© 2017. Published by The Company of Biologists Ltd | Development (2017) 144, 4476-4480 doi:10.1242/dev.159756

MEETING REVIEW

The TGFβ superfamily in Lisbon: navigating through development and disease

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ABSTRACT

The 10th FASEB meeting 'The TGFβ Superfamily: Signaling in Development and Disease' took place in Lisbon, Portugal, in July 2017. As we review here, the findings presented at the meeting highlighted the important contributions of TGFβ family signaling to normal development, adult homeostasis and disease, and also revealed novel mechanisms by which TGFβ signals are transduced.

KEY WORDS: BMP, Cancer, TGFβ, Signal transduction

Introduction

The transforming growth factor β (TGFβ) superfamily consists of over 30 ligands that can be roughly divided into two arms: the TGFβ arm [TGFβs, activins, nodals and some growth and differentiation factors (GDFs)] and the bone morphogenetic protein (BMP) arm (BMPs and most GDFs). TGFβ superfamily ligands activate a complex of two type I and two type II transmembrane serine/threonine kinase receptors that phosphorylate pathway-specific SMADs (usually SMAD1/5/8 for the BMP arm, SMAD2/3 for the TGFβ arm). Phosphorylated SMADs (pSMADs) then form complexes with SMAD4 that accumulate in the nucleus to induce target gene expression (Fig. 1A). TGFβ family receptors can also activate non-SMAD signaling pathways (Fig. 1B-E). These seemingly straightforward signal transduction cascades impact virtually every developmental process, from cell fate specification to morphogenesis.

In July 2017, researchers from across the world gathered in Lisbon, Portugal, for the 10th Federation of American Societies for Experimental Biology (FASEB) meeting 'The TGFβ Superfamily: Signaling in Development and Disease' to discuss the latest findings in the field. A recurrent theme of the meeting, which was organized by Akiko Hata (UCSF, San Francisco, USA) and Mary Mullins (University of Pennsylvania, Philadelphia, USA), was the discovery of novel mechanisms used by cells to fine-tune TGFβ superfamily signaling to maintain proper output in a spatially and temporally controlled manner. A second theme was the involvement of TGFβ family members in various diseases, including cancers; in particular, the mechanisms and clinical relevance of the tumor-suppressing and tumor-promoting activities of TGFβ were discussed.

Regulation of TGFβ family signaling

Ligand delivery and signal spread

TGFβ family ligands function as morphogens to provide positional information in a concentration-dependent manner, but how activity gradients are established remains controversial. New studies suggest that the distance over which ligands travel and the mechanism of ligand delivery vary in a context- and ligand-dependent manner. For example, several groups reported findings consistent with the possibility that gradients form by ligand diffusion. David Umulis (Purdue University, West Lafayette, USA) used mathematical modeling to test whether BMP gradient formation in fish requires transport with the BMP-binding protein Chordin, as has been proposed in *Drosophila* (Shimmi et al., 2005; Wang and Ferguson, 2005). The resulting models were incompatible with a Chordin-based shuttling mechanism, but instead suggest that BMPs travel by free diffusion in the extracellular space and are removed at the end of the gradient by a sink of dorsally expressed Chordin (Zinski et al., 2017). Similarly, Ali Brivanlou (Rockefeller University, New York City, USA) reported that, in human gastruloids generated by BMP4-induced differentiation of embryonic stem cells, BMP4 induces the expression of its own inhibitor, noggin, to generate a reaction-diffusion mechanism that might underlie patterning (Etoc et al., 2016). In this context, BMP4 is secreted from, and can act only on, the basal side of cells, whereas noggin is present only on, and can inhibit BMP only if applied to, the apical side. These findings raise the possibility that the ligand and inhibitor meet intracellularly, in endosomes.

By contrast, Tom Kornberg (UCSF, San Francisco, USA) posits that Dpp (the fly ortholog of BMP4) does not move by diffusion, nor is it presented to receiving cells from the extracellular space. Instead, he presented data demonstrating that morphogens move along specialized filopodia, termed cytonemes, and are presented to receiving cells at sites of direct contact, termed morphogenetic synapses. Cytonemes have been shown to transfer ligands in fly, fish and chick embryos (Kornberg, 2017). Cellular protrusions also function in signal reception in the *Drosophila* ovary, where it is crucial that Dpp generated by niche cells can only access the immediately adjacent, and not the more distal, germline stem cell (GSC) (Chen et al., 2011). Hilary Ashe (University of Manchester, Manchester, UK) reported that Dpp protein is restricted to the side of the niche cell opposite the GSC-niche interface, and that the GSC extends protrusions that reach around the niche cell to access Dpp. Thus, cellular extensions can enable Dpp signal reception at a distance, or can restrict Dpp signaling to the local environment.

Not all TGFβ signaling occurs locally. Michael O'Connor (University of Minnesota, Minneapolis, USA) presented evidence that, in *Drosophila*, members of the activin family of ligands are delivered systemically through the circulation and act as both agonists and antagonists of different Babo type I receptor isoforms to regulate body size. Nodal signals only at short range to induce mesoderm and endoderm in fish, but within this domain it induces the expression of fibroblast growth factors (Fgfs) that signal over a longer range to induce mesoderm (van Boxtel et al., 2015). Paradoxically, Fgfs are potent inhibitors of endoderm formation, and Caroline Hill (Francis Crick Institute, London, UK) described work in zebrafish exploring a

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Development (2017) 144, 4476-4480 doi:10.1242/dev.159756

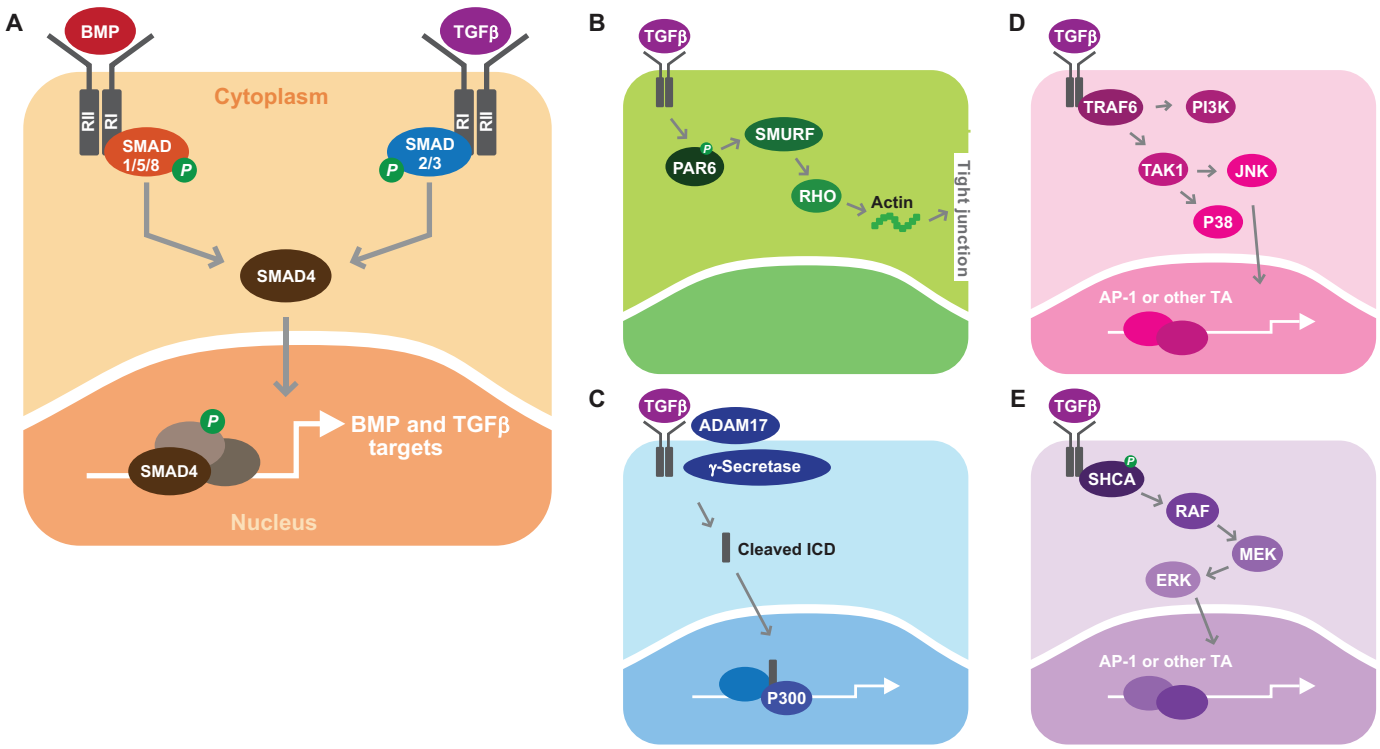

Fig. 1. Summary of TGFβ family signaling cascades. (A) BMP or TGFβ ligands bind and activate distinct sets of type I (RI) and type II (RII) transmembrane receptor serine/threonine kinases that then phosphorylate receptor-activated Smad1/5/8 or Smad2/3, respectively. These phosphorylated SMADs associate with a common SMAD, Smad4, and accumulate in the nucleus. The SMAD complex functions to activate or repress the expression of target genes, usually in conjunction with additional sequence-specific transcriptional regulators. (B) Activated type II TGFβ receptors phosphorylate Par6, leading to recruitment of Smurf1, which mediates localized ubiquitylation and turnover of RhoA. This leads to the dissolution of tight junctions. (C) The intracellular domain (ICD) of the type I TGFβ receptor can be released from the membrane following sequential cleavages mediated by ADAM17 and gamma-secretase. The cleaved ICD translocates into the nucleus to regulate transcription in association with p300. (D) Activated TGFβ receptors bind TRAF6, inducing autopolyubiquitylation. TRAF6 recruits TAK1 (MAP3K7) to activate JNK/p38, and these kinases phosphorylate and control the activities of AP-1 and other downstream transcriptional activators (TAs). JNK/p38 are also activated downstream of BMP receptors in some contexts (not shown). TRAF6 can also activate PI3K. (E) The type I TGFβ receptor recruits and phosphorylates ShcA (SHC1) to activate Erk through Ras, Raf and downstream MAPK cascades. Erk phosphorylates downstream TAs, such as AP-1, which then regulate target gene transcription.

mechanism that allows endoderm to be specified within the overlapping expression domain of *nodal* and Fgf family genes.

Signaling by heterodimeric ligands

Although it has long been known that TGFβ family ligands can form either homodimers or heterodimers, with the latter showing significantly greater specific activity, only recently has it been shown that, in some contexts, heterodimers are the only physiologically relevant ligand. For example, Bmp2/7 heterodimers are the obligate ligand that confers ventral patterning in zebrafish (Little and Mullins, 2009), and GDF9/15 heterodimers are the major active form of BMPs that regulate ovarian function (Peng et al., 2013). Martin Matzuk (Baylor College of Medicine, Houston, USA) reported that GDF9/15 heterodimers signal through a heterodimeric receptor consisting of two distinct type I receptor kinases but, surprisingly, the kinase activity of only one of the receptors is required. In addition, Mary Mullins (University of Pennsylvania, Philadelphia, USA) described work designed to test the hypothesis that the inability of homodimers to signal in the early embryo is caused by more efficient binding of BMP antagonists to homodimers than to heterodimers.

Activation of latent TGFβ family members

TGFβ is secreted as a complex in which the mature TGFβ ligand is kept inactive by association with the cleaved, amino-terminal part of its own precursor, the latency-associated peptide (LAP). Additional proteins associate with the complex, such as latent TGFβ binding protein 1 (LTBP1) and LRRC32, and mediate association with extracellular matrix components and the cell surface. Timothy Springer (Harvard Medical School, Boston, USA) described how TGFβ is activated by interaction between certain integrins and the LAP molecule; mechanical force unfolds LAP and releases active TGFβ (Dong et al., 2017). Dean Sheppard (UCSF, San Francisco, USA) reported that brain-specific deletion of the TGFβ-activating integrin aVβ8 leads to astrocytosis, impaired maturation of oligodendrocytes, loss of GABAergic inhibitory interneurons and postnatal spasticity in mice. The same defects are observed when TGFβ receptors are deleted from microglia. Surprisingly, postnatal depletion of microglia in mice mutant for integrin aVβ8 rescues myelination and motor function. This raises the possibility that postnatal interventions targeting microglial products might improve function in human neuropathologies where progression of motor defects is observed after birth. Collectively, these studies highlight the potential to modulate TGFβ signaling at the level of ligand activation, which might have therapeutic implications in diseases in which TGFβ activity is misregulated.

Timothy Springer and Tom Thompson (University of Cincinnati, Cincinnati, USA) also compared the structure of the GDF8 (myostatin) precursor with that of TGFβ and BMP9. Unlike TGFβ, in which the two arms of the prodomain encircle the mature domain to

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keep it in a closed, inactive conformation (Shi et al., 2011), or BMP9, in which the arms of the prodomain appear completely open (Mi et al., 2015), the GDF8/prodomain complex adopts an intermediate, partially open conformation and the prodomain remains bound to the mature domain even after cleavage. This might indicate an evolutionary adaptation to different degrees of ligand activation. It will be interesting to correlate ligand conformation to the evolution of extracellular ligand traps.

Regulation of receptor trafficking and turnover

A number of talks highlighted how membrane trafficking functions to propagate TGFβ signals. For instance, Rik Derynck (UCSF, San Francisco, USA) showed that most TGFβ receptors reside intracellularly and are transported to the plasma membrane in response to specific stimuli such as high glucose or insulin (Budi et al., 2015; Wu and Derynck, 2009) that induce activation of Akt signaling. Consequently, insulin stimulation greatly enhances the number of TGFβ receptors at the cell surface through an Akt-controlled transport mechanism, and this increases TGFβ responsiveness while also enabling TGFβ signaling to participate in the response to insulin (Budi et al., 2015). Tamara Alliston (UCSF, San Francisco, USA) described how reduced cytoskeletal tension releases TGFβ type I receptors from focal adhesions, enabling them to colocalize with type II receptors to form a functional signaling complex (Rys et al., 2015).

Interestingly, the subcellular localization of SMADs is also mechanosensitive (Allen et al., 2012). Jeff Wrana (Mount Sinai Hospital and University, Toronto, Canada) reported that, in cells grown on a soft matrix, TGFβ-induced pSmad2 resides in the cytoplasm, whereas in cells grown on a stiff surface pSmad2 translocates to the nucleus, where it can exert its transcription factor function. Jun (Kelly) Liu (Cornell University, Ithaca, USA) reported the identification of two tetraspanins (TSP-12 and TSP-14) that function redundantly in *C. elegans* to promote cell surface localization of the type II BMP receptor and an ADAM10 ortholog, the latter of which is thought to cleave neogenin to promote BMP signaling (Wang et al., 2017).

After internalization, TGFβ family receptors are sorted to exocytic vesicles for recycling or to lysosomes for degradation. Hilary Ashe reported that, in the *Drosophila* embryo, integrins and sorting nexin 17 function together to direct trafficking of type I receptors toward recycling endosomes and away from lysosomes. This facilitates Dpp signaling both by preventing receptor degradation and by increasing the dwell time of the active signaling complex in endosomes.

TGFβ family signaling in disease

Heightened TGFβ signaling in Marfan syndrome and fibrodysplasia ossificans progressive

In Marfan syndrome (a connective tissue disorder), the deficiency of fibrillin 1 (FBN1), a constituent of elastic fibers, leads to impairment of TGFβ retention in the matrix and is associated with overactivity of TGFβ. This causes emphysema, aortic aneurysms and death at an early age (Andelfinger et al., 2016). Thus, although mutations in the *FBN1* gene initially lead to weaker elastic fibers, a feedback loop involving overactive TGFβ plays a major role in disease progression. Harry Dietz (Johns Hopkins University School of Medicine, Baltimore, USA) now reported that activation of ERK1/2 MAP kinase (MAPK3/1) is a crucial event in the development of aortic disease. The importance of this pathway is illustrated by identification of protective modifier loci in both humans and mice with Marfan syndrome; genetic and functional analyses implicate the *MAP3K4* and *MAP2K6* genes, which encode MAPK kinases. In Marfan mice, protection from aneurysm progression is associated with abrogation of pathological SMAD, ERK1/2 and p38 (MAPK14) activation. These findings provide a basis for treatment of patients with Marfan syndrome with inhibitors of TGFβ or of the ERK1/2 MAP kinase pathway.

Several talks highlighted new findings related to the cause of fibrodysplasia ossificans progressiva (FOP), a disabling disease in which a mutation in the intracellular domain of the BMP type I receptor ACVR1 leads to heterotopic ossification (HO) (Shore et al., 2006). Studies reported by Aris Economides (Regeneron Pharmaceuticals, Tarrytown, USA) showed that ligand is required to activate the mutant receptor to trigger HO, and that the relevant ligand is activin, which normally functions as a competitive inhibitor of BMP-activated ACVR1 to keep bone growth under control (Hatsell et al., 2015). Since immune cells are a rich source of activins, these findings might explain the observation that there is a strong immunological component to FOP. Highlighting the role of the immune system in FOP, Eileen Shore (University of Pennsylvania, Philadelphia, USA) reported that inflammatory cytokines are upregulated in mouse models of FOP, and that HO is attenuated in mice lacking macrophages and/or mast cells.

TGFβ in tumor suppression and progression

TGFβ suppresses most cancers at early stages of tumorigenesis, but later promotes tumorigenesis (Massagué, 2012). The tumor-promoting effects of TGFβ include direct influences on tumor cells, as well as effects on cells in the tumor stroma, for example stimulation of angiogenesis or immune system suppression (Fig. 2). The tumor-suppressive and tumor-promoting roles of TGFβ were discussed by several speakers.

A demonstration of the tumorigenic effects of TGFβ was described by Joan Seoane (Vall d'Hebron Institute of Oncology, Barcelona, Spain), who reported that mutations in *FBN2* are common driver mutations in lung cancer, occurring in ∼5% of all cases. These mutations lead to activation of TGFβ, similar to what was found for mutations in the related *FBN1* gene in Marfan syndrome, and this promotes tumorigenesis. In the case of colorectal cancers, it is known that activating mutations in the Wnt pathway drive expression of the oncoprotein Myc, which then crosstalks with TGFβ to promote tumorigenesis. Following on from this, Andrei Thomas-Tikhonenko (University of Pennsylvania, Pennsylvania, USA) reported that Myc drives transcription of miR-17-92, which downregulates both thrombospondin 1 and components of the TGFβ signaling cascade to promote angiogenesis.

An important part of the tumorigenic effects of TGFβ is the induction of epithelial-mesenchymal transition (EMT), which involves the loss of epithelial and gain of mesenchymal characters and leads to increased invasiveness and metastasis (Moustakas and Heldin, 2016). TGFβ induces expression of members of the Snail, Twist and Zeb families of transcriptional regulators that drive EMT. Aristidis Moustakas (Uppsala University, Uppsala, Sweden) described work implicating the chromatin modulator high mobility group A2 (HMGA2) in TGFβ-mediated induction of EMT transcription factors. The mechanism involves suppression of Dicer and the recruitment of DNA methyl transferase 3A (DNMT3A), both of which promote EMT. Rik Derynck reported that upon TGFβ stimulation, Smad3 binding to the *Snail1* promoter in association with the histone methyltransferase SETDB1 represses Snail expression, in contrast to activation of Snail expression when Smad3 combines with the histone acetyl transferase p300. Thus, levels of SETDB1, and competition between SETDB1 and

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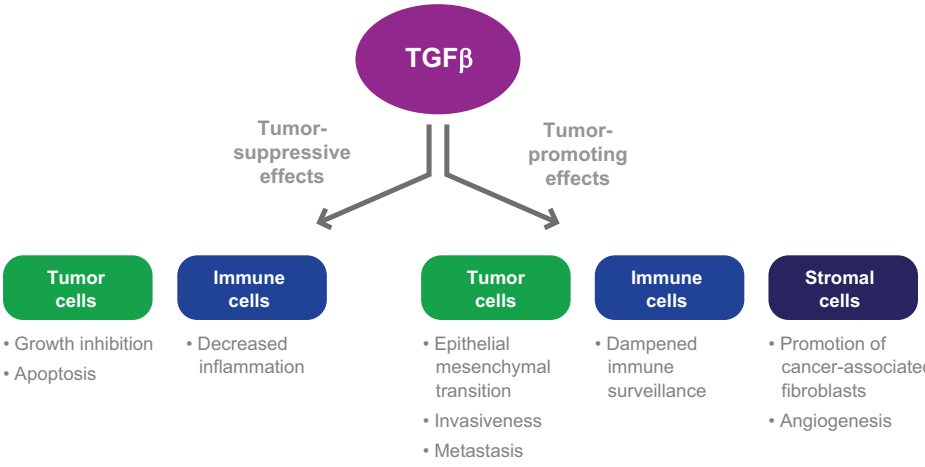

Fig. 2. TGFβ can function to promote or suppress tumorigenesis. TGFβ can act directly on tumor cells, or can function indirectly through immune or stromal cells to promote or suppress tumorigenesis through a variety of mechanisms, as illustrated.

p300 for binding to pSmad3, determine whether TGFβ suppresses or promotes Snail1 expression and EMT. Using a mouse model in which pancreatic cancer is driven by a $Kras^{G12D}$ mutation, Joan Massagué (Memorial Sloan-Kettering Cancer Center, New York, USA) showed that Ras-responsive element binding protein 1 (Rreb1), which is activated by phosphorylation downstream of $Kras^{G12D}$, is prebound to Smad2/3 target sites and is needed for TGFβ-induced EMT. Thus, the switch from tumor suppressor to tumor promoter activity of TGFβ is dependent on Rreb1 as a binding partner of SMADs. Kohei Miyazono (Tokyo University, Tokyo, Japan) described a method that enables visualization of metastases at single-cell resolution (Kubota et al., 2017). He showed that pretreatment of A549 lung carcinoma cells with TGFβ significantly increases metastasis in the lungs after intracardiac injection.

Loss-of-function mutations in components of the TGFβ pathway are associated with many cancers, illustrating the tumor suppressor abilities of TGFβ. Gareth Inman (University of Dundee, Dundee, UK) reported that loss-of-function mutations in TGFβ pathway components occur in ∼25% of squamous cell carcinomas of the skin; in particular, inactivation of the TGFβ pathway in Lgr5-positive stem cells of hair follicles drives tumorigenesis (Cammareri et al., 2016). David Wotton (University of Virginia, Charlottesville, USA) showed that mutations in *Pten* and *Tgfbr2* lead to rapid progression to invasive metastatic cancer in a mouse model of prostate cancer. Increased chromatin accessibility, with high histone H3 K27 acetylation, and increased expression of a number of transcription factors, including Sox2, EGR2 and Klf10, was observed in these tumors. Lopa Mishra (George Washington University, Washington DC, USA) also showed that mice deficient in β2-spectrin (β2SP) and Smad3 phenocopy Beckwith-Wiedemann syndrome (BWS), a human stem cell syndrome that is characterized by loss of imprinting, high levels of insulin-like growth factor 2 (IGF2), and aberrant signaling by CCCTC-binding factor (CTCF). She showed that Smad3, β2SP and CTCF bind to each other and repress expression of IGF2 in response to TGFβ treatment. She also reported that mutations in genes encoding TGFβ pathway proteins are observed in 24% of hepatocellular carcinomas, a cancer associated with BWS, and that this correlates with poor prognosis (Chen et al., 2017).

Inhibition of TGFβ activity in the treatment of malignancies

There has been substantial progress in the application of checkpoint inhibitors, such as antibodies against CTLA4, PD-1 (PDCD1) or PD-L1 (CD274), in cancer immunotherapy (Topalian et al., 2015), and TGFβ inhibitors hold similar promise. Rosemary Akhurst (UCSF, San Francisco, USA) reported that anti-TGFβ antibodies are more effective than anti-PD-1 treatment in a mouse model of squamous cell carcinoma. Anti-PD-1 treatment leads to increased levels of immunosuppressive regulatory T cells (Tregs), whereas anti-TGFβ treatment enhances the ratio between cytotoxic T cells and Tregs; combining the two treatments leads to a cure of 50% of the tumor-bearing animals. Stephen Nishimura (UCSF, San Francisco, USA) designed anti-integrin antibodies that block the activation of TGFβ, and these show therapeutic promise in inhibiting metastasis in lung cancer models. Ravindra Kumar (Acceleron Pharma, Cambridge, USA) reported the design of selective TGFβ family ligand traps (IntelliTrap™) consisting of the extracellular domain of naturally cooperative pairs of type I and type II receptors, fused to the IgG Fc intracellular domain. Engineered heterodimers of ActRIIb/ALK4 (Acvr2b/Acvr1b) selectively trap negative regulators of muscle mass such as activins, GDF8 and GDF11, but not BMP9, and might have therapeutic value for diseases associated with muscle atrophy. A similar strategy might target TGFβ ligands in cancer treatment.

Many clinical trials are underway to determine whether blocking TGFβ activity can reduce tumor burden, as discussed by Michael Lahn (Incyte, Geneva, Switzerland) (Herbertz et al., 2015), but an outstanding question is whether these treatments might have unintended negative consequences due to loss of TGFβ tumor-suppressive effects. Lalage Wakefield (NCI/NIH, Bethesda, USA) reported that, in some models of metastasis, tumor-suppressive effects of TGFβ are retained even in advanced disease, most likely due to the ability of TGFβ to inhibit cancer stem cell proliferation. This raises a cautionary note that effective biomarkers are needed to decide who will benefit from anti-TGFβ therapy.

In summary, the meeting emphasized that the TGFβ field has matured to a dramatic degree, as evidenced by the diverse and cutting-edge research presented at the meeting. We look forward with great enthusiasm to the next FASEB meeting on TGFβ signaling, where we expect to learn more about the roles and regulation of TGFβ signaling in many contexts.

Acknowledgements

We thank all of the speakers, participants, sponsors and organizers of the meeting. We apologize to those whose talks were not covered here owing to space limitations. We thank Anne Martin for designing and creating the illustrations and Aristidis Moustakas and Caroline Hill for providing constructive feedback on this report.

Competing interests

The authors declare no competing or financial interests.

Funding

J.L.C. is funded by the National Institute of Child Health and Human Development (RO1HD067473) and the American Heart Association (15GRNT21800007). C.-H.H.

is supported by the Swedish Research Council (Vetenskapsrådet; 2015-02757) and the Swedish Cancer Society (Cancerfonden; 2016/445).

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