Current topic**Fetal growth signals**

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Human fetal growth is not uniform. Tissue patterns and organ primordia are established during embryogenesis, then from the end of the first trimester and throughout the second the fetus undergoes massive hyperplasia. In the third trimester further organ modelling and functional maturation occur in preparation for extrauterine life. Each aspect of development requires orchestrated intercellular signalling at two levels. The release of peptide growth factors and the modulation of an extracellular matrix are paracrine actions that occur within cell populations and between adjacent germ layers. In contrast, endocrine hormones may stimulate growth non-specifically or promote specific maturational events. The interactions between paracrinology, endocrinology, and environmental constraints to growth during normal and abnormal fetal development have been reviewed in detail recently.^{1 2} In this commentary emphasis has been placed on new concepts of embryonic and fetal growth control.

Paracrinology

Peptide growth factors act on cell cultures in vitro to stimulate differentiation, functional activity, and chemotaxis as well as causing cell proliferation.³ Though this has led to speculation that peptide growth factors are fundamental to prenatal development, the technology and purified peptides to test the relevant hypotheses in vivo have only recently become available.

FIBROBLAST GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR β

It is now clear that at least two peptide growth factors, fibroblast growth factor and transforming growth factor β are present early in embryogenesis and are potentially concerned with germ layer separation. Fibroblast growth factor has been identified in chick embryo from day 11 of incubation and messenger ribonucleic acid (mRNA) for fibroblast growth factor has been located in the *Xenopus* blastula.⁴ Messenger RNA encoding a transforming

growth factor β like peptide is found in the vegetal pole ectoderm of the early *Xenopus* embryo and is transcribed by a maternally derived gene designated *Vgl*. An ectodermal cell line, XTC, from the metamorphosing tadpole releases a transforming growth factor β like peptide in vitro.⁵ In the 11–18 day fetal mouse transforming growth factor β can be detected in bone and connective tissue, particularly that derived from neural crest such as palate, larynx, facial mesenchyme, and teeth.⁶ Staining was most intense at sites of tissue morphogenesis affecting mesodermal and epithelial interaction such as hair follicles, teeth, and secondary palate.

Recently both fibroblast growth factor and transforming growth factor β have been shown to exert remarkable effects on embryonal morphology. Induction of mesoderm in the amphibian embryo depends on morphogens from the ectoderm of the vegetal pole diffusing to cause mesodermal development in the ectoderm of the animal pole. Fibroblast growth factor induces mesoderm in *Xenopus* animal pole ectoderm in vitro, the structures developing being mainly mesenchyme, mesothelium, and blood cells enveloped in ectoderm.⁷ Transforming growth factor β also induces mesoderm in *Xenopus* ectoderm, especially muscle, which was identified by the presence of α actin mRNA.⁵ The most potent natural source of mesoderm inducing activity is conditioned medium from *Xenopus* XTC cell cultures, and this can be blocked by transforming growth factor β antiserum. Fibroblast growth factor promotes mitosis, cell migration, invasion, and production of plasminogen activator by vascular endothelial cells, which are all necessary features of angiogenesis in vivo.⁸ It also induces the differentiation of chondrocytes, preadipocytes, astrocytes, and oligodendrocytes. Not all actions of fibroblast growth factor on tissue differentiation are augmentative, as it attenuates the differentiation and fusion of fetal myoblasts and decreases concentrations of muscle specific enzymes such as myokinase. Of particular relevance to embryogenesis is the avid association of fibroblast growth factor with heparin sulphate, a

glycosaminoglycan produced in large amounts by vascular and corneal endothelium and a structural component of their extracellular matrix.

Fibroblast growth factor may act in a novel way because its gene does not encode the conventional peptide sequence that is necessary for vesicle associated exocytosis.⁸ The peptide may leave the cell together with matrix molecules, and either react immediately with fibroblast growth factor receptors as an autocrine stimulus, or remain stored within the matrix. Since organogenesis entails cells interacting with newly formed matrix to promote stem cell growth and stabilise phenotype, it is possible that fibroblast growth factor might influence differentiation from a matrix store.

INSULIN LIKE GROWTH FACTORS

There seems to be no report of insulin like growth factor being present in embryos before separation of the germ layers. Studies with embryonal mouse carcinoma cell lines suggest that insulin like growth factor II occurs only after differentiation of the pluripotential ectodermal cells by exposure to retinoic acid to yield cells with mesodermal or endodermal phenotypes. Insulin like growth factor and epidermal growth factor may have a fundamentally different role from transforming growth factor β and fibroblast growth factor. They are expressed during and after the condensation of tissues and may influence expansion and differentiation of stem cell populations.

In the human fetus in the early second trimester, insulin like growth factors have been localised by immunohistochemistry to epithelia within the gut, kidney, and lung, and to hepatocytes, fetal zone adrenal cells, skeletal, and cardiac muscle fibres, haemopoietic cells, and dermis.⁹ This distribution may be partly due to a complex of insulin like growth factor with specific binding protein attaching avidly to certain cell membranes. The binding protein has been shown to potentiate the biological actions of insulin like growth factors *in vitro*.¹⁰ The tissue distribution of insulin like growth factor binding protein in the human fetus mirrors that of insulin like growth factor itself suggesting that the two coexist as a complex in or on certain cells.¹¹ This may reflect sequestration of insulin like growth factor, as *in situ* hybridisation studies show most insulin like growth factor mRNA to be in fibrous mesenchyme adjacent to the cell types positive for insulin like growth factor.¹²

Most insulin like growth factor mRNA in rat or human fetuses encodes insulin like growth factor II, but insulin like growth factor I mRNA is detectable in the rat embryo from day 11 and in the human fetus from late in the first trimester. In both chick

and human development insulin like growth factors are strongly associated with populations of differentiated functional cells, suggesting that their role is to support this phenotype. Both insulin like growth factors are not only mitogenic but also induce differentiation.² Insulin like growth factor I induces the fusion of myoblasts and the appearance of muscle specific enzymes in postmitotic contractile myotubes; and is 100 times more potent than insulin like growth factor II in this respect. Insulin like growth factor I augments the action of follicle stimulating hormone during differentiation of ovarian granulosa cells, leading to an increase in luteinising hormone receptors and sex steroid accumulation. Whereas insulin like growth factor I promotes the differentiation of fetal brain astrocytes into oligodendrocytes, insulin like growth factor II synergises with nerve growth factor to promote neurite outgrowth from sensory and sympathetic ganglia. Both insulin like growth factor I and II increase extracellular matrix synthesis by connective tissues, particularly chondrocytes.

Plasma insulin like growth factor I and II in the human fetus rise gradually until full term, when insulin like growth factor I, but not insulin like growth factor II correlates with birth weight. If we accept that the main action of insulin like growth factors is a local one, the biological interpretation of circulating concentrations is difficult. Nevertheless there is a sharp rise in plasma insulin like growth factor II in the fetal rat, lamb, and guinea pig that is coincident with the onset of gluconeogenesis.

EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR α

Epidermal growth factor is also associated with cell differentiation and maturation during embryogenesis. In the mouse embryo the first differentiated cell type, primary trophoectoderm, possesses epidermal growth factor receptors that may be activated by either epidermal growth factor or its analogue transforming growth factor α . Although transforming growth factor α concentration is highest in the mouse embryo at day 7, transforming growth factor α mRNA cannot be detected at this time but is abundant in the maternal decidua, especially adjacent to the embryo.¹³ Decidual expression began after implantation, peaked at day 8, then slowly declined with decidual reabsorption. Transforming growth factor α may therefore be available to the embryo from a maternal source during gastrulation and neurulation. By early in the second trimester, epidermal growth factor is found in the human fetus in the gastrointestinal tract, kidney, pituitary gland, trachea, and placenta.¹⁴

In the fetus epidermal growth factor affects the

growth, differentiation, and function of epithelial cells.² When given parenterally to the fetal lamb, it stimulates skin hypertrophy and growth of the viscera. In both the rabbit and the lamb it causes lung epithelial maturation and surfactant production. In vitro, it is mitogenic and can influence differentiation; it inhibits glucocorticoid induced keratinisation of embryonic chick skin. Receptors for epidermal growth factor are abundant in human placenta, especially on the microvillous plasma membranes facing the maternal circulation and the basolateral membranes facing the fetal circulation. It is not surprising, therefore, that studies with isolated trophoblasts or placental cultures have shown that epidermal growth factor modulates trophoblast differentiation and function. In homogenous cultures of full term trophoblasts it caused a dose related release of human placental lactogen and human chorionic gonadotrophin.¹⁵ Trophoblasts differentiated into a syncytium of cytotrophoblasts but did not multiply. It is not known if the epidermal growth factor acting on placenta in vivo is placental, or comes from elsewhere in the fetus or mother.

Endocrinology

Because endocrinology has been with us for much longer than paracrinology the role of the endocrines in fetal growth control is understood in a more balanced manner and has been the subject of regular detailed review. Conceptual changes concerning endocrine growth control are evolving more slowly than those in paracrinology and arguably the most topical facet of fetal endocrinology is placental hormone production. Otherwise most of the points made here count as fine tuning of established dogma.

Whereas fetal growth continues in the absence of hormones such as thyroxine, insulin, or the pituitary hormones (none of which cross the placenta in physiologically significant amounts), optimal growth depends on all of these. Insulin is necessary to promote anabolism, thyroid hormones stimulate neuronal maturation, and pituitary hormones are necessary for enzymic and gonadal development.

INSULIN

Insulin is detectable in the human pancreas from eight weeks', and in fetal plasma from 12 weeks' gestation but may well be present in the early embryo because mRNA for proinsulin has been identified in embryonic chick yolk sac. During embryogenesis insulin may be both a mitogen and a metabolic hormone. At physiological concentrations of 1–10 nM insulin stimulated tissue growth, metabolism, and muscle differentiation in four day

chick embryos, and stimulated the appearance of choline acetyltransferase, a differentiation marker in retinal neurones, at seven days.^{16 17} Insulin stimulated glucose oxidation in chick embryos as early as gastrulation at 18 hours, and neurulation at 24 hours.¹⁸

The role of insulin as a fetal metabolic hormone varies between species and depends as much on the ontogeny of receptor and post receptor links as on the presence of insulin itself. Despite abundant insulin receptors on full term fetal rat hepatocytes, insulin had little effect on glucose incorporation into glycogen or glycogen synthetase activity. When fetal lambs in the third trimester were placed on a glucose clamp and infused with insulin there was a 13% rise in oxygen consumption, a 106% increase in glucose uptake, and an 83% increase in glucose utilisation.¹⁹ The ability of insulin to promote fetal adiposity is well recognised clinically, and has been confirmed experimentally. Insulin infusion into the monkey fetus caused a fall in plasma free fatty acids and an increase in hepatic lipogenic enzyme activity.

Recent experiments have added to our sparse knowledge of human fetal insulin secretion. Perfusion of isolated islet clusters from fetuses of less than 16 weeks' gestation caused a small monophasic rise in insulin secretion within 30 minutes of a glucose challenge.²⁰ The response increased at 17 weeks' gestation, but only became biphasic in the perinatal period. Other secretagogues that may be more physiologically relevant to the fetus, such as amino acids, were not tested.

The mitogenic action of insulin in physiological concentrations seen during embryogenesis is retained by only a few tissues (such as liver) in later fetal life. Hypoinsulinaemia is, however, invariably associated with a reduction in circulating insulin like growth factor I irrespective of whether the availability of nutrients is also decreased, as seen after ligation of the uterine vessels in the rat,²¹ or if fetal glycaemia is maintained, as in the fetal lamb that has undergone pancreatectomy.²² Because insulin has no direct action on release of insulin like growth factor from fetal human or rat connective tissues or hepatocytes in vitro, it seems likely that the effect of insulin on insulin like growth factor expression is through stimulation of nutrient uptake and utilisation.

The idea that insulin is permissive for fetal growth is supported by the clinical evidence that fetal hyperinsulinaemia, though it causes adiposity, has little effect on lean body mass.²³ Experiments in which fetal monkeys were made chronically hyperinsulinaemic led to similar conclusions. These observations in no way detract from the impact of insulin on fetal morbidity. Hyperinsulinaemia inhibits development of surfactant, which contributes

to the increased incidence of respiratory distress syndrome in infants of diabetic mothers. Fetal polycythaemia can result in neonatal problems caused by hyperviscosity and hyperbilirubinaemia. The polycythaemia may arise from the comparative hypoxia caused by increased glucose utilisation that in turn causes increased erythropoietin synthesis or direct stimulation of erythropoiesis by insulin. Human fetal and neonatal erythroid progenitor cells depend less on erythropoietin for growth in vitro than those from adults²⁴ and insulin, in the presence of erythropoietin, stimulated late erythroid progenitor cell growth in vitro from fetal blood taken between 21 weeks' gestation and full term.²⁵

GROWTH HORMONE

Growth hormone is found in the human fetal pituitary gland and circulation from at least 10 weeks' gestation, and by mid pregnancy plasma growth hormone concentrations are at a peak of more than 200 mU/l. Despite this plethora, pituitary growth hormone seems to have little influence on fetal development, possibly because growth hormone receptors do not appear in connective tissue before birth in the mouse, rat, or sheep and not until at least the second trimester in humans. Growth hormone is without effect on human fetal muscle cell growth in vitro, but does stimulate DNA synthesis together with release of insulin like growth factor I, by isolated human fetal hepatocytes from as early as 12 weeks' gestation.^{26, 27} Immunological neutralisation of the insulin like growth factor release blocked the mitogenic effect of the growth hormone, showing an indirect mechanism of action. Growth hormone binding sites are found on hepatic cell membranes from the end of the first trimester.²⁸

Growth hormone and insulin may cooperate to augment fetal growth indirectly. Growth hormone promotes β cell replication and insulin release in pancreatic islet cultures from human fetuses of 12 to 25 weeks' gestation.²⁹ The β cell is known to contain and release insulin like growth factor I at this time, but it is not known whether insulin like growth factor I mediates the mitogenic action of growth hormone. If growth hormone interacts with islet cells in utero, the growth hormone molecule concerned might be pituitary or placental in origin.

PLACENTAL HORMONES

The human placenta contains a growth hormone like molecule which differs from pituitary growth hormone at 13 amino acids and is a product of the variant growth hormone gene, human growth hormone V that remains dormant in the pituitary. Placental growth hormone is secreted copiously into the maternal circulation towards full term, but

disappears within one hour of delivery.³⁰ Attempts to measure placental growth hormone in the fetus have been bedevilled by the abundance of pituitary growth hormone, and it is not yet appropriate to speculate on its role in fetal life.

Human placental lactogen has 85% homology in amino acid sequence with growth hormone and is released into both maternal and fetal compartments. A role for human placental lactogen in fetal development seemed unlikely because of its low somatotrophic activity in classical growth hormone bioassays, such as tibial growth in the rat after hypophysectomy. Such test systems may be inappropriate because they measure the interaction of human placental lactogen and growth hormone receptors, and take no account of the possibility of specific human placental lactogen receptors in fetal tissues.

Random measurements of fetal serum human placental lactogen in the second trimester are between 50 and 200 ng/ml. Human placental lactogen in this concentration range promotes DNA synthesis and cellular anabolism in isolated human connective tissues and hepatocytes.^{26, 27} The actions on DNA synthesis were mediated by a paracrine release of insulin like growth factor peptides. Specific binding sites for human placental lactogen are present on cell membranes from second trimester fetal liver and skeletal muscle, and receptor numbers increase with body weight between 12 and 19 weeks' gestation.²⁸ Although the mechanisms for human placental lactogen to potentiate fetal growth are there, its role in utero remains uncertain because reports exist of healthy term infants born to women with placentas lacking the two human placental lactogen genes. In these circumstances other related peptides, such as placental growth hormone, may play a compensatory part.

Comment

Fetal growth is coordinated by spatial and temporal interactions of cell populations with extracellular matrix components and peptide growth factors. In the early embryo the main prerequisites for success are adequate nutrition and gaseous exchange. By the end of the first trimester a complex signalling network has developed, in which fetal and placental hormones interact with tissue growth factors. Hormones act in two ways: they may stimulate growth diffusely or they may act specifically to induce cellular maturation for extrauterine life. What is less clear is the degree of interdependence of endocrine and paracrine pathways. Postnatally they can be hierarchical, as in the control of insulin like growth factor expression by growth hormone,

or synergistic as in the interaction between follicle stimulating hormone and insulin like growth factor I during granulosa cell differentiation.

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