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Survey

Unchaining the beast; insights from structural and evolutionary studies on TGFβ secretion, sequestration, and activation

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ABSTRACT

TGFβ is secreted in a latent state and must be "activated" by molecules that facilitate its release from a latent complex and allow binding to high affinity cell surface receptors. Numerous molecules have been implicated as potential mediators of this activation process, but only a limited number of these activators have been demonstrated to play a role in TGFβ mobilisation *in vivo*. Here we review the process of TGFβ secretion and activation using evolutionary data, sequence conservation and structural information to examine the molecular mechanisms by which TGFβ is secreted, sequestered and released. This allows the separation of more ancient TGFβ activators from those factors that emerged more recently, and helps to define a potential hierarchy of activation mechanisms.

Contents

1. Introduction to TGFβ

The three TGFβ isoforms encoded by the human genome (TGFβ 1, 2 and 3) are part of a much larger group of 38 human growth factors described as the "TGFβ superfamily". This group includes growth and differentiation factors (GDFs), bone morphogenic proteins (BMPs), and several other important cytokines [1,2]. TGFβ plays a significant role in the development and homeostasis of many tissues. For example it performs crucial roles in wound healing, immunity, muscle differentiation, palate closure, bone growth, and control of cellular proliferation [3–5]. However, TGFβ is a beast that can bring devastation to numerous tissues if not properly controlled, and excess TGFβ activity is associated with

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Table 1
Mouse knock outs of TGFβ.

Mutation/ disruption	Heterozygous mouse phenotype	Homozygous mouse phenotype	Other Notes	Refs
TGFβ1	Normal and gain weight at same rate as WT mice	Die after 3–4 weeks due to multi-organ inflammation Infiltration of lymphocytes and macrophages, especially the heart and lungs High embryonic lethality Mice exhibit "dishevelled" appearance, and wasting Lack langerhans cells in epidermis Defective haematopoiesis and vasculogenesis	Develop gastrointestinal tumours when exposed to pathogens or combined with $Rag2^{-/-}$ mutation [155,156] Elimination of CD4+ and CD8+ T cells prevents inflammation and prolongs survival [157] No significant inflammation seen in small intestine, thymus, most connective tissues, brain, skin, or bone marrow	[3]
TGFβ2	No immediately apparent defects or embryonic lethality Closer analysis showed aortic root dissection and increased phosphorylation of Smad2, Smad3 and Erk1/2 [78]	Two thirds die shortly before or during birth Congenital heart defects; thin walled aorta, ventricular septation defects, hypercellular myocardium Skeletal defects; reduction in skull bone size and less ossification, retrognathia, complete cleft palate, shortened forelimbs, spina bifida, abnormal ribs and sternum Eye defects Inner ear defects Urogenital defects Degeneration of kidney tubular epithelium	Lungs exhibit no major defects, except collapsed airways in PN animals Brain, gut, tooth bud, and Thyroid glands normal	[4]
TGFβ3	Normal	No prenatal lethality Offspring did not suckle and died within 20 hours of birth Similar birth weight to WT animals but deteriorated rapidly and showed respiratory distress Severely disrupted lung development including mesenchymal thickening, hypercellularity, and pleural haemorrhage Cleft palate, (although at E14.5 shelves had grown out and were adherent, but did not fuse) No skeletal or cardiovascular abnormalities, or changes in tooth bud and whisker follicle development, despite high TGFβ3 expression in these tissues	$TGFβ2^{-/-}$ $TGFβ3^{-/-}$ double nulls display early embryonic death, with failure of midline fusion [5] $TGFβ2^{-/-}$ $TGFβ3^{-/+}$ also still display severe malformations while $TGFβ2^{-/+}$ $TGFβ3^{-/-}$ mice do not TGFβ1 knocked into the TGFβ3 locus gives partial but not complete rescue of palate closure [158] $TGFβ1^{RGE/RGE}$ $TGFβ3^{-/-}$ mice have defects in brain vascular morphogenesis [18]	[16,17]

liver cirrhosis [6], pulmonary fibrosis [7], arthritis [8], muscular dystrophy [9], aortic aneurysm [10], Alzheimer's disease [11], inflammation [12], and cancer [13,14], making TGFβ a major target for therapeutic research [15].

The importance of TGFβ in development is clearly demonstrated by the phenotypes of knock out mice (Table 1). TGFβ1 null mice die within three to four weeks of birth due to multiorgan inflammation [3]. TGFβ2 null mice display severe disruption in the development of many tissues, yielding congenital heart defects, skeletal defects, cleft palate, and urogenital defects, with most mice dying at birth [4]. TGFβ3 null mice die shortly after birth displaying significantly disrupted lung development and cleft palate [16,17]. Redundancy between TGFβ2 and TGFβ3 has been demonstrated by double knock out mice, which display early embryonic death due to failure of midline fusion [5], while $Tgfb1^{RGE/RGE}$ and $Tgfb3^{-/-}$ double mutant mice display cerebral haemorrhages and other defects in vasculogenesis [18].

Signalling by TGFβ is regulated at several levels; extracellular factors control TGFβ activation and receptor binding and intracellular factors modulate the downstream signalling pathways. The main features of the TGFβ pathway are summarised in Fig. 1. Within the ER, pairs of TGFβ precursor proteins form a dimeric complex, which is subsequently processed into the mature TGFβ dimer and the latency associated peptide (LAP) dimer by furin cleavage in the trans-Golgi network [19]. LAP binds TGFβ with high affinity, with the result that LAP and TGFβ remain associated after cleavage. This assemblage is referred to as the small latent complex (SLC), as when in this complex TGFβ cannot bind to its cell surface receptors [20]. Structural studies have shown that TGFβ adopts a similar structure in the free, latent, and receptor bound states, but when bound to LAP, the TGFβ receptor binding sites are shielded by regions of the propeptide [21]. Simultaneously with pro-TGFβ dimerisation in the ER, a single latent TGFβ binding protein (LTBP) also binds the pro-TGFβ complex *via* disulfide bonds with LAP [22,23]. The assemblage of LTBP and the SLC is referred to as the large latent complex (LLC).

Before TGFβ signalling occurs, the growth factor must be released from its latent complex; a process referred to as activation, and a variety of molecules have been implicated as catalysing this process (discussed later) [20]. Once released from