Growth factors and their cellular actions

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Introduction

Normal tissue growth involves a complex interaction of cell proliferation, hypertrophy, migration, differentiation and senescence which proceeds within strict temporal and anatomical constraints. While classical endocrine hormones such as growth hormone and thyroxine can influence the overall rate of growth and maturation they do not contribute to the fundamental intercellular signalling within tissues that underlies these processes. This appears to be the primary role of peptide growth factors. During the rapid development of the embryo and fetus peptide growth factors are associated with tissue induction, the clonal growth of stem-cell populations, and the appearance of differentiated function in the presumptive organs. In post-natal life the actions of growth factors are less obvious although notable exceptions demonstrate that the same biological pathways exist; the best studied examples are the role of somatomedins during longitudinal skeletal growth and the coordinated actions of several growth factors within the ovarian cycle, during testicular function, and during peripheral wound healing. The aim of this article is to present a broad overview of the diverse presence and actions of peptide growth factors during human development and reproduction.

Physical form and anatomical location

Those peptide growth factors that have been best characterized are shown in Table 1. They are typically proteins of molecular weight < 30 000 which are synthesized and act within the local tissue environment as paracrine or autocrine hormones. Unlike classical endocrine hormones each growth factor may be synthesized by many anatomically diffuse tissues. They are not stored intracellularly in granules and release is largely dependent on de-novo synthesis. While acute release is not common this has been overcome during wound healing by the rapid delivery of several growth factors to the sites of tissue damage by platelet lysis. Peptide growth factors interact with receptors located on the cell membrane which are usually glycoproteins and which communicate with secondary messenger systems by conformational changes. This often involves the autophosphorylation of tyrosine residues located on the intracellular domain of the receptor. The second messenger systems utilized are diverse and include changes to intracellular calcium levels, cyclic AMP, cellular alkalization, and phosphoinositol metabolites. The net biological events induced by growth factors are, however, remarkably consistent and include a rapid stimulation of amino acid transport, glucose uptake and utilization, and RNA and protein synthesis. For many, but not all, growth factors this is followed by DNA synthesis and cell replication.

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The somatomedins or insulin-like growth factors (IGFs) are single chain polypeptides of $M_r \sim 7500$ which share structural and functional similarities with insulin. Two peptide species exist; IGF-I which has 70 amino acids and is encoded by a gene on human chromosome 12, and IGF-II which has 67 amino acids and derives from a gene on the short arm of chromosome 11 (Tricoli *et al.*, 1984). The IGFs are potent mitogens for cells derived from all primitive germ layers. In post-natal life the IGF-I gene is under tight transcriptional control by growth hormone, circulating concentrations being severely reduced in hypopituitarism and elevated during acromegaly. While the major source of IGFs was originally thought to be liver it is now recognized that they are synthesized by most body tissues both before and after birth (D'Ercole *et al.*, 1984; Rotwein *et al.*, 1987).

The IGFs appear early in embryogenesis during and after the condensation of presumptive organs and tissues, and their primary role may be to promote the clonal expansion and subsequent differentiation of stem cell populations. Messenger RNA for IGF-II was identified in embryonic chick eye, heart and wing bud mesenchyme while IGF peptides have been localized, using immunohistochemistry, to the mesencephalon, lens, neural tube, myotome and developing wing buds at 3–4 days incubation (Engstrom *et al.*, 1987; Rolfe *et al.*, 1988). In the rat embryo mRNA for IGF-II was localized by cDNA/mRNA in-situ hybridization to yolk sac, hepatic bud, branchial arch mesoderm, and dermato-myotome between 10.5 and 14.5 days of gestation, and was additionally present in differentiated muscle, perichondrium, chondrocytes and gut epithelium in later gestation (Beck *et al.*, 1987). In the human fetus in the early second trimester, IGFs were localized by immunohistochemistry to epithelia within the gut, kidney and lung; hepatocytes, adrenal cortical cells from the fetal zone, differentiated skeletal and cardiac muscle fibres, haemopoietic tissue and dermal skin (Han *et al.*, 1987b). This distribution may depend on an association with specific IGF binding proteins which share a similar cellular distribution (Hill *et al.*, 1988). The binding proteins may contribute to a sequestration of IGF peptides within certain cell populations, since analysis of IGF-I and -II mRNA distribution in human fetal tissues by in-situ hybridization suggested that the majority was present in fibrous mesenchymal tissues adjacent to the cell types positive for IGF peptides (Han *et al.*, 1987a).

In post-natal life IGF peptide presence is less apparent in many tissues by immunohistochemistry compared to the fetus, but can be re-expressed when local damage is inflicted on skeletal muscle, vascular endothelium, hyaline cartilage or peripheral nerves (Hansson *et al.*, 1986, 1987; Jennische & Oliverecrone, 1987). However, a strong expression of IGF peptides is retained in those tissues which continue to undergo rapid growth and differentiation, including the epiphyseal growth plate cartilage, testis and ovary. Within the cartilage growth plates of the long bones IGF-I is synthesized by proliferative chondrocytes but this ceases once cells have hypertrophied and become post-mitotic before matrix calcification (Isaksson *et al.*, 1987). The expression of IGF-I within growth plate cartilage is largely dependent on the presence of growth hormone. IGF-I is likely to act as a paracrine or autocrine agent within growth plate cartilage to promote the clonal expansion of newly-formed chondrocytes and their subsequent differentiation. Within the rat testis IGF-I is released from Sertoli cells and is also located within spermatocytes (Andersson *et al.*, 1986; Smith *et al.*, 1987). In the rat ovary IGF-I mRNA has been localized by in-situ hybridization to the granulosa cells surrounding the follicles, expression being greatest around the dominant follicles (Oliver *et al.*, 1988). Human follicular fluid is a rich source of IGF-I which, in cohort follicles, correlates with that in the circulation. Concentrations are greatest in the dominant follicles and increase further following the LH surge, after which they correlate positively with oestrogen concentrations (Eden *et al.*, 1988). IGF-I is released by isolated rat granulosa cells, this being enhanced by GH or FSH and further augmented by oestradiol (Hsu & Hammond, 1987).

Epidermal growth factor (EGF) is a single chain polypeptide of 53 amino acids with molecular weight of 6000. Mouse EGF was first isolated from male submaxillary glands and the equivalent human peptide, urogastrone, from urine (Cohen, 1962; Gregory, 1975). A structural analogue of EGF is transforming growth factor- α (TGF- α). This has a molecular weight of 7500 and shows a

Table 1. Peptide growth factors

Insulin-like growth factors/ somatomedins	IGF-I and -II	Mitogen and differentiating agent for cells derived from all primitive germ layers.
Epidermal growth factor	EGF	Mitogens for epithelial and mesodermal tissues. Inhibit differentiated function in ovary. Enhances maturation of skin and teeth. Promotes release of hCG and placental lactogen from placenta.
Transforming growth factor α	TGF- α	
Fibroblast growth factor	Acidic FGF Basic FGF	Mitogen for mesodermal and endodermal cell types. Induces mesoderm formation in <i>Xenopus</i> embryo. Inhibits differentiation in muscle but potentiates hormone release in pituitary. Causes angiogenesis.
Platelet-derived growth factor	PDGF	Mitogen for mesodermal cell types. Chemotactic for fibroblasts, smooth muscle cells, monocytes and neutrophils.
Transforming growth factor β	TGF- β	Inhibitor of epithelial cell proliferation but biphasic action on mesodermal cell types. Induces mesoderm formation on <i>Xenopus</i> embryo. Inhibits differentiation in muscle. Chemotactic for monocytes, and fibroblasts. Promotes fibronectin and collagen synthesis.

30–40% amino acid homology with EGF (Marquardt *et al.*, 1984). TGF- α reproduces all of the biological actions of EGF and interacts with the EGF receptor. EGF is a potent mitogen for epithelial and mesodermal cell types *in vitro*, including connective tissues, glial cells and ovarian granulosa cells. In the mouse embryo EGF receptors appear with the first differentiated cell type, trophoctoderm (Adamson & Meek, 1984). It is unclear whether the peptide that might activate these receptors is EGF or TGF- α . Although TGF- α was detected in the mouse embryo with the highest levels on Day 7 of gestation, *in-situ* hybridization studies in rats suggested that the embryo does not express TGF- α at this time (Han *et al.*, 1987c). However, abundant TGF- α mRNA was present in maternal decidua, especially adjacent to the embryo. Decidual expression appeared after implantation, peaked at Day 8 and slowly declined to Day 15 in parallel with decidual resorption. Peak levels of TGF- α may therefore be available to the embryo from a maternal source during gastrulation and neurulation. Early in the second trimester, EGF is present in the human fetus within cells at the base of the gastric and pyloric glands in the stomach, the Brunner's glands in the duodenum, the epithelium of the distal convoluted kidney tubules, anterior pituitary, pseudostratified columnar epithelium of the trachea and the placenta (Kasselberg *et al.*, 1985). In post-natal life also, EGF is detectable by immunohistochemistry in association with epithelial layers demonstrating rapid turnover, such as in gut and skin. Additionally, it is stored in large quantities in platelets and released during tissue injury. Similarly, its presence within monocytes may be related to wound healing.

Fibroblast growth factor (FGF) has been isolated from bovine pituitary and brain in both an acidic (pI 4.5) and basic (pI 9.6) form, each being of $M_r \sim 16\,000$ – $17\,000$ (Gospodarowicz *et al.*, 1984; Esch *et al.*, 1985). FGFs are potent mitogens for tissues derived from mesenchyme or neuroectoderm such as chondrocytes, fibroblasts, myoblasts, glial cells, adrenal cortex and ovarian granulosa cells (Gospodarowicz *et al.*, 1987). They are the most effective mitogens so far identified for vascular endothelial cells, and additionally promote cell migration, invasion and the production of plasminogen activator; all necessary features of angiogenesis *in vivo*. FGF mRNA is detectable in the *Xenopus* blastula (Kimelman & Kirschner, 1987) while the peptides are present in chick embryo brain, retina and vitreum from Day 11 of gestation. In adult life FGF is diversely distributed among tissues including ovary, adrenal, kidney and the anterior pituitary.

Platelet-derived growth factor (PDGF) was first identified within the α storage granules of platelets. Human PDGF is a highly basic glycoprotein (pI 10.2) with two biologically equipotent variants, PDGF 1 which contains 7% carbohydrate and has a molecular weight of 31 000, and PDGF 2 with 4% carbohydrate and molecular weight 28 000 (Deuel & Huang, 1984). In platelets

both forms contain an A-chain of M_r 15 000–17 000 and a B-chain of 14 000 linked by disulphide bridges. The B-chain of PDGF shows extensive sequence homology with the viral p28 protein encoded by the *v-sis* oncogene (Waterfield *et al.*, 1983), and the cellular *sis* gene was subsequently shown to encode the precursor protein for PDGF B-chain (Johnsson *et al.*, 1984). In tissues other than platelets PDGF usually consists of a homodimer of two B-chains. PDGF stimulates proliferation in many cell types derived from mesoderm including fibroblasts, smooth muscle cells, glial cells and ovarian granulosa cells (Deuel & Huang, 1984).

Goustin *et al.* (1985) found expression of PDGF in human placenta from as early as 21 days after conception. By in-situ hybridization the expression of the *c-sis* gene was localized to trophoblasts. Explants of first trimester placenta released radioreceptor-assayable PDGF, while cultured trophoblast cell lines were rich in high affinity PDGF receptors, and responded to exogenous PDGF with an increased expression of the proto-oncogene *c-myc* accompanied by DNA synthesis. Since the expression of *c-myc*, which encodes a DNA binding protein and is one of the first intracellular events to take place following interaction of PDGF with its receptor, was also localized to trophoblasts this may represent an autocrine action of PDGF on trophoblast proliferation during the early invasive growth of the placenta. In later fetal development mRNA for PDGF was identified in the human kidney (Fraizer *et al.*, 1987). While PDGF continues to be present in aortic smooth muscle in the immature rat, expression in later life appears to be limited, in normal circumstances, to platelets where it contributes to wound healing following platelet lysis.

Transforming growth factor β (TGF- β) differs from the other growth factors outlined in that it predominantly acts as an inhibitor of cell proliferation. It is a homodimer of M_r 25 000 found in abundance in, and purified from, platelets. TGF- β is a potent inhibitor of epithelial cell replication *in vitro* at picomolar concentrations (Strain *et al.*, 1986). For mesodermal cells it can act as a bifunctional growth regulator, either enhancing the actions of other growth factors on cell replication or serving as a growth inhibitor depending on the cellular substratum, the age of development of the tissue, and whether the cells are virally transformed (Roberts *et al.*, 1985). TGF- β stimulates fibronectin and collagen release, major constituents of extracellular matrix, by some fibroblast types; increases the release of plasminogen activator from other fibroblasts leading to a degradation of extracellular matrix glycoproteins; and modulates cell adhesion molecule receptors. These properties suggest a potential role in tissue modelling and this is supported by the anatomical distribution of TGF- β in the embryo and fetus.

TGF- β mRNA was found in the vegetal pole ectoderm of *Xenopus* embryos and is transcribed by a maternally-derived gene *Vg1* (Weeks & Melton, 1987). An ectodermal cell line, XTC, obtained from a metamorphosing tadpole releases a TGF- β -like peptide *in vitro* (Rosa *et al.*, 1988). In the 11–18-day mouse embryo TGF- β has been localized immunohistochemically to differentiated and undifferentiated mesenchyme including bone and connective tissues, particularly that derived from neural crest such as palate, larynx, facial mesenchyme and teeth (Heine *et al.*, 1987). Staining was most intense around sites of tissue morphogenesis involving mesodermal interaction with adjacent epithelia, including hair follicles, teeth and secondary palate. In late fetal rat development TGF- β bioactivity was identified in multiple tissues, levels being greatest in muscle, lung, liver and kidney (Hill *et al.*, 1986), while TGF- β was released by isolated fetal rat calvaria and by myoblasts (Centrella & Canalis, 1985; Hill *et al.*, 1986). After birth, TGF- β concentrations in rat tissues decline rapidly to barely detectable levels (Hill *et al.*, 1986), although in the human TGF- β remains widely distributed amongst cells of the immune system including monocytes and T-lymphocytes. It has been proposed that its presence in the latter constitutes an autocrine negative feedback system on the clonal expansion of lymphocytes in response to interleukin 2 (Kehrl *et al.*, 1986).

Mitogenesis

The overlapping biological properties of growth factors such as IGFs, EGF, PDGF and FGF as cell mitogens may represent, at first sight, gross evolutionary overcapacity. However, some

selectivity exists since differing cell types may recognize each growth factor with different relative sensitivity. Additionally, each growth factor appears to fulfil a particular role within the cycle of cell replication.

Many fundamental studies on cell cycle control have been performed using BALB/c-3T3 fibroblasts which were originally derived from an embryonic mouse. It was found that PDGF alone and at concentrations similar to those found in tissues was unable to initiate proliferation in quiescent, density arrested BALB/c-3T3 cells and that an addition of plasma was needed for cells to enter S phase and DNA synthesis (O'Keefe & Pledger, 1983). Similarly, platelet-poor plasma alone could not stimulate cell replication although it maintained cell survival. A sequential addition of PDGF followed by platelet-poor plasma enabled cells to progress to S phase with a lag period of 12 h following exposure to PDGF. The percentage of nuclei stimulated to initiate DNA synthesis depended on the concentrations of PDGF and plasma. Only a transient exposure to PDGF was necessary and the sensitizing effect lasted for 3–5 h. In contrast, plasma was required for the whole of the remaining period of G_1 . It was concluded that PDGF induced a state of 'competence' which rendered quiescent cells capable of traversing the cell cycle from G_0 to G_1 in response to other plasma factors. FGF fulfilled a similar role for this cell type.

The need for plasma to enable synchronized BALB/c-3T3 cells to progress through G_1 could be replaced by a mixture of EGF and IGF-I, although the latter could be substituted by pharmacological concentrations of insulin (Van Wyk *et al.*, 1981). The temporal order of exposure to these 'progression' factors was important for subsequent entry into DNA synthesis, EGF being necessary for the first 6 h of G_1 and IGF-I for the remaining period until about 2.5 h before the G_1 /S boundary when cell replication becomes growth factor-independent. The ability of cells to traverse the final part of G_1 without growth factor stimulation is probably due to the transcription of DNA-synthesizing enzymes such as thymidine kinase and thymidylate synthase having been completed by this time. Their transcription earlier in G_1 is stimulated by IGF-I (Yang & Pardee, 1986). Growth-arrested BALB/c-3T3 cells could therefore be induced to traverse the full cell cycle in defined culture medium containing only PDGF, EGF and IGF-I in sequential order. Each growth factor may render the cells responsive to the next since exposure of cells to PDGF caused a change in affinity of the EGF receptor for EGF (Sinnott-Smith & Rozengurt, 1985), while plasma caused a doubling of IGF-I receptor number about 3 h into G_1 and before the onset of IGF sensitivity (Van Wyk *et al.*, 1981).

The ability of BALB/c-3T3 cells to traverse the cell cycle in growth factor-supplemented medium was dependent on the availability of amino acids, especially those in group B such as cysteine, isoleucine, lysine, phenylalanine and tyrosine (Cooper & Wharton, 1985). In medium deficient in amino acids cells become arrested at a point midway through G_1 coincident with the time from which IGF-I is necessary. An additional amino acid-dependent restriction point was found in human fibroblasts 2–3 h before the G_1 /S boundary coincident with the completion of DNA-synthesizing enzyme transcription. This suggests that two periods within G_1 in particular require protein synthesis and that both may be dependent on IGF-I.

This model of growth factor action during BALB/c-3T3 cell proliferation does not hold true for all cell types although the concept on 'competence' and 'progression' factors appears to be universal. Human fibroblasts have a greater endogenous ability to release IGFs *in vitro* than do the embryonic mouse fibroblasts (Clemmons *et al.*, 1981). Consequently, this obviates the absolute need for IGFs during the G_1 phase. Additionally, PDGF is necessary throughout G_1 as well as at the G_0 / G_1 interface for optimal proliferation to occur (Westermarck & Heldin, 1985). Other cell types differ more radically; for instance, EGF appears to act as a competence factor for isolated hepatocytes while IGF-I fulfils this role for chondrocytes.

The mitogenic actions of some growth factors are influenced by associations with specific binding proteins or extracellular matrix components. An IGF binding protein of M_r 26 000 is released by multiple cell types *in vitro* and is found throughout extracellular fluids and the circulation (Ooi & Herington, 1988). It greatly extends the biological half-life of the IGF peptides. One form of this

binding protein, purified from human amniotic fluid, avidly attaches to some cell membranes *in vitro* including those of fibroblasts (Clemmons *et al.*, 1986). Exogenous binding protein enhanced the mitogenic capacity of IGF-I on adult human fibroblasts, while other closely related species, also isolated from amniotic fluid, did not associate with cell membranes and antagonized IGF-I biological activity (Elgin *et al.*, 1987). This may represent an additional level of growth control by which cells can up- or down-regulate IGF action by the synthesis of specific binding proteins.

A different mechanism may be responsible for prolonging the biological half-life of FGF within tissues. FGF avidly associates with heparin sulphate, a glycosaminoglycan produced in large amounts by vascular and corneal endothelium and a structural component of their extracellular matrix. The FGF gene does not encode a conventional signal peptide sequence which is necessary for vesicle-associated exocytosis (Gospodarowicz *et al.*, 1987). The peptide may therefore leave the cell associated with extracellular matrix molecules and either interact immediately with FGF receptors as an autocrine stimulus or remain stored within the matrix: the FGF bound to matrix components retains full biological activity. Since organogenesis involves an interaction of cells with newly synthesized extracellular matrix to promote stem-cell growth and stabilize their specialized phenotype it is possible that FGF could exert mitogenic and differentiating effects from a matrix store.

Tissue induction

Two peptide growth factors, FGF and TGF- β , have been shown to promote the induction of mesoderm from primitive ectoderm in the early embryo. Such induction in the amphibian embryo depends on diffusible morphogens from the vegetal pole ectoderm causing mesodermal development from the animal pole ectoderm. FGF is a mesoderm-inducing factor for cultured animal pole ectoderm from *Xenopus* embryos; the structures which develop being primarily derived from ventral mesoderm and consisting of concentric patterns of loose mesenchyme, mesothelium and blood cells enclosed by ectoderm. Some dorsal mesodermal structures, such as muscle cells, were also identified (Slack *et al.*, 1987). TGF- β also induced mesoderm formation in *Xenopus* ectoderm, especially muscle which was identified by the presence of α -actin mRNA (Rosa *et al.*, 1988). A synergism existed between FGF and TGF- β in this respect (Kimelman & Kirschner, 1987). The most potent natural source of mesoderm-inducing activity is conditioned medium from *Xenopus* XTC cell cultures, a known source of TGF- β . The ability of TGF- β antiserum to block the mesoderm-inductive capacity of XTC-conditioned medium suggests that an amphibian TGF- β may be the major bioactive moiety (Rosa *et al.*, 1988).

Tissue differentiation

Growth factors can augment or antagonize the onset of differentiated cell function. A potentiation of differentiation need not exclude a mitogenic role since an immediate action on proliferation may be followed by a later tendency to promote terminal differentiation. Separate growth factors can co-operate or compete during the induction of differentiated function, while some show synergistic interaction with endocrine hormones. While growth factor action is most obvious during the progressive differentiation of the fetus and neonate, considerable interest has been shown in tissues which retain a high degree of anatomical and functional rearrangement in adult life, such as the ovarian follicles.

A well-studied model of tissue differentiation *in vitro* is the fusion of myoblasts into polynuclear, post-mitotic myotubes. This process can be quantitated by measuring the rise in myotube-associated enzymes such as myokinase and creatinine phosphokinase, or the acquisition of acetyl choline receptors. Muscle differentiation largely occurs *in utero* in man but can still be observed in the first

week *post partum* in the rat. Physiological concentrations of IGF-I or pharmacological concentrations of insulin promoted the proliferation of newly isolated myoblasts and of established myoblast cell lines (Ewton & Florini, 1980; Hill *et al.*, 1985). However, after 1 or 2 replicative cycles myoblasts ceased to proliferate in response to IGF-I and were instead stimulated to differentiate terminally (Ewton & Florini, 1981). This action of IGF-I on myoblast differentiation was still apparent when proliferation was abolished by exposure of cells to cytosine arabinoside. Primary and established myoblast cultures also proliferated in response to FGF. However, FGF inhibited myoblast differentiation to maintain an expanding population of cells (Linkhardt *et al.*, 1981). TGF- β exerted rapid anabolic actions on isolated myoblasts, such as an increase in amino acid transport, but had little effect on myoblast proliferation and inhibited myoblast differentiation (Massague *et al.*, 1986). When myoblasts differentiated spontaneously at high cell density the number of cellular receptors for TGF- β was greatly reduced, a process also noted for EGF and FGF receptors (Ewton *et al.*, 1988). Differentiation of tissues may therefore be associated with gross changes in growth factor responsiveness.

The myoblast model demonstrates that separate growth factors, which in the short term have similar mitogenic actions, can have opposite effects on long-term tissue phenotype, FGF blocking and IGF-I stimulating differentiation. Two observations suggest that IGFs are also involved in the differentiation of brain cell types. Firstly, when isolated cells from 1-day-old rat cerebellum were exposed to IGF-I glial cell differentiation was directed towards the production of oligodendrocytes rather than astrocytes (McMorris *et al.*, 1986). Secondly, IGF-II synergized with nerve growth factor to promote neurite outgrowth from rat sensory and sympathetic ganglia (Recio-Pinto & Ishii, 1984). IGF-I has also been reported to potentiate the differentiation of fetal rat calvaria, chondrocytes, and erythroid precursor cells.

EGF modulates the differentiation of epithelial structures in the fetus and neonate. When administered to the fetal lamb for 2 weeks in the last third of pregnancy EGF caused hypertrophy of skin epithelium and the growth of the adrenal, thyroid, liver and kidney (Thorburn *et al.*, 1981). In the fetal rabbit and lamb exogenous EGF induced lung epithelium maturation and surfactant production (Catterton *et al.*, 1979; Sundell *et al.*, 1980), while in cultures of embryonic chick skin EGF inhibited glucocorticoid-induced epidermal keratinization (Obinata *et al.*, 1987). Receptors for EGF are abundant in human placenta, especially on the microvillus plasma membranes in contact with the maternal circulation, and on the basolateral membranes adjacent to the fetal circulation (Roa *et al.*, 1984). This distribution implies an interaction between EGF and the syncytiotrophoblast, and studies with isolated trophoblasts and placental cultures have shown that EGF modulates trophoblast differentiation and function. In homogeneous cultures of term trophoblasts, EGF caused a dose-related release of human placental lactogen and chorionic gonadotrophin (Morrish *et al.*, 1987). There was no change in trophoblast cell number within these experiments but EGF induced a differentiation of cytotrophoblasts to form a syncytium. Parallel experiments with early and late placental explants yielded similar results for hormone release (Maruo *et al.*, 1987). It is not known whether the EGF that interacts with placenta *in vivo* is fetal, maternal or placental in origin. However, infusion of EGF into the fetal lamb caused an increased release of placental lactogen into the maternal circulation. EGF is abundant in milk, especially colostrum, and may contribute to the proliferation and maturation of the neonatal gut.

The cyclic nature of the female reproductive system relies on the ability of ovarian follicles to change rapidly in structure and function. While gonadotrophins are the primary initiators of follicular development, growth factors have a permissive or mediatory role in these responses during follicular growth and maturation, and also during ovulation. Granulosa cells from human and animal species have specific receptors for IGF-I, the binding capacities of which are elevated by FSH or LH and further augmented by oestrogens (Adashi *et al.*, 1988). IGF-I was reported to stimulate directly granulosa cell proliferation, but not that of thecal cells (Hernandez *et al.*, 1988). It had little effect alone on steroidogenesis by isolated rat granulosa cells but synergized with FSH to increase LH receptor number and, consequently, progesterone synthesis, aromatase activity and

oestrogen accumulation (Adashi *et al.*, 1985; Veldhuis *et al.*, 1986). The ability of IGF-I to enhance the appearance of LH receptors was augmented by oestrogens and/or thecal cell-derived androgens. In turn, IGF-I was reported to increase basal and LH-stimulated androstenedione release by thecal cells. On isolated porcine granulosa cells IGF-I increased granulosa cell differentiated function without the presence of FSH (Veldhuis *et al.*, 1986).

Thecal-interstitial cells within the follicle release TGF- β (Skinner *et al.*, 1987), which has little action on granulosa cell proliferation but, like IGF-I, synergizes with FSH to increase LH receptor number and steroidogenesis by granulosa cells (Knecht *et al.*, 1987). These actions are attenuated by FGF. A molecule which is structurally related to TGF- β is inhibin which is reported to be released from granulosa cells under FSH control and to block steroidogenesis by these cells (Hillier *et al.*, 1987; Bicsak *et al.*, 1988). The FSH-stimulated release of inhibin was potentiated by IGF-I, oestradiol and androstenedione, but blocked by EGF, or its analogue TGF- α , which is expressed by the thecal-interstitial cells (Kudlow *et al.*, 1987). EGF stimulated granulosa cell proliferation in most species examined except rat, and this action was suppressed by FSH. While some species variation exists, EGF generally blocked FSH-stimulated granulosa cell differentiation (Franchimont *et al.*, 1986). Partly pure preparations of PDGF were reported to enhance granulosa cell proliferation and differentiated function but the physiological presence of PDGF in ovary is uncertain. Peptide growth factors have been implicated in the maturation of the rat oocyte, assessed by the breakdown of nuclear germinal vesicles as a marker of meiotic re-activation. TGF- β caused an acceleration of maturation in follicle-enclosed oocytes and cumulus-oocyte complexes (Feng *et al.*, 1988). This action was probably indirect since TGF- β had no influence on cumulus-free oocytes. IGF-I and -II also stimulated oocyte maturation while PDGF and insulin had no effect.

Within the male reproductive system IGF-I and an EGF-like molecule, both released by Sertoli cells, can influence Leydig cell activity. Exogenous IGF-I increased LH/hCG binding and hCG-stimulated testosterone release by isolated rat Leydig cells (Morera *et al.*, 1987). These actions were independent of the proliferative action of IGF-I on Leydig cells.

Chemotaxis

The potent chemotactic properties of growth factors such as PDGF and TGF- β for mesenchymal tissues suggests a possible role in the cell migration of embryogenesis, but this remains unproven. However, growth factor-induced chemotaxis may be central to the cascade of paracrine interactions which accompany wound healing. Following the disruption of the vascular endothelium platelet lysis occurs within damaged tissues to release high local concentrations of PDGF, TGF- β and EGF. Additionally, damaged endothelium itself expresses the *c-sis* oncogene and may release PDGF. PDGF is a potent chemotactic agent *in vivo* for inflammatory cells such as monocytes and neutrophils, and mesenchymal cell types including fibroblasts and smooth muscle cells (Deuel *et al.*, 1987). At higher concentrations PDGF activated superoxide generation, specific granule release and mobilization of calcium stores by neutrophils and monocytes. Such mobilizing actions of PDGF on inflammatory cells are reinforced by TGF- β , which when included in wound chambers implanted into rats exerted chemoattractant properties for neutrophils, monocytes and fibroblasts (Sporn *et al.*, 1983). Monocytes and macrophages are further potential sources of PDGF, TGF- β and FGF release.

Chemoattraction may therefore lead to extremely high local concentrations of several growth factors at injury sites. When coupled with an abundant presence of IGFs derived from the circulation this may create an optimal synergistic interaction of growth factors necessary to initiate mesenchymal and epithelial proliferation and promote wound healing. In addition, TGF- β promoted the synthesis of collagens and fibronectin, creating fresh extracellular matrix (Ignatz *et al.*, 1987). Ectopic application of EGF in saliva could potentiate epithelial regeneration. Once the vascular endothelium is again intact platelet lysis will cease, the chemoattraction of inflammatory

cells will decrease and the tissue growth factor presence will return to equilibrium. Support for such a model of wound healing is provided by the ability of exogenous, PDGF, TGF- β or EGF/TGF- α to reduce wound healing time and increase the tensile strength of new tissue (Roberts *et al.*, 1986; Lynch *et al.*, 1987; Mustoe *et al.*, 1987; Schultz *et al.*, 1987).

Comment

Peptide growth factors provide an infinitely variable, fundamental level of intercellular communication influencing all aspects of cell behaviour throughout life and preceding the appearance of the endocrine system. Their sphere of influence is predominantly within each local tissue environment and this level of control, coupled with interactions between cells and extracellular matrix components, provides the fundamental drive to embryonic development. In later fetal and post-natal life peptide growth factor presence or action may become sub-ordinate to endocrine hormones. The finding of peptides analogous to IGFs, EGF and TGF- β in invertebrates suggests that growth factors represent a primaeval control system which has been retained throughout animal evolution.

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