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In silico prediction of buffalo (Bubalus bubalis) pregnancy associated glycoprotein-1 signaling pathway

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Abstract

The present study was conducted to predict and analyze signaling pathway of buffalo pregnancy associated glycoprotein-1 in silico. Various databases viz. String database, Panther pathways, Biocarta pathways were used for deducing signaling of buffalo pregnancy associated glycoprotein-1. Analysis reveals buffalo pregnancy associated glycoprotein-1 exerts their biological function by interacting with cytokines viz. placenta growth factor and SP1 transcription factor. Placental growth factor belongs to the vascular endothelial growth factor superfamily. Vascular endothelial growth factor is mainly involved in cellular proliferation, migration, differentiation, angiogenesis acting through protein kinase-C signaling pathway. SP1 transcription factor is mostly involved in mediators of transcription and signal transduction during cellular process acting through the SMAD factors. In toto pregnancy associated glycoprotein-1 through these factors may exert its biological functions of angiogenesis, endothelial cell growth, proliferation, migration and differentiation enhancing embryonic growth and development. In conclusion from this study the signaling pathway of buffalo pregnancy associated glycoprotein-1 (PAG-1) was predicted in silico.

Keywords: Buffalo**,** Pregnancy associated glycoprotein, Signaling pathway

Introduction

During pregnancy there are varied cascade of cytokines and their interactions to accomplish their biological functions (Schäfer-Somi, 2003). This involves various

biomolecules predominatly protein essential for uterine receptivity (Spencer and Bazer, 2004). Pregnancy associated glycoproteins (PAGs) are acidic glycoprotein belonging to aspartic proteinase superfamily expressed in the placenta of eutherian mammals. They have role in the placentogenesis, placental modeling and embryogenesis during pregnancy in domestic species as described in earlier reports (Xie *et al.*, 1991; Barbato *et al.*, **2014**) . Biological function of cytokines involve cascade of numerous signaling pathways and their mutual interaction and bovine pregnancy is not an exception (Szilagyi *et al.*, 2005). Studies in buffalo pregnancy associated glycoprotein-1 (PAG-1) *in silico* modeling revealed this molecule associates other cytokines for exerting its biological function but its plausible functional pathways needs to be delienated. Therefore the present study was conducted to predict and analyze signaling pathway of buffalo pregnancy associated glycoprotein-1 with the associating molcules *in silico.*

Materials and Methods

The signaling pathway of buffalo pregnancy associated glycoprotein-1 was deduced in silico, using databases viz. KEGG, Panther and Biocarta pathways (Kanehisa and Goto, 2000; Nishimura, 2001; Mi and Thomas, 2009). Buffalo PAG-1 were marked out using various signalling pathway servers along with other ligands and associating molecules. By these servers the predominant associating molecules of buffalo pregnancy associated glycoprotein-1 were alpha feto-protein (AFP), placental growth factor (PGF) and SP1 transcription factor as reported in earlier studies (Jerome *et al.,* 2013). Association of these molecules was used for predicting the cellular action of buffalo PAG-1 in silico.

Results and Discussion

Alpha feto-protein (AFP) is a glycoprotein having endodermal origin produced predominantly in liver of mammals possessing role in developmental biology. Other tissues which predominately produce this factor include yolk sac, fetal liver and gut. Alpha feto-protein belong to growth factors which has its role in ontogenic and tumorogenic growth in addition to cellular differentiation, growth and regulation. Studies revealed AFP helps to maintain pregnancy by modeling fetus as allograft and elevated levels of AFP have been found in multiple pregnancy, congenital nephrosis and other fetal disorders endorsing its role during pregnancy (Li *et al.,* 2011). In addition, AFP can act as as transcription associated regulatory factor owing to its property of binding to the nuclear receptors including trans-retinoic acid (ATRA; Trans-retinoic acid) receptors. Thus it can be speculated that biological action of buffalo PAG-1 can be exerted through this pathway

owing its its association with alpha feto-protein as reported in earlier studies (Jerome *et al.,* 2013). Studies revealed that intracellular AFP has the capability to bind RAR (*Retinoic acid receptor)* and lead to the activation or blockage of RA-RAR signaling which inturn act on DNA and promote cellular proliferation (Soltani,1979; Li *et al.,* 2011).

Placenta growth factor (PlGF) is another associating molecule with PAG-1. This growth factor belongs to vascular endothelial growth factor (VEGF) family and this factor gets its name as it was cloned from human placenta. Placenta growth factor is a glycosylated homodimer with cysteine residues. PlGF is abundant in trophoblastic giant cells associated with the parietal yolk sac at early stages of embryogenesis suggesting a role to coordinate vascularization in the placenta during early embryogenesis (**Sandro De Falco, 2012**) The PlGF aids in the transfer of nutrient to the fetus through the syncytial surface. This growth factor remodels the placental layer as the pregnancy by continual proliferation, differentiation, and fusion of cytotrophoblasts augmenting placental growth and nutrient supply to the fetus. Considering its nature of function its association with PAG-1 is justified as during pregnancy interaction of these molecules is imperative for pregnancy maintenance. PlGF exerts its action directly or indirectly by binding with VEGFR1 (vascular endothelial growth factor-receptor) whose main function is angiogenesis. Angiogenesis is important in embryonic development commenced by vascular endothelial cells and involves their orderly proliferation, migration and morphogenesis of new blood vesssels. Binding of VEGF to its receptor VEGFR activates endothelial cell growth through PKC (Protein Kinase-C) and ERK1/2 (Extracellular-Signal-Regulated Kinase) which inturn stimulates SphK1 (Sphingosine kinase 1). This is promoted through several sphingolipids acting as second messengers and playing the important role in the regulation of cell proliferation, survival, and cell death (Park *et al.,*1994; Ferrara, 2004).

Another important factor interacting with buffalo PAG-1 is SP1 transcription factor. SP1 also known as Specificity Protein 1, is a transcription factor involved in gene expression during fetal stages. It belongs to the Sp/KLF (Specificity protein/ Krüppel-like factor) family of transcription factors with a molecular weight of 81 kDA. The SP1 transcription factor possesses zinc finger protein (Cys_2/His_2 type) motif, by which it binds directly to DNA i.e. consensus equence (GC box element) and promotes gene transcription. It is usually found in relation to the transcriptional control in cell cycle regulation, hormonal activation and early fetal developmental. Activation of SP1 occures through triggering of TGF Beta receptor by its ligand *viz*. transforming growth factor-beta. It is polypeptide growth factor playing an important role in not only in angiogenesis and vascular remodeling, but also

regulating cellular growth, proliferation, differentiation, migration, and adhesion during embryonic stages. TGF-beta (Transforming growth factor-beta) activates SMAD (Sma and Mad Related Family; Mediator of signal transduction) signal transduction pathway for trigeering SP1 factor. These SMADs are ubiquitously expressed throughout development and in all adult tissues and many of them (SMAD2, SMAD4/DPC4, SMAD5, SMAD6 and SMAD8) are produced from alternatively spliced mRNAs. SMAD2 and SMAD4/DPC4 are important for transcriptional and antiproliferative responses to TGF-Beta (Suske,1999).

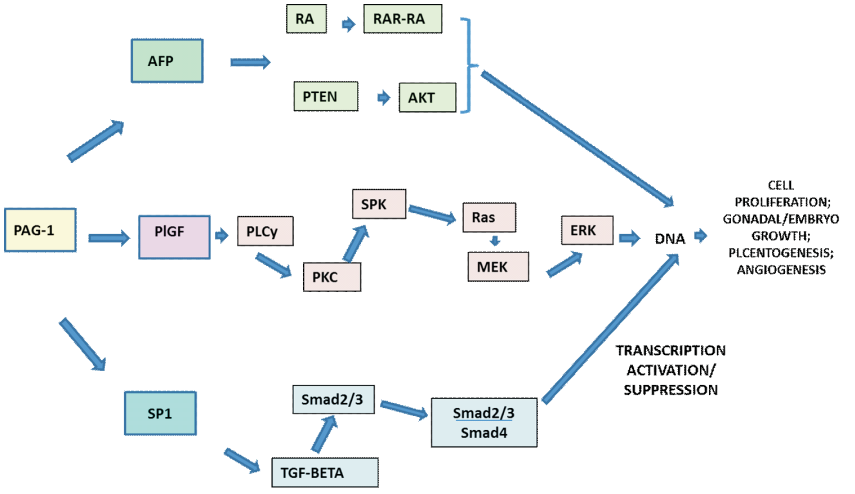

Figure 1. Schematic diagram depicting possible mode of action of buffalo pregnancy associated glycoprotein-1

Based on their structures and known functional roles, the SMAD family members consists of three classes: Co-SMADs (Co-mediator SMADs; SMAD4/DPC4 and SMAD10), participating in signaling by diverse TGF-Beta family members; R-SMADs (Receptor-regulated SMADs; SMAD1, SMAD2, SMAD3, SMAD5, and SMAD8), involving in specific signaling pathways; and antagonistic SMADs *viz*. SMAD6 and SMAD7. Thus on activation and phosphorylation, R-Smads associate with the co-mediator Smad, Smad4, and the heteromeric complex then translocates into the nucleus activate specific genes through cooperative interactions with other DNA-binding and coactivator proteins. The binding of R-SMADs (SMAD2 and SMAD3) to the phosphorylated GS domain via their phosphoserine-binding MH2 (Mad Homology-2) domain leads to its dissociation

from the receptor. This triggers SMAD4/DPC4 anchored to the cytoplasm by TRAP1 proteins (TGF-Beta Receptor Type-I Associated Protein-1). Thus post TGF-Beta stimulation, SMAD4/DPC4 enters the nucleus in complex with R-SMADs (R-SMAD/Co-SMAD complexes) and regulates gene expression (Suske,1999) (Fig. 1).

In silico analysis reveals buffalo pregnancy associated glycoprotein-1 exerts their biological function by interacting with cytokines *viz*. alpha fetoprotein, placenta growth factor and SP1 transcription factor. Alpha fetoprotein exerts its role through RAR receptor for cell proliferation whereas placental growth factor belonging to the vascular endothelial growth factor superfamily is mainly involved in cellular proliferation, migration, differentiation, angiogenesis acting through protein kinase-C signaling pathway. SP1 transcription factor is mostly involved in mediators of transcription and signal transduction during cellular process acting through the SMAD factors. Thus pregnancy associated glycoprotein-1 through these factors exerts not only its biological functions of angiogenesis, endothelial cell growth, proliferation, migration and differentiation enhancing embryonic growth and development but also activates or suppresses the genomic reualtion of cell cycle through transcriptional facotors. In conclusion this study delineates the plausible signaling pathway of buffalo pregnancy associated glycoprotein-1 *in silico*. Thus association of pregnancy associated glycoprotein-1 with these factors facilictate angiogenesis, endothelial cell growth, proliferation, migration and genomic activation during embryonic growth.

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