• Review Article •

The role of cell mediated immunopathogenesis in thyroid-associated ophthalmopathy

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Abstract

• Currently, thyroid-associated ophthalmopathy (TAO) lacks effective treatment due to our lack of clarity in its immunopathogenesis. Orbital fibroblasts play a key role in altering inflammation and immune response in TAO, and are considered as the key target and effector cells in its pathogenesis. The orbit infiltrating CD34+ fibrocytes add on to the process by expressing high levels of autoantigens and inflammatory cytokines, while also differentiating into myofibroblasts or adipocytes. This review focuses on the role of orbital fibroblasts and CD34+ fibrocytes in the pathogenesis of TAO, highlighting the basis of emerging treatments.

• KEYWORDS: thyroid-associated ophthalmopathy; orbital fibroblast; fibrocytes; immunopathogenesis
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INTRODUCTION

Thyroid-associated ophthalmopathy (TAO) is an autoimmune inflammatory disorder, which is a part of Graves' disease (GD)[1]. Environmental, genetic, and immune factors play an important role in TAO[2]. OrbitaI fibroblasts are considered the key target and effector cells in the pathogenesis of TAO[3]. CD34+ fibrocytes, derived from B cell lineages and monocyte, are found circulating as peripheral blood mononuclear cells (PBMCs)[4]. These cells, expressing CD34, CD45, CXCR4 and collagen I, reportedly involved in inflammation, tissue remodeling and wound healing[5-7]. The number of circulating CD34+ fibrocytes was observed to be significantly increased in TAO[8]. Further, these cells have been discovered to infiltrate orbital tissues in TAO individuals, where they turn into CD34+ fibroblasts and thus included into the population of orbital fibroblasts[9]. Moreover, these CD34+ fibroblasts are also found to express thyrotropin receptor (TSHR), produce inflammatory cytokines, and then can terminally differentiate into adipocytes or myofibroblasts, all of which are the reasons for TAO tissue reconstruction[9].

Association of Orbital Fibroblasts and CD34+ Fibrocytes with TSHR Several studies have demonstrated that TSHR is an autoantigen shared by the orbit and the thyroid glan[9-10]. TSHR mRNA has been found in cultured orbital fibroblasts[11], the expression of which can be enhanced by inducing adipocyte differentiation of these cells[12-13]. Immunofluorescence could localize intense TSHR staining to the perinuclear areas of orbital fibroblasts[14]. Recent studies have suggested that the bTSH or M22 mediated ligation of TSHR on CD34+ fibrocytes produce copious inflammatory cytokines, such as IL-6, IL-8, IL-1β, and TNF-α[8,15-18]. Unlike CD34+ fibrocytes, orbital fibroblasts from TAO patients have an extremely low response to thyroid stimulating hormone (TSH)[19]. Orbital fibroblasts, when activated *via* TSHR signaling, produce cAMP, pAkt, and hyaluronan, which play an important role in the pathogenesis of TAO[20]. Thus, TSHR inhibitors may demonstrate to be effective in the treatment or prevention of TAO in future[20].

Relationship Between Orbital Fibroblasts and IGF-1R The insulin-like growth factor-1 receptor (IGF-1R) is a second autoantigen in GD and TAO[21]. It is a tyrosine kinase receptor comprising of two subunits; IGF-1Rα with ligand-binding domain and IGF-1Rβ with tyrosine phosphorylation sites. Overexpression of IGF-1R has been involved in the pathogenesis of many malignant diseases and autoimmune diseases, such as crohn's disease and multiple sclerosis[10]. Autoantibodies directed against IGF-1R have been detected in most GD patients, while the same is uncommon in individuals without the disease[22]. Studies have shown that orbital fibroblasts in TAO patients overexpress IGF-1R when

compared with orbital fibroblasts from normal controls[10]. On exposing to either IGF-1 or GD-IgG, fibroblasts from TAO patients release IL-16 and RANTES, two powerful T cell chemoattractants synthesized by activating the AKT/mTOR/p70S6K signaling pathway, as well as produce hyaluronan[23-25]. Research demonstrated that there was extensive overlap between TSHR and IGF-1R downstream signaling. When the IGF-1R signaling pathway is blocked by monoclonal antibodies, the downstream signaling of TSHR is also attenuated, suggesting a physiological signaling between the two. Further, TSHR and IGF-1R can potentially synergize to form a physical and functional complex that could activate abnormal signaling pathway, such as that related to GD[14].

Association of Orbital Fibroblasts and CD34+ Fibrocytes with Thyroid Antigens Fernando *et al*[26] have reported that CD34+ fibrocytes co-express considerably high levels of thyroglobulin (TG) and TSHR. Further, they also demonstrated that these fibrocytes infiltrated thyroid gland of GD patients, suggesting them as a bridge between TAO and GD. Besides TSHR and TG, the CD34+ fibrocytes and cultured fibrocytes are shown to abundantly express other thyroid-specific proteins, such sodium/iodide symporter (NIS) and thyroid peroxidase (TPO)[27], necessary for thyroid hormone production. The mRNAs and the respective proteins of TSHR, TG, NIS and TPO are extremely higher in fibrocytes from TAO patients and normal individuals, than in orbital fibroblasts of TAO[26-27].

Role of Orbital Fibroblasts in Hyaluronan Production The clinical feature of TAO is enlargement of extraocular muscles. This is mainly due to edema caused by hydrophilic glycosaminoglycans (GAGs), produced by the orbital fibroblasts[28]. Hyaluronic acid (HA) is the main component GAG in TAO orbit[29]. HA can be produced by hyaluronan synthases, HAS1, HSA2 and HAS3[30] and UDP-glucose dehydrogenase (UGDH)[31]. Tsui *et al*[31] reported that higher levels of UGDH was found in an anatomic-specific manner by orbital fibroblasts, due to enhanced activity of UGDH gene promoter and more abundant stability of UGDH mRNA in the orbit, which may be the cause of excessive hyaluronan in the orbit in GD. *In vitro*, orbital fibroblasts response to many inflammation mediators, such as IL-1β, IFN-γ and leukoregulin, by producing excessive amounts of hyaluronan[32-34]. In addition, when induced by CD40L, they produced substantial hyaluronan and prostaglandin E2 (PGE2) synthesis, and PGHS-2 and IL-1α mediated the latter[35]. These cells also produced hyaluronan regulated by TGF-β[36]. Zhang *et al*[37-38] introduced a functional mutant TSHR into orbital fibroblasts, which resulted in increased expression of cAMP and hyaluronan. Guo *et al*[39] reported HA biosynthesis in orbital fibroblasts *via* DP1 activation by mast cell-derived PGD2. Recent studies have demonstrated TSH and IGF-1 to synergistically increase HA secretion in orbital fibroblasts. M22 mediated induction of HA production in TAO fibroblasts/preadipocytes involve cross talk between TSHR and IGF-1R, leads to synergistic stimulation of HA production[40].

Role of Orbital Fibroblasts and CD34+ Fibrocytes in Adipogenesis of TAO Computed tomography (CT) of GD patients indicate pathological changes in TAO including extraocular muscles and orbital fat tissues[41]. Proptosis in TAO is mainly due to enlargement of extraocular muscles and increased orbital fat tissue[42]. Regensburg *et al*[43] reported that the increased orbital fat volume contributed more towards the observed proptosis in TAO patients, than the enlargement of extraocular muscles. Based on the heterogenous expression of Thy-1, orbital fibroblasts can be divided into Thy-1+ and Thy-1- subsets, of which Thy-1- subset underwent adipogenesis in response to peroxisome proliferator-activated receptor (PPAR)-γ agonist[44-45]. During the process, the TSHR levels are elevated in these differentiating orbital fibroblasts. When co-incubated with activated T lymphocytes that produce PPAR-γ ligands, PPAR-γ expressing orbital fibroblasts underwent adipogenesis, and this process could be abated by cyclooxygenase (COX) inhibitors[46]. Further, cigarette smoke extract (CSE) reportedly stimulated HA production and adipogenesis in a dosage-dependent approach in orbital fibroblasts from TAO patients[47]. In addition, IL-1 doubled the magnitude of the effect of CSE on adipogenesis, indicating a synergistic activity between the two[47]. Hypoxia is also found to induce adipogenesis in TAO orbital fibroblasts, and may represent another mechanism by which smoking contributes to deterioration of TAO[48]. CD34+ fibrocytes derive from the bone marrow and infiltrate into the orbit as circulating where they transition into CD34+ fibroblasts. *In vitro*, they can differentiate into adipocytes depending on the microenvironment of their location, where exposure to PPAR-γ agonist will result in adipocytic differentiation[9,49].

Association of Orbital Fibroblasts with Cytokines Infiltration of T cells, B cells, macrophages, monocytes and mast cells were found in orbital fat and extraocular muscle in TAO patients[50-52]. It seems that cytokine-dependent fibroblast activation leads to TAO tissue remodeling. This might be due to the abnormal susceptibility of orbital fibroblasts to the induction of proinflammatory cytokines[53]. Hwang *et al*[54] treated TAO orbital fibroblasts with IFN-γ and observed an upregulation of CD40 expression, which could be blocked in the presence of dexamethasone. On further exposure of these cells to CD40 ligand, an upregulation in the production of IL-6, IL-8, and MCP-1 was observed. On exposure to IL-1β and IgGs from GD patients, the TAO orbital fibroblasts also produce IL-16 and RANTES, *via* IGF-1R signaling[55-57,23].

Consequently, TAO orbital fibroblasts might play crucial role in T cell infiltration of the orbit and B cell differentiation. This observed effect of differential expression of cytokines and its receptors on TAO orbital fibroblasts might be of use in the future research towards its treatment.

Association of Orbital Fibroblasts with Inflammatory Mediators and Adhesion Molecules Orbital fibroblasts express high levels of prostaglandins, lipoxygenase, and chemokines under the stimulation of cytokines, thereby initiating a series of inflammatory reactions. B cell class-switching[58], T cell differentiation[59], and mast cell degranulation are influenced by PGE2[60], all of which may play a role in TAO. Excess production of PGE2 is probably an autocrine process of TAO orbital fibroblasts, and could be related to the immune response and inflammation of the orbital tissue. Adhesion molecules mediate contact and adhesion between the cells, and are related to the aggregation and migration of leukocytes[61]. Orbital fibroblasts of TAO express high levels of intercellular adhesion molecule-1 (ICAM-1, CD54) when induced by cytokines, such as IL-1α, TNF-α and IFN-γ. This response is observed both in fibroblasts from TAO patients and normal individuals[62]. The percentage of ICAM-1+ conjunctival epithelial cells in active TAO patients can be used as a marker of local inflammation of the disease[63].

Role of Orbital Fibroblasts and CD34+ Fibrocytes in the Treatment of TAO The clinically available treatment of TAO is limited to systemic corticosteroids and orbital radiation. Immunomodulation, targeting antigen receptors, inflammatory cytokines and immune cell depletion, is a new approach in the treatment of TAO. Teprotumumab, an IGF-IR inhibitory monoclonal antibody can inhibit both antigen (TSHR and IGF-1R) expression on CD34+ fibrocytes and TSH-induced cytokine (IL-6 and IL-8) production, by partially inhibiting phosphorylation of AKT[64] and has recently demonstrated substantial therapeutic benefit in active, moderate to severe TAO[65]. Recent human studies using anti-CD20 monoclonal antibody, which targets CD20 and its precursors on B cells, has shown improvement in disease activity and severity of TAO[66-67]. Other drugs, such as anti- CD52 antibody alemtuzumab, TNF-α blocking adalimumab, IL-6 and IL-17 receptor blockers, small molecule antagonists of TSHR, and PPAR-γ antagonists are possible potential treatments to TAO, and may hold promise in the near future[68].

CONCLUSION

Orbital fibroblasts are the key target and effector cells in the pathogenesis of TAO, which shows complex biological activities in the development of the condition. These cells can not only recognize autoantigen, but also secret cytokines and inflammatory mediators, produce GAGs and even can differentiate into adipocytes. CD34+ fibrocytes, circulating in the peripheral blood, will infiltrate the orbital tissues in TAO and produce many inflammatory cytokines, while also co-express TG and TSHR autoantigens.

In summary, orbital fibroblasts and CD34+ fibrocytes play major role in the pathogenesis of TAO by altering immune response, increasing inflammation and remodeling of orbits in TAO patients, targeting which might aid in developing potential new treatment to the condition.

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REFERENCES

1 Smith TJ, Hegedüs L. Graves' disease. *N Engl J Med* 2017;376(2):185.

2 Bahn RS. Current insights into the pathogenesis of Graves' ophthalmopathy. *Horm Metab Res* 2015;47(10):773-778.

3 Lu Y, Atkins SJ, Fernando R, Trierweiler A, Mester T, Grisolia ABD, Mou P, Novaes P, Smith TJ. CD34- orbital fibroblasts from patients with thyroid-associated ophthalmopathy modulate TNF-α expression in CD34+ fibroblasts and fibrocytes. *Invest Ophthalmol Vis Sci* 2018;59(6): 2615-2622.

4 Tai WL, Zhou ZP, Zheng BY, Li JN, Ding JW, Wu HX, Gao L, Dong ZX. Inhibitory effect of circulating fibrocytes on injury repair in acute lung injury/acute respiratory distress syndrome mice model. *J Cell Biochem* 2018;119(10):7982-7990.

5 Nielepkowicz-Goździńska A, Fendler W, Robak E, Kulczycka-Siennicka L, Górski P, Pietras T, Brzeziańska E, Pietrusińska M, Antczak A. The role of CXC chemokines in pulmonary fibrosis of systemic lupus erythematosus patients. *Arch Immunol Ther Exp* 2015;63(6):465-473.

6 Heukels P, van Hulst JAC, van Nimwegen M, Boorsma CE, Melgert BN, van den Toorn LM, Boomars KAT, Wijsenbeek MS, Hoogsteden H, von der Thüsen JH, Hendriks RW, Kool M, van den Blink B. Fibrocytes are increased in lung and peripheral blood of patients with idiopathic pulmonary fibrosis. *Respir Res* 2018;19(1):90.

7 García de Alba C, Buendia-Roldán I, Salgado A, Becerril C, Ramírez R, González Y, Checa M, Navarro C, Ruiz V, Pardo A, Selman M. Fibrocytes contribute to inflammation and fibrosis in chronic hypersensitivity pneumonitis through paracrine effects. *Am J Respir Crit Care Med* 2015;191(4):427-436.

8 Douglas RS, Afifiyan NF, Hwang CJ, Chong K, Haider U, Richards P, Gianoukakis AG, Smith TJ. Increased generation of fibrocytes in thyroid-associated ophthalmopathy. *J Clin Endocrinol Metab* 2010;95(1):430-438.

9 Smith TJ. TSH-receptor-expressing fibrocytes and thyroid-associated ophthalmopathy. *Nat Rev Endocrinol* 2015;11(3):171-181.

10 Mohyi M, Smith TJ. IGF1 receptor and thyroid-associated ophthalmopathy. *J Mol Endocrinol* 2018;61(1):T29-T43.

11 Heufelder AE, Dutton CM, Sarkar G, Donovan KA, Bahn RS. Detection of TSH receptor RNA in cultured fibroblasts from patients with Graves' ophthalmopathy and pretibial dermopathy. *Thyroid* 1993;3(4):297-300.

12 Valyasevi RW, Erickson DZ, Harteneck DA, Dutton CM, Heufelder AE, Jyonouchi SC, Bahn RS. Differentiation of human orbital preadipocyte fibroblasts induces expression of functional thyrotropin receptor. *J Clin Endocrinol Metab* 1999;84(7):2557-2562.

13 van Zeijl CJ, Fliers E, van Koppen CJ, Surovtseva OV, de Gooyer ME, Mourits MP, Wiersinga WM, Miltenburg AM, Boelen A. Thyrotropin receptor-stimulating Graves' disease immunoglobulins induce hyaluronan synthesis by differentiated orbital fibroblasts from patients with Graves' ophthalmopathy not only via cyclic adenosine monophosphate signaling pathways. *Thyroid* 2011;21(2):169-176.

14 Tsui S, Naik V, Hoa N, Hwang CJ, Afifiyan NF, Sinha Hikim A, Gianoukakis AG, Douglas RS, Smith TJ. Evidence for an association between thyroid-stimulating hormone and insulin-like growth factor 1 receptors: a tale of two antigens implicated in Graves' disease. *J Immunol* 2008;181(6):4397-4405.

15 Gillespie EF, Papageorgiou KI, Fernando R, Raychaudhuri N, Cockerham KP, Charara LK, Goncalves AC, Zhao SX, Ginter A, Lu Y, Smith TJ, Douglas RS. Increased expression of TSH receptor by fibrocytes in thyroid-associated ophthalmopathy leads to chemokine production. *J Clin Endocrinol Metab* 2012;97(5):E740-E746.

16 Smith TJ. Potential role for bone marrow-derived fibrocytes in the orbital fibroblast heterogeneity associated with thyroid-associated ophthalmopathy. *Clin Exp Immunol* 2010;162(1):24-31.

17 Li B, Smith TJ. Regulation of IL-1 receptor antagonist by TSH in fibrocytes and orbital fibroblasts. *J Clin Endocrinol Metab* 2014;99(4): E625-E633.

18 Douglas RS, Mester T, Ginter A, Kim DS. Thyrotropin receptor and CD40 mediate interleukin-8 expression in fibrocytes: implications for thyroid-associated ophthalmopathy (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 2014;112:26-37.

19 Raychaudhuri N, Fernando R, Smith TJ. Thyrotropin regulates IL-6 expression in CD34+ fibrocytes: clear delineation of its cAMP-independent actions. *PLoS One* 2013;8(9):e75100.

20 Turcu AF, Kumar S, Neumann S, Coenen M, Iyer S, Chiriboga P, Gershengorn MC, Bahn RS. A small molecule antagonist inhibits thyrotropin receptor antibody-induced orbital fibroblast functions involved in the pathogenesis of Graves ophthalmopathy. *J Clin Endocrinol Metab* 2013;98(5):2153-2159.

21 Smith TJ. Rationale for therapeutic targeting insulin-like growth factor-1 receptor and bone marrow-derived fibrocytes in thyroid-associated ophthalmopathy. *Expert Rev Ophthalmol* 2016;11(2):77-79.

22 Douglas RS, Gianoukakis AG, Kamat S, Smith TJ. Aberrant expression of the insulin-like growth factor-1 receptor by T cells from patients with Graves' disease may carry functional consequences for disease pathogenesis. *J Immunol* 2007;178(5):3281-3287.

23 Pritchard J, Horst N, Cruikshank W, Smith TJ. Igs from patients with Graves' disease induce the expression of T cell chemoattractants in their fibroblasts. *J Immunol* 2002;168(2):942-950.

24 Smith TJ, Hoa N. Immunoglobulins from patients with Graves' disease induce hyaluronan synthesis in their orbital fibroblasts through the self-antigen, insulin-like growth factor-I receptor. *J Clin Endocrinol Metab* 2004;89(10):5076-5080.

25 Smith TJ. The insulin-like growth factor-I receptor and its role in thyroid-associated ophthalmopathy. *Eye (Lond)* 2019;33(2):200-205.

26 Fernando R, Atkins S, Raychaudhuri N, Lu Y, Li B, Douglas RS, Smith TJ. Human fibrocytes coexpress thyroglobulin and thyrotropin receptor. *Proc Natl Acad Sci U S A* 2012;109(19):7427-7432.

27 Fernando R, Lu Y, Atkins SJ, Mester T, Branham K, Smith TJ. Expression of thyrotropin receptor, thyroglobulin, sodium-iodide symporter, and thyroperoxidase by fibrocytes depends on AIRE. *J Clin Endocrinol Metab* 2014;99(7):E1236-E1244.

28 Fang SJ, Huang YZ, Zhong SS, Li YY, Zhang YD, Li YW, Sun J, Liu XT, Wang Y, Zhang S, Xu TL, Sun XD, Gu P, Li D, Zhou HF, Li B, Fan XQ. Regulation of orbital fibrosis and adipogenesis by pathogenic Th17 cells in graves orbitopathy. *J Clin Endocrinol Metab* 2017;102(11): 4273-4283.

29 Dik WA, Virakul S, van Steensel L. Current perspectives on the role of orbital fibroblasts in the pathogenesis of Graves' ophthalmopathy. *Exp Eye Res* 2016;142:83-91.

30 van Zeijl CJ, Fliers E, van Koppen CJ, Surovtseva OV, de Gooyer ME, Mourits MP, Wiersinga WM, Miltenburg AM, Boelen A. Effects of thyrotropin and thyrotropin-receptor-stimulating Graves' disease immunoglobulin G on cyclic adenosine monophosphate and hyaluronan production in nondifferentiated orbital fibroblasts of Graves' ophthalmopathy patients. *Thyroid* 2010;20(5):535-544.

31 Tsui S, Fernando R, Chen BL, Smith TJ. Divergent Sp1 protein levels may underlie differential expression of UDP-glucose dehydrogenase by fibroblasts: role in susceptibility to orbital Graves disease. *J Biol Chem* 2011;286(27):24487-24499.

32 Wang HS, Cao HJ, Winn VD, Rezanka LJ, Frobert Y, Evans CH, Sciaky D, Young DA, Smith TJ. Leukoregulin induction of prostaglandin-endoperoxide H synthase-2 in human orbital fibroblasts. An in vitro model for connective tissue inflammation. *J Biol Chem* 1996;271(37): 22718-22728.

33 Spicer AP, Kaback LA, Smith TJ, Seldin MF. Molecular cloning and characterization of the human and mouse UDP-glucose dehydrogenase genes. *J Biol Chem* 1998;273(39):25117-25124.

34 Smith TJ, Bahn RS, Gorman CA, Cheavens M. Stimulation of glycosaminoglycan accumulation by interferon gamma in cultured human retroocular fibroblasts. *J Clin Endocrinol Metab* 1991;72(5):1169-1171.

35 Cao HJ, Wang HS, Zhang Y, Lin HY, Phipps RP, Smith TJ. Activation of human orbital fibroblasts through CD40 engagement results in a dramatic induction of hyaluronan synthesis and prostaglandin endoperoxide H synthase-2 expression. Insights into potential pathogenic mechanisms of thyroid-associated ophthalmopathy. *J Biol Chem* 1998;273(45):29615-29625.

36 Guo NX, Woeller CF, Feldon SE, Phipps RP. Peroxisome proliferator-activated receptor gamma ligands inhibit transforming growth factor-beta-induced, hyaluronan-dependent, T cell adhesion to orbital fibroblasts. *J Biol Chem* 2011;286(21):18856-18867.

37 Zhang L, Baker G, Janus D, Paddon CA, Fuhrer D, Ludgate M. Biological effects of thyrotropin receptor activation on human orbital preadipocytes. *Invest Ophthalmol Vis Sci* 2006;47(12):5197-5203.

38 Zhang L, Bowen T, Grennan-Jones F, Paddon C, Giles P, Webber J, Steadman R, Ludgate M. Thyrotropin receptor activation increases hyaluronan production in preadipocyte fibroblasts: contributory role in hyaluronan accumulation in thyroid dysfunction. *J Biol Chem* 2009;284(39):26447-26455.

39 Guo NX, Baglole CJ, O'Loughlin CW, Feldon SE, Phipps RP. Mast cell-derived prostaglandin D2 controls hyaluronan synthesis in human orbital fibroblasts via DP1 activation: implications for thyroid eye disease. *J Biol Chem* 2010;285(21):15794-15804.

40 Krieger CC, Neumann S, Place RF, Marcus-Samuels B, Gershengorn MC. Bidirectional TSH and IGF-1 receptor cross talk mediates stimulation of hyaluronan secretion by Graves' disease immunoglobins. *J Clin Endocrinol Metab* 2015;100(3):1071-1077.

41 Wiersinga WM. Advances in treatment of active, moderate-to-severe Graves' ophthalmopathy. *Lancet Diabetes Endocrinol* 2017;5(2):134-142.

42 Wagner LH, Seiff SR. New antibody-based therapies for thyroid-associated ophthalmopathy. *Surv Ophthalmol* 2018;63(3):447.

43 Regensburg NI, Wiersinga WM, Berendschot TT, Potgieser P, Mourits MP. Do subtypes of Graves' orbitopathy exist? *Ophthalmology* 2011;118(1):191-196.

44 Koumas L, Smith TJ, Feldon S, Blumberg N, Phipps RP. Thy-1 expression in human fibroblast subsets defines myofibroblastic or lipofibroblastic phenotypes. *Am J Pathol* 2003;163(4):1291-1300.

45 Koumas L, Smith TJ, Phipps RP. Fibroblast subsets in the human orbit: Thy-1+ and Thy-1- subpopulations exhibit distinct phenotypes. *Eur J Immunol* 2002;32(2):477-485.

46 Feldon SE, O'loughlin CW, Ray DM, Landskroner-Eiger S, Seweryniak KE, Phipps RP. Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. *Am J Pathol* 2006;169(4):1183-1193.

47 Cawood TJ, Moriarty P, O'Farrelly C, O'Shea D. Smoking and thyroid-associated ophthalmopathy: a novel explanation of the biological link. *J Clin Endocrinol Metab* 2007;92(1):59-64.

48 Chng CL, Lai OF, Chew CS, Peh YP, Fook-Chong SM, Seah LL, Khoo DH. Hypoxia increases adipogenesis and affects adipocytokine production in orbital fibroblasts-a possible explanation of the link between smoking and Graves' ophthalmopathy. *Int J Ophthalmol* 2014;7(3):403-407.

49 Hong KM, Belperio JA, Keane MP, Burdick MD, Strieter RM. Differentiation of human circulating fibrocytes as mediated by transforming growth factor-beta and peroxisome proliferator-activated receptor gamma. *J Biol Chem* 2007;282(31):22910-22920.

50 Förster G, Otto E, Hansen C, Ochs K, Kahaly G. Analysis of orbital T cells in thyroid-associated ophthalmopathy. *Clin Exp Immunol* 1998;112(3):427-434.

51 van Steensel L, Paridaens D, van Meurs M, van Hagen PM, van den Bosch WA, Kuijpers RW, Drexhage HA, Hooijkaas H, Dik WA. Orbit-infiltrating mast cells, monocytes, and macrophages produce PDGF isoforms that orchestrate orbital fibroblast activation in Graves' ophthalmopathy. *J Clin Endocrinol Metab* 2012;97(3):E400-E408.

52 Chen MH, Chen MH, Liao SL, Chang TC, Chuang LM. Role of macrophage infiltration in the orbital fat of patients with Graves' ophthalmopathy. *Clin Endocrinol (Oxf)* 2008;69(2):332-337.

53 Smith TJ. Insights into the role of fibroblasts in human autoimmune diseases. *Clin Exp Immunol* 2005;141(3):388-397.

54 Hwang CJ, Afifiyan N, Sand D, Naik V, Said J, Pollock SJ, Chen BL, Phipps RP, Goldberg RA, Smith TJ, Douglas RS. Orbital fibroblasts from patients with thyroid-associated ophthalmopathy overexpress CD40: CD154 hyperinduces IL-6, IL-8, and MCP-1. *Invest Ophthalmol Vis Sci* 2009;50(5):2262-2268.

55 Gianoukakis AG, Douglas RS, King CS, Cruikshank WW, Smith TJ. Immunoglobulin G from patients with Graves' disease induces interleukin-16 and RANTES expression in cultured human thyrocytes: a putative mechanism for T-cell infiltration of the thyroid in autoimmune disease. *Endocrinology* 2006;147(4):1941-1949.

56 Sciaky D, Brazer W, Center DM, Cruikshank WW, Smith TJ. Cultured human fibroblasts express constitutive IL-16 mRNA: cytokine induction of active IL-16 protein synthesis through a caspase-3-dependent mechanism. *J Immunol* 2000;164(7):3806-3814.

57 Pritchard J, Han R, Horst N, Cruikshank WW, Smith TJ. Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway. *J Immunol* 2003;170(12):6348-6354.

58 Brown DM, Warner GL, Alés-Martínez JE, Scott DW, Phipps RP. Prostaglandin E2 induces apoptosis in immature normal and malignant B lymphocytes. *Clin Immunol Immunopathol* 1992;63(3):221-229.

59 Betz M, Fox BS. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J Immunol* 1991;146(1):108-113.

60 Torres R, Picado C, de Mora F. The PGE2-EP_2-mast cell axis: an antiasthma mechanism. *Mol Immunol* 2015;63(1):61-68.

61 Khong JJ, McNab AA, Ebeling PR, Craig JE, Selva D. Pathogenesis of thyroid eye disease: review and update on molecular mechanisms. *Br J Ophthalmol* 2016;100(1):142-150.

62 Heufelder AE, Bahn RS. Graves' immunoglobulins and cytokines stimulate the expression of intercellular adhesion molecule-1 (ICAM-1) in cultured Graves' orbital fibroblasts. *Eur J Clin Invest* 1992;22(8):529-537.

63 Pawlowski P, Mysliwiec J, Mrugacz M, Zak J, Bakunowicz-Lazarczyk A, Rejdak R, Wysocka J, Gorska M. Elevated percentage of HLA-DR^+ and ICAM-1^+ conjunctival epithelial cells in active Graves' orbitopathy. *Graefes Arch Clin Exp Ophthalmol* 2014;252(4):641-645.

64 Chen H, Mester T, Raychaudhuri N, Kauh CY, Gupta S, Smith TJ, Douglas RS. Teprotumumab, an IGF-1R blocking monoclonal antibody inhibits TSH and IGF-1 action in fibrocytes. *J Clin Endocrinol Metab* 2014;99(9):E1635-E1640.

65 Smith TJ, Kahaly GJ, Ezra DG, Fleming JC, Dailey RA, Tang RA, Harris GJ, Antonelli A, Salvi M, Goldberg RA, Gigantelli JW, Couch SM, Shriver EM, Hayek BR, Hink EM, Woodward RM, Gabriel K, Magni G, Douglas RS. Teprotumumab for thyroid-associated ophthalmopathy. *N Engl J Med* 2017;376(18):1748-1761.

66 Salvi M, Vannucchi G, Currò N, Campi I, Covelli D, Dazzi D, Simonetta S, Guastella C, Pignataro L, Avignone S, Beck-Peccoz P. Efficacy of B-cell targeted therapy with rituximab in patients with active moderate to severe Graves' orbitopathy: a randomized controlled study. *J Clin Endocrinol Metab* 2015;100(2):422-431.

67 Stan MN, Garrity JA, Carranza Leon BG, Prabin T, Bradley EA, Bahn RS. Randomized controlled trial of rituximab in patients with Graves' orbitopathy. *J Clin Endocrinol Metab* 2015;100(2):432-441.

68 Rajaii F, McCoy AN, Smith TJ. Cytokines are both villains and potential therapeutic targets in thyroid-associated ophthalmopathy: from bench to bedside. *Expert Rev Ophthalmol* 2014;9(3):227-234.