*Review Article*

# Activin A: Its Role and Involvement in Inflammatory Diseases

**Tie Tung Hing[1], Rusliza Basir[1*], Chuah Yaw Kuang[1], Herni Talib[2] and Norshariza Nordin[3]**

[1]*Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

[2]*Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

[3]*Department of Obstetric and Gynaecology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

## ABSTRACT

Activin proteins are members of the transforming growth factor-β family. Activin A is involved in several biological responses including wound repair, cell death, proliferation and differentiation of many cell types. Biologically active activins consist of homodimers or heterodimers of two beta (β) subunits that are linked together by a single covalent disulphide bond. The subunits in humans are βA, βB, βC and βE. As an example, a combination of two βA subunits will produce a unit of activin A. These proteins are found in most cells of body such as macrophage and activated circulating monocytes. Their role in inflammation can be categorised into two types, either pro- or anti-inflammatory agents, depending on the cell type and phase. Activin signals are kept in balance by antagonist follistatin (Fst), which is a glycoprotein expressed in tissues and encoded by the follistatin gene in humans.

*Keywords*: Activin, transforming growth factor, activin subunit, inflammation, follistatin.

## INTRODUCTION

Activin A is a multifunctional growth factor that is a functionally and structurally different member of the transforming growth factor-beta (TGF-β) superfamily of proteins (Chang, Brown & Matzuk, 2002; Griselda, Anayansi, & Iván, 2012). Precursor proteins are the forms of activin produced after cleavage from bioactive ligands. They were given the name activin after the first identification as

activators of follicle-stimulating hormone (FSH) secreted from the pituitary gland (Ling *et al*., 1986). These proteins also play an important role in regulating various biological functions in a many types of tissues and cells which include apoptosis, wound repair, differentiation of cells, survival of neural cell and inflammation (Yndestad *et al*., 2004).

Activins are involved in inducing mesodermal tissue differentiation in embryonic *Xenopus* explants (Griselda *et al*., 2012). Given their ability to accumulate and stimulate haemoglobin differentiation in erythroleukemia cell lines in vitro, these proteins were discovered through isolation from a monocytic leukaemic cell line derived from human bone marrow. The very first name given to activins was erythroid differentiating factor (EDF). Description of activin and its ligand as survival factors for nerve cells was the result of a search for nerve growth factors. Activin A was able to support the survival of the central nervous system (CNS) neurons in culture, as well as to protect neuronal injury from neurotoxicity due to its role as a neurotrophic and neuroprotective factor (Woodruff, 2000). Moreover, activin A affects liver homeostasis and may stimulate hepatocyte cell death (Woodruff, 1998). It is produced and secreted quickly into the bloodstream from the endothelium of the vascular system and other tissues during inflammation. Mediators such as nitric oxide and pro-inflammatory cytokines, as well as leucocyte activation, play a crucial role in numerous aspects of the inflammatory response. As seen in patients with either acute or chronic inflammation (e.g., septicaemia and inflammatory bowel disease), the concentrations of activin A were observed to have increased in the serum and tissues (Hubner, Brauchle, Gregor & Werner, 1997; Michel, Ebert, Phillips & Nau, 2003a).

Bioactive activins comprise either homodimers or heterodimers of two beta (β) subunits, with four distinct subunits of activin identified to date as being involved in the development of these proteins. These subunits in humans are βA, βB, βC and βE, which are able to produce activin A (βA/βA), activin B (βB/βB) and activin AB (βA/βB). Activin A homodimer is produced by merging two subunits of βA. This homodimer is produced by the creation of either an intermolecular disulphide bond or a covalently dimerised bond between the 6th (of the nine) conserved cysteines in the established proteins. The entire nine cysteines, excluding the 6th, are implicated in the production of a cysteine knot by means of disulphide bonds within the molecule. The disulphide bonds are typical for members of the TGF-α family and are fundamental for their effects on living tissues (Griselda *et al*., 2012; Kreidl, Oztürk, Metzner, Berger & Grusch, 2009).

## SOURCE OF ACTIVIN A

Activin A is synthesised and secreted in almost every cell type and tissue. For the action of an autocrine or paracrine mechanism to be carried out, the tissues must be equipped with many receptors of activin A. Tissue macrophages and activated circulating monocytes can synthesise and release activin A after stimulation by inflammatory stimuli. A large amount of activin A is secreted by bone marrow stromal cells in response to inflammatory mediators (Phillips, Jones, Scheerlinck, Hedger & de Kretser, 2001; Welt, Sidis, Keutmann & Schneyer, 2002). Activin A is released from bone marrow stromal cells after stimulation of the vascular endothelium by monocytes and bone marrow stromal fibroblasts once T-cells have interacted with cytokine.

Therefore, Bone marrow is another effective source of activin A and also a source of activin A during inflammation (Wu, Chen, Winnall, Phillips & Hedger, 2013). Additionally, strong stimulation by lipopolysaccharide (LPS), interleukin (IL)-1β or IL-6 also leads to the production of activin A by macrophages, monocytes and endothelial cells. In systemic inflammation, endothelial cells may be an essential depot of activin A activity (Phillips, Jones, Clarke, Scheerlinck & de Kretser, 2005; Phillips *et al*., 2001). Furthermore, secretion of activin A can be enhanced by mutual action between monocytes and activated T-cells via CD-40 (Abe, Shintani, Eto, Harada, Kosaka & Matsumoto, 2002). Moreover, mast cells and neutrophils are other potential sources of activin A during inflammation, while activin A can be produced after treatment with interferon (IFN)-γ and CD40L, or LPS when dendritic cells interact with activated T-cells (Phillips *et al*., 2005; de Kretser, O'Hehir, Hardy & Hedger, 2011).

## ACTIVIN A SUBUNIT
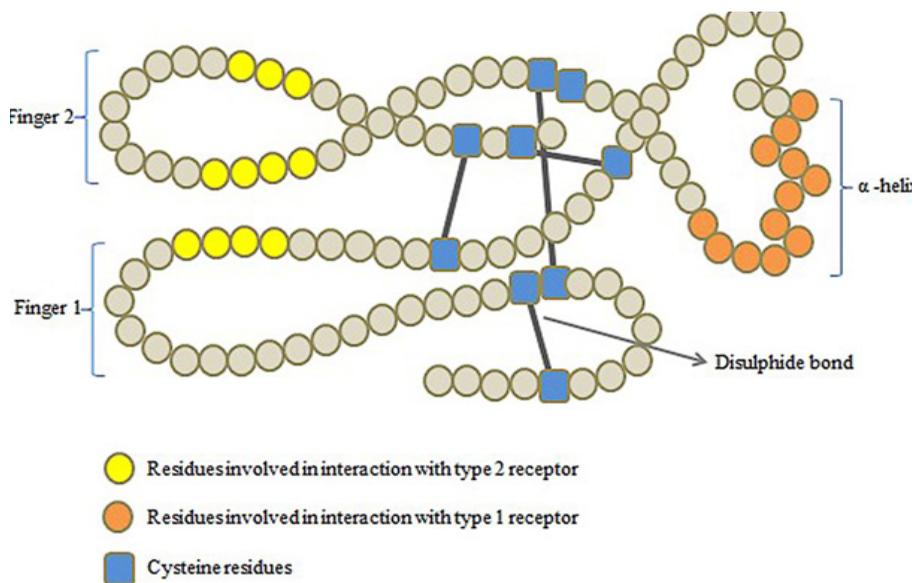


Fig.1: A subunit of activin A (Adapted from *Activin-A Binds Follistatin and Type II Receptors through Overlapping Binding Sites: Generation of Mutants with Isolated Binding Activities* by Harrison, C. A. & Chan, K. L., 2008, *Endocrinology, 147*(6), 2744-2753).

The binding sites for type II receptor are located at finger 1 (Phe[17], Ile[30], AL[31], Pro[32], His[36]) and finger 2 (Arg[87], Pro[88], Ser[90], Leu[92], Tyr[94], Ile[100], Lys[102], Glu[111]) of activin A. Follisatin can bind to contiguous site on activin A. The first is the convex outer side of activin β strand (Ile[30], AL[31], Pro[32], Leu[92], Tyr[9], Ile[100] and Lys[102]) and in β strand fingertip (Asp[32] – Asn[99]). The second is the binding site of type I receptor that is formed by the concave activin β strand of one subunit (Trp[25], Trp[28], Met[91], Tyr[93] and Ile[105]) and helical wrist region on the other β subunit (His[47], Ile[48], Gly[50], Thr[51], Ser[52], Phe[58,] Thr[61] and His[65]) (Harrison, Chan & Robertson, 2006).

## ACTIVIN A SIGNALING PATHWAY

Since activins are members of the TGF-β family of signaling molecules, mature activins are believed to signal through single-pass transmembrane serine-threonine kinase receptors type I and type II, both of which are crucial for activin-mediated biological activities. The cytoplasmic region of both type I and type II receptors is the region where the serine-threonine activity takes place, which also serves as the docking stations. The signalling pathway started by the attachment of activin A to dimers of the activin type-II receptors ActR-II, which are also known as ACVR2 or ActR-IIB (ACVR2B).

Consequently, dimers of the activin type I receptor (activin receptor-like kinase [ALK] 4) are recruited and phosphorylated in their type-II serine-threonine kinase domain. The primary residues within a glycine- and serine-rich (GS) domain of the type I receptor are phosphorylated by the assembled active type II receptor, as a consequence of the formation of the heteromeric ligand-receptor complex, which brings together the cytoplasmic region of the two types of receptors. The activation of intracellular protein Smads is the result of the activation of activin type I receptors. It was found that the mutually dependent characteristic of activin type I and type II receptors is due to the need of activin type I receptors to bind their ligand to activin type II receptors, whilst the association of type II receptors to type I receptors is important to signal the nucleus (Bilezikjian & Vale, 2011). Although the binding of type II receptors to ligands can take place without the presence of type I receptors, they still need the latter to signal and function well.

Receptors of activin are regularly internalized immediately after the binding of ligand. Immediately after receptor activation, phosphorylation of receptor-regulated Smad (R-Smad) proteins by the activin type I receptors takes place after their recruitment to the receptor complex. Molecular complexes are formed after the common mediator Smad 4 approaches the recruited and phosphorylated R-Smads (Smads 2 & 3). Modulation of gene expression is initiated by direct binding to DNA or association with other transcription factors by the molecular complexes that come together with cofactors. The phosphatidylinositol-3′-phosphate-binding protein, known as Smad anchor for activation of the receptor (SARA), is responsible for phosphorylation of Smads 2 and 3. Smads 2 and 3 are bound together to the receptor complex with the aid of SARA. R-Smads and SARA are cleaved from the receptor complex once R-Smad proteins are phosphorylated. Gene expression is initiated following the translocation of phosphorylated R-Smads after the recruitment of the cytoplasmic common mediator Smad, the co-Smad 4 (Schmierer, Schuster, Shkumatava & Kuchler, 2003; Kreidl *et al.*, 2009; Griselda *et al.*, 2012; Cárcamo *et al.*, 1994; Wieser, Attisano, Wrana & Massagué, 1993).

The Smad proteins are generally divided into two subcategories: R-Smads (receptor-activated) or co-Smad (not receptor-activated) (Bilezikjian & Vale, 2011). Smad proteins have an N-terminal against decapentaplegic homology domain 1 (MH1) and a C-terminal against decapentaplegic homology 2 (MH2) linked by a proline-rich region. Binding of DNA to the CAGA sequence, as well as to some GC-rich sequences, is mediated by the MH1 domain, which contains a nuclear localisation signal. Transcription activation only happens when the MH2 domain is fused to the Gal4 DNA-binding domain. For MH2-specific association with the L45 loop of activin type I receptors, as well as in the homo- or hetero-oligomerisation of Smads,
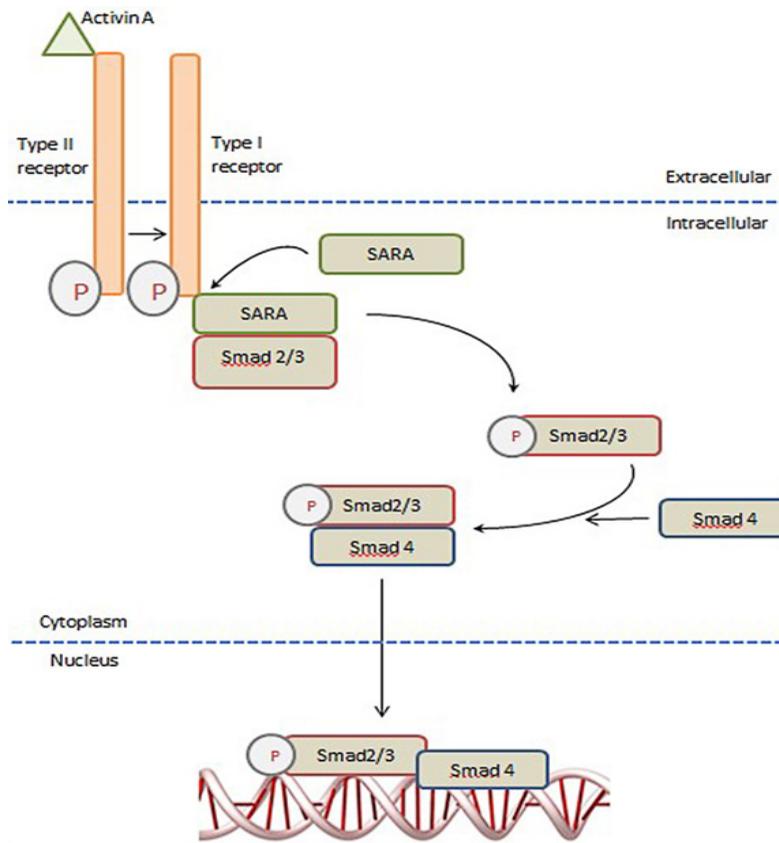
Fig.2: Activin/ Smad signalling pathway

the presence of L3 loop containing in MH2 is important (Bilezikjian & Vale, 2011; Ten Dijke, Miyazono & Heldin, 2000). The conserved C-terminal SSXS motif in R-Smads is recognized by the tail of activin type I receptors in the cytoplasmic region. The association between Smad 4 and R-Smad is initiated after the cleavage of R-Smad from activin type I receptors due to the phosphorylation of the C-terminal SSXS. Consequently, this initiates translocation of the heteromeric Smad complex to the nucleus (Attisano & Wrana, 2000; Bilezikjian & Vale, 2011).
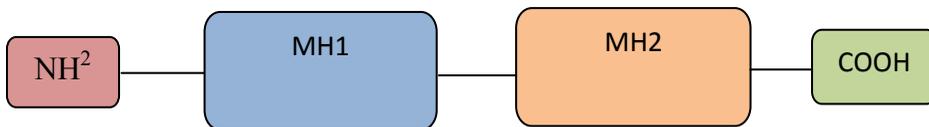


Fig.3: A schematic diagram of the Smad protein with an N-terminal Mad Homology 1 (MH1) and a C-terminal Mad Homology 2 (MH2) domain linked by a proline-rich region.

Smad 3 recognises the two inverted repeats of GTCT on DNA. However, the single copy of this core sequence only bound by the MH1 of Smad 3. The MH1 of Smad 2 cannot bind to DNA by itself. In addition, the repeat sequence of GNCN in the promoter are not sufficient for binding to Smad-dependent targeting of specific genes, but it is enough for Smad-DNA binding.

The most comprehensively characterised DNA-binding partners of the Smad proteins are the forkhead activin signal transducer (FAST) family of DNA-binding proteins. Furthermore, the family has been identified in humans and mice as well. The TGF-β activin-responsive region of promoters acts mutually with FAST in order to activate gene transcription downstream of activin signaling (Attisano & Wrana, 2000).

## ACTIVIN AS A MEDIATOR IN INFLAMMATION: ACUTE AND CHRONIC

At the site of injury, inflammation quickly develops as the consequence of the secretion of inflammatory cytokines (IL-1, IL-6 and tumour necrosis factor-alpha (TNF-α)] from cells such as macrophages and stromal cells. In succession, acute phase response (APR) is stimulated after these cytokines act at systemic sites such as the liver to trigger febrile responses and gene expression (de Kretser, Hedger & Phillips, 1999). Sites of the injury are protected by innate response by APR to prevent host from continuous tissue damage. Some physical changes are protected by innate response, which in turn, suppresses actions of serum acute phase proteins and cytokines (Steel & Whitehead, 1994). Damage of lung during LPS-induced inflammation occurs after neutrophils translocate from bone marrow. Activin A is secreted during inflammation by neutrophils following direct stimulation by TNF-α; serum TNF-α is reduced if activin A is blocked. After 4 and 5 hours of LPS introduction, serum activin A level is greatly elevated (Wu *et al*., 2013; de Kretser *et al*., 1999). The activin A released during acute inflammation is very early and biphasic. Its release during early acute inflammation is rapid and therefore from pre-stored materials. Its secretion during the later phases of inflammatory responses is from newly synthesised inactive material hours after stimulation such as by LPS. The cells that are responsible for the production of activin A at the later stage are monocytes, macrophages, dendritic cells and endothelial cells. Neutrophils and epithelial cells are responsible for activin A secretion such as those from the stomach and lungs (Philips *et al*., 2009).

Upregulation of activin A expression at the sites of injury is either as protein or mRNA in synovial fluid or in chronic inflammatory bowel disease (IBD) in patients with inflammatory arthropathy (de Kretser *et al*., 1999). In chronic inflammation, comparing patients with gout and rheumatoid arthritis with those with non-inflammatory osteoarthritis, activin A levels were elevated in synovial fluid, as well as expression of activin βA in synovial membranes. Activin A has been implicated in IBD. The ulcerative colitis or Crohn's disease from IBD patients, inflamed mucosal and sub-mucosal tissues in the intestinal wall were observed, together with high IL-1β and activin βA subunit mRNA levels. In normal tissues, expression of IL-1β was low (Philips *et al*., 2009). This same group of patients also displayed very high level of plasma activin A level and βA mRNA in the intestinal wall. Moreover, in areas where IL-1β mRNA was highly expressed, activin βA subunit mRNA was found in the mucosa and sub-mucosa of tissues at sites of inflammation, whereas low levels of expression were found in healthy tissues; for example, the activin A-stimulated production of IL-6 and IL-8 in amniotic membranes. The action of activin A is believed to have two phases; inhibitory as the activin A concentration is high and vice versa (Wu *et al*., 2013).

## ACTIVIN A: A PRO-INFLAMMATORY AGENT

A pro-inflammatory role for activin A has been observed in diverse cell types (Jones, de Kretser, Patella, & Phillips, 2004). The role of activin A as a pro-inflammatory mediator is proved by the upregulation of activin expression by pro-inflammatory cytokines (granulocyte monocyte colony-stimulating factor (GM-CSF) and interferon γ) and/or LPS, as well as down regulation by glucocorticoids. The role of activin A during pro-inflammatory activities was confirmed by its pro-migratory on monocytes. Additionally, metalloproteinase expression can be induced by activin A in macrophages during the inflammatory response. Migration and infiltration by macrophages through the basement membrane during inflammation are also triggered by activin A. Taking malarial infection as an example, it is important that cells are first activated by parasite-specific molecules to initiate immune responses against the infection. This is then followed by the activation of *mitogen-activated protein kinase* (MAPK) and nuclear factor (NFκB) signalling pathways, resulting in expression of cytokine genes (El-Gendi *et al.*, 2010; Zhu, Krishnegowda, Li & Gowda, 2010).

In malarial infection, macrophages phagocytose the invading parasites. During this process, the release of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β can be stimulated by activin A (Rapeah, Dhaniah, Nurul & Norazmi, 2010; Phillips *et al.*, 2005). In bone marrow-derived macrophages, TNF-α and IL-1β are also produced following the stimulation of activin A. Moreover, activin A increases the expression of pro-inflammatory mediators such as prostanoids, prostaglandin and thromboxane, cyclo-oxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS) (Jones *et al.*, 2004; Nusing & Barsig, 1999). The pro-inflammatory effects of activin A can also be seen in LPS-induced inflammation process in sheep. This involves the pro-inflammatory cytokines TNF-α and IL-6, which are secreted following the release of activin A. Activin A has also been implicated in stimulating the production of IL-6 and IL-8 in amniotic membranes, where its role as a pro- or anti-inflammatory agent depends on its concentration. When the activin concentration is low, it acts as a pro-inflammatory factor, and when it is high, an anti-inflammatory agent (Jones *et al.*, 2004). Additionally, IκB degradation, nuclear translocation of NFκB and phosphorylation of Extracellular signal-regulated kinases 1/2 (ERK 1/2) and p38 MAPK lead to the production of TNF-α, IL-1β and IL-6 by activin A (Phillips, de Kretser & Hedger, 2009).

## ACTIVIN A: AN ANTI-INFLAMMATORY AGENT

Activin A has been well documented an anti-inflammatory agent, with evidence that activin A can limit the secretion and actions of IL-1β and IL-6 (Phillips *et al.*, 2001). A study on monocytic cultures uncovered the ability of activin A to keep down the production of IL-1β by inhibiting the alteration of the IL-1β precursor into its secreted biologically active form, most likely by blocking caspase-1 (Philips *et al.*, 2009; Phillips *et al.*, 2011). In LPS-stimulated macrophages, activin A can suppress the secretion and expression of IL-1β and IL-10 (de Kretser *et al.*, 2011; Wang *et al.*, 2008). During the early stage of inflammation induced by macrophage, the pro-inflammatory effect of activin A is turned into anti-inflammatory at the later stage (de Kretser *et al.*, 2011). During APRs, activin A is able to initiate anti-inflammatory effects at local site of infection or injury and to repress unnecessary inflammation at the pheripheral sites such as

liver (Smith *et al.*, 2004). Moreover, theactivity of activin A in a hepatoma cell line (HepG2) can be inhibited by a secretion of acute phase inflammatory proteins mediated by IL-6 (Werner & Alzheimer, 2006). With the aid of activin A, the pancreatic islet cells and endothelial cells of humans are able to produce insulin and anti-inflammatory effects, as well as anti-oxidative and anti-inflammatory effects. In patients with coronary artery disease (CAD), anti-inflammatory effects on previously activated peripheral blood mononuclear cells are also displayed by activin A (Andersen *et al.*, 2011). The anti-inflammatory effects of activin A are also proven, with antagonistic action on the secretion of IL-6 and IL-11 in myeloblasts (Sugama, Takenouchi, Kitani, Fujita & Hashimoto, 2007). During the early phase of cardiopulmonary bypass (CPB), the anti-inflammatory effects of activin A only occur after the production of pro-inflammatory cytokines such as TNF-α and IL-8 (Sablotzki *et al.*, 1997).

## ACTIVIN IN ORGAN SPECIFIC FUNCTIONS

In the brain, differentiation of cerebrocorticol neural progenitor cells (NPC) into neuronal phenotype is driven by activin A. In proliferating NPC, activin can induce discrete but significant rises in the proportion of neurons. The neurogenesis by activin is not due to its role as regulator but as an instructor on NPC. In addition, inhibition of activin can cause reduced neurogenesis and development of anxiety-related behavior (Rodríguez-Martínez, Molina-Hernández & Velasco, 2012).

Activin A is upregulated in heart failure but treatment with anti-activin A antibody can restore growth hormone (GH) which improves heart function by normalizing GH level (Fukushima *et al.*, 2011). Activin A has also been identified to play a role in healing after myocardial infarction, but a continuous increase in activin A levels can lead to myocardial remodelling and eventually heart failure (Yndestad *et al.*, 2004). Recovery could be due to the presence of high serum activin levels that cause a reduction by upregulating expression of B-cell lymphoma 2 (Bcl-2) protein (Oshima *et al.*, 2009).

Activin A has been proven to function as a negative regulator for liver growth. It can inhibit mitogen-induced DNA synthesis and induce apoptosis in cell lines of hepatoma and in hepatocytes *in vivo* as well as *in vitro*. Overproduction of activin A due to inhibin deficiency can cause hepatocyte destruction. Activin A remained at low levels at the first 12 hours of partial hepatectomy (PHX) and increased by three times higher at 168 hours, which suggests that it acts as regulator of liver regeneration. Furthermore, hepatocyte replication increased following the elevation of follistatin at 24-48 hours of post PHX (Rodgarkia-Dara *et al.*, 2006).

In kidney, activin A is important as a negative regulator in the growth of ureter bud (UB) from Wolffian duct (WD) during the development of metanephric kidney. UB outgrowth is in response to glial-cell-derived neurotrophic growth factor (GDNF). Thus, ectopic bud formation can be controlled by inhibiting GDNF by activin A. Activin A is also involves in branching morphogenesis of UB by acting as negative regulator (Maeshima *et al.*, 2004). Activin A in pancreas is crucial for glucose metabolism by stimulating differentiation of β cells to produce insulin, which allows insulin target cells to act efficiently in glucose uptake (Hashimoto & Funaba, 2011; Ueland *et al.*, 2012).

## ROLE OF ACTIVIN IN DISEASES

During malaria parasite infection, pro-inflammatory cytokines like TNF-α and IFN-γ, and anti-inflammatory cytokine like IL-10 are produced to provide protection against the parasites. In malarial infection, activin A initiates the release of pro-inflammatory cytokines such as TNF-α, and IFN-γ and IL-10 production is activated upon enhancement of inflammation. These cytokines are important in protection against malaria parasites (Semitekolou *et al.*, 2009; Phillips *et al.*, 2005; Gribi, Tanaka, Harper-Summers & Yu, 2005; Robinson *et al.*, 2009).

Activin A has been identified to be involved in the pathogenesis of inflammation bowel disease (IBD), enhancing the migration instead of proliferation of intestinal epithelial cells, as well as stimulating inflammation during colitis (Huber *et al.*, 1997; Zhang, Resta, Jung, Barrett & Sarvetnick, 2009). In patients with ulcerative colitis or Crohn's disease, strong expression of the activin βA subunit was detected, but not in people with a healthy digestive tract. Moreover, the higher the expression of activin βA mRNA was, the more severe the degree of inflammation would be. Increased expression of activin A mRNA has been identified in highly inflamed tissues such as the mucosa and sub-mucosa of injured intestinal epithelium (Hubner *et al.*, 1997). Furthermore, increased expression of type I and type II activin receptors has also been reported in patients with IBD but not in healthy people, indicating that activin signalling contributes to the enhanced receptor expression (Zhang *et al.*, 2009).

In patients with asthma, increased concentration of activin A in bronchoalveolar lavage fluid is due to its secretion from cells lining the alveoli, which are epithelial cells and activated human lung mast cells (Philips *et al.*, 2009; Werner & Alzheimer, 2006). Alveolar cells, endothelial cells and fibroblasts were found to respond to activin in inflamed lung tissues, which was supported by the strong ActR-IB expression in these cells. Mice deficient in mast cells shows lower secretion of activin A; therefore, mast cells are an important source of activin in the airway of mice with asthma. It is believed that mast cell-derived activin A could promote airway tissue remodelling, as activin A was found to enhance the proliferation of airway smooth muscle (ASM) cells in humans. The action is completed with paracrine signalling to enhance the proliferation of ASM cells. Activin increases the differentiation and migration of mast cell progenitors but inhibits growth. For that reason, activin A may regulate mast cells as the effector cell of the immune system, which could further contribute to the pathogenesis of asthma (Werner & Alzheimer, 2006).

## CURRENT RESEARCH FINDINGS AND NEW TREND FOR ACTIVIN RESEARCH

Activin has been identified to be involved in the control of biological systems and wide interaction with other members of TGF-β superfamily, as well as other hormones/peptides. The motivation underlying the study by Makanji *et al.* (2011) is the apparent role of activin in promoting cachexia in patients with a variety of tumours and promoting development of gonadal tumours. However, the value of this study is much broader.

The non-specific interaction of activin and other TGF-β superfamily, together with regulation and signalling of other family members, makes the identification of specific action and the development of specific agonists and antagonists very challenging. In this study, genetic

manipulations (transgenic animals or in vitro small interfering RNA gene knockdown) are useful only with the aid of specific agonistic and antagonistic compounds for the peptide of interest. The system's biology framework was used to create specific antagonist (activin propetide [AT propetide]) that inhibits activin A stimulation of FSH release. Specific domains for protein folding were determined by study on the molecular two- and three-dimensional structures of activin and TGF-β1 peptide so that the structure that binds and inhibits mature TGF-β1 can be created. The inhibition of activin action by chimeric peptide was achieved through the linking of C-terminal portion of activin with the N-terminal portion of TGF-β1. The specificity of AT propetide was important in genetic manipulation. The AT propetide allows insight into functions of activin and mechanisms of interaction with other TGF-β1 members such as inhibin. New therapies for cancer and other diseases could be developed from this chimeric propeptide. Hence, this study is valuable and may be rewarding upon the proper application of the 21st century systems biology thinking (Nielsen & Torday, 2011).

## FOLLISTATIN: AN ANTAGONIST OF ACTIVIN A

The single-chain glycoprotein and structurally different follistatin was recognised as an extracellular antagonist of activin that could reduce its biological activities. The LPS- or IL-1β dosed sheep has proven the involvement of follistatin during inflammation. Follistatin is a monomeric protein and is structurally different to the TGF-β superfamily of proteins. Most organs that express follistatin also express activin; consequencely, follistatin activities in nature likely to have an autocrine or paracrine characteristic. The bond between follistatin and activin is very strong, with Kd 50-680 pmol/L. In addition, the local diffusion of activin A from the site of action can be controlled by follistatin (Deli *et al*., 2008; Welt *et al*., 2002; Oshima *et al*., 2009). The bond between follistatin and activin is virtually irreversible; however, activin must be cleaved by proteolytic mechanism to reveal its endocrine signalling effects. Such a mechanism has been identified for the related bone marrow macrophage protein (BMP), in which bioactive BMP is released from chordin, its binding protein, by metalloproteinase (Welt *et al*., 2002).

A third of activin residues are buried at the binding sites of receptor as soon as one activin dimer binds to two follistatin molecules. Three distinct types of follistatins are produced through the protein processing and splicing of a single follistatin gene. They are proteins with 288, 303 and 315 amino acids. Three homologous domains are contained in each of these three follistatins. In case of inhibition of activin, only two domains are involved in the binding process (Deli *et al*., 2008). The inhibitory action occurs only when the receptor binding site of activin A is obstructed by complex formation with follistatin (Kreidl *et al*., 2009). Follistatin has an important role in suppressing activin A, as it can reduce mortality rate by reducing the excessive production of activin A during inflammation (Wu *et al*., 2013; Jones *et al*., 2004). For example, over scarring as well as formation of granule and re-epithelisation on wounded skin is under the influence of a high concentration of activin A but healing quality can be restored with the aid of follistatin (Werner & Alzheimer, 2006). Furthermore, liver apoptosis induced by activin A can be inhibited with follstatin as well (Oshima *et al*., 2009).

## CONCLUSION

Activin A has been linked to inflammation, cells growth, tissue repair, metabolism and apoptosis. The focus of this review is on its role in inflammatory response. Its expression is significantly upregulated during acute and chronic inflammation. Activin can be involved in both pro- and anti-inflammatory responses depending on the cell type and stage of its expression. Inflammation has always been a major biological response related to activin, such as that in malaria infection, IBD and asthma. Besides, activin has different functions in organs, while in brain, it has a role in neurogenesis. It also improves heart failure and healing after myocardial infarction and acts as a regulator of liver regeneration after PHX in the liver, growth of UB from WD and branching of UB, and glucose metabolism in pancreas. Nonetheless, further studies are required to identify and understand the roles of activin in other pathological conditions.

## REFERENCES

Abe, M., Shintani, Y., Eto, Y., Harada, K., Kosaka, M., & Matsumoto, T. (2002). Potent induction of activin A secretion from monocytes and bone marrow stromal fibroblasts by cognate interaction with activated T cells. *Journal of Leukocyte Biology, 72*(2), 347-352.

Andersen, G.Ø., Ueland, T., Knudsen, E. C., Scholz, H., Yndestad, A., Sahraoui, A., Smith, C., Lekva, T., Otterdal, K., Halvorsen, B., Seljeflot, I., & Aukrust, P. (2011). Activin A Levels Are Associated With Abnormal Glucose Regulation in Patients With Myocardial Infarction. Potential Counteracting Effects of Activin A on Inflammation. *Diabetes, 60*(5), 1544-1551. doi: 10.2337/db10-1493.

Attisano, L. & Wrana, J. L. (2000). Smads as transcriptional co-modulators. *Current Opinion in Cell Biology, 12*(2), 235–243.

Bilezikjian, L. M. & Vale, W. W. (2011). The Local Control of the Pituitary by Activin Signaling and Modulation. *Open Neuroendocrinol J., 4*, 90–101. doi: 10.2174/1876528901104010090.

Bonfield, T. L., Barna, B. P., John, N., Malur, A., Culver, D. A., Kavuru, M. S., & Thomassen, M. J. (2006). Suppression of activin A in autoimmune lung disease associated with anti-GM-CSF. *Journal of Autoimmunity, 26*, 37-41.

Cárcamo, J., Weis, F. M., Ventura, F., Wieser, R., Wrana, J. L., Attisano, L., & Massagué, J. (1994). Type I Receptors Specify Growth-Inhibitory and Transcriptional Responses to Transforming Growth Factor β and Activin. *Molecular and Cellular Biology,* 3810-3821.

Chang, H., Brown, C. W., & Matzuk, M. M. (2002). Genetic analysis of the mammalian transforming growth factor-β superfamily. *Endocrine Revision, 23*, 787–823.

de Kretser, D.M., Hedger, M.P., & Phillips, D.J. (1999). Activin A and follistatin: their role in the acute phase reaction and inflammation. *J Endocrinol, 161*(2), 195-8.

de Kretser, D. M., O'Hehir, R. E., Hardy, C.L., & Hedger, M. P. (2011). The roles of activin A and its binding protein, follistatin, in inflammation and tissue repair. *Molecular and Cellular Endocrinology, 359*(1–2), 101-106.

Deli, A., Kreidl, E., Santifaller, S., Trotter, B., Seir, K., Berger, W., Schulte-Hermann, R., Rodgarkia-Dara, C., & Grusch, M. (2008). Activins and activin antagonists in hepatocellular carcinoma. World *J. Gastroenterol, 14(*11), 1699–1709. doi: 10.3748/wjg.14.1699.

El-Gendi, S. S., Moniem, A. E., Tawfik, N. M., Ashmawy, M. M., Mohammed, O. A., Mostafa, A. K., Zakhari, M. M., & Herdan, O. M. (2010). Value of serum and synovial fluid activin A and inhibin A in some rheumatic diseases. *Int. J. Rheum. Dis., 13*(3), 273-9. doi: 10.1111/j.1756-185X.2010.01532.x.

Fukushima, N., Matsuura, K., Akazawa, H., Honda, A., Nagai, T., Takahashi, T., Seki, A., Murasaki, K. M., Shimizu, T., Okano, T., Hagiwara, N., & Komuro, I. (2011). A crucial role of activin A-mediated growth hormone suppression in mouse and human heart failure. *PLoS One, 6*(12), e27901. doi: 10.1371/journal.pone.0027901.

Gressner, O.A., Lahme, B., Siluschek, M., Rehbein, K., Weiskirchen, R., & Gressner, A.M. (2008). Intracrine signalling of activin A in hepatocytes upregulates connective tissue growth factor (CTGF/CCN2) expression. *Liver International, 28,* 1207–1216. Doi: 10.1111/j.1478-3231.2008.01729.x.

Gribi, R., Tanaka, T., Harper-Summers, R., & Yu, J. (2001. Expression of activin A in inflammatory arthropathies. Mol. Cell. Endocrinol., 180(1-2), 163-7.

Griselda, R. M., Anayansi, M. H., & Iván, V. (2012). Activin A Promotes Neuronal Differentiation of Cerebrocortical Neural Progenitor Cells. *PLoS One, 7*(8), e43797. doi: 10.1371/journal.pone.0043797.

Harrison, C. A., Chan, K. L., & Robertson, D. M. (2006). Activin-A Binds Follistatin and Type II Receptors through Overlapping Binding Sites: Generation of Mutants with Isolated Binding Activities. *Endocrinology, 147*(6), 2744-2753. doi: 10.1210/en.2006-0131.

Hashimoto, O. & Funaba, M. (2011). Activin in glucose metabolism. *Vitam Horm, 85,* 217-34. doi: 10.1016/B978-0-12-385961-7.00011-1.

Hubner, G., Brauchle, M., Gregor, M., & Werner, S. (1997). Activin A: a novel player and inflammatory marker in inflammatory bowel disease? *Laboratory Investigations, 77,* 311-318.

Hubner, G., Brauchle, M., Gregor, M., & Werner, S. (1997). Activin A: a novel player and inflammatory marker in inflammatory bowel disease. *Lab. Invest., 77*, 311–318.

Hubner, G., Hu, Q. J., Smola, H., & Werner, S. (1996) Strong Induction of Activin Expression after Injury Suggests an Important Role of Activin in Wound Repair. *Developmental Biology, 173*, 490-498.

Jones K.L., de Kretser, D. M., Patella, S., & Phillips, D. J. (2004). Activin A and follistatin in systemic inflammation. *Molecular and Cellular Endocrinology, 225,* 119-125.

Kreidl, E., Oztürk, D., Metzner, T., Berger, W., & Grusch, M. (2009). Activins and follistatins: Emerging roles in liver physiology and cancer. *World J. Hepatol., 1*(1), 17-27. doi:10.4254/wjh.v1.i1.17.

Ling, N., Ying, S. Y., Ueno, N., Shimasaki, S., Esch, F., Hotta, M., & Guillemin, R. (1986). A homodimer of the beta-subunits of inhibin A stimulates the secretion of pituitary follicle stimulating hormone. *Biochem. Biophys. Res. Commun.*, 1129-1137.

Maeshima, A., Miya, M., Mishima, K., Yamashita, S., Kojima, I., & Nojima, Y. (2008). Activin A: autocrine regulator of kidney development and repair. *Endocr J., 55(*1), 1-9.

Michel, U., Ebert, S., Phillips, D., & Nau, R. (2003a). Serum concentrations of activin and follistatin are elevated and run in parallel in patients with septicemia. *European Journal of Clinical Endocrinology, 148*, 559-564.

Michel, U., Gerber, J., E O'Connor, A., Bunkowski, S., Brück, W., Nau, R., & Phillips, D. J. (2003b). Increased activin levels in cerebrospinal fluid of rabbits with bacterial meningitis are associated with activation of microglia. *Journal of Neurochemistry, 86*, 238-245. doi: 10.1046/j.1471-4159.2003.01834.x.

Nielsen, H. C. & Torday, J. S. (2011). A new compass for activin research--a triumph for systems biology. *Endocrinology, 152*(10), 3587-8. doi: 10.1210/en.2011-1620.

Nusing, R. M. & Barsig, J. (1999). Induction of prostanoid, nitric oxide, and cytokine formation in rat bone marrow derived macrophages by activin A. *Br. J. Pharmacol., 127*(4), 919–926. doi: 10.1038/sj.bjp.0702626.

Oshima, Y., Ouchi, N., Shimano, M., Pimentel, D. R., Papanicolaou, K. N., Panse, K. D., Tsuchida, K., Lara-Pezzi, E., Lee, S. J., & Walsh, K. (2009). Activin A and Follistatin-like 3 determine the susceptibility of heart to ischemic injury. *Circulation, 120*(16), 1606-1615. doi: 10.1161/CIRCULATIONAHA.109.872200.

Phillips, D. J., de Kretser, D. M., & Hedger, M. P. (2009). Activin and related proteins in inflammation: Not just interested bystanders. *Cytokine & Growth Factor Reviews, 2*, 153-164.

Phillips, D. J., Jones, K. L., Clarke, I. J., Scheerlinck, J. P., & de Kretser, D. M. (2005). Activin A: From sometime reproductive factor to genuine cytokine. *Veterinary Immunology and Immunopathology, 108*(1-2), 23-27. doi: 10.1016/j.vetimm.2005.08.011.

Phillips, D. J., Jones, K. L., Scheerlinck, J. Y., Hedger, M. P., & de Kretser, D. M. (2001). Evidence for activin A and follistatin involvement in the systemic inflammatory response. Molecular and Cellular *Endocrinology, 180*(1-2), 155-162. doi: 10.1016/S0303-7207(01)00516-0.

Rapeah, S., Dhaniah, M., Nurul, A. A., Norazmi, M. N. (2010). Phagocytic activity and pro-inflammatory cytokines production by the murine macrophage cell line J774A.1 stimulated by a recombinant BCG (rBCG) expressing the MSP1-C of Plasmodium falciparum. *Tropical Biomedicine, 27*(3), 461-469.

Robinson, L. J., D'Ombrain, M. C., Stanisic, D. I., Taraika, J., Bernard, N., Richards, J. S., Beeson, J. G., Tavul, L., Michon, P., Mueller, I., & Schofield, L. (2009). Cellular tumor necrosis factor, gamma interferon, and interleukin-6 responses as correlates of immunity and risk of clinical Plasmodium falciparum malaria in children from Papua New Guinea. *Infect. Immun., 77*(7), 3033-43. doi: 10.1128/IAI.00211-09.

Rodgarkia-Dara, C., Vejda, S., Erlach, N., Losert, A., Bursch, W., Berger, W., Schulte-Hermann, R., & Grusch, M. (2006). The activin axis in liver biology and disease. *Mutat. Res., 613*(2-3), 123-37.

Rodríguez-Martínez, G., Molina-Hernández, A., & Velasco, I. (2012). Activin A promotes neuronal differentiation of cerebrocortical neural progenitor cells. *PLoS One, 7(*8), e43797.

Sablotzki, A., Welters, I., Lehmann, N., Menges, T., Giorlach, G., Dehne, M., & Hempelmann, G. (1997). Plasma levels of immunoinhibitory cytokines interleukin-10 and transforming growth factor-beta in patients undergoing coronary artery bypass grafting. *Eur. J. Cardiothorac. Surg., 11*(4), 763-8.

Schmierer, B., Schuster, M. K., Shkumatava, A., & Kuchler, K. (2003). Activin A Signaling Induces Smad2, but Not Smad3, Requiring Protein Kinase A Activity in Granulosa Cells from the Avian Ovary. *The Journal of Biological Chemistry, 278,* 21197-21203. doi: 10.1074/jbc.M212425200.

Semitekolou, M., .Alissafi, T., Aggelakopoulou, M., Kourepini, E., Kariyawasam, H. H., Kay, A. B., Robinson, D. S., Lloyd, C. M., Panoutsakopoulou, V., & Xanthou, Activin-A induces regulatory T cells that suppress T helper cell immune responses and protect from allergic airway disease. *J. Exp. Med., 206*(8), 1769-85. doi: 10.1084/jem.20082603.

Smith, C., Yndestad, A., Halvorsen, B., Ueland, T., Waehre, T., Otterdal, K., Scholz, H., Endresen, K., Gullestad, L., Froland, S. S., Damas J. K., & Aukrust, F. (2004). Potential anti-inflammatory role of activin A in acute coronary syndromes. *J. Am. Coll. Cardiol., 44*(2), 369-375.

Steel, D. M. & Whitehead, A. S. (1994). The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunology Today, 15*(2), 81-88.

Sugama, S., Takenouchi, T., Kitani, H., Fujita, M., & Hashimoto, M. (2007). Activin as an anti-inflammatory cytokine produced by microglia. *Journal of Neuroimmunology, 192*(1–2), 31–39.

Ten Dijke, P., Miyazono, K., & Heldin, C. H. (2000). Signaling inputs converge on nuclear effectors in TGF-β signaling. *Trends in Biochemical Sciences, 25,* 64-70.

Ueland, T., Aukrust, P., Aakhus, S., Smith, C., Endresen, K., Birkeland, K. I., Gullestad, L., & Johansen, O. E. (2012). Activin A and cardiovascular disease in type 2 diabetes mellitus. Diabetes and Vascular *Disease Research, 9(*3), 234-247.

Wang, S. Y., Tai, G. X., Zhang, P. Y., Mu, D. P., Zhang, X. J., & Liu, Z. H. (2008). Inhibitory effect of activin A on activation of lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells. *Cytokine, 42(*1), 85-91. doi: 10.1016/j.cyto.2008.01.010.

Wang, T., Lazar, C. A, Fishbein, M. C., & Lynch, J. P. (2012). Pulmonary alveolar proteinosis. S*emin Respir Crit Care Med., 33*(5), 498-508.

Welt, C., Sidis, Y., Keutmann, H., & Schneyer, A. (2002). Activins, Inhibins, and Follistatins: From Endocrinology to Signaling. A Paradigm for the New Millennium. *Exp. Biol. Med., 227(*9), 724-752.

Werner, S. & Alzheimer, C. (2006). Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine & Growth Factor Reviews, 17,* 157-171.

Wieser, R., Attisano, L., Wrana, J. L., & Massagué, J. (1993). Signaling Activity of Transforming Growth Factor 3 Receptors Lacking Specific Domains in the Cytoplasmic Region. *Molecualr and Cellular Biology*, 7239-7247.

Woodruff, T. K. (1998). Regulation of cellular and system function by activin. *Biochem Pharmaco., 55*(7), 953-963.

Woodruff, T. K. (2000). Activin. *Neurobiology and Physiology*, 60208-285. DOI: 10.1006/rwcy.2000.08008.

Yndestad, A., Ueland, T., Øie, E., Florholmen, G., Halvorsen, B., Attramadal, H., Simonsen, S., Frøland, S. S., Gullestad, L., Christensen, G., Damås, J. K., & Aukrust, P. (2004). Elevated Levels of Activin A in Heart Failure: Potential Role in Myocardial Remodeling. *Circulation, 109*, 1379-1385. doi: 10.1161/01.CIR.0000120704.97934.41.

Zhang, Y. Q., Resta, S., Jung, B., Barrett, K. E., & Sarvetnick, N. (2009). Upregulation of activin signaling in experimental colitis. Am. *J. Physiol. Gastrointest Liver Physiol., 297*(4), G768-80. doi: 10.1152/ajpgi.90631.2008.

Zhu, J., Krishnegowda, G., Li, G., & Gowda, D. C. (2010). Proinflammatory responses by glycosylphosphatidylinositols (GPIs) of Plasmodium falciparum are mainly mediated through the recognition of TLR2/TLR. *Exp. Parasitol., 128*(3), 205-11. doi: 10.1016/j.exppara.2011.03.010.