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Focus on molecules: Transforming growth factor beta induced protein (TGFB1p)

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1. Structure

The transforming growth factor-beta induced (*TGFB1*) gene (also known as β ig-h3), was first identified in an adenocarcinoma cell line where it was found to be up-regulated upon addition of transforming growth factor-beta (TGF- β) (Skonier *et al.*, 1992). The *TGFB1* gene encodes the transforming growth factor beta induced protein (TGFB1p, pseudonyms; β ig-h3, kerato-epithelin, RGD-CAP, MP78. Accession numbers: human, NP_000349, mouse, NP_033395, porcine, O11780, chick, 990367), a 72 kDa extracellular protein composed of 683 amino acid residues, an N-terminal cysteine-rich domain (EMI), four consecutive fasciclin 1 (FAS) repeats and an Arg-Gly-Asp (RGD) motif situated near the C-terminus (Figure 1).

The FAS repeats are seen in a range of different proteins including periostin (also known as osteoblast specific factor 2) to which TGFB1p shows a high degree of overall similarity. The order of the FAS repeats in these proteins is highly conserved, and the homology between the first repeat of TGFB1p and the first repeat of periostin is greater than between the individual repeats in TGFB1p. This indicates duplication of the FAS repeat prior to the separation of the genes. Other homologues of TGFB1p include stabilin-1 and stabilin-2.

No TGFB1p post translational additions have been reported but when purified from human or porcine corneas, the C-terminal is truncated in a fraction of the purified material (Andersen *et al.*, 2004). This truncation differs in human and porcine corneas and in porcine tissue two isoforms are found. Accordingly, the mature TGFB1p comprises residues from amino acids 24–657 in humans and 24–602 or 24–647 in porcine corneas. At present, the three dimensional structure of the full-length protein is unknown although the structure of the several fasciclin I repeats has been solved (Protein data bank (PDB) IDs: 1O70, 1NYO, 1X3B).

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2. Function

Since the discovery of the *TGFBI* gene, its expression has been observed in a wide range of tissues in several species. Elevated expression levels are particularly seen in zones of active growth, and high levels of TGFBIp are found in skin, bone, kidney and cornea.

TGFBIp is thought to function as a cell adhesion protein partly because of structural elements like the homology to FAS domains which are known to possess cell binding properties and partly because of the presence of the integrin binding RGD motif. Furthermore, *in vitro* studies have shown that TGFBIp mediates cell adhesion and/or spreading through integrins $\alpha1\beta1$, $\alpha3\beta1$, $\alpha v\beta3$, $\alpha v\beta5$, $\alpha6\beta4$ and $\alpha7\beta1$.

In addition to integrins TGFBIp interacts with several components of the extracellular matrix (ECM), including structural proteins such as collagens, fibronectin and proteoglycans like decorin and biglycan.

The exact biochemical role of these interactions remains largely unknown, but it seems likely that TGFBIp works as a regulatory mediator between the ECM and the cells embedded therein.

3. Disease involvement

Mutations in *TGFBI* or TGFBIp have been found to several distinct autosomal dominant genetically determined corneal disorders (Munier *et al.*, 1997). These diseases are characterized by the progressive accumulation of cloudy material in the cornea leading to impaired vision. The phenotypes depend on the particular mutation. Opaque protein deposits appears within the first decade of life in the most severe phenotypes in the third or fourth decade in less progressive cases.

To date 38 different mutations leading to corneal dystrophies have been attributed to mutations in the *TGFBI* gene. Some of the mutations are caused by intragenic insertions and deletions, but by far most of those affecting the cornea are missense mutations. They mainly affect codons Arg124 and Arg555, which are considered mutational hotspots, but it is noteworthy that the majority of the remaining disease causing mutations are located in the fourth FAS domain of TGFBIp. The FAS domain structure (PDB ID: 1O70) predicts the Arg124 and Arg555 residues to be located on the surface of the domain (i.e. solvent exposed), and mutations in these residues may therefore affect protein solubility or stability thus leading to aggregation (Clout and Hohenester, 2003).

It is particularly remarkable that different TGFBIp mutations cause completely different clinicopathologic phenotypes. These can be divided into four groups; lattice corneal dystrophy (LCD) type 1 and its variants with amyloid deposition in the cornea, granular corneal dystrophies (GCDs) characterized by crystalloid amorphous aggregates, a combination of GCD deposits with amyloid deposition (GCD type II) and Thiel-Behnke corneal dystrophy (TBCD) in which curious curly fibers deposit in the superficial cornea.

Diseased corneas have been shown to contain significantly increased levels of TGFBIp, but the exact molecular composition of the aggregates still remains to be elucidated.

At this point the only efficient remedy for the *TGFBI* corneal dystrophies is a full-thickness corneal transplant in cases with TGFBIp deposits throughout the corneal stroma or a lamellar keratoplasty or phototherapeutic keratectomy in those disorders that only affect the superficial cornea. The effect of this is only transient, however, since the donor cornea may accumulate new deposits of TGFBIp over time.

Aside from its involvement in the cornea, TGFB β may also be important in certain cancers as serum TGFB β levels have been found to be elevated in some cancer types in which TGFB β could be involved in tumor invasion and metastasis.

4. Future studies

Much still remains to be discovered about the functions of TGFB β . Although many ECM components interact with TGFB β , the precise roles of these interactions in cell adhesion and migration still need to be elucidated. This could be of value in research related to the *TGFB β* corneal dystrophies and in other corneal diseases, but also in cancer, where TGFB β may be a key component in tumor invasion and distant metastases. Insight into the mechanisms of protein aggregation and fibrillation will also be important for research on protein misfolding diseases, including those that affect the cornea.

Solving the three dimensional structure of wild-type and mutant TGFB β will help understand the mechanisms leading to protein aggregation in the *TGFB β* corneal dystrophies. This will possibly aid in the development of therapeutic agents capable of dissolving and/or inhibiting the formation of mutant TGFB β aggregates in the cornea.

Abbreviations

TGFB β , transforming growth factor beta induced protein; ECM, extracellular matrix; GCD, granular corneal dystrophy; LCD, lattice corneal dystrophy; TBCD, Thiel-Behnke corneal dystrophy.

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References

- Andersen RB, Karring H, Moller-Pedersen T, Valnickova Z, Thogersen IB, Hedegaard CJ, Kristensen T, Klintworth GK, Enghild JJ. Purification and structural characterization of transforming growth factor beta induced protein (TGFB β) from porcine and human corneas. *Biochemistry* 2004;43:16374–16384. [PubMed: 15610032]
- Clout NJ, Hohenester E. A model of FAS1 domain 4 of the corneal protein beta(ig)-h3 gives a clearer view on corneal dystrophies. *Mol Vis* 2003;9:440–448. [PubMed: 14502125]
- Munier FL, Korvatska E, Djemai A, Le Paslier D, Zografos L, Pescia G, Schorderet DF. Kerato-epithelin mutations in four 5q31-linked corneal dystrophies. *Nat Genet* 1997;15:247–251. [PubMed: 9054935]
- Skonier J, Neubauer M, Madisen L, Bennett K, Plowman GD, Purchio AF. cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta. *DNA Cell Biol* 1992;11:511–522. [PubMed: 1388724]

Figure 1. Schematic representation of TGFBIp

A; The secreted TGFBIp comprises residues 24–683. It contains an N-terminal cysteine rich domain (EMI), four fasciclin 1 domains (FAS 1–4) and a C-terminal RGD-motif. When purified from human corneas, TGFBIp has an alternative C-terminus (at residue 657) indicating a proteolytic processing of the protein, which might have a regulatory function. Mutational 'hot spots' are found at positions Arg124 and Arg555. **B;** Cartoon representation of FAS 4 from *Drosophila* (PDB ID: 1O70, modified from Clout and Hohenester, 2003). The FAS 4 domain consists of an N-terminal linker alpha-helix (α_L), five alpha-helices ($\alpha 1$ – $\alpha 5$) and five beta sheets ($\beta 1$ – $\beta 7$).