J Mol Hist (2006) 37: 43–52
DOI 10.1007/s10735-006-9029-6

ORIGINAL PAPER

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

I. Mylonas · B. Schiessl · U. Jeschke · J. Vogl · A. Makrigiannakis · C. Kuhn · S. Kunze · S. Schulze · F. Kainer · K. Friese

Received: 29 January 2006 / Accepted: 29 March 2006 / Published online: 3 May 2006

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 feto-placental unit. Inhibins are dimeric glycoproteins, composed of an α-subunit and one of two possible β-subunits (β_A or β_B). 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, -β_A, -β_B. 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-β_B 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-β_A · Inhibin-β_B · Intrauterine growth restriction (IUGR) · Extravillous trophoblast · Syncytiotrophoblast

I. Mylonas (✉) · B. Schiessl · U. Jeschke · J. Vogl · C. Kuhn · S. Kunze · S. Schulze · F. Kainer · K. Friese
1st Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University Munich, Maistrasse 11, Munich 80337, Germany
e-mail: ioannis.mylonas@med.uni-muenchen.de

B. Schiessl
School of Surgical and Reproductive Sciences (Obstetrics & Gynaecology), University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

A. Makrigiannakis
Department of Obstetrics and Gynecology, University of Crete, Heraklion, Crete, Greece

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 (β_A- and β_B-subunits). The α-subunit can dimerize with either β_A or β_B to form inhibin A (α-β_A) or B (α-β_B), respectively. Activins are homodimers of β-subunits linked by a disulfide bond. Depending on the combination of the subunits, there are three isoforms of activin, namely activin A (β_A–β_A), activin B (β_B–β_B) and activin AB (β_A–β_B)

(de Kretser et al. 2002; Vale et al. 1988). Recently, two additional β-subunits have been identified in human tissue, determined as β_C (Hötten et al. 1995) and β_E (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 (β_A- and β_B-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/H_2O_2 (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-Vectastain 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 distillated 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

	Control	IUGR
Weeks of delivery	38.2 ± 3.9	33.0 ± 3.0
Birth weight	3435 ± 412.2	1213.6 ± 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

Table 2 Antibodies used for immunohistochemical characterisation of placental tissue samples by immunohistochemistry and immunofluorescence

Antibody	Clone	Isotype	Dilution	Source
CK 7	Polyclonal	Rabbit IgG	1:200	Dianova, Hamburg, Germany
Inhibin-α	R1	Mouse IgG_{2a}	1:50	Serotec, Oxford, UK
Inhibin-α	Polyclonal	Rabbit IgG	1:40	Signet, Dedham, MA, USA
Inhibin-β_A	E4	Mouse IgG_{2b}	1:50	Serotec, Oxford, UK
Inhibin-β_B	C5	Mouse IgG_{2a}	1:10	Serotec, Oxford, UK
Cy2 and Cy3		Goat anti-rabbit IgG	1:200	Dianova, Hamburg, Germany
Cy2 and Cy3		Goat anti-mouse IgM	1:200	Dianova, Hamburg, Germany

temperature. After incubation with the avidin–biotin peroxidase complex for another 30 min and repeated washing steps with PBS, visualisation was performed with substrate and chromagen 3,3′-diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8–10 min. The slides where counterstained further with Mayer's acidic hematoxylin and washed in an alcohol row (50–98%). After xylol treatment the slides were covered. Negative controls were performed by replacing the primary antibody. Positive cells showed a brownish colour and negative control as well as unstained cells appeared blue. The standardisation, dilution and optimisation of this protocol were primarily tested on normal premenopausal ovary tissue, while negative controls included postmenopausal ovarian tissue.

Immunofluorescence double staining reaction

For the immunohistochemical characterisation cryosections from normal placental tissue and from placentas of IUGR pregnancies were examined as previously described (Jeschke et al. 2002; Mylonas et al. 2004a, 2006). The used antibodies are listed in Table 2, whereas the inhibin-α polyclonal antibody was applied. Briefly, all samples were fixed in 5% buffered formalin. They were diluted to 10 μg/ml with PBS and incubated with the slides overnight at 4°C. After washing, Cy2-labelled goat anti-rabbit IgG and Cy3-labelled goat anti-mouse IgG, diluted 1:200, served as second antibody. The slides were finally embedded in mounting buffer containing 4,6-diamino-2-phenylindole (DAPI) resulting in blue staining of the nucleus. Slides were examined with a Zeiss (Jena, Germany) Axiophot photomicroscope. Digital images were obtained with a digital camera system (CF20DXC; KAPPA Messtechnik, Gleichen, Germany) and saved on computer.

Immunohistochemical evaluation and statistical analysis

The intensity and distribution patterns of specific inhibin/activin subunit immunohistochemical staining reaction was evaluated by two blinded, independent observers, including a gynaecological pathologist, using a semi-quantitative score (IRS score) as previously described (Mylonas et al. 2000) and used in the evaluation of inhibin/activin subunit expression in human endometrial specimens (Mylonas et al. 2004a, 2006). Briefly, the IRS score was calculated as follows: IRS = SI×PP, where SI is the optical staining intensity (graded as 0 = no staining; 1 = weak staining; 2 = moderate staining and 3 = strong staining) and PP the percentage of positively stained cells. The PP was estimated by counting approx. 200 cells (defined as 0 = no staining; 1 = < 10% staining; 2 = 11–50% staining; 3 = 51–80% staining and 4 = >81% staining). Digital images were obtained with a digital camera system (Olympus, Tokyo, Japan) and were saved on computer. The Mann–Whitney rank-sum test was used to compare the means of the different IRS scores (SPSS; Chicago, IL, USA). Significance of differences was assumed at $P \leq 0.05$.

Results

Immunohistochemical expression of inhibin-α, -β_A and -β_B

Immunohistochemical staining was performed using an appropriate positive control comprising ovaries containing follicular cysts. Inhibin-α stained positive with ovarian granulosa cells and theca interna cells, while inhibin-β_A and -β_B subunits showed also a positive immunohistochemical staining reaction with human ovarian tissue as previously described (Mylonas et al. 2004a).

All three inhibin/activin subunits were detected in human extravillous trophoblast and syncytiotrophoblast of placental tissue of normal and IUGR pregnancies. In normal placental tissue inhibin-α was primarily expressed in extravillous trophoblast cells, while immunostaining in syncytiotrophoblast cells was weaker. In IUGR placental tissue the inhibin-α expression was decreased in extravillous trophoblast cells, while syncytiotrophoblast showed a stronger immunoreactive reaction (Fig. 1a–d).

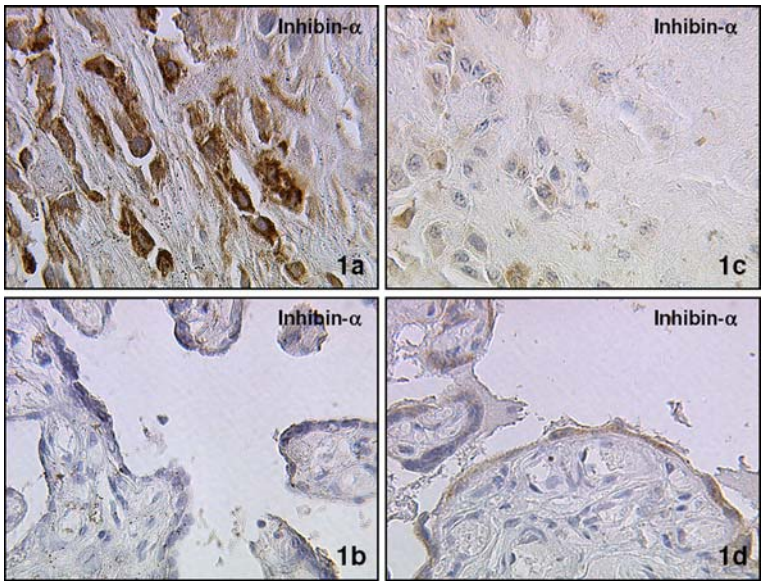

Fig. 1 (**a**–**d**) Immunohistochemical staining reaction of inhibin-α in placental tissue. In normal placental tissue inhibin-α was primarily expressed in extravillous trophoblast cells (**a**), while immunostaining in syncytiotrophoblast cells was weaker (**b**). In IUGR placental tissue the inhibin-α expression was weaker in extravillous trophoblast cells (**c**), while syncytiotrophoblast showed an intense immunoreactive staining reaction (**d**). Magnifications: extravillous trophoblast 250×; syncytiotrophoblast 125×

The β_A-subunit was also detected in placental extravillous trophoblast and syncytiotrophoblast cells. While a diffuse immunohistochemical staining reaction could be observed in IUGR extravillous trophoblast cells an increased staining intensity reaction was observed in syncytiotrophoblast placenta (Fig. 2a–d). Interestingly, a decrease in the β_B-subunit staining reaction was observed between normal and IUGR extravillous trophoblast cells. In the syncytiotrophoblast the staining intensity presented without a distinct expression pattern between normal and IUGR cells (Fig. 3a–d).

Expression of inhibin-α, -β_A and -β_B demonstrated by immunofluorescence

All three inhibin/activin subunits were detected in placental tissue of normal and IUGR pregnancies, using immunofluorescence, with a polyclonal inhibin-α subunit antibody and monoclonal antibodies for the

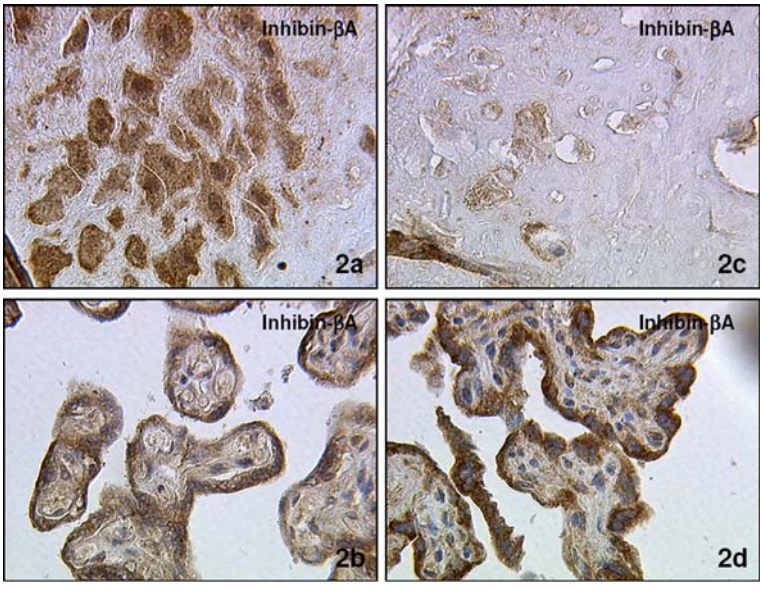

Fig. 2 (**a**–**d**) Immunohistochemical staining reaction of inhibin-β_A in placental tissue. The β_A-subunit was also detected in normal placenta tissue. Extravillous trophoblast (**a**) and syncytiotrophoblast cells (**b**) showed intense staining reaction against the inhibin-β_A antibody. While a diffuse immunohistochemical staining reaction could be observed in IUGR extravillous trophoblast cells (**c**) an increased immunohistochemical staining reaction was observed in syncytiotrophoblast placenta (**d**). Magnifications: extravillous trophoblast 250×; syncytiotrophoblast 125×

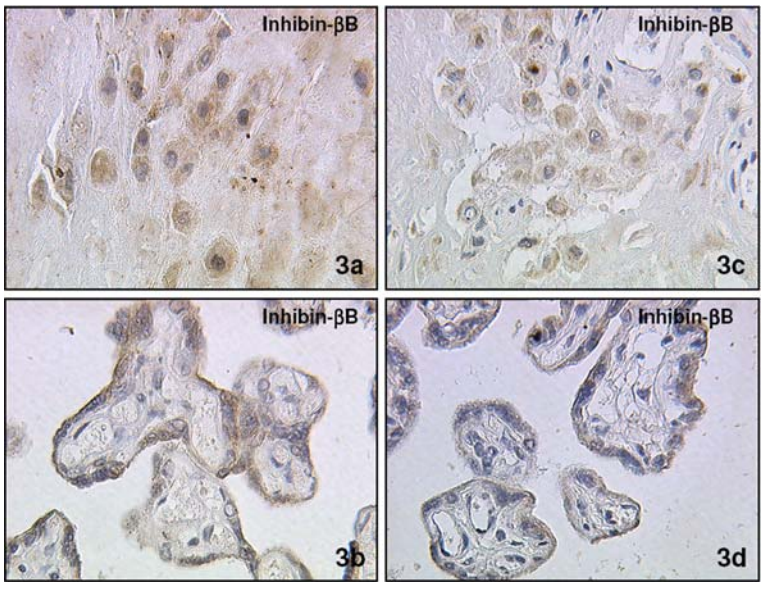

Fig. 3 (**a**–**d**) Immunohistochemical staining reaction of inhibin-β_B in placental tissue. Interestingly, a decrease in the staining reaction was observed between normal (**a**) and IUGR (**c**) extravillous trophoblast cells. In the syncytiotrophoblast the staining intensity presented without a distinct expression pattern between normal (**b**) and IUGR cells (**d**). Magnifications: extravillous trophoblast 250×; syncytiotrophoblast 125×

two distinct inhibin-β subunits. The inhibin/activin subunits were primarily expressed in normal human extravillous trophoblast as confirmed by co-expression with cytokeratin antibody (Figs. 4, 5).

In IUGR placental tissue the inhibin-α subunit was expressed in extravillous trophoblast and syncytiotrophoblast cells. Inhibin-β_A was also expressed in both placental cell types. A co-expression of inhibin-α and -β_A subunits could be demonstrated suggesting a secretion of inhibin A (Fig. 6a–c). The inhibin-β_B subunit could also be demonstrated in extravillous trophoblast cells, while the co-expression showed minimal co-localisation of the α- and β_B-subunit (Fig. 6d–f).

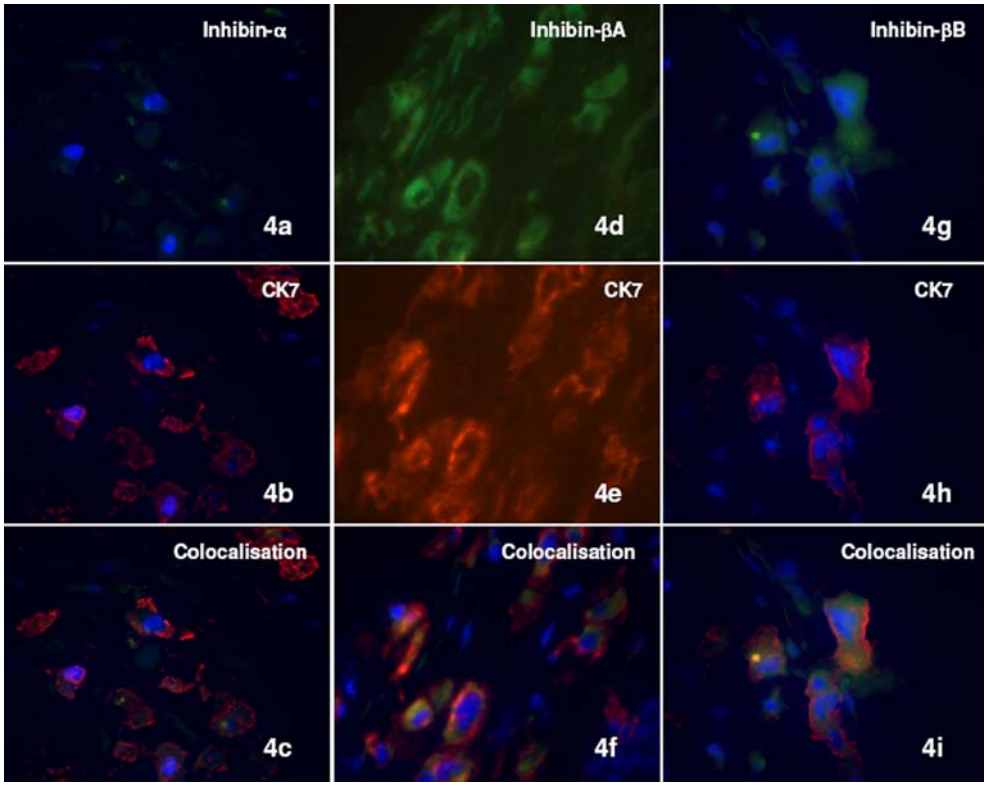

Fig. 4 Expression of inhibin-α, -β_A and -β_B demonstrated by immunofluorescence in extravillous trophoblast cells of normal and IUGR placental tissue. The inhibin-α (**a**–**c**; 125×), inhibin-β_A (**d**–**f**; 250×) and inhibin-β_B (**g**–**i**; 250×) were primarily expressed in normal human extravillous trophoblast as confirmed by co-expression with cytokeratin 7 antibody

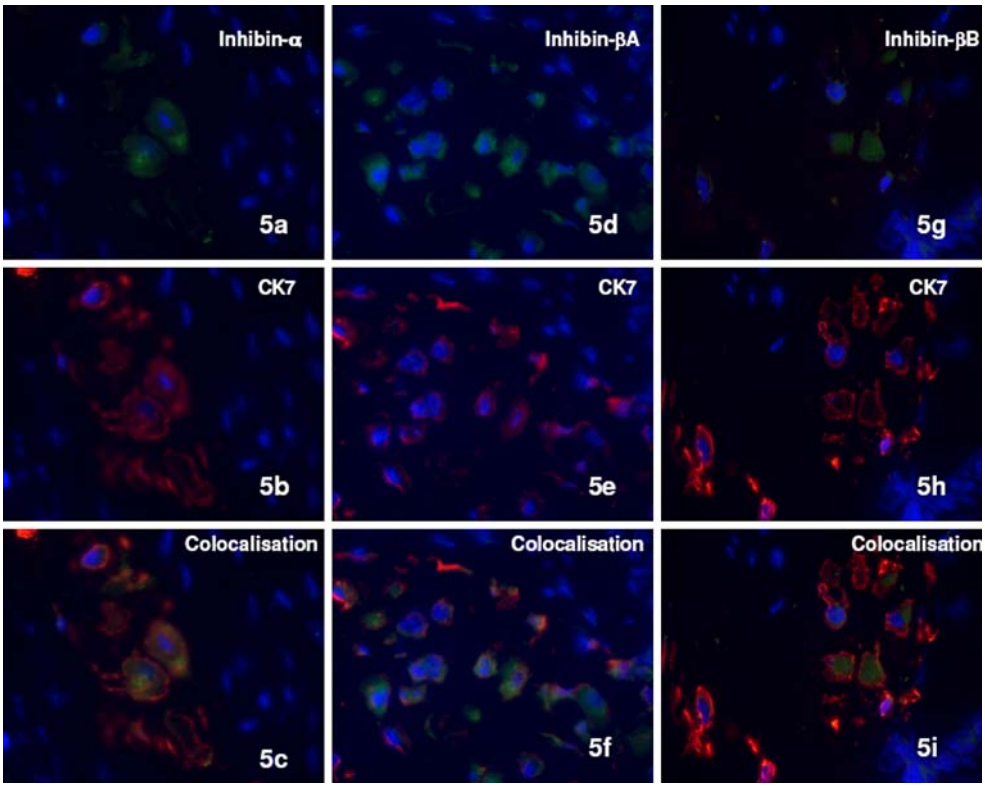

Fig. 5 Expression of inhibin-α, -β_A and -β_B demonstrated by immunofluorescence in extravillous trophoblast cells of normal and IUGR placental tissue. The inhibin-α (**a**–**c**; 250×), inhibin-β_A (**d**–**f**; 125×) and inhibin-β_B (**g**–**i**; 125×) were also expressed in human extravillous trophoblast of IUGR pregnancies as confirmed by co-expression with cytokeratin 7 antibody

Immunohistochemical evaluation of inhibin-α, -β_A and -β_B

The immunoreactive score for inhibin-α decreased significantly between normal and IUGR placenta in extravillous trophoblast tissue ($P < 0.05$), while the inhibin-β_A staining reaction did not show any significant differences. Interestingly, inhibin-β_B-subunit also demonstrated a statistical significant decline between normal and IUGR extravillous trophoblast cells (Fig. 7a).

In syncytiotrophoblast cells the inhibin-α subunit showed a significant increase between normal and IUGR tissue ($P < 0.05$). Although a more intensive staining reaction was observed for inhibin-β_A in IUGR syncytiotrophoblast compared to normal control tissue, no significant differences could be demonstrated. The immunoreactive score for inhibin-β_B did also show no statistical differences between normal and IUGR syncytiotrophoblast cells (Fig. 7b).

Discussion

While inhibins/activins were initially characterised as endocrine and paracrine hormonal regulators of the hypothalamic–pituitary–gonadal axis, it is now clear that they are expressed in a wide range of tissues including human endometrium (Mylonas et al. 2003, 2004b, 2006) and human placenta (Florio et al. 2001; McCluggage et al. 1998; Petraglia et al. 1991). The human placenta expresses inhibin/activin mRNA (Petraglia et al. 1991) and inhibin/activin subunit protein throughout pregnancy, being the primary source of maternal circulating inhibin and activin levels (Florio et al. 2001). We demonstrated by using immunohistochemistry and immunofluorescence double staining technique an expression of inhibin/activin-subunit in normal and IUGR placental tissues. Additionally we observed a significant lower expression of the inhibin-α subunit in extravillous trophoblast of IUGR compared to normal pregnancies, while the inhibin-α immunostaining was significantly upregulated in syncytiotrophoblast. A significant down-regulation of inhibin-β_B subunit in extravillas trophoblast cells was also demonstrated, while inhibin-β_A-subunit was more intense in IUGR synctiotrophoblast cells but without any statistical differences. We also showed a co-localisation of inhibin-α and the β-subunits, suggesting a production and secretion of intact inhibin A and inhibin B.

In preeclampsia and IUGR pregnancies, cytotrophoblast invasion is restrained, resulting in a reduced uteroplacental perfusion (Lim et al. 1997). Despite

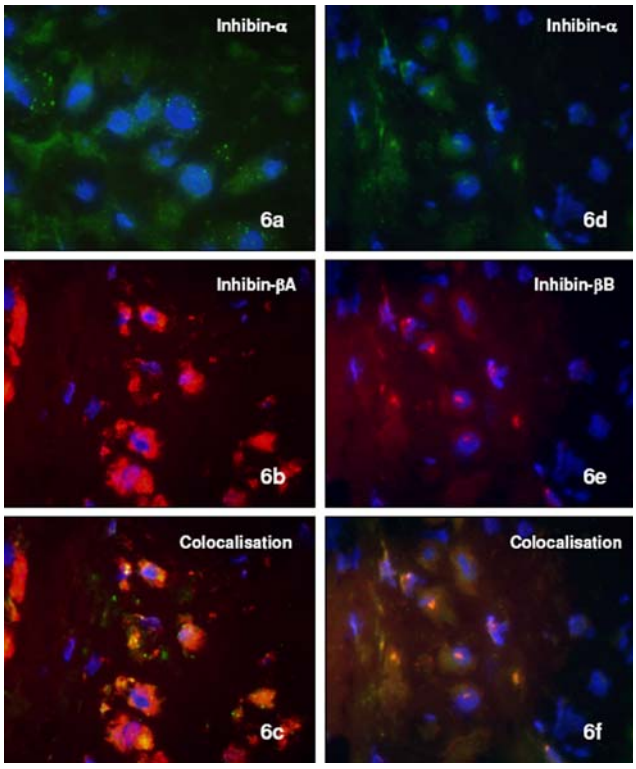

Fig. 6 (**a**–**f**) Expression of inhibin-α, -β_A and -β_B as demonstrated by immunofluorescence in placental tissue of IUGR pregnancies. In IUGR placental tissue the inhibin-α subunit was expressed in extravillous trophoblast and syncytiotrophoblast cells (**a**, **d**; green). Inhibin-β_A was also expressed in both placental cell types (**b**; red). A co-expression of inhibin-α and -β_A subunits could be demonstrated suggesting a secretion of inhibin A (**c**; yellow). The inhibin-β_B subunit could also be demonstrated in extravillous trophoblast cells (**e**; red), while the co-expression showed co-localisation of the α- and β_B-subunit, suggesting a secretion of intact inhibin B molecule (**f**; yellow). Magnifications: 250×

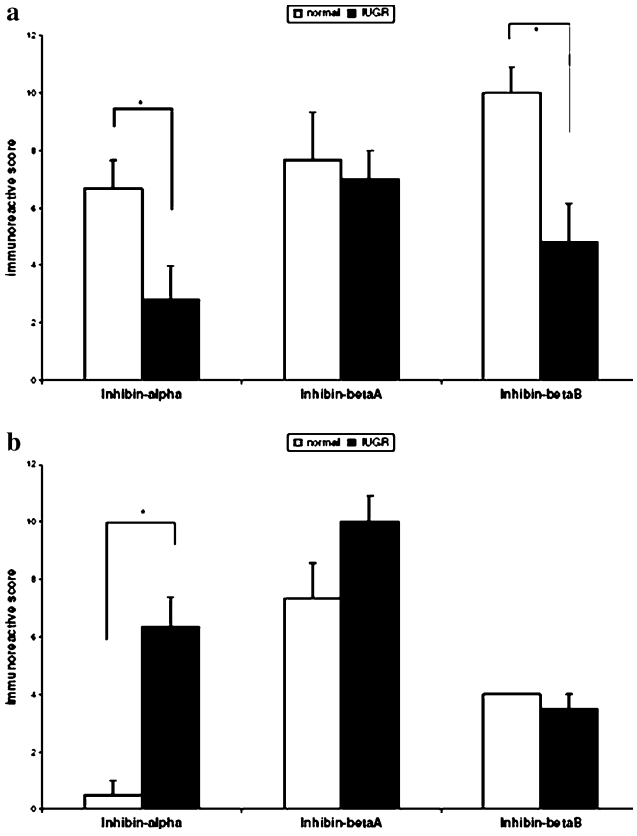

Fig. 7 (**a**, **b**) Immunohistochemical expression of inhibin/activin subunits in extravillous trophoblast (**a**) and syncytiotrophoblast (**b**). The immunoreactive score for inhibin-α decreased significantly in extravillous trophoblast cells between normal and IUGR ($P < 0.05$, *), while it increased significantly between normal and IUGR syncytiotrophoblast ($P < 0.05$, *). Inhibin-β_A did not show any significant differences between normal and IUGR tissue in extravillous trophoblast and syncytiotrophoblast cells. The immunoreactive score for inhibin-β_B-subunit showed a statistical significance difference between normal and IUGR extravillous trophoblast cells ($P < 0.05$, *), while no statistical differences could be observed by analyzing the syncytiotrophoblast. Data represent mean ± SEM. Significance was assumed at $P < 0.05$ (*)

several similarities in placental pathology, preeclampsia and IUGR pregnancies show marked differences in maternal pathophysiology. While preeclampsia results in maternal disease with hypertension and massive proteinuria, patients with IUGR remain normotensive and healthy. Recently, levels of activin A and inhibin A were demonstrated to be significantly elevated in the blood circulation of women who have developed preeclampsia (Bersinger et al. 2002; Muttukrishna et al. 1997, 2000). The expression of inhibin-α subunit, β_A, β_B and β_C subunits, follistatin, betaglycan and activin receptor genes were demonstrated in placental tissue from both uncomplicated term pregnancies and term pregnancies with preeclampsia (Casagrandi et al. 2003), supporting the results of elevated of inhibin A and activin A levels in maternal serum (Muttukrishna et al. 2000) and placental homogenates (Bersinger et al. 2002).

However, data on IUGR pregnancies are limited. In a recent publication, high maternal serum inhibin A and activin A levels were reported in an IUGR pregnancy (Greenwood et al. 2001). Although no markedly elevated circulating levels of activin A and inhibin A were observed in women with IUGR, analysis of the longitudinal data suggested that activin A levels may be modestly increased (Keelan et al. 2002). Additionally, small fetuses did not significantly modify the inhibin and activin concentrations in pregnancies complicated by preeclampsia (D'Antona et al. 2000; Florio et al. 2002). Furthermore, inhibin and activin concentrations in fetal circulation levels did not change in presence of IUGR (Debieve et al. 2000). However,

comparing activin A levels in constitutionally small fetuses, IUGR restricted fetuses and preeclampsia associated with IUGR, significantly lower activin A was observed in constitutionally small fetuses pregnancies (Wallace et al. 2003). However, it seems that activin A is increased in IUGR pregnancies, mainly in presence of abnormal umbilical artery Doppler waveforms (Bobrow et al. 2002). Our histological data suggest an increased production of the inhibin-α subunit in syncytiotrophoblast, thus resulting in higher inhibin secretion. In extravillous trophoblast cells an opposite phenomenon is observed with a lower α-subunit production resulting probably in a higher activin formation. The lower expression of inhibin-β_B subunit in extravillous trophoblast cells suggests an important function of inhibin B and/or activin B during pregnancy, although serological data regarding these proteins are still missing. Although the combined down-regulation of the α- and β_B-subunits most likely indicates a down-regulation of inhibin B in the extravillous trophoblast, other possibilities cannot be excluded. It is for example still quite unclear if and to what extent the newly cloned additional β-subunits (β_C and β_E) might bind to inhibin-α, resulting in a putative inhibin C and inhibin E molecule. Additionally, it also unclear to what extent activin secretion is being modulated by the distinct subunits. Since there are three known activins (activin A, B and AB) and further seven theoretically possible activins (activin C, E, AC, AE, BC; BE or CE), an assumption on which activin form is primarily being produced is quite impossible. Additionally studies are needed to clarify the binding capacities of these subunits and also elucidate mechanisms that might influence the selective inhibin and activin formation.

Since inhibin A and activin A play a crucial role in the differentiation of proliferative cytotrophoblast into invasive trophoblast (Caniggia et al. 1997), the increased inhibin A and activin A production and secretion might have a compensatory role (Florio et al. 2002). This hypothesis is very intriguing, since activin A has been associated with fetal hypoxia and modulation of vascular endothelial cells, since vascular endothelium is a potent source of activin A (Phillips et al. 2001). The possibility that activin may influence angiogenesis is based on its function as a growth inhibitor of vascular endothelial cells (McCarthy and Bicknell 1993). Recently, a differentially expression of eNOS, iNOS, ERα and ERβ by human extravillous trophoblast cells was associated with lower NO-output and impaired trophoblast invasion, providing evidence that the reduced expression of these proteins is related to IUGR (Schiessl et al. 2005). Therefore, activin A might be compensatory secreted from human placental tissue to remodel feto-placental blood flow, especially within the view that in IUGR pregnancies the cytotrophoblast invasion is restricted and limited remodelling of the spiral arteries occurs (Lim et al. 1997). Additionally, preterm newborns with signs of perinatal hypoxia at birth have increased activin-A levels, suggesting that activin-A may reflect indirectly intrauterine hypoxia (Florio et al. 2003). Interestingly, placental and/or fetal activin secretion is increased as a result of feto-placental hypoxia in vitro in sheep (Jenkin et al. 2001a, 2001b). Therefore, an increase of activin A might be a mechanism regulating feto-placental blood flow, suggesting that activin A could be a useful marker for assessing fetal hypoxemia, at least in sheep species (Jenkin et al. 2001b). However, it is quite unclear if this is also true for humans.

One of the difficulties of this study was to obtain placental tissue from normal and IUGR pregnancies adjusted to gestational age. Pregnancies complicated with IUGR, in contrast to small-for-gestational-age (SGA) pregnancies, are delivered preterm due to susceptibility of fetal cardiotocography (CTG) or abnormal umbilical artery Doppler waveforms. Since it is still quite unclear if placental gestation can influence inhibin/activin production, carefully acquirement of normal placental tissue samples was envisaged. Therefore, tissue specimens of normal pregnancies were obtained at the course of an elective cesarean section for breech presentation during the 38th week of gestation to avoid any influencing factors due to the physiological ''stress'' during normal delivery. However, this is a major issue, since the observed differences can be either due to the disease or due to the gestation of the tissue and further investigations are warranted to elucidate these questions.

In conclusion, all three inhibin/activin subunits were expressed by normal and IUGR placental tissue. We observed a significant lower expression of the inhibin-α subunit in IUGR extravillous trophoblast cells compared to normal pregnancies, while the inhibin-α immunostaining was significantly upregulated in syncytiotrophoblast. Additionally, we demonstrated a significant down-regulation of inhibin-β_B subunit in extravillous trophoblast cells. We also observed a co-localisation of inhibin-α and the β-subunits, 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 local aspects like angiogenesis and tissue remodelling.

Acknowledgements We would like to thank the medical doctors of the 1st Department of Obstetrics and Gynaecology—Ludwig-Maximilians-University Munich for obtaining the placental samples. Additionally, we would like to thank Dr. N. Shabani—1st Department of Obsterics and Gynaecology—-Ludwig-Maximilians-University Munich for evaluating the placental tissue samples and Dr. U. Bierl—DSL Deutschland GmbH—Sinsheim Germany for his support in conducting this study. This study was financially supported in part by FöFoLe (Nr. 297/03)—Ludwig-Maximilians-University Munich, Munich, Germany and the Weigland Foundation Ludwig-Maximilians-University Munich, Munich, Germany for I. Mylonas.

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