Temporal changes in the expression of the insulin-like growth factor II gene associated with tissue maturation in the human fetus

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Summary

The insulin-like growth factors are broadly distributed in the human conceptus and are thought to play a role in the growth and differentiation of tissues during development. Using in situ hybridization we have shown that a wide variety of specific cell types within tissues express the gene for insulin-like growth factor II at times of development from 18 days to 14 weeks of gestation. Examination of blastocysts produced by in vitro fertilization showed no expression, thus bracketing the time of first accumulation of IGF-II mRNA to between 5 and 18 days postfertilization. The pattern of IGF-II expression shows specific age-related differences in different tissues. In the kidney, for example, expression is found in the cells of the metanephric blastema which is dramatically reduced as the blastema differentiates. The reverse is also seen, and we have noted an increase in expression of IGF-II in the cytotrophoblast layer of the placenta with gestational age. The sites of expression do not correlate with areas of either high mitotic activity or specific types of differentiation, but the observed pattern of expression in the kidney, adrenal glands and liver suggests an explanation for the abnormally high IGF-II mRNA expression in developmental tumours such as Wilms' tumour.

Key words: insulin-like growth factor II, somatomedins, human fetus, organogenesis.

Introduction

The somatomedins, or insulin-like growth factors, are small peptide hormones with a relative molecular mass of approximately 7x10^3, which exert a wide range of pleiotropic effects on many cell types (Froesch et al. 1985). These effects include processes essential to normal fetal growth, such as the promotion of cellular differentiation and maturation (e.g. Schmidt et al. 1983, and see Hill et al. (1987) for review) and the stimulation of growth and cell division (Biddle et al. 1988; Adams et al. 1983; Nagarajan et al. 1982; Hill et al. 1986; Hiraki et al. 1986; Mattson et al. 1986; Strain et al. 1987; Vetter et al. 1986). Evidence that both IGF-I and IGF-II might be important to embryogenesis has recently been accumulating. On an organ basis, messenger RNA for both IGF-I and IGF-II is widely distributed in the first and second trimester human fetus (Scott et al. 1985; Schofield & Tate, 1987; Han et al. 1987; Gray et al. 1987; de Pagter-Holthuizen et al. 1987) and IGF-II is similarly distributed in the rat and mouse (Brown et al. 1986; Beck et al. 1987; Smith et al. 1987), as demonstrated both by Northern blotting and in situ hybridization. Further, tissues explanted from second trimester fetuses retain the ability to synthesize and secrete IGFs both in organ culture and as primary cell cultures (Hill et al. 1985). The demonstration of functional receptors for IGFs in these tissues (Hill et al. 1986; Vetter et al. 1986; Weidmann & Bala, 1980) strongly suggests that the mode of action of these growth factors in the fetus might be autocrine or paracrine (Hill et al. 1987).

Levels of mRNA and serum IGFs have been measured throughout rat gestation, and between 6 and 26 weeks of gestation in the human (Ashton et al. 1985; D'Ercole et al. 1986; Sara & Hall, 1984). Whilst IGF-II mRNA and protein are abundant in both rat and human fetuses over this period, there is a marked interspecific difference in that IGF-II becomes almost undetectable by a few weeks after birth in the rat (Lund et al. 1986), whereas, in the human, serum levels continue to rise postpartum (Enberg & Hall, 1984).

The cellular distribution of IGF-II gene expression has been investigated using in situ hybridization, in late (16–20 weeks postfertilization) human fetal tissues
(Han et al. 1987) and in rat tissues (Beck et al. 1987, 1988a) through gestation, and the results have demonstrated that, whilst there are similarities in the pattern of expression in the two species, there are also differences which might be attributable to species-specific features of organogenesis and tissue maturation.

The tissues expressing the IGF-II gene at its highest level in the late second trimester human fetus are connective tissues and fibroblasts. Immunocytochemistry carried out on slightly earlier (12–16 weeks) human material (Han et al. 1988) suggested that the cell types expressing mRNA were not always identical with those that contained the protein and, in order to explain this discrepancy, the authors proposed that the immunoreactive cells were accumulating IGF secreted by neighbouring cells. An alternative explanation would be that IGF-II gene expression is elevated earlier in development and decreases with gestational age. Consequently we undertook a study, using as wide a range of stages of gestation as was available, to see if there might be critical, time-dependant changes occurring in the course of tissue maturation.

Tissue-specific temporal changes in the pattern of IGF-II expression have already been described by Beck et al. (1988a), who showed that IGF-II mRNAs are lost from the liver 18–20 days postpartum in the rat, and from all other visceral organs before birth. A reduction in IGF-II mRNA between 13 and 26 weeks of gestation has also been reported for the human fetal testis (Voutilainen & Miller, 1988).

We report here that IGF-II mRNA accumulation begins between the blastocyst and 18 days of development and that at least in the kidney and adrenal glands there is a reduction in the expression of IGF-II in maturing tissue. This suggests that the expression of IGF-II mRNA is suppressed in a developmentally regulated fashion in mature tissues.

Materials and methods

Fetal tissues

First trimester fetal tissues were collected and processed, and the age estimated as previously described by Hopkins et al. (1987). Samples of trophoblast up to 40 days of gestation were obtained from medical terminations of pregnancy using a combination of mifepristone and prostaglandin E1 (Cervagen) as described in Rodger & Baird (1987). Second trimester tissues were collected and processed, and the age estimated as previously described by Hopkins et al. (1987). Fresh tissues were normally fixed for 12 h in 4% paraformaldehyde in Ca2+-, Mg2+-free phosphate-buffered saline and cryostat sectioned as previously described (Hopkins et al. 1987). Blastocysts were fixed for five minutes in the same solution and then either embedded in a small piece of mouse uterus to facilitate handling or in a small block of molten agarose as described by Bonduelle et al. (1988). Subsequently they were set in wax and cut into 5 micron sections onto slides subbed with TESPA (3-aminopropyltriethoxy silane (Sigma, Poole Dorset (Rentrop et al. 1986))).

In situ hybridization

In situ hybridization was carried out essentially according to Holland as described in Hopkins et al. (1987), with the following modifications. (a) Hybridization was carried out at 52°C to minimize background, and b) during the posthybridization washes the 50% formamide/1x salts/10mM-DTT overnight wash was reduced to 4h. This was found to give a reasonable background and reduced the loss of sections from the slides. Grain counting and photography were carried out as described in Hopkins et al. (1987).

Probes

The hybridization probes were transcripts produced by T3 or T7 RNA polymerase transcription from the HinfI–SalI fragment of pHGF-II (Bell et al. 1984), cloned into pGEM3, in the presence of 35S-UTP (1000–1500Ci:mmol−1, NEN, DuPont UK). The human chorionic gonadotropin (hCG) β subunit probe, (the kind gift of J. Fiddes, Calbiotech, CA USA) has been previously described. (Bonduelle et al. 1988; Fiddes & Goodman, 1980).

Filter hybridization

RNA was prepared from first trimester fetal tissues by the guanidinium/caesium chloride method and blotted onto nitrocellulose as previously described (Hydahl et al. 1986). Filters were hybridized with either sense or antisense probes transcribed in the presence of 35P-GTP (Specific activity 800Ci:mmol−1, NEN DuPont, UK) according to Krumlauf et al. (1987), and washed to a final stringency of 50°C in 2×SSC after RNase treatment with 2μg/ml−1 RNAse A in 2×SSC at 37°C. The RNAse treatment was essential to ensure low background in the filter hybridized with the control probe.

Results

Probe specificity

The region of the IGF-II gene used to detect IGF-II mRNA covered the HinfI–SalI region of pHGF-II, corresponding to a region of the propeptide covering all of the B and some of the C peptide (de Pagter-Holthuizen et al. 1987). Consequently this probe will detect mRNA that has the potential to code for functional IGF peptide. The position of alternative splicing within the peptide previously noted by us is contained within this probe. However, as pHGF-II does not have the inserted sequence and the sequence difference is only 12 nucleotides, we do not consider that we are preferentially detecting one alternatively spliced form over the other. Use of plus and minus strands in hybridization to Northern blots of fetal liver demonstrate that the RNA probe specifically detects...
IGF-II and the sense probe does not cross hybridize to other mRNAs (Fig. 1A).

Use of the probe on Northern blot filters with RNA derived from a range of first trimester tissues demonstrated that it detects the mRNAs previously reported (Fig. 1B), at levels quantitatively similar to those reported by ourselves and others (Scott et al. 1985; Schofield & Tate 1987; de Pagter-Holthuizen et al. 1987).

**Cellular distribution of IGF-II coding mRNAs**

**Extraembryonic membranes**

*In situ* hybridization carried out on sections of three expanded blastocysts revealed no detectable hybridization to either trophectoderm or inner cell mass (Fig. 2H). Control hybridizations carried out with a probe to the β subunit of human chorionic gonadotropin showed a strong hybridization signal localized over the trophectoderm (Fig. 2E). We consequently place the time of appearance of IGF-II expression at the time between blastocyst and 18 days of development, when expression is first visible in the Langhans layer of trophoblastic villi and the cytotrophoblast layer of the amniotic plate (Fig. 2B). Similar data are reported in the accompanying paper by Ohlsson et al. (1989).

Comparison of grain densities between 18-, 30- and 69-day-old placentas showed an increase of specific grain density in the proliferating cytotrophoblast layer, doubling between 18 and 69 days. (Figs 4A–C, 5, and Table 2). Over the same period the grain density over the syncytiotrophoblast also increased slightly but this apparent increase may be due to the spread of labelling from the cytotrophoblast in this region. No expression was detected in the mesenchyme, Hofbauer cells, or in fetal blood present in the sections.

Control hybridization with a probe from the β subunit of human chorionic gonadotropin routinely localized to the trophectoderm of the blastocysts and to the syncytiotrophoblast (Fig. 2E, K). The expression in the syncytiotrophoblast at 30 days postfertilization (dpf) was patchy and can be seen in the lower power magnification in Fig. 2. Distinct boundaries between expressing and nonexpressing cells in the same villus were observed at high power. Later material (55 days and onwards) expressed the RNA much more evenly.

Grain densities above background could be detected in all the individual yolk sacs hybridized with the IGF-II probe, with a slight preponderance over the mesodermal cell layer. However, this was not marked, and was similar in all the samples studied. No hybridization could be detected to the small haematopoietic islands in the yolk sacs.

**Kidney**

The major site of expression of IGF-II was located in the metanephric blastema, levels being highest at the earliest stages studied (6 weeks). The epithelial component of the blastema, composed of nephrogenic tubules, showed 70% lower labelling than the blastema at this time, but expression could be detected throughout the nephric mesenchyme and collecting tubules (Fig. 3G, H; Table 1). As nephrogenesis proceeds, with increasing gestational age, mature glomeruli can be seen along the periphery of the organ. The blastema at 15 weeks is much more narrow and is associated with approximately 50% less grains than at ten weeks (Fig. 5C, D). Concomitant with the decrease in labelling in the blastema region is a decrease in the labelling of the rest of the nephric mesenchyme. At these stages all epithelia are labelled to the same extent as mesenchyme, while collecting ducts show higher grain densities.

**Adrenal glands**

Whilst both the fetal and definitive cortex of the adrenal express IGF-II, grain counting demonstrated a near twofold increased level in the fetal cortex (Table 1). Between 70 and 80% of fetal cortical cells are heavily labelled (see Fig. 3K, L), the remainder being weakly labelled. As maturation proceeds and the definitive cortex increases in size, so the pattern of lower labelling associated with it contributes more to the overall pattern. Medullary invasion is only just beginning at the end of this study, and where small medullary islands can be seen they are negative. The adrenal capsule, which is thicker and more obvious by 14 weeks, is always devoid.
Fig. 2. Expression of IGF-II in the blastocyst and early trophoblast. (A) 30 days postfertilization trophoblast (s; Syncytiotrophoblast, c; Cytotrophoblast); Phase contrast. (B) Same section as (A) under dark-field illumination hybridized to an antisense probe from IGF-II. (C) Adjacent section hybridized with 'sense' probe from IGF-II; (D) 5 days postfertilization human blastocyst, light haematoxylin and eosin stain; (E) same section as (D) under dark-field illumination, βHCG antisense probe; (F) adjacent section of same blastocyst, hybridized with the sense strand of βHCG; (G) 6 days postfertilization human blastocyst, (light haematoxylin and eosin). This particular specimen was embedded in a small piece of mouse uterus visible in the bottom of the frame. (H) Dark field of the same section with antisense IGF-II probe; (I) serial section with sense probe for IGF-II; (J) 30 days postfertilization trophoblast; (K) same section hybridized with antisense probe to βHCG; (L) adjacent section hybridized with sense probe to βHCG. Scale bars are for panels A, B, C, 70 μm; D, E, F, 20 μm; G, H, I, 20 μm; and J, K, L, 50 μm.
Table 1. Variation in labelling between different cell types within an organ*^†

<table>
<thead>
<tr>
<th>Organ cell type</th>
<th>Probe for message (antisense)</th>
<th>Probe for control (sense)</th>
<th>Difference (message-control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 days Blasema</td>
<td>40.82 ± 2.16</td>
<td>6.82 ± 0.38</td>
<td>34.00</td>
</tr>
<tr>
<td>T. epithelium</td>
<td>15.95 ± 0.56</td>
<td>6.06 ± 0.51</td>
<td>9.91</td>
</tr>
<tr>
<td>Mesenchyme</td>
<td>12.31 ± 0.37</td>
<td>4.68 ± 0.28</td>
<td>15.32</td>
</tr>
<tr>
<td>105 days Blasema</td>
<td>31.06 ± 0.49</td>
<td>14.32 ± 1.31</td>
<td>16.74</td>
</tr>
<tr>
<td>T. epithelium</td>
<td>20.67 ± 0.69</td>
<td>11.89 ± 1.29</td>
<td>8.78</td>
</tr>
<tr>
<td>Mesenchyme</td>
<td>12.31 ± 0.37</td>
<td>8.60 ± 0.93</td>
<td>3.71</td>
</tr>
<tr>
<td>Adrenal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57 days D. cortex</td>
<td>43.42 ± 0.77</td>
<td>6.50 ± 0.32</td>
<td>36.92</td>
</tr>
<tr>
<td>Fetal cortex</td>
<td>67.24 ± 4.24</td>
<td>5.50 ± 0.27</td>
<td>61.74</td>
</tr>
<tr>
<td>105 days D. cortex</td>
<td>46.13 ± 1.80</td>
<td>16.74 ± 0.27</td>
<td>29.39</td>
</tr>
<tr>
<td>Fetal cortex</td>
<td>63.68 ± 3.35</td>
<td>14.11 ± 0.44</td>
<td>49.57</td>
</tr>
</tbody>
</table>

* Values are X ± s.e.m. where X is the average number of grains per square.
† All organs are 3 day exposures.
Six different transects were counted in each of three sections for both experimental and control, D, definitive; T, tubule.
Grain counting was carried out as described in the legend to Fig. 5.
Statistical analysis by Student's t-test demonstrated that the decrease in the labelling of kidney blastema, mesenchyme, and both fetal and definitive adrenal cortices was highly significant (P<0.01). The changes seen in the kidney tubule labelling were not significant.

of label. Quantification of grain density in the fetal and definitive cortices suggested that the definitive cortex maintains a 40–60% lower grain density than the fetal cortex, and that the level of expression in both components dropped slightly and by a similar amount between the time points counted.

Liver
The majority of cells in the liver, in all ages studied, expressed mRNA for IGF-II. This included the parenchyma, hepatocytes and haematopoietic tissue. In livers of both ages, the vasculature was uniformly negative as were erythroid precursor cells undergoing diapedesis (Fig. 3A, B, and 3E, F). In the second trimester, the hepatic portal tracts were much better developed and both these and the mesenchyme associated with them were negative. Our impression was that levels of expression did not change over the time period studied.

Lungs
Weak labelling was detected over the airway epithelia at stages up to 12 weeks, with no labelling over the connective tissue of the pulmonary parenchyma (Fig. 3I, J).

Skin
Dermal fibroblasts expressed levels of IGF-II mRNA above background, but the major site, seen uniformly in limb and trunk skin was in the stratum germinativum and to a lesser extent in the cells in the immediate outside of this layer – the periderm (Fig. 4B, D). Little change was seen between 56 and 84 days. Most connective tissue and mesenchyme was seen to be labelled at a significant but very low level throughout the time period examined, and it is possible that this basal level of expression may account for the bulk of the mRNA in 16- to 20-week fetuses (Han et al. 1987).

Musculoskeletal system
The highest labelling density observed was in the perichondrium, particularly in primary ossification centres where the perichondrium is developing osteogenic potential. In regions where ossification was beginning in older material, osteoblasts were labelled to a similar extent and the peristeum maintained its level of labelling throughout the period examined, up to 14 weeks. Defined epiphyseal growth plates are not visible before this stage of development. Striated muscle showed above background labelling, and this was no more marked in fusing myoblasts than in mature muscle fibres.

Gut
Weak labelling of the prospective submucosa of the intestine was observed at all stages, although it is unclear from our material at what level sections were examined, histology suggests the midgut, or small intestine. The smooth muscle layers and connective tissue sheath are unlabelled in all sections.

Vascular system
Major blood vessels could be seen in several sections, notably in the liver. These showed no labelling of either the smooth muscle component or the endothelial cells lining the lumen.

Central nervous system
No significant labelling of the central nervous system was ever detected, although samples did cover serial sections throughout the tissue.

Discussion
The overall pattern of labelling we observe differs
Fig. 3. Expression of IGF-II mRNA in human fetal tissues. All sections show the results of hybridization to an antisense probe derived from pGEM IGF-II photographed under phase contrast and dark-field illumination. In some cases the phase image is of a section lightly stained in haematoxylin and eosin to enhance the definition. (A,B) 57-day-old fetal liver. P, parenchymal cells; b, immature erythrocytes. Bright- and dark-field of antisense IGF-II hybridization, respectively. Small case letters mark the same feature in each field. (E,F) The same liver showing part of the hepatic portal tract with blood (b), parenchyma, (p) and mesenchyme, (I,J) fetal lung aged 55 days. Phase image. a, airway epithelium; p, parenchyma. (C,D) 15-week-old kidney phase image. g, glomerulus; (G H) 10-week-old kidney, e, tubular epithelium; b, metanephric blastema; (K,L) 57-day-old adrenal gland; f, fetal cortex; d, definitive cortex. Scale bars are for A and B, 20 μm and for all other panels 50 μm.
markedly from that described by Han et al. (1987), who found expression mainly in connective tissues and organ capsules. Our data suggest that the reason for this is the temporal dynamics of the pattern as Han et al. (1987) used much older fetuses than we, at 16–20 weeks, and that with increasing maturation levels of IGF-II mRNA decrease in several tissues. This is probably most clear in the case of the adrenal cortex and kidney where we have demonstrated that the decrease in expression of IGF-II mRNA is associated with the appearance in that tissue of a particular differentiated cell type. That such expression is not seen by Han et al. (1987) in older tissues suggests that this process continues in later development until the level of mRNA becomes that appropriate to adult tissues (Scott et al. 1985; Gray et al. 1987). Similar maturation-related suppression of IGF-II gene expression has also been reported at around the time of birth in the rat, being especially apparent in the liver (Beck et al. 1988a), and in the human fetal testis (Voutilainen & Miller, 1988).

Table 2. Variation in labelling between different cell types within the trophoblast of 18, 30 and 55 days postfertilization human embryos*†

<table>
<thead>
<tr>
<th>Age</th>
<th>Cell type</th>
<th>Probe for message (anti-sense)</th>
<th>Probe for control (sense)</th>
<th>Difference (message-control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 days</td>
<td>Syncytio.</td>
<td>2-50 ± 0-08</td>
<td>1-25 ± 0-18</td>
<td>1-25</td>
</tr>
<tr>
<td></td>
<td>Cyto.</td>
<td>6-36 ± 0-42</td>
<td>1-39 ± 0-13</td>
<td>4-97</td>
</tr>
<tr>
<td>30 days</td>
<td>Syncytio.</td>
<td>3-72 ± 0-14</td>
<td>1-78 ± 0-16</td>
<td>1-94</td>
</tr>
<tr>
<td></td>
<td>Cyto.</td>
<td>12-42 ± 0-58</td>
<td>2-14 ± 0-21</td>
<td>10-28</td>
</tr>
<tr>
<td>55 days</td>
<td>Syncytio.</td>
<td>4-31 ± 0-81</td>
<td>1-28 ± 0-29</td>
<td>3-03</td>
</tr>
<tr>
<td></td>
<td>Cyto.</td>
<td>29-97 ± 1-63</td>
<td>1-50 ± 0-30</td>
<td>28-47</td>
</tr>
</tbody>
</table>

* Values are X ± s.e.m. where X is the average number of grains per square.
† All ages are 3 day exposures.
Syncytio =syncytiotrophoblast.
Cyto =cytotrophoblast.

Grain counting was carried out as described in the legend to Fig. 5. Statistical analysis using Student’s t-test showed that the level of labelling of the cytotrophoblast increased significantly (P < 0.01) between each gestational age, whereas the changes in syncytiotrophoblast labelling were not significant.
Fig. 5. Distribution of IGF-II mRNA as determined by grain counting of autoradiographs produced from *in situ* hybridization of first and second trimester tissues. (A) Adrenal, (B) Kidney and (C) Trophoblast. *In situ* hybridization to 7 μm cryostat sections was carried out exactly as described in Materials and methods. Autoradiographic grains were counted in the emulsion which was over and adjacent to parts of flat tissue sections lying in a background of even grain density. A graticule of squares was placed in a ×8 eyepiece and with a ×40 phase objective, it was possible to count grains in 17×68 μm contiguous rectangles (transects) which ran in a straight path from views of the slide alone into views of the tissue. The adjacent serial section was hybridized with the opposite sense strand of the probe and grains counted in the same 17×68 μm area. The exposure times were all three days and slide were lightly stained with haematoxylin and eosin to facilitate cell type recognition. The figures given here are derived from ten counts over the same area of each pair of slides and are representative of five serial pairs of transects taken within each organ. Each symbol represents the average number of grains in one square. Panel (A): Open triangles; 57 days postfertilization, open circles; 116 days postfertilization. Panel (B): Open triangles; 57 days postfertilization, open circles; 116 days postfertilization. Panel (C): Open squares; 55 days postfertilization, open triangles; 33 days postfertilization, open circles, 18 days postfertilization. In all cases, filled symbols represent the equivalent control hybridizations with sense strand RNA probes to adjacent sections. Topographical boundaries are marked on the underlying bars. Panel (A): dc = definitive cortex, m = fetal cortex. Panel (B): mb = metanephric blastema, t = tubule, m = mesenchyme. Panel (C): Syn = syncytiotrophoblast, cyt = cytotrophoblast.
Cell specificity of expression

We have demonstrated that expression of the IGF-II gene can be first detected in the placenta at 18 days of development. Due to lack of suitable material we cannot exclude the possibility that it is expressed in the embryo proper before then, but no expression can be detected in expanded blastocysts cultured for 5 days postfertilization. The conclusion that no expression occurs in the blastocyst is based on the assumption that culture of the blastocysts does not affect their growth factor expression. It was noted that the number of cells identifiable from position as inner cell mass components was rather small in our material.

In the placenta, expression of IGF-II is limited to the cytotrophoblast, being present both in the Langhans layer of the villous cytotrophoblast and the chorionic plate cytotrophoblast. Areas of cytotrophoblastic shell were not present in our material. Control hybridization with a probe from the β subunit of human chorionic gonadotropin routinely localized to the trophectoderm of the blastocysts and to the syncytiotrophoblast demonstrating that the technique worked well for the blastocyst material. The expression of beta hCG in the syncytiotrophoblast was patchy and distinct boundaries between expressing and nonexpressing cells in the same villus were observed. This is somewhat at variance with the observed pattern of immunostaining seen at later stages (Gaspard et al., 1980), which might indicate that the peptide diffuses and binds over the extravillous surface. In contrast, expression of IGF-II was even and uniform in all cells that could be identified as cytotrophoblast and increased significantly with the progress of gestation. Our data represent the second assignment of growth factor expression to the cytotrophoblast, previous workers finding expression of PDGF in the same layer (Goustin et al., 1985). It is striking that the growth factor expression so far seen is in the mitotically active and invasive cytotrophoblast, whereas the expression of the endocrine hormones associated with the maintenance of pregnancy, hCG, placental lactogen and steroid hormones, is in the differentiated syncytiotrophoblast (Gaspard et al., 1980). As the placenta contains both type 1 and type 2 IGF receptors (Bhaumick et al., 1981), expression of IGF-II at the sites reported may in principle have a local effect on proliferation of the placenta.

We have consistently failed to demonstrate high levels of expression in the yolk sac. This is in contrast both to earlier findings using Northern blotting and extensive evidence for production of IGF-II in both rat and mouse yolk sac (Beck et al., 1987; Heath & Shi, 1986). We conclude that as the previously used yolk sac samples were pooled from many individuals it may have contained tissue of an age in which transient expression occurred. An alternative explanation would be contamination with trophoblast. However, this suggests that human and rodent yolk sacs might have a slightly different spectrum of functions, perhaps underlying differences in the physiology of the organ in the three species.

It is now clear that apparently homogeneous popu-

lations of liver cells consist of immature hepatocytes, bile duct progenitors and haematopoietic cells, (Holmes et al., 1986) and they show differences as regards enzymic activity and surface antigen presentation. Greater than 90% of the cells of the liver were shown to be expressing IGF-II transcripts at a high level, and these include the above cell types, along with the parenchyma. No expression was detected in the identifiable erythropoietic tissue of the liver, the smooth muscle or endothelial cell lining of blood vessels, or the hepatic portal tracts and their associated mesenchyme. This pattern of expression corresponds to that seen in midgestation rat embryos, where the expression was mainly seen in parenchyma. Strain et al. (1987) have demonstrated the ability of hepatocytes to secrete at least IGF-I when explanted into culture, and the immunocytochemical detection of IGF-II peptide in parenchymal cells of 16-week-old livers (Han et al., 1988). This suggests that the mRNA that we see might be translated into a peptide product.

The pattern that we observe corresponds well to that obtained by immunocytochemistry, but differs markedly from that seen in 16- to 26-week fetuses described by Han et al. (1987), who found the liver ranked relatively low in overall mRNA levels, expression being limited to perisinusoidal cells. We suggest that the differences are due to a decrease in either transcription or messenger stability related to the appearance of differentiated liver functions and structure. However, for reasons discussed earlier, it is not possible to implicate individual processes. For example, sinusoids are not apparent until 6–7 weeks of gestation and bile acids are only detectable at 12 weeks, enzymes associated with glycogen metabolism appearing at 12–14 weeks (Peters, 1983). Previous data suggest that both erythroleukaemia cells and normal erythropoietic cells possess receptors and respond to IGFs (Kurtz et al. 1988; Tally et al. 1987) suggesting that an autocrine or paracrine action of IGFs on haematopoiesis in the liver may be significant.

Hepatic IGF-II expression is known to continue in adult life in humans as opposed to rodents where there is a dramatic shut off of expression in the first 18 days of life. The hepatic expression in humans is controlled by a specific promoter, which is only active in adult liver and is absent from the rat, whilst the ‘fetal’ promoter is suppressed (de Pagter Holthuizen et al., 1987; Schofield & Tate, 1987). We suggest that the change in pattern from parenchymal expression to perisinusoidal cell expression may be related to a switch in promoter usage to the adult promoter. This could be tested using exon specific probes for in situ hybridization.

Both the mesodermally derived fetal and definitive adrenal cortices were found to express IGF-II at a high level, the fetal cortex expressing at a 40–60% higher level than the definitive. As maturation progresses and the definitive cortex grows, so the overall level of expression drops. Han et al. show that, by the late second trimester, expression is mainly at the adrenal capsule. Comparison to the levels of mRNA in the adult adrenal suggests that the decrease in expression is
associated with maturation although this does not reach completion until several months postpartum. Evidence has been presented that in the rat, glucocorticoid expression reduces levels of IGF-II transcription (Beck et al. 1988b). However, active steroidogenesis and cortisol production is underway in the human fetal adrenal cortex by six weeks (Winter, 1985), and an age-dependent decrease in the activity of the steroidogenic enzyme P450scc has been interpreted as strong evidence for a positive correlation between IGF-II expression and corticosteroidogenesis (Voutilainen & Miller, 1987). Immunocytochemistry is in agreement with this distribution showing a higher concentration of peptide in the fetal cortex than the definitive. Whilst some faint immunoreactivity was reported in the medulla, we have never seen IGF-II mRNA in the developing medulla.

The most dramatic changes over the time period examined are in the kidney. The major site of IGF-II expression is in the metanephric blastema. At 6–7 weeks, when the first nephrogenic vesicles begin to form, expression is at its highest. As differentiation proceeds the S-shaped bodies, and later the proximal and distal convoluted tubule progenitors, are characterized by a low level (approx. 30% of that seen in the blastema) of expression. This is also true of the ureteric-bud-derived collecting tubules. By 14 weeks, it is estimated that 20% of the glomeruli have a mature aspect, but arcade formation and maturation again continue to early postnatal life (Hamilton et al. 1978; Olsanondh & Potter, 1963). Han et al. (1988), found immunoreactive IGF only in the epithelial component of the kidney, but it is clear that examination of much earlier stages would have been necessary to demonstrate the level of expression reported here in the blastema at 10 weeks. Similarly Han et al. (1987) reported only low levels of expression in 16–26 weeks of gestation, substantially later than our observations, when there is little or no overt blastema remaining, reinforcing our conclusions that the loss of IGF-II expression by the kidney is progressive and associated with maturation.

IGF-II expression and developmental tumours

It is now well established that several childhood tumours are characterized by inappropriate expression of IGF-II mRNA. These include Wilms' tumour (embryonal nephroblastoma), hepatoblastoma, adrenal cortical carcinoma and rhabdomyosarcoma (Scott et al. 1985; Haselbacher et al. 1987). It is very clear for the first three of these that the cell types that characterize these tumours express large amounts of IGF-II in the fetus, for example the metanephric blastema, which in histopathology is thought to resemble the stem cells of Wilms' tumour (Gonzalez-Crussi, 1984). It has been hypothesized that failure of such tissues to differentiate appropriately leads to the formation of a tumour which is manifest at or soon after birth and shows fetal characteristics, such as the ability to differentiate. The data presented here support such an hypothesis, but do not address the question of whether the aberrant IGF-II expression is responsible for the neoplasia. It may be simply a concomitant of the fetal phenotype. The expression of IGF-II in other types of tumour such as phaeochromocytoma clearly does not conform to such an analysis (Haselbacher et al. 1987), as we have never seen expression in developing adrenal medullary cells. We cannot rule out transient expression not detected in the timed material available. However, inappropriate expression of a growth factor could have several disparate causes, and the two types of tumour clearly have very different origins and characteristics.

Evidence for potential autocrine or paracrine action of IGF-II in the fetus is strong as both peptides and receptors are present in either the same tissue or juxtaposed tissue in many organs (Sara et al. 1983) but relies on assuming that responsiveness in vitro equates with responsiveness in vivo, and there is insufficient evidence yet to draw further conclusions.

We have here identified distinct age-dependent changes in the pattern of IGF-II gene expression. In several tissues, with the exception of placenta, these changes are associated with the disappearance of a less differentiated cell type and the appearance of more mature tissues. As organ maturation reflects cell division, differentiation and frequently cell recruitment, specific to individual organs, it has not proved possible to identify a common process in which the IGFs might be involved. IGF-II expression is most frequently, but not exclusively, associated with areas of high mitotic activity, nor does it correlate with maturation of all tissue types, therefore it is probably not acting as a simple mitogen in a straightforward autocrine loop, and is perhaps affecting specific aspects of differentiation.

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References


Insulin-like growth factors in the human fetus

553


A. L. Brice and others


