Blastocyst implantation precedes induction of insulin-like growth factor II gene expression in human trophoblasts

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Summary

The cytotrophoblast cell population of the human embryonic conceptus proliferates rapidly during the first month following blastocyst implantation. Since the trophectoderm lineage is established in preimplantation morula/blastocysts, the scenario underlying initiation and maintenance of the rapid proliferative phenotype of cytotrophoblasts is a central issue. The insulin-like growth factor II (IGF-II) gene is highly expressed in proliferative cytotrophoblasts of first trimester placenta and performs as a placenta growth factor. To establish a temporal correlation between IGF-II expression and initiation of highly proliferative trophoblasts in human development, we employed in situ hybridization analysis of the expression of the IGF-II and human chorionic gonadotropin β-subunit (β-HCG) genes in human pre- and postimplantation development. The data show that the appearance of high steady-state levels of IGF-II transcripts in trophoblasts is a postimplantation event, whereas β-HCG transcripts can already be detected in preimplantation development. This observation makes a role for endogenously produced IGF-II in the normal development of preimplantation embryos unlikely, but suggests that endogenously produced IGF-II participates in the formation and subsequent expansion of the rapid proliferative phenotype of the trophoblastic shell, following implantation.

Key words: in situ hybridization, trophoblastic shell, cell proliferation.

Introduction

In order to establish the future placental bed, the trophoblastic shell structures of early human embryonic conceptus displays both highly invasive and proliferative properties (Langman, 1969; Hamilton & Hamilton, 1977; Beaconsfield et al. 1980; Ohlsson et al. 1989a). The term ‘pseudomalignant’ has been coined to describe these and other tumour-like properties, which appear in the absence of circulating embryonic blood during the histiotrophic phase (Beaconsfield et al. 1980; Ohlsson et al. 1989a). The rapid expansion of cytotrophoblastic cell populations is retarded, however, later in the placental development (Langman, 1969; Hamilton & Hamilton, 1977). A few questions, which are pertinent to central issues in developmental biology, are readily apparent: for instance, what are the underlying mechanisms for the maintenance of the highly proliferative phase of the cytotrophoblasts during early human embryogenesis; how is it regulated?

The endogenous production of growth factors to establish autocrine/short-range paracrine stimulatory loops has been invoked to explain the ‘pseudomalignant’ properties of the early human placenta (Scott et al. 1983; Pfeifer-Ohlsson et al. 1984; Goustin et al. 1985; Ohlsson & Pfeifer-Ohlsson, 1986, 1987; Schofield & Tate, 1987; Ohlsson et al. 1989b). We have previously provided evidence to show that the platelet-derived growth factor B (PDGF-B) and PDGF-B receptor genes are transiently coexpressed and potentially contribute to the proliferative properties of the human cytotrophoblasts in trophoblastic shell structures (Goustin et al. 1985; Ohlsson & Pfeifer-Ohlsson, 1986; Holmgren et al. unpublished observation). It is clear, however, that the spatiotemporal patterns of expression for PDGF-B and its receptor are segregated; proliferat-
ive cytotrophoblast express the highest levels of PDGF-B mRNA while cells of the mesenchymal stroma express highest levels of PDGF-B receptor mRNA (Holmgren et al. unpublished data). A similar reciprocity in the expression patterns for the ligand and its receptor has been shown for IGF-II: the most proliferative cytotrophoblasts express the highest levels of IGF-II mRNA but little of type 1 or type 2 receptor mRNA (Ohlsson et al. 1989b). In contrast, villous cytotrophoblasts express little IGF-II transcripts, but express significant amounts of type 1 and 2 receptor mRNAs (Ohlsson et al. 1989b). As a direct role for IGF-II in the proliferative cytotrophoblastic phenotype has been envisaged in first trimester pregnancies (Ohlsson et al. 1989b), the relative positioning of the cells producing the ligand and target cells with high affinity receptors will ultimately control short-range stimulatory loops. It is therefore essential to establish the spatiotemporal timing of IGF-II gene expression.

We now address whether a role for IGF-II in the development of the extraembryonic tissue in particular, and embryogenesis in general, is a postimplantation event. In the end, such information bears upon the changes in inductive microenvironments as well as the identity of inductive signals that accompany the process of blastocyst implantation.

Materials and methods

Histological specimens

Human postimplantation conceptuses were provided as thin (4 μm) sections of paraffin-embedded and formalin-fixed patient material. These samples have been kept for a minimum of 4 years and were recovered from routine curettage. The two samples shown in this report were judged to be 12 and 20 days postconception, based upon histology and patient information (refer to text). Human preimplantation morulae/blastocysts were obtained as spare specimens from in vitro fertilization treatments (Wranby et al. 1987) at the department of Gynecology of Malmö University Hospital. Only a small fraction of such samples could be recovered as morula/blastocysts from in vitro fertilization for 5 to 7 days (Wranby et al. 1987). To ascertain a generality in the conclusions drawn, we have analyzed three different murula/blastocyst preparations, which were developed on three different occasions. In addition, three different placental samples with a well-developed trophoblastic shell, dated 20, 24 and 28 days postconception (on account of morphology and patient history), were simultaneously analyzed with the morula/blastocyst samples. Only one specimen, dated 12 ±2 days postconception, covered the period between implantation and 20 days postconception.

In situ hybridization analysis

Antisense and sense riboprobes were generated from pIGF2RW and pHCGB3 plasmids, using SP6 and T7 polymerases (Promega Biotec) and [32P]-UTP (Amersham). The pHCGβ3 and IGFIIRW plasmids contain the pGEM3 vector (Promega Biotec) and the inserts of 0-579 kb (HindIII/HindIII) and 0-581 kb (HinHI/PstI), respectively. These two probes were selected to cover coding regions only (Ohlsson et al. 1989b; Fiddes & Talmadge, 1984). The specific radioactivity of the different template-free probes were essentially identical (approximately 350 Ci mmol–1). The hybridization of these probes to thin tissue sections followed previously published protocols (Pfeifer-Ohlsson et al. 1984; Goustin et al. 1985; Ohlsson et al. 1989b). Following removal of excess probe, the tissue sections were exposed to stripping film (Kodak AR10) and counterstained by water-based hematoxylin to visualize hybridization signal and tissue morphology.

Results

The spatial distribution of active IGF-II genes to the proliferative cytotrophoblasts, in particular, of the trophoblastic shell structure, supports the contention that the role of IGF-II in early development is correlated with trophoblastic shell formation/maintenance (Ohlsson et al. 1989b). Therefore, we have applied in situ hybridization analysis to determine the spatial pattern of IGF-II gene expression during early human development. For comparison, we found the β-HCG mRNA level to be a suitable internal marker since β-HCG transcripts are found in both trophoblast cell types (primarily the syncytiotrophoblasts) throughout pregnancy (Hoshina et al. 1983; Fischel et al. 1984). Moreover, HCG production and β-HCG mRNA have been detected in preimplantation human conceptuses (Fischel et al. 1984; Bonduelle et al. 1988). To compare directly the spatial patterns of the different gene transcripts in thin sections of human pre- and postimplantation conceptuses, we performed simultaneous analysis in adjacent sections, with essentially identical input of radioactive riboprobes of identical specific activity (Fig. 1). In Fig. 1 C, no silver grains are detectable above background when a 35S-labeled IGF-II riboprobe was hybridized to formalin-fixed early human morula/blastocysts, fertilized and maintained in vitro. On the other hand, the trophectoderm in the adjacent section of the same blastocyst displays high steady-state levels of both α- and β-HCG mRNA (Fig. 1 A and B). Since both gene products have high steady-state mRNA levels in trophoblasts of the cytotrophoblastic shell from placenta dated 20 days postconception (Pfeifer-Ohlsson et al. 1984; Goustin et al. 1985; Pfeifer-Ohlsson et al. 1985) (Fig. 1 J and K), we deduced that induction of high steady-state levels of IGF-II mRNA, in contrast to β-HCG mRNA, is a postimplantation event. To substantiate this deduction further, we analyzed the spatial expression of both genes in an early postimplantation conceptus (Fig. 1 D). In this sample, which was estimated to be 12 (±2) days postconception based upon parameters such as lacunar formation, lack of trophoblastic shell structure, breakdown of maternal blood sinuses, over-all size of the implanted conceptus and patient history, we found generally high levels of the β-HCG mRNA (Fig. 1 E–G). Conversely, only single cells of an adjacent section display a potential IGF-II signal over background (Fig. 1 H). To rule out that the clinical samples have been subjected to substantial variation during experimental handling, we have analyzed three different murula/blastocysts for spatiotemporal expression of the two genes, with the same qualitative information reported above (Fig. 1)
Fig. 1. Analysis of spatiotemporal pattern of IGF-II and β-HCG gene expression in the trophoderm of human pre- and postimplantation conceptuses. A–C are serial sections of a human morula/blastocyst, which have been subjected to simultaneous in situ hybridization analysis of expression of α-HCG, β-HCG and IGF-2 genes, respectively. An early human conceptus, which has been dated 12 ± 2 days post-conception, was similarly analyzed. An overall light-microscopic view of extraembryonic tissue is shown in D. The boxed areas in D were magnified by dark-field- or bright-field light microscopy to show expression of β-HCG in both mononuclear and multinuclear trophoblasts (E–G). An adjacent section shows potential IGF-II expression (arrows) in single cells. Dark-field views depict the exclusive expression of IGF-II in part of the trophoblastic shell (ts) and β-HCG in villous syncytiotrophoblasts (vs) in J and K, respectively, in a placenta which has been dated 20 days postconception (I shows an over-all light-microscopic view of this sample). Magnifications A–C, G & H, ×343; D, I–K, ×65; E, F, ×163.
and data not shown). Moreover, we have subjected at least 15 different placenta, including patient material dated 12, 20, 25 and 28 days postconception, as well as term placenta, to in situ hybridization analysis. These results consistently reveal very high steady-state levels of IGF-II mRNA in trophoblastic shell from early placenta (earlier than 5 weeks postconception) and in columnar trophoblasts during the whole first trimester pregnancy (Fig. 1 and data not shown) (Ohlsson et al. 1989b).

Discussion

In this study, we have focussed upon the spatiotemporal pattern of IGF-II expression, in order to establish a potential correlation to the initiation of the highly proliferative phenotype of cytotrophobasts and to determine whether IGF-II functions were restricted to postimplantation development. With the approximation that the single sample of 12 ± 2 days postconception represents a proper developmental stage for the period around 7 (implantation) and 20 days (earliest available placental sample with well-developed trophoblastic shell) postconception, we conclude that induction of high IGF-II mRNA steady-state levels in human trophoblasts is a postimplantation event, which correlates with the formation and/or expansion of cytotrophoblastic shell. Our studies have not, however, focussed upon all aspects of preimplantation development. It remains to be established, therefore, whether the maternal or zygotic IGF-II gene is transiently expressed in early cleavages of fertilized eggs or IGF-II expression commences only in association with the laying down of the proliferative trophoblast lineage. In addition, because of the sensitivity of the in situ hybridization technique, we cannot rule out low steady-state levels of IGF-II gene expression in preimplantation embryos. The transcription of the β-HCG gene in mononuclear cells of preimplantation embryos (Fig. 1 B) must represent one of the earliest inductive events in human embryogenesis, since a subset of human embryonic genes appears to be activated initially in the 4- to 8-cell stage of in vitro fertilized embryos (Braude et al. 1988). Similar observations have been reported in an accompanying contribution (Brice et al. 1989).

One could argue that the activation of growth factor genes and the formation of confined tissue compartments goes hand in hand (to eventually establish high local concentrations of growth factors, which interact with target cells in specified positions). Such a reasoning appears particularly attractive in explaining the formation of the trophoblastic shell, where the controlled diffusion of endogenous growth-promoting factors in a confined compartment should govern proliferation, in the absence of embryonic blood circulation. In this context, a role for growth factors and autostimulatory loops in governing the development of mammalian preimplantation embryos is less obvious. The recent demonstration, however, that a number of different growth factors, like PDGF-A and TGFα, are expressed in mouse preimplantation embryos (Rappolee et al. 1984) suggests that endogenous growth factors may play a role in the early cleavage pattern of fertilized eggs. Although the functional involvement of these factors in preimplantation development remains to be established, it is interesting to learn that difficulties in the creation of mice strains, transgenic with IGF-II, relates to failure of in vitro fertilized embryo to develop beyond the 4- to 8-cell stage (Davison et al. personal communication). Following removal of the coding information in IGF-II mRNA (through a point mutation), the transgenic embryos would readily implant, and express the transgene (Davison et al. personal communication). Since IGF-I and IGF-II interact with both type 1 and 2 receptors, albeit with differential affinities (Hall & Sara, 1983), it is noteworthy that the problems encountered with preimplantation embryos, transgenic with IGF-II, have not been reported with the successful establishment of mice strains transgenic with an IGF-I construct (Mathews et al. 1988). Whether the use of different promoters (the IGF-II promoter for the IGF-II transgenes and the metallothein promoter for the IGF-I transgenes) or the differential expression of type 1 or 2 IGF receptors could explain the discrepancy in these two cases, remains to be established. Nonetheless, this and other reports document regulatory roles of IGF-II that necessitate tight developmental control of the spatiotemporal pattern of expression during normal pre- and postimplantation human development.

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References


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