Expression of Messenger RNA of Insulin-Like Growth Factors (IGFs) and IGF Binding Proteins (IGFBP1–6) in Placenta of Normal and Diabetic Pregnancy

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INSULIN-LIKE growth factors (IGF-I and IGF-II) play a central role in regulating placental, embryonic and fetal growth and development [1, 2]. The placenta not only transports nutrients from maternal to fetal circulation but also produces a number of growth factors including IGFs, which may regulate the growth and the functions of placenta in an autocrine or paracrine fashion. The effects of IGFs are modified by one or more of the six of IGF binding proteins (IGFBPs) in a complex and incompletely understood manner [3], but there are few data available on the expression of IGFBPs in human placental tissues.

The incidence of placental hypertrophy or macrosomia of the newborn is higher in gestational as well as in preexisting diabetes. We have shown that concentrations of IGF-I and IGF-II in the cord blood of the newborns of diabetic mothers are higher than in infants born to normal mothers [4]. Furthermore, there was a positive correlation between the cord IGF-I or IGF-II level and birth weight of newborns, suggesting that both IGF-I and IGF-II are involved in fetal growth, but the role of IGFs and their binding proteins produced in placental tissues in regulating human fetal or placental growth is unknown. We therefore measured the expression of mRNA of IGFs and IGFBPs in the placenta of normal and diabetic pregnancy and correlated them to placental and fetal weight.

Materials and Methods

Human placentas were obtained from 5 normal and 9 diabetic mothers at the time of delivery (38.2–40.0 week gestational period). The serum level of HbA1c in diabetic mothers was 5.31 ± 1.05% (mean ± SD). Total cellular RNA was isolated according to the method described by Chomczynski and Sacchi [5]. Human IGF-I and IGF-II cDNA probes were obtained from ATCC, and human IGFBP probes were gifts from Dr. S. Shimasaki (Whittier Institute, CA). Northern blot was carried out with cDNA probes labeled with a random primer labeling kit (New England Nuclear). Hybridization signals on the blots were exposed to film (Fuji-Film Co., Tokyo) at −70 °C. The relative abundance of mRNAs was determined by densitometry and expressed in arbitrary units.

Results and Discussion

Messenger RNAs of IGF-I, IGF-II and all species of IGFBPs (IGFBP-1–6) were expressed in human placenta. There was a higher expression of GF-II mRNA in placentas from diabetic mothers than in those from normal mothers (P<0.05), which is consistent with a previous report stating that insulin-related gene expression in the placenta is higher in diabetic patients [6]. IGF-I mRNA expression was also higher in diabetic mothers, but the difference was not significant. Both IGF-I and -II mRNA expression positively correlated with maternal HbA1c levels (r=0.708, P<0.05 and r=0.744,
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Hyperglycemia or resultant hyperinsulinemia may be responsible for higher expression of IGF-I mRNA. Among IGFBPs, only IGFBP-3 mRNA positively correlated with maternal HbA1c levels ($r=0.883$, $P<0.05$). No significant difference in the expression of IGFBP-1 mRNA was found between normal and diabetes group despite fact that the gene expression of IGFBP-1 is affected by metabolic disturbances such as diabetes [3]. The failure to detect difference in IGFBP-1 mRNA expression between the two groups may be due to relatively good control of diabetes in the patients examined.

There was a positive correlation ($r=0.550$, $P<0.05$) between the abundance of IGF-II mRNA and that of IGFBP-3 mRNA. Both IGFBP-1 and BP-2 mRNA expression negatively correlated with that of IGFBP-3 ($r=-0.707$, $P<0.05$, and $r=-0.567$, $P<0.05$, respectively), suggesting that IGFBP-1 and -2 on the one hand, and IGFBP-3 on the other hand are oppositely regulated by a possibly common regulator(s) in human placenta. Among IGFs and IGFBPs, only IGF-II mRNA abundance positively correlated with placental weight ($r=0.761$, $P<0.01$) as shown in Fig. 1. This observation strongly suggests that locally produced IGF-II plays an important role in placental growth since placental tissues are rich in IGF-II receptors. Supporting this, a recent report by Baker et al. [2] has shown that IGF-II gene disruption results in retardation of placental growth in mice. We have also recently shown a positive correlation between IGF-II levels in cord blood and placental weight [3]. IGF-II produced by placenta and/or fetus therefore appears to contribute to placental growth.

Abundance of IGFBP-1 mRNA and IGFBP-2 mRNA negatively correlated with the birth weight of infants ($r=-0.573$, $P<0.05$, and $r=-0.605$, $P<0.05$), whereas IGFBP-3 mRNA abundance positively correlated with body height but not with placental weight. In one study [7], the umbilical IGFBP-3 level was positively correlated to birth weight, but neither the BP-1 nor the BP-2 level was related to fetal growth. Another report [8], however, has shown that both cord IGFBP-1 and IGFBP-2 levels are inversely correlated with birth weight. Furthermore, maternal IGFBP-1 levels have been shown to inversely correlated with birth weight [9]. The data presented here suggest that both locally produced IGFBP-1 and IGFBP-2 are negative regulators, and IGFBP-3 is one of the positive regulators in regulating fetal growth, but the mechanism by which placental IGFBP-1, BP-2 and BP-3 modify fetal growth is unknown. It would be possible that these BPs modify the bioavailability of IGFs which are produced by the placenta itself or by maternal IGFs and affect placental functions and thereby fetal growth.

We also detected expression of IGFBP-4, -5 and -6 in placental tissues. Interestingly, we found a positive correlation between the abundance of IGFBP-3 mRNA and that of IGFBP-6 mRNA, which was in turn positively related to body height ($r=0.701$, $P<0.05$). A positive correlation between the expression of IGFBP-6 mRNA and that of IGFBP-3 mRNA is difficult to explain, because previous in vivo studies have shown that serum levels of the two BPs change in different ways [3]: serum IGFBP-3 levels increase and IGFBP-6 levels decrease in patients with acromegaly. Regulation of the two BPs may be tissue-specific. The present study failed to show any effect of maternal glucose metabolism on placental expression of IGFBP-6 mRNA. Presumably IGFBP-6 is not sensitive to change in glucose metabolism. IGFBP-6 is unique in its higher affinity for IGF-II than for IGF-I. Taken together with the abundance IGF-II receptors in placenta, it could be speculated that IGFBP-6 plays a role in regulating placental functions and fetal growth.
We found a positive correlation between the abundance of IGFBP-4 and that of IGFBP-5 mRNA. In contrast to IGFBP-3 mRNA, there was a negative correlation between both IGFBP-4 and IGFBP-5 mRNA levels and maternal HbA1c ($r=-0.745$, $P<0.05$, and $r=-0.656$, $P<0.05$). Recent in vitro studies have revealed that both IGFBPs production is increased by insulin [3], which is consistent with the present observations, but the physiological role of BP-4 and -5 remains to be determined. In conclusion, the data presented here demonstrate that human placenta expresses all species of IGFBPs along with IGF-I and -II. The present study suggests that locally produced IGFs and IGFBPs are involved in the regulation of placental and fetal growth.

Acknowledgments

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References