are seen 48 h later as a significant decrease in the rate of cavitation of the embryos in the antisense groups compared with controls. There is little effect on the rate of cell proliferation of the embryo either by antisense RNA or antibodies [Brice et al., 1993]. The conclusion is that the EGF-receptor is concerned more with differentiation than with cell proliferation.

Table III summarizes some studies of the effects of EGF and other growth factors on preimplantation embryos. Accumulated results indicate that when ligands or antibodies bind to the EGF-receptor, the signal leads to the stimulation of differentiation as measured by earlier onset and increased rate of cavitation and increased blastocoel expansion. This presumably occurs through increases in receptor activities and activation of genes such as those coding for ion transporters which are important in trophectoderm differentiation and the formation of the blastocoel cavity. Conversely, when the synthesis and relocation of receptor protein is prevented by antisense EGF-receptor RNA or DNA, the differentiation of the embryo is inhibited [Brice et al., 1993].

We speculate that the EGF-receptor plays an equally if not more important role in cell differentiation of the embryo than as a growth stimulator [Adamson et al., 1991; Adamson, 1993], and the data in Table III supports this hypothesis. EGF-receptor protein levels appear to increase markedly up to the precompacted 8-cell embryo stage and therefore could be involved in the process of epithelialization of trophectoderm that becomes increasingly evident after compaction. The location of receptor protein in the morula stages is predominantly apical, although it can also be seen on the inner blastomere membranes after disaggregating the embryo with Ca-free medium containing EDTA. By the time the blastocyst forms, the receptor is now observed predominantly on the basal surfaces of the trophectoderm with less on the apical surfaces and on the inner cell mass cells [Dardik et al., 1992]. This shift in location suggests that the function of the EGF-receptor in the early stages is to receive signals from maternal EGF in the oviduct. We speculate that when the receptors start to receive signals from endogenous TGFα they shift to an internal location. It

TABLE III. Effect of Growth Factors on Preimplantation Mouse Embryos

Growth factor	Assay	Reference
EGF + TGFα	1. Increased frequency of GVBD	Downs et al., 1988
	2. Increased protein synthesis in trophoblast cells	Wood and Kaye, 1989
	3. Increased number of blastocysts; increased hatching rate	Paria and Dey, 1990
	4. Phos. of 170 kDa protein inhibited by tyrphostins; tyrphostins inhibit number of bc and number of cells	Paria et al., 1991
	5. Stimulation of blastocoel expansion	Dardik and Schultz, 1991
	6. Stimulation of cavitation by Ab to EGF-R; Inhibition of cavitation by antisense RNA and oligos to EGF-R	Brice et al., 1993
Insulin	1. Stimulation of protein synthesis in 8-cell	Harvey and Kaye, 1988
	2. Stimulation of cell number of ICM and increased protein synthesis	Harvey and Kaye, 1990, 1991a
	3. Decreased number of morulae and increased number of blastocysts	Gardner and Kaye, 1991
	4. Increased cell number from 8-cell, increased nucleic acid synthesis	Rao et al., 1990
IGF-I	1. Stimulation of growth and metabolism	Harvey and Kaye, 1991a, 1992a
	2. Increased cell number; increased protein synthesis	Rappolee et al., 1992
	3. Stimulation of proliferation of ICM cells	
IGF-II	1. Stimulation of growth and metabolism	Harvey and Kaye, 1992b.
	2. Increased cell number in blastocyst; antisense oligos decreased rate of development; decreased cell number	Rappolee et al. 1992
PDGF-AB	1. Increased number of embryos with endoderm; increased number of endoderm cells	Rizzino and Bowen-Pope, 1985
PDGF-A	1. Stimulates tyrosine kinase activity of 3.5 day embryo	Palmieri et al., 1992a
TGFβ	1. Increased number of blastocysts develop	Paria and Dey, 1990
	2. Ab to TGFβ2 inhibits implantation	Slager et al., 1993
CSF-1	1. Blocks normal development	Tartakovsky and Ben-Yair, 1991
	2. Restored by TNFα, GM-CSF, and IL-2α and not by TGFβ	Tartakovsky and Ben-Yair, 1991

is known that the membrane proteins of the apical surface of an epithelial layer become less laterally mobile after the formation of tight junctions. The EGF-receptors could contribute to the polarization of the outer blastomeres by their inability to move to all cell surfaces after the formation of tight junctions.

When endogenous TGFα binds and stimulates a signal, one effect is to increase the further synthesis of its own receptor, a process documented in vitro in several cell types [Kudlow et al., 1986]. We suggest that the autocrine loop results in increases in the receptor predominantly in the basal and lateral surfaces of the blastomeres. This relocation of membrane components is a feature of epithelial cell polarization, although we do not know if the contribution of EGF-receptor is a cause or an effect.

The EGF-receptor protein that remains on the outer surfaces of the blastocyst could also help in the process of implantation. Paria et al. [1993] have observed that the high levels of EGF-receptor mRNA and protein in the blastocyst rapidly decrease upon ovariectomy of the mother, a process that leads to the inhibition of implantation. These levels remain low until the administration of estrogen and progesterone to the mother reinitiates implantation. Eight to twenty-four hours later, EGF-receptor mRNA and protein levels rise prior to implantation. The effect of steroid hormones is likely indirect since no such change is seen in the isolated blastocyst by the direct application of hormones. Since TGFα is present on the uterine epithelial surface and the membrane bound precursor form of the ligand can bind to the receptor on the blastocyst, there is a potential role of the receptor in the specific adhesion of the embryo. This putative activity of the TGFα is juxtacrine in nature since two adjacent cell types are in-

volved. However, there is no evidence for this effect yet.

The final proof for the necessity of a gene product in a biological or physiological process is to show that its loss leads to specific defect. There are two recent reports of the ablation of the TGFα gene by homologous recombination with a targeting vector in embryo stem cells and in vivo in mice [Luetteke et al., 1993; Mann et al., 1993]. The TGFα (−/−) mice are smaller, have smaller eyes, are born with their eyes open, etc., but the major defect is in the organization of the hair and whisker follicles, which are kinky and lead to the production of wavy hair. This mutation has been recognized as a well-known mutation, *waved-1,* that is clearly not lethal. The conclusion is that TGFα is not necessary for preimplantation or later developmental viability. We still do not know whether EGF or the EGF-receptor are necessary until these products are similarly tested. The "gene knock-out" technique has led to quite a few surprising results [Erickson, 1993] and actually may distort our views of developmental roles of genes in cases where there is redundancy and overlapping activity.

PLATELET-DERIVED GROWTH FACTOR (PDGF) FAMILY MEMBERS AND THEIR RECEPTORS IN PREIMPLANTATION EMBRYOS

Platelet-derived growth factor family members and their receptors in preimplantation embryos are reviewed by Heldin and Westermark [1989], Bowen-Pope et al. [1991], and Palmieri et al. [1992b].

The multiplicity of related genes seen above in the members of a growth factor family occurs to a lesser degree within the PDGF family. There are two ligands, A and B; in addition, the A isoform undergoes alternate splicing to yield polypeptides of slightly different sizes. These ligands are produced as precursors and are extensively processed before secretion as dimers or placement on the cell surface where they can bind to receptors. The two chains associate in homo- or heterodimers that depend on disulfide bonds to achieve their active forms. Two receptor genes have been cloned so far. The α-receptor binds all forms of PDGF ligands, while the β-form binds only the BB dimer. Ligand binding stimulates the dimerization of the receptors, and these can be homo- or heterodimers also. Several differences in the activities of the ligands have been described depending upon the target cell. Both forms can be potent mitogens, but BB has more effect on chemotaxis. PDGF-AA is secreted into the culture medium of cells producing it in contrast to PDGF-BB, which remains close to the membrane.

PDGF-B is detectable in the human blastocyst, and since it is absent from follicular fluid it appears to be an embryo-derived product [Svalander et al., 1991]. A clear difference between species occurs because in contrast to humans, in mouse the B chain is only detected at a later stage in the postimplantation 8-day embryo at the same time as the β-receptor is synthesized. The PDGF-A ligand and the α-receptor appear much earlier and are found at all stages from 2-cell to blastocyst in the preimplantation embryo [Palmieri et al., 1992a]. There is good evidence that the receptor could function in an autocrine fashion at this stage in the mouse. In postimplantation stages, the α-receptor and PDGF-A are products of different cell types that could therefore act in a paracrine mode [Palmieri et al., 1992a]. In the cow there is a developmental block at the 8-cell stage in embryos in culture, and this is alleviated by the addition of PDGF-BB to the culture medium. Beyond the 16-cell stage, TGFα is effective in stimulating blastulation, but PDGF is inhibitory [Larson et al., 1992]. Other work suggests that the addition of PDGF to preimplantation rabbit embryos has no effect on development [Carney and Foote, 1991].

Although a gene knock-out has not been reported for PDGF or receptors, there is a mutation, *Patch,* that almost certainly represents the loss of the PDGF-receptor-α gene. The recessive dominant mutation is lethal during the latter half of gestation, but there is no suggestion that preimplantation development is compromised [Schatteman et al., 1992; Morrison-Graham et al., 1992; Orr-Urtreger et al., 1992]. One must conclude that it is unlikely that the preimplantation embryo relies on PDGF-A or its receptor for promotion through the first 5 days of development.

INSULIN AND THE INSULIN-LIKE GROWTH FACTORS IN PREIMPLANTATION DEVELOPMENT

Members of this family of ligands include insulin and insulin-like growth factor I and II (IGF-I and IGF-II) which are all capable of binding (but with different affinities) to three receptors: insulin-receptors (IRs), IGF-I-Rs, and IGF-II-Rs. Their roles appear to be predominantly

growth promoting in all cell types. The role of the insulin family of growth factors in preimplantation embryos has been extensively studied by at least three groups [see, for example, Heyner et al., 1989; Harvey and Kaye, 1991a,b; Rappolee et al., 1992]. The first appearance of the insulin receptor was demonstrated at the late 8 cell stage. The addition of insulin stimulates embryonic metabolism and cellular proliferation. Neither insulin nor IGF-I is produced by the preimplantation embryo, but insulin, IGF-I, and IGF-II can all be detected in the reproductive tract. IGF-I mRNA is present in bovine preimplantation embryos, however [Schultz et al., 1993]. IGF-II mRNA and protein can be detected in the 2 cell embryo and, like IGF-I, IGF-II stimulates the growth and metabolism of preimplantation embryos [Harvey and Kaye, 1992a,b]. The reduction of IGF-II expression by the presence of antisense IGF-II oligonucleotides inhibits the developmental rate of embryos. Interestingly, only the maternal IGF-II receptor gene is active in preimplantation embryos, while the paternal copy is inactivated in a process known as "genomic imprinting" [Barlow et al., 1991], while the opposite is true for the IGF-II ligand [DeChiara et al., 1991] and IGF-I receptor genes [Rappolee et al., 1992]. Such clear regulatory mechanisms strongly suggest that the insulin family plays essential roles in normal developmental and growth processes in the preimplantation mouse [Rappolee et al., 1992]. Although the loss of IGF-II is not lethal, the offspring are smaller in its absence [DeChiara et al., 1990], and this affects fitness to survive in the competitive conditions in nature.

OTHER GROWTH FACTORS IN PREIMPLANTATION DEVELOPMENT

For a review of other growth factors in preimplantation development, see Rappolee et al. [1990].

Cleavage stage embryos from the 4 cell to blastocyst stage transcribe and translate the mRNA of three members of the TGF-β family [Rappolee et al., 1988; Paria et al., 1992]. Embryos from the 8-cell stage bind TGF-βs and express all three classes of TGF-β binding proteins or receptors. Some stage differences between the isoforms may indicate that they may play different roles [Paria et al., 1992]. The 8-cell block (a stage when development frequently stops) in bovine embryos is alleviated by the addition of TGF-β, and this is synergized by the presence of basic fibroblast growth factor or bFGF [Larson et al., 1992]. Active TGFs are secreted by mouse blastocysts [Rizzino, 1985] and may play a role, direct or indirect, in implantation, since the injection of antibodies to TGF-β2 into the uterine cavity on the fourth day of gestation results in a lower implantation frequency (32% compared to 71% in controls) [Slager et al., 1993]. However, the uterus also expresses several TGF-β isoforms, and therefore the target for antibody inhibition of implantation is not clear [Das et al., 1992]. The stimulatory activity of the TGFβs on development could also be acting through the mediation of fibronectin which is known to assist embryonic development [Larson et al., 1992].

Other growth factors have not been explored extensively in preimplantation embryos, and these are listed in Table II.

CYTOKINES IN PREIMPLANTATION DEVELOPMENT

For reviews of cytokines in preimplantation development, see Pampfer et al. [1991] and Lee [1992].

Macrophages and lymphocytes in the reproductive tract and in the uterine epithelium and glands are probably involved in the developmental process as participants of a paracrine mechanism. A possible role is to synchronize the process of implantation through production of cytokines and growth factors elicited or mediated by the sex steroid hormones. Leukemia inhibitory factor (LIF) is particularly suspect for a role since its expression fluctuates with the estrus cycle and is highest after ovulation and on the day of implantation in the pregnant uterus [Bhatt et al., 1991]. Furthermore, female mice lacking both LIF genes fail to implant their blastocysts even though these are viable. Such blastocysts will implant when transferred into normal host pseudopregnant females [Stewart et al., 1992]. Another influence of LIF may be to preserve a high rate of proliferation in the primitive ectoderm cells (inner cell mass, or ICM, cells). This would account for the improved developmental capability of in vivo compared to in vitro cultured embryos. Presumably, the low level of LIF expressed by the blastocyst itself is insufficient to maintain a high rate of ICM cell proliferation [Murray et al., 1990], and therefore the uterus must be the major source. The expression of LIF by embryo stem cells (ES) is also insufficient to maintain ES cells in an undif-

ferentiated form in vitro, and it is necessary to add LIF either from feeder cells or from external recombinant sources to ES cell cultures.

Preimplantation mouse embryos do not express colony stimulating factor-1 (CSF-1), but they are exposed to CSF-1 throughout this period. CSF-1 mRNA and protein are found in the oviduct from day 1 of pregnancy and from day 3 in the uterus, where levels peak on day 5 [Bartocci et al., 1986]. The embryo is transcribing the receptor gene for CSF-1 since transcripts for c-*fms* are found from the 2-cell stage, and receptors for *CSF-1* are found concentrated on the trophectoderm cells. It has been suggested that the receptor on trophectoderm cells and CSF-1 on the uterine epithelial cells could play a role in implantation by specific binding, and indeed many of the growth factors discussed here could also act similarly. Another example is the receptor c-*kit* found in the preimplantation mouse embryo while its ligand, the *steel* factor, occurs in the oviduct from day 2 [Pampfer et al., 1991; Arceci et al., 1992].

Some of the cytokines also appear to have a beneficial effect on the development of the preimplantation embryo. For example, CSF-1 and LIF stimulate but GM-CSF arrests the growth of 2-cell embryos. IL-1 and IL-2 have no measurable effect on embryo development or on trophoblast outgrowth in vitro [Schneider et al., 1989]. CSF-1 and LIF are secreted into the medium by blastocysts in an active form and in sufficient amounts to be detected in a biological assay. The evidence that LIF plays an essential role in implantation is clear, but for CSF-1 and GM-CSF the evidence is inconsistent. The administration of CSF-1 for the first 5 days of pregnancy appears to impair the capacity of the embryos of certain mouse strains to develop and implant. Tartakovsky and Ben-Yair [1991] were able to rescue both the experimental nonimplanting embryos as well as those of the CBA/J strain of females that have a high level of spontaneous failure of preimplantation development by the administration of TNFα or GM-CSF, but not with TGF-β. These in vivo experiments differ from in vitro culture results and appear to act through different mechanisms.

FUTURE STUDIES

Most observations on preimplantation embryos have been made on the embryo removed from the uterus and cultured in an artificial medium. Whether these observations have any bearing on the activities and roles of the growth factors and their receptors in vivo can only be determined by other approaches such as the ablation of the each gene to determine the effect of its loss. Even this rather direct method of studying the function of receptors such as the EGF-receptor or its ligands may fail to reveal their normal roles because of the redundancy of the ErbB polypeptides and the number of ligands with overlapping functions in vivo. It is likely that at least some of the activities of the genes discussed here will be circumvented by related genes that can be up-regulated in the absence of the targeted gene. Perhaps we may measure the importance of a gene by the number of its surrogates.

I believe the roles of each receptor may be further blurred by a second level of redundancy. It is possible that to some extent the activity of a certain receptor kinase (A) may be replaceable by an unrelated kinase (B) which becomes more active in the absence of A. This may work because all that is needed in the preimplantation embryo is the gradual increase in unspecified tyrosine kinase activity in order to elevate the levels of the signal transducing intermediates. Subsequently, increasing levels of the common transcription factors lead to the secondary activation of genes in the developmental pathway. This could readily occur because the signal transducing pathways converge and overlap in order to elicit the expression of c-Jun, c-Fos (AP-1), c-Myc, or other early growth response transcription factors such as Egr-1. Conceivably, the studies described here which show that there is a measurable biological effect upon the inhibition of a receptor or by stimulation of a receptor work only because the culture system in vitro is suboptimal and is not exposed to maternal influences and regulatory responses. Thus each of the components that appears to have a role in preimplantation development will have to be tested by gene ablation and cross-breeding studies. Homozygous gene knock-out mice bearing different deletions could then be crossbred to combine the effect of their mutant genes. These sorts of experiments will be increasingly performed in the next few years, and we should see a great advance in our understanding of the roles of growth factor genes in every aspect of normal development as well as on abnormal growth patterns.

ACKNOWLEDGMENTS

I am grateful to Drs. Dan Mercola and Lynn Wiley for critical reading of the manuscript. This work was supported by grants from the PHS, namely NIH grants CA-28427, P30 CA-30199, and HD-28025.

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