Original Paper

Expression of inhibin/activin subunits alpha (-α), beta A (-βA) and beta B (-βB) in placental tissue of normal and intrauterine growth restricted (IUGR) pregnancies

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Abstract During human pregnancy the placenta produces a variety of proteins like steroid hormones and their receptors that are responsible for the establishment and ongoing of the fetoplacental unit. Inhibins are dimeric glycoproteins, composed of an α-subunit and one of two possible β-subunits (βA or βB). Aims of the present study were the determination of the frequency and tissue distribution patterns of the inhibin/activin subunits in human placental tissue of normal pregnancies and pregnancies complicated with fetal growth restriction (IUGR). Slides of paraffin embedded placental tissue were obtained after delivery from patients diagnosed with IUGR (n = 6) and normal term placentas (n = 8). Tissue samples were fixed and incubated with monoclonal antibodies inhibin/activin-subunits -α, -βA, -βB. Intensity of immunohistochemical reaction on the slides was analysed using a semi-quantitative score and statistical analysis was performed (P < 0.05). A significant lower expression of the inhibin-α subunit in IUGR extravillous trophoblast compared to normal pregnancies was observed, while the inhibin-α immunostaining was significantly upregulated in syncytiotrophoblast. Additionally, a significant down-regulation of inhibin-βB subunit in extravillous trophoblast cells in IUGR syncytiotrophoblast cells was demonstrated. A co-localisation of inhibin-α and the β-subunits was also observed, suggesting a production and secretion of intact inhibin A and inhibin B. Although the precise role of these inhibin/activin subunits in human placenta and IUGR pregnancies is still unclear, they could be involved in autocrine/paracrine signalling, contributing to several aspects like angiogenesis and tissue remodelling.

Keywords Inhibin/activin subunits · Inhibin-α · Inhibin-βA · Inhibin-βB · Intrauterine growth restriction (IUGR) · Extravillous trophoblast · Syncytiotrophoblast

Introduction

Inhibins are dimeric disulphide-linked glycoproteins and belong to the transforming growth factor beta (TGF-β) family of cytokines. They were initially isolated from the gonads and identified as modulators of FSH production from the anterior pituitary gland (de Kretser et al. 2002; Vale et al. 1988). These molecules are heterodimers consisting of one α-subunit and one of two possible β-subunits (βA and βB subunits). The α-subunit can dimerize with either βA or βB to form inhibin A (α-βA) or B (α-βB), respectively. Activins are homodimers of β-subunits linked by a disulphide bond. Depending on the combination of the subunits, there are three isoforms of activin, namely activin A (βA-βA), activin B (βB-βB) and activin AB (βA-βB)
(de Kretser et al. 2002; Vale et al. 1988). Recently, two additional β-subunits have been identified in human tissue, determined as β₁C (Hötten et al. 1995) and β₁E (Fang et al. 1996), although their precise role and functional relationship to the existing subunits remains still unknown.

The expression of inhibin/activin subunits have been described in different female tissues, including normal and pathological human endometrium (Mylonas et al. 2003, 2004a, 2006) and placenta (Caniggia et al. 1997; McCluggage et al. 1998; Petraglia et al. 1991), suggesting different roles such as paracrine modulators of reproductive function (de Kretser et al. 2002; Welt 2002). During pregnancy, inhibin/activin subunits are also expressed in placental decidua, the syncytiotrophoblast (Petraglia et al. 1991) and the trophoblast (McCluggage et al. 1998). Interestingly, higher inhibin levels in human serum have been described in preeclampsia (Muttukrishna et al. 1997) and down-syndrome (Aitken et al. 1996), suggesting that inhibin/activin production by placental cells might play a major and crucial role in pregnancy-related pathogenesis.

Normal fetal growth depends on several factors modulated by the fetus, the placenta and the mother. In preeclampsia and idiopathic small for gestational age (SGA) pregnancies, cytotrophoblast invasion is restricted with a limited remodelling of spiral arteries, thus resulting in reduced uteroplacental perfusion (Lim et al. 1997). Small fetuses due of intrauterine growth restriction (IUGR) are at higher risk for poor perinatal and long-term outcome (Baschat 2004; Tjoa et al. 2004), being associated with an increased risk of heart diseases and type 2 diabetes mellitus (Barker 1998). The most common definition of IUGR is a birth weight lower than the 10th percentile when adjusted to gestational age. In the past years several molecules have been suggested as predictive markers of IUGR, including cytokines, neuropeptides, adhesion molecules and glycoproteins such like inhibin A and activin A (Tjoa et al. 2004). However, limited data on histological expression of inhibin/activin subunits expression exists. Therefore, aims of the present study were:

(a) The determination of the frequency and tissue distribution patterns of the inhibin/activin subunits in human placental tissue of normal pregnancies and pregnancies complicated with fetal growth restriction (IUGR).

(b) The assessment of a combined expression of inhibin-α- and both β-subunits (β₁A- and β₁B-subunits) using double immunofluorescence technique.

Materials and methods

Tissue samples

Placental tissues were obtained from 12 placentas of women giving birth at the 1st Department of Obstetrics and Gynaecology of the LMU Munich. Tissue samples were obtained from patients diagnosed with IUGR (n = 6) and normal pregnancies (n = 6) after delivery (Table 1).

Immunohistochemistry

Immunohistochemistry on paraffin sections (7 μm) of the different placental tissue specimens was performed by incubating the slides in methanol/H₂O₂ (30 min) to inhibit endogenous peroxidase activity. Immunohistochemistry with inhibin-subunits was performed using a combination of pressure cooker heating and the standard streptavidin–biotin–peroxidase complex with the use of the mouse-IgG-Vector stain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) as previously described (Mylonas et al. 2004a). Briefly, paraffin-fixed tissue sections were dewaxed using xylol for 15 min, rehydrated in an ascending series of alcohol row (70, 96 and 100%), and subjected to antigen retrieval on a high setting for 10 min in a pressure cooker in sodium citrate buffer (pH 6.0), containing citrate acid 0.1 M and sodium citrate 0.1 M in distilled water. After cooling, the slides were washed twice in PBS. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with diluted normal serum (10 ml PBS containing 150 μl horse serum; provided by Vector Laboratories) for 20 min at room temperature. Sections were then incubated at room temperature for 120 min with the primary antibodies (Table 2). After washing with PBS, the slides were incubated in diluted biotinylated serum (10 ml PBS containing 50 μl horse serum; provided by Vector Laboratories) for another 30 min at room

| Table 1 Clinical data of the examined placental tissue. Mean±SD |
|-----------------|-----------------|
| Weeks of delivery | Control | IUGR |
| 38.2 ± 3.9 | 33.0 ± 3.0 |
| Birth weight | 34.25 ± 4.12 | 121.6 ± 45.3 |
| pH umbilical artery | 7.27 ± 0.09 | 7.26 ± 0.09 |
| APGAR score < 7 at 5 min | 9.4 ± 0.9 | 9.0 ± 0.7 |
| APGAR score < 7 at 10 min | 10 ± 0 | 9.6 ± 0.5 |