ROLE OF TRANSFORMING GROWTH FACTOR – BETA IN OPHTHALMOLOGY

Essay
Submitted for fulfillment of Master Degree in Ophthalmology

By

Taher Kamel Eleiwa
(M.B.B.Ch., Faculty of Medicine, Benha University)

Supervised by

Professor Dr. Waheed Mahmoud Orouk
(Professor of Ophthalmology; Faculty of Medicine, Benha University)

Professor Dr. Amr Ibrahim Sharawy
(Professor of Ophthalmology; Faculty of Medicine, Benha University)

Faculty of Medicine, Benha University
2015
Benha
دور عامل النمو المُحَوّل – بِيتما
فـى طـب وجـراحة العين
الرسالة
تمثـيـدًا للحصول على درجة الماجستير في طب وجراحة العين
مقدمة من الطبيب/ طاهر كامل عليوة
بكلاوريوس الطب والجراحة – جامعة بنها
إشراف
الأستاذ الدكتور/ وحيد محمود عروق
أستاذ طب وجراحة العين – كلية الطب – جامعة بنها
الأستاذ الدكتور/ عمرو إبراهيم شعراوي
أستاذ طب وجراحة العين – كلية الطب – جامعة بنها
كلية الطب
جامعة بنها
2015
بنها
بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

اقْرَأْ وَرَبُّكَ الَّذِي عَلَّمَ بِالْقَلَمِ عَلَّمَ الْنَّاسَ مَا لَمْ يَعْلَمُ

بالْقَلَمِ عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمُ

سورة العلق الآية 3-5

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ
Acknowledgement

First and foremost, deep thanks to ALLAH "The most merciful" for HIS grace and mercy and for giving me the effort to complete this work.

Words are few to speak and do fail to express my deepest gratitude to Professor Dr. Waheed Mahmoud Orouk, Professor of ophthalmology, Faculty of medicine, Benha University, for his continuous attention, follow up and providence of all facilities possible to complete this work, without his honest assistance and abundant patience, this work would have never come to light.

I would like to express my deep appreciation and most gratefulness for Professor Dr. Amr Ibrahim Sharawy, Professor of ophthalmology, Faculty of medicine, Benha University, for his unlimited support, keen supervision and continuous guidance not only through this essay, but also throughout my residency years.
Also special thanks to my family, colleagues and friends for their help in this work.
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>ii. Aim of the Work</td>
<td>4</td>
</tr>
<tr>
<td>iii. Chapter (1): Overview of TGF-Beta Pathobiology</td>
<td>5</td>
</tr>
<tr>
<td>A. Biochemistry of TGF-Beta</td>
<td></td>
</tr>
<tr>
<td>B. Isoforms of TGF-Beta Subfamily</td>
<td></td>
</tr>
<tr>
<td>C. Structure of TGF-Beta</td>
<td></td>
</tr>
<tr>
<td>D. TGF-Beta Signal Transduction</td>
<td></td>
</tr>
<tr>
<td>E. Central Role of TGF-Beta in Fibrogenic Reaction</td>
<td></td>
</tr>
<tr>
<td>F. Generation of Myofibroblasts by TGF-Beta</td>
<td></td>
</tr>
<tr>
<td>G. TGF-Beta and Epithelial-Mesenchymal Transition (EMT)</td>
<td></td>
</tr>
<tr>
<td>iv. Chapter (2): Outline of Ocular Fibrotic Disorders</td>
<td>12</td>
</tr>
<tr>
<td>v. Chapter (3): TGF-Beta and Ocular Surface Disorders</td>
<td>14</td>
</tr>
<tr>
<td>A. Fibrosis / scarring in ocular surface; cornea&amp; conjunctiva</td>
<td></td>
</tr>
<tr>
<td>B. Strategies to prevent excess fibrogenic reaction in ocular surface tissue by targeting TGF-beta signals</td>
<td></td>
</tr>
<tr>
<td>vi. Chapter (4): TGF-Beta and Lens Epithelium EMT</td>
<td>20</td>
</tr>
<tr>
<td>A. Anterior Subcapsular Cataract</td>
<td></td>
</tr>
<tr>
<td>B. Posterior Capsular Opacification</td>
<td></td>
</tr>
<tr>
<td>vii. Chapter (5): TGF-Beta and Glaucoma</td>
<td>24</td>
</tr>
<tr>
<td>A. Role of TGF-Beta in Pathogenesis of Glaucoma</td>
<td></td>
</tr>
<tr>
<td>B. TGF-Beta and Postoperative scarring</td>
<td></td>
</tr>
<tr>
<td>C. Anti-TGF-Beta&lt;sub&gt;2&lt;/sub&gt; Monoclonal Antibodies</td>
<td></td>
</tr>
<tr>
<td>D. Possible Future Alternatives to Antimetabolites</td>
<td></td>
</tr>
<tr>
<td>viii. Chapter (6): TGF-Beta and Retinal Diseases</td>
<td>28</td>
</tr>
<tr>
<td>A. Retinal Pigment Epithelium EMT and Proliferative Vitreoretinopathy</td>
<td></td>
</tr>
<tr>
<td>B. TGF-Beta and Diabetic Retinopathy</td>
<td></td>
</tr>
<tr>
<td>ix. Chapter (7): TGF-Beta and Choroidal Neovascularization</td>
<td>31</td>
</tr>
<tr>
<td>x. Summary</td>
<td>32</td>
</tr>
<tr>
<td>xi. References</td>
<td>34</td>
</tr>
<tr>
<td>xii. Arabic Summary</td>
<td>1</td>
</tr>
<tr>
<td>xiii. Aim of the Work (Arabic)</td>
<td>3</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ag</td>
<td>Antigen</td>
</tr>
<tr>
<td>AH</td>
<td>Aqueous humor</td>
</tr>
<tr>
<td>ALK-1</td>
<td>Activin receptor like kinase-1</td>
</tr>
<tr>
<td>ATF-2</td>
<td>activating transcription factor-2</td>
</tr>
<tr>
<td>ASCs</td>
<td>Adipose-derived stem cells</td>
</tr>
<tr>
<td>b-FGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone morphogenetic protein</td>
</tr>
<tr>
<td>BRVO</td>
<td>Branch Retinal Vein Occlusion</td>
</tr>
<tr>
<td>CAT-152</td>
<td>Cambridge Antibody Technology-152</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CTGF</td>
<td>Connective tissue growth factor</td>
</tr>
<tr>
<td>CTL</td>
<td>Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>CNV</td>
<td>Choroidal Neovascularization</td>
</tr>
<tr>
<td>CRVO</td>
<td>Central Retinal Vein Occlusion</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTHR</td>
<td>Delayed type hypersensitivity response</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>ERK</td>
<td>External regulated kinase</td>
</tr>
<tr>
<td>ERM</td>
<td>Epiretinal membranes</td>
</tr>
<tr>
<td>ES cells</td>
<td>Embryonic stem cells</td>
</tr>
<tr>
<td>EMT</td>
<td>Epithelial Mesenchymal Transition</td>
</tr>
<tr>
<td>FasL</td>
<td>Fas ligand</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast growth factor</td>
</tr>
<tr>
<td>FHC</td>
<td>Fuch’s heterochromic cyclitis</td>
</tr>
<tr>
<td>GMCSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>Hydroxy-methyl-glutaryl coenzyme A</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>HTFs</td>
<td>Human Tenon’s fibroblasts</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun N-terminal kinase</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo-dalton</td>
</tr>
<tr>
<td>KGF</td>
<td>Keratinocyte growth factor</td>
</tr>
<tr>
<td>LAP</td>
<td>Latency-associated protein</td>
</tr>
<tr>
<td>LC</td>
<td>Lamina cribrosa</td>
</tr>
<tr>
<td>LECs</td>
<td>Lens epithelial cells</td>
</tr>
<tr>
<td>LIF</td>
<td>Leukemia inhibitory factor</td>
</tr>
<tr>
<td>LLC</td>
<td>Large latent complex</td>
</tr>
<tr>
<td>LP</td>
<td>Lipoprotein</td>
</tr>
<tr>
<td>LTBPs</td>
<td>Latent transforming growth factor-β binding proteins</td>
</tr>
<tr>
<td>M</td>
<td>Macrophage</td>
</tr>
<tr>
<td>M6P</td>
<td>Mannose-6 phosphate</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>MLC</td>
<td>Myosin light chain</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>My</td>
<td>Myofibroblast</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian Target of Rapamycin</td>
</tr>
<tr>
<td>ng</td>
<td>Nano-gram</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NF-KappaB</td>
<td>Nuclear factor-KappaB</td>
</tr>
<tr>
<td>OCP</td>
<td>Ocular cicatricial pemphigoid</td>
</tr>
<tr>
<td>ONH</td>
<td>Optic nerve head</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet derived growth factor</td>
</tr>
<tr>
<td>PDGF-AB</td>
<td>Platelet derived growth factor-ab subunits heterodimer</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>Platelet derived growth factor-b subunit homodimer</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>PK</td>
<td>Penetrating keratoplasty</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PVD</td>
<td>Posterior vitreous detachment</td>
</tr>
<tr>
<td>PVR</td>
<td>Proliferative vitreo-retinopathy</td>
</tr>
<tr>
<td>R</td>
<td>Receptor</td>
</tr>
<tr>
<td>RGC</td>
<td>Retinal ganglion cell</td>
</tr>
<tr>
<td>Rho-GTPase</td>
<td>Rho-guanosinetriphosphatase</td>
</tr>
<tr>
<td>RODK</td>
<td>Rho-dependent Kinase</td>
</tr>
<tr>
<td>RPE</td>
<td>Retinal pigment epithelium</td>
</tr>
<tr>
<td>SCFs</td>
<td>Subconjunctival fibroblasts</td>
</tr>
<tr>
<td>SLC</td>
<td>Small latent complex</td>
</tr>
<tr>
<td>SMA</td>
<td>smooth muscle actin</td>
</tr>
<tr>
<td>SS</td>
<td>Sjögren’s syndrome</td>
</tr>
<tr>
<td>T reg</td>
<td>Regulatory T cells</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Transforming growth factor-α</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor-β</td>
</tr>
<tr>
<td>Th</td>
<td>T-helper</td>
</tr>
<tr>
<td>TIMP</td>
<td>Tissue inhibitor of the metalloproteinase</td>
</tr>
<tr>
<td>TM</td>
<td>Trabecular Meshwork</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>TSP</td>
<td>Thrombospondin</td>
</tr>
<tr>
<td>tTGase</td>
<td>Tissue transglutaminase</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VF</td>
<td>Vitreous fluid</td>
</tr>
<tr>
<td>VKC</td>
<td>Vernal Keratoconjunctivitis</td>
</tr>
</tbody>
</table>
# TABLE OF FIGURES

<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Fig. title</th>
<th>Fig. page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>The TGF-β fold</td>
<td>6</td>
</tr>
<tr>
<td>1-2</td>
<td>Cytokine signaling cascades.</td>
<td>7</td>
</tr>
<tr>
<td>1-3</td>
<td>TGFβ/Smad signal.</td>
<td>9</td>
</tr>
<tr>
<td>1-4</td>
<td>A myofibroblast is the main player in the process of tissue fibrosis.</td>
<td>10</td>
</tr>
<tr>
<td>4-1</td>
<td>Anti-TGF-β2 antibody multilayers are successfully fabricated on IOL surfaces.</td>
<td>23</td>
</tr>
</tbody>
</table>
INTRODUCTION

Transforming growth factor β (TGF-β) is one of the most important ligands involved in modulation of cell behavior in ocular tissues. This includes modulation of cell migration and proliferation, cell death, and protein synthesis during development, tissue repair, collagen gel contraction and other physiological or pathological processes. (Saika, 2004).

TGF-β is a multifunctional growth factor that either stimulates or inhibits cell proliferation and differentiation, depending on the cell type or environment (August et al., 2006).

Overactivation of TGF-β underlies the pathogenesis of wound healing-related fibrotic diseases in ocular tissues which impair vision & ocular tissue homeostasis (Saika, 2006).

The secretion of TGF-β into the aqueous humour is important for maintaining the immunological privilege of the anterior ocular segment that promotes corneal allograft survival. The activated form of TGF-beta is decreased in eyes with immune reactions following penetrating keratoplasty (Maier et al., 2008).

TGF-β plays a critical role in the contractile effect and its underlying mechanisms mediating cicatricial contraction in proliferative vitreoretinal diseases (Kita, 2010).

Disruption of TGF-β signaling improves ocular surface epithelial disease in experimental autoimmune keratoconjunctivitis sicca (De Paiva et al, 2011).
Inhibition of TGF-β expression by lithium is used as TGF-β linked corneal dystrophy therapy (Choi, et al., 2011).

TGF-β is produced in high levels in the fetal and glaucomatous optic nerve heads, perhaps by a mechanism of post-transcriptional regulation. TGF-β is important during development of the optic nerve head and in glaucoma. TGF-β may be a mediator of astrocyte reactivation and extracellular matrix re-modelling in the lamina cribrosa (Rudolf Fuchshofer 2011).

Post-cataract surgery fibrosis in the lens capsule is caused by epithelial to mesenchymal transition (EMT) of the lens epithelium. Mammalian target of rapamycin (mTOR) is involved in the regulation of TGF-β-induced EMT and may contribute to the development of posterior capsule opacification (Meng et al., 2013).

Sun CB et al fabricate anti-TGF-β2 antibody functionalized intraocular lens for in situ capture and neutralization of TGF-β2 in the capsular bag, which might be a possible solution to preventing posterior capsule opacification after cataract surgery (Sun et al., 2013).

Rapamycin significantly inhibited TGF-β-induced collagen gel contraction & inhibit proliferation and differentiation of corneal myofibroblasts and, thus, may provide an effective therapeutic measure for preventing corneal scarring after photorefractive keratectomy (Milani et al., 2013).

The use of TGF-β inhibitor peptides (P17 & P144) decrease the development of early choroidal neovascularization (CNV) lesions by targeting different mediators than those typically affected using current anti-angiogenic therapies. Its potential role in the treatment of early CNV
appears promising as a single therapy or adjuvant to anti-VEGF drugs (Zarranz-Ventura et al., 2013).

TGF-β is implicated in several ocular scarring processes including conjunctival wound healing especially that occurring after glaucoma filtration surgery which reduces surgical success. Subconjunctival anti-transforming growth factor-beta monoclonal antibody treatment effectively reduces subconjunctival scarring after glaucoma surgery being safer than Mitomycin-C and 5-Flurouracil (Prendes et al., 2013).

Anti-TGF-β agent (pirfenidone) may be effective as an adjunctive treatment to decrease inflammation and fibrosis resulting from strabismus surgery (Jung et al., 2013).

Significantly higher concentrations of TGF-β and VEGF were found in the aqueous humor of CRVO and BRVO patients than in the aqueous humor of control patients (Feng et al., 2013).

TGF-β1 treated Adipose-derived stem cells (ASCs) may represent an innovative cellular therapy for protection against and repair of diabetic retinopathy (DR) and other retinal vascular diseases (Mendel et al., 2013).

The ophthalmic solution of a peroxisome proliferator-activated receptor gamma agonist (PPARγ) inhibited TGF-beta mediated inflammation, decreased the fibrotic reaction, and prevented neovascularization in the cornea from the early phase after alkali burn injury (Uchiyama et al., 2013).
The aim of the work is to highlight the role of transforming growth factor-beta and its signaling pathways in the pathogenesis of ocular disorders and its possible applications.
Chapter I

OVERVIEW OF TGF-BETA PATHOBIOLOGY

A- Biochemistry of TGF-B

The TGF superfamily encompasses extracellular ligands involved in a diverse range of biological Functions. Family members include TGF-beta, bone morphogenetic proteins (BMPs) and growth and differentiation factors along with activins and inhibins. Found in almost all cell types, these proteins are involved in numerous cell processes including bone and joint development, cell proliferation and differentiation (Massague et al., 2000 & Zhang et al., 2013).

Because of their ubiquitous nature TGF-beta proteins are associated with a variety of diseases ranging from skeletal abnormalities and differentiation to metabolic disorders and play critical roles in neoplastic development and stem cell differentiation (Chen et al., 2003 & Rosemary et al., 2012).

To this date, nearly 40 TGF-beta family members have been isolated in the human genome, with which five type II and seven type I receptors interact (Allendorph et al., 2006).

B- Isoforms of TGF-β Subfamily

TGF-β isoforms possess similar biological activities to other members of TGF-β superfamily. These isoforms share approximately 75% sequence identity (Mittl et al., 1996 & Gao et al., 2014).

Three isoforms have been described in humans (TGF-β1 to TGF-β3) that are closely related to one another structurally and functionally (Blobe et al., 2000).
During development all three isoforms are highly conserved suggesting a critical biologic function for each isoform. These isoforms differ in their binding affinity for TGF-β receptors and the deletion of individual isoforms in mice results in different phenotypes (Blobe et al., 2000).

C- Structure

Ligand members of the TGF-β superfamily consist of both homodimers and heterodimers, containing an ordered set of seven cysteine residues forming disulfide bonds (Lin et al., 2006).

A typical TGF-β monomer consists a cysteine knot motif with two pairs of antiparallel β-strands (fingers) extending from an α-helix (‘wrist’ region) (Figure 1-1). The β-strands are curved to form both a concave and convex surface for receptor interaction (Minlong et al., 2011).

Figure (1-1): The TGF-β fold. A typical TGF-β monomer consists a cysteine knot motif with two pairs of antiparallel β-strands (fingers) extending from an α-helix (‘wrist’ region). The β-strands are curved to form both a concave and convex surface for receptor interaction (Lin et al., 2006).
D- TGF-B Signal Transduction

Binding of a cytokine/growth factor to its specific cell surface receptor is followed by activation of intracellular signaling cascades. The ligand/receptor signaling cascades often converge via mitogen-activated protein kinases (MAPK) that include three subfamilies, i.e. p42/44 ERK (external regulated kinase, MAP kinase), c-Jun N-terminal kinase (JNK), and p38 MAP kinase (Figure 1-2). These signaling pathways mediate various biological responses by regulating expression of genes. For example, in general, pP42/44 MAP kinase is involved in cell proliferation regulation whereas JNK and p38 are activated upon cellular stresses and modulate cell survival/cell death or control expression of stress-response genes (Shi et al., 2003 & Jack D. Thatcher, 2010).

Figure (1-2): Cytokine signaling cascades. Binding a ligand, including transforming growth factor b to its specific receptor activates signaling cascades of Ras/mitogen-activated protein kinase (MAP kinase or p42/p44), c-Jun N-terminal kinase (JNK) or p38 MAP kinase. The first pathway is mainly involved in cell proliferation regulation, whereas the latter two modulate cell stress responses or cell survival/cell death. (Saika et al., 2009).
TGF-b1 to b3 utilize the Smad signaling pathway which is specific to the members of TGF-b superfamily. Upon TGF-b binding to its receptor, a pair of transmembrane receptor serine-threonine kinases are activated. Receptor-activated Smad proteins, Smad2 and Smad3, are phosphorylated directly by the TGF-b receptor type I kinase. They then partner with the common mediator, Smad4, and translocate to the nucleus where they play a significant role in modulation of expression TGF-b-dependent gene targets (Figure 1-3). Details of differences between Smad2 and Smad3 were recently investigated by using a gene expression array made of embryonic fibroblasts obtained from embryos lacking either Smad2 or Smad3 (Massague, J., 2003).

In vivo, the roles of Smad2 and Smad3 differ because the lack of Smad2 is lethal for mice at the embryonic stage whereas those lacking Smad3 survive (Massague et al., 2006 & Itoh et al., 2007).

The bone morphogenetic proteins (BMPs), which are members of the TGF-B superfamily, bind to their own receptors and phosphorylate Smads1, 5 and 8 which then bind to Smad4 for translocation to the nucleus. Smads6/7 are known to be inhibitory Smads, that block phosphorylation of Smads2/3 and thus suppresses Smads2/3-mediated gene expression (Tin Dijke et al., 2002 & Shi et al., 2003).
Figure (1-3): TGFβ/Smad signal. Smad2 or Smad3 is phosphorylated at its C-terminal region upon TGFβ binding to receptor. Phosphorylated Smad2 or Smad3 forms a complex with the common Smad (Smad4) and translocates to the nucleus to bind to gene promoters. Co-factors modulate Smad-dependent gene expression.

(Saika et al., 2009).

**E- Role of TGF-B in Fibrogenic Reaction**

TGF-B upregulates fibrogenic ECM genes that are involved in both initial tissue repair and long-term remodeling, but in turn in unfavorable scarring when the activity is disregulated, which often lead to failure of tissue remodeling and dysfunction of tissues due to excess accumulation and contraction of ECM (Martin et al., 2004).

**F- Generation of Myofibroblasts by TGF-B**

Beside excess accumulation of ECM, a fibrotic lesion is characterized by the presence of myofibroblasts, the key player in fibrogenic reaction, in association with persistence of inflammation, both of which must decline for the healing process to be complete and the
restoration of normal tissue functions (Figure 1-4) (Gabbiani et al., 2003 & Friedman et al., 2004 & Wynn et al., 2007).

This cell type is derived from both activated fibroblasts and epithelial cell types. Myofibroblasts are usually derived from fibroblasts that are activated by various cytokines upon tissue injury. Expression of alpha-smooth muscle actin (alphaSMA), the hallmark for fibroblast-myofibroblast conversion, is mediated by Smad2 (Flanders et al., 2003 & Roberts et al., 2003). AlphaSMA produces a contractile force in a scarred tissue (Hinz et al., 2003).

Some reports suggest that circulating bone marrow derived cell types (so-called fibrocytes) could differentiate and become myofibroblasts in a local healing tissue, but this is still a controversial issue (Willis et al., 2006 & Li et al., 2007).

**Figure (1-4):** A myofibroblast is the main player in the process of tissue fibrosis. A myofibroblast is derived from either a fibroblast, an epithelial cell or a bone-marrow derived cell (a fibrocyte), and exerts a central role in cell repopulation, inflammation and extracellular matrix reconstruction in the process of formation of a fibrotic lesion. The process of production of a myofibroblast from an epithelial cell is called as "Epithelial-mesenchymal transition, EMT"

(Saika et al., 2009).
G- TGF-B and Epithelial-Mesenchymal Transition (EMT)

The process of transdifferentiation in which an epithelial cell changes its phenotype to a (myo)fibroblast is called an EMT (Nito, 2002) (Figure 1-4).

Transcription factors involved in EMT includes ZEB (Sip1/EF1), bHLH (E47/Twist) and Snail1/2 (Huber et al., 2004 & Lee et al., 2006).

Expression of these molecules seems to be strictly regulated by various signaling pathways, i.e., Smads, p42/p44 ERK or p38MAP kinase, and nuclear factor-KappaB (NF-KappaB), etc. Expression of Snail; the master transcription factor involved in an early step of the EMT is an important step in the process of tissue fibrosis, and is controlled by Smad3 signaling (Saika, 2004 & Samy Lamouille et al., 2014).

These transcription factors are up-regulated by TGF-B and directly suppress E-cadherin expression which is essential in the maintenance of epithelial phenotype. Acquisition of motility and metastasis in neoplastic epithelial cells (cancer cells) is also considered to be the result of EMT (Saika, 2007).
OUTLINE OF OCULAR FIBROTIC DISORDERS

The eye is a unique tissue composed of surface ectodermal tissues and neuronal tissues. The former includes cornea and conjunctiva that exhibit a structure similar to skin except for the avascularity of the cornea. However, The essence of fibrotic diseases in the eye is quite similar to that seen in fibrotic disorders in other tissues of the human body.

The diseases include scarring in the cornea and conjunctiva, the fibrosis in the lens capsule post-cataract surgery, proliferative vitreoretinopathy (PVR) which is characterized by excess scarring tissue formed on the detached retina. In proliferative stage of diabetic retinopathy; fibrogenic reaction adjacent to the retinal neovascularization has a significant role in the development of tractional retinal detachment. Excess accumulation of ECM and appearance of myofibroblasts, in association with inflammatory cells, are observed in all the cases in the eye.

Myofibroblasts are derived from mesenchymal cells, i. e., subconjunctival fibroblasts or keratocytes (corneal fibroblasts) or epithelial cell types, i. e., lens or retinal pigment epithelium at each specific injured site. Although it has long been believed that the main component of proliferative diabetic retinopathy is retinal neovascularization, the fibrogenic process that occurs around such new vessels causes a traction force and the resultant detachment of the retina making this condition a fibrotic disease as well. In an adult human eye, TGF-B2 predominates in the aqueous humor (Saika, 2006).
The importance of TGF-B2 in ocular physiology is also demonstrated in embryonic development; by the observation that embryos of a TGF-B2-null mouse, but not TGF-B1 or TGFB3-null mouse, have multiple ocular abnormalities, i.e., loss of corneal endothelium and the anterior chamber, as well as corneal stroma and retinal and vitreous hypercellularity, all of which are due to impaired immigration of neural crest cells (*Saika et al.,* 2001).

Ocular surface epithelium express TGF-B1,2&3 receptors. The cells inside the eye also express all the TGF-B members during disease process, i.e., inflammation or tissue repair. TGF-B also upregulates other growth factors/cytokines, e.g., connective tissue growth factor or fibroblast growth factor, involved in tissue fibrosis (*Saika et al.,* 2009).
TGF-B and Ocular Surface Disorders

A- Fibrosis/Scarring in Ocular Surface;

Cornea and Conjunctiva

The ocular surface is covered with stratified epithelium and underlying connective tissue serving as a protective outer coat of the globe against external stimuli of chemical components, microbial attack or mechanical trauma. Ocular surface scarring diseases include Stevens-Johnson's syndrome or post-alkaline burn scarring etc. In the majority of these diseases, the components of the disease process include inflammation, fibroblast activation and ECM accumulation. Once the tissues are injured, infiltrating inflammatory cells, i.e., macrophages secrete various growth factors/ cytokines including TGF-B; one of the most important fibrogenic growth factors. Although TGF-B is critical in the maintenance of the tissue integrity, it also promotes fibrogenic reaction in the healing subepithelial tissue (Imanishi et al., 2000 & Suzuki et al., 2003).

The avascularity, transparency and the regular curvature are all essential for proper light refraction and, therefore, vision. Although the it lacks vasculature, the main components involved in tissue repair of the cornea are quite similar to those of skin; stratified epithelium and a collagenous matrix containing mesenchymal cells (corneal fibroblasts) lying beneath it. An organized ECM structure of collagen fibers of types I, III, and V and proteoglycans among the fibers is essential to the maintenance of its transparency and the regular shape. Transparency of the cornea is reduced by stromal fibrosis/scarring, leading to the impairment of the patients' vision. Such severe corneal fibrosis could be
surgically treated by using transplantation of an epithelial cell sheet grown on a membrane (Nishida et al., 2004).

Although conjunctiva is much vascularized, the behaviors of epithelial cells and mesenchymal cells (fibroblasts) in a healing injured conjunctiva are similar to those seen in a healing cornea. Scarring of conjunctiva potentially causes problems of reduction in filtration efficacy following glaucoma filtering surgery (Jinza et al., 2000). Topical mitomycin C is applied to local tissue after trabeculectomy in order to suppress excess proliferation of subconjunctival fibroblasts. Although it is markedly effective in the majority of cases, we do encounter patients for whom such effectiveness is limited (Kaimori et al., 2007). Similarly to the wound healing process in cornea, the TGF-B family is believed to have important roles in wound healing in conjunctiva.

A neutralizing antibody against TGF-B2 was tested to try to suppress excess fibrosis/scarring in filtering blebs, but no significant effect was observed (Siriwardena et al., 2002). Although aqueous humor contains abundant TGF-B2 (Tripathi et al., 1994), TGF-B1 and TGF-B2 are also expressed in local cells (conjunctival epithelium and fibroblasts) in the filtering bleb tissue, that might account for the failure of the trial of TGF-B2 antibody for suppression of conjunctival scarring (Saika et al., 2001).

Disruption of TGF-β signaling was found to improve ocular surface epithelial disease in experimental autoimmune keratoconjunctivitis sicca (Maier et al., 2008).

Recent studies show extracellular matrix components osteopontin and tenascin-C which are ligands of α9 integrin, play
roles in corneal wound fibrosis and neovascularization. Although loss of osteopontin reduces macrophage invasion and myofibroblast differentiation in the healing stroma by suppression of fibrogenic gene expression in response to injury, it impairs closure of incisional wounds in the mouse cornea. *(Saika et al., 2013)*

**B- Strategies to prevent excess fibrogenic reaction in ocular surface tissue by targeting TGF-B signals**

Blocking unfavorable cytokine activity; TGF-B has therapeutic effects on fibrogenic or scarring diseases. Gene introduction is one of the efficicent routes for blocking signaling cascades in the cytoplasm, while other protein-based agents might not be effective. It has been reported that blocking type II TGF-B receptor by expression of soluble receptor by adenoviral gene expression in muscles suppressed scarring and neovascularization in a healing rat cornea post-alkali burn *(Sakamoto et al., 2000)*.

TGF-B activated p38MAP kinase is critical for the migratory activity of corneal epithelial cells during tissue repair & blocking it suppresses fibrogenic reaction in ocular fibroblasts independently from Smad signal. Therefore, it is beneficial to maintain p38 signaling for cell migration, but to specifically block signals that yielded unfavorable scarring and development of neovascularization in a healing cornea. TGF-B/Smad signal is suitable for this purpose. However, this strategy might potentially impair the healing of conjunctival epithelium, in that epithelial migration is regulated by TGF-B/p38 signaling *(Saika et al., 2004)*.

It's showed that deletion of Smad3 gene or Smad7 overexpression had a similar anti-profibrogenic/ proinflammatory effect in the healing of
an alkali-burned mouse cornea or of mechanically injured mouse conjunctiva (Saika et al., 2005).

Smad also inhibited macrophage recruitment in the inflamed tissue, resulting in a reduction of local macrophage-derived growth factors. Smad7 gene transfer attenuates the fibrogenic reaction in a healing mouse cornea and conjunctiva, suggesting that blocking TGF-B/Smad signal might have a therapeutic potential in the prevention of excess scarring in these tissues in humans (Saika et al., 2005).

Such anti-Smad signal strategy includes gene transfer of BMP-7, Id2/3, or The peroxisome proliferator-activated receptor gamma (PPAR-g). PPAR-g is a member of PPAR family which is involved in modulation of adipose metabolism and inflammatory cell function as well as behaviors of non-inflammatory cells i.e. fibrogenic reaction or cell proliferation during wound healing (Clark, 2002). Like other tissues, gene transfer of PPAR-g blocks injury-induced fibrogenic reaction in mouse cornea and conjunctiva as well as in cultured fibroblasts (Yamanaka et al., 2007).

It's also showed that over-expressed Smad7 does not inhibit phosphorylation of the p65 subunit of NF-KappaB, a signal transmitter related to inflammation, but does block its nuclear translocation (Saika et al., 2007).

This might explains why Smad7 gene transfer is more effective in the suppression of inflammation and excess fibrogenic reaction in an alkali-burned cornea as compared with Smad3 gene deletion (Saika et al., 2009).
It was confirmed that inhibiting NF-KappaB by a peptide inhibitor, SN50, produces a therapeutic effect on alkali-burned corneas in mice. The mechanism of action of SN50 includes suppression of the inflammatory response and also acceleration of epithelial cell proliferation through over-activation of TNFalpha/JNK signal (Saika et al., 2005).

TNF alpha antagonizes TGF-B effects in vivo in healing tissues. In TNF alpha-null mice; inflammation, tissue scarring and stromal neovascularization all were less severe in the earlier phase of healing, but later such events were more marked in association with over-expression of inflammation/fibrosis-related growth factors in cornea and other tissues of TNF alpha-null mice (Yamanaka et al., 2006). Experiments in these reports revealed that TNF alpha expressed in macrophages, but not resident mesenchymal cells, antagonized fibrogenic effects by TGF-B (Fujita et al., 2007).

Topical use of antibody to TGF-beta can also be considered to suppress recurrence of corneal opacities after PTK or lamellar keratectomy (Lee & Kim, 2003).

Rapamycin significantly inhibited TGF-β-induced collagen gel contraction & inhibit proliferation and differentiation of corneal myofibroblasts and, thus, may provide an effective therapeutic measure for preventing corneal scarring after photorefractive keratectomy (Milani et al., 2013).

Jung et al., (2012), studied the effects of an anti-transforming growth factor-β agent (pirfenidone"PFD") on strabismus surgery in rabbits and found that intraoperative injection of PFD may be effective as
an adjunctive treatment to decrease inflammation and fibrosis resulting from strabismus surgery (Jung, et al., 2012).

Several studies have reported different approaches for prevention of corneal fibrosis. These therapeutic strategies include selectively killing the myofibroblasts (Gupta et al. 2011), gene targeting to silence TGF beta 1 with BMP 7 (Tandon et al. 2013), triple combination targeting of TGF beta1, TGF beta R2, CTGF (Sriram et al. 2013), vimentin inhibition (Das et al. 2014), histone deacetylase inhibitors (Tandon et al 2012), peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist (Huxlin et al. 2013), rapamycin (Milani 2013), infliximab (Ferrari et al. 2013) and also novel approaches by pharmaceutical inhibition of TGF beta 1 (Chowdhury et al. 2013).

A volume of knowledge has been gained from several studies on the understanding of pathobiology of EMT fibrosis and corneal opacity, but still remains to be understood when and why wound fibroblasts decide to become myofibroblasts and identifying such mechanism may help in reversing EMT. This will perhaps give hope for not just prevention but also treatment of established corneal fibrosis.
TGF-B and Lens Epithelial Mesenchymal Transition

A-Anterior Subcapsular Cataract

Anterior subcapsular type of cataract shows fibrous plaque of opacity just beneath the anterior lens capsule. The opaque tissue consists of myofibroblasts and accumulation of fibrous ECM inside the lens capsule. Because the lens tissue contains epithelial cells and lens fiber cells, the myofibroblasts seen inside the capsule must be originated from epithelial cells through EMT (Saika et al., 2009).

The cells actively proliferate to form multilayers and undergo transdifferentiation leading to plaque formation. Surgery is the only solution for anterior subcapsular cataract. Researchers are trying to find out methods that can reverse the process and inhibition of TGF-B activity by its inhibitors has shown promise (Dwivedi et al., 2006).

B-Posterior Capsular Opacification

Posterior capsular opacification remains a major complication following cataract surgery causing significant vision loss (Roh et al., 2010). The incidence of PCO is still 8~34.3% in adults, and nearly 100% in children (Rönbeck et al., 2009).

In modern cataract surgery, an artificial intraocular lens (IOL) made of a hard or soft plastic material is implanted in the residual bag of the lens capsule after removing the opaque lens content. Lens epithelial cells start to proliferate and to migrate toward the behind of the IOL and then transform into the myofibroblast through the process of EMT. Aftercataract (post-operative capsular opacification), the most common complication, is caused by myofibroblast generation through EMT of lens
epithelial cells and subsequent accumulation of fibrous ECM components, i.e., collagen types, proteoglycans and basement membrane components on the inner surface of the residual lens capsule. Lens fiber regeneration also contributes to the development of secondary cataracts by forming Sommerring's ring and Elschnig's pearls (Olson et al., 2003 & Saika et al., 2004). One of the most critical growth factors involved in the development of secondary cataracts is reportedly TGF-B (de Iong et al., 2005).

Lens epithelial cell EMT in post-operative secondary cataracts is mimicked by puncture of the lens in animals (Okoda et al., 2001). In the mouse model, Smad signal is activated in lens epithelial cells adjacent to the wound in 12 hrs. Smad4 nuclear translocation is readily blocked by intraocular administration of anti-TGF-B2 neutralizing antibody, indicating that TGF-B2 is the TGF-B family member that activates lens epithelium post-injury in mice. Then the cells begin to express alphaSMA, the hallmark of EMT, mRNA and protein at 3 and 5 days respectively when the cells exhibit a fibroblastic appearance (Valcourt et al., 2005).

However, non-Smad signals can transmit EMT signals when cells receive strong stimuli, such as adenoviral over-expression of the active form of TGF-B1 in lens epithelium or marked intraocular inflammation induced by ocular alkali exposure (Robertson et al., 2007). This explained why the signal toward EMT can bypass Smad3 in such circumstances, although the signal is not fully transmitted (Shirai et al., 2006).
Other signals such as Rho-kinase, PI3-kinase or SRC are also reported to modulate the process of EMT in lens cells (Cho et al., 2007 & Walker et al., 2007).

Prevention of secondary cataract formation is necessary to maintain good vision for patients and also for proper examination of the ocular fundus of these patients. The latter is critical in patients with retinal diseases i.e. diabetic retinopathy or various macular diseases.

Drugs or surface improvement/modification of an intraocular lens that are effective in prevention of development of secondary cataracts must be clinically tested, although some are reported to have a potential in vitro or in animals (Maloof et al., 2005).

Chuan, et al., in 2014 studied the effect of anti-TGF-β2 antibody functionalized intraocular lens on lens epithelial cell (LEC) migration and epithelial–mesenchymal transition & found transient inhibition to LECs adhesion and no inhibition to LECs proliferation demonstrating a simple, inexpensive, and feasible method to fabricate surface functionalized IOL for in situ capture and neutralization of TGF-β2 in the capsular bag, which might be a possible solution to preventing posterior capsule opacification after cataract surgery.
Figure (4-1): Anti-TGF-β2 antibody multilayers are successfully fabricated on IOL surfaces

(Chuan, et al., 2014).
Chapter V

TGF-B AND GLAUCOMA

A-Role of TGF-Beta in Pathogenesis of Glaucoma

The Lamina Cribrosa (LC) region of the Optic Nerve Head consists of a characteristic sieve-like structure through which Retinal Ganglion Cells' axons exit the eye. These laminar plates contain extracellular matrix proteins such as elastin and collagens (I, III, V, and VI). Correct organization and assembly of the collagen and elastin fibers in the LC provides both a supportive framework and elasticity to the ONH, which is believed to protect RGC axons from mechanical stress (Kirwan et al., 2005). Major cell types present in the human ONH include ONH astrocytes and LC cells (Oyama et al., 2006).

These cells support RGC axons by synthesizing growth factors (e.g., neurotrophins) and extracellular matrix (ECM) proteins (Agrawal et al., 2001). Increased synthesis and deposition of ECM proteins in the LC region may disrupt nutritional and mechanical support to RGC axons, resulting in RGC atrophy (Jhonson et al., 2007). Several studies suggest that ONH astrocytes and LC cells respond to elevated IOP by increasing transforming growth factor-β2 (TGF-β2) synthesis in the LC region, which in turn causes altered ECM protein expression. TGF-β2 appears to be involved in the pathogenesis of POAG (Zode et al., 2009).

Patients with glaucoma have higher levels of TGF-β2 in their aqueous humor (Tripathi et al., 1994), and TGF-β2 increased IOP in cultured human perfused anterior eye segments (Gottanaka et al., 2004). TGF-β2 has been shown to increase ECM protein in human trabecular meshwork (TM) cells (Wordinger et al., 2007).
Modulation of BMP 7 by pharmacological means might be a potential strategy for prevention of POAG (Fuchshofer et al. 2007).

Furthermore, adenoviral gene transfer of active TGF-β2 elevates IOP in mice and rats and reduces outflow facility in mice (Shepard et al., 2010). Robertson et al. also reported that gene transfer of TGF-β1 into the anterior chamber of rats elevated IOP (Robertson et al., 2010).

Gulab Zode, et al. in 2011 studied both in vivo and in vitro evidence to support the conclusion that TGF-β2 is involved in ECM remodeling by cells of the human ONH. In addition, TGF-β2-driven ECM stimulation requires activation of the Smad signaling pathway via Smad2/3. TGF-β2 stimulation of ECM protein synthesis and secretion by ONH astrocytes or LC cells. Inhibition of the type I TGF-β receptor or knockdown of either Smad2 or Smad3 reversed TGF-β2 stimulated ECM proteins in ONH astrocytes and LC cells. Therefore, inhibition of these downstream signals may provide a therapeutic target to prevent ECM remodeling in the glaucomatous ONH.

Further research initiative can divulge the complex relationship among TGF beta, BMPs and BMP inhibitors and can provide insight regarding possible target for prevention of POGA.

B- Transforming Growth Factor-β and post-operative scarring

The cytokine TGF-β is pivotal in wound healing and scar formation in general (Tomasek et al., 2002). Of all the growth factors involved in the wound healing cascade, TGF-β is shown to be one of the most potent stimulators of scarring in the eye and is involved in the pathogenesis of conjunctival scarring. Transforming Growth Factor-β2-
the most predominant of the three mammalian isoforms in the eye-is the most potent growth factor in the aqueous at stimulating conjunctival fibroblast function (Mead et al., 2003). Scar formation is a serious problem following filtering glaucoma surgery. TGF-β₂ is the most potent stimulator of human Tenon’s fibroblasts (HTF) activity (Han et al., 2008). It remains the commonest cause of late bleb failure after glaucoma filtration surgery, but the pathophysiological mechanisms of filtering bleb scarring are not fully elucidated (Meyer-ter-Vehn T et al., 2008).

TGF-β induces the transition of fibroblasts to myofibroblasts characterized by α-smooth muscle actin (α-SMA) expression. Myofibroblasts deposit extracellular matrix proteins and exert contractile forces to drive the remodeling phase of wound healing. Excessive or abnormal contraction of granulation tissue leads to pathologic scarring (Han et al., 2008 & Meyer-ter-Vehn et al., 2008).

C- Anti-TGF-β₂ monoclonal antibodies

Cambridge Antibody Technology has developed a human monoclonal antibody called lerdelimumab [CAT 152], which is directed against transforming growth factor-β₂. CAT 152 is a novel human monoclonal antibody that was isolated and developed in vitro by the technique of antibody phage display. In the first human trial of CAT-152 in patients undergoing trabeculectomy, good tolerance and safety were reported with a treatment regimen of both intra- and postoperative injections (Siriwardena D et al., 2002).

It displays high affinity and specificity for the active form of TGF-β₂ and has been designed for therapeutic use (Wong et al., 2003).
However, this antibody was found to have no significant advantage over placebo in a phase III clinical trial (Khaw et al., 2007).

**D- Other possible future alternatives to antimetabolites**

Tissue transglutaminase (tTgase) is present and functionally active in failed blebs. Expression and activity of tTgase appeared to be stimulated by TGF-β2. Intervention at this pathway might open a new approach to prevent scarring after glaucoma filtration surgery (Priglinger et al., 2006).

Rho-dependent Kinase inhibitors release TGF-induced HTF contraction and block subsequent TGF-β–induced myofibroblast transdifferentiation and may therefore serve to modulate postoperative scarring after glaucoma filtering surgery (Sierprath S et al., 2006).

Statins inhibit Rho-guanosinetriphosphatase (Rho-GTPase) signaling. They inhibit TGF-β–induced connective tissue growth factor (CTGF) transcription, α-SMA expression and incorporation into actin stress fibers, and subsequent collagen gel contraction. These effects are reversed by mevalonate. Statins may, therefore serve to inhibit scarring after filtering glaucoma surgery (Grehn et al., 2008).

The p38 signaling pathway mediates the TGF-β1-induced transdifferentiation of human Tenon fibroblasts to myofibroblasts. Rosiglitazone can exert anti-fibrotic activity by interfering with the TGF-β/p38 signaling pathway and might be useful for modulating scar formation after glaucoma filtration surgery (Luo et al., 2014).
A- Retinal Pigment Epithelium EMT and Proliferative Vitreoretinopathy (PVR)

In rhegmatogenous retinal detachment liquefied vitreous fluid or aqueous humor influxes beneath the retina through a break in the peripheral retina and the overlying retina is detached from the pigment epithelium. Once neuronal retina is detached, pigment epithelial cells (RPEs) began to be dispersed in subretinal fluid. The RPE is the most critical contributor to the development of fibrous tissue on the retina (Bochaton-piallat et al., 2000). Cultured RPE cells undergo transformation to fibroblast-like cells through the process of EMT, proliferate and produce extracellular matrix components in response to exposure to exogenous TGF-b. A similar phenomenon might occur on the detached retina, participating in this fibrotic sequelae of PVR. PVR is a disease caused by the formation of fibrotic tissue on the detached retina, which reduces the flexibility of the retina and may potentially make it difficult to reattach to the retina (Kroll et al., 2007).

Like EMT in lens epithelium in the formation of secondary cataracts, TGF-b is likely a key player in the development of PVR (Hinton et al., 2002). The concentration of TGF-b2 in the vitreous humor of the eye correlates with the severity of the PVR, supporting its importance in the pathogenesis. Similar to other cell types, i.e., lens epithelial cells, EMT of RPE cells is also suppressed by the loss of Smad3 & Smad7 gene introduction in vivo, resulting in the attenuation of development of PVR (Kitano et al., 2007).
Nassar et al., in 2011 evaluated the effect of decorin, a naturally occurring TGF-β inhibitor, in an experimental rabbit model for proliferative vitreoretinopathy and concluded that adjuvant decorin application during vitrectomy effectively reduced fibrosis and TRD development with no obvious histopathological toxicity signs, decorin represents a promising substance to inhibit PVR reactions (Nassar et al., 2011).

Nassar et al., in 2014 studied the effect of intravitreal injection of TGF-β receptor 1 inhibitor LY-364947 (LY) to prevent proliferative vitreoretinopathy and concluded that treatment with the TGF-β receptor 1 inhibitor LY reduces RPE transdifferentiation in vitro and prevents proliferative vitreoretinopathy and subsequent tractional retinal detachment in vivo (Nassar et al., 2014).

B- TGF-B and Diabetic Retinopathy

The main phenomenon in the non-proliferative stage includes damage of the pericytes and obstruction of the retinal microvasculature. Along with spreading of the area of the retina with microvascular obstruction, ischemic retinal tissue expresses various cytokines/growth factors, i.e., vascular endothelial growth factor (VEGF) involved in new vessel formation (Simo et al., 2006). Neovascularization grows on the posterior surface of the vitreous from the retinal vessels. Then, fibrogenic reaction occurs in cells (such as pericytes) around naked new vessels to form "fibrovascular" proliferative tissue, contraction of which potentially cause tractional retinal detachment (Ban et al., 2008). Zhang., et al study (2012), concluded that Both VEGF and TGF-β2 can induce the collagen gel contraction, partly by means of inducing the expression of α-SMA
and RPE contraction, which may thus contribute to the explanations of \textit{vitro}-retinal diseases (Zhang et al., 2012).

TGF-β signaling in retinal endothelial cells and pericytes show that these cells, and in particular the pericytes, have the essential characteristics to allow for a role of TGF-β in Basal Lamina thickening in preclinical diabetic retinopathy (\textit{Rob Van Geest et al., 2010}).

\textit{Katarzyna Zorena, et al., in 2013} found that out of nine tested parameters (age, duration of diabetes, systolic and diastolic blood pressure, albumin excretion rate, serum HbA1c, CRP, TGF-β1, and creatinine), it turned out that TGF-β1 in serum had the most discriminative power in predicting the occurrence of DR in juvenile patients with Type 1 Diabetes Mellitus (\textit{KatarzynaZorena, et al., 2013}).
TGF-B and CHORIDAL NEOVASCULARIZATION

Recent evidence suggests that adenoviral vector-mediated ocular gene transfer is a viable approach for the treatment of ocular disorders in a human trial for the treatment of age-related macular degeneration (Campochiaro et al., 2006).

The use of TGF-β inhibitor peptides (P17 & P144) decrease the development of early CNV lesions by targeting different mediators than those typically affected using current anti-angiogenic therapies. Its potential role in the treatment of early CNV appears promising as a single therapy or adjuvant to anti-VEGF drugs (Zarranz-Ventura et al., 2013).

Bai et al., (2014), investigated the antiangiogenic effects of semaphorin3A (Sema3A), which is a chemorepellent guidance molecule that inhibited the formation of retina neovascularization (Yu et al., 2013), on transforming growth factor beta (TGF-β) in vitro and in vivo. They verified that the vitreous TGF-β level was higher in patients with neovascular AMD than in the controls. Sema3A inhibited the response of TGF-β in vitro. In vivo, the TGF-β level was increased in the CNV mouse model. Sema3A not only inhibited laser-induced CNV formation but also inhibited the uptake of VEGF and TGF-β.

In the western blot analysis, Sema3A was shown to inhibit the phosphorylation of p38 MAPK, ERK1/2, and JNK and to inhibit the SMAD2/3 signaling pathway after Sema3A treatment in CNV mice. They concluded that Sema3A can be applied as a useful, adjunctive therapeutic strategy for preventing CNV formation (Bai et al., 2014).
SUMMARY

Transforming growth factor-β (TFG-β) is a universal growth factor that plays multiple roles in different parts of the body.

Physiologically, it is important for regulation of cell cycle, differentiation of cells and regulation of immunity. It also plays a vital role in wound healing and scar formation.

TFG-β has also been implicated in the pathology of vernal keratoconjunctivitis, scarring with ocular cicatricial pemphigoid, alkali burn and dryness of Sjögren’s syndrome.

TFG-β has been linked to posterior capsular opacification after cataract surgery.

Identifying TFG-β as a potent stimulator of scarring after glaucoma surgery may open a wide door for using anti-TFG-β antibodies as an alternative to the currently used antimetabolites. They appear much safer and less destructive.

In diabetic retinopathy, TFG-β intravitreal levels are highest in insulin-treated patient with active neovascular membrane, implicating a role of this factor. It may induce posterior vitreous detachment, epiretinal membrane contraction, retinal detachment and proliferative vitreoretinopathy formation. Statins, through cutting down TFG-β signaling pathway may play a protective role.
It's showed that the each part of the eye is susceptible to the fibrotic diseases, that are characterized by the appearance of myofibroblasts and accumulation of ECM. The process of generation of myofibroblasts from either fibroblasts or epithelial cell types are mediated by the growth factors including TGF-b, one of the most potent factors involved in tissue fibrosis. Blocking the signal activated by TGF-b is one of the powerful tool to prevent or treat the diseases.
**References**


References


Mittl, et al., (1996): The crystal structure of TGF-β3 and comparison to TGF-β2: Implications for receptor binding. Protein Science; 5: 1261-1271.


**References**


*Roh, et al.,(2010)*: Comparison of posterior capsular opacification between a combined procedure and a sequential procedure of pars plana vitrectomy and cataract between a combined procedure and a sequential procedure of pars plana vitrectomy and cataract surgery. Ophthalmologica; 224:42-6.


*Rudolf Fuchshafer (2011)*: The pathogenic role of transforming growth factor-β2 in glaucomatous damage to the optic nerve head; Experimental Eye Research Volume 93, Issue 2, August, Pages 165–169.


(B) Intraocular lens: Biocompatibility related to lens epithelial cells. Prog Retinal Eye Res 23, 283-305.


References


(B)Critical role and involvement of vascular endothelial growth factor and transforming growth factor-β2 in collagen gel contraction induced by retinal pigment epithelial cells]. Zhonghua Yan KeZaZhi. Jan.;48(1):52-60.
الملخص العربي

بعد عامل النمو المحول - بيتا عاملًا واسع الانتشار، ويلعب دورًا هاماً في جميع أجزاء الجسم.

وبعد هذا العامل في البيولوجيا عاملًا هاماً لتنظيم دورة الخلايا، ولحدوث تمييز الخلايا، ولتنظيم المناعة، كما يلعب دوراً حيوياً في التئام الجروح وتكوين الندبات.

ويلعب عامل النمو المحول - بيتا دوراً في الاعتلال المرضي المصاحب للزمن الربيعي، والتندب المصاحب لمرض العين التندبي النقاعي، والحرق القموى، والجفاف في حالات متلازمة جوغرن.

وأيضاً يشارك في تكون العتامة في الحافظة الخلفية للمعدسة المزروعة داخل العين بعد إزالة المياه البيضاء.

ويعرف عامل النمو المحول - بيتا كمحفز قوي لحدوث التندب بعد جراحة المياه الزرقاء، وقد يفتح هذا الباب لاستخدام أجسام مضادة له كبديل لمضادات الآيض خاصة أنها أكثر أماناً وأقل تدميراً.

وفي حالات اعتلال الشبكية السكرى يكون عامل النمو المحول - بيتا في أعلى مستوياته في المرضى الذين يعالجون بالانسولين ويعانون من أغشية دموية نشطة، بما يعد دليلاً على دور هذا العامل. وقد يستحث هذا العامل حدوث انقسام خلفي للجسم الزجاجي، وانقباض للإغشية فوق الشبكية، وحدود الانفصال الشبكي، واعتلال تزعرع في الجسم الزجاجي والشبكي، وربما تلعب أدوية "الستاتين" دوراً وقائياً عن طريق منع إشارات عامل النمو المحول - بيتا.
وقد وجد أن كل جزء من أجزاء العين هو عرضة للتليف أو التندب والذي يتميز بظهور خلايا ليفية عضلية وتراكم الأنسجة خارج الخلايا. ونشأة هذه الخلايا من الخلايا الليفية أو الطلائية يتورط عوامل نمو تشمل عامل النمو المحول - بيتا، والذي يعد واحداً من أقوى العوامل في احداث التليف النسيجي.

واعد حجب الإشارات المنشطة بواسطة عامل النمو المحول - بيتا واحدة من أقوى الأدوية لعلاج هذه الأمراض.
الهدف من العمل

الهدف من العمل هو تسليط الضوء على عامل النمو المُخول - بيئة في الحالات الفسيولوجية والمرضية للعين، وعرض الاستخدامات المحتملة لهذه المعلومات وتطبيقها في طب العين وجرجاحاتها.