Update on Immunopathogenesis of Graves' Ophthalmopathy

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Speaker Disclosures

Inventor of the following issued US Patents

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7998681
8153121
8178304

Consultant: Novartis
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Learning Objectives

Review recent developments in solving the pathogenesis of TAO

Introduce the concept that bone marrow derived fibrocytes express high levels of TSHR and infiltrate the orbit in TAO.

Discuss the concept that fibrocytes express several “thyroid-specific” proteins that become suppressed by native orbital fibroblasts

Introduce the concept that the orbit in TAO represents a recapitulation of the thyroid
Graves’ Disease

- Thyrotoxicosis
- Ophthalmopathy
- Dermopathy

PRESENTATION FROM THE 83rd ANNUAL MEETING OF THE AMERICAN THYROID ASSOCIATION, OCTOBER 16-20, 2013 (Terry J. Smith)
Disease

Active Phase

Ideal Immunomodulatory Therapy

Time

18-36 months

5-7 years

Stable Phase

Surgery

PRESENTATION FROM THE 83rd ANNUAL MEETING OF THE AMERICAN THYROID ASSOCIATION, OCTOBER 16-20, 2013 (Terry J. Smith)
Orbital fibroblasts exhibit a unique phenotype

Differential Gene Expression in Orbital Fibroblasts

<table>
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<tr>
<th>Gene/Response</th>
<th>Reference</th>
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<tbody>
<tr>
<td>PAI-I</td>
<td>Smith et al AJP 1992</td>
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<td>HLA-DR</td>
<td>Heufelder et al JCEM 1991</td>
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<td>Endothelin-I</td>
<td>Smith et al AJP 1993</td>
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<td>Actin- Rearrangement</td>
<td>Smith et al PNAS 1994</td>
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<td>HA</td>
<td>Smith et al AJP 1995</td>
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<td>Adipogenesis</td>
<td>Sorisky et al JCEM 1996</td>
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<tr>
<td>COX-2, PGE₂</td>
<td>Wang et al JBC 1996</td>
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<tr>
<td>IL-6, IL-8, CD40</td>
<td>Sempowski et al AJP 1998</td>
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<tr>
<td>Actin Neighbor Protein</td>
<td>Young et al PNAS 1998</td>
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<td>HA, HAS-2</td>
<td>Cao et al JBC 1998</td>
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<tr>
<td>HAS 1, HAS-2, HAS 3</td>
<td>Kaback et al JCEM 1999</td>
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<td>IL-16, RANTES</td>
<td>Sciaky et al J Immunol 2000</td>
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<tr>
<td>PPAR-α</td>
<td>Smith et al JCEM 2002</td>
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<td>PGHS-2, mPGS</td>
<td>Han et al JBC 2002</td>
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<tr>
<td>IL-IRA</td>
<td>Cao et al AJP 2003</td>
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<tr>
<td>IGF-IR</td>
<td>Pritchard et al J Immunol 2003</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Han &amp; Smith J Immunol 2005</td>
</tr>
<tr>
<td>15-Lipoxygenase</td>
<td>Chen et al JBC 2006</td>
</tr>
<tr>
<td>UDP-glucose dehydrogenase</td>
<td>Tsui et al JBC 2011</td>
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Identifying an Orbital Antigen Explaining TAO

• While TSHR represents the central antigen in GD, the mechanisms through which orbital tissues become activated remains uncertain.

• Multiple antigens have been proposed as potentially important to the pathogenesis of TAO
  • TSHR (Fenzi et al, Bahn et al, several others)
  • Tg (Kriss, Marino et al)
  • Muscle antigens (Wall et al)
  • IGF-1R (Kendall-Taylor, Smith)

To date, no convincing evidence substantiates antigen-specific T cell infiltration of the orbit in TAO
Electroporation of TSHR-Expressing Plasmid Results in Orbital Inflammation

• Electroporation with TSHR A subunit containing plasmid leads to a phenotype both typical and atypical of TAO
  + • Orbital congestion
  + • Muscle enlargement
  + • Orbital fat expansion
  + • Infiltration of T cells, F4/80+ macrophages, and mast cells
  - • Predominance of TSHR blocking Abs and hypothyroidism
  - • Dramatic mononuclear infiltration of the optic nerve

• IGF-1R containing plasmids failed to elicit pathology
• Anti-IGF-1R Abs became detectable in many animals immunized with TSHR.

Moshkelgosha et al Endocrinology 2013
Electroporation of TSHR-Expressing Plasmid Results in Orbital Inflammation

Moshkelgosha et al
Endocrinol 2013
Conundrum: TSHR or IGF-1R. Which is the relevant orbital antigen?
IGF-1R Mediates Induction of T Cell Chemokines TAO Fibroblasts

- IGF-1
- GD-IgG
- Anti-IGF-1R Abs
TSHR and IGF-1R Co-Localize and Form a Signaling Complex

IGF-1Rβ (red) TSHR (green) demonstrates Co-localization (yellow/orange)

(A–C) TAO orbital fibroblasts
(D–F) Thyrocytes
(G–I) TAO orbital fibroblasts
(J–L) TAO orbital fibroblasts IGF-1Rα vs TSHR
(M–O) TAO orbital fat

IGF-1R Inhibition Attenuates TSHR Signaling

- Inhibition of IGF-1R with 1H7, a blocking mAb, attenuates signaling through both IGF-1R and TSHR

TAO Fibroblast Heterogeneity-Divergent Capacity to Differentiate into Adipocytes and Myofibroblasts

Thy-1⁺

Myofibroblast

Thy-1⁻

Adipocyte

Smith and Phipps 1995

TGF-β

PPARγ
TAO Orbital Fibroblasts Can be Further Segregated into CD34\(^+\) and CD34\(^-\) Cells
CD34+ Fibrocytes

Hematopoietic Stem cells

Multipotent progenitors

Circulating CD14+ Precursors

Circulating Fibrocytes

Recruitment Chemokine signals

Migrate to sites of injury

Myofibroblast

Fibroblast

Fibroadipocyte

Bucala et al 1994

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Peripheral Blood Fibrocytes Increased in GD

p<0.001

Control: n=25
Graves': n=70

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TAO fibrocytes display a typical phenotype

- CD34
- CXCR4
- CD11b
- CD14
- Col1
- α-actin
- GD
- ISO

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Unlike TAO Fibroblasts, IGF-1 Receptor Levels on Fibrocytes are Relatively Low
But, TSHR Levels on Fibrocytes Exceed Those on TAO Orbital Fibroblasts

Gillespie et al JCEM (2012)
Fibrocytes Express Cytokines in Response to TSH

Douglas et al., 2010
Fibrocytes Infiltrate the Orbit in TAO

A. Graves’ CD34

B. Healthy CD34

C. Graves’ LSP

D. Healthy LSP

E. CD 31

F.
Do Fibrocytes Express Other “Thyroid-Specific” Proteins?

Fernando et al PNAS 2012
Tg mRNA Expression Results from an Active Gene Promoter

Fernando et al PNAS 2012
Fibrocytes Express NIS and TPO

NIS mRNA (fold-change)

TPO mRNA (fold-change)

69 kDa

103 kDa

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New Graves’ Paradigm

CD34+ Fibrocyte

T cell Activation
MHC-Class II

CD34
TSHR
CD86

Iodine
Tg
TPO

CD80

CD40

UP IGF-1R
DOWN TSHR
Negative signals

CD34- Fibroblast

CD34+ Fibroblast

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Circulation

CD34+ Fibrocytes
IGF-1 Receptor
TSH Receptor

Orbital Tissue

CD34+ Fibroblasts
IGF-1 Receptor
TSH Receptor
Conclusions

Orbital fibroblasts in TAO exhibit unique phenotypes.

TSHR/IGF-1R complexes form in fibroblasts and fibrocytes. Interrupting IGF-1R function attenuates Erk and Akt signaling initiated by TSHR and blocks cytokine induction.

CD34$^+$ fibrocytes express functional TSHR, Tg, NIS, and TPO and infiltrate the orbit in TAO where levels drop. This results from negative influence from native CD34$^-$ orbital fibroblasts.

These insights might lead to more specific therapies.

Could the TAO orbit represent a recapitulation of the thyroid?
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