REVIEW ARTICLE

The latent transforming growth factor β binding protein (LTBP) family

Rahmi ÖKLÜ* and Robin HESKETH†[1]

*Northwestern University Medical School, Lake Shore Center, Room 1707, 850 North Lake Shore Drive, Chicago, IL 60611, U.S.A., and †Section of Cardiovascular Biology, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

The transforming growth factor β (TGFβ) cytokines are a multifunctional family that exert a wide variety of effects on both normal and transformed mammalian cells. The secretion and activation of TGFβs is regulated by their association with latency-associated proteins and latent TGFβ binding proteins (LTBPs). Over the past few years, three members of the LTBP family have been identified, in addition to the protoype LTBP1 first sequenced in 1990. Three of the LTBP family are expressed in a variety of isoforms as a consequence of alternative splicing. This review summarizes the differences between the isoforms in terms of the effects on domain structure and hence possible function. The close identity between LTBPs and members of the fibrillin family, mutations in which have been linked directly to Marfan's syndrome, suggests that anomalous expression of LTBPs may be associated with disease. Recent data indicating that differential expression of LTBP1 isoforms occurs during the development of coronary heart disease is considered, together with evidence that modulation of LTBP function, and hence of TGFβ activity, is associated with a variety of cancers.

Key words: atherosclerosis, cancer, fibrillin, Marfan's syndrome, TGFβ.

INTRODUCTION

Transforming growth factor β (TGFβ) exists as three mammalian isoforms (TGFβ1, TGFβ2 and TGFβ3). Each of these is usually secreted in large latent complexes (LLCs) which have no biological activity and comprise three components: a disulphide-bonded homodimer of mature TGFβ associated non-covalently with latentcy-associated proteins (LAPs; homodimers of the N-terminal fragment of precursor TGFβ) and a covalently attached molecule of latent TGFβ binding protein (LTBP) (Figure 1) [1–6]. Four LTBP genes have been identified: *LTBP1* [4,6,7], *LTBP2* [8–11], *LTBP*3 [12,13] and *LTBP4* [14]. LAPs are sufficient to render the mature homodimer inactive, and removal of both the LAPs and LTBP or modulation of their interaction is essential for any of the TGFβ isoforms to function.

The TGFβ cytokines modulate the growth and functions of a wide variety of mammalian cell types. TGFβ inhibits the proliferation of most types of cells [15], although it was first identified as sarcoma growth factor, synthesized by virally or chemically transformed fibroblasts [16–18]. It is now established that TGFβ isoforms can act as growth-promoting factors in some cell types [19,20], in addition to their more familiar role as growth inhibitors of many normal and transformed cells, with lung epithelial cells and keratinocytes being particularly susceptible [15]. TGFβ can also stimulate extracellular matrix (ECM) biosynthesis [21,22], induce monocyte chemotaxis [23], suppress lymphocyte function [24], and regulate both angiogenesis [22,25,26] and bone formation [27]. The implication of TGFβ in such a wide range of biological responses suggests that it plays important roles in many normal cellular functions. Consistent with these multiple roles, anomalous regulation of TGFβ activity has been associated with the development of a number of diseases, most notably several forms of cancer [28–33]. In breast and colon carcinomas in particular, elevated levels of immunoreactive TGFβ and/or mutations in TGFβ signal transduction components have been detected [34–37]. Indeed, it is possible that in some pathological tissues overexpression of the protein represents an attempt to compensate for loss of signal transduction [38,39]. The role of TGFβ in human cardiovascular development and disease is unclear and controversial, although it has been hypothesized that expression of TGFβ is necessary for the maintenance of vascular homoeostasis [40–42]. The study of TGFβ function has been confounded by the fact that antibodies raised against mature TGFβ may also bind to the small latent complex (SLC) of TGFβ associated with LAPs ([43]; R. Öklü, P. Ellis, A. Grace and J. Metcalfe, unpublished work). Thus it has been difficult to determine the level of active TGFβ in tissues and samples, as distinct from the amount of total (active plus sequestered) TGFβ present.

It has become evident in recent years that LTBPs may be involved in the assembly, secretion and targeting of TGFβ to sites at which it is stored and/or activated (Table 1). Thus these proteins may play critical roles in controlling and directing the activity of TGFβs. LTBPs may also exert effects independently of those associated with TGFβ, for example as structural matrix proteins. In this review we describe the family of LTBP proteins and the diverse roles that they appear to play, before discussing the evidence linking changes in the expression of LTBPs with cancer and atherosclerosis.

Abbreviations used: 8Cys domain, domain containing a conserved pattern of eight cysteine residues; ECM, extracellular matrix; EGF, epidermal growth factor; HEL, human erythroleukaemia; IGF2R, insulin-like growth factor 2 receptor; LAP, latentcy-associated protein; LLC, large latent complex; LTBP, latent transforming growth factor β binding protein; mLTBP, mouse LTBP; M6P, mannose 6-phosphate; M6PR, mannose 6-phosphate receptor; SLC, small latent complex; SMC, smooth muscle cells; SMC/EC, smooth muscle cells/endothelial cells; TGFβ, transforming growth factor β; TSP1, thrombospondin 1; VSMC, vascular smooth muscle cells.

[1] To whom correspondence should be addressed (e-mail t.r.hesketh@bioc.cam.ac.uk).

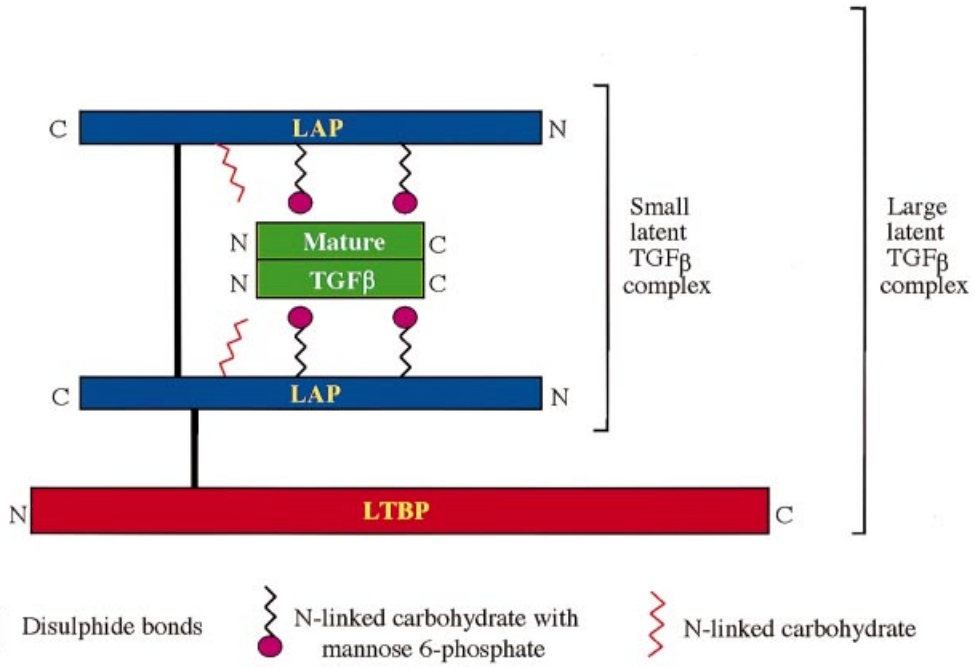

Figure 1 Structure of TGFβ latent complexes

The SLC comprises an active TGFβ homodimer associated non-covalently with a LAP homodimer. The LLC contains an additional protein, LTBP, which is disulphide-bonded to LAP.

Table 1 Tissue distribution of LTBP mRNAs

Gene	Tissue	Refs
LTBP1	Widely expressed.	[4,6,7,57,58]
LTBP2	Lung, heart, testes, placenta, spleen, liver and skeletal muscle. Expressed during elastic fibre formation in the aorta and cartilage perichondrium and blood vessels of mouse embryo.	[8,9,11,99]
LTBP3	Mainly liver; some in heart, central nervous system, bone, pancreas, artery and kidney.	[12]
LTBP4	Heart, skeletal muscle, pancreas, aorta, uterus and small intestine. Some expression in lung, placenta, liver and kidney.	[14,56]

Table 2 LTBP gene family

Gene	Species/chromosome	mRNA (kb)	No. of residues	Molecular mass (kDa)	EGF-like domains	8Cys domains	Refs
LTBP1	Human/2p22–24	5.2/7.0	1394, 1740	125–210	16–17	3	[4,7,131]
LTBP1	Rat	5.3/6.2	1712	186	18	4	[6]
LTBP2	Human/14q22–q33	7.5/9.0	1842	240–310	20	4	[9,10]
	Mouse/12D	6.8	1810	197	20	4	[11]
LTBP3	Human/11q12						
	Mouse/19B	4.6	1251	135	15	3	[12,13]
LTBP4	Human/19q13.1	5.0	1587	160	20	4	[14,56]

STRUCTURES OF LTBPs

LTBPs may be regarded structurally as a subfamily of the extracellular microfibrillar proteins fibrillin 1 and 2. LTBP and fibrillin proteins have several domains that contain a conserved pattern of eight cysteine residues (8Cys), found thus far only in this family (three to four in LTBPs, and seven to nine in fibrillins) [4,44]. Alternative use of promoters produces two major isoforms of LTBP1, defined as LTBP1S and LTBP1L [45]. LTBP1L contains an additional 346 amino acids from the N-terminal region of LTBP1S [7]. The third 8Cys domain of human LTBP1, which includes a putative N-glycosylation site, is necessary and sufficient for disulphide bonding to the TGFβ SLC [46,47]. The second and third 8Cys domains of mouse LTBP2 (mLTBP2) and mLTBP3 are also reported to bind covalently to TGFβ SLCs [48]. The solution structure of the 8Cys domain of fibrillin suggests that hydrophobic contacts may be important in the recognition of LAP by LTBP1 [49].

In addition to the 8Cys domains, LTBPs and fibrillins contain long tandem arrays of epidermal growth factor (EGF)-like domains (Table 2). Some EGF-like domains include a consensus sequence for calcium binding [50–52], and the effect of calcium binding to LTBP and fibrillin may be to confer resistance to proteolysis [53–55]. Human LTBPs 1, 2 and 4 and bovine LTBP2 contain an Arg-Gly-Asp (RGD) sequence that potentially promotes interaction with integrin(s), although interaction of LTBPs with integrins has not been detected *in vitro* [4,8,9,56]. LTBP1 also contains a single consensus heparin binding site, but interaction between LTBP1 and heparin sulphates has not been reported [57].

ALTERNATIVE SPLICING OF LTBP RNA

Alternatively spliced forms of LTBP1, LTBP3 and LTBP4 have been identified, with the variations between isoforms mainly involving exons encoding the 8Cys and EGF-like domains. LTBP1 RNA is alternatively spliced to give three shorter forms, denoted as LTBP1Δ41, LTBP1Δ53 and LTBP1Δ55 [57–59]. LTBP1Δ41 has the twelfth EGF-like domain completely deleted; the sequence deleted in LTBP1Δ53 encodes the eighth cysteine of the first 8Cys domain (Figure 2a). A single consensus heparin binding sequence is also deleted in LTBP1Δ53. The deleted region in LTBP1Δ55 encodes two putative N-glycosylation sites that have no known functional significance (Figure 2a). Two alternatively spliced variants of mLTBP3 RNA have been described, in which 51 bp and 159 bp respectively of the full-length gene are deleted (Figure 2b) [60]. The 51 bp deleted from the N-terminal region of mLTBP3 encode a sequence rich in proline and glycine. The 159 bp deletion encodes seven cysteines of the third 8Cys domain. LTBP4 has two alternatively spliced forms, generating LTBP4ΔEGF and LTBP4Δ2EGF (Figure 2c). LTBP4ΔEGF has the 14th EGF-like domain (which contains a calcium binding site) deleted; in LTBP4Δ2EGF the 14th and 15th EGF-like domains are deleted [56].

FUNCTIONAL EFFECTS OF ALTERNATIVE SPLICING

There is no experimental evidence for the functional effects of the deletion of EGF-like domains or the truncation of 8Cys domains in alternatively spliced LTBPs. Structural studies of an isolated 8Cys domain from fibrillin have indicated that the cysteine repeat is stabilized by four intradomain disulphide bonds [49]. Deletion of the eighth cysteine residue of the first 8Cys domain in LTBP1Δ53 may affect disulphide bonding and have important consequences for LTBP1 function, since loss of the eighth cysteine in 8Cys domains of fibrillin 1, arising from point mutations [61] or from exon skipping [62,63], results in Marfan's syndrome, a severe form of connective tissue disease. Although there are no

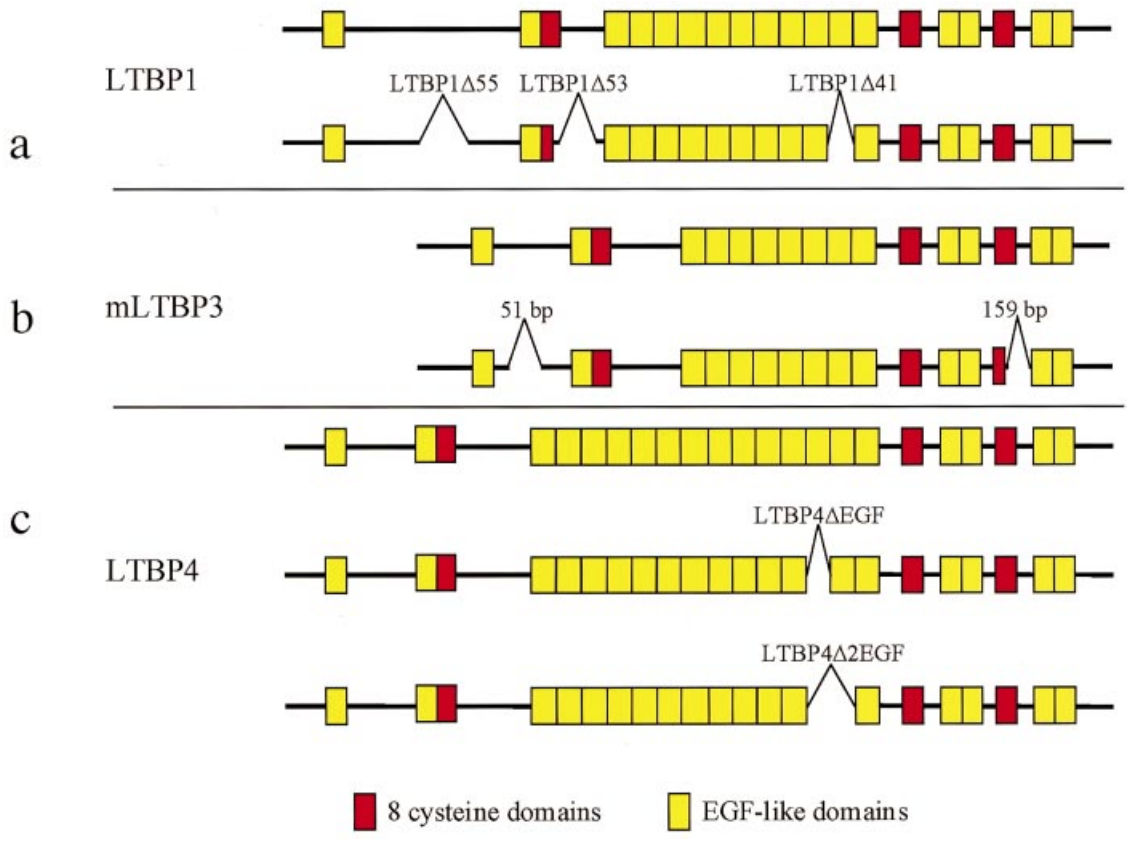

Figure 2 Alternative splicing of LTBP1, mLTBP3 and LTBP4

(**a**) LTBP1 RNA can be alternatively spliced to produce isoforms defined as LTBP1Δ55, LTBP1Δ53 and LTBP1Δ41. LTBP1Δ41 and LTBP1Δ53 are common to both the LTBP1L and LTBP1S isoforms. (**b**) mLTBP3 RNA can be alternatively spliced to produce transcripts with deletions of 51 bp and 159 bp. (**c**) LTBP4 RNA is alternatively spliced to produce isoforms defined as LTBP4ΔEGF and LTBP4Δ2EGF.

reports of mutations in LTBP1 in patients with Marfan's syndrome, mutations in LTBP2 have been reported in these patients [64], but their functional significance has not been resolved. In LTBP1Δ53 there is no neighbouring cysteine to replace the residue deleted by alternative splicing, and it is therefore probable that the structure in this region of the protein will be altered.

Deletion of the region containing the single consensus heparin binding site in LTBP1S blocks binding to the ECM, although specific inactivation of the binding site by site-directed mutagenesis, to demonstrate that it is required for interaction with the ECM, has not been reported [65]. Similar studies of heparin binding sites in growth factors such as vascular endothelial growth factor suggest, but do not prove, that these sites are required for localization to the ECM. For example, deletion of the exon that encodes the heparin binding site in vascular endothelial growth factor results in a protein that does not bind efficiently to the ECM [66]. By analogy, LTBP1Δ53, which lacks the heparin binding site, may differ from LTBP1 in its interactions with the ECM.

Alternative splicing of mLTBP3 results in deletion of seven cysteine residues in the third 8Cys domain that are necessary for covalent binding to LAP. This truncated form of mLTBP3 may therefore be secreted unattached to TGFβ SLCs [60]. Analogous deletions of the LAP-binding cysteine in the other isoforms of LTBP have not been reported.

Removal of calcium-binding EGF-like domains due to alternative splicing, which occurs in LTBP1 and LTBP4, may reduce the resistance to proteolytic degradation normally conferred by calcium. EGF-like domains may also be important for protein–protein interactions, as exemplified by the association of Notch and its ligands Delta and Serrate via EGF domains [67]. Furthermore, a number of other genes (e.g. those encoding aggrecan and PREF-1) give rise to alternatively spliced variants in which EGF-like domains are lost or modified [68,69], although the functional consequences of this are not known. However, in addition to the cysteine deletion in fibrillin in Marfan's syndrome, severe forms of this disease also result from mutations within EGF-like domains or from the skipping of exons that encode EGF-like domains [70]. These findings suggest that it will be of interest to determine whether the variant forms of LTBP1 and LTBP4 that lack EGF-like repeats present in the full-length proteins play a role in normal tissues or whether they are involved in the development of disease (see the section on 'Latent TGFβ complexes and human and atherosclerosis' below).

ASSEMBLY AND SECRETION OF TGFβ

Expression of TGFβ1 and LTBP1 mRNAs and proteins are co-ordinated in human erythroleukaemia (HEL) cells in response to PMA treatment [71]. In human foreskin fibroblast cells, expression of TGFβ1 and LTBP1 occurs with a similar time course after treatment with exogenous TGFβ1 (5 ng/ml; [72]). Furthermore, the addition of 1,25-dihydroxyvitamin D_3 to the oestrogen-receptor-negative breast cancer cell line, BT-20, also increases LTBP1 protein expression in parallel with that of TGFβ1, suggesting that they are co-ordinately regulated [73]. In contrast, the addition of TGFβ1 protein (0.1–5 ng/ml) to the osteoblast-like cell lines MG63, ROS 17/2.8 and UMR-106 suggests that the expression of LTBP1 and TGFβ1 is not co-ordinately regulated in these cell types. Furthermore, LTBP1 is undetectable in UMR-106 cells, which secretes almost exclusively the SLC [72].

There is evidence from pulse–chase studies (e.g. in HEL cells) that LTBP1 becomes covalently associated with the SLC, which comprises TGFβ1 associated non-covalently with LAP, within 15 min of its synthesis, and that secretion of the LLC occurs within 30 min of the synthesis of LTBP1 [71]. Secretion and folding of the SLC not associated with LTBP1 occurs much more slowly, with the complex being mainly retained in an immature form in the Golgi apparatus [71,74]. In addition, immunoprecipitation studies of cell lysates and conditioned media show that the TGFβ precursor may form anomalous disulphide bonds, suggesting that LTBP1 may be essential for the normal assembly and secretion of latent TGFβ1 [71]. It is clear from such studies that, in some cells, the assembly and secretion of TGFβ may require the co-expression of LTBP. However, in other cell types the expression of TGFβ and LTBP do not correlate. For example, in UMR-106 cells (which secrete almost exclusively the SLC), the folding, processing and rapid secretion of TGFβ does not depend on the co-expression of LTBP1, since LTBP1 is not expressed in these cells [72]. Furthermore, human glioblastoma cells (U-1240 MG) also secrete the SLC and active TGFβ in the absence of LTBP [1].

TISSUE TARGETING AND ACTIVATION

Activation of latent TGFβ *in vitro* has been studied in a variety of cellular systems. Some systems require co-cultures for the activation of TGFβ, e.g. co-cultures of smooth muscle cells and endothelial cells (SMC/EC), whereas in other cellular systems an isolated cell line is sufficient for the activation of TGFβ, e.g. human aortic SMC [75,76].

Endogenous activation of latent TGFβ occurs in bovine SMC/EC co-cultures [77,78]. Activation in this cell system *in vitro* requires the TGFβ LLC to be targeted to a cell surface, where it is activated proteolytically by plasmin [77,78]. Both mannose 6-phosphate (M6P; 100 μM) and antibodies directed against the insulin-like growth factor 2 receptor (IGF2R)/M6P receptor (M6PR) inhibit the activation of TGFβ in SMC/EC co-cultures [79]. In these experiments, activation of TGFβ in conditioned medium from the co-cultures was assayed by the suppression of TGFβ-dependent migration and protease production of bovine aortic endothelial cells [79]. As these responses were not inhibited when exogenous active TGFβ was added with M6P or anti-M6PR antibody to the SMC/EC co-cultures, it appears that binding of latent TGFβ to the IGF2R/M6PR is required for activation [79]. Furthermore, latent TGFβ complexes purified from human platelets can also bind isolated IGF2Rs/M6PRs [80]. In some human gastrointestinal tumours, mutation of this receptor prevents LAP binding, giving rise to reduced levels of active TGFβ1 [81]. These findings are consistent with a model in which latent TGFβ is localized specifically to the IGF2R/M6PR by binding M6P on LAP.

The oligosaccharide moieties at Asn^{82} and Asn^{136} of LAP contain M6P and sialic acid [82,83], and their enzymic removal or the addition of free M6P or sialic acid generates active TGFβ [84]. However, the M6PR is widely expressed at high levels, and this mechanism would therefore appear to lack specificity as well as possibly promoting the endocytosis of TGFβ.

Activation of the LLC may also involve targeting by LTBP1 through its RGD domain, the eight-amino-acid region identical to the cell binding domain of laminin α2, or its heparin binding domain [4,57]. The addition of excess free LTBP1, antibody directed against native platelet LTBP1 or a peptide fragment of LTBP1 to bovine SMC/EC co-cultures inhibits TGFβ activation in a dose-dependent manner [78]. This suggests that LTBP1 plays

a role in regulating the activation of TGFβ by concentrating the latent complex at appropriate cell surface sites. There is also evidence that LTBP1 targets the SLC to the ECM, and that LTBP1 is present with TGFβ as an extracellular fibrillar structure [85,86]. Subsequent proteolytic cleavage of LTBP1 by plasmin releases the TGFβ complex [87].

In contrast with these suggestions, other studies using bovine SMC/EC co-cultures indicate that anti-LTBP1 antibodies, M6P (300 μM) or a synthetic RGDS peptide do not affect TGFβ activation, but that an antibody directed against LAP blocks the binding of ^{125}I-labelled LLCs to SMC and inhibits TGFβ1 activation [88]. Thus the domain of LAP involved in cell surface targeting may not be related to M6P or the RGD sequence [88,89], despite the evidence that such regions comprise the cellular binding domains within LAP.

Another mechanism that appears to contribute to the localization of latent TGFβ is cross-linking of LTBP1 in the LLC to the ECM. *In vitro* studies with human lung fibroblast and fibrosarcoma cells have shown that the majority of secreted LTBP1 associates rapidly and covalently with the ECM [90]. The N-terminal amino acids of LTBP1 (residues 294–441) are cross-linked by the action of transglutaminase [65,91]. The plasmin-mediated activation of TGFβ by SMC/EC co-cultures or by bovine aortic endothelial cells treated with retinoids is blocked either by inhibitors of transglutaminase or by antibodies that neutralize bovine endothelial cell type II transglutaminase [92]. Inhibition of transglutaminase activity reduces the generation of active TGFβ, measured by the mink lung epithelial cell luciferase assay [93], but the transglutaminase inactivators do not themselves interfere with the direct action of TGFβ, the release of latent TGFβ from cells, or its activation by plasmin or by transient acidification [92]. Furthermore, the expression of transglutaminase increases in bovine aortic endothelial cells following retinoid addition, in parallel with the activation of TGFβ, consistent with the conclusion that type II transglutaminase is required for cell surface activation of latent TGFβ by plasmin [92].

A functional role for LTBP in regulating the local activity of TGFβ has emerged from several studies using antibodies to LTBP1. Foetal rat calvarial cells form mineralized bone-like nodules in long-term cultures, a development that is inhibited either by anti-LTBP1 antibodies or by antisense oligonucleotides directed against *LTBP1* [94]. This suggests that, in osteoblast-like cells at least, modulation of the LTBP1 content of latent TGFβ complexes may act to regulate their function. LTBP1 also surrounds the endocardial cushion mesenchymal cells of the mouse embryonic heart and, as anti-LTBP1 antiserum inhibits the endothelial–mesenchymal transformation in atrio-ventricular endocardial cells (an effect that is reversed by TGFβ), LTBP1 appears to act as a regulator of TGFβ availability during embryonic development [95].

These observations suggest that LTBPs may play important roles in the localization and consequent activation of TGFβ *in vitro*. Furthermore, *in vitro* experiments suggest that plasmin is required in the latent TGFβ activation model [15,41,87,96]. However, *in vivo* studies have indicated that other growth factors and proteins regulate the release of active TGFβ from latent complexes. Deletion of the murine plasminogen gene by homologous recombination does not reproduce the phenotype of TGFβ1 knock-out mice [97,98]. *In vivo* experiments are therefore consistent with either a minor or a redundant role for plasmin in the activation of latent TGFβ1. The only LTBP-null mice to have been generated, LTBP2$^{-/-}$, die at the implantation stage [99], a phenotype that differs markedly from that of TGFβ1-null mice [98]. This indicates that LTBP2 has an unidentified activity in early development that cannot be provided by other members of the family. This function may or may not involve TGFβ.

Recently, thrombospondin 1 (TSP1) was described as an important activator of TGFβ1 under normal physiological conditions [100]. The phenotypes of TGFβ1-null mice and TSP1-null mice are similar in many organs. An increase in mitosis occurs in the testes and the gastric epithelium of both strains of null mice, which may be a consequence of loss of TGFβ1-mediated inhibition of cell proliferation [100]. In the bronchiolar arteries of both strains, the vessel wall is thickened and laden with vascular smooth muscle cells (VSMC), suggesting an important role for TGFβ1 in regulating the proliferation of VSMC [100]. Furthermore, TGFβ1–3 immunostaining is markedly reduced in the vessel wall of TSP1-null mice compared with the vessel wall of wild-type mice [100]. These pathological changes are correlated with the loss of TSP1 and the consequent decrease in active TGFβ.

Treatment of TSP1-null mice with a peptide fragment derived from TSP1 partially restored the normal phenotype, presumably due to the fragment interacting with the latent complexes to enable the generation of active TGFβ1 [100]. Moreover, treatment of wild-type mice with a peptide fragment derived from LAP1, which inhibits activation of TGFβ1 by TSP1 *in vitro*, produced multiple organ pathologies similar to those observed in TSP1- and TGFβ1-null mice [100]. These observations strongly suggest that TSP1 is an important activator of TGFβ1 from latent complexes *in vivo*.

As summarized above, a number of LTBPs have been shown to associate directly with the ECM, including LTBP1 secreted from fibroblasts and fibrosarcoma cells [85,91], LTBP2 secreted from human fibroblast cells [9,101], and LTBP4 also secreted from human fibroblast cells [56]. Furthermore, in adult rat bone and cultured human fibroblasts, LTBP1 shows a fibrillar staining pattern [85,94], which has prompted speculation that LTBP may have a structural role in the ECM.

LATENT TGFβ COMPLEXES AND HUMAN ATHEROSCLEROSIS

Data relating to the function of TGFβ in the arterial wall are variable and often contradictory. The data from some animal studies suggest that TGFβ1 is pro-atherogenic [102–107]. In contrast with these animal studies, the data from the TSP1-null mice described above [100] suggest that reduced TGFβ1 activity resulted in VSMC hyperplasia in the arterial wall. Grainger and co-workers proposed a 'protective cytokine hypothesis', in which TGFβ1 is postulated to be an inhibitor of lesion development and lesion progression [42,96,108,109]. There is evidence suggesting that abnormal levels of TGFβ1 in serum and in the vessel wall are associated with the development of atherosclerosis [42]. Furthemore, the protective properties of drugs such as tamoxifen for the cardiovascular system of mice and monkeys [110–112] and in breast cancer clinical trials [113] may be derived in part from their capacity to increase the concentration of active TGFβ1 in the vessel wall. Control of TGFβ1 activity is therefore likely to be important in regulating its effect on the arterial wall.

The studies of immunoreactive TGFβ1 and its receptors in atherosclerotic plaques and in the vessel walls of animal models described above have not addressed the question of whether the immunoreactive TGFβ1 that is detected is active or whether it is present in latent complexes. Activity of the cytokine was generally inferred indirectly from the distribution of the TGFβ1 antigen and its receptors. The assumption that TGFβ1 activity can be inferred from co-localization of the TGFβ1 antigen and its

receptors may well be misleading. However, in some studies the presence of active TGFβ1 in arterial tissue was assayed by immunostaining using the fluorescein isothiocyanate-labelled truncated extracellular domain of the TGFβ type II receptor [41].

The role of TGFβ1 in human atherosclerosis will depend on (i) whether the cytokine is active, and (ii) whether it activates the TGFβ signalling pathways, neither of which can be inferred from immunohistochemistry, since TGFβ is normally secreted in inactive complexes covalently associated with the LTBPs. We have shown that LTBP1 mRNA and protein are detected at all of the sites in the neointima of coronary atherosclerotic plaques where TGFβ1 is detected (R. Oklü and J. Metcalfe, unpublished work).

Expression of human LTBPΔ41 mRNA, as assayed by quantitative competitor reverse transcription–PCR, is increased in diseased coronary arteries, although the splice variants LTBP1Δ53 and LTBP1Δ55 show no association with atherosclerotic disease (R. Öklü and J. Metcalfe, unpublished work). The finding that a variant of LTBP1 (LTBP1Δ41) is associated with arterial disease raises the question of the function of this isoform. There is currently no direct evidence concerning the roles of LTBP1; however, as the deletion in LTBPΔ41 removes a consensus calcium binding site, LTBP1Δ41 may be degraded more rapidly than LTBP1. Alternatively, as the deletions in LTBP1Δ41 include an EGF-like domain, it is possible that overexpression of LTBP1Δ41 causes anomalous targeting of TGFβ.

Defining the role of TGFβ in human atherosclerosis will be considerably more challenging, given the limited opportunities for experimental interventions. Indeed, it remains entirely possible that TGFβ1 may have opposing effects on lesion development in the media and the neointima. Definitive experiments to resolve this issue will be possible in transgenic mice in which TGFβ1 expression is regulated in a vascular-cell-specific manner. However, no definitive experiments for human atherosclerosis appear feasible at present, and it is by no means certain that TGFβ1 activity is coupled to atherogenesis in the same way in mice and humans.

LTBPs AND CANCER

Data relating to the anomalous expression of LTBPs in human cancers are derived mainly from comparative immunoassays. The general pattern appears to be one of reduced expression of LTBPs in malignant tissue, with increased amounts being present in the surrounding ECM. In colorectal adenomas (the presumed precursors of most colorectal adenocarcinomas) and in normal colonic mucosa, TGFβ1–LAP is expressed in both epithelial cells and stromal cells. However, LTBP1 is expressed only in stromal cells and in the ECM. There is no evident correlation between TGFβ1–LAP expression and the grade of dysplasia, but LTBP1 expression in the ECM is closely associated with regions of higher dysplasia [114]. In prostatic carcinoma there is immunohistochemical evidence that TGFβ1 is produced without associated LTBP1 in malignant cells, although TGFβ1–LTBP1 complexes are present in cystectomized prostatic and benign prostatic hyperplastic tissues [115]. In neuroendocrine tumours of the digestive system (midgut carcinoid tumours and endocrine pancreatic tumours), expression of all three TGFβ isoforms occurs, but LTBP1 is expressed only weakly compared with the pattern in surrounding stromal tissues, where there is high expression of LTBP1 and TGFβ1 but low levels of TGFβ2 and TGFβ3 [116]. In pancreatic neoplasms, expression of TGFβ1–LAP has been detected in the cytoplasm of tumour cells, while LTBP1 is found only in stromal cells and the surrounding ECM [117]. These patterns are consistent with the possibility that TGFβ might exert differential effects on tumour cells and adjacent stromal cells in terms of the induction of ECM-modulating enzymes and angiogenic factors. Human malignant ovarian tumours show increased expression of all three TGFβ isoforms compared with normal epithelial cells, whereas in the blood vessels of such tumours TGFβ1 is increased and TGFβ2 is decreased. However, LTBP1 immunoreactivity is generally greater in normal epithelium with respect to that in tumour cells [118]. In this latter study, patients with malignant tumours having blood vessels in which TGFβ, the TGFβ type I receptor or endoglin was expressed had a better prognosis than those with vessels negative for these proteins. Taken together, these data indicate the probable importance of the TGFβ system in modulating ovarian tumorigenesis. LTBP1 is not expressed in normal epithelial cells or in cancer cells in gastrointestinal tumours, but is detectable in the stroma cells surrounding the tumour [119]. We know of no data relating to LTBP1 expression in breast cancer.

Finally, levels of LTBP1 are also altered in other pathological conditions, including solar elastosis, solar keratosis and pseudoxanthoma elasticum [120], pancreatitis [121], rheumatoid arthritis [122], glomerulosclerosis [123–125], hypertension [126,127], muscular dystrophy [128], carcinoid heart disease [129] and tuberculous pleurisy [130]. However, the role of LTBP1 in these diseases remains to be determined.

CONCLUSIONS

LTBPs are emerging as a substantial and complex group within the extracellular microfibrillar protein family. LTBPs are secreted covalently bound to an LAP in complexes with TGFβ, and removal of LTBP is essential for the activation of TGFβ. LTBPs thus normally act to sequester TGFβ during its assembly and secretion, although TGFβ expression can occur in the absence of LTBPs, most notably in some types of tumour cell. However, there is evidence that LTBPs may play a more direct role in the activation of TGFβ by directing secreted complexes to localized cell surface or ECM sites through the action of RGD or heparin binding domains. In addition to regulating TGFβ function, LTBPs may also function as structural components of the ECM. Of the four known members of the LTBP family, three (LTBP1, LTBP3 and LTBP4) can be expressed in a variety of alternatively spliced forms, the variable regions comprising conserved EGF-like and Cys8 domains, putative N-glycosylation sites and a consensus heparin binding sequence. The functional consequences of the loss of one or more of these regions are unknown, but analogy with fibrillin suggests that differing forms may exhibit variations in location, protein–protein interactions and/or proteolytic susceptibility.

The role of LTBPs in disease remains unclear, but the evidence that the level of active TGFβ is critical in maintaining homoeostasis in the vascular wall is consistent with the suggestion that anomalous expression of LTBPs plays a role in arterial disease. Evidence that one isoform (LTBPΔ41) is expressed specifically in human atherosclerotic tissues suggests that, as with the closely related fibrillins, loss of critical domains in LTBP proteins may promote the development of disease.

The limited data presently available for human cancers indicate that levels of LTBP proteins tend to be reduced in malignant cells by comparison with corresponding normal tissue. Whether this

relates to the frequently observed increase in activity of TGFβ isoforms in tumour tissue remains to be established.

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