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The mechanism and significance of synergistic induction of the expression of plasminogen activator inhibitor-1 by glucocorticoid and transforming growth factor beta in human ovarian cancer cells

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ABSTRACT

Plasminogen activator inhibitor-1 (PAI-1) plays a key role in tissue remodeling and tumor development by suppression of plasminogen activator function. Glucocorticoids (GCs) and transforming growth factor beta (TGF-β) signal pathways cross-talk to regulate gene expression, but the mechanism is poorly understood. Here we investigated the mechanism and significance of co-regulation of PAI-1 by TGF-β and dexamethasone (DEX), a synthetic glucocorticoid in ovarian cancer cells. We found that TGF-β and DEX showed rapidly synergistic induction of PAI-1 expression, which contributed to the early pro-adhesion effects. The synergistic induction effect was accomplished by several signal pathways, including GC receptor (GR) pathway and TGF-β-activated p38MAPK, ERK and Smad3 pathways. TGF-β-activated p38MAPK and ERK pathways cross-talked with GR pathway to augment the expression of PAI-1 through enhancing DEX-induced GR phosphorylation at Ser211 in ovarian cancer cells. These findings reveal possible novel mechanisms by which TGF-β pathways cooperatively cross-talk with GR pathway to regulate gene expression.

1. Introduction

Glucocorticoids (GCs) and transforming growth factor-β (TGF-β) play important roles in regulation of cell growth, differentiation, extracellular matrix (ECM) formation, immune function, inflammation and tumor metastasis (Baschant and Tuckermann, 2010; Derynck et al., 2001; Gordon and Blobe, 2008; Phuc Le et al., 2005; Sheen et al., 2013). Synthetic GCs are widely prescribed therapeutics for the treatment of numerous inflammatory disorders and cancers. Increasing evidences have indicated that TGF-β and GCs signaling pathways cross-talk positively and negatively in regulating a variety of physiologic and pathologic processes through regulation of gene expression, although the molecular mechanisms involved remain to be established (Beck et al., 1993; Derynck et al., 2001; Kanatani et al., 1996; Kassel and Herrlich, 2007; Periyasamy and Sanchez, 2002; Pierce et al., 1989; Takuma et al., 2003).

The effect of GCs is mediated by the GC receptor (GR). As a transcription factor, liganded/activated GR regulates gene expression through either a direct interaction with GC response element (GRE) in the promoter region of target genes or through interference of other transcription factors to inhibit their transcriptional activity (Baschant and Tuckermann, 2010; Kassel and Herrlich, 2007; Phuc Le et al., 2005). The signaling and transcriptional activity of GR can be modulated by various post-transcriptional modifications including phosphorylation (Anbalagan et al., 2012; Davies et al., 2008; Housley and Pratt, 1983). GR is subject to hormone-dependent and -independent phosphorylation on several serines (such as S113, S141, S203, S211, S226, and S404) in its N terminus. The role of phosphorylation in regulating the transcriptional function of GR varies with different phospho-related sites within GR, different target genes and cell types (Beck et al., 2009; Galliher-Beckley and Cidlowski, 2009; Housley and Pratt, 1983). The phosphorylations of GR are regulated by protein kinases and phosphatases. Several cell-specific kinases such as cyclin-dependent kinases (CDKs), glycogen synthase kinase 3β (GSK3β), casein kinase II and mitogen-activated protein kinases

Abbreviations: PAI-1, plasminogen activator inhibitor-1; TGF-β, transforming growth factor beta; GC, glucocorticoid; DEX, dexamethasone; GR, glucocorticoid receptor; GRE, glucocorticoid response element; ECM, extracellular matrix; pGR-S211, phosphorylation of GR at Ser211; MAPK, mitogen-activated protein kinase; ERK, extra-cellular-signal regulated kinase; OSE, ovarian surface epithelium; siRNA, small interfere RNA; Smad3, mothers against decapentaplegic homolog 3; GAPDH, glyceraldehydes-3-phosphate dehydrogenase.

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(MAPKs) have been reported to phosphorylate GR (Beck et al., 2009; Bouazza et al., 2012; Galliher-Beckley and Cidlowski, 2009; Kino et al., 2007; Krstic et al., 1997; Miller et al., 2005, 2007; Wang et al., 2007). In the classical TGF-β pathway, TGF-β binding induces activation of its two types of trans-membrane serine/threonine kinase receptors which then phosphorylate and activate cytoplasmic proteins called Smads (Smad2/3), which form heteromeric Smad complexes with Smad4, and subsequently translocate to the nucleus. Once in the nucleus, the Smad complexes control gene transcription directly or in cooperation with other transcription factors (Gordon and Blobe, 2008; Sheen et al., 2013). TGF-β also activates non-Smad pathways, including PI3K-AKT, Rho-ROCK and members of the MAPK family, such as p38 MAPK, ERK and JNK (Derynck and Zhang, 2003; Gordon and Blobe, 2008; Massague, 2008; Sheen et al., 2013; Zhang, 2009). TGF-β activated non-Smad pathways can interact with Smad pathway to regulate gene expression (Derynck and Zhang, 2003; Dziembowska et al., 2007; Ohshima and Shimotohno, 2003; Vasilaki et al., 2010). TGF-β and GC modulate each other's activities (Almawi and Irani-Hakime, 1998; AyanlarBatuman et al., 1991; Chen et al., 2010; Li et al., 2006; Oursler et al., 1993; Peltier et al., 2003). For example TGF-β increases GC binding and signaling in macrophages (Peltier et al., 2003). Our previous work demonstrated that GC up-regulates the expression of type II TGF-β receptor (TβRII) and enhances TGF-β signaling in prostate cancer PC3 cells (Li et al., 2006) and ovarian cancer HO-8910 cells (Chen et al., 2010). However, whether TGF-β cross-talk with GR signaling pathways through altering GR phosphorylation to regulate gene expression has not been investigated before.

Plasminogen activator inhibitor-1(PAI-1) plays a key role in hemostatic balance, tissue remodeling, tumor invasion, angiogenesis and reproduction by virtue of suppression of plasminogen activator function (Durand et al., 2004; Ghosh and Vaughan, 2012; Rerolle et al., 2000; Zorio et al., 2008). PAI-1 is a target gene of TGF-β, and PAI-1 promoter contains binding elements of the Smads (Smad3 and Smad4) (Dennler et al., 1998; Lund et al., 1987). GC can also up-regulate the expression of PAI-1 (Bruzdzinski et al., 1993; Halleux et al., 1999). GC can cross-talk with TGF-β to regulate the expression of PAI-1 in different kinds of cells (Hamilton et al., 1993; Kimura et al., 2011; Ma et al., 2002; Song et al., 1999; Wickert et al., 2007). For example, previous studies demonstrated that liganded GR represses TGF-β/Smad3 transactivation of the PAI-1 gene in Hep3B human hepatoma cells by directly targeting the transcriptional activation function of Smad3 (Song et al., 1999). Conversely, GC and TGF-β were found to co-induce PAI-1 expression in human trophoblast cell (Ma et al., 2002), human monocytes (Hamilton et al., 1993) and human proximal tubular epithelial cells (Kimura et al., 2011). However, the mechanism by which TGF-β and GC/GR pathways interact to co-induce the expression of PAI-1 is unclear.

Epithelial ovarian cancer is the leading cause of cancer-related death in women diagnosed with gynecologic malignancies. At least 85% of ovarian cancers come from the human ovarian surface epithelium (OSE). OSE can not only secrete TGF-β1 (Peng, 2003) but also express 11β-hydroxysteroid dehydrogenase-1 (HSD1) which converts cortisone to the receptor active cortisol (Rae et al., 2004). Ovulation-associated inflammation factors, such as IL-1, could stimulate activity of HSD1, thereby producing GCs locally besides circulating GCs (Rae et al., 2004). PAI-1 is expressed in ovarian tissue, and plays an important role in ovulation and ovulation-associated wound healing (Liu, 1988, 2004; Ny et al., 1985). Moreover, high PAI-1 expression levels have been found to be associated with malignancy and metastasis of epithelial ovarian cancer, therefore PAI-1 is considered as an independent factor for overall survival and a strong predictor of metastasis in ovarian cancer (Cai et al., 2007; Koensgen et al., 2006).

Our previous studies found that DEX and TGF-β1 cooperatively promote cell adhesion to extracellular matrix (ECM) in ovarian cancer cells, which enhances resistance of cells to chemotherapeutics (Chen et al., 2010). Since PAI-1 is an endogenous target gene of both TGF-β1 and GC, and plays important roles in ECM formation and cell adherence by inhibiting the plasmin-mediated ECM degradation system (Durand et al., 2004; Ghosh and Vaughan, 2012; Zorio et al., 2008), it is interesting to explore whether TGF-β signaling pathways cross-talk with GR to regulate the expression of PAI-1, thereby contributing the synergistic pro-adhesion effect of DEX and TGF-β1 in ovarian cancer cells. In this study, we found that there is a rapid synergistic induction effect of GC and TGF-β1 on the expression of PAI-1. Then we further investigated its mechanism and significance in ovarian cancer cells.

2. Materials and methods

2.1. Cell culture

Human ovarian cancer cell line HO-8910 was established and kindly provided by Dr. Xu Shenhua (Zhejiang Cancer Research Institute, Zhejiang Cancer Hospital, China) at passage number 26 (Mou et al., 1994). SKOV3 cell line was obtained from National Infrastruture of Cell Line Resourse (Beijing, China) at passage number 30. The two cell lines were cultured in RPMI 1640 medium (Gibco, USA), supplemented with 10% newborn bovine serum (NBS; PAA Laboratories, Canada) at 37 °C under a humidified atmosphere of air containing 5% CO_2. Cells (less than 60 passages) were grown to ~70% confluence, and rinsed thrice with PBS, then cultured in medium containing 5% Dextran-coated charcoal (DCC) treated NBS to avoid possible interference by serum steroids, and the cells were incubated with 100 nM DEX (Sigma, Aldrich Saint Louis, Missouri, USA) or 10 ng/ml recombinant human TGF-β1 (PeproTech Inc., Rocky Hill, USA) or both of them for different periods of time. Control cells were incubated with ethanol (1‰). P38MAPK inhibitor SB203580 (10 μM), ERK inhibitor PD98059 (20 μM), and RU486 (1 μM) (Sigma-Aldrich, Saint Louis, Missouri, USA) were added 1 h before the administration of DEX and/or TGFβ1.

2.2. RNA extraction and quantitative real-time RT-PCR analysis

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and 2 μg of total RNA was reverse transcribed using Reverse Transcription Reagents (Fermentas, Lithuania, USA) in accordance with the manufacturer's instructions. The mRNA levels of the indicated genes were analyzed in triplicate using SYBR Green PCR Master Mix (Toyobo, Japan) on Mastercycler ep realplex (Eppendorf, Germany). The primer sequences used in the PCR reactions were: 5′-GCCTCCAAAGACCGAAATGTG-3′ (forward) and 5′-GTCGTTGATGATG AATCTGGCTC-3′ (reverse) for human PAI-1, and 5′-TAGCCCA GGATGCCCTTTAGT-3′ (forward), 5′-CCCCCAATGTATCCGTTGTG-3′ (reverse) for human GAPDH. Thermal cycling conditions consisted of an initial denaturing step (95 °C, 2 min) followed by 40 cycles of denaturing (95 °C, 20 s), annealing (60 °C, 20 s), and extending (72 °C, 45 s). The specified mode of reaction was controlled with the melting curve. The mRNA levels were normalized to GAPDH (internal control) using the formula ΔCT = CT target-CT reference. The differential expression signal was calculated as ΔΔCt = ΔCt (gene of DEX or/and TGF-β1 treated group) – ΔCt (gene of untreated group) and expressed as relative fold of change using the formula: $2^{\Delta\Delta CT}$.

2.3. Western blot analysis

Whole cells were prepared in SDS lysis buffer and protein extracts were equally loaded on SDS–polyacrylamide gel, and transferred to nitrocellulose membrane (Millipore, Ireland). The membranes were blocked in TBST (tris-buffered saline with Tween-20) containing 5% nonfat milk and probed with specific anti-phospho