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Placental endoglin levels in diamniotic-monochorionic twin gestations: Correlation with clinical and placental characteristics

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ABSTRACT

Objective: While endoglin has been implicated in the pathogenesis of various complications in singleton pregnancies, its potential contribution to complications of monochorionic twinning remains largely undetermined. The aim of this study was to determine the correlation between relevant clinical and pathological variables and placental endoglin levels in diamniotic-monochorionic twin pregnancies.
Methods: Endoglin expression was studied by immunohistochemistry and Western blot in a prospective cohort of 68 non-TTTS and 7 TTTS monochorionic twin placentas. Placental endoglin levels were correlated with clinical and placental characteristics associated with twin-to-twin transfusion syndrome (TTTS) and selective growth restriction, including birth weight discordance, uneven placental sharing, peripheral cord insertion and choriovascular anatomy.
Results: In non-TTTS gestations discordant for these criteria, placental endoglin levels were significantly higher for the twin with smaller birth weight, intrauterine growth restriction, and/or abnormal ultrasound Doppler studies than for the more normal co-twin. Similarly, placental endoglin levels were significantly higher in the placental territory with smaller share and/or peripheral cord insertion in cases discordant for these placental characteristics. In TTTS gestations, placental endoglin levels tended to be higher for donor twins than for recipients. There was no correlation between endoglin levels and superficial choriovascular anastomoses.
Conclusions: While the exact functional implications remain to be determined, our findings suggest a strong correlation between unbalanced placental endoglin levels and intertwin growth discordance in monochorionic twins.

1. Introduction

Endoglin (CD105), a transmembrane accessory receptor for transforming growth factor (TGF)-β, has emerged as a pivotal regulatory component of TGF-β signaling in vascular endothelial cells [1,2] and is implicated as an important regulator of cardiovascular development, angiogenesis and vascular remodeling [2–4]. Endoglin is predominantly expressed in proliferating endothelial cells [5–7]. In addition, endoglin is weakly expressed in selected non-endothelial cell types including stromal cells [8], mesenchymal cells (fibroblasts and vascular smooth muscle cells) [9], and some hematopoietic cells [10,11]. In the placenta, membrane-bound endoglin is expressed at high levels in the syncytiotrophoblast and intermediate type invading cytotrophoblasts and, to lesser extent, capillary endothelium [12,13].

Endoglin exists in membrane-bound and soluble forms. The most abundant form, membrane-bound full length L-endoglin, is pro-angiogenic and has a role in regulation of vascular tone. Another membrane-bound isoform, S-endoglin, is generated by alternative splicing of the same gene [14]. A soluble form of endoglin, sEng, represents an NH_2-terminal cleavage product of full length membrane-bound endoglin [15]. Both the membrane-bound short isoform (S-endoglin) and the soluble endoglin (sEng) are involved in several pathological conditions and play opposite antiangiogenic roles with respect to the predominant membrane-bound L-endoglin isoform [1,16].

Recent clinical and experimental studies suggest endoglin, in particular in the form of sEng, may play a role in the pathophysiology of pregnancy complications such as preeclampsia and small for gestational age/intrauterine growth restriction (IUGR) [15,17–22][reviewed in [23]]. The potential role of endoglin in the

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pathogenesis of complications of monochorionic twinning remains largely undetermined. Monochorionic twin gestations are associated with a high risk of poor pregnancy and perinatal outcome compared with dichorionic gestations [24,25]. A significant portion of the excess perinatal or neonatal morbidity and mortality in monochorionic pregnancies is attributable to severe chronic twin-to-twin transfusion syndrome (TTTS), a serious condition characterized by a gradual shift of blood from donor twin to recipient twin through placental vascular connections between the fetuses. TTTS complicates approximately 9–15% of diamniotic-monochorionic twin pregnancies [25,26] and is clinically defined by amniotic fluid discordance (twin oligohydramnios-polyhydramnios sequence, TOPS), often associated with body weight discordance between donor and recipient twins. In addition to TTTS, 12.5–36% of all monochorionic pregnancies are affected by 'selective' or isolated severe intertwin birth weight discordance, not occurring in the context of TTTS. Selective intrauterine growth restriction is associated with increased perinatal mortality and morbidity rates, most often involving the smaller twin [26–28].

Placental anatomic features have been linked to an increased risk of TTTS and birth weight discordance in monochorionic pregnancies. We [29] and others [30–33] have previously described the placental anatomic markers of TTTS, which include peripheral cord insertion of at least one twin, uneven placental sharing, and characteristic choriovascular patterns (absence of superficial artery-to-artery (AA) anastomoses and presence of vein-to-vein (VV) anastomoses). Birth weight discordant placentas similarly have an increased frequency of peripheral cord insertion and uneven placental sharing [32–35], whereas their placental angioarchitecture appears to be similar to that of control monochorionic twin placentas [34].

While their association with an increased risk of TTTS and selective birth weight discordance has been well documented, the exact mechanisms whereby these placental anatomic and choriovascular features predispose to these twin pregnancy complications remain incompletely understood. Furthermore, not all cases of TTTS and selective growth restriction display aberrant placental or choriovascular anatomy [29,34], prompting the need to consider potential alternative pathogenic mechanisms for these conditions. Urged by recent reports implicating endoglin dysregulation in severe complications in singleton pregnancies [15,17–22], the aim of the present study was to perform a comprehensive analysis of placental endoglin levels in diamniotic-monochorionic twin pregnancies, focusing on the placental and clinical correlates of intertwin endoglin discordance. Elucidation of the correlation between critical clinical and placental characteristics of monochorionic twinning complications and placental endoglin levels may provide a deeper insight into the regulation and potential function of placental endoglin expression in monochorionic twin gestations in general and, more specifically, in twin gestations complicated by TTTS or selective growth restriction.

2. Materials and methods

2.1. Patient population

A prospective cohort of 88 consecutive diamniotic-monochorionic twin placentas was examined at the Department of Pathology at Women and Infants Hospital between late 2009 and mid-2011. Placentas with remote (>48 h prior to delivery) fetal demise of one twin (4, of which 2 from TTTS pregnancies), laser treatment for TTTS (5) or incomplete clinical information (4) were excluded from this study. The remaining 75 placentas (68 non-TTTS and 7 non-laser-treated TTTS) form the basis of this study. The accompanying charts were reviewed for relevant maternal and fetal information, such as gestational age, maternal age, pregnancy complications (eg preeclampsia), ultrasound Doppler velocimetry results, presence of intrauterine growth restriction (<10th percentile for age) [36], and birth weights of both twins. Severe birth weight discordance was defined as a birth weight difference of ≥20%. The presence or absence of TTTS, defined by the sonographic determination of twin oligohydramnios/polyhydramnios sequence (TOPS) in a monochorionic gestation, was based on well-established criteria [37]. TTTS severity was graded according to Quintero staging criteria [38]. This study was approved by the Institutional Review Board.

2.2. Processing of the placenta

Gross examination of the placenta was performed as previously described in detail [34,39,40]. The type of cord insertion was categorized as paracentral, marginal or velamentous. Velamentous cord insertion was defined as cord insertion into the fetal membranes rather than onto the placental disc. Marginal cord insertion was defined as cord insertion at the edge of the placental disc. Paracentral cord insertion was defined as cord insertion on the placental disc proper, and is used as an umbrella term for central, paracentral and eccentric (non-marginal) types of cord insertion. Chorionicity was confirmed by microscopic examination of the dividing membrane in all cases.

Immediately upon receipt in the Department of Pathology, placental parenchyma from three to five randomly selected areas from each individual twin territory was sampled for subsequent Western blot analyses. These samples were obtained from villous parenchyma lateral to the respective cord insertions, at a distance from the vascular equator to avoid inclusion of shared cotyledons. Placental samples were placed in RNA*later* (Ambion Inc., Austin, TX) and stored at −20 °C. Areas of placental parenchyma with calcification, infarction, fibrin deposition or hemorrhage were avoided. Further examination of the placenta, including injection of the chorionic vasculature, proceeded as described previously [34,39,40]. For histological and immunohistochemical studies, tissues were obtained from at least 5 randomly selected areas per twin territory. These tissues were formalin-fixed and paraffin-embedded according to standard methods.

2.3. Immunohistochemical analysis

Sections of placenta were processed for avidin-biotin-immunoperoxidase staining using anti-endoglin antibody (clone SN6h; DakoCytomation, Inc., Carpinteria, CA). Binding was detected with 3,3′-diaminobenzidine tetrachloride (DAB). Sections were counterstained with Mayer's hematoxylin, cleared and mounted. Controls for specificity consisted of incubation with isotype IgG instead of anti-endoglin antibody, which abolished all immunoreactivity.

2.4. Western blot analysis

Placental endoglin protein levels were evaluated by Western blot analysis of placental lysates according to methods described in detail elsewhere [41], using anti-endoglin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA; and Epitomics, Burlingame, CA) under reducing or non-reducing conditions.

2.5. Data analysis

Values are expressed as mean ± standard deviation (SD) or median (range). The significance of differences between groups was determined by Student *t*-test, Mann–Whitney *U*-test, ANOVA with post-hoc Scheffe test, Fisher's exact test, or Wilcoxon matched pairs signed rank test, where applicable. Data were analyzed and graphically represented using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA). Data depicted in modified (Tukey) box plots reflect group median, upper and lower quartiles (box), maximum and minimum values excluding outliers (whiskers), and outliers (more than 3/2 times upper quartile). The significance level was set at $P < 0.05$.

3. Results

3.1. Western blot analysis of placental endoglin levels

Placental endoglin levels were assessed by Western blot analysis of lysates of placental tissue sampled from each twin territory. Western blot analysis under non-reducing conditions and using a monoclonal anti-endoglin antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) produced a 180 kDa band (Fig. 1A), as previously reported by others [42] and consistent with the dimeric membrane-bound form of endoglin. Incubation with a different anti-endoglin antibody (rabbit polyclonal anti-endoglin antibody, Epitomics, Burlingame, CA) under reducing conditions produced a 90 kDa band, corresponding to the monomeric integral membrane-bound protein (Fig. 1B), as previously reported by others [15,21,43]. Robust correlation in immunoreactivity between the 180 kDa band in non-reducing conditions and the 90 kDa band in reducing conditions was found in all samples studied (Fig. 1A–B). The polyclonal