Transformation Growth Factor β (TGF-β) Superfamily Signaling and Their Novel Candidate Antagonist, High-Temperature Requirement Factor A (HtrA3)

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Summary

Members of Transformation Growth Factor (TGF-β) superfamily, which include TGF-βs, growth differentiation factors, bone morphogenetic proteins, activin, inhibin, and glial cell line-derived neurotrophic factor, are multifunctional cytokines. Most ligands of the family signal through transmembrane serine/theronine kinase receptors and Smad proteins to regulate cellular functions, including proliferation, apoptosis, extracellular matrix secretion and adhesion, terminal differentiation and specification of developmental fate. The regulation of each of these functions of TGF-βs superfamily factors is important for embryonic development. Extracellularly multiple binding proteins for the TGF-β family have been characterized as regulators of TGF-β signaling. This review focused on a novel candidate antagonist of TGF-β superfamily signaling termed HtrA3. high-temperature requirement factor A (HtrA3) binds to a broad range of TGF-β proteins such as TGF-β1, TGF-β2, BMP4 and GDF5 and also inhibit at least TGF-β1 and BMP4 signaling.

Introduction

The transformation growth factor β (TGF-β) superfamily comprises a large and diverse group of polypeptide morphogens over 30 different cytokines including transformation growth factors β (TGF-βs), bone morphogenic proteins (BMPs), growth and differentiation factors (GDFs), activin, inhibin and others (Kingsley, 1994; Hogan, 1996). Members of TGF-βs superfamily regulate a broad range of cellular functions such as embryogenesis, organogenesis, morphogenesis of tissues like bone and cartilage, vasculogenesis, wound repair and angiogenesis, hematopoiesis and immune regulation (Massague et al., 1992; Massague and Chen, 2000; Moses and Serra, 1996; Roberts and Sporn, 1992; Wall and Hogan, 1994). The members of TGF-β superfamily are synthesized as prepropeptide precursor with an N-terminal signal peptide followed by the prodomain and the mature domain. The precursor molecules are proteolytically processed into mature TGF-β proteins in golgi apparatus by convertase family of endoprotease. The active forms of TGF-βs are composed of 2 mature peptide chains linked by disulfide bonds. TGF-βs ligands are typically found as homodimers although heterodimers can also forms. The members of this superfamily exhibit structural variation in their C-terminal amino acid sequences and share similar biological activities (Burt and Law, 1994).

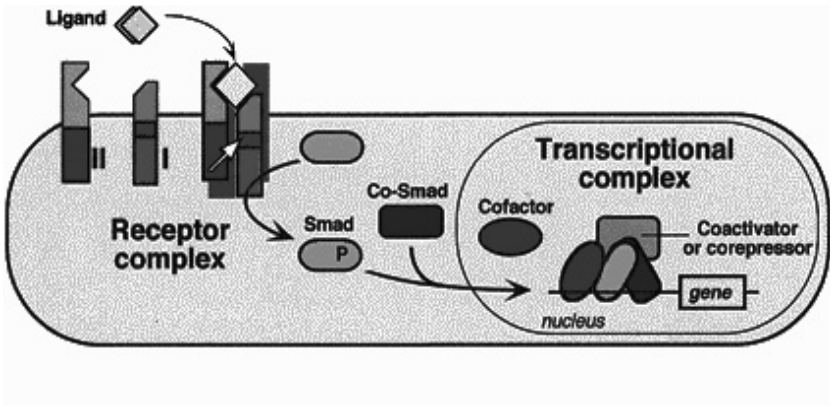
Signal Transduction

TGF-βs family exert their biological effects through binding to a heteromeric complex of type I and type II receptors (Heldin et al., 1997). Both receptors are

serine-threonine-specific protein kinase; upon ligand binding, the type II receptors then phosphorylates the type I receptors, which in turn phosphorylate activating intracellular signaling via Smads proteins that propagate the signal from the cytoplasm to nucleus (Massague and Chen, 2000; Massague and Wotton, 2000; Miyazono et al., 2001) (Fig.1). Smads are a family of intracellular proteins that participate in an evolutionarily conserved signal transduction for TGF-βs (Massague, 1998). At lease 10 vertebrate Smad proteins have been identified (Massague and Chen, 2000). Members of the Smad family play different roles in TGF-β superfamily signaling. (Baker and Harland, 1996; Eppert et al., 1996; Hoodless et al., 1996; Liu et al., 1996; Susuki et al., 1997; Thomsen, 1996; Yingling et al., 1996) (Table 1). For instance, R-Smads (Receptor Smads) include Smad2 and Smad3 (which are activated in response to TGF-β and activins) and Smad1, Smad5 and Smad8 (which are specifically regulated by BMP and GDF factors). Smads have been classified into 3 subtypes, e.g. receptor-regulated Smads (R-Smads), common-partner smads (Co-Smads), and inhibitory smads (I-Smads). Of the eight different Smad proteins in mammals, Smad1, Smad2 and smad3, Smad5, and Smad8 serve as R-Smads for TGF-β signaling pathways, Smad4 acts as a Co-Smad, and Smad6, Smad7 functions as an I-Smad for TGF-βsignaling. Upon binding TGF-β to type II and type I receptors, R-Smad bind Smad 4 and the complexes translocate to the nucleus. In the nucleus, the R-Smad-Co-Smad heteromers interact with various transcription factors and transcriptional co-activator or co-repressors, resulting in transduction of a wide variety of intracellular signals in target cells (Attisano and Wrana, 2002; Massague and Wotton, 2000; Miyazono et al., 2001). Thus, Smads and other transcription factors cooperatively regulate transcription of target genes through binding to their promoters. Transcriptional co-activators, including p300, CBP contain histone acetyl transferase (HAT) domains. Through acetylation of histones and probably other proteins, these transcriptional co-activators help Smads activate the transcription of target genes and in conjunction with other nuclear cofactors, regulate the transcription of target genes. A third group of Smad proteins, I-Smads negatively regulate TGF-β signaling by competing with R-Smads for receptor or Co-Smad and by targeting the receptors for degradation (Attisano and Wrana, 2002). I-Smads inhibit the signaling activities induced by TGF-β superfamily proteins. I-Smads physically interact with type I receptors activated by type II receptor kinases. Thus, I-Smads compete with R-Smads for activation by type I receptors, and inhibit signaling by the TGF-β superfamily proteins. In addition to inhibiting TGF-β superfamily signaling by preventing activation of R-Smads by the receptors, I-Smads also interact with R-Smads activated by the receptors, and interfere with the complex formation of R-Smads with co-Smads.

Table 1 Molecular components of TGF-β superfamily signal transduction

Ligands	Receptor		Receptor-regulated Smad	Common Smad
	Type II	Type I		
Activin →	ActRII ActRIIB →	ActRIB →	Smad2 Smad3 →	Smad4
TGF-β →	TβRII →	TβRI →	Smad2 Smad3 Smad1 →	Smad4
BMP2/4 →	BMPRII ActRII →	BMPRI BMPRIB →	Smad5 Smad8 →	Smad4



Source: Massague and Chen, 2000

Figure 1 A general scheme of smads as TGF-β superfamily signal transducers

TGF-β Superfamily Signaling and Development

Gene inactivation studies in mice during the past decade have greatly expanded the understanding of TGF-β superfamily signaling in animal development. Members of TGF-β superfamily have been shown to play essential roles in development in mammals as well as in *Caenorhabditis elegans* and *Drosophila melanogaster*. They can be found in virtually any cell types and throughout the development of all stages of any given species. BMPs were first discovered due to their ability to induce bone and cartilage formation in vivo by regulating the function of osteoblasts and chondroblasts. Mutation of BMP genes lead to various skeletal abnormalities. The various forms of TGF-β and activin are known for their roles in late stages of embryogenesis and in the mature organism. The activins have important roles in the mammalian endocrine reproductive axis. Expression of the signaling Smads appears to be wide spread dembryogenesis. In

mice, Smad1 is expressed first in the early embryonic mesoderm, and later ubiquitously, whereas Smad2 and Smad4 are ubiquitous in both embryonic and extraembryonic tissues in embryonic day 8.5. During organogenesis in the mouse, increased Smad1 and Smad2 expression is found in a variety of developing organs at sites of epithelial-mesenchymal interactions (Dick et al., 1998). The broad spatial and temporal expression of signaling Smads seem to indicate that the differential expression is not a critical determinant of cell type specific responses to TGF-β superfamily signals. The tissues-specific enrichment of Smad expression is important in the development of distinct responses to TGF-β superfamily signaling. However, the cell type-specific responses to TGF-β superfamily seems to involve differential expression of mediators downstream of Smads rather than differential expression of the signaling Smads themselves. In almost every developmental and physiological process, TGF-β signaling proteins are involved. Examining the signal transduction pathway of TGF-β superfamily members will continue to provide important information for understanding fundamental and developmental processes, as well as genetic causes of human disease.

Controlling the TGF-β superfamily proteins

In addition to tissue-specific expression of TGF-β superfamily ligands, a crucial regulatory step of TGF-β signaling is modulation by specific TGF-β antagonists. The TGF-β antagonists regulate the activity and cellular signaling of the TGF-β superfamily through multiple mechanisms. Intracellularly, for example, inhibitory Smad proteins, Smad 6 and Smad7, act as ligand-inducible inhibitors of signal transduction (Heldin et al., 1997). Extracellularly, multiple binding proteins for the TGF-β family have been characterized as regulators of TGF-β signaling. In vertebrate, the antagonist of TGF-β superfamily proteins comprises more than seven proteins, including noggin, chordin, chordin-like, follistatin, FSRP, the DAN/Cerberus protein and sclerostin. These proteins prevent ligand access to the signaling receptors (Dale and Jones, 1999; De Robertis et al., 2000; Wharton et al., 1993). These proteins may contribute to the formation of morphogen gradients during embryogenesis, to the relay of signals by extracellular signal transduction pathway, and to homeostasis of signaling in tissues (Barth et al., 1999; Liem et al., 2000; Hama and Weinstein, 2001). Follistatin regulates all aspects of biological activities of activin (Hemmati-Brivanlou et al., 1994; Nakamura et al., 1990) by preventing activin binds to its receptor. Activin signaling has been implicated in early limb development (Stern et al., 1995) and in skeletal muscle differentiation (Link and Nishi, 1997), and probably also in limb skeletogenesis. The activities of bone morphogenetic protein (BMP) are regulated by BMP-binding proteins such as chordin, noggin, the Cerberus/Dan families, and follistatin (Pearce et al., 1999). Noggin and chordin also inhibited BMP-4 signaling in a competitive manner by binding to BMP-4 and consequently interfering with the ability of BMP-4 to bind to cell-surface receptors (Zimmermann et al., 1996).

Recently, a novel candidate of TGF-β antagonist has been identified; HtrA3. HtrA3 (High temperature requirement A) is a member of mammalian HtrA. HtrA was initially identified in *E.coli* by two phenotypes of corresponding null mutants and named accordingly. Mutant did not grow at either elevate temperature (HtrA) (Lipinska et al., 1988) or failed to digest misfold protein in the periplasm (Deg P) (Strauch and Beckwith, 1988). HtrA shows an ATP -independent proteolytic activity and plays an important role in the degradation of misfolded proteins accumulated by heat shock of other stresses (Clausen et al., 2002). Therefore, its activity seems to be essential for bacterial thermotolerance and for cell survival at high temperatures.

HtrA is also involved in pathogenesis of Gram-negative and Gram-positive bacterial by degrading damage proteins that are produced by reactive oxygen species released from the host defense. In addition to proteolytic activity, HtrA is known to have a molecular chaperon activity (Spiess et al., 1999; Misra et al., 2000). The chaperon function is dominant at low temperatures, whereas the proteolytic activity is turned on at elevated temperatures (Spiess et al., 1999). HtrA is a highly conserved protein found in species ranging from bacteria to humans (Fig. 2). Similar to bacterial HtrA, the mammalian HtrA3 shares a modular architecture composed of a conserved trypsin-like protease domain, and PDZ domain at C-terminal. PDZ domain is a protein module that mediates specific protein-protein interactions and binds preferentially to the C-terminal three to four residues of membrane receptor and ion channels. The N-terminal region of HtrA3 shares high degree of sequence homologies with follistatin, an activin binding protein (Oh et al., 1995).

HtrA3 may regulate biological process by modulating growth factor systems that mediated by the transforming growth factor-β (TGF-β) family to which activin belongs. The expression pattern of mouse HtrA3 during embryogenesis has been investigated in detail and found that HtrA3 was characteristically expressed in a distinct set of embryo tissues where the development was largely regulated by TGF-β family proteins (Tocharus et al., 2004). For instance, HtrA3 is expressed in skeletal tissues, such as rudimentary tendons and ligaments and cells in future joint areas. Development of these tissues is regulated by BMPs, GDFs, and TGF-βs (Brunet et al., 1998; Capdevila and Belmonte, 2001; Francis-West et al., 1999; Schweitzer et al., 2001).

It has been proposed that signaling mediated by TGF- β superfamily participates in the maintenance of adult joints. TGF-β is regulated to keep articular chondrocytes in their normal, undifferentiated state. Transgenic mice carrying a dominant negative kinase-defective TGF-β type II receptor gene (Serra et al., 1997) or Smad3 knockout mice (Yang et al., 2001) showed phenotypes very similar to human osteoarthritis. In these mice, suppression of TGF-β signaling probably promoted terminal differentiation of articular chondrocytes, leading to osteoarthritis-like phenotypes. It was reported that the expression of HtrA3 increased substantially in articular cartilage cells of osteoarthritic mice (Tocharus et al., 2004). It is possible that HtrA3 antagonized TGF-β and aggravated osteoarthritis. The previous investigation has proved that HtrA3 binds to various

TGF-β proteins, and inhibit signaling in C2C12 myoblast cells.

All these findings suggest close association between HtrA3 and TGF-β signaling. HtrA3 may act to modulate the activity of TGF-β family members during development in normal physiology and in the altered conditions of disease states (Fig. 3). The actual mechanism of how HtrA3 inhibits TGF-β signaling remains elusive.

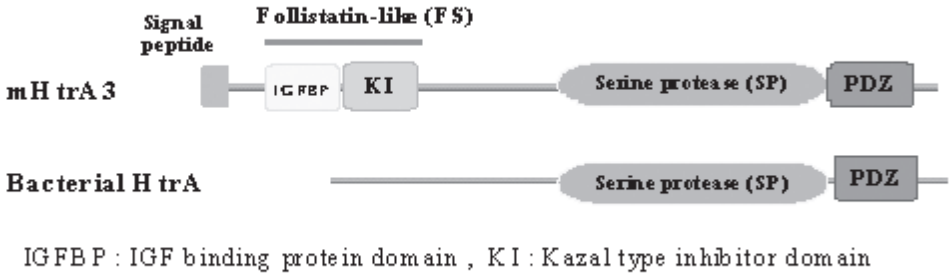


Figure 2 Domain structure of mammalian HtrA3 and bacterial HtrA

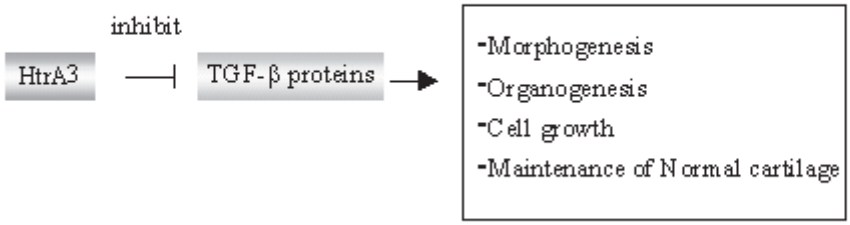


Figure 3 Possible role of HtrA3 in normal and pathological condition

Conclusion

Transforming growth factor-β (TGF-β) superfamily members are multifunctional cell-cell signaling proteins that play pivotal roles in the development of multicellular animals, but their signal transduction pathways are based on a relatively simple central signaling engine. The progressive elucidation of the mechanism that control this system is shedding light on the antagonist that controlled TGF-β signaling and its integration with regulatory networks of the cell. The diverse biological activities of TGF-βs are regulated by multiple TGF-β binding proteins. This review focused on the newly identified TGF-β antagonist

term HtrA3. The expression of HtrA3 is developmentally regulated and restricted in embryo tissues which depend largely on TGF-β signaling for their differentiation. Moreover, HtrA3 bound to various TGF-β proteins and inhibited the signaling of at least BMP-4 and TGF-β1. A much better understanding of the mechanism of HtrA3 will contribute to the more effective treatment of joint disease and also a variety of diseases regulated by this protein. Clearly, HtrA3 becomes a potential drug target and is awaited to be elucidated.

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