Placenta 35, Supplement A, Trophoblast Research, Vol. 28 (2014) S93–S99

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Review: The enigmatic role of endoglin in the placenta☆

A.L. Gregory [a,b], G. Xu [a,b,1], V. Sotov [a,b], M. Letarte [a,b,*]

[a] Molecular Structure and Function Program, Hospital for Sick Children, Department of Immunology, Canada
[b] Heart & Stroke Richard Lewar Centre of Excellence, University of Toronto, Canada

ARTICLE INFO

Article history:
Accepted 31 October 2013

Keywords:
Vascular endothelium
TGF-beta
BMP9
Preeclampsia
Endoglin
Endothelin-1

ABSTRACT

The cellular expression, structure and function of endoglin, and its implication in several vascular disorders remain enigmatic, even 30 years after its discovery. Endoglin (CD105) is a homodimeric glycoprotein (180 kDa) constitutively expressed in the vascular endothelium. It is essential for cardiovascular development and mutations in the *ENG* gene lead to Hereditary Hemorrhagic Telangiectasia, a disorder characterized by arteriovenous malformations. Endoglin is also expressed in the syncytiotrophoblast throughout pregnancy, but transiently upregulated in the extravillous trophoblast of anchoring villi. Endoglin modulates responses to several TGF-β superfamily ligands and is essential for the negative regulation by TGF-β isoforms 1 and 3 of extravillous trophoblast differentiation. Membrane endoglin binds endothelial NO synthase and regulates its activation and vasomotor tone. There is also a circulating soluble form of endoglin (sEng; 65 kDa); its levels in the serum of women with preeclampsia are increased and correlated with disease severity. The exact sequence of sEng is still unresolved and the proposed mechanism of release from the syncytium by metalloproteases would not yield the expected size protein. The nature of the ligand sequestered by sEng is also an enigma. sEng is said to block the effects of TGF-β on NO-mediated vasorelaxation. However, sEng alone cannot scavenge these ligands for which it has very low affinity. sEng binds with high affinity to BMP9, which stimulates secretion from endothelial cells of the vascoconstrictor endothelin-1, also implicated in endothelial cell stabilization. It remains to be determined if scavenging of circulating BMP9 by sEng is important in preeclampsia and regulation of hypertension.

1. Introduction

There are now close to 1300 papers on endoglin listed on PubMed, thirty years after its discovery. Its constant versus transient expression pattern on a restricted number of cell types, the ability of membrane endoglin to interact with multiple ligands of the TGF-β superfamily, the exact sequence of the soluble protein (sEng), its mechanism of production from the placenta and its ligand specificity, are all fascinating but remain controversial aspects of endoglin biology. We will review endoglin distribution, structure, ligand specificity, and role in the vascular endothelium, particularly in the context of normal pregnancy and preeclampsia.

2. Distribution and function of endoglin

2.1. Vascular endothelium

We first identified endoglin with the monoclonal antibody 44G4, raised against cell surface proteins prepared from a childhood leukemia cell line [1]. It soon became apparent that this glycoprotein was found in blood vessels and it was later classified as the endothelial marker CD105 [2]. Activation of endothelial cells leads to increased expression of endoglin, in response to an angiogenic or inflammatory stimulus [3,4]. Endoglin is in fact widely used as a marker of tumor angiogenesis [3,5]. Although there is higher endoglin on dividing cells and none on senescent cells, it is not required for proliferation as endoglin-null endothelial cells divide faster than normal cells [6]. Endoglin is likely important for maintaining vascular homeostasis and quiescence of the vascular endothelium. In fact, mutations in the *ENG* gene lead to Hereditary Hemorrhagic Telangiectasia (HHT) type 1 [7], a vascular

☆ This research was funded by grant # NA 7093 from the Heart and Stroke Foundation of Canada.

* Corresponding author. Hospital for Sick Children, 555 University Ave, Toronto M5G1X8, Canada. Tel.: +1 416 813 6258; fax: +1 416 813 7877.
E-mail addresses: allison.gregory@medportal.ca (A.L. Gregory), guoxiongxu@hotmail.com (G. Xu), valentin.sotov@mail.utoronto.ca (V. Sotov), michelle.letarte@sickkids.ca (M. Letarte).
[1] Present address: Center Laboratory, Jinshan Hospital, Fudan University, Shanghai 201508, China.

0143-4004/$ – see front matter © 2013 Published by IFPA and Elsevier Ltd.
http://dx.doi.org/10.1016/j.placenta.2013.10.020

dysplasia characterized by arteriovenous malformations and severe and frequent bleeding episodes. The disease is due to haploinsufficiency and reduced functional levels of endoglin [8]. We demonstrated that the underlying defect is at least in part due to endothelial dysfunction caused by disrupted association of endoglin with endothelial NO synthase (eNOS), leading to generation of superoxide and impaired vasomotor function [9].

2.2. Mesenchymal and hematopoietic cells

Endoglin is expressed in mesenchymal stem cells [10] and is required for hemangioblast and early hematopoietic development [11,12]. Endoglin is absent from most immune cell subsets but is induced by activation of monocytes into macrophages [13]. The role of endoglin may be to conserve stem cell potential, versus differentiation of precursor cells, via its modulation of TGF-β and BMP effects on lineage determination. TGF-β acts as a potent immunosuppressive agent by inhibiting cell growth, inducing apoptosis and contributing to the generation of T regulatory cells. Therefore the role of endoglin may be to tightly regulate cell differentiation and activation by TGF-β superfamily members.

A striking example of transient expression of endoglin is during the endocardial-mesenchymal transformation that leads to heart septation and valve formation [14]. Endoglin-null embryos die at mid-gestation due to defects in vessel and heart development [15], indicating that endoglin is essential for both angiogenesis and heart development. Endoglin is also present on fibroblasts at the edge of a wound and on perivascular stromal cells involved in vascular remodeling [16]. Additionally, endoglin is found on vascular smooth muscle cells, particularly after arterial injury, and could mediate the effects of TGF-β on their migration and tissue repair [17].

2.3. Placenta

The human placenta is an abundant source of endoglin. We first purified endoglin from human term placenta, using monoclonal antibody 44G4; this led to cloning of the *ENG* gene from a placental library [18]. Immunostaining revealed expression of endoglin in the syncytiotrophoblast of term placenta [19], where it is actually present throughout pregnancy. Endoglin is also transiently expressed in extravillous trophoblast (EVT) in the proximal columns of anchoring villi [20]. We showed previously that monoclonal antibody 44G4, or antisense oligonucleotides to endoglin, stimulated budding and outgrowth of EVT from villous explants at 5–8 weeks of gestation [21], a process that is negatively regulated by TGF-β1/β3 [22]. Fibronectin synthesis and changes in integrin expression also occur during EVT differentiation. Furthermore, the distribution of endoglin on EVT parallels that of the α5β1 integrin [20], which was recently shown to interact with endoglin [23]. The interaction is mediated by the RGD peptide of human endoglin or the equivalent TDD motif found in pig and mouse endoglin [18,23]. We propose that the concurrent induction of endoglin and α5β1 integrin on budding EVT and their RGD – mediated interaction may contribute to the regulation of EVT differentiation. The subsequent decrease in these proteins in the distal columns may facilitate remodeling of spiral arteries, as reduced endoglin expression is associated with vasorelaxation [9].

3. Structure of membrane and soluble endoglin

3.1. Membrane endoglin

Human endoglin is an integral membrane glycoprotein, composed of an N-terminal signal peptide, an orphan domain, a zona pellucida (ZP) domain, a juxtamembrane region, a transmembrane region and a short cytoplasmic domain [18,24] as shown in Fig. 1. The protein is highly glycosylated with five potential N-linked sites within the orphan domain and O-glycan sites mostly in the ZP domain. Enzymatic removal of all the sugars leads to an observed reduction of about 25 kDa in molecular mass [25]. Membrane endoglin exists as a disulphide-linked homodimer of 180 kDa that contains 17 cysteine residues [18]. However the position of the inter-chain disulphide bond(s) has yet to be established. It was suggested that Cys582 is responsible for inter-chain bonding although this residue is not conserved in all species. Studies of a CD31-endoglin hybrid molecule indicated that cysteine residues within the fragment Cys330–Cys412 were implicated [26]. Our own studies showed that Eng1–357 is expressed as a dimer, leaving Cys330 or Cys350 as the most likely residues involved in dimer formation (unpublished data).

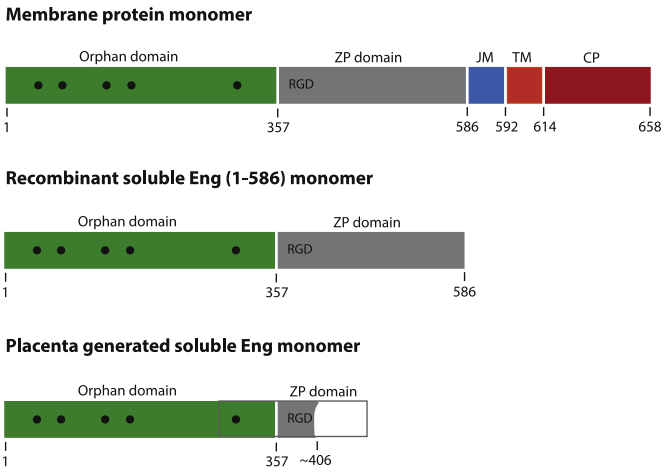

Fig. 1. Schematic diagram of membrane, recombinant and soluble endoglin. Membrane endoglin is an integral dimeric glycoprotein composed of two identical monomers of 90 kDa linked by disulphide bonds. The polypeptide is composed of an orphan domain with no known structural features (27–357; residues 1–26 representing the signal peptide) and contains five N-linked glycosylation sites (●). The orphan domain is followed by a zona pellucida domain (ZP; 358–586), and by juxtamembrane, transmembrane and short cytoplasmic domains. The recombinant soluble monomeric protein used in most studies corresponds to the complete extracellular domain of endoglin and has a molecular mass of 80 kDa (1–586). The soluble endoglin (sEng) monomer released from placenta into the maternal serum has a molecular mass of 65 kDa and its C terminal residue is likely shortly after Arg 406, within the ZP domain.

3.2. Soluble endoglin

Several studies reported the increased expression of a circulating form of endoglin in serum, plasma or other fluids from cancer patients, as summarized in a review by Bernabeu et al. [5]. This form is referred to as soluble endoglin (sEng), although its true solubility has not been established and it could be associated with other proteins [27]. A 80 kDa sEng was recently shown to be cleaved from the surface of human umbilical vein endothelial cells by MMP14 (or MT1-MMP); the cleavage site was shown to be at position 586, implying that the whole extracellular domain (Eng1–586) was released [28]. However it remains to be proven that a similar mechanism occurs in tumors. For several years, we (and others) [24,29–31] have used the Eng1–586 construct (Fig. 1) to generate recombinant sEng; this form is soluble to some degree but has not proven satisfactory for structural studies, implying that it might not represent a functional physiological sEng, with a stable structure.

A soluble form of endoglin is also present in increased amounts in the serum of women with preeclampsia (PE) [29]. It begins to rise