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Signaling Cross-Talk between Transforming Growth Factor-Beta and Bone Morphogenic Protein pathways in Human Endometrial and Endometriotic Cells

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Abstract: ***Transforming growth factor-beta (TGF-β) and bone morphogenic protein (BMP) signaling pathways are involved in the vast majority of cellular processes and are fundamentally important during the entire life of all metazoans. TGF-βs and BMPs transduce their signals via canonical Smad-dependent pathways which involve TGF-β/BMP ligands, receptors and Smad molecules. Also, non-canonical Smad-independent signalling pathways are involved in TGF-β/BMP signal transduction. Here we investigated signalling cross-talk between the pathways downstream of TGF-β and BMP signalling in endometrial and endometriotic cells. Treatment of endometrial and endometriotic stromal and epithelial cells with TGF-β1 or TGF-β2 increased secretion of plasminogen activator inhibitor 1 (PAI-1) dramatically in all cell lines. Of note, higher PAI-1 secretion was observed in endometriotic cells compared to endometrial cells. Both a transforming growth factor-beta receptor-1 (TβRI) and a Bone morphogenic protein receptor-1(BMPR1) inhibitors completely blocked the TGF-β-induced PAI-1 secretion in all cell lines while a specific inhibitor of Smad3 (SiS3) had a partial effect on PAI-1 secretion in all cell lines. Additionally, we showed that Activin Receptor-Like kinase (ALK-2) is the main BMP receptor that is responsible for complete blockage of TGF-β-induced PAI-1 secretion whereas, ALK-3 and ALK-6 exhibited partial effects on PAI-1 secretion. In summary, the complete inhibition of TGF-β-induced PAI-1 secretion by a general BMPR1 and ALK-2 inhibitors suggest a possible cross-talk between TGF-β and BMP pathways. Since only ALK-2 had a complete decrease of TGF-β-induced PAI-1 secretion compared to ALK-3 and ALK-6 in all cells, then our results demonstrate the importance of ALK-2 as a point of cross-talk of TGF-β and BMP pathways. Furthermore, we showed TGF-β-induced phosphorylation of Smad1 in all cells upon TGF-beta treatment. Our result further confirms that the Smads are the most important intracellular transducers of TGF-β and BMP signals***

Keywords: Endometriosis, Transforming growth factor-betas, Bone morphogenic protein, Activin Receptor-Like kinase

Introduction

The transforming growth factor-beta (TGF-β) family of cytokines, including TGF-β, bone morphogenic proteins (BMPs), and activin/inhibin, plays crucial roles in embryonic development, adult tissue homeostasis and the pathogenesis of a variety of diseases (Chang et al., 2002; Peng, 2003). TGF-βs comprise three isoforms namely TGF-β1, TGF-β2 and TGF-β3. Biologically, TGF-βs regulate cell motility, proliferation, apoptosis, gene expression and differentiation. In addition, TGF-βs tightly regulate production of the extracellular matrix (ECM) and are involved in wound healing and immunosupression (Roberts and Sporn, 1993; Roberts, 1998; Kaminska et al., 2005; Taylor, 2009). They are also involved in tumorigenesis and inflammation (Padua and Massagué, 2009; Santibañez et al., 2011).

The TGF-β family receptors are divided into three groups, namely type I, type II and type III receptors. The three receptor types have distinct properties (Heldin et al., 1997; Chang et al., 2002). TGF-β receptor type I (TβRI) and type II (TβRII) are transmembrane serine/threonine kinases. The type III receptors, betaglycan (TβRIII) and endoglin are accessory receptors and have high affinity to all three TGF-β isoforms (Wrana et al., 1994; Gordon et al., 2008).

The general mechanism of TGF-β signaling starts by TGF-β binding either to TβRIII, which presents it to TβRII, or binding to TβRII directly, which then binds to and transphosphorylates TβRI. Then the activated TβRI phosphorylates Smad2 or Smad3, which bind to Smad4 in the cytoplasm or the nucleus forming a Smad complex. The Smad complex interacts with transcription factors in the nucleus to regulate TGF-β responsive genes (Chen et al., 2003; Guglielmo et al., 2003; Biondi et al., 2007; Wrighton et al., 2009).

The BMPs are multifunctional proteins that regulate functions such as proliferation, apoptosis and differentiation of a large variety of cell types (Reddi, 1997). BMPs mediate their cellular functions through binding to a combination of type I and type II receptor serine/threonine kinases (Kawabata et al., 1998). The BMP ligands can bind to any of the three type II receptors (BMPRII, ActRIIa and ActRIIb) which then bind to one of the three type 1 receptors (ALK-2, ALK-3 and ALK-6). Upon binding, the constitutively active type II receptor phosphorylates type 1 receptor then phosphorylates the BMP-responsive Smad proteins namely Smad1, Smad5 and Smad 8. The activated Smads bind Smad4 either in the cytoplasm or in the nucleus for signaling (Yu et al., 2008). In addition, BMP signals have been found to activate other intracellular effectors like mitogen-activated protein kinase (MAPK) p38 via the Smad pathway (Nohe et al., 2004).

Although TGF-βs transduce their signals through activation of Smad2 and Smad3 (Chen et al., 2003), recent studies have indicated that they can also strongly but only transiently induce phosphorylation and activation of Smad1, Smad5 and Smad8

(BMP-responsive Smads) in endothelial cells, epithelial cells, fibroblasts and epithelium derived cancer cells (Bharathy et al., 2008; Daly et al., 2008; Liu et al., 2009). These observations have raised several questions of how the activation of Smads1/5/8 by TGF-βs affect BMP responses (Grönroos et al., 2012).

Several hypothesis have been put forward to explain the possible crosstalk of TGF-βs/BMP pathways, for example Grönroos et al., 2012 suggested the involvement of ALK-5 and formation of pSmad3-pSmad1/5 complexes. Recently, knockdown of Smad3 phosphorylation in mice abolished the ability of TGF-β to inhibit BMP-induced transcription (Grönroos et al., 2012), further supporting a possible crosstalk between TGF-β and BMP pathways.

Perturbations of both BMP and TGF-β signalling have been reported to cause distinct bone diseases (Jansens et al., 2000). In endothelial cells, ALK-1 together with ALK-5 can activate TGF-β-responsive Smads (Smad2/3) and also phosphorylate BMP-responsive Smads (Smad1/ 5/ 8; Miyazono and Kusanagi, 2001). Furthermore, Smad5-deficient mice exhibit defects in vascular tissues (Chang et al., 1999), which are similar to those observed in ALK-1 deficient mice. These observations suggest that Smad5 is a downstream target of ALK-1 (Miyazono and Kusanagi, 2001). In addition, molecules that repress both TGF-βs and BMPs have been found to be involved in the pathogenesis of vascular diseases (Miyazono and Kusanagi, 2001), hence indicating a possible crosstalk of both pathways. What remains to be investigated is the exact location of cross-talk of the TGF-β and BMP pathways (e.g. at the extracellular membrane, in the cytoplasm or in the nucleus) and which molecules are involved (e.g. the individual BMP/TGF-β receptors or Plasminogen Activator Inhibitor 1 (PAI-1) secretion among other proteins. These will be helpful in determining the cross-talk of TGF-β and BMP pathways and understand their possible role in the pathophysiology of endometriosis.

Materials and Methods

Cell lines

We utilized four cell lines which represent the stromal and epithelial compartment of human eutopic and ectopic endometrium. The HES and T-HESC cells which represents the epithelial and the stromal cells have been isolated from normal endometrium and they show typical endometrial characteristics (Desai et al., 1994; Krikun et al., 2004; Lee et al., 2010). The 12Z and 22B cells which represents epithelial and stromal cells have been obtained from active peritoneal lesions and they show typical features of active phase of endometriosis (Zeitvogel et al., 2001). Thus, both endometrial and endometriotic cells lines above are suitable for studying the pathogenesis of endometriosis (Lee et al., 2010).

PAI-1 secretion and Smad1 phosphorylation

$4x10^5$ cells were seeded into 6-well plates (TPP, Switzerland) in DMEM high glucose or DMEM/F12 media (+ 10% FCS). After culturing overnight (37°C, 5% CO_2), cells were starved in fresh medium (+ 1% FCS) for 6 hours. After removal of the old medium, fresh medium containing 5μM a BMP inhibitor, LDN 193189 (Stegment, USA; Yu et al., 2008), 5μM LY364947 (Sigma-Aldrich, USA; Sawyer et al., 2003), 2μM SiS3 (Calbiochem, USA; Masatoshi et al., 2005), 10 μg/ml ALK-2 inhibitor (R&D Systems, USA; Wu and Hill, 2009), 4 μg/ml ALK-3 inhibitor (R&D Systems, USA; Wu and Hill, 2009), 6 μg/ml ALK-6 inhibitor(R&D Systems, USA; Kawabata et al., 1998) and 2 μg/ml IgG1 inhibitor (R&D Systems, USA; Kawabata et al., 1998), respectively, were prepared inhibitors, respectively, was added into the corresponding wells, respectively. After cells were incubated for 2 hours (37°C, 5% CO_2), they were stimulated with 10 ng/ml TGF-β1 or TGF-β2.

For Smad1 phosphorylation experiments, After 2 hours incubation with inhibitors as described above, the cells were stimulated with or without TGF-β1 or TGF-β2 (10ng/ml) for 30 minutes (37°C, 5% CO_2) and then the media was removed and the cells washed with ice-cold PBS. Cells were lysed and then used for phospho-Smad1 ELISA (eBioscience, USA) according to the manufacturer's instructions and quantitated with the Benchmark Reader infinite M2000 (Tecan).

For PAI-1 secretion experiments, after 2 hours incubation with inhibitors as described above, the cells were stimulated with or without TGF-β1 or TGF-β2 (10ng/ml), respectively. In the untreated controls only 1x PBS was added to the medium. Cells were cultured for up to three days (37°C, 5% CO_2).

Supernatants were collected and mixed with a Protease Inhibitor cocktail (Sigma-Aldrich, USA). After centrifugation (5000 xg, 10 min, 4°C) supernatants were aliquoted and stored at -20°C until use. Quantitation of PAI-1 protein secretion was performed by TECHNOZYM® PAI-1 Antigen ELISA Reagent Kit (Technoclone, Germany). Each ELISA was performed according to the manufacturer`s instructions and quantitated with the Benchmark Reader infinite M2000 (Tecan). Cell numbers were used for standardization and determined as described below.

Quantification of Cell Numbers

After the supernatants were removed, the cells were detached at 37°C by use of accutase. The accutase reaction was stopped by addition of equal amounts of fresh media and then 10 μl of the cell suspension was transferred to a CASY tube containing 10 ml CASY ton solution and mixed thoroughly. Quantification of cells was done with a CASY-counter (Schaerfe System, Germany).

Statistical Analysis

Each experiment was repeated at least three times in duplicate. Values are expressed as means±SEM. The significance in the data was analysed by use of non-parametric Kruskal-Wallis test (Instat GraphPad®).

Results

In order to study the signaling cross-talk between the TGF-β and BMP pathways and their role in the pathogenesis of endometriosis in vitro, we have used four cell lines which represent the stromal and epithelial compartment of human eutopic and ectopic endometrium. PAI-1 acts as one of the gold standards to study the effects of TGF-β signalling. Thus, in order to elucidate the signaling pathways involved PAI-1 secretion, we investigated Smad-dependent pathways (TGF-β and BMP) with pharmacological inhibitors. Our results showed that both TGF-β1 and TGF-β2 both strongly increased secretion of PAI-1 in all cell lines studied (Fig. 1). We utilized the inhibitors LY364947 and SiS3 which blocks the phosphorylation TBR1 and Smad 3 respectively (Peng et al., 2005). Upon addition of LY364947 to the cells together with TGF-β1 or TGF-β2 strongly decreased PAI-1 secretion completely while a SiS3 inhibitor blocked PAI-1

secretion partially in endometrial cells (Fig. 2), thus demonstrating the involvement of the Smad pathway in TGF-β-induced PAI-1 secretion in endometrial cells. Similar results were observed in the other cell lines (data not shown).

In order to investigate the signaling cross-talk between TGF-β and BMP pathways, we further investigated the effects of BMP inhibitor (LDN 193189) on secretion of PAI-1. LDN 193189 inhibits the phosphorylation of Smad1, Smad5 and Smad8 by blocking the kinase activity of BMPR1 (Yu et al., 2008). Addition of LDN 193189 to the cells together with TGF-β1 or TGF-β2 strongly decreased PAI-1 secretion to levels comparable to the untreated controls (Fig. 3), thus demonstrating that BMP pathway is involved in TGF-β-induced PAI-1 secretion in endometrial cells.

To further analyse the individual BMP receptors involved in TGF-β-induced PAI-1 secretion in endometrial cells, we utilized three ALK inhibitors (ALK-2, ALK-3 and ALK-6) separately. Our results showed that the TGF-β1-induced or TGF-β2-induced PAI-1 secretion was reduced completely by ALK-2 inhibitor in 12Z cell line (Fig. 4). ALK-3 and ALK-6 reduced TGF-β1-induced or TGF-β2-induced PAI-1 secretion by 25-40% in 12Z cell line (Fig. 5). ALK-2, ALK-3 and ALK-6 inhibitors had similar effects in all the other cell lines HES, T-HESC and 22B (data not shown). Furthermore, our results showed that stimulation with TGF-β1 or TGF-β2 strongly enhanced phosphorylation of Smad1 in all cell lines (data not shown).

Discussion

The BMPs can bind to any of the three type II receptors (BMPRII, ActRIIa and ActRIIb) and the three type 1 receptors (ALK-2, ALK-3 and ALK-6). Upon binding, the constitutively active type II receptor phosphorylates type 1 receptor. The activated type 1 receptor phosphorylates the BMP-responsive Smad proteins namely Smad1, Smad5 and Smad8. The activated Smads bind Smad4 either in the cytoplasm or in the nucleus to facilitate signaling (Yu et al., 2008).

On the other hand, TGF-βs can bind either to TβRIII, which presents it to TβRII, or binds to TβRII directly, which then binds to and transphosphorylates TβRI. Then the activated TβRI phosphorylates Smad2 or Smad3, which bind to Smad4 in the cytoplasm or the nucleus forming a Smad complex. The Smad complex interacts with transcription factors in the nucleus to regulate TGF-β responsive genes (Chen et al., 2003; Guglielmo et al., 2003; Biondi et al., 2007; Wrighton et al., 2009).

TGF-βs have been shown to play an important role in the process of menstruation and endometriosis (Pizzo et al., 2002; Gaide Chevronnay et al., 2008). Thus, TGF-βs and their receptors (TβRs) are suspected to be involved in establishment and maintenance of endometriosis (Omwandho et al., 2010). Many genes have been found to be targeted by TGF-βs but plasminogen activator inhibitor-1 (PAI-1) is most commonly analyzed and it acts as one of the gold standards to study the effects of TGF-β signalling.

Perturbations of both BMP and TGF-β signaling have been reported to cause distinct and also overlapping phenotypic bone diseases (Jansens et al., 2000). Furthermore, alterations of both TGF-β and BMP pathways have been associated with vascular diseases like pulmonary hypertension (Boeck and Dijke, 2011). These two observations clearly indicate that the two pathways might be cross-talking at some point. Thus, it might be crucial to determine whether or not a cross-talk exists between the two pathways in endometrial and endometriotic cells. This will enhance further understanding of the roles of the two pathways in the pathogenesis of endometriosis.

In our experiments, we used a general BMP inhibitor LDN-193189 selectively inhibiting the BMP type 1 receptors ALK-2, ALK-3 and ALK-6 and thus blocking BMP-mediated phosphorylation of Smad1/5/8. In addition, we used distinct inhibitors for ALK-2, ALK-3 and ALK-6 separately to determine the possible cross-talk of the TGF-β and BMP pathways upon TGF-β1 or TGF-β2 stimulation.

Our results showed that LDN-193189 as well as the ALK-2 inhibitor completely inhibited the TGF-β-induced secretion of PAI-1 in endometrial and endometriotic cells. Interestingly, both ALK-3- and ALK-6-inhibitors reduced the TGF-β-induced secretion of PAI-1 only partially. Our results suggest a cross-talk between the TGF-β and BMP pathways and also that the Smads are the most important intracellular transducers of TGF-β and BMP signals from the receptors to the nucleus.

Furthermore, we also showed for the first time that an ALK-2 inhibitor completely blocked the TGF-β-induced secretion of PAI-1 in endometrial and endometriotic cells. Our results are in agreement with those of Chen et al. (2003) and Bharathy et al. (2008) who showed that TGF-βs can also strongly but only transiently phosphorylate Smad1, Smad5 and Smad8 in endothelial, epithelial, fibroblasts and cancer-derived cells. Our results are further supported by Barnet et al. (2002) who showed that TGF-βs signals via ALK-2 and ALK-5 in chick atrial cells and Olivey et al. (2006) who implicated ALK-2 in the TGF-βs stimulated epithelial-mesenchymal transformation in mammary glands of the mouse.

Despite many studies on Smad proteins (Miyazono al., 2000; Wu et al., 2001; Itoh et al., 2001; Chen et al., 2003), there is so far no direct evidence showing the Smad1 as an intermediate protein in TGF-β signaling pathway in endometrial and endometriotic cells. Our results demonstrated that TGF-βs stimulated phosphorylation of Smad1 in endometrial and endometriotic cell lines. Thus, our results provide strong evidence that Smad1 is involved in TGF-β-dependent responses and we suppose that a possible cross-talk exists between TGF-β and BMP pathways in endometrial and endometriotic cells.

In conclusion, we were able to demonstrate ALK-2 as a possible point of cross-talk between the BMP and TGF-β pathways besides ALK-3 and ALK-6. However, further studies are required to clarify this connection between the two pathways. Nevertheless, our findings might provide new insights in understanding the potential role of TGF-β and BMP pathways in the pathophysiology of endometriosis and possible therapeutic interventions.

References

i. Barnet J.V, Desgrosellier J,S (2002). Earlier events in valvulogenesis: a signaling perspective. Birth Defects Res C. Embryo Today. 69:58-72.

ii. Bharathy S, Xie W, Yingling J, Reiss M (2008). Cancer-associated transforming growth factor β type II receptor gene mutant causes activation of bone morphogenetic protein-Smads and invasive phenotype. Cancer Res 68:1656-66

iii. Biondi CA, Das D, Howell M, Islam A, Bikoff EK, Hill CS, Robertson EJ (2007). Mice develop normally in the absence of Smad4 nucleocytoplasmic shuttling. J Biochem 404: 235-45.

iv. Boeck M, Dijke P (2011). Deregulation of BMP signalling in the pathogenesis of pulmonary hypertension. Pulmonary Hypertension-From Bench research to clinical challenges. In: Tech, (Ed) ISBN: 835-9.

v. Chang H, Huylebroeck D, Verschueren K, Guo Q, Matzuk M (1999). Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. Development 126:1631-42.

vi. Chang H, Brown CW, Matzuk MM (2002). Genetic analysis of the mammalian transforming growth factor-beta superfamily. Endocr Rev 23:787-823.

vii. Peng C (2003). The TGF-beta superfamily and its roles in the human ovary and placenta. J Obstet Gynaecol 25:834-44.

viii. Chen W, Jin W, Hardegen A (2003). Conversion of peripheral CD4+CD252 naïve T Cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 198:1875-86.

ix. Daly A, Randall R, Hill C (2008). Transforming growth factor β-induced Smad1/5 phosphorylation in epithelial cells is mediated by novel receptor complexes and is essential for anchorage-independent growth. Mol Cell Biol 28:6889-902.

x. Desai NN, Kennard EA, Kniss DA, Friedman CI (1994). Novel human endometrial cell line promotes blastocyst development. Fertil Steril 61:760-6.

xi. Gaide Chevronnay HP, Cornet PB, Delvaux D, Lemoine P, Courtoy PJ, Henriet P, Marbaix E (2008). Opposite regulation of transforming growth factors-beta2 and -beta3 expression in the human endometrium. Endocrinology 149:1015-25.

xii. Gordon KJ, Dong M, Chislock EM, Fields TA, Blobe GC (2008). Loss of type III transforming growth factor beta receptor expression increases motility and invasiveness associated with epithelial to mesenchymal transition during pancreatic cancer progression. Carcinogenesis 29:252-62.

xiii. Grönroos E, Kingston I, Ramachandran A, Randall R, Visan P, Hill C (2012). Transforming growth factor β inhibits bone morphogenetic protein-induced transcription through novel phosphorylated Smad1/5-Smad complexes. Mol Cell Biol 32:2904.

xiv. Guglielmo G, Roy C, Goodfellow A, Wrana J (2003). Distinct endocytic pathways regulate TGF-beta receptor signaling and turnover. Nat Cell Biol 5:410-21.

xv. Heldin C, Miyazano K, Tendijke P (1997). TGF-β signaling from cell membrane to nucleus through smad proteins. Nature 390:465-71.

xvi. Itoh F, Horonobu A, Kasua S, Carl H, Dijke P, Itoh S (2001). Promoting bone morphogenetic protein signaling through negative regulation of inhibitory Smads. EMBO J 15:4132-42.

xvii. Jansens K, Gershoni-Baruch R, Guanabens N, Migone N, Ralston S, Bonduelle M, Lissens W, Van Maldergen L, Vanhoenacker F (2000). Mutations in the gene encoding the latency-associated peptide of transforming growth factor β1 cause Camurati-1 Engelmann disease. Nat Genet 26:273-5.

xviii. Kaminska B, Wesolowska A, Danilkiewicz M (2005). TGF beta signalling and its role in tumour pathogenesis. Acta Biochem Pol 52:329-37.

xix. Kawabata M, Imamura T, Miyazono K (1998). Signal transduction by bone morphogenetic proteins. Cytokine Growth Factor Rev 9:49-61.

xx. Krikun G, Mor G, Alvero A, Guller S, Schatz F, Sapi E, Rahman M, Caze R, Qumsiyeh M, Lockwood CJ (2004). A novel immortalized human endometrial stromal cell line with normal progestational response. Endocrinology 145:2291-6.

xxi. Lee MK, Pardoux C, Hall MC, Lee PS, Warburton D, Qing J, Smith SM, Derynck R (2007). TGF-beta activates Erk MAP kinase signalling through direct phosphorylation of ShcA. EMBO 26:3957-67.

xxii. Liu M, Schilling H, Knouse K, Choy L, Derynck R, Wang X (2009). Transforming growth factor β-stimulated Smad1/5 phosphorylation requires the ALK-5L45 loop and mediates the pro-migratory transforming growth factor β switch. EMBO 28:88-98.

xxiii. Masatoshi J, Hironobu I, Kunuhiko T (2005). Characterization of SiS3, a novel specific inhibitor of Smad3 and its effect on TGF-β1 induced extracellular matrix expression. Molecular Pharm 69:597-607.

xxiv. Miyazono K, Duke P, Heldin C (2000). TGF-β signaling by Smad proteins. Adv Immunol 75:115-57.

xxv. Miyazono K, Kusanagi K (2001). Divergence and convergence of TGF-β/BMP signaling. J Cell Physiol 187:265-76.

xxvi. Nohe A, Keating E, Knaus P, Peterson N (2004). Signal transduction of bone morphogenetic protein receptors. Cell Signal 16:531-9.

xxvii. Olivey H, Mundell N, Austin A, Barnet J (2006). Transforming growth factor-β stimulates epithelial-mesenchymal transformation in the proepicardium. Dev Dyn 235:50-9.

xxviii. Omwandho CO, Konrad L, Halis G, Oehmke F, Tinneberg HR (2010). Role of TGF-betas in normal human endometrium and endometriosis. Hum Reprod 25:101-9.

xxix. Padua D, Massagué J (2009). Roles of TGF-beta in metastasis. Cell Res 19:89-102.

xxx. Peng C (2003). The TGF-beta superfamily and its roles in the human ovary and placenta. J Obstet Gynaecol 25:834-44.

xxxi. Pizzo A, Salmeri FM, Ardita FV, Sofo V, Tripepi M, Marsico S (2002). Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. Gynecol Obstet Invest 54:82-7.

xxxii. Reddi AH (1997). Bone morphogenetic proteins: unconventional approach to isolation of first mammalian morphogens. Cytokine Growth Factor Rev 8:11-20.

xxxiii. Roberts AB, Sporn MB (1993). A major advance in the use of growth factors to enhance wound healing. J Clin Invest 92:2565-66.

xxxiv. Roberts AB (1998). Molecular and cell biology of TGF-beta. Miner Electrolyte Metab 94: 111-19.

xxxv. Santibañez JF, Quintanilla M, Bernabeu C (2011). TGF-β/TGF-β receptor system and its role in physiological and pathological conditions. Clin Sci 121:233-51.

xxxvi. Sawyer JS, Anderson BD, Beight DW, Campbell RM, Jones ML, Herron DK, Lampe JW, McCowan JR, McMillen WT, Mort N, Parsons S, Smith EC, Vieth M, Weir LC, Yan L, Zhang F, Yingling JM (2003). Synthesis and activity of new aryl- and heteroaryl-substituted pyrazole inhibitors of the transforming growth factor-beta type I receptor kinase domain. J Med Chem 46:3953-6.

xxxvii. Taylor AW (2009). Review of the activation of TGF-beta in immunity. J Leukoc Biol 85:29-33.

xxxviii. Wrana J, Attisano L, Wieser R, Ventura F, Massague J (1994). Mechanism of activation of the TGF-beta receptor. Nature 370:341-7.

xxxix. Wrighton KH, Lin X, Feng XH (2009). Phospho-control of TGF-beta superfamily signaling. Cell Res 19:8-20.

xl. Wu S, Hu M, Chai J, Seore J, Huse M, Li C, Rigotti D, Kyin S, Muur T, Fairman R, Massague J (2001). Crystal structure of a phosphorylated Smad2. Recognition of phosphoserine by the MH2 domain and insights on Smad function in TGF-β signaling. Mol Cell 8:1277-89.

xli. Wu M, Hill CS (2009). TGF-beta superfamily signaling in embryonic development and homeostasis. Dev Cell 16:3294-43.

xlii. Yu P, Deng D, Lai C, Hong C. Cuny G, Bouxsein M, Hong D, McManus P, Katagiri T, Sachidanandan C, Kamiya N, Fukuda T. Mishina Y, Peterson R, Bloch K (2008). BMP type 1 receptor inhibition reduces heterotopic ossification. Nat Med 12:1363-9.

xliii. Zeitvogel A, Baumann R, Starzinski-Powitz A (2001). Identification of an invasive, N-cadherin-expressing epithelial cell type in endometriosis using a new cell culture model. Am J Pathol 159:1839-52.

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Figures

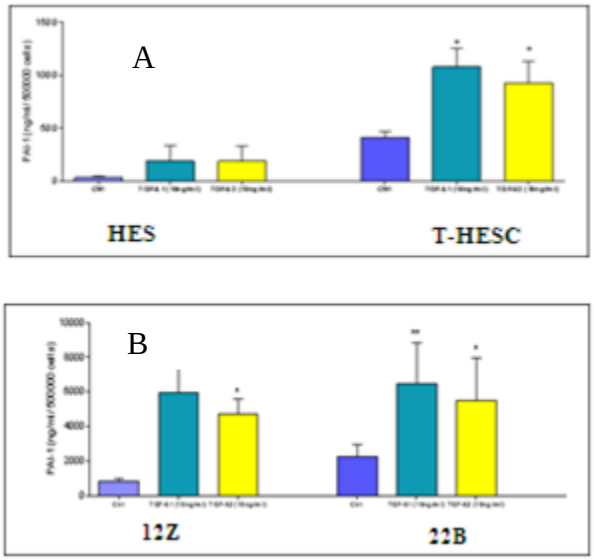

Figure 1 *Treatment of cells with TGF-β1 or TGF-β2 (10ng/ml), respectively, induced PAI-1 secretion in endometrial cells* ***(A)*** *and endometriotic cells* ***(B)*** *(*=P<0.05, **=P<0.01, n=9).*

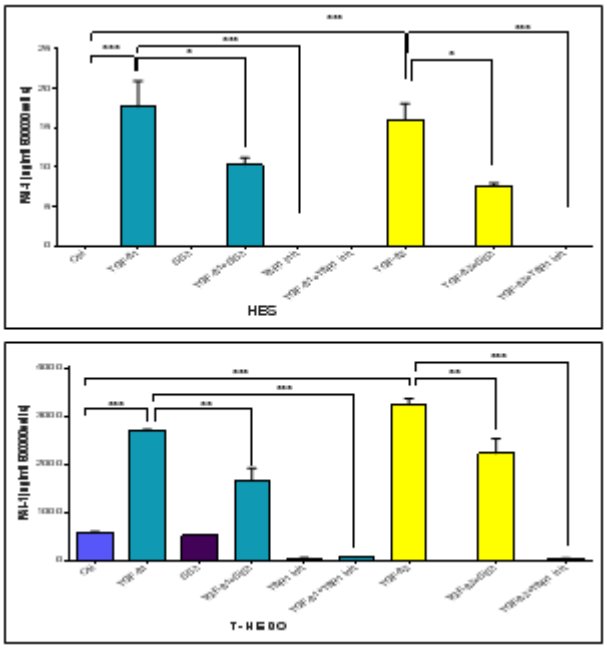

Figure 2 *Treatment of cells with TGF-β1 or TGF-β2 (10ng/ml), respectively, induced PAI-1 secretion in endometrial epithelial cells, HES and endometrial stromal cells T-HESC. The TBR1 inhibitor (LY364947) blocked TGF- β-induced PAI-1 secretion of all cells completely to control levels whereas a Smad3 (SiS3) partially inhibited the PAI-1 secretion in all cell lines (*=P<0.05, **=P<0.01, ***=P<0.001, n=9).*

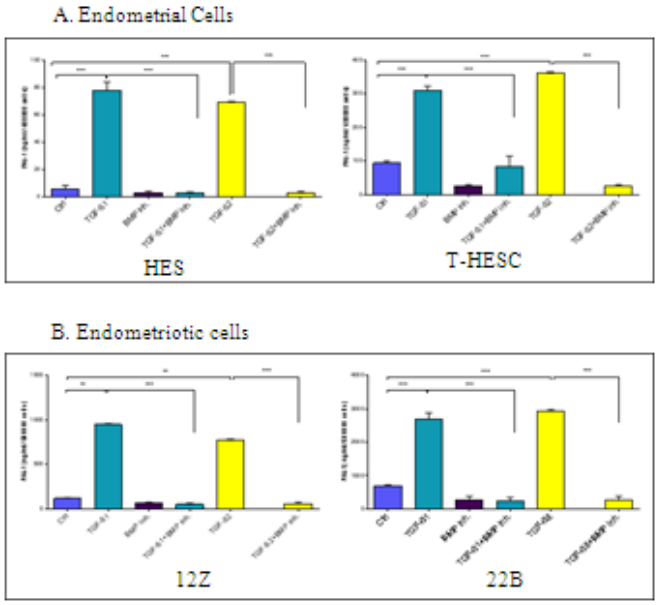

Figure 3 *Treatment of cells with TGF-β1 or TGF-β2 (10ng/ml), respectively, induced PAI-1 secretion in endometrial cells* ***(A)*** *and endometriotic cells* ***(B)****. The BMP inhibitor (LDN 193189) blocked TGF-β-induced PAI-1 secretion of all cells completely to control levels (*=P<0.05, **=P<0.01, ***=P<0.001, n=9).*

Endometriotic epithelial Cells **(12Z)**

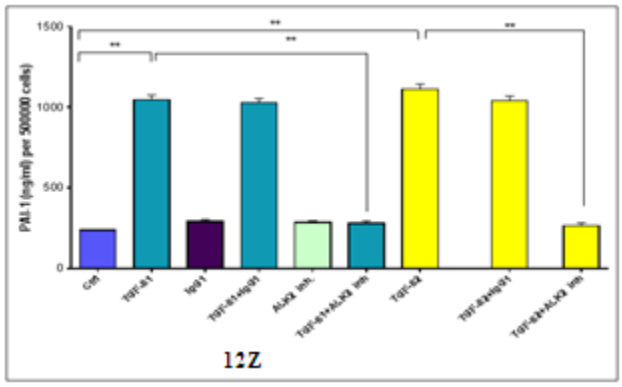

Figure 4 *Treatment of cells with TGF-β1 or TGF-β2 (10ng/ml), respectively, induced PAI-1 secretion in endometriotic epithelial cells 12Z. The ALK-2 inhibitor blocked TGF-β-induced PAI-1 secretion completely to control levels, whereas IgG1 which acted as a positive control had no effect on TGF-β-induced PAI-1 secretion (**=P<0.01, n=9). Similar effects were observed in the other cell lines (data not shown).*

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Endometriotic epithelial Cells (12Z)

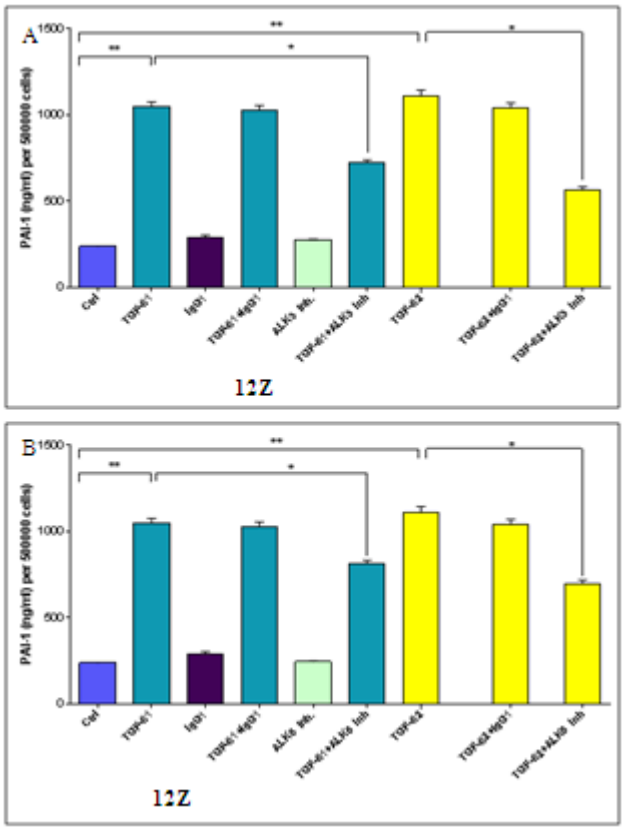

Figure 5 *Treatment of cells with TGF-β1 or TGF-β2 (10ng/ml), respectively, induced PAI-1 secretion in endometriotic epithelial cells 12Z. The ALK-3 and ALK-6 inhibitors partially blocked TGF-β-induced PAI-1 secretion, whereas IgG1, a positive control had no effect on TGF-β-induced PAI-1 secretion (*=P<0.05, **=P<0.01, n=9). Similar effects were observed in the other cell lines (data not shown).*

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