The role of cell mediated immunopathogenesis in thyroid-associated ophthalmopathy

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Received: 2019-01-03 Accepted: 2019-05-21

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

DOI:10.18240/ijo.2019.07.24


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]. Orbital 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 gland [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 exposure 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 with Cytokines 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, HAS2 and HAS3[30] and UDP-glucose dehydrogenase (UDGDH)[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-β1[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-1β 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[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, lipooxygenase, and chemokines under the stimulation of cytokines, thereby initiating a series of inflammatory reactions. B cell class-switching,[59] 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 IGFR1) 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 cannot 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.

**ACKNOWLEDGEMENTS**

**Foundations:** Supported by National Natural Science Foundation of China (No.81200719); China Postdoctoral Science Foundation (No.2013M543579; No.2014T71013); Key Specialized Projects in Nanjing (No.SZDZK2016008).

**Conflicts of Interest:** Wang ZM, None; Wang ZY, None; Lu Y, None.

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